ORIGINAL RESEARCH

The epidemiology of hepatitis delta virus infection in Cameroon

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ABSTRACT

Objective To investigate the distribution and risk factors of hepatitis delta virus (HDV) infection in Cameroon.

Design We tested for hepatitis B virus (HBV) surface antigen (HBsAg) and anti-HDV antibody 14 150 samples collected during a survey whose participants were representative of the Cameroonian adult population. The samples had already been tested for hepatitis C virus and HIV antibodies.

Results Overall, 1621/14 150 (weighted prevalence=11.9%) participants were HBsAg positive, among whom 224/1621 (10.6%) were anti-HDV positive. In 2011, the estimated numbers of HBsAg positive and HDV seropositives were 1 160 799 and 122 910 in the 15–49 years age group, respectively. There were substantial regional variations in prevalence of chronic HBV infection, but even more so for HDV (from 1% to 54%). In multivariable analysis, HDV seropositivity was independently associated with living with an HDV-seropositive person (OR=8.80; 95% CI: 3.23 to 24.0), being HIV infected (OR=2.82; 95% CI: 1.32 to 6.02) and living in the South (latitude <4°N) while having rural/outdoor work (OR=15.2; 95% CI: 8.35 to 27.6, when compared with living on latitude ≥4°N and not having rural/outdoor work).

Conclusion We found evidence for effective intra-household transmission of HDV in Cameroon. We also identified large differences in prevalence between regions, with cases concentrated in forested areas close to the Equator, as described in other tropical areas. The reasons underlying these geographical variations in HDV prevalence deserve further investigation.

Significance of this study

What is already known on this subject

► A recent systematic review summarised current knowledge of hepatitis delta virus (HDV) distribution in the African continent, with high prevalence in Central Africa, intermediate prevalence in West Africa and low prevalence in East and Southern Africa. The reason for these important disparities remains unclear as the routes of transmission of HDV in this part of the world are largely unknown. Also, many of the studies used in this review were small and based on convenience samples.

What are the new findings

► This epidemiological study, based on a large nationwide representative sample of the adult population of Cameroon, included 224 HDV-seropositive individuals. It is also one of the few studies that have examined risk factors for HDV infection in the African context. It showed that intra-household transmission was very effective in households where this was possible (at least two hepatitis B virus (HBV) surface antigen (HBsAg)-positive members). There were profound disparities between regions in HDV prevalence, and, like in other tropical areas, cases were concentrated in forested areas close to the Equator. The reasons underlying these geographic variations in HDV prevalence deserve further investigations.

How might it impact on clinical practice in the foreseeable future

► Even if ultimately HBV immunisation will control HDV spread, concomitant HBV/HDV infections will continue to have important consequences in the next decades as close to 1 200 000 and 120 000 Cameroonians aged 15–49 years were HBs-antigen-positive and HDV seropositives, respectively, in 2011. New treatments, like bulevirtide, may represent life-saving therapeutic options for these patients at high risk of progression to cirrhosis, provided they are made available at affordable cost for low-income countries.

INTRODUCTION

Central Africa has the unfortunate peculiarity of being highly endemic for infection with HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis delta virus (HDV) so that concomitant infections with more than one of these pathogens occur frequently. While much attention has been paid to the first three, relatively little is known about the epidemiology of HDV and its interactions with the other blood-borne viruses. In its 2017 Global Hepatitis Report, the WHO provided worldwide estimates for the number of individuals infected with HBV (256 million), HCV (71 million), HIV and HBV (2.7 million), HIV and HCV (2.3 million) but declined to quantify the global burden of HDV, for lack of data.[1] If, as suggested empirically, 5% of persons with chronic HBV are HDV infected, then the latter's reservoir would correspond to 13 million individuals. With a patchy distribution, the virus is endemic in several regions

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of the world, in particular parts of Africa and of Asia, the Amazon basin and some Pacific islands.[2 3]

A recent systematic review and meta-analysis summarised current knowledge of HDV in the continent: high prevalence in Central Africa (26% in persons with chronic HBV infection, 38% among those with liver disease), intermediate prevalence in West Africa (respectively 7% in general population with chronic HBV infection and 10% in liver-disease population), and low prevalence in East and Southern Africa (only 0.05% in chronic HBV infection).[4] However, this assessment was based on a small number of studies in a few countries, which generally used samples of convenience. The reasons for this huge variation in HDV prevalence between regions (and between countries within a given region) are unknown. Unmet needs include a better description of the epidemiology of the virus, its natural history and the modes of transmission in Africa.[4 5] Demographic Health Surveys (DHS) offer a unique opportunity for the mapping of various pathogens on a very large nationwide representative sample. We recently described the epidemiology of HCV in Cameroon using the specimens obtained during the 2011 DHS.[6] Here, we extend this work to HBV and HDV, and investigate the distribution and risk factors of HDV infection in Cameroon.

MATERIALS AND METHODS

Sampling strategy and data sources

Methods used in the Cameroon 2011 DHS are described elsewhere.[6] In brief, 15 050 households were selected, with urban/rural and administrative stratification so as to yield a representative sample of the entire country. In each household, a standardised questionnaire was administered, designed to evaluate health-related knowledge and behaviours, fertility issues and health-seeking patterns. In half of these households, capillary blood was obtained from consenting women aged 15–49 and men aged 15–59 years to perform anonymous HIV serology.[7] In 14 150 of the 14 202 participants tested for HIV serology, there were sufficient leftover samples for HBV, HCV and HDV testing. We used cluster geolocalisation of each participant to estimate altitude and latitude/longitude coordinates. There was no patient or public involvement in the design, conduct, reporting or dissemination of the research.

Laboratory assays

Details of the processing of capillary blood specimens and methods for HCV serology are described elsewhere; for HIV, we used results determined by the DHS.[6 7] The presence of HBsAg in the eluted blood was measured with an automated chemiluminescent microplate immunoassay (CMIA Architect Plus, Abbott) at the Centre Pasteur du Cameroun. The results were expressed with a signal-to-cut-off ratio calculated by dividing a formalised signal by a threshold value. A sample was considered positive if the ratio was >1 and negative if the ratio was <1. HBsAg-positive samples were tested for HDV antibodies using a competitive ELISA (HDV-Ab, Dia.Pro Diagnostic Bioprobes). A sample was scored as positive if the signal-to-cut-off ratio was >1, and negative if the ratio was <1. In a validation study using paired samples, dried blood spots analysis had a sensitivity of 99% for HBsAg and 98% for HDV antibodies and a specificity of 100% for both viruses when compared with plasma samples (data not shown), in line with a recent meta-analysis.[8]

Statistical analysis

The data were analysed with Stata V.14. National and regional HBV and HDV prevalence were estimated using samples weights provided by the DHS programme, based on the probability of a subject being included in the survey. More information about the weighting procedure is available elsewhere.[9] The estimate of the total number of HBS-antigen and HDV seropositive individuals in the age group 15–49 years was obtained by multiplying the weighted 5-year age-specific and sex-specific prevalence by the number of Cameroonians in each of these age and sex categories in 2011 and summing up all the products. The HDV analyses were performed among the subgroup of HBs antigen-positive individuals, using the subpop command of Stata adapted to the analysis of subpopulations.[10] Weighted proportions were displayed and compared using a Pearson χ^2 statistic adapted to the survey design using the second-order Rao and Scott correction and converted into an F statistic.[11 12] Factors associated with HDV infection among HBV-infected individuals were explored from the information available in the DHS questionnaires, in univariable and multivariable logistic regression analyses adapted to the survey design. Variables with p values <0.25 in univariable analyses were introduced simultaneously and dropped one by one until only variables significant ($p<0.05$) as per the Wald χ^2 test remained in the model. Three variables, regions, ethnic groups and latitude, could have each been kept in the final model, but without the two other ones as they were correlated with each other. We chose to retain in the final model the variable common to other geographical areas experiencing high endemic HDV prevalence worldwide, that is, latitude as a measure of proximity to equatorial regions. Choosing any of these three for the final model had little impact on the magnitude of the ORs of the other variables kept in the model. We noticed an interaction between latitude and having a rural outdoor work (farming, hunting and fishing). To simplify the presentation of the interaction in the multivariable model, we expressed latitude as a two-category variable (<4°N and ≥4°N), rather than the four-category variable (<4°N, 4–5.5°N, 5.6–9.5°N,and >9.5°N) used in the univariable analysis.

To document the intrafamilial clustering of HDV infections, we tested whether HDV infections were more clustered within HBV-infected households than expected by chance, restricting the analysis to households with at least two HBV-infected individuals (ie, households where intra-household transmission of HDV was possible).[13] We randomly distributed the HDV-infected individuals among the population of HBV-infected individuals, preserving the observed household structure (same number of households and of HBV infections per household). We repeated this procedure 1000 times, summarised the total number of HDV-infected households (households with at least one HDV infection) over the 1000 simulations by taking the median and 95% (based on the 2.5 and 97.5 percentiles) CI, and compared it to the observed number of HDV-infected households. The same approach was used to document the intra-household clustering of HBV, HIV and HCV infections, restricting the analysis to households with at least two individuals. To compare the level of clustering among viruses, we estimated for each virus the ratio of observed number of infected households versus expected had the distribution of infections been random.

To model intra-household exposure to HDV infection, we created a new variable taking the value one for individuals exposed to an HDV-infected household member, and 0 otherwise. When two or more HDV-infected individuals were living in the same household, we hypothesised that one of them introduced into the household the virus to which others were exposed, rather than having simultaneous infections of several members of the household through an external source. Since it was not possible to know which household member got infected first by an external source, we randomly attributed the external infection to one of the infected individuals of the household. We repeated the same procedure 100 times, and obtained 100 different datasets. We then ran the multivariable analysis with these 100 datasets, and obtained

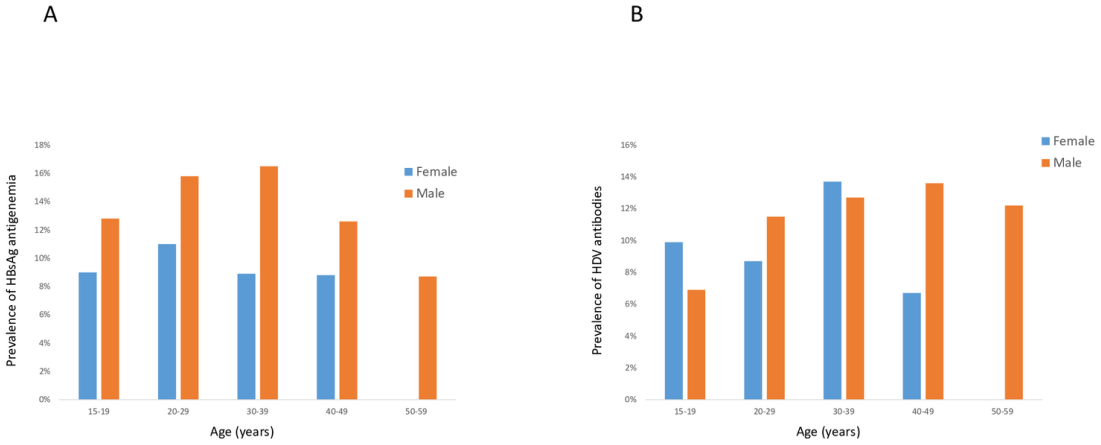

Figure 1 Prevalence of chronic HBV infection (A) and of HDV antibody among patients with chronic HBV infection (B) by age and gender, DHS 2011, Cameroon. DHS, Demographic Health Surveys; HBV, hepatitis B virus; HDV, hepatitis delta virus.

100 estimates for each beta coefficient of the variables included in the final model of the multivariable analysis. To combine the results obtained from these 100 completed-data analyses into a single multiple-imputation result, we averaged the 100 beta coefficients, and computed the 95% CI, using the mi commands of Stata, thus performing the same averaging and 95% CIs computations than during multiple imputation.[14] To appropriately compute the 95% CIs, the pooling step took into account both the within-imputation variability and the between-imputation variability.

To create smoothed interpolated maps of HBV and HDV prevalence in Cameroon not constrained by administrative boundaries, we developed a Bayesian geostatistical model[15] as described elsewhere.[16 17] This model assumed that the number of infections at each spatial location (DHS cluster) followed a binomial distribution, taking into account the population denominator (total number of individuals for HBV prevalence and total number of HBV-infected individuals for HDV prevalence) at each location. It accounted for the spatial dependence in the data, using a Matérn covariance function, and included an unstructured random error, to create a continuous surface of prevalence where the random noise has been properly removed. The model was implemented using the integrated nested Laplace approximation (INLA) method in the R-INLA package.[18] HBV and HDV prevalence was predicted on a grid of 2×2 km resolution, and mapped using QGIS.

RESULTS

Age, gender and regional distribution of HBV and HDV infections

Overall, 1621/14 150 (weighted prevalence=11.9%) participants were HBsAg-antigenemic, leading to an estimate of 1 160 799 HBs-antigen positives in the 15–49 years age group in 2011. Figure 1A displays the prevalence of chronic HBV infection according to age and sex. Prevalence among women peaked at 11.0% among those aged 20–29 years, and then slowly declined with age. In men, prevalence was higher, peaking at 16.5% in those aged 30–39 years but then dropping considerably to reach 8.7% in the oldest cohort. The prevalence of chronic HBV infection varied between regions, from 7.0% in Nord-Ouest up to 17.7% in Extrême-Nord (figure 2A). Overall, 224/1621 (10.6%) of participants with chronic HBV infection were also HDV seropositives, leading to an estimate of 122 910 HDV seropositives in the 15–49 years age group in 2011. HDV antibody prevalence did not vary with gender or age (figure 1B), but there were profound variations between regions (table 1/figure 2B) and ethnic groups (table 1). In two regions, Sud and Est, HDV prevalence was 50% and 54%, respectively, whereas it was only 1%–19% in the remaining 10 regions (p<0.0001). Regarding ethnic groups, HDV prevalence was 49% and 25% among Eastern and Southern Bantus, respectively, whereas it was

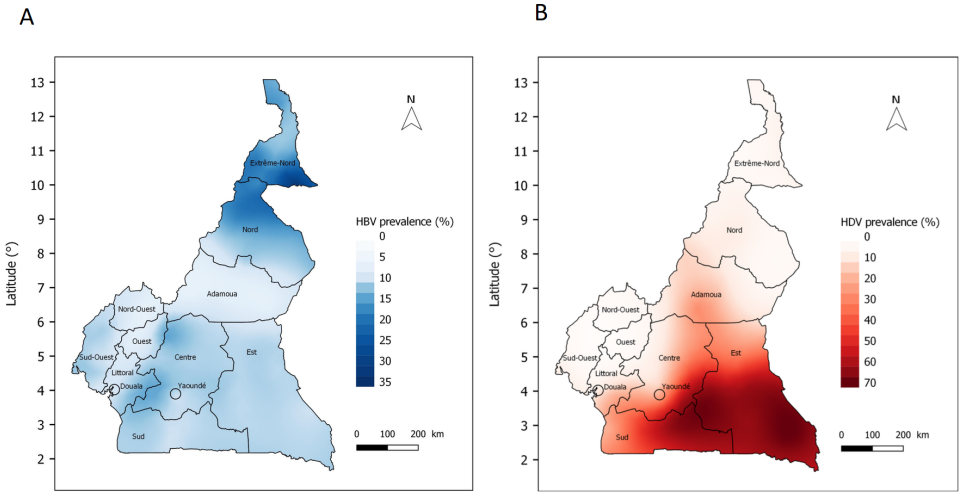

Figure 2 Spatial distribution of the prevalence of chronic HBV infection (A) and of HDV antibodies among patients with chronic HBV infection (B), DHS 2011, Cameroon. DHS, Demographic Health Surveys; HBV, hepatitis B virus; HDV, hepatitis delta virus.

Table 1 Risk factors for HDV seropositivity among individuals with chronic hepatitis B virus infection in univariable analyses

	HDV+/total (%)*	ORs (95% CI)	P value
Gender			0.4
Female	91/664 (9.8%)	1	
Male	133/957 (11.2%)	1.16 (0.82 to 1.65)	
Region			<0.001
Littoral	13/91 (15.3%)	1	
Adamaoua	11/89 (12.5%)	0.79 (0.21 to 3.01)	
Centre	33/158 (18.6%)	1.26 (0.49 to 3.29)	
Est	54/102 (53.7%)	6.44 (2.40 to 17.3)	
Extreme Nord	9/267 (2.9%)	0.17 (0.05 to 0.52)	
Nord	17/265 (7.5%)	0.45 (0.14 to 1.38)	
Nord-Ouest	3/100 (3.4%)	0.19 (0.05 to 0.79)	
Ouest	1/110 (0.8%)	0.04 (0.005 to 0.37)	
Sud	48/97 (48.5%)	5.24 (2.12 to 12.9)	
Sud-Ouest	5/122 (3.8%)	0.22 (0.06 to 0.78)	
Douala	8/112 (5.8%)	0.34 (0.10 to 1.19)	
Yaounde	22/108 (19.3%)	1.32 (0.47 to 3.69)	
Ethnicity			<0.001
Pygmies/others/foreigners	5/43 (7.7%)	1	
Non-natives northerners	12/135 (6.4%)	0.82 (0.21 to 3.26)	
Autochtonous northerners	17/312 (5.9%)	0.75 (0.18 to 3.06)	
Eastern bantus/bantoids	44/82 (49.2%)	11.6 (3.01 to 44.8)	
Grassfields peoples	6/192 (3.1%)	0.39 (0.09 to 1.67)	
Western bantoids	20/458 (4.1%)	0.51 (0.13 to 1.99)	
Southern bantus	120/399 (24.8%)	3.95 (1.15 to 13.5)	
Altitude, metres			<0.001
<300	44/460 (8.3%)	1	
300–599	47/440 (7.7%)	0.92 (0.48 to 1.76)	
600–899	124/454 (19.9%)	2.75 (1.49 to 5.06)	
>900	9/267 (3.2%)	0.37 (0.15 to 0.87)	
Latitude			<0.001
<4° N	129/345 (28.3%)	9.09 (3.52 to 23.5)	
4–5.5 ° N	52/460 (9.3%)	2.36 (0.91 to 6.15)	
5.6–9.5 ° N	27/467 (5.5%)	1.34 (0.48 to 3.73)	
9.6–13 ° N	16/349 (4.2%)	1	
Forested area			0.03
No	151/1265 (9.6%)	1	
Yes	73/356 (14.8%)	1.63 (1.05 to 2.55)	
Education			<0.001
None	12/246 (3.1%)	1	
Primary	76/586 (9.9%)	3.43 (1.64 to 7.2)	
Secondary or higher	136/789 (13.7%)	4.92 (2.43 to 9.93)	
Wealth index			0.44
Poorest	32/363 (6.3%)	0.49 (0.22 to 1.11)	
Poor	57/345 (12.0%)	1.00 (0.56 to 1.77)	
Intermediate	45/293 (11.1%)	0.91 (0.52 to 1.58)	
Rich	45/301 (12.2%)	1.02 (0.56 to 1.86)	
Richest	45/319 (12.1%)	1	
Rural and outdoor work†			0.95
No	132/1041 (10.5%)	1	
Yes	92/580 (10.7%)	1.01 (0.68 to 1.50)	
Intra-household HDV infection			<0.001
No	204/1585 (9.8%)	1	
Yes	20/36 (48.8%)	8.80 (3.80 to 20.4)	
Injections, last year			0.007
0	124/989 (9.2%)	1	
1–2	44/310 (11.7%)	1.30 (0.84 to 2.03)	
3–9	35/250 (11.3%)	1.25 (0.81 to 1.94)	
≥10	21/72 (23.5%)	3.03 (1.60 to 5.72)	
All injections by HCW, last year			0.01
No injections	124/989 (9.2%)	1	
All injections by HCW	94/613 (12.2%)	1.36 (1.00 to 1.86)	
Not all injections by HCW	6/19 (32.5%)	4.75 (1.52 to 14.8)	
Marital status			0.09
Never married	80/627 (10.8%)	1	
Married	123/889 (9.7%)	0.89 (0.59 to 1.34)	
Divorced/separated/widowed	21/105 (16.6%)	1.64 (0.88 to 3.06)	
Lifetime number of sex partners			<0.001
None	19/243 (7.1%)	1	
1	19/335 (4.2%)	0.57 (0.28 to 1.17)	
2–9	116/763 (12.0%)	1.78 (0.86 to 3.67)	
≥10	69/259 (18.3%)	2.93 (1.33 to 6.44)	
Sexual partnerships, last year			<0.001
Only spouse	117/1077 (8.2%)	1	
Other partner	107/537 (15.8%)	2.08 (1.51 to 2.88)	
HIV			0.003
Seronegative	205/1553 (10.0%)	1	
Seropositive	19/68 (24.4%)	2.92 (1.45 to 5.88)	
Hepatitis C virus			0.51
Seronegative	224/1616 (10.6%)	1	
Seropositive	0/5 (0.0%)	0.93 (0 to 6.82)‡	
Males only			
Paid for sex in last year			0.11
No	106/684 (11.8%)	1	
Yes	9/31 (21.1%)	2.01 (0.86 to 4.68)	
Circumcision			0.05
Not circumcised	3/84 (3.9%)	1	
Circumcised	130/873 (12.0%)	3.33 (0.98 to 11.4)	
Place of circumcision			0.3
Health facility	46/425 (11.0%)	1	
Ritual site	11/81 (9.6%)	0.86 (0.35 to 2.14)	
Home	70/335 (14.5%)	1.37 (0.79 to 2.39)	
Person who circumcised			0.34
Health worker	63/523 (11.1%)	1	
Traditional practitioner	63/310 (14.1%)	1.31 (0.75 to 2.30)	
Age at circumcision			0.03
<5 years	41/385 (8.9%)	1	
≥5 years	83/460 (14.5%)	1.73 (1.06 to 2.83)	

Missing values: lifetime number of sex partner 21, sexual partnerships last year 7, paid for sex in last year 242, place for circumcision 116, person who circumcised 124, age at circumcision 112.
*Weighted-proportion.
†Farming, hunting, fishing.
‡Exact logistic regression, ignoring the sampling desing.
HCW, healthcare worker; HDV, hepatitis delta virus.

only 3%–8% in the remaining ethnic groups (p<0.0001). There was a pronounced South–North gradient in HDV prevalence, from 28.3% under 4°N down to 4.2% above 9.6° N (figure 2B). In univariable analysis, HDV prevalence was higher in forested areas, and at altitudes between 600 and 900 m.

Sociodemographic and behavioural activities associated with HDV seropositivity—univariable analysis

As shown in table 1, HDV seropositivity was significantly more common among participants who had received ≥10 injections in the last year or injections made in the informal sector. It was also more frequent among the HIV infected, in those who had had recently a sex partner other than the spouse, and in men and women with higher number of partners in their lifetime. For instance, among men and women reporting ≥10 lifetime partners, respectively, 58/240 (16.4%) and 11/19 (40.1%) were HDV seropositive. It was also more frequent in males who had been

Table 2 HDV antibody weighted-prevalence (%) by latitude and rural/outdoor work

	Latitude			
	<4 °N	4–5.5 °N	5.6–9.5 °N	>9.5 °N
Rural/outdoor work				
No	23.0	7.8	5.9	5.8
Yes	52.5	13.9	4.7	2.4

HDV, hepatitis delta virus.

circumcised after 5 years of age, but it was less common among the poorest socioeconomic stratum. Rural and outdoor (hunting, fishing and farming) workers were more at risk of HDV infection compared with other occupational category, but only in the Southern regions (p value for the interaction term=0.01; table 2). HDV seropositivity did not vary according to a history of genital ulcer or discharge, occupation, type of housing, number of people in the household, and rural or urban residence (data not shown).

Intra-household clustering of HDV infection

Table 3 shows the distribution of the 224 HDV seropositives according to the number of HBV infections in the household. Among the 239 households with at least two HBV-infected individuals, if HDV seropositivity had been randomly distributed across individuals of these households, the expected number of households with at least one HDV seropositive would have been 48 (95% CI, 44 to 50). The observed number was 31, significantly lower than expected, suggesting an intra-household clustering of cases (the lower the number of households infected, the larger the number of infected individuals within infected households). Likewise, the observed number of households with two or more HDV seropositives was significantly higher than expected by chance (16 vs 3; 95% CI, 1 to 7) (thus indicating clustering of infected individuals within households). It is noteworthy that in the family with four chronically infected with HBV, all four were co-infected with HDV. Likewise, of the three families with three chronically infected with HBV, all three were co-infected with HDV in two families, and two out of three were co-infected with HDV in the remaining. The median age of HDV-positive individuals in households where intrafamilial transmission occurred was significantly lower compared with that in households were there was no intrafamilial transmission (22 vs 27 years, respectively, p=0.04). In univariable analysis, living with an HDV-seropositive individual was strongly associated with the risk of being HDV seropositive (OR=8.80; 95%CI, 3.80 to 20.4) (table 1).

Table 3 HDV seropositivity distribution according to the number of HBV infections in the household

	Number of HDV infections in the household				
	0	1	2	3	4
Number of HBV infections in the household					
1	898	173	0	0	0
2	160	15	12	0	0
3	38	0	1	2	0
4	4	0	0	0	1
5	4	0	0	0	0
6	1	0	0	0	0
7	1	0	0	0	0

HBV, hepatitis B virus; HDV, hepatitis delta virus.

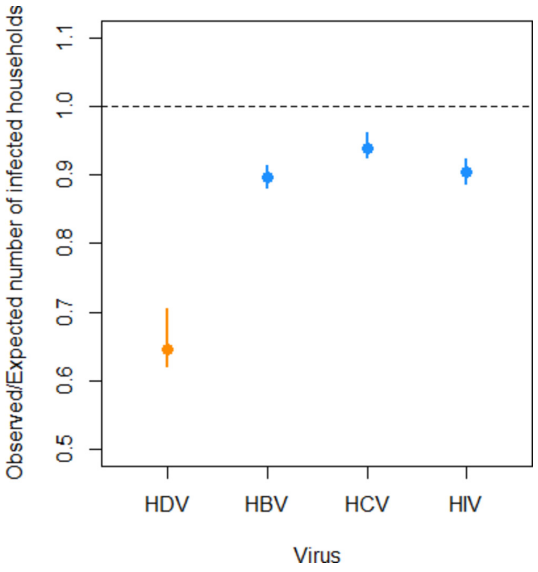

Figure 3 Ratio of observed number of infected households versus expected, had the distribution of infections been random for HDV, HBV, HCV, and HIV, DHS 2011, Cameroon. DHS, Demographic Health Surveys; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus.

We repeated the same analysis for the other viruses, and found the following: The observed number of infected households was 1085 for HBV versus 1209 (1187–1231) had the distribution of infections been random; for HCV, the figures were 123 for observed, and 131 (128–133) for expected; and for HIV 442 for observed, and 489 (479-498) for expected. To compare the level of clustering among viruses, we computed the ratio of observed versus expected, and displayed it on figure 3. Clustering was stronger for HDV compared with the three other viruses.

Multivariable analysis of factors associated with HDV seropositivity

Table 4 shows the factors independently associated with HDV seropositivity in multivariable analysis. HDV seropositivity was independently associated with living with an HDV-seropositive person (OR=8.80; 95%CI: 3.23 to 24.0), being HIV infected (OR=2.82; 95%CI: 1.32 to 6.02) and living in the South (latitude <4°N) while having rural/outdoor work (OR=15.2; 95%CI: 8.35 to 27.6, when compared with living on latitude ≥4°N and not having rural/outdoor work).

Table 4 Risk factors for HDV seropositivity among individuals with chronic HBV infection in multivariable analysis

	Adjusted ORs (95% CI)
Latitude and rural/outdoor work*	
<4° N with rural/outdoor work	15.2 (8.35 to 27.6)
<4° N without rural/outdoor work	4.23 (2.48 to 7.22)
4–13 ° N with rural/outdoor work	0.92 (0.53 to 1.59)
4–13 ° N without rural/outdoor work	1
Intra-household HDV infection	
No	1
Yes	8.80 (3.23 to 24.0)
HIV	
Seronegative	1
Seropositive	2.82 (1.32 to 6.02)

*Farming, hunting, fishing.
HBV, hepatitis B virus; HDV, hepatitis delta virus.

DISCUSSION

Little is known about the epidemiology of HDV in Africa. The requirement of a prior infection with HBV before HDV can become infectious means that only a fraction of the population, even in HBV-endemic countries, is susceptible. Some previous large-scale studies of HDV in Africa were conducted in The Gambia and Burkina Faso, where only ≈1% of HBsAg carriers are HDV-seropositive, precluding the evaluation of risk factors for HDV infection.[19 20] In Gabon, a study of 4107 persons living in rural villages identified 303 HBsAg antigenemic participants, of whom 84 (28%) were HDV seropositives.[21] With a large representative sample of the adult population of Cameroon, this study revealed an extreme degree of heterogeneity of HDV distribution across Cameroon, ranging from 1% to 54% according to regions, with the highest prevalence found in the Southern regions close to the Equator. While we did not attempt to detect HDV RNA in our dried blood samples, we believe that around two-thirds of them would be positive for HDV RNA as was observed in similar populations of Cameroon and the Central African Republic.[22–24] With 224 HDV-seropositive individuals out of 1621 at risk, our study also provides one of the most complete description of risk factors for HDV infection in Africa. With this large sample size, we were able to document intrahousehold clustering of HDV infection, and estimated a ninefold increase in HDV risk for those exposed to someone infected with HDV in the household. Finally, we found some evidence for HDV transmission through parenteral and sexual exposures, and a threefold increase in risk of HDV infection among those infected with HIV.

HDV infection: a peri-equatorial distribution

In Cameroon, the highest HDV prevalence was seen in the Southern part of the country, under latitude 4° N, with a subequatorial climate and largely forested areas. Similar high HDV prevalence were observed through populational studies in neighbouring regions of Gabon (63%–66% in the Northern region called Woleu-Ntem),[21 25 26] and investigation of icteric patients in Democratic Republic of Congo (33% and 48% in the Northern regions of Equator and Orientale, respectively).[27] In a similar manner, migrants from Equatorial Guinea in Spain presented high rates of HDV seropositivity (24%).[28] The same pattern of HDV hyperendemicity is observed in adjacent regions of Brazil, Peru and Colombia, corresponding to the peri-equatorial and forested areas of the Amazon basin.[29–32] There is no known explanation for the increased HDV prevalence observed in these regions. In our study, individuals practising rural and outdoor work, such as farming, fishing or hunting, in the Southern part of the country were at increased risk of HDV infection, as already observed in Amazon Basin,[33] suggesting that higher exposition to outdoor environments such as forest or plantations may play a role in contracting the infection. Such observations, as well as the high prevalence seen among children in Southern Cameroon,[24] and the lower prevalence seen in our study for altitudes higher than 900 m, raises the possibility of a role for mosquitoes or other insects in transmission. Entomological studies and isolation of infectious viruses from insects would be required to document this provocative hypothesis. Of note, a recent publication identified viruses other than HBV, such as HCV or dengue virus, as potential helpers for HDV infection, so that means of HDV circulation in population may be broader than initially thought.[34]

Household clustering of HDV infection

With its large number of HDV seropositives identified through household sampling during the DHS, this study offered a unique opportunity to estimate the increase in risk associated with intra-household exposure to HDV. Such studies are difficult to conduct, since it requires to have several household members chronically infected with HBV, and the introduction of HDV in the household, before such observation can be made. Molecular evidence of HDV intra-household transmission is already available from families in Italy,[35] and more recently between two grandsons in Gabon[25]; however, these studies could not quantify the increase in risk associated with intrahousehold exposure to HDV. In this study, in households with three or four HBV-infected members, once HDV was introduced, almost all HBV-infected household members became co-infected with HDV (table 3). Through logistic regression analysis, we were able to document a ninefold increase in HDV risk for HBV carriers living in an household with at least one HDV-seropositive individual. This estimate was little affected in multivariable analysis, suggesting that intra-household HDV transmission was independent of other factors found associated with HDV seropositivity in this study. Prevalence of HDV is already high at age 15 years, as for HBV (figure 1), in contrast to HIV (online supplementary figure S1) and HCV (online supplementary figure S2) whose prevalence are low at that age. Thus, one may speculate that the same modes of horizontal transmission operate during childhood for HDV as for HBV and involve routes of transmission other than parenteral or sexual. The preselection of households with substantial HBV transmission for HDV transmission to occur, and the possible shared routes of transmission between the two viruses, may explain the high level of intra-household clustering of HDV.

Sexual and parenteral transmission of HDV infection

Until now, the modes of propagation of HDV in central Africa were poorly understood,[4] but we found some evidence for its sexual spread. HDV prevalence increased along with the number of lifetime sexual partners, and was especially high among participants who reported ≥10 partners. We also found some evidence for increased risk of HDV infection among participants who received injections, but these findings were no longer significant in multivariable analysis. It is noteworthy that high number of partners and high number of injections in the last year were strongly associated with living in the Southern part (latitude <4°N) of the country, where HDV prevalence was much higher so that one cannot exclude some confounding of the association. Still, sexual and parenteral transmission of HDV has already been documented in other regions of the world,[35–38] and may have contributed to some of the cases identified in this study. The association found with HIV infection may be the result of sexual and parenteral transmission modes shared by the two viruses, with residual confounding since the control for these modes of transmission in multivariable analysis was incomplete. One cannot exclude though an increased susceptibility to HDV infection among those immunosuppressed by HIV infection, or the possibility of false positive HDV antibody results among HIV-infected individuals.

This study has several limitations, among which its cross-sectional design so that the temporal relationship between studied exposures and HDV infection could not be ascertained; the restricted age group of surveyed women to the reproductive years, that is, 15–49 years so that no epidemiological information beyond that age was available for women; the dearth of questions in the DHS survey which did explore sexual and parenteral modes of transmission as part of the planned HIV epidemiological analysis, but did not specifically envisioned an HDV epidemiological study; and the lack of data regarding children which would have been useful to complete the household transmission analysis. Finally, the use of dried blood spots made HDV RNA testing and extraction difficult, and we were not able to perform them as part of this study. HDV

RNA testing is not essential to the study of HDV transmission in the context of a cross-sectional survey, since transmission may have taken place before HDV RNA clearance in the infecting individual. However, when both infected individuals remain viraemic, the analysis of sequences is the best approach to confirm transmission between two individuals.

In conclusion, we found evidence for effective intrafamilial transmission of HDV in Cameroon, but mostly large differences in prevalence between regions and, like in other tropical areas, a concentration of cases in forested areas close to the Equator. The reasons underlying these geographic variations in HDV prevalence deserve further investigation.

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