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CASE REPORT

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Transfusion-transmitted hepatitis B virus (HBV) infection from an individual-donation nucleic acid (ID-NAT) non-reactive donor

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Lookback was initiated upon notification of an acute HBV infection in a repeat Irish donor, 108 days post-donation. The donation screened non-reactive by individual-donation nucleic acid testing (ID-NAT) using the Procleix Ultrio Elite multiplex assay and again when the archived sample was retested, but the discriminatory assay for HBV was reactive. The immunocompromised recipient of the implicated red cell component was tested 110 days post-transfusion, revealing a HBV DNA viral load of 470 IU/ml. Genotype C2 sequences identical across two regions of the HBV genome were found in samples from the donor and recipient.

Key words: blood donation testing, blood safety, NAT testing, transfusion - transmissible infections, transfusion medicine (in general).

Introduction

Among the major blood-borne pathogens, transfusion-transmitted HBV (TT-HBV) carries the highest residual risk [1]. ID-NAT for HBV DNA was introduced by the Irish Blood Transfusion Service (IBTS) in May 2009 in addition to the existing serological assays for hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) (Abbott Prism). ID-NAT screening is conducted using the Procleix Ultrio Elite (UE) multiplex assay on the Panther platform (Grifols Diagnostic Solutions Inc.). The first reported case of a TT-HBV infection from a donation screened by ID-NAT was published in 2012 [2]. We report the first case of TT-HBV in a HBV low-endemic country using the UE assay.

Case history

The IBTS was notified by the Public Health Service of a blood donor who presented with a short history of jaundice and fatigue due to acute HBV infection. The donor screened negative for HBV markers (HBsAg, anti-HBc and DNA) at the time of their most recent donation 108 days earlier. Although the archive sample from this donation tested non-reactive with the UE assay (S/CO = 0·08), the UE discriminatory (d) HBV DNA assay was reactive (S/CO = 24·04, Table 1). Viral load estimation was not possible. The donor was compliant with all donation guidelines on the index and ten preceding donations. A follow-up interview with the unvaccinated donor revealed one casual protected heterosexual contact 7 months prior to the day of the implicated donation. No other classical risk exposures for HBV were elicited including any high-risk sexual activity, injecting drug - use, or contact with HBV-infected household members.

Two days into standard treatment for acute myeloid leukaemia (day 0), the recipient was transfused with a unit of five-day-old red blood cells from the only component of the implicated donation (average plasma volume, 6·5 ml), as well as one other red cell unit and three doses of apheresis platelets (from two donors). The recipient was recalled 110 days post-transfusion (PT) when HBsAg, anti-HBc IgM and anti-HBc total antibodies (Abbott Architect) were negative (Table 1). However, HBV DNA was detected (Abbott Real*time* PCR) with a viral load of 470 IU/ml.

The asymptomatic patient then commenced treatment with entecavir 0·5 mg once daily. Serological and

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Table 1 Summary of donor and recipient test results

	Donor (repeat blood donor)			Recipient		
Marker	**Index Donation (ID)**	**Symptomatic Phase (post-donation day + 108)**	**Donor Archive (retrospective repeat testing of index donation)**	**Pre-Transfusion (day - 4)**	**PT[d] (day + 110)**	**PT (day + 121)**
HBsAg	Negative[b]	Positive[c]	Negative[b]	Negative[c]	Negative[c]	Positive[c]=(Murex HBsAg neutralization positive)
Anti-HBc (total)	Negative[b]	Positive[c]	Negative[b]	Negative[c]	Negative[c]	Negative[c]
Anti-HBc IgM		Positive[c]		Negative[c]	Negative[c]	Negative[c]
Anti-HBe		Positive[c]				Negative[c]
Ultrio Elite[a] (Multiplex HIV-1/-2, HCV, HBV)	Negative (0·07)		Negative (0·08)			
dHBV DNA UE assay			Positive (24·04)			
HBV DNA Viral load (Abbott real-time PCR)		Detected 4225 IU/ml	Insufficient sample volume	Not[e] Detected	Detected 470 IU/ml	Detected <10 IU/ml (on entecavir)

[a]S/CO = Sample to cut-off ratio.
[b]Abbott Prism.
[c]Abbott Architect®.
[d]PT = Post-Transfusion.
[e]Diluted sample - lower limit of detection 100 IU/ml.

molecular testing of a serum sample taken 4 days before the implicated transfusion excluded a pre-existing HBV infection (Table 1).

From day −2 to day +110 post the implicated transfusion, the recipient received blood components from sixty-one other donors – RBCs (n = 18), platelets: apheresis (n = 14), pooled (n = 8). As part of the investigation, fifty-eight donors were retested for HBsAg, anti-HBc total (Abbott Architect) and HBV DNA (Procleix UE, Grifols) a minimum of 6 months from their donation(s) to the recipient. The archive from the three non-returning donors was negative for HBV DNA (Procleix UE), HBsAg and anti-HBc total (Abbott Architect). Retrospective anti-HBs testing was undertaken in the twelve apheresis donors (average plasma volume per apheresis platelet unit, 240 ml) five of whom had detectable anti-HBs levels ranging from 10 to 121 mIU/ml. Six platelet doses from these donors were infused on day 0 (n = 3), day 5 (n = 1), day 8(n = 1) and day 11(n = 1). No other risk factor for HBV infection was identified in the recipient.

Sequence and phylogenetic analysis across two regions of the HBV genome was undertaken on HBV DNA extracted from the donor and recipient samples. Analysis indicated the virus in samples from both donor and recipient to belong to genotype C2 and to be, 99·9% and 100% identical at the nucleotide level across the surface/polymerase and the x/precore/core regions. The virus strains clustered together when analysed against contemporaneous HBV C2 sequences from Ireland and the UK (Fig. 1).

The manufacturer challenged different master lots of the UE and dHBV assays with a low copy number HBV panel (4 IU/ml); no statistically significant variation in sensitivity was observed. Dilutions of a sample from the implicated donor (post-donation day +123, viral load 120 IU/ml Aptima HBV Quant Assay, Hologic) of 20, 6 and 2 IU/ml were tested in replicates (r) using the UE assay (n = 10r, 10r, and 20r, respectively); reactivity was observed in all replicates. Sequencing analysis of the donor sample did not reveal any mismatches or mutations that would have impacted assay sensitivity, personal communication [3].

Discussion

Over 1·2 million donations have been screened for HBV DNA at the IBTS by ID-NAT since 2009. Of the 30 confirmed HBV infections identified, 22 have been in first-time donors born outside of Ireland, five in first-time Irish donors and only three (now four) in repeat Irish donors. In the latter group, two cases were NAT-yield window period (WP) cases (HBV DNA positive, HBsAg and anti-HBc negative). Using the incidence rate/window period model (WP of UE assay of 16·3 days), the per unit residual risk for HBV in IBTS donations was estimated at one in two million donations [4, 5].

This is the first reported case of a TT-HBV in which the UE assay was used for screening blood donations. Although the package insert (PI) suggests reduced

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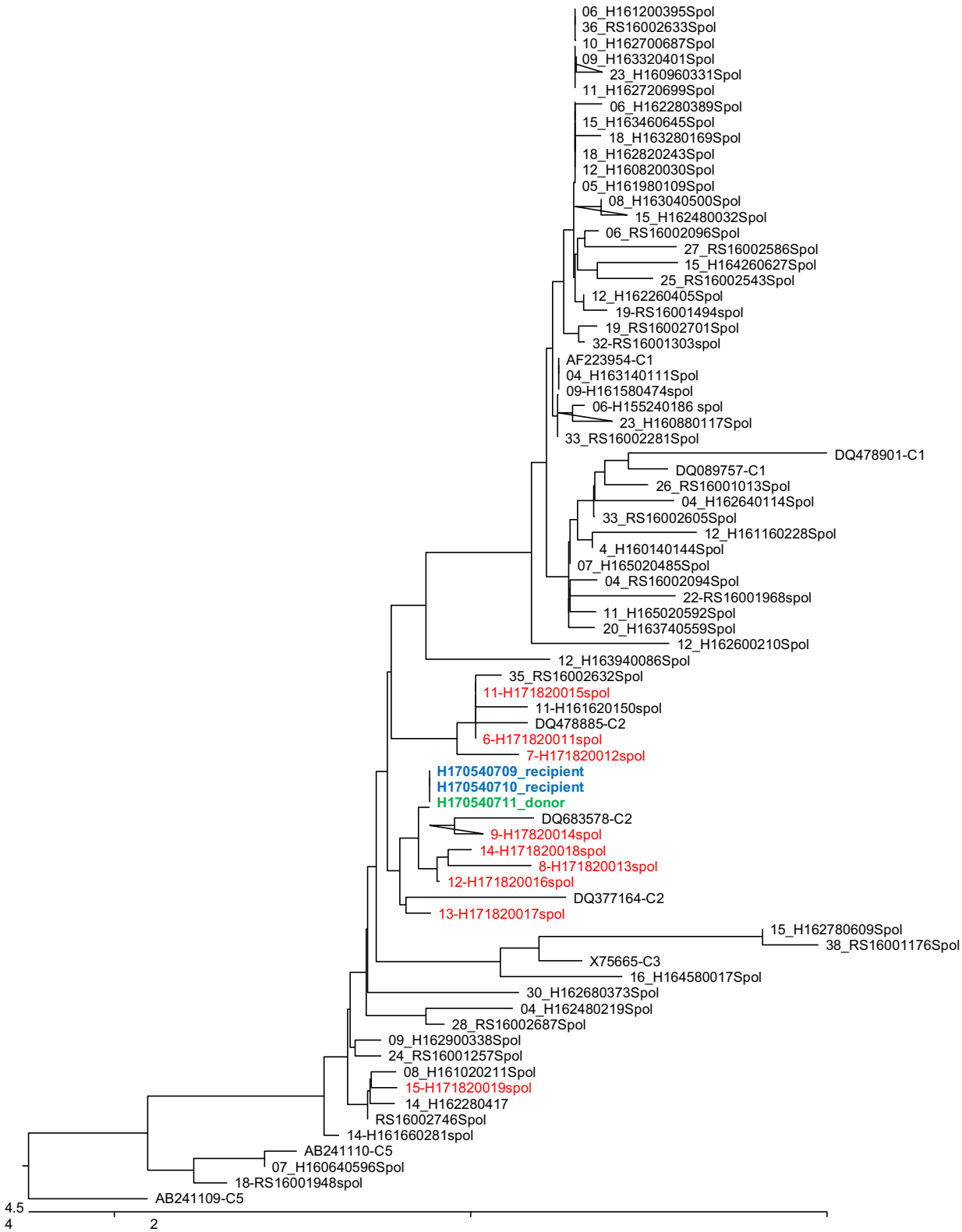

Fig. 1 Genotype C specific phylogenetic tree of ~1 Kb region of the HBV surface/polymerase region. The donor sequences are shown in green and recipient sequences in blue. Genotype C sequences generated in Blood-borne Virus Unit (BBVU) from January 2016 onwards are indicated in black, genotype C sequences from National Virus Reference Laboratory (NVRL, Dublin) are shown in red.

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sensitivity for HBV genotype C for both UE and UE dHBV assays, the manufacturer's investigation demonstrated satisfactory assay performance at a low HBV concentration. [6]. Our results suggest that the implicated donation in our case contained a very low viral load and was obtained in the pre-ID-NAT window period for HBV.The detection of HBV DNA in one of three tests is therefore consistent with stochastic sampling variation in a sample with a very low viral load. The TTI was facilitated by the transmissibility of HBV which approaches 100% even with a very low copy number in the unit [7]. The viral load in the recovered plasma unit associated with the first reported case of TT-HBV infection from an RBC unit screened using ID-NAT (Ultrio) was estimated by probit analysis to be approximately 1·6 (1·1–2·1) copies/ml. Reactivity was observed in 10% (3/30) replicate Ultrio and 33% replicate Ultrio Plus assays [2]. The archived sample from a donation that screened PCR negative in a minipool, which was implicated in the transmission of HBV to two recipients reported in the UK, was positive for HBV DNA in only one of two individual sample tests by PCR [8]. Although the course of HBV infection in our case was probably modified by the contemporaneous passive transfusion of anti-HBs and the low viral inoculum, as has been previously reported transmission was not averted [9–11]. Robust public health measures however allowed prompt investigation, diagnosis and management of HBV infection in the recipient.

References

1 Candotti D, Allain JP: Transfusion-transmitted hepatitis B virus infection. *J Hepatol* 2009; **51**:798–809

2 Vermeulen M, Dickens C, Lelie N, *et al.*: Hepatitis B virus transmission by blood transfusion during 4 years of individual-donation nucleic acid testing in South Africa: estimated and observed window period risk. *Transfusion* 2012; **52**:880–892

3 Linnen JM: PhD, VP, Product Development. Personal communication, R& D. Grifols Diagnostic Solutions Inc., 2017

4 Schreiber GB, Busch MP, Kleinman SH, *et al.*: The risk of transfusion-transmitted viral infections. *N Engl J Med* 1996; **334**:1685–1690

5 Grifols Procleix Ultrio Elite Assay. Available at: http://164.109.68.1/products/procleixassays/ultrio-elite.shtml. Accessed 13 May 2016

6 Procleix Ultrio Elite Assay Package Insert 503049EN Rev. 002 (Accessed August 2017)

7 Weusten J, Vermeulen M, van Drimmelen H: Refinement of a viral transmission risk model for blood donations in seroconversion window phase screened by nucleic acid testing in different pool sizes and repeat test algorithms. *Transfusion* 2011; **51**:203–15

8 *Serious Hazards of Transfusion* Annual report 2012. Available at: https://www.shotuk.org/wp-content/uploads/SHOT-Annual-Report-20121.pdf. Accessed August 2017

9 Gerlich W, Wagner F, Chudy M, *et al.*: HBsAg non-reactive HBV infection in blood donors: transmission and Pathogenicity. *J Med Virol* 2007; **79**: S32–S36

10 Wendel S, Levi JE, Biagini S: A probable case of hepatitis B virus transfusion transmission revealed after a 13 month long window period. *Transfusion* 2008; **48**:1602–1608

11 Hollinger FB, Dodd RY: Hepatitis B virus traceback and lookback: factors to consider. *Transfusion* 2009; **49**:176–184

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