



## Case report

# Transmission of hepatitis D virus between spouses: A longitudinal study of the first reported Canadian case



Carla Osiowy<sup>a,\*</sup>, Anton Andonov<sup>a</sup>, Kevin Fonseca<sup>b</sup>, Ken Swidinsky<sup>a</sup>, Elizabeth Giles<sup>a</sup>, Andrew Mason<sup>c</sup>, Carla S. Coffin<sup>d</sup>

<sup>a</sup> Viral Hepatitis and Bloodborne Pathogens, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba, R3E 3R2, Canada

<sup>b</sup> Provincial Laboratory for Public Health, 3030 Hospital Dr. NW, Calgary, Alberta, T2N 4W4, Canada

<sup>c</sup> Division of Gastroenterology, Zeidler Leducor Centre, University of Alberta, Edmonton, Alberta, T6G 2X8, Canada

<sup>d</sup> Calgary Liver Unit, Division of Gastroenterology and Hepatology, Cumming School of Medicine, University of Calgary, 3280 Hospital Dr. NW, Calgary, Alberta, T2N 4Z6, Canada

## ARTICLE INFO

## Article history:

Received 28 February 2017

Received in revised form 2 March 2017

Accepted 2 March 2017

## Keywords:

Hepatitis B  
Hepatitis D  
Superinfection  
Acute liver failure  
Spousal transmission  
Tenofovir

## ABSTRACT

In chronic hepatitis B (CHB), hepatitis D virus (HDV) superinfection can lead to acute liver failure. The incidence of HDV superinfection is unknown, but is often detected in immigrants from HDV endemic countries. In this report, we characterize long-term clinical and virological outcomes in a hepatitis B virus (HBV) infected carrier before and after HDV superinfection, acquired from their spouse having HBV/HDV co-infection. A 38 year-old Mongolian male with CHB on anti-HBV therapy developed acute liver failure following HDV superinfection. Although he recovered, avoiding the need for liver transplant, HDV serological and molecular markers of infection persisted for the subsequent 16-month follow-up period, suggesting the development of CHB/HDV co-infection. The source of his HDV was from his wife of 10 years, a 34-year old Mongolian female known to have inactive CHB/HDV co-infection but who was not on anti-HBV therapy. Phylogenetic analysis of the complete HDV genome from the couple showed >99% similarity, with post-transmission longitudinal sequence revealing specific nucleotide substitutions between both spouse's HDV genome sequences. This study highlights the ongoing risk of HDV superinfection due to long-term co-habitation or sexual transmission in CHB patients. The fact that transmission occurred after almost a decade of marriage may be due to host immune or environmental factors that created a more favorable condition for transmission.

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## Introduction

Approximately 5%–10% of chronic hepatitis B (CHB) patients worldwide are co-infected with hepatitis D virus (HDV) [1]. HDV requires the hepatitis B surface antigen (HBsAg) for a complete replication cycle; therefore, infection is only possible in individuals with CHB, known as superinfection, or in the context of co-

infection with hepatitis B virus (HBV) and HDV together. The incidence of HBV/HDV co-infection and HDV superinfection is unknown in most countries, including Canada.

Although co-infection with HBV and HDV is often self-limiting in adults [2], over 70% of CHB patients with HDV superinfection develop chronic HBV/HDV infection [3] and are at higher risk of cirrhosis and hepatocellular carcinoma compared to HBV mono-infection [4]. HDV superinfection also is associated with fulminant hepatitis [5] and is a significant concern in HBV/HDV endemic regions and immigrant populations from such regions [6]. This study is the first detailed longitudinal clinical and virological characterization of HDV superinfection in Canada, with the surprising finding of transmission occurring between spouses despite almost a decade of marriage, with 2 children that had been successfully immunized against HBV.

**Abbreviations:** CHB, chronic hepatitis B infection; HBV, hepatitis B virus; HDV, hepatitis D virus; IFN, interferon; IU, international units; kPA, kilopascals; LSM, liver stiffness measurement; PCR, polymerase chain reaction; qHBsAg, quantitative HBsAg; TDF, tenofovir dioxoproxil fumarate; TND, target not detected.

\* Corresponding author.

E-mail addresses: [carla.osiowy@phac-aspc.gc.ca](mailto:carla.osiowy@phac-aspc.gc.ca) (C. Osiowy),

[anton.andonov@phac-aspc.gc.ca](mailto:anton.andonov@phac-aspc.gc.ca) (A. Andonov),

[kevin.fonseca@albertahealthservices.ca](mailto:kevin.fonseca@albertahealthservices.ca) (K. Fonseca),

[ken.swidinsky@phac-aspc.gc.ca](mailto:ken.swidinsky@phac-aspc.gc.ca) (K. Swidinsky), [elizabeth.giles@phac-aspc.gc.ca](mailto:elizabeth.giles@phac-aspc.gc.ca)

(E. Giles), [andy.mason@ualberta.ca](mailto:andy.mason@ualberta.ca) (A. Mason), [cscoffin@ucalgary.ca](mailto:cscoffin@ucalgary.ca) (C.S. Coffin).

<http://dx.doi.org/10.1016/j.idcr.2017.03.001>

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## Case report

A 38-year old genotype D CHB male presented to the hospital in July 2015 with a 1-week history of jaundice, fever, epigastric pain, and rash. The patient was not cirrhotic and had been treated for approximately 2 years with tenofovir dioxoproxil fumarate (TDF), with a quantitative HBV DNA measurement of 2.12 log<sub>10</sub> IU/mL. Due to progressive liver dysfunction the patient was urgently transferred to the regional liver transplantation unit. Testing was done to determine the etiology of fulminant liver failure, including acetaminophen levels, septicemia, and abdominal ultrasound with Doppler ultrasonography of the hepatic vessels, all of which were unremarkable or normal. Possible infectious disease causes, including herpes simplex virus, Epstein Barr virus, cytomegalovirus, and other hepatitis viruses were all found to be negative, with the exception of positive antibody and RNA results for hepatitis D virus. It was noted that he had tested negative for HDV at 6 months and 2 years prior (Table 1). He subsequently stabilized and after 3 months he had improved liver function and by 16 months follow-up he remained clinically stable with Child-Pugh Class A cirrhosis, normal liver function, and undetectable HBV DNA, although his quantitative HBsAg (qHBsAg) and HDV RNA continued to be detectable.

The patient's primary risk factor for HDV transmission was exposure to his wife, who had known HBeAg negative, anti-HBe positive, genotype A CHB (diagnosed during routine prenatal screen). At presentation (5 years prior), she had low-level HBV DNA (2.11 log<sub>10</sub> IU/mL) and normal liver stiffness measurement (LSM; 4.4 kPa). Approximately 30 months after initial assessment, she was diagnosed with chronic HDV infection, with high levels of HDV RNA ( $\geq 6$  log<sub>10</sub> copies/mL), which was also found following retrospective testing of an archived sample previously only tested for HBV markers. Over 5 years of follow-up, she continued to have low-level HBV DNA (Table 1), but significant qHBsAg levels (4.5 log<sub>10</sub> IU/mL) and fluctuating LSM values (4.4 – 9.3 kPa). She

was treated with 48 weeks of Pegylated IFN/TDF therapy, (initiated ~6 months after her husband developed acute liver failure). The wife had Peg-IFN-related side effects but liver function remained normal. Following completion of IFN treatment, HBV DNA and HDV RNA were not detected and she remains on TDF for CHB. The couple had emigrated independently from Mongolia and met in Canada approximately 10 years previously. The couple has 2 Canadian-born children who received HBV immunization and have HBV immunity. Both subjects provided written informed consent to participate and for testing of archived and prospectively collected blood samples for research (University of Calgary Conjoint Ethics Research Board, ID 16636).

To investigate possible interspousal transmission, HDV was extracted from serum obtained from both the patient and his wife and the full HDV genome was PCR-amplified [7]. The complete genomes were approximately 1670 bp in length (GenBank accession numbers KY379246 and KY379247) and differed by <1% nucleotide distance (0.51% ± 0.17%; MEGA7) [8]. Both sequences were HDV genotype 1 (Fig. 1a). Phylogenetic analysis of longitudinal HDV delta antigen (DAg) coding sequence (357 bp, GenBank accession numbers KY379238 to KY379245, KY458181, KY458182; Fig. 1b) from the couple demonstrate HDV evolution following transmission, with 2 nucleotide substitutions apparent in the husband's DAg sequence (at nt 1000 and nt 1246) differing from the DAg sequence of the wife close to the 6-month time of suspected transmission (Fig. 2). The husband's DAg sequence remained nearly identical throughout the 16-month follow-up (over 357 bp; Fig. 2), while the wife's DAg sequence displayed increased heterogeneity, particularly following initiation of treatment (16-07-06 sequence; Figs. 1 b and 2).

## Discussion

This is the first reported case in Canada, confirmed by HDV complete genome phylogenetic analysis, of spousal transmission

**Table 1**  
Longitudinal serological and molecular markers of HBV and HDV infection in the wife and husband over time.

Date (month-YY)	WIFE					HUSBAND				
	HBV DNA <sup>a</sup> (log <sub>10</sub> IU/mL)	HBsAg <sup>b</sup> (log <sub>10</sub> IU/mL)	HDV Ab <sup>c</sup>	HDV RNA <sup>d</sup>	HDV RNA <sup>e</sup> (log <sub>10</sub> copies/mL)	HBV DNA <sup>a</sup> (log <sub>10</sub> IU/mL)	HBsAg <sup>b</sup> (log <sub>10</sub> IU/mL)	HDV Ab <sup>c</sup>	HDV RNA <sup>d</sup>	HDV RNA <sup>e</sup> (log <sub>10</sub> copies/mL)
Mar-11						6.53				
May-11		2.03		POS	6.55					
Dec-11						6.26				
Aug-11	2.11									
Mar-12						5.85				
Nov-12	2.20									
Jun-13	1.78					3.30		NEG		
Nov-13	1.08	2.08	POS							
Jan-14		1.97		POS	5.93					
Sep-14	<1									
Jan-15	<1	4.59	POS	POS	6.02			NEG		
Jul-15	<1	1.97		POS	6.39	2.12	1.10	POS	POS	4.39
Oct-15						<1	3.16		NEG	
Jan-16	<1	4.52				<1			POS	3.11
Apr-16						TND <sup>f</sup>				
Jul-16	<1	1.97	POS	POS	4.29	TND			POS	TND
Oct-16						TND	2.80		POS	3.65
Jan-17			POS	NEG						

<sup>a</sup> HBV DNA measured by the Cobas AmpliPrep/Cobas TaqMan v.2 assay (Roche Diagnostics, Laval, QC) from 2011 to 12 and by the RealTime HBV assay (Abbott Laboratories, Mississauga, ON) 2013 and beyond.

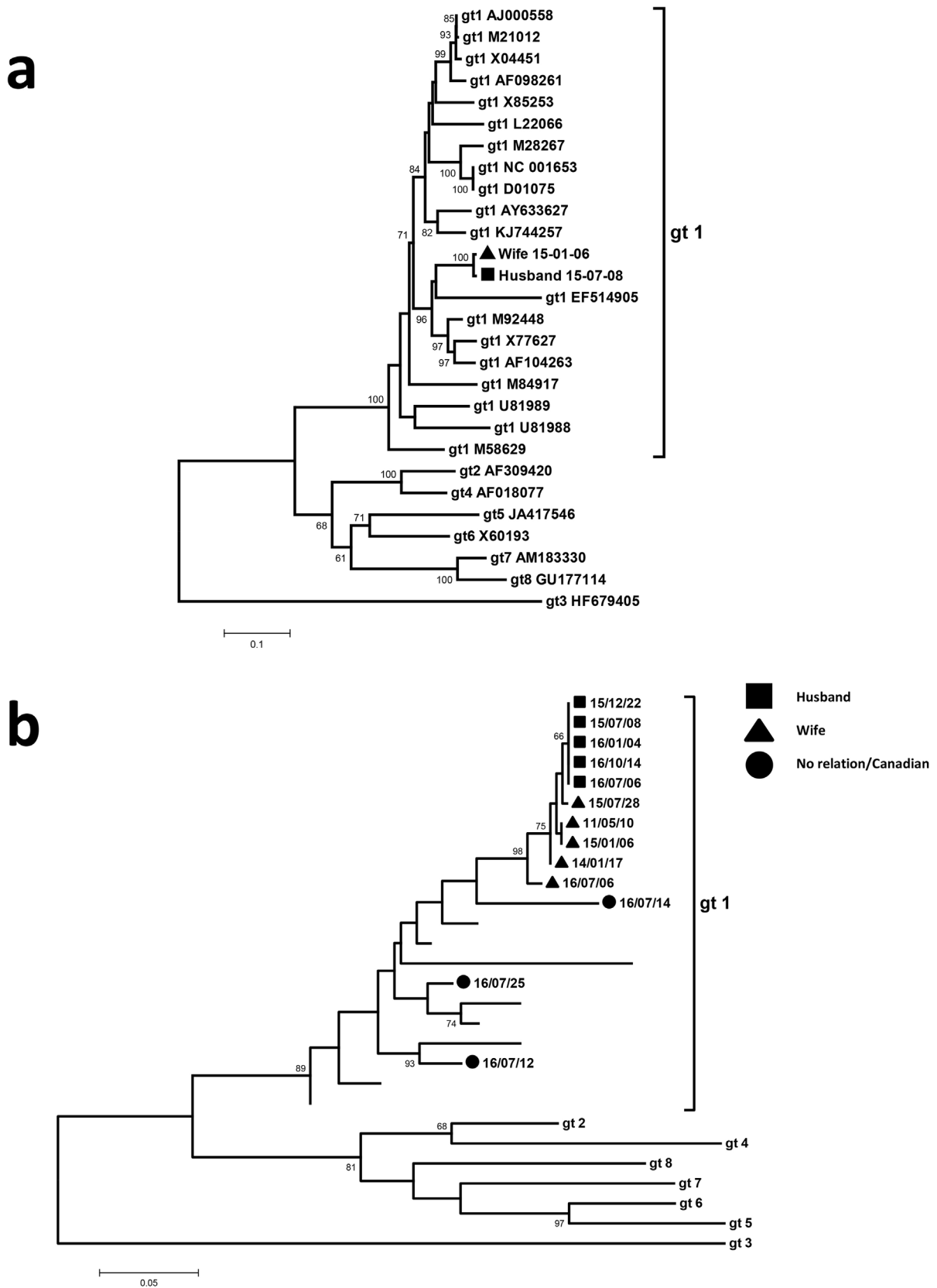
<sup>b</sup> Quantitative HBsAg was measured by the Abbott Architect HBsAg assay (limit of detection  $\leq 0.05$  IU/mL).

<sup>c</sup> HDV antibodies were measured by the Wantai HDV-IgG ELISA assay (Beijing Wantai Biological Pharmacy Enterprise Co., Beijing, China).

<sup>d</sup> HDV RNA detection was performed by nested PCR [18].

<sup>e</sup> Quantitative HDV RNA was measured by an in-house real-time RT-PCR method having a linear range of 3.11 log<sub>10</sub> to 10.44 log<sub>10</sub> copies/mL and a limit of detection of 3.11 log<sub>10</sub> copies/mL, based on previously described methods [19,20].

<sup>f</sup> TND, target not detected.



**Fig. 1.** (a) Phylogenetic analysis of full genome HDV sequence of husband (■) and wife (▲) with GenBank reference sequences for all HDV genotypes. The date of specimen collection is provided (Y-M-D). (b) Phylogenetic analysis of the delta antigen coding region (357 bp, approximately nt 906–1262 based on the numbering of GenBank accession no. M58629) from longitudinal specimens of husband (■) and wife (▲). The date of specimen collection is provided beside each sequence (Y/M/D). Sequenced diagnostic reference samples unrelated to the couple, but residing in the same Canadian location are shown (●). Maximum likelihood phylogenetic analysis was performed using MEGA7 with 500 bootstrap replicates and the Tamura-3-parameter + Gamma substitution model as the most appropriate model for the alignment data. Bootstrap confidence values  $\geq 60\%$  are shown. The ruler shows the branch length for a pairwise distance equal to (a) 0.1 or (b) 0.05. The GenBank accession numbers for the sequences reported are KY379238 to KY379247, KY458181, KY458182.

Spouse (date)	HDV nt 998-1012*	HDV nt 1055-1070*	HDV nt 1086-1090*	HDV nt 1244-1248*
Wife 11-05-10	...AAAAGCATATCCTAT...	...CTAGAGAAGGGGGACT...	...TGCTG...	...CCCGC...
Wife 14-01-17	...AAAAGCATATCCYAT...	...CTAGAGAAGGGGGACT...	...TGCTG...	...CCCGC...
Wife 15-01-06	...AAAAGCATATCCYAT...	...CTRGAGAAGGGGGACT...	...TGCTG...	...CCCGC...
Wife 15-07-28	...AAAAGCATATCCYAT...	...CTAGAGAAGGGGGACT...	...TGCTG...	...CCTGC...
Wife 16-07-06	...AAGAGTATATCCYAT...	...CTGGTGAAGGGGGACT...	...TGCTG...	...CCCGC...
Husband 15-07-08	...AAGAGCATATCCTAT...	...CTAGAGAAGGGGGACT...	...TGCTG...	...CCTGC...
Husband 15-12-22	...AAGAGCATATCCTAT...	...CTAGAGAAGGGGGACT...	...TGCTG...	...CCTGC...
Husband 16-01-04	...AAGAGCATATCCCAT...	...CTAGAGAAGGGGGACT...	...TGTTG...	...CCTGC...
Husband 16-07-06	...AAGAGCATATCCTAT...	...CTAGAGAAGGGGGATT...	...TGTTG...	...CCTGC...
Husband 16-10-14	...AAGAGCATATCCTAT...	...CTAGAGAAGGGGGACT...	...TGTTG...	...CCTGC...

**Fig. 2.** Longitudinal HDV delta antigen region sequence (357 bp) from spousal couple specimens, by date (Y-M-D). The major nucleotide substitutions observed between husband and wife or among longitudinal samples are denoted by the boxes. \*Nucleotide numbering is based on GenBank accession no. M58629 (357 bp region is found at nt 906 to nt 1262).

after nearly 10 years of marriage and ongoing transmission risk factors (co-habitation and sexual contact) [9–11]. The couple's country of origin, Mongolia, is highly endemic for HDV [12], thus it is assumed that the wife has been HBV/HDV co-infected since childhood or as a young adult [13]. Prior to HDV transmission to her spouse, the wife experienced high HDV RNA ( $\geq 6 \log_{10}$  copies/mL), which likely contributed to liver fibrosis progression and increased infectious risk. Despite the high genomic variability within HDV genotype 1 (up to 12% nucleotide distance), there was near-complete sequence identity (99.5%), with nucleotide shifts or substitutions observed within the DAg coding region with increasing time from transmission.

HDV superinfection of the husband appears to have subsequently led to chronic HDV infection, with suppressed HBV DNA a few months following HDV superinfection. Although HBV suppression in HDV chronic co-infection is common, several longitudinal studies have shown dynamic fluctuations between HBV DNA and HDV RNA over time [14], possibly due to immune or environmental factors or changes in HBsAg [15], suggesting ongoing risk of HBV DNA flares. HDV is dependent on HBsAg for viral packaging and release from infected cells and on PreS1 protein expression for receptor binding [5]. Thus, the detection of qHBsAg in the husband over the clinical follow-up period portends a higher likelihood of ongoing HDV replication.

This study illustrates the continuing risk of HDV transmission in long-term HBV-infected co-habitants/spouses. HDV superinfection is associated with a high risk of severe disease, including fulminant hepatitis as was observed in our patient. Although, co-habitants/spouses may consider preventative measures to reduce the risk of transmission, it is interesting that the couple had both a long-term relationship and 2 children without HDV superinfection. Thus, this study raises several clinical questions; namely, (i) should couples use barrier protection and conception with assisted reproduction methods, similar to recommendations for HIV prior to the advent of highly suppressive antiviral therapy [16]?; (ii) is therapy a consideration even in inactive CHB mono-infected carriers, if they have a close HBV/HDV co-infected contact, in order to potentially lower qHBsAg levels and the risk of HDV transmission?; (iii) should Peg-IFN and/or suppressive nucleos(t)ide antiviral therapy be considered with the HBV/HDV co-infected partner [17], even though lowering HBV DNA could lead to an increase in HDV

viremia and transmission risk, due to the complex viral interactions that occur during co-infection? This study illustrates the clinical challenge regarding optimal management of HDV and HBV co-infection, and the need for detailed long-term epidemiological and virological studies.

#### Funding

None. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### Competing interests

None declared.

#### Ethical approval

Study subjects provided written informed consent to participate and for testing of archived and prospectively collected blood samples for research (University of Calgary Conjoint Ethics Research Board, ID 16636).

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