Hepatitis B virus infection in blood donors in Argentina: prevalence of infection, genotype distribution and frequency of occult HBV infection

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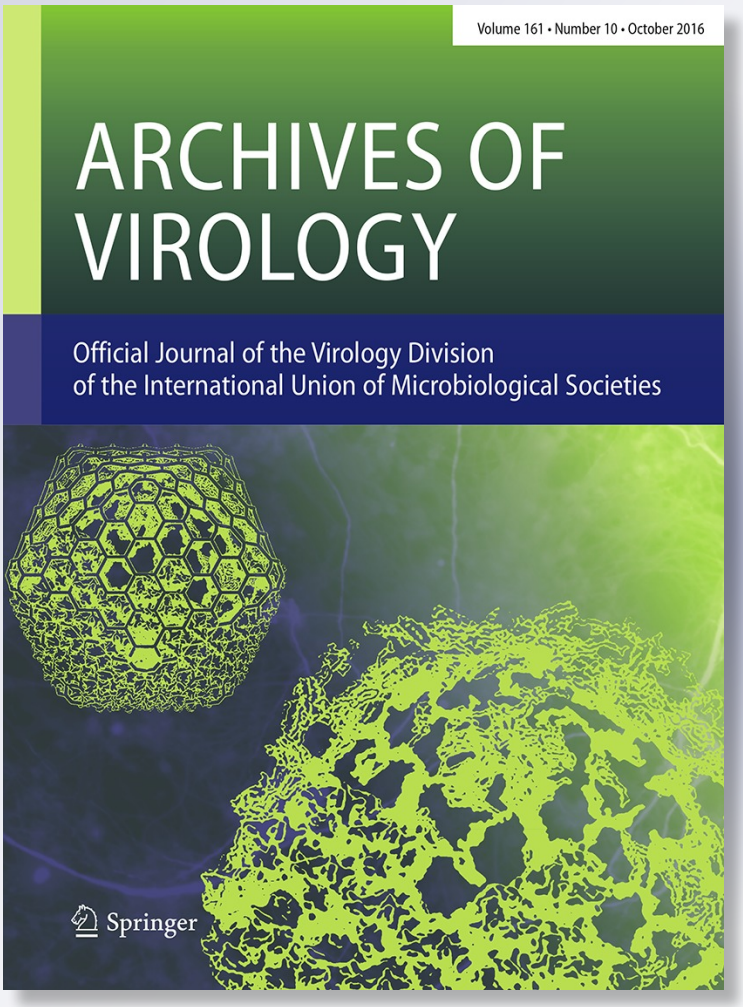

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BRIEF REPORT

Hepatitis B virus infection in blood donors in Argentina: prevalence of infection, genotype distribution and frequency of occult HBV infection

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Abstract This study describes the prevalence of HBV infection based on detection of HBsAg and HBV-DNA by NAT in 70,102 blood donors in Argentina (Córdoba province) and shows the viral genotype distribution and frequency of occult HBV infection (OBI) in this population. Forty-two donors were confirmed positive for HBV infection (0.06 %), and four had OBI. Genotype F was the most prevalent (71.4 %), followed by A (14.3 %), C (7.1 %) and D (7.1 %). This is the first report of the prevalence of confirmed HBV infection and the high frequency of occult HBV infection in a blood bank in Argentina.

Keywords HBV · Blood bank · Genotype · Prevalence · NAT

Hepatitis B virus (HBV) (family *Hepadnaviridae, genus Orthohepadnavirus*) is one of the most important human pathogens. It is estimated that more than 2 billion people have been infected with this virus, [1] and 240 million individuals are chronically infected worldwide [2]. Genetic analysis based on comparison of complete HBV genomes has identified at least nine different genotypes (gts) (A-J), which differ in 8 % or more on their entire nucleotide sequences [3–5].

M. B. Pisano and S. Blanco contributed equally to this work.

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HBV has been identified as one of the most important agents responsible for transfusion-transmitted infections (TTIs), together with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) [6]. To prevent HBV transmission by transfusion, donor selection is carried out using serological assays to detect antibodies against the virus, as well as viral antigens, using different screening immunoassays. Serological screening have greatly reduced, but not eliminated, the risk of transmission of HBV by transfusion of blood and blood products [7].

Nucleic acid amplification testing (NAT) has also been included for HCV, HBV and HIV screening in blood banks in some countries, since this technique shortens the window period [8]. For HBV, NAT is also useful for detecting occult HBV infections (OBI), defined according to the consensus definition of OBI as the persistence of HBV DNA in liver tissue (and in some cases also in serum) in the absence of HBs antigen (HBsAg), with or without anti-HBc and/or anti-HBsAg antibodies [9, 10]. Because of the introduction of NAT, the risk of acquiring HBV infections through transfusions has progressively declined in recent years [8].

Argentina is considered a low-endemic country for HBV (<2 %) [11], with a genotype distribution that varies by region among the general population: gts A, D and F are the most prevalent in the city of Buenos Aires with similar proportions (approximately 30 %) [12]; gt F represents more than 90 % of HBV infections in the Northwest region (provinces of Salta, Chaco and Formosa) [12]; gt D shows a high prevalence (58 %) in Misiones province (Northeast) [13]; and for the central area of the country, gts F (52 %), A (37.5 %), C (5 %) and D (5 %) have been reported recently [14].

There are few data about the situation of HBV in blood banks of Argentina. Recently, Flichman et al. [6] reported a

decreasing trend in HBV prevalence, which went from 0.336 % in 2004 to 0.198 % in 2011 based on screening and non-confirmed serology assays. Another recent study, carried out with samples obtained in the city of Buenos Aires and Misiones province, revealed an HBsAg prevalence of 0.12 % and 0.73 %, respectively [15]. The prevalence rates reported in these studies, based only on the detection of HBsAg, could not be accurate enough because infections during the window period or occult infections were not detected, and false positive results of serology assays are not taken into account. In this sense, a consensus document from the Committee for Transfusion-Transmitted Infections of the Argentine Association of Hemotherapy and Immunohematology announced the detection of five blood units from the window period for HBV by NAT among 568,973 donors [16].

Even though molecular screening of HBV, HIV and HCV in blood banks is not mandatory for most provinces in Argentina, in the province of Córdoba, the main Mediterranean province of the country, it has been required by law since 2010 (Ministry of Health decree o.N 1047/Resolution No. 618) [16].

The aim of this study was to determine the prevalence of confirmed HBV infection (based on detection of HBsAg and HBV-DNA by NAT), the frequency of OBI, and the viral genotype distribution of HBV in blood donors in central Argentina.

A total of 70,102 samples obtained from blood donors at the Fundación Banco Central de Sangre in the city of Córdoba between July 2011 and February 2014 were included in this study. Blood samples were collected in BD Vacutainers (Becton Dickinson), at the time of donation. Serological screening for HBV was performed using chemiluminescence assays for HBsAg (ARCHITECT®-AgHBs Reagent, Abbott ARCHITECT System, Wiesbaden, Germany) and anti-HBc (CORE Reagent kit ARCHITECT, Abbott ARCHITECT System, Wiesbaden, Germany). Detection of HBV DNA by NAT was carried out using a COBAS TaqScreen MPX test followed by COBAS TaqScreen MPX v2.0 test (Roche Molecular Systems, Branchburg, NJ, USA) in an automated real-time PCR analyzer (COBAS® TaqMan®, Roche Instrument Center, Rotkreuz, Switzerland).

In HBV-positive samples, DNA quantification was performed by COBAS®Taqman® HBV Test (Roche Molecular Systems, Pleasanton, CA) or Versant HBV DNA 3.0 Assay (bDNA; Siemens Medical Solutions, Mississauga, Ontario, Canada).

To determine the viral genotype, NAT-positive samples were amplified by nested PCR and then sequenced. For that, HBV DNA was extracted from serum samples using a QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany), and two HBV genomic sequences were amplified by nested PCR, corresponding to the S gene and the BCP-pC gene, as described previously [17]. For the S gene, the primers used were 5′-CCTGCTGGTGGCTCCAGTTC-3′ (nucleotides 57–75) and 5′-AGAAAATTGGTAA-CAGMGGYA-3′ (nucleotides 815–795) in the first round, and 5′-GCGGKGTKTTTCTTGTTGACAA-3′ (nucleotides 203–224) and 5′-GGGACTCAAGATGYTGYACAG-3′ (nucleotides 787–767) in the second round (product of 585 bp). For the amplification of the BCP-pC region, the primers utilized were 5′-ATGGAGACCACCGTG AACGC-3′ (nucleotides 1608–1627) and 5′-CCCAC CTTATGAGTCCAAGG-3′ (nucleotides 2484–2465), and 5′-TGCCAACAGTCTTACATAAGMG-3′ (nucleotides 1639–1660) and 5′-GAGTTCTTCTTCTAGGGGACCTG-3′ (nucleotides 2381–2359) for the first and second round, respectively (product of 742 bp).

The resulting amplicons were purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA) and subjected to direct nucleotide sequencing in both directions (Macrogen, Inc. Seoul, Korea).

For genotype and subgenotype assignation, a combined phylogenetic analysis of the S gene and BCP-pC regions using reference sequences obtained from the GenBank database was performed. In samples where it was not possible to sequence some of the fragments, the genotyping was carried out using only one of the genes but was not included in the final analysis.

Sequences were edited and aligned with MEGA v.5 [18]. Phylogenetic analysis was performed using the maximum-likelihood method with the same program under the appropriate model of nucleotide substitution selected by jModeltest v2.1 [19] according to the Akaike information criterion. The robustness of the phylogenetic grouping was evaluated by bootstrap analysis with 1000 replicates.

Nucleotide sequences obtained in this work were deposited in the GenBank database under accession numbers KU212148 to KU212159 for the S gene and KU212160 to KU212171 for the BCP-pC genomic region.

Of the 70,102 samples analyzed, 42 were positive for HBV infection, which represents 0.06 % of the studied population. Thirty-five were positive for HBsAg, anti-HBc and NAT; three were positive for anti-HBc and NAT, one was positive for HBsAg and NAT, and three were only positive for NAT. HBV infection was confirmed in these blood donors by detection of anti -HBs, anti-HBe and/or viral load.

For 25 HBV-infected blood donors (23 males and 2 females; mean age, 37.6 years; range, 20-61 years), enough

Table 1 Serological and molecular HBV markers in NAT-positive blood donors

Sample no.	HBsAg	Anti-HBc	Viral load (UI/mL)	ORF-S sequence	BCP-pC sequence	Genotype/subgenotype
1	+	+	ND	+	+	F1b
2	+	+	>110,000,000	+	+	F1b
3	+	+	612	−	−	−
4	−	−	99	−	+	F1b
5	+	+	1860	+	+[a]	F[b]
6	+	+	304	+	+	F4a
7	+	+	251	+	+	A2
8	+	+	5460	+	+	F4a
9	+	+	56	−	−	−
10	+	+	ND	+	+	F1b
11	+	+	107	−	−	−
12	+	+	1930	−	−	−
13	+	+	<29	−	−	−
14	−	+	<29	−	−	−
15	+	+	5460	+[a]	+	F4a
16	+	+	1290	+	+[a]	D2
17	−	+	<29	−	−	−
18	−	+	<29	−	−	−
19	+	+	208	−	−	−
20	+	+	66,000	+	+	A2
21	+	+	6540	+	+	F1b
22	+	+	30.1	−	−	−
23	+	+	118	−	−	−
24	+	−	>110,000,000	+	+	F1b
25	+	+	>110,000,000	+	+	C[b]

ND, no data; +, positive; −, negative

[a] Samples could not be sequenced due to very weak bands

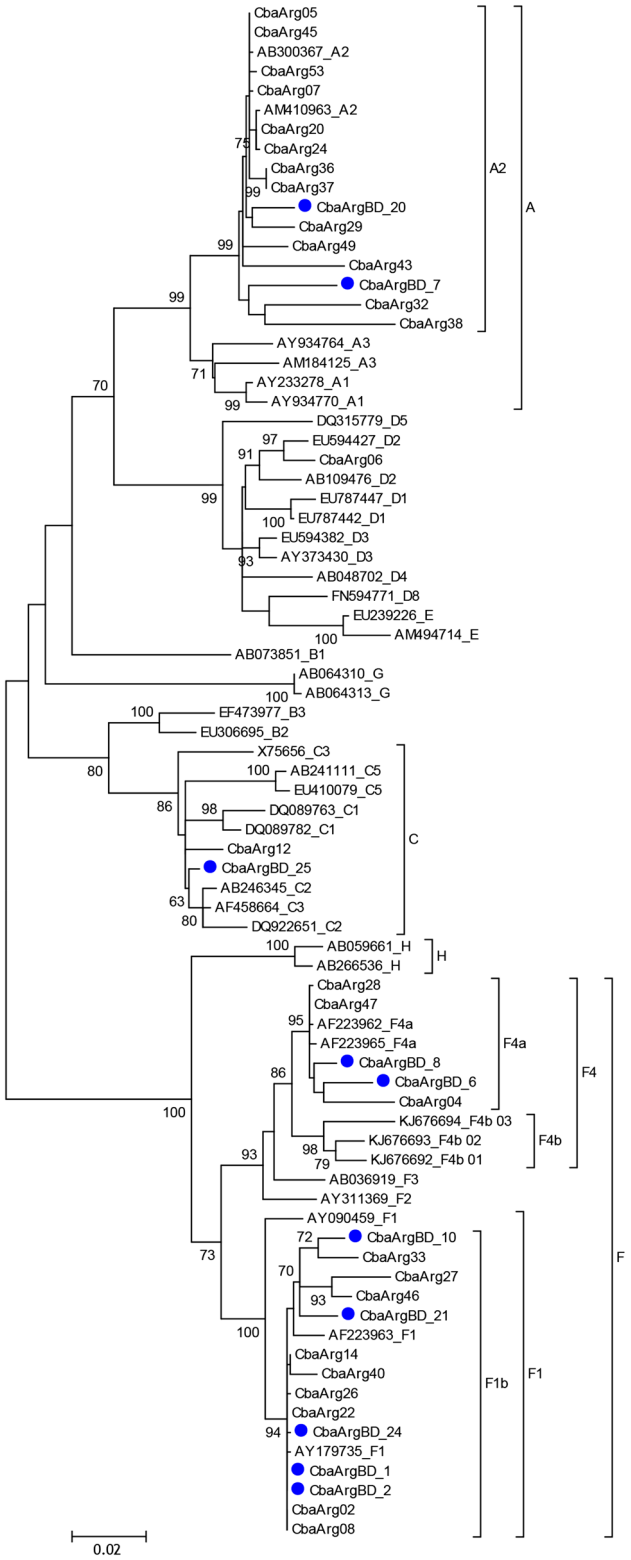
[b] It was not possible to determine the subgenotype

blood sample was available for complementary nested PCR for genotyping. Results for serological and molecular HBV markers in HBV-infected blood donors are shown in Table 1. Samples that were HBsAg negative and NAT positive in this group of samples had a low viral load, and one was negative for anti-HBc (sample 4) (Table 1).

Genotype F was the most prevalent (71.4 %, 10/14, sgts F1b, F4a), followed by gt A (14.3 %, 2/14, sgt A2), C (7.1 %, 1/14) and D (7.1 %, 1/14, sgt D2) (Fig. 1). Although all samples were subjected to amplification of the ORF-S and BCP-pC genomic regions, 10 of them could be sequenced in both cases, revealing the same gt when the amplicons were analyzed; two samples could only be sequenced in the ORF-S region (samples 5 and 16), and two only in the BCP-pC region (samples 4 and 15) (Table 1).

This study reports for the first time the prevalence of confirmed HBV infection (by serological and molecular assays) in a blood bank in Argentina and the current HBV genotype distribution in blood donors of the central area of the country. Until now, knowledge about the HBV prevalence in blood banks in Argentina was based on screening assays and unconfirmed serology. The HBV prevalence obtained in this study (0.06 %) is lower than that reported for the same population in other regions, such as the northern provinces (0.45 %) and the city of Buenos Aires (0.18 %) [6]. For Córdoba, a previous study reported a prevalence of 0.155 %, based only on the detection of HBsAg in 54,370 donors at a public blood bank [6]. This reported prevalence was probably overestimated, due to false positive results in serology assays that were not taken into account.

The obtained genotype distribution in blood donors was very similar to that reported for the general population in the same region [14], with a highest prevalence of gt F, which has an American origin, followed by gt A, and low prevalence of gts C and D. Moreover, the subgenotypes identified in blood donors are the same as described



◀**Fig. 1** Maximum-likelihood phylogenetic tree obtained using sequences of 1186 nucleotides, resulting from concatenated sequences of the S gene (525 nucleotides) and the BCP-pC region (661 nucleotides) of 10 HBV samples from blood donors of Córdoba province, Argentina, named "CbaArgBD" followed by the sample number (labeled with a circle). Reference sequences from each genotype and subgenotype available in the GenBank database were included with their accession number. Sequences previously obtained from Córdoba were also included in the analysis, named "CbaArg" followed by the sample number [14]. The numbers next to each node correspond to bootstrap values obtained with 1000 replicates

previously (F4, F1b and A2) for our region [14]. A new clade within the subgenotype F4 has been identified recently in Argentina (Buenos Aires city), dividing it into subgenotypes F4a and F4b [20]. All subgenotype F4 samples detected in this study grouped within subgroup F4a.

Due to the introduction of screening by NAT and after a long time, the residual risk of transmission of HBV by transfusion has decreased progressively in other countries [21–23]. However, it remains higher than the estimated risks for HIV and HCV [24]. This residual risk of HBV transmission by transfusion is mainly related to donations of HBsAg-negative blood collected during the window period or from donors with OBI [9, 10, 25]. In this study, four cases of OBI were detected, all of them with low viral load (<200 UI/mL) (Table 1). One of these samples (sample 4) could probably be an ORF-S HBV mutant: it tested positive for HBV DNA by NAT and positive for the BCP-pC sequence, but it was negative for both the amplification of the S gene and the corresponding HBsAg detection. To clarify this finding, other attempts to amplify the S gene targeting other regions or the complete genome would be necessary.

Our results indicate that NAT has been of great help in detecting HBV infections, especially in donors with OBI. In accordance with other blood banks [7, 8] occult HBV infection was considerably more frequently detected than those in the window period. In addition, the low viral load found in these cases (below the limit of viral load detection kits), highlights the need to use very sensitive NAT assays for HBV screening in blood banks.

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Compliance with ethical standards

This study was performed in accordance with provisions of the 1964 Declaration of Helsinki, its later amendments, and Good Clinical Practice guidelines, as well as the additional requirements of local and national authorities.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lai CL, Ratziu V, Yuen MF et al (2003) Viral hepatitis B. Lancet 362(9401):2089–2094
2. World Health Organization (WHO). Fact sheet No 204, March 2015. http://www.who.int/mediacentre/factsheets/fs204/en/
3. Norder H, Couroucé AM, Coursaget P et al (2004) Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology 47(6):289–309
4. Alvarado Mora M, Romano CM, Gomez-Gouvea MS et al (2012) Phylogenetic analyses of complete genome sequences of hepatitis B virus from an Afro-Colombian community: presence of HBV F3/A1 recombinant strain. Virol J 9:244
5. Moura IF, Lopes EP, Alvarado-Mora MV et al (2013) Phylogenetic analysis and subgenotypic distribution of the hepatitis B virus in Recife, Brazil. Infect Genet Evol 14:195–199
6. Flichman D, Blejer JL, Livellara BI et al (2014) Prevalence and trends of markers of hepatitis B virus, hepatitis C virus and human Immunodeficiency virus in Argentine blood donors. BMC Infect Dis 14:218
7. Stramer SL, Wend U, Candotti D et al (2011) Nucleic acid testing to detect HBV infection in blood donors. N Engl J Med 364:236–247
8. Velati C, Romanó L, Fomiatti L et al (2008) Impact of nucleic acid testing for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus on the safety of blood supply in Italy: 6-year survey. Transfusion 48:2205–2213
9. Said ZNA (2011) An overview of occult hepatitis B virus infection. World J Gastroenterol 17(15):1927–1938
10. Raimondo G, Pollicino T, Romano L et al (2010) Update on occult Hepatitis B infection. Path Biol 58:254–257
11. Paraná R, Almeida D (2005) HBV epidemiology in Latin America. J Clin Virol 34(1):130–133
12. y Leone FGP, Pezzano SC, Torres C et al (2008) Hepatitis B virus genetic deversity in Argentina: dissimilar genotype distribution in two different geographical regions; description of hepatitis B surface antigen variants. J Clin Virol 42(4):381–388
13. Mojsiejczuk LN, y Leone FGP, Torres C et al (2013) Análisis filodinámico del virus de la hepatitis B circulante en la provincia de Misiones, Argentina. In: XXXIII Annual Meeting of Argentine Society of Virology, pp 26–27
14. Gallego F, Pisano MB, Torres C et al (2014) Molecular epidemiology of hepatitis B virus in Córdoba, Argentina. J Clin Virol 61(2):204–210
15. Delfino CM, Gentile EA, Castillo AI et al (2014) Hepatitis B virus and hepatitis D virus in blood donors from Argentina: circulation of HBsAg and reverse transcriptase mutants. Arch Virol 159:1109–1117
16. Asociación Argentina de Hemoterapia e Inmunohematología: Las Pruebas de Biología Molecular (NAT) y la Seguridad Transfusional. Comité de Infecciones Transmisibles por Transfusión 2012; Documento consenso 002-2012
17. Pezzano SC, Torres C, Fainboim HA et al (2011) Hepatitis B virus in Buenos Aires, Argentina: genotypes, virological characteristics and clinical outcomes. Clin Microbiol Infect 17(2):223–231
18. Tamura K, Peterson D, Peterson N et al (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739
19. Darriba D, Taboada GL, Doallo R et al (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9(8):772
20. Mojsiejczuk LN, Torres C, Fainboin H et al (2015) Identification of a new clade of hepatitis B virus genotype F. Inf Gen Evol 34:122–125
21. ÓBrian SF, Xi G, Fan W et al (2008) Epidemiology of hepatitis B in Canadian blood donors. Transfusion 48:2323–2330
22. Daniels D, Grytdal S, Wasley A (2009) Surveillance for acute viral hepatitis—United States, 2007. MMWR Surveill Summ 58:1–27
23. Zou S, Stramer SL, Notari EP et al (2009) Current incidence and residual risk of hepatitis B infection among blood donors in the United States. Transfusion 49:1609–1620
24. Zou S, Dorsey KA, Notari EP et al (2010) Prevalence, incidence and residual risk of human immunodeficiency virus and hepatitis C virus infections among United States blood donors since the introduction of nucleic acid testing. Transfusion 50(7):1495–1504
25. Allain JP, Mihaljevic I, Gonzalez-Fraile MI et al (2013) Infectivity of blood products from donors with occult hepatitis B virus infection. Transfusion 53(7):1405–1415