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Molecular epidemiology of a large community-based outbreak of hepatitis B in Bristol, UK

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ABSTRACT

Background: A large outbreak of hepatitis B virus (HBV) infection in the UK occurred between 2001 and 2005 in Bristol, UK.
Objectives: To identify HBV strains circulating amongst risk groups in the HBV outbreak cohort.
Study design: Cross-sectional study of acute HBV outbreak cases in Bristol.
Results: HBV sequences from sera of 95 of the 237 cases (40%) were characterised. The majority of cases (77%) were found to carry an HBV variant belonging to genotype D, designated HBV^{BV}. Eighty-eight percent (36/41) of sequences from injection drug users were HBV^{BV} as were 70% (19/27) from those with heterosexual intercourse as the primary identified risk factor. Of 15 sequences characterised from cases of pre-outbreak acute or chronic hepatitis B residing in Bristol, 40% also carried HBV^{BV}; the earliest was from a case identified in 1994.
Conclusion: The findings from this study link the spread of HBV^{BV} from injecting drug users to the general population through heterosexual intercourse during the outbreak. The molecular sequencing of specimens from this outbreak reports the emergence of HBV^{BV}, a HBV strain circulating in Bristol and South West England, as the cause of one of the largest outbreaks of acute hepatitis B in the UK.

1. Background

The United Kingdom (UK) has amongst the lowest incidences of acute hepatitis B virus (HBV) infection in the world, with an average incidence of 1.3 per 100,000. Nonetheless, outbreaks of acute HBV infection occur, mostly associated with nosocomial transmission.[1] The molecular epidemiology of the more prominent point-source hepatitis B outbreaks in health-care settings has been reported.[2,3] Outside of these settings, community outbreaks of HBV infection tend to occur amongst high-risk subpopulations, in whom vaccine coverage is often poor.[4] Many of the strains described from these outbreaks and up to a third of reported sporadic HBV infection are associated with the prisoners' variant of HBV (HBV^{PV}). This is a group of closely related variants of genotype A,[5] which was responsible for a number of outbreaks in prisons in the 1990s, and remains widespread in the UK a decade after its identification.[6]

2. Objectives

Here we describe the molecular epidemiology of a large epidemic of hepatitis B in the UK, which was associated with another genotype D HBV variant, transmitted predominantly through injecting drug use and heterosexual sex.

3. Study design

During the period of study, the Specialist Virology Centre of the Health Protection Agency South West Regional Virus Laboratory, located in Bristol, South West England, was performing laboratory testing for viral hepatitis for Bristol (which has a population of 1.1 million) and confirmatory testing for South West England. Serum samples from patients with suspected viral hepatitis were

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tested for hepatitis A virus, hepatitis B surface antigen (HBsAg), and anti-hepatitis C virus (HCV) IgG by enzyme immunoassay (Ortho-Clinical Diagnostics, Amersham, UK). Samples found reactive for HBsAg were tested for HBsAg in a second assay (Biokit, Kent, UK) as well as for total hepatitis B core antibody (anti-HBc), anti-HBc IgM, hepatitis B e antigen and anti-HBe (Ortho-Clinical Diagnostics, Amersham, UK).

3.1. The outbreak

The detailed epidemiology of this outbreak has been described elsewhere.[7] In brief, the annual rate in Avon of acute hepatitis B was noted to rise from 16 to 19 cases per year between 1998 and 2001 to 77 for 2002. From July 2001 through December 2005, 237 cases were notified. The median age of the cases was 33 years (interquartile range, 29–41 years), and males made up 168 of the cases (71%). Injecting drug use in the year prior to infection was identified as the primary risk factor in 104 cases (44%). High-risk heterosexual contact, defined as sexual contact in the 12 months before onset of acute hepatitis with an injecting drug user, a jaundiced person or a female sex worker, or with two or more partners, was reported in 71 cases (30%). Thirteen cases (5%) reported male-to-male or bisexual sex as a risk factor. For three cases (1%) another risk factor was identified (blood transfusion, a fight and needlestick injury). For 46 cases (20%) no risk factor could be determined.

3.2. Case definition

Cases were defined as those who were HBsAg-seropositive and anti-HBc IgM seropositive, or seroconverted to total anti-HBc, with or without recent history of discrete onset jaundice or other symptoms compatible with acute viral hepatitis. When a case was notified, patients were contacted by a public health nurse or doctor by telephone and asked about potential exposures to HBV. Contacts were followed up, counselled and offered immunisation. The data were further supplemented by information provided by general practitioners, review of case notes, a case-control study in injecting drug users and a descriptive study of all other cases, as described elsewhere.[7]

3.3. Study serum specimens

Study samples were derived from the 237 cases, of which 103 samples that retained sufficient sample volume for HBV sequencing studies, were included. In addition, samples from two cases with acute and 13 cases with chronic HBV infection previously diagnosed by the laboratory prior to July 2001 were analysed. Six samples received during the outbreak period from outside Bristol, but from within South West England, were also sequenced.

3.4. PCR amplification of HBV DNA

Two millilitres of clotted blood were collected from each patient, separated and then stored at −70 °C until processing for sequencing. DNA extraction was performed using the QIAamp DNA mini kit (QIAGEN, Crawley, UK) in accordance with the manufacturer's instructions. Part of the HBsAg-coding region (476 base-pairs (bps), positions 1551–2027) and core-coding region (242 bp, positions 6–248) were amplified as previously described.[8] In a proportion of the samples, the entire HBsAg-coding region was amplified (1013 bp, positions 1292–2306).[9] Genomic positioning is according to Pugh et al.[10]

3.5. Sequence analysis

Nucleotide sequences amplicons from the HBsAg- and core-coding regions were multiple-aligned using Clustal X version 1.83.[11] The sequences were edited using Bioedit version 7.0.5.3.[12] Genetic distances were calculated using the Kimura-two-parameter method and trees were constructed by neighbour-joining method using Mega4 version 4.0.2.[13] Corresponding sequences lodged with Genbank that represent the major HBV genotypes were included in the phylogenetic analyses. A search was also conducted amongst sequences deposited in HepSEQ, a UK national HBV sequence database,[14] to determine if sequences in the current study had been characterised previously.

3.6. Statistical analysis

Descriptive analyses were conducted, using chi-squared tests to compare categorical variables and a test to compare median ages between groups.

4. Results

4.1. Molecular epidemiology of the study cases

Sequence data were available for 95 cases (hereafter referred to as the study cases). Table 1 compares the demographic and risk characteristics of the study cases with those in the outbreak cases not included in this study. There was no statistical evidence of differences between the two groups for any of the variables examined.

Table 1
Characteristics of acute hepatitis B cases in the Bristol outbreak with and without genetic sequence data ($N = 237$).

Characteristic	Total outbreak $N = 237$ (%)	Study cases $n = 95$ (%)	Non-study cases $n = 142$ (%)	p-Value
Median age (years) [inter-quartile range]	33 [27–41]	31.5 [28–43]	33 [27–40]	0.701
Sex				0.502
Male	168 (71)	71 (79)	97 (68) 0.04	
Female	69 (29)	24 (21)	45 (32)	
Hepatitis C positive[a]				0.314
Yes	48 (20)	18 (19)	30 (21)	
No	134 (56)	62 (66)	72 (50)	
Primary risk factor:				0.719
Injecting drug use	104 (44)	41 (43)	63 (44)	
High risk heterosexual contact[b]	71 (30)	27 (28)	44 (31)	
MSM[c]	13 (5)	4 (4)	9 (6)	
Unknown	46 (20)	21 (22)	25 (17.5)	
Other	3 (1)	2 (2)	1 (0.5)	

[a] Anti-HCV-IgG-seropositive.
[b] Sex in previous 12 months with two or more new partners or a high-risk partner (i.e., an IDU, a jaundiced person, or a female CSW)
[c] MSM, men who have sex with men.