

erosion. Pathologic diagnosis of *Mycobacterium* infection from bony specimens was recorded for 35 (92%) patients. For 29 (76%), diagnosis was conducted by molecular study, including 25 (66%) by the national reference mycobacterial laboratory. For 4 patients, diagnosis was confirmed by culture of *M. bovis*. Osteomyelitis/osteitis for 5 patients was considered BCG related according to pathologic diagnosis of *Mycobacterium* infection, BCG vaccination history, and lack of a history of contact with a person with tuberculosis.

Thirty-two (84%) children underwent surgery (excision, debridement, open biopsy), 4 children received arthrotomy (3 ankle and knee joint), and 2 children underwent only aspiration biopsy. All patients received isoniazid and rifampin therapy; 33 patients also received pyrazinamide, and 6 received additional ethambutol therapy. Medications were adjusted after diagnoses changed from tuberculosis to BCG infection. Two patients had major sequelae, both involving the thoracic spine and causing severe kyphosis.

Adverse reactions after BCG vaccination depend on the BCG dose, vaccine strain, vaccine administration method, injection technique, and recipient's underlying immune status (5). The vaccine strain and manufacturing process in Taiwan did not change during the study period. Findings were not associated with a specific batch of vaccine, inoculation age, underlying disease, or *Salmonella* spp. infection. Patients had no common birth place, hospital, or area of residence. We believe the increased number of cases resulted mainly from policy changes and laboratory facility improvements.

A surgical approach to obtain a specimen is indicated. However, because medical treatment usually yields a good outcome (6), extensive debridement should be avoided. Although some patients with lower extremity involvement initially limped, most were able to walk well later. Vertebral involvement is rare. Unlike previously reported cases (7,8), both patients reported here who had vertebral involvement had sequelae. For young children with suspected vertebral tuberculosis but no tuberculosis contact history, a biopsy specimen for BCG studies is preferable to spondylectomy. Although no definite immunologic deficit was found in these BCG osteomyelitis/osteitis patients, 2 other compensated infants with disseminated BCG during the same period in Taiwan had identified immunodeficiency (9). Studies are ongoing by the Taiwan Centers for Disease Control to evaluate medical treatment duration, long-term outcomes, and more detailed immune genetic tests.

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High Prevalence of Hepatitis Delta Virus among Persons Who Inject Drugs, Vietnam

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To the Editor: Hepatitis delta virus (HDV) is a small RNA virus that infects and persists only in persons whose samples test positive for hepatitis B surface antigen (HBsAg) (1). Phylogenetic analysis has revealed 8 HDV genotypes (2) with evidence of distinct global geographic distributions and pathogenicity (3,4). The implications of HDV infection in Vietnam have been unclear. Studies of persons who have chronic illness caused by HBV in populations of southern and northern Vietnam reported no cases or low prevalence (1.3%), respectively (5,6). In contrast, our multicenter study of chronically HBV-infected persons in 2009 showed a higher overall HDV seroprevalence rate of 10.7% (34/318) (7). These rates varied among regions of Vietnam and groups that had varying risk factors for infection. Higher rates were observed among persons who inject drugs (PWIDs) (20/78, 25.6%), commercial sex workers (5/57, 8.8%), and military recruits (8/45, 17.8%). A 2013

study, in which PCR-based methods were used, reported a high rate of HDV RNA detection (41/266, 15.4%) in a cohort of HBV-infected persons in the city of Ha Noi (also known as Hanoi) collected during 2000–2009 (8). Illnesses of these patients ranged from acute hepatitis to severe liver disease, but injection of drugs was not reported. To better clarify the prevalence of HDV, we conducted serologic and molecular testing focusing on PWIDs from different geographic regions of Vietnam.

During 2010–2011, we screened consecutive samples ($n = 1,999$) from PWIDs from 5 centers (Ha Noi and Hai Phong in northern, Da Nang and Khanh Hoa in central, and Can Tho in southern Vietnam) for HBsAg. In each center, we recruited PWIDs to obtain 200 participants per year following national guidelines for annual sentinel surveillance of HIV (http://www.vaac.gov.vn/Download.aspx/C64DBE4B-B9074A489283056ACF639780/1/Huong_dan_giam_sat_

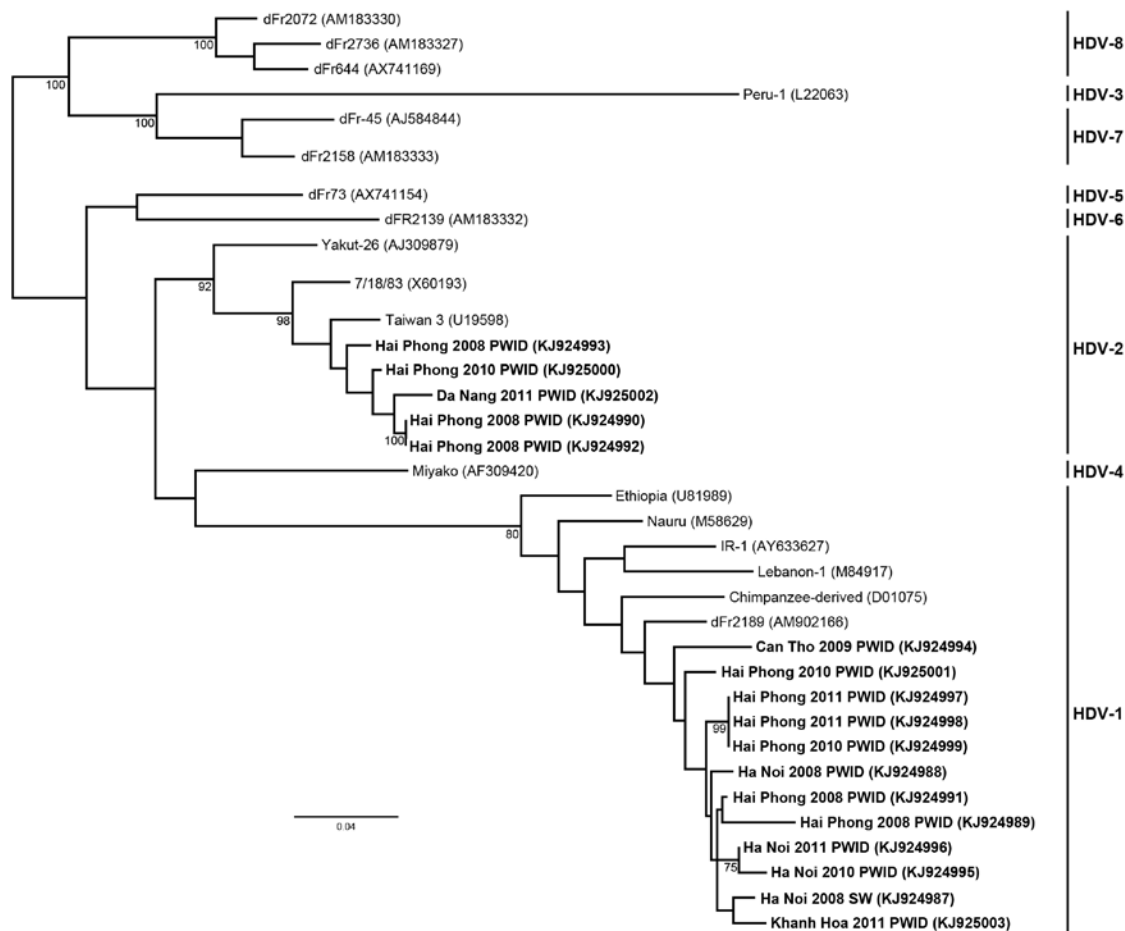


Figure. Maximum-likelihood phylogenetic tree of hepatitis delta virus (HDV) genotypes 1 and 2 from Vietnam. A 472-nt fragment (corresponding to nucleotides 802–1,273 from HDV isolate C15; Genbank accession no. KF660600) was used to construct the phylogram. HDV genotyping was done by using amplification and bidirectional sequencing of the R_0 region as described by Le Gal et al. (2). Bootstrap resampling was done for 1,000 replicates of the dataset using the neighbor-joining algorithm; values >70% are shown at the nodes. Bold text indicates samples from patients in Vietnam; location, year, and risk group are indicated. Genbank accession numbers are shown in parentheses. Scale indicates substitutions per position. PWID, person who injects drugs; SW, sex worker.

trong_diem_2010.doc). Ethical approval for the study was obtained from the National Institute of Hygiene and Epidemiology in Ha Noi. Samples collected from 300 (15%) persons were HBsAg positive, consistent with our previous study (7). Of these, 294 were subsequently screened by using ELISA for anti-HDV IgG; reactive samples were tested for HDV IgM. HDV IgG was detected in 45/294 (15.3%) samples; 20 were also HDV IgM positive (6.8% total; 44.4% of IgG-positive samples). Serologic analysis revealed considerable differences in prevalence by geographic region. HDV seroprevalence rates were high among PWIDs from northern Vietnam (30.2% and 29.4% in Ha Noi and Hai Phong, respectively), but a lower seroprevalence rate was observed in Da Nang (5.3%), and intermediate rates were found in Khanh Hoa (8.1%) and Can Tho (12.5%) in southern Vietnam (online Technical Appendix Tables 1, 2, <http://wwwnc.cdc.gov/EID/article/21/3/14-1147-Techapp1.pdf>).

We analyzed anti-HDV-positive samples ($n = 41$) for the presence of HDV RNA using a quantitative real-time PCR. HDV RNA was detected in 25/41 (61%) of IgG-seropositive samples (median 1.2×10^4 copies/mL, range 0– 1.8×10^7 copies/mL) and 19/19 (100%) of IgM-seropositive samples (median 1.2×10^6 copies/mL, range 4.3×10^2 – 1.7×10^7 copies/mL). The viral loads of HDV IgM-positive samples were significantly higher than those of IgM-negative samples ($p < 0.0001$) (online Technical Appendix Figure 1); however, when only samples with detectable HDV RNA from the IgM negative and positive groups were analyzed, there was no statistically significant difference in viral titer ($p = 0.45$; online Technical Appendix Figure 2). Comparison of HDV RNA and HDV IgM seroresponses showed evidence of superinfection with HDV persistence in 6 cases (HDV IgM negative/RNA positive; 6/22, 27.3%; online Technical Appendix Figure 1). The 6 samples that were IgM negative for detectable RNA (median 2.9×10^5 copies/mL, range 1.1×10^3 – 1.8×10^7 copies/mL) highlight the limitation of using IgM as a surrogate marker for HDV replication; therefore, HDV RNA investigation is more appropriate for IgG-positive samples.

To identify the genotypes of HDV involved, we completed nucleotide sequencing and phylogenetic analysis of HDV from 17 viremic patient samples from Ha Noi, Hai Phong, Da Nang, Khanh Hoa, and Can Tho collected from another study cohort during 2008–2011 (Figure) (7). Most (12/17, 71%) samples were HDV genotype 1 from both northern and southern Vietnam; 5 (29%) HDV genotype 2 species were identified in 4 samples from Hai Phong in northern and 1 sample from Da Nang in central Vietnam. The finding that HDV-1 was the predominant genotype is consistent with reports by Sy et al. (19/21 HDV-1; 2/21 HDV-2) (8), suggesting that HDV-1 is the predominant genotype in all parts of the country.

This study, the previous report from the National Institute of Hygiene and Epidemiology laboratory (7), and data

from Sy et al. (8,9) indicate that HDV is highly prevalent in Vietnam, particularly in the northern part of the country, contrary to previous reports (5,6,10). In particular, our findings indicate that increased efforts are needed to improve HBV vaccination rates among PWIDs and others with risk factors for infection. Over time, these interventions may help reduce the effects of hepatitis virus-related liver disease. We also intend to study HDV in other high-risk groups, including commercial sex workers and men who have sex with men.

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Cholera in Yangon, Myanmar, 2012–2013

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To the Editor: *Vibrio cholerae* O1, a causative agent of cholera, is classified into 2 biotypes, classical and El Tor. Since 1817, cholera has spread from the Indian subcontinent to other regions of the globe 7 times (1). However, little information on the occurrence of cholera and *V. cholerae* in Myanmar has been published. Here, we report cholera cases and characterization of 58 clinical isolates of *V. cholerae* O1 serotype Ogawa recovered from patients with diarrhea in Yangon, Myanmar, during February 2012–June 2013.

During February and August 2012, rectal swab specimens were collected from patients suspected of having cholera in 4 hospitals in Yangon: New Yangon General Hospital, North Okkalapa General Hospital, Thingangyun Sanpya General Hospital, and Yangon Children Hospital. The specimens were cultured on thiosulfate citrate bile salts sucrose agar plates. After overnight incubation, several colonies that resembled to those of *V. cholerae* were confirmed as serogroup O1 by using slide agglutination tests with specific monoclonal antibodies (2).

Of the tested specimens, 34 isolates carried the *tcpA* gene, encoding the structural subunit of toxin-coregulated pilus, and the *rstR* gene, the repressor gene in the cholera toxin encoding (CTX) phage (3); these results may indicate that these strains belonged to the El Tor biotype. However, identification of the sequence type of the cholera toxin B subunit gene in these isolates revealed that they were of classical type (*ctxB*^{Cl_a}). Thus, these isolates were classified as atypical El Tor *V. cholerae* O1 (4)

carrying *ctxB*^{Cl_a} and *rstR*^{El}. Currently, the predominant clones causing cholera in Asia and Africa are atypical El Tor *V. cholerae*, CIRS101, and CIRS101-like variants (5,6). Myanmar isolates from 2012 and the CIRS101 strain contained a single nucleotide polymorphism in the *tcpA* gene at nt 266 (A→G) of the prototype seventh pandemic El Tor (N16961) strain.

Pulsed-field gel electrophoresis (PFGE) (7) using the 2012 isolates revealed 9 patterns (Table, <http://wwwnc.cdc.gov/EID/article/21/3/14-1309-T1.htm>). During the initial phase of cholera occurrences, *V. cholerae* O1 was mainly isolated from adults, and 9 pulsotypes were observed. During the later period (May–August), most isolates were from children <5 years of age, and pulsotype Y6 predominated.

We carried out multilocus variable-number tandem-repeat analysis (MLVA) (8) of the 2012 isolates to resolve distinct populations. MLVA yielded 13 isolate types, and all 18 isolates of pulsotype Y6 exhibited either MLVA profile 11.6.6.17.17 or a closely related profile that differed only by 1 repeat number. These data suggest that cholera was contracted mainly in adults and was caused by multiclonal *V. cholerae* O1. However, in children, *V. cholerae* has transformed from single clonal expansion since May 2012.

During March–June 2013, we extended our studies to characterize cholera organisms isolated from patients with severe diarrhea who were admitted to the original 4 hospitals as well as 2 additional hospitals, Yankin Children Hospital and Insein General Hospital. Of 24 cases, 16 patients showed symptoms of severe dehydration, including 1 patient who experienced shock. Other common symptoms in this patient population included fever (50%, 12/24), vomiting (92%, 22/24), and abdominal pain (33%, 8/24). Although fever is less common among patients with cholera-associated diarrhea (9), the frequency of fever was considerably high in this study. PFGE revealed 23 of the 24 isolates were identical to pulsotype Y7, which was the second-most prevalent pattern in 2012; MLVA profiles were also similar to those from 2012. Thus, the occurrences of cholera in 2013 may have been related to persistent transmission of a clone from 2012.

According to surveillance records from the Yangon Regional Health Center, the reported number of diarrhea cases in Yangon increased from 11,651 in 2010 and 11,016 in 2011 to 15,540 in 2012 and 13,919 in 2013. Although there were no reports of cholera outbreaks in Yangon, PFGE/MLVA results revealed that most of the cholera cases in this study were caused by isolates belonging to identical or closely related types. Thus, cholera outbreaks could have occurred in Yangon, and the related clone may have persisted.

In Myanmar, the illness rate for severe diarrhea is estimated to be 2.6–3.5 per 100,000 persons and the mortality

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Technical Appendix

Tabular summary of frequency of cases of hepatitis delta virus among persons who inject drugs and statistical analysis of data in study regions of Vietnam

Technical Appendix Table 1. Hepatitis B surface antigen seropositivity in persons who inject drugs from 5 study regions in Vietnam*

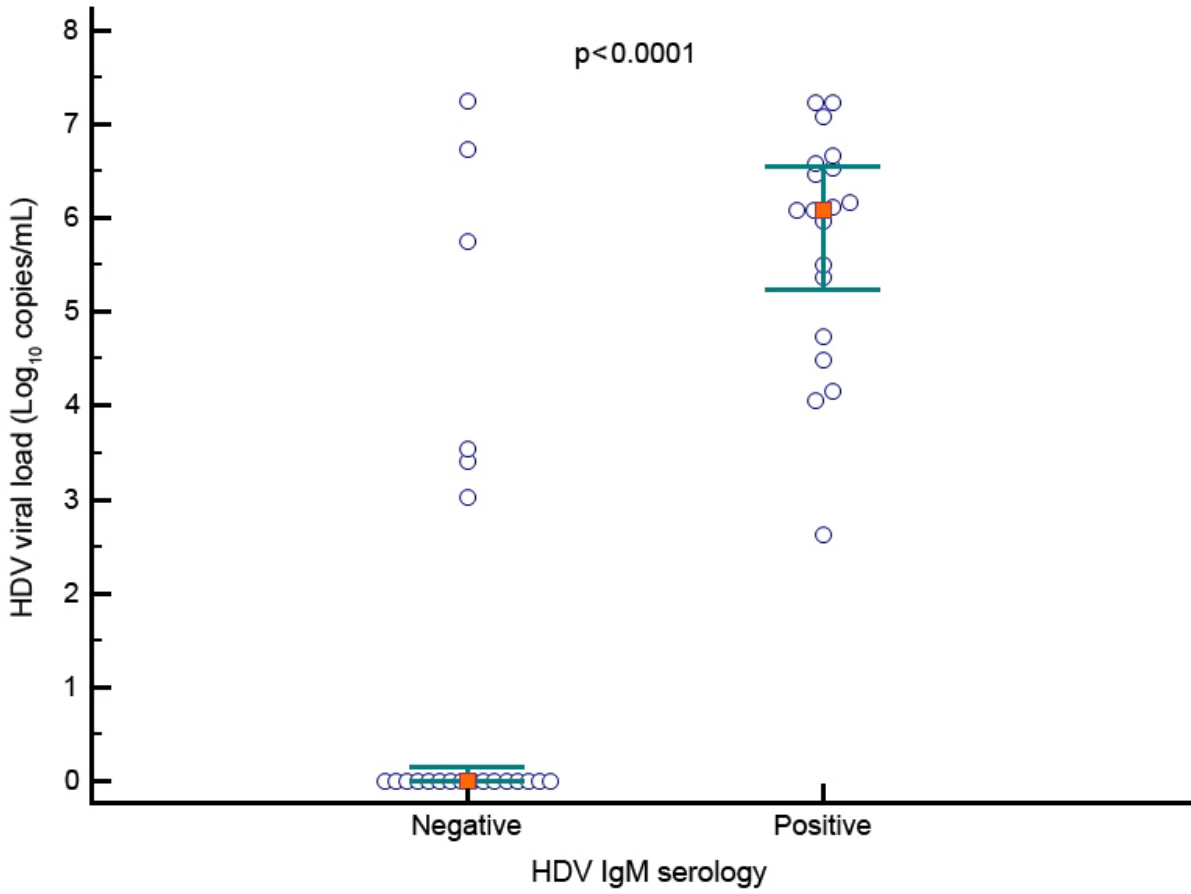
Group/HBV Serostatus	Northern no. (%)		Central no. (%)	Southern no. (%)		Total no. (%)
	Ha Noi	Hai Phong	Da Nang	Khanh Hoa	Can Tho	
PWIDs	400	400	400	400	399	1,999
HBsAg (+)	43 (10.8)	51 (12.8)	76 (19)	74 (18.5)	56 (14)	300 (15)

*HBV, hepatitis B virus; PWIDs, persons who inject drugs; HBsAg, hepatitis B surface antigen.

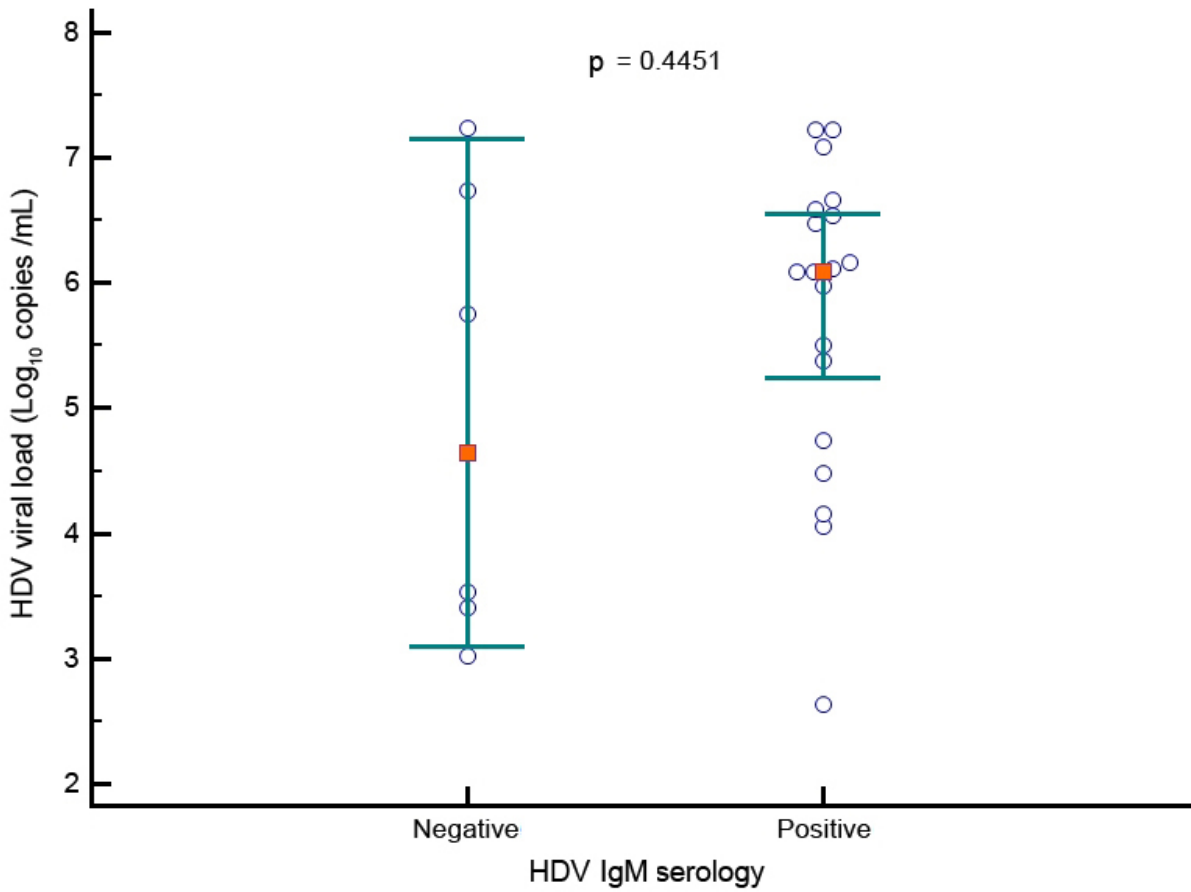
Technical Appendix Table 2. Hepatitis delta virus IgG and IgM seropositivity in hepatitis B surface antigen-positive persons who inject drugs in 5 study regions in Vietnam*

Characteristics	Northern, no. (%)		Central, no. (%)	Southern, no. (%)		Total, no. (%)
	Ha Noi, 43	Hai Phong, 51	Da Nang, 76	Khanh Hoa, 74	Can Tho, 56	
Serologic Markers						294*
HDV IgG (+)	13 (30.2)	15 (29.4)	4 (5.3)	6 (8.1)	7 (12.5)	45 (15.3)
HDV IgM (+)	5 (11.6)	8 (15.6)	2 (2.6)	5 (6.7)	0 (0)	20 (6.8)

*Of the 300 hepatitis B surface antigen-positive samples (Table 1), 294 were available for analysis; the remaining 6 had insufficient sample volumes. HDV, hepatitis delta virus.



Technical Appendix Figure 1. HDV RNA log_{10} copies/mL in IgM-negative ($n=22$) and IgM-positive ($n=19$) samples. The orange square indicates the median and the error bars indicate 95% CI.



Technical Appendix Figure 2. HDV RNA log₁₀ copies/mL in IgM-negative/HDV RNA positive (n=6) and IgM-positive/HDV RNA positive (n=19) samples. The orange square indicates the median and the error bars indicate 95% CI.