after central venous catheter or fistula handling; preparation of material, connection, disconnection, dressing, and manipulation of lines and before and after direct contact with a patient; handling of other invasive devices, if present; measurement of temperature; measurement of arterial pressure; etc.). The handling of catheter and fistula were considered to be activities with high risk of HCV transmission. Overall, 2382 opportunities were observed during 197 shifts, with a total of 98 h of observation.

Glove use was observed during the same periods as was hand hygiene. For each care activity, the following variables were collected on the same standardized questionnaire as that used for hand hygiene: type of contact, wearing gloves during contact, and glove removal immediately after contact. Wearing gloves is recommended in the unit when exposure to body fluids is anticipated.

With consideration that the nurse-to-patient ratio (including nurses and nurse assistants) may influence the risk of HCV transmission, the ratio was recorded during each observation period, and the average nurse-to-patient ratio per shift (morning, afternoon, and night) was determined by calculating the median ratio for all the relevant observation periods. Hand hygiene compliance was also calculated for each of the 3 shifts.

Statistical analysis. Percentages and 95% CIs were calculated. The χ^2 test or Fisher's exact test was used, as appropriate, to compare proportions. The Mann-Whitney nonparametric test was used to compare continuous variables. Each potential risk factor for environmental hemoglobin contamination (i.e., nurse-to-patient ratio and hand hygiene compliance) was tested in a univariate model, and results were then entered in a logistic regression model. Variables were not dichotomized. To take into account the interdependence of observations made during the same shift, we used robust estimates of variance (generalized estimating equations) in which each shift observation was included as a cluster. Goodness of fit was assessed using the Hosmer-Lemeshow χ^2 test, and discrimination was determined from the area under the receiver operating characteristics curve. Accuracy was considered to be good when the area under the receiver operating characteristics curve had a range of 0.70–0.80 and was considered to be excellent when it was >0.80. The adjusted OR and 95% CI were calculated for each factor that was statistically significant in the logistic regression model. *P* values <.05 were considered to be statistically significant. All tests were 2 tailed. Statistical tests were performed using Intercooled Stata software, version 8.2 (Stata).

RESULTS

Virological study of environmental surfaces. A total of 740 surface samples were collected in the dialysis unit during June–August 2005, comprising 663 (90%) from dialysis machines and 77 (10%) from other surfaces (table 1). Hemoglobin was found in 82 samples (11%), including 71 (10%) from surfaces where blood was not evident. Among the 25 hemoglobin-positive samples collected from dialysis machines, 5 had been obtained after external disinfection of the machine. Six (7%) of the 82 hemoglobin-positive samples contained detectable levels of HCV RNA, comprising 4 samples taken from a dialysis machine and 2 from a shared waste cart (table 1). The HVR1-coding region could be PCR-amplified and sequenced in 5 of these 6 samples, designated S1–S5. These sequences were compared with HVR1 sequences recovered from patients 1–8 during the at-risk period (except for patient 5, in whom HVR1 could not be amplified) and also from patient 3 at the time of surface sampling (figure 2). As shown in figure 2, phylogenetic analysis revealed that all sequences found in environmental samples were closely related to those isolated from patient 2 when he was infected in 2004 and to those from patient 3, from whom samples were obtained both in 2004 and in 2005. Note also in figure 2 the very slow genetic evolution of the HVR1 in patient 3 (only 4 nucleotide substitutions accumulated in 14 months; data not shown), probably because of hemodialysis-associated immune suppression. Interestingly, the same HCV strain was isolated from 2 environmental samples taken at a 6-h interval from the same machine that had been used to treat 2 different patients.

Assessment of practices. Compliance with local precautions for machine use and internal disinfection was adequate. Multidose vials were never shared between patients. The finding that patients 2 and 3, who were infected with closely related HCV strains (figures 1 and 2), had always undergone dialysis during the same sessions but had never shared the same machine strongly suggested that patient 2 had been infected by patient 3 via the hands of a health care worker.

Compliance with standard precautions during the investigation is shown in figure 3. Overall, 2382 opportunities for hand hygiene were observed (2358 [99%] for nurses; 24 [1%]

Table 1. Environmental samples containing hemoglobin and/or hepatitis C virus (HCV) RNA.

Sample site	No. of samples	Positive samples, no. (%)	
		Hemoglobin	HCV RNA
Dialysis machine	663	36 (5)	4 (11)
Shared waste cart	27	24 (89)	2 (8)
Patients' removable table	9	6 (67)	0 (0)
Miscellaneous[a]	41	16 (39)	0 (0)
Total	740	82 (11)	6 (7)

NOTE. HCV RNA–positive findings are percentages of the number of hemoglobin-positive samples.

[a] Including nursing preparation area, wheelchairs, and patient file cart.

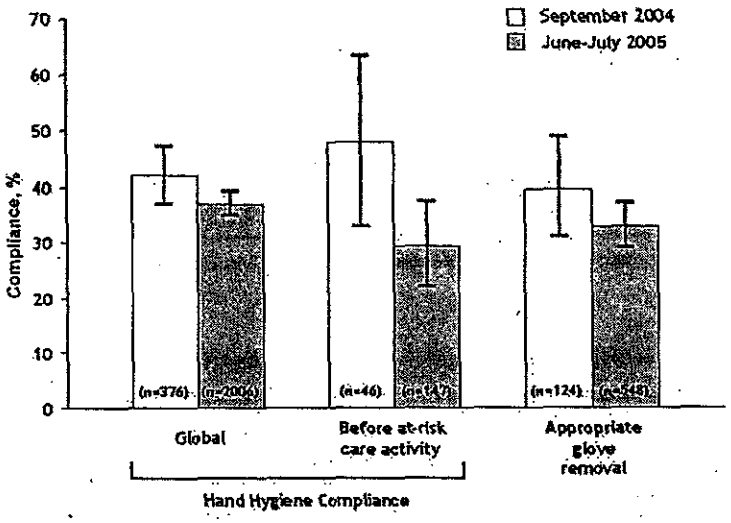

Figure 3. Compliance with guidelines for health care worker hand hygiene and appropriate glove use during dialysis. At-risk care activities consisted of handling dialysis catheters or fistulas. Whiskers, 95% CIs.

for nurse assistants). Immediately after the infection alert (September 2004), compliance with hand hygiene immediately before handling a dialysis catheter or fistula was significantly higher ($P < .001$) than it was several months later (figure 3). Globally, gloves were worn in 857 (36%) of observed contacts with a patient or the environment. When worn, gloves were removed immediately after a contact in only 672 (34.1%) of cases (95% CI, 30.5%–37.8%). There was no statistically significant difference between the findings of the 2 periods of observation. As shown in table 2, a low nurse-to-patient ratio and a poor rate of hand hygiene compliance were independently associated with the detection of hemoglobin on environmental surfaces.

DISCUSSION

Several reports of nosocomial HCV transmission in the hemodialysis setting have been published, but the investigations were incomplete and the routes of transmission remained unclear [13, 17, 18, 26]. Allander et al. [26] reported nosocomial HCV transmission in a series of patients who underwent dialysis at the same time but who did not share dialysis equipment. Those authors postulated, but did not show, that the environment was contaminated. Compliance with standard precautions was not studied.

To our knowledge, ours is the first study to demonstrate that a low nurse-to-patient ratio and poor compliance with guidelines for hand hygiene and glove use are independent predictors of environmental contamination by blood and HCV. By combining genetic and phylogenetic analyses of HCV recovered from patients' blood and the environment with measurements of compliance with standard precautions, we showed that: (1) 2 sporadic cases of HCV transmission occurred in the dialysis unit during the study period, 1 of which was unequivocally due to patient-to-patient transmission within the unit; (2) the dialysis environment was frequently contaminated by blood, including HCV-infected patients' blood, as shown by the detection of hemoglobin, sometimes associated with detectable levels of HCV RNA in a substantial proportion of swabs; and (3) compliance with guidelines for hand hygiene and glove use during patient care was poor, raising the possibility of HCV transmission via the hands of health care workers. Interestingly, all HCV-infected blood found in environmental samples belonged to the patient who indirectly infected another patient undergoing dialysis.

In our study, hemoglobin was found in 11% of environmental samples, and 7% of those positive samples contained detectable HCV RNA. Hepatitis B virus transmission has been linked to the presence of the virus on environmental surfaces—in the absence of visible blood [27]. Hepatitis B virus has been reported to remain viable on environmental surfaces for at least 7 days at room temperature [28, 29]. HCV RNA has been shown to be resistant for at least 48 h on inert surfaces at room temperature [24, 30, 31]. A robust cell culture system for HCV was recently developed, but it cannot be infected with viruses other than those produced after cell culture transfection of a specific HCV clone [32–34]. Cell culture systems that can be directly infected by HCV-infected patients' blood will be needed to determine how long HCV remains infective in the environment. Even in the absence of such data, our results strongly suggest that infectious HCV is present in the dialysis environment and that HCV can be transmitted by the hands of health care workers. We did not, however, sample health care workers'

Table 2. Factors independently associated with environmental blood contamination during nursing shifts.

Variable	Univariate analysis of environmental hemoglobin, by daily shifts		Multivariate analysis	
	Hemoglobin found (n = 28)	Hemoglobin not found (n = 14)	OR (95% CI)	P
Nurse-to-patient ratio, mean ± SD	0.55 ± 0.23	0.78 ± 0.50	0.03 (0.002–0.39)	.008
Hand hygiene compliance, mean % ± SD	39 ± 15	44 ± 17	0.93 (0.88–0.99)	.036

NOTE. Performance of the model, Hosmer-Lemeshow goodness-of-fit; P = .386; area under receiver operating characteristics curve, 0.768.

gloved or ungloved hands during care activities, because this would have hindered the assessment of compliance with standard precautions by increasing the Hawthorne effect.

The rate of compliance with standard precautions in our study was similar to that reported elsewhere about a similar setting [35, 36]. A recent survey of hand hygiene practices in 9 Spanish hemodialysis units showed poor compliance, both before and after contact with patients (14% and 36%, respectively) [36].

Permanent glove use can impair compliance with hand hygiene [37] and may thus lead to cross-transmission of infectious agents. This is the first time that glove use and removal have been studied in relation to the risk of environmental contamination. Gloves are worn mainly for health care worker self-protection, rather than to prevent patient cross-infection. The recommendation that gloves always be worn in the hemodialysis setting, whatever the type of contact (environment or patient) [38], therefore, may be confusing and may expose patients to HCV transmission if not followed properly, with systematic glove removal and hand hygiene between care procedures.

We found that a nurse-to-patient ratio <0.60 was independently associated with hemoglobin contamination of environmental surfaces. Understaffing is a recognized major risk factor for nosocomial infection [39–41]. Recently, a Brazilian study of 22 dialysis centers showed that the number of patients per health care worker was independently related to the risk of hepatitis B virus infection [16]. Petrosillo et al. [42] showed, in a prospective multicenter study in Italian hemodialysis units, that a low staff-to-patient ratio is an independent predictor of the risk of HCV nosocomial transmission. Therefore, to limit the spread of blood in the dialysis environment, we recommend that at-risk care procedures, such as connection and disconnection of equipment to the patient, be performed by a pair of nurses: one working with the patient and the other working with the machine.

In conclusion, blood-contaminated surfaces may represent a source of HCV transmission, via health care workers' hands or gloves. Environmental contamination is mainly a consequence of poor adherence to standard precautions in the hemodialysis setting. Strict adherence to guidelines for hand hygiene and glove use and strict organization of care procedures, with an adequate nurse-to-patient ratio, should help to reduce the risk of environmental contamination and, thus, HCV transmission in patients undergoing dialysis.

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Potential conflicts of interest. All authors: no conflicts.

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研究報告の概要

○養豚業従事者のE型肝炎ウイルス(HEV)への職業的曝露
本研究の目的は、ブタ接触群(養豚業従事者)と非接触群のHEV陽性率とウイルス感染リスク因子を調べることであった。合計198名[非接触者97名(49%)、接触者101名(51%)]を対象にHEV感染の有無を調べた。抗HEV IgG抗体陽性率はブタ接触群が18.8%、非接触群が4.1%であった。ブタ接触者の抗HEV IgG抗体陽性リスクは5.4倍(P = 0.03)であった。IgG抗体陽性者10名(52.6%)は、未処理水の摂取およびブタへの接触という2つの汚染リスク因子を示した。以上のデータは、HEV感染を養豚従事者の職業病として扱うべきことを裏付けるものである。したがって、当該ウイルスへの曝露を予防するために、当該集団における包括的な衛生措置の適用が強く推奨される。

報告企業の意見	今後の対応
職業上のブタ接触群と非接触群のHEVの陽性率とウイルス感染リスク因子を調べたところ、抗HEV IgG抗体陽性率はブタ接触群が有意に高く、陽性者は未処理水の摂取およびブタへの接触という2つのリスクファクターを示したとの報告である。HEV感染については血液の安全対策上だけではなく、公衆衛生及び食品衛生上の問題でもある。	日本赤十字社では、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以上の血液を排除している。また、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。加えて、北海道における輸血後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。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-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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Short Report: Occupational Exposure to Hepatitis E Virus (HEV) in Swine Workers

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Abstract. The aim of this work was to study the prevalence of hepatitis E virus (HEV) and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine. A total of 198 individuals, 97 unexposed (49%) and 101 exposed (51%) to swine, were tested for the presence of HEV infection. The prevalence of anti-HEV IgG in the exposed group was 18.8% versus 4.1% in the unexposed to swine group. People exposed to swine were observed to be 5.4 times ($P = 0.03$) at risk of having anti-HEV IgG. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this collective is highly recommended to avoid the exposition to this virus.

Hepatitis E virus (HEV) is the main causative agent of enterically transmitted non-A non-B hepatitis and self-limiting clinical presentation in humans.[1] It is a non-enveloped virus with a positive-sense, single-stranded RNA genome of ~7,200 nucleotides in length and contains three open reading frames (ORFs). Nowadays, HEV is classified into the family *Hepeviridae*, genus *Hepevirus*. Regarding the phylogeny, HEV has been divided into four genotypes,[2] although only one serotype of HEV is recognized.[3] Transmission of HEV infection primarily occurs through contaminated water, although person to person transmission and sexual transmission occur infrequently.

Hepatitis E has been considered an infectious endemic in developing areas such as India, Africa, and Southeast Asia, because of poor sanitary conditions in drinking water.[4] The mortality rate of hepatitis E in the normal population is generally < 1%, but it can be as high as 20–25% among pregnant women.

In industrialized countries, HEV has been found mainly in individuals who had traveled to endemic zones. Actually, the increasing number of autochthonous cases of hepatitis E[5] and the recent findings of HEV in domestic animals such as swine give rise to the suspicion that HEV is underdetected in idiopathic non-A non-B hepatitis. Therefore, the transmission pathways from animals to humans remain obscure. However, in developed countries, seroprevalence ranges varying from 1–18% have been reported. In the last years, several studies have been published describing differences in the prevalence of anti-HEV antibodies between people exposed and not exposed to swine,[6–12] but the risk factors for the acquisition of the virus have not been studied.

Accordingly, the aim of this work was to study the prevalence of HEV and the risk factors for the acquisition of the virus in healthy Spanish people distributed in exposed and unexposed to swine groups.

A retrospective study was carried out to determine the prevalence of HEV during the period from October 2004 to July 2007 in Spain.

A total number of 198 healthy individuals, 101 (51%) men and 97 (49%) women, were included in this study to detect the prevalence of HEV. Participants filled out an epidemiologic questionnaire including name, age, area of residence, travel abroad, exposure to swine, and consumption of raw vegetables, raw shellfish, and untreated water. Informed approval was obtained from all participants. Individuals were divided into two separate groups taking into consideration exposition to swine: 97 unexposed (NE; 27 men and 70 women) and 101 exposed (E; 74 men and 27 women). Individuals included in the E group were made up of swine farmers, pig handlers, and swine veterinarians, whereas the NE group was made up of volunteers with no contact with swine.

Blood samples were obtained from all the participants by venipuncture, and sera were obtained and frozen at −20°C until used. RNA was extracted from 140 μL of each serum using a commercial kit following the manufacturer's instructions (QIampViral RNA Kit; Qiagen, Valencia, CA). Two pairs of degenerate oligonucleotide primers[13] were used to amplify a 348-bp fragment of ORF-2 of HEV using a reverse transcriptase–nested polymerase chain reaction (PCR).[14] These primers were based on 18 human HEV sequences and the swine HEV prototype strain from the United States. A positive control from a naturally infected pig (GenBank accession number AY323506) was included in each procedure. Different stages of assay were performed in different places to avoid the possibility of cross-contamination. The PCR products were separated by electrophoresis in 2% agarose and were detected by staining with ethidium bromide.

Sera from all individuals were tested for the presence of HEV antibodies (anti-HEV IgG and IgM) using a commercial ELISA (Fortress Diagnostics, Antrim, UK) according to the manufacturer's instructions. This kit used polystyrene microwell strips precoated with recombinant HEV antigens (HEV-Ag) corresponding to structural proteins ORF2, derived from genotype 1. The sensitivity and specificity of the ELISA assay use in this study were determined by the manufacturer as 92% and 88%, respectively. Positive results obtained using this assay were confirmed by means of an HEV immunoblot test (Recomblot HEV-IgG/IgM; Mikrogen, Martinsried, Germany). Antigens used in this kit were the N-terminal part of the capsid antigen (GST fusion protein O2N, 50 kd), the C-terminal part of the capsid antigen (triple band; O2C 38–41 kd), the middle part of the capsid antigen (O2M; 28 kd), and the ORF3 protein (O3; 15 kd) of genotypes 1 and 2.

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