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Genotype D amongst injection drug users with acute hepatitis B virus infection in British Columbia

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SUMMARY. The eight genotypes of hepatitis B virus (HBV) exhibit distinct geographical distributions. This study identified HBV genotypes and transmission modes associated with acute infection in British Columbia (BC), Canada, from 2001 to 2005. Seventy cases of acute HBV in BC were identified from laboratory reports using a standardized case definition. Interviews for risk factors and hepatitis history were conducted for each case. HBV genotypes were determined by BLAST comparison analysis of the surface (S) or preS gene sequence. To illustrate the distribution of genotypes identified amongst acute cases in BC, an annotated map was produced showing the global occurrence of HBV genotypes. The majority of acute HBV cases occurred in Caucasian, Canadian-born males, with 30% of cases reporting injection drug use (IDU) and 21% reporting incarceration. The most common genotype observed was genotype D (62.9%), followed by genotypes A (18.6%), C (11.4%), B (4.3%), and E (1.4%). A significant association was observed between Genotype D and IDU ($P = 0.0025$) and previous incarceration ($P = 0.0067$). Phylogenetic analysis of the S gene sequence demonstrated identical or high genetic relatedness amongst genotype D viral strains (86% sub-genotype D3), thus verifying transmission clustering amongst BC injection drug users. The association between acute HBV genotype and reported transmission modes has not been previously described in North America. Tracking of genotypes can help identify disease transmission patterns and target at-risk populations for preventive immunization.

Keywords: genotype, incarceration, transmission risk factor.

INTRODUCTION

Hepatitis B virus (HBV) is a global problem, with an estimated 350–400 million chronic cases worldwide [1,2]. HBV disease burden varies by geographical region, with regions classified as having low (<2%), intermediate (2–7%) and high (>8%) endemicity according to the proportion of individuals with detectable HBsAg. Approximately 60% of HBV cases occur in high endemicity regions, where most infections are transmitted vertically from infected mothers [3]. In low endemicity regions, most HBV infections are acquired sexually or through injection drug use (IDU), despite the availability of a safe and effective vaccine [4].

Hepatitis B virus is classified into eight genotypes (designated A–H) based on a genomic nucleotide sequence divergence of greater than or equal to 8% [5]. The infecting genotype may affect the clinical progression of chronic HBV disease and its sequelae, such as hepatocellular carcinoma [6–9]. Response to HBV therapy may also vary according to genotype. For example, individuals with genotypes A and B respond more favourably on treatment with interferon, as manifested by loss of HBeAg, than do individuals infected with genotypes C and D [10].

Hepatitis B virus genotypes exhibit distinct geographical distributions, reflecting ethnographic patterns of disease transmission and immigration [11]. Compared to the general population, immigrants to Canada from regions of high HBV endemicity have a high prevalence of chronic HBV concordant with the genotype(s) prevalent in their countries of origin [12–16]. The number of HBV infections acquired in Canada and the distribution of their respective genotypes is unknown. In areas with universal HBV vaccination programmes, such as British Columbia (BC), the distribution of HBV genotypes amongst acute infections may differ from that amongst chronic infections, since immunization interrupts the cycle of vertical transmission seen in HBV endemic regions. The distribution of genotypes amongst acute HBV infection and its relationship to patterns of disease transmission has only been described in several European countries [17,18] and Japan [19,20], and has not yet been described in North America.

Abbreviations: BC, British Columbia; BCCDC, BC Centre for Disease Control; HBV, hepatitis B virus; IDU, injection drug use.

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The objectives of this study were to determine the distribution of HBV genotypes and sub-genotypes associated with acute infection in BC and their relationship to patterns of disease transmission. To accurately compare the findings of the present study to the global distribution of HBV genotypes, an annotated map was created from published studies.

METHODS

Acute case definition

Positive tests for HBsAg and anti-HBcIgM (i.e. positive serology indicative of HBV acute infection) identified at private laboratories or the BC Centre for Disease Control (BCCDC) were reported to the site investigator, who reviewed clinical history and/or contacted public health or primary health care providers. Since anti-HBcIgM is detected in approximately 20% of individuals with chronic HBV [21], case confirmation required a patient clinical history and/or exposure compatible with acute HBV infection. Individuals who received a positive test for HBsAg within 6 months of a documented negative HBsAg test were also considered acute. Markers used to define and clinically confirm acute HBV cases included elevated liver enzymes (i.e. ALT and AST), and/or symptoms of acute infection (e.g. abdominal pain, dark urine, fatigue, jaundice, loss of appetite, nausea and/or malaise), and/or high-risk exposure (i.e. sexual contact with an HBV carrier and/or IDU).

Epidemiological data collection

Clinically confirmed acute cases were contacted as part of the mandatory BC public health follow-up and contact tracing of acute HBV cases. Patients were asked to consent to a voluntary, confidential interview with a public health nurse to collect additional information on their risk factors for HBV. This interview was offered as part of an ongoing Public Health Agency of Canada enhanced surveillance project that investigates transmission modes of acute HBV in Canada. The BCCDC surveillance site collects risk factor and genotype data on acute HBV cases in all BC regions except Vancouver. The resulting catchment area comprises approximately 3.3 million people, 78% of which are Non-Aboriginal Caucasian (17% visible minorities, 5% Aboriginal) and 76% of which are Canadian-born (23% foreign-born, 1% non-permanent residents).

After giving informed consent, participants completed by telephone or in person a structured interview on demographics, ethnicity, birthplace, laboratory testing results, symptoms, vaccination history prior to diagnosis, and HBV risk factors (i.e. high-risk sexual behaviour [i.e. men who have sex with men, sexual partner who is an injection drug user (IDU), multiple sexual partners], sexual or blood contact with a known case of HBV infection, IDU, or tattoo/body piercing) in the participant's lifetime and within 6 months of diagnosis. All interview data were kept confidential and identifying information was available only to the BCCDC site investigator.

Hepatitis B virus genotyping and phylogenetic analysis

Blood samples from clinically confirmed acute cases were analysed for HBV genotype at the National Microbiology Laboratory. HBV DNA was extracted from 150 μL of sera by sodium dodecyl sulphate-proteinase K and phenol chloroform extraction methods [22]. Extracted DNA pellets were re-suspended in 30 μL of sterile distilled water and stored at −20 °C until use. Extracted DNA was amplified by PCR using primers specific for either the preS region [23] or the HBsAg and polymerase overlapping coding region: Spr1A (5′-GTTCAGGAACAGTAAG-CCC-3′) and DRv2as (5′-GAAAG GCCTTGTAAGTTGGCG-3′). If necessary, the following primers were used for nested PCR: DRv2s (5′-GGTGGACTTCT CTCAA-TTTTCTAGG-3′) and Spr2A (5′-ACTTTCCAA TCAATAGGCC-3′) [16]. Amplicon products were gel purified prior to cycle sequencing with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using BigDye v3.1 terminator chemistry.

Sequence data obtained were used to determine the HBsAg subtype and the genotype of each viral sample was determined by using the NCBI genotyping tool [24]. Further analysis of the HBsAg/polymerase coding region of all genotype D (HBV/D) strains was performed to determine their phylogenetic relatedness to GenBank-derived HBV sequences of known sub-genotype (D1–D4). Sequence alignments were performed using CLUSTALX software, version 1.81 (http://bips.u-strasbg.fr/fr/Documentation/ClustalX/). Genetic distances were estimated by Kimura two-parameter analysis, and a phylogenetic tree was constructed by the neighbour-joining method with 500 bootstrap replicates using MEGA software, version 4 (http://www.megasoftware.net/).

Geographical distribution of hepatitis B virus genotypes

A review was undertaken of 106 studies, including one unpublished conference presentation, describing the distribution of both acute and chronic HBV genotypes in a specified geographical region. For each of these studies, the HBV genotypes identified were displayed as a pie chart. Three sizes of pie charts were made to correspond to study sample size (i.e. N = 1–50, 51–150, >150) and a unique colour was assigned to each genotype and to mixed genotype infections. The pie charts were subsequently plotted on a map to illustrate world HBV genotype prevalence according to the regions studied. The province of BC is on the west coast of Canada and is shown on the map outlined in black.

Risk factor and genotype statistical analysis

Statistical analysis was conducted using SAS version 9.1.3 for Windows (SAS Institute, Cary NC, USA). Contingency tables

were constructed for genotypes and risk factors. Statistical significance of association between genotype (D *vs* Non-D) and risk factors was assessed using Fisher's exact test.

RESULTS

Acute cases

Between 2001 and 2005, 70 cases of clinically confirmed acute HBV within the BCCDC enhanced surveillance catchment area completed interviews and submitted blood specimens. Group demographic data for all acute cases is provided in Table 1. The mean age of cases was 42 years and the majority of cases were Caucasian, Canadian-born males. Fifty-three per cent of cases had symptoms at the time of diagnosis (Table 1). Sexual contact with an HBV carrier of the opposite sex was the most frequently reported risk factor (49%). IDU was reported as a risk factor by 30% of the cases, with 12/21 (57%) reporting IDU within the previous 6 months. Incarceration in a correctional facility was also reported by 21% of cases. Cases infected with HBV/D reported IDU and incarceration as risk factors more frequently than did cases infected with other genotypes, as detailed below.

Table 1 Demographic information and presence of symptoms in HBV genotype D *vs* non-genotype D acute HBV in BC

	Genotype		
	D	Non-D	Total
Age			
N	44	26	70
Mean	41.3	44.4	42.4
Gender			
Male	28 (63.6%)	19 (73.1%)	47 (67.1%)
Female	16 (36.4%)	7 (26.9%)	23 (32.9%)
Ethnicity			
Caucasian	28 (63.6%)	16 (61.5%)	44 (62.9%)
Asian	2 (4.5%)	5 (19.2%)	7 (10.0%)
Aboriginal	5 (11.4%)	1 (3.8%)	6 (8.6%)
South Indian	5 (11.4%)	1 (3.8%)	6 (8.6%)
Unknown	4 (9.1%)	1 (3.8%)	5 (7.1%)
Other	0 (0.0%)	2 (7.7%)	2 (2.9%)
Birth place			
Canada	36 (81.8%)	18 (69.2%)	54 (77.1%)
Other	5 (11.3%)	3 (11.5%)	8 (11.5%)
India	3 (6.8%)	1 (3.8%)	4 (5.7%)
Hong Kong	0 (0.0%)	3 (11.5%)	3 (4.3%)
Unknown	0 (0.0%)	1 (3.8%)	1 (1.4%)
Symptoms[†]			
Yes	25 (56.8%)	12 (46.2%)	37 (52.9%)
No	19 (43.2%)	14 (53.8%)	33 (47.1%)

[†]Symptoms of acute infection at or up to 6 months before diagnosis (i.e. abdominal pain, dark urine, fatigue, jaundice, loss of appetite, nausea and/or malaise).

Molecular epidemiology

Amongst the 70 acute HBV cases, genotype D was the most frequently observed genotype (44/70, 62.9%), followed by A (13/70, 18.6%), C (8/70, 11.4%), B (3/70, 4.3%), and E (1/70, 1.4%). HBV from one sample could not be genotyped (1/70; 1.4%). Non-D genotype cases were comprised of 18 Canadian-born cases (nine HBV/A, two HBV/B, six HBV/C, and one HBV/E), one Indian-born case (HBV/A), three Hong Kong-born cases (one HBV/B and two HBV/C), one Honduran-born case (HBV/A), one Korean-born case (unknown genotype), and one case born in the Philippines (HBV/A). The birthplace of all HBV/D-infected cases is shown in Table 1 (birthplace of 'Other': Fiji ($n = 1$), Great Britain ($n = 2$), Pakistan ($n = 1$), and Yugoslavia ($n = 1$).

Heterosexual sex was reported as the main mode of potential sexual transmission of HBV for all genotypes: 10 cases HBV/A (76.9%), one case HBV/B (33.3%), seven cases HBV/C (87.5%), and 42 cases HBV/D (95.5%); sexual orientation not specified for the HBV/E case. IDU was reported by 7.7% HBV/A ($n = 1$), 12.5% HBV/C ($n = 1$), and 43.2% HBV/D ($n = 19$) acute cases, while incarceration as a risk factor for potential transmission was only reported by cases infected with HBV/A (one case; 7.7%) and HBV/D (14 cases; 31.8%). A significant association was observed between HBV/D and IDU ($P = 0.0025$) and HBV/D and prior incarceration ($P = 0.0067$; Fig. 1). Due to this result and the observed high prevalence of HBV/D amongst acute HBV cases in BC, this association was focused upon for the remainder of the study.

Phylogenetic analysis

A phylogenetic tree of the BC HBV/D strains was prepared to determine genetic relatedness (Fig. 2). Representative HBV genotype and sub-genotype HBsAg coding region sequences from GenBank (522 bp; nucleotides 156–677 of the HBsAg gene) were aligned with all HBV/D acute cases, except one, for which only PreS sequence was available. The code number and year for each acute case is shown for the HBV/D sequences investigated (Fig. 2). The majority of HBV/D strains clustered with sub-genotype D3 representative sequences (37/43, 86%), with very few D1 and D2 strains observed (1/43 and 3/43, respectively). The majority of HBV/D strains were observed to share high HBsAg sequence similarity, with several outliers noted (range of sequence divergence, 0–3.2%). For example, two acute cases (1340_05 and 1911_03) were found to cluster apart from the D1 to D3 clusters, supported by a 92% bootstrap value. All HBV/D3 strains were subtype *ayw3* (data not shown).

All sub-genotype D3 sequences, except one (137_01), were very closely related with an intragroup nucleotide

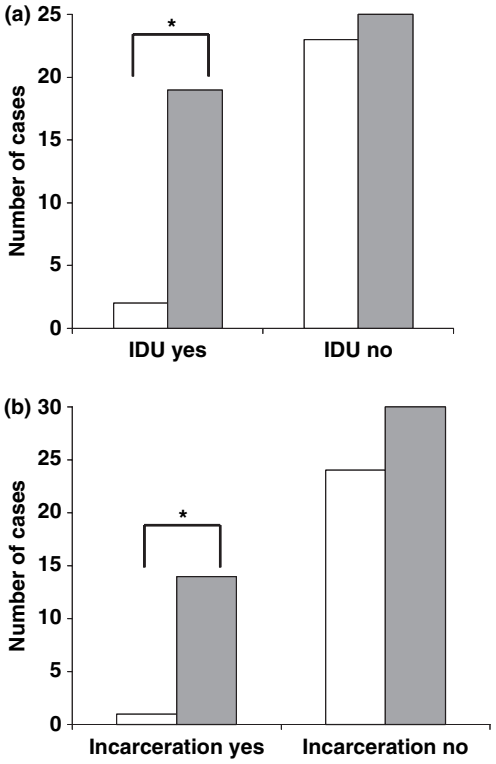

Fig. 1 Risk factor association with acute cases of HBV genotype D. (a) HBV genotype association with IDU as a transmission risk factor. (b) HBV genotype association with prior incarceration as a transmission risk factor. □: Non-HBV/D genotypes; ■: HBV/D. *The significance of the association between IDU or prior incarceration and HBV/D is shown ($P = 0.0025$ and $P = 0.0067$, respectively).

divergence ≤1.0%. The observation of highly similar sequences throughout the 5-year period of study suggests that these viral strains are relatively stable and were persistently circulating, likely within a specific, high-risk community. This observation is confirmed by the finding of a significant association between BC HBV/D and IDU or previous incarceration.

Global distribution of HBV genotypes

In order to compare and contrast the genotype distribution amongst BC acute HBV cases to other regions, a world map of HBV genotype distribution amongst chronic and acute HBV infection, annotated with source references, was prepared (Fig. 3). In most parts of the world, with the exception of Canada, the United States, and parts of Europe, one or two genotypes tend to dominate chronic HBV infections. For example, genotypes B and C predominate within parts of Asia, genotype D predominates throughout the Indian subcontinent, the Middle East and parts of Europe and North Africa, while genotypes A and E predominate throughout Southeast and West Africa, respectively.

The distribution of HBV genotypes amongst chronic infections in Canada and the contiguous United States is clearly different from the distribution of genotypes amongst acute infections in BC. The latter distribution is similar to that detected in AK, parts of Europe, the Middle East, and the Indian subcontinent. Therefore, there appears to be a distinct difference amongst the distribution of chronic and acute HBV genotypes in Canada, likely due to the different risk factors associated with each. The predominant HBV genotypes observed in chronic patients living in BC are known to be HBV/B and C [115], due to a large Asian immigrant population, and this chronic HBV genotypic prevalence is similar to that observed in other parts of Canada and the United States (Fig. 3). When comparing amongst the few reports of genotypic distribution of acute HBV, the results from the present study most closely match the distribution observed in Denmark [17].

DISCUSSION

Hepatitis B virus genotypes show distinct geographical distributions. In Canada, the majority of chronic HBV occurs in immigrants whose infections are assumed to have been acquired in their countries of origin. In BC, where over 370 000 immigrants are from Asia [116], the predominant HBV genotypes observed are B and C [115]. Therefore, incident cases of HBV would be expected to be genotype B or C if the infections were being transmitted from chronic cases. However, only a small number of cases in this sample are of genotypes B and C, suggesting that the majority of acute HBV infections were not transmitted from chronic cases whose infections were acquired in Asia. Instead, the most common genotype in this sample is D and the majority of acute cases are white, Canadian-born males.

As the majority of these acute infections occurred in adults, who are likely to resolve their infection, it is unknown what long-term effect these cases will have on the overall distribution of genotypes in BC. However, it is an important finding that 30% of cases reported IDU and 21% reported incarceration, as this indicates that there are important risk factors linked with acute HBV transmission other than sexual exposure.

Cases reporting lifetime IDU were almost exclusively infected with genotype D (19/21, 90.5%) and all 12 cases who reported injecting within 6 months prior to diagnosis were infected with genotype D. It should be noted that because social desirability or recall biases may cause an underreporting of recent IDU [117,118] the number of cases reporting lifetime IDU may be more indicative of risk than the number reporting recent drug use. Regardless, these findings indicate that IDU is a major route of spread for HBV in BC and that based on phylogenetic analysis clustering exists. To this end, targeted vaccination may be warranted to reduce the transmission of HBV (genotype D) in such high risk populations as IDU and incarcerated individuals.

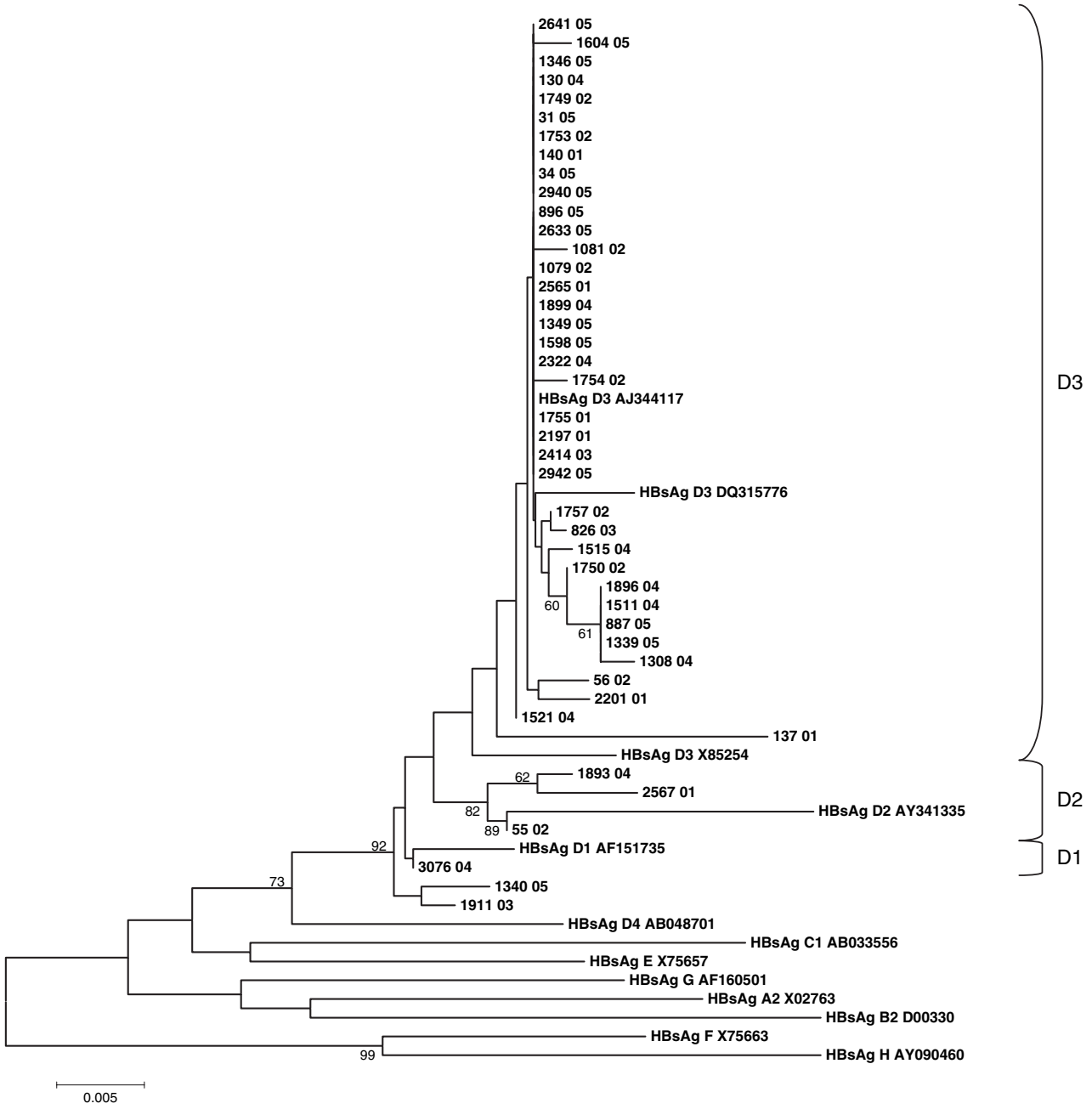

Fig. 2 Phylogenetic analysis of 43 HBsAg coding region sequences from British Columbians acutely infected with HBV/D, 2001–2005. Each acute sequence is denoted by a code number and the year (01–05). Sequence alignment was performed using ClustalX v.1.8. Evolutionary distances were calculated using the Kimura-2 parameter model, and the phylogenetic tree topology was evaluated by bootstrap analysis (500 replicates) using the neighbour-joining method (confidence values of 60% or greater are shown). The ruler shows the branch length for a pairwise distance equal to 0.005.

Discordance in genotype distributions between acute and chronic HBV cases has been documented. In Denmark, HBV/D was linked with acute infection acquired through IDU and in Japan HBV/A was linked with acute infection by sexual transmission in younger age groups [17,20]. This discordance is also found in BC, as a substantially lower prevalence of HBV/D (9/199; 4.5%) was observed amongst chronic HBV cases [115] in comparison to the acute cases (62.9%) observed in this analysis. Furthermore, Canadian HBV/D strains amongst those chronically infected show an equal D1–D3 sub-genotype distribution (data not shown), which is not observed with acute HBV/D strains in BC. The elevated HBV/D prevalence in this sample of acute cases suggests that the transmission of HBV in BC is occurring in a subpopu-

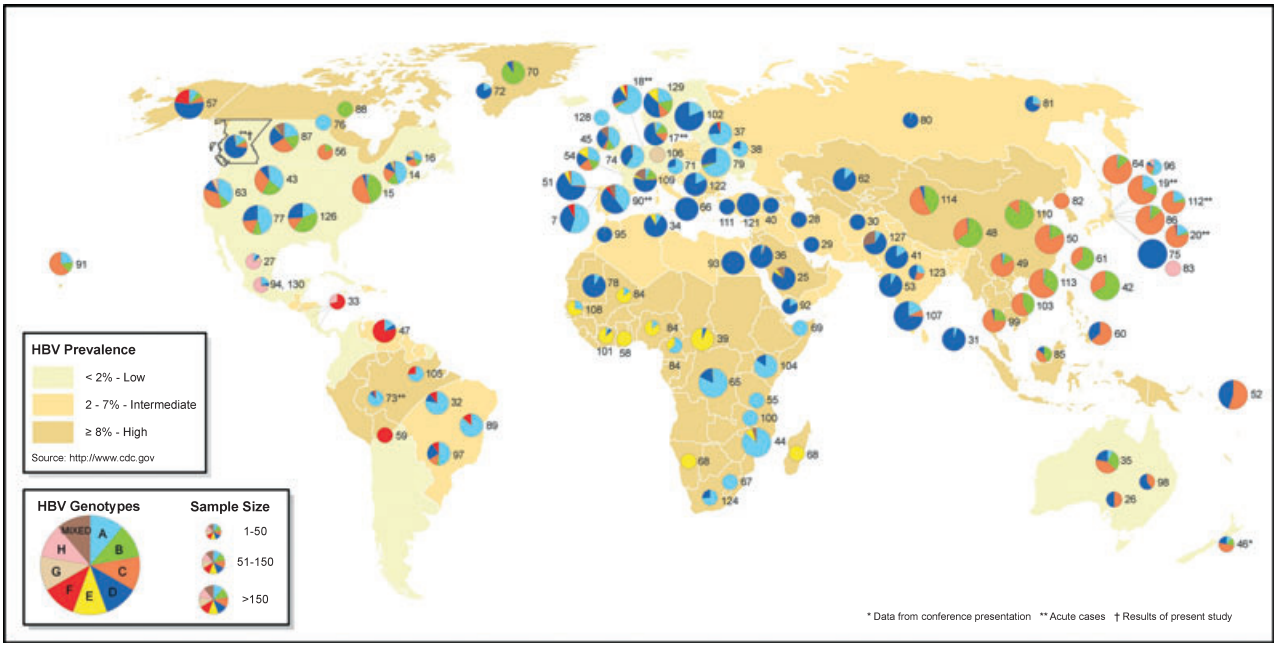

Fig. 3 Global geographical distribution of HBV genotypes. A compilation of 106 literature reports of HBV genotype prevalence is shown. For each study, a pie chart was produced showing the HBV genotypes identified for the associated geographical region. Three sizes of pie charts were made to correspond to study sample size (i.e. N = 1–50, 51–150, >150) and a unique colour was assigned to each genotype as well as to mixed genotype infections. All pie charts shown represent chronic HBV, except when indicated (**). All data are from published articles (reference numbers are given) except when indicated (*data from a conference presentation). The results of the present study are indicated (†) [130].

lation that is characterized by distinct risk factors, i.e. IDU and incarceration.

Genotype D has a worldwide distribution, although it is found to be more prevalent in the Mediterranean, and regions of the near and Middle East and Indian subcontinent [119]. Similarly, sub-genotypes of HBV/D, characterized by a ≥4% genomic nucleotide divergence, have been described throughout the world [120]. Sub-genotype D3 has been observed in parts of Europe [5,121,122], India [123], and South Africa [124], but has not yet been fully described in North America. Previous studies have shown either HBV genotype A [18,125] or D [17,126,127] to be prevalent amongst IDU. The observation of a high prevalence of HBV/D3 (*ayw3*) strains in BC IDU corroborates with reports from Denmark [17] and the USA [126], describing the association of HBV/D and/or subtype *ayw3* with IDU in both countries.

In general, data are lacking on how genotypes relate to transmission modes in low endemicity regions, where acute infections do not generally reflect vertical transmission. Characterizing the relationship between genotypes and transmission mode can help identify at-risk subpopulations. In this analysis, sexual contact with an HBV carrier was the most frequently reported risk factor amongst all cases. Only by linking genotype and risk factor data were IDU and incarceration identified as special markers of an emerging transmission pattern. Phylogenetic analysis of HBV/D strains revealed a cluster of highly similar sequences. As these strains were present throughout the 5-year collection period, it suggests that these viral strains are relatively stable and were persistently circulating within the IDU community in BC. Similarly, Hallett *et al.*, have described the stable and persistent circulation of a genotype A HBV strain, largely transmitted amongst prison inmates participating in high-risk behaviours (i.e. IDU) [128]. In each case, phylogenetic analysis correlated to epidemiological data to provide insight into transmission patterns.

This association between acute HBV genotype and reported transmission modes has not been previously described in North America. While the clinical importance of HBV genotypes continues to be elucidated, genotypes of acute HBV can be used to identify emerging transmission patterns and help target at-risk subpopulations for preventive immunization.

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