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Phylogenetic analysis reveals three distinct epidemiological profiles in Dutch and Flemish blood donors with hepatitis B virus infection

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ABSTRACT

During 2006–2016, hepatitis B virus (HBV) was detected in nearly 400 blood donors in the Netherlands and Flanders. Donor demographics and self-reported risk factors as disclosed during the donor exit interview were compared to HBV phylogenies of donor and reference sequences. First-time donors with chronic HBV-infection were often immigrants (67%) infected with genetically highly diverse strains of genotypes A (32%), B (8%), C (6%), D (53%) and E to H (1%). Each subtype was strongly associated with donor ethnicity. In contrast, 57/62 (93%) of acute/recent HBV infections occurred among indigenous donors, of whom 67% was infected with one specific widely circulating epidemic HBV-A2 lineage. HBV typing identified three distinct epidemiological profiles: the import of chronic HBV infections through migration, longstanding transmission of non-epidemic HBV-A2 strains within western-Europe, and the active transmission of one epidemic HBV-A2 strain most likely fueled by sexual risk behavior.

1. Introduction

Blood services apply multiple strategies to protect recipients from transfusion transmissible infections (TTIs), including laboratory testing for TTIs, pathogen reduction technologies and donor selection criteria Germain and Goldman, 2002). In the Netherlands and Flanders, all donations are tested for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis (Slot et al., 2016; Vandewalle et al., 2014). Nonetheless, a small risk of acquiring TTIs from donors with (very) recent infection remains (Kleinman et al., 2009). To avoid infectious window-period donations, blood services have enforced risk-behavior based donor deferral (RBDD) criteria (Offergeld et al., 2014). RBDD is not 100% effective as donors might not disclose (recent) risk behavior out of social discomfort, disagreement with donor deferral criteria, test-seeking behavior, the underestimation of own risk behavior, and the genuine belief that nondisclosure poses no real risk to the recipient since all blood is tested (Goldman and O'Brien, 2016; Lucky et al., 2014; Wong et al., 2015). The estimated non-compliance rate of male donors with undeclared male-to-male sex, probably the most debated donor deferral criterion, varies between 0.23–2.6% (Goldman and O'Brien, 2016).

The residual risk for acquiring TTIs through blood or blood products in the Netherlands and Flanders is extremely low (Slot et al., 2016; Vandewalle et al., 2014). The highest risk remains for HBV as (i) HBV has a relatively long infectious window-period compared to HIV and HCV, (ii) HBV accounts for the majority of viral TTIs in first-time and repeat donors, and (iii) detection of occult HBV characterized by low viral loads and absence of HBsAg remains problematic (Slot et al., 2016; Kleinman et al., 2009; van de Laar et al., 2015). Post-donation risk questionnaires are frequently used to study determinants of TTIs in donor populations. However, only 9–22% of donors with recent HBV infection report risk factors, which if revealed prior to donation, would have resulted in donor deferral (Slot et al., 2016; Custer et al., 2015; Lucky et al., 2013). Phylogenetic analysis provides an alternative approach to study transmission patterns of viruses with extensive genetic diversity like HBV. In a previous study, molecular typing of HIV-strains obtained from Dutch and Flemish donors suggested severe under-reporting of male-to-male sex among HIV-1 infected male donors, and confirmed heterosexual contact with partners from HIV-endemic areas as the predominant mode of HIV-1 transmission in female donors (van

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de Laar et al., 2017). Like HIV-1, HBV is divided in multiple genotypes (A to H) and subgenotypes (e.g. A1, A2) (Pourkarim et al., 2014). Different HBV (sub)genotypes have evolved around the world and spread via (at least partly) separated transmission networks, causing the genomes of individual HBV strains to carry information on geographic origin, transmission route and time of infection (Paraskevis et al., 2013; Zehender et al., 2015; Soetens et al., 2015; Lin et al., 2016). The current study aims to improve our understanding of the underlying risk factors of HBV-infections among Dutch and Flemish donors by shifting the focus from the donor to the infecting viral strain.

2. Materials and methods

2.1. Study population: HBV infected Dutch and Flemish donors

This study includes all HBV DNA positive first-time and repeat blood donors in the Netherlands and Flanders in the period 2006–2016. Before each donation, donors complete a standardized donor health questionnaire (DHQ). If risk factors leading to lifetime donor deferral (e.g. male-to-male sex, commercial sex work or injecting drug use (IDU)) were reported in the DHQ, donors were not tested for TTIs. In December 2015, the lifetime deferral of men who have sex with men (MSM) in the Netherlands was changed to a deferral period of 12 months after the last male-to-male sexual contact. Donors with confirmed HBV-infection were invited to the blood bank for medical counseling, and for repeat testing to exclude mistaken donor identities and laboratory errors. A trained medical counselor conducted a face-to-face interview using a standardized posttest questionnaire dealing with potential risk factors for sexually transmitted infections (STIs) and blood-borne infections to identify the most likely route of transmission (Slot et al., 2016). Risk factors were categorized as blood-related (e.g. IDU, transfusion, needle-stick injuries, potential blood-contact, tattoo/piercing, acupuncture, medical procedures), sexual (e.g. number of (new) sexual partners, context and nature of sexual encounters, history of STIs, and potential risk factors present among sexual partners) or endemic (country of birth, ethnicity, travel). For each self-reported risk factor, the country of potential HBV exposure was documented.

2.2. Routine HBV donor screening and confirmation testing

Routine HBV donor screening in the Netherlands and Flanders was highly similar (Slot et al., 2016; Vandewalle et al., 2014). All donations were individually tested for HBsAg (PRISM HBsAg, Abbott Diagnostics). HBV DNA screening was performed in pools of 6 donations (Cobas Taqscreen MPX, Roche Diagnostics) and was introduced in November 2008 (the Netherlands) and January 2010 (Flanders). HBV DNA positive pools were deconstructed to identify the individual HBV DNA positive donation. HBV confirmation testing occurred for all HBV DNA reactive nd/or HBsAg repeat reactive donations, and included an alternative HBV DNA assay, HBsAg neutralization and anti-HBcore testing. Various commercial assays have been used over time; all tests were performed as described by the manufacturer. In the Netherlands, but not in Flanders, universal anti-HBcore screening was implemented in November 2011 to prevent transmission of occult HBV infection. Dutch testing algorithms and donor deferral protocols with regards to anti-HBcore reactivity in the absence of HBV DNA and HBsAg have been described in detail previously (van de Laar et al., 2015).

2.3. Definitions of acute, recent and chronic HBV infection

Acute HBV infection was defined as the presence of HBV DNA (with or without HBsAg) in the absence of anti-HBcore. HBV infections in anti-HBcore reactive donors were defined as ‘recent’ or ‘chronic’ depending on the presence (or absence) of a documented HBV-negative test result in the two years preceding the HBV-positive visit.

2.4. HBV genotyping and phylogenetic analysis

HBV DNA isolation was performed on 400 µl of plasma using QIAmp MinElute® Virus Spin Kits. Reverse transcription, PCR and sequencing were performed as described previously (Lieshout-Krikke et al., 2013). Two nested PCR assays were used to amplify a 1010 nt (Pre-Core/Core) and a 967 nt fragment (Polymerase/pre-S1/pre-S2/Surface) of the HBV genome. The HBV genotype was determined by phylogenetic analysis of the concatenated HBV fragment (1977 nt) along with well-established HBV reference sequences from GenBank (Pourkarim et al., 2014). Phylogenetic trees of donor sequences were constructed using the maximum-likelihood (ML) approach in MEGA (v6.0). Evolutionary model selection was performed after comparison of the corrected Aikaike information criterion (AICc) scores for each model. The general time reversed model with a γ-distribution of among-site rate assuming a certain fraction of evolutionary invariable sites (GTR + G + I) provided the best fit. Bootstrap values (n = 500) were calculated to analyze the stability of the tree topology. HBV donor sequences were submitted to Genbank (KX354997, KX372028-KX372218, MG383595-MG383625).

2.5. Statistical analysis

Standard binomial regression models were used to assess trends in the HBV prevalence in first-time donors (expressed per 100,000 donors) and the HBV incidence in repeat donors (expressed per 100,000 donor years [DY]). The HBV incidence was not adjusted for transient antigenemia (i.e. anti-HBcore seroconversion without detectable HBV DNA). Different models were applied to detect continuous, stepwise or monotonic changes in the proportion of infected donors over time using R software (https://cran.r-project.org/bin/windows/base/old/3.1.1) (Janssen et al., 2009). Chi-squared and Mann-Whitney *U* tests were used to evaluate differences in proportions and continuous variables between subsets of donors, respectively. Analyses were performed using the IBM SPSS (v23.0); P-values less than 0.050 were considered statistically significant.

3. Results

3.1. HBV prevalence and incidence in Dutch and Flemish donors

During 2006–2016, 362,814 and 286,013 first-time donors were screened for TTIs in the Netherlands and Flanders, respectively. HBV-infection was detected in 159 Dutch and 181 Flemish first-time donors. The HBV prevalence decreased by 3.71 per 100,000 donors ($p < 0.001$) and 6.11 per 100,000 donors ($p < 0.001$) each year in the Netherlands and Flanders, respectively. The maximum and minimum HBV prevalence were 65.8 vs. 24.8 per 100,000 first-time donors in the Netherlands, and 99.1 vs. 29.5 per 100,000 first-time donors in Flanders (Fig. 1). HBV screening in repeat donors equaled 3,432,396 DYs and 1,497,492 DYs in the Netherlands and in Flanders, respectively. HBV-infection was detected in 35 Dutch and 21 Flemish repeat donors. The overall HBV incidence rate was low and remained stable over time: 1.02 vs. 1.40 per 100,000 DYs in the Netherlands and Flanders, respectively.

3.2. Acute, recent and chronic HBV infections

In total 396 donors (340 first-time, 56 repeat) tested positive for HBV DNA in the period 2006–2016. Of the 56 HBV-infections among repeat donors, 40 were classified as ‘acute’ and 16 were classified as ‘recent’. The median interval between the last HBV-negative and the first HBV-positive visit was 87 days [Inter quartile range (IQR): 69–138 days]. In first-time donors, 6 HBV-infections were defined as ‘acute’, and 334 HBV-infections were defined as ‘chronic’. After the implementation of NAT screening (November 2008 in the Netherlands;