Hepatitis B Virus (HBV)/Hepatitis D Virus (HDV) Coinfection in Outbreaks of Acute Hepatitis in the Peruvian Amazon Basin: The Roles of HDV Genotype III and HBV Genotype F

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Recurring outbreaks of acute hepatitis have been a significant cause of morbidity and mortality among Peruvian military personnel stationed in the Amazon Basin region of Peru. The role of hepatitis B virus (HBV) and hepatitis D virus (HDV) infection was investigated as the possible cause of acute hepatitis among 88 military patients stationed at four different jungle outposts during 1992–1993. Analysis of serum markers indicated that 95% (84/88) had evidence of acute HBV infection; 64% (54/84) were also infected with HDV. Genetic analysis of PCR-amplified HDV and HBV fragments showed exclusively HDV genotype III and HBV genotype F. Furthermore, HDV RNA sequences were similar among patients from the same outpost but different from those at other jungle locations. The data suggested focal sources of HDV infection in the jungle environment of the outposts and, further, confirmed the unique association of HDV genotype III with severe cases of human disease in northern South America.

The pattern of hepatitis D virus (HDV) infection and disease varies considerably worldwide [1, 2]. In developed countries, the rate of HDV infection is generally low, and the severity of disease ranges from moderate to severe. In contrast, HDV infection has been associated more commonly with outbreaks of severe and frequently fatal fulminant hepatitis in northern South America for at least 50 years [3, 4]. Mortality as high as 70% among acutely jaundiced patients was associated with HDV infection during 1980–1983 in the Santa Marta mountains of northern Colombia [5, 6], as was severe and fatal acute and chronic hepatitis during an outbreak in 1979 among the Yucpa Indians in Venezuela [7, 8]. HDV also has been found to be endemic in remote jungle areas of Brazil and Peru [9–12]. Similarities in the disease patterns and the particular histologic features associated with hepatitis in these areas have suggested that HDV was the predominant etiologic agent responsible for numerous hyperendemic foci of severe hepatitis throughout the region [3, 11].

Observations derived from genetic analysis of the HDV genome suggested that a particular genotype of HDV, genotype III, was unique to northern South America [13]. This genotype, which is distinct from those found in other areas of the world, was initially isolated in the town of Julio Zawady in the Santa Marta region of Colombia and among military personnel stationed at remote outposts in the northern Peruvian jungle in 1986. The recognition of the same unique HDV genotype in different areas of South America suggested that it could be an important determinant of the characteristically severe form of HDV-related disease in this region. The virus genetics of hepatitis B virus (HBV) in South America have not been well characterized; however, available data indicated that the most common serotype was *adw4,* which is uncommon in most other areas of the world except parts of French Polynesia [14, 15]. The sequence of an isolate obtained in Rio de Janeiro was consistent with that of the *adw4* serotype and was designated HBV genotype F [16–18].

Outbreaks of acute hepatitis have been a recurring problem among Peruvian military personnel following deployment to remote outposts in the Amazon Basin region of Peru. The roles of HBV and HDV, and in particular their respective genotypes, F and III, were investigated as possible causes of acute hepatitis that occurred during 1992–1993 among troops stationed at four such jungle outposts.

Materials and Methods

Study subjects. Patients were Peruvian military troops who had been stationed at four outposts along the northern and northeastern border of Peru in the Amazon jungle. Cohorts of military recruits from all areas of Peru were periodically deployed to these outposts,

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The research protocol employing human subjects in this study has been reviewed and approved by the Naval Medical Research Institute's Committee for the Protection of Human Subjects.

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Primer	Target	Location	Sequence
5414	HDV	883 - 906	5' - GAG ATG CCA TGC CGA CCC GAA GAG - 3'
5415	HDV	1288 - 1265	5' - GAA GGA AGG CCC TCG AGA ACA AGA - 3'
8180	HDV	1057 - 1077	5' - CGT TCG GGA AAA GGG GGA CTC - 3'
1835	HDV	1077 - 1057	5' - GAG TCC CCC TTT TCC CGA ACG - 3'
1754	HBV	56 - 75	5' - CCT GCT GGT GGC TCC AGT TC - 3'
1755	HBV	1202 - 1183	5' - GTG GGG GTT GCG TCA GCA AA - 3'

Figure 1. Oligonucleotide primers used for polymerase chain reaction amplification and sequencing of HBV and HDV nucleic acids.

which are remote and difficult to access. Skin lesions due to insect bites and poor hygiene in the tropical environment were common among all troops. Soldiers with symptoms of acute hepatitis were evacuated by helicopter to Peruvian Santa Rosa Military Hospital in Iquitos for evaluation, medical care, and treatment. Patients were hospitalized for an average of 30 days (range, 7–77) with a clinical diagnosis of severe acute hepatitis. All were men, ranging in age from 17 to 26 years. All experienced jaundice, with maximum serum total bilirubin averaging 6.6 mg/dL (range, 1.12–17.9). Sera were obtained during the acute phase of disease, a mean of 14.3 days after onset of illness; in addition, convalescent sera were available for 72 (82%) of the patients before discharge from the hospital.

Serology. Serum samples were collected from the patients on admission to the hospital and tested by EIA for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), IgM anti-HBc, and antibody to hepatitis delta antigen (anti-HD) (all Abbott, Abbott Park, IL). Also, sera obtained from patients during the convalescent phase of illness were tested for anti-HD.

Viral nucleic acid assay and analysis. Samples were tested for HBV DNA and HDV RNA by polymerase chain reaction (PCR) assays. Briefly, 50 μL of each serum sample was incubated in 500 μL of lysing solution (50 m*M* EDTA, 50 m*M* HEPES, pH 7.4, 0.2 *M* NaCl, 2% SDS, and 1 mg/mL proteinase K) overnight at 37°C. The lysate was extracted once with phenol and once with phenol–chloroform–isoamyl alcohol (25:24:1). RNA was precipitated by the addition of 50 μL of 3 *M* sodium acetate and 400 μL of isopropanol. After the pellet was washed with 70% ethanol, nucleic acids were dissolved in 50 μL of water.

Samples were analyzed for HDV RNA by adding 1 μL of serum nucleic acids to a 10-μL reverse transcription reaction containing 0.02 absorbance units of random hexamers (Pharmacia, Piscataway, NJ), 0.5 U of InhibitAce (5′-3′, Boulder, CO), 1 m*M* dNTPs, and 100 U of Moloney murine leukemia virus reverse transcriptase (Life Technologies GIBCO BRL, Gaithersburg, MD) in the buffer supplied by the manufacturer. After 15 min of incubation at 37°C and 15 min at 42°C, the reaction was stopped by incubation at 95°C for 5 min. PCR reaction mix (40 μL), containing 25 pmol of primers 5414 and 5415 (figure 1) and 0.5 U of Taq polymerase (Boehringer Mannheim, Indianapolis) in the buffer supplied by the manufacturer, was added to the 10-μL reverse transcription reaction and covered with 50 μL of mineral oil. PCR was done in a thermocycler (model 480; Perkin-Elmer Cetus, Norwalk, CT) as follows: 2 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, then 5 min at 72°C.

For HBV DNA amplification, a 1123-bp fragment encompassing the major surface antigen–coding region was amplified from 1 μL of the extracted nucleic acids in a 50-μL reaction containing 50 m*M* TRIS, pH 8.3, 3 m*M* $MgCl_2$, 0.2 m*M* dNTPs, and 50 pmol of primers 1754 and 1755 (figure 1). The thermocycler conditions were 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Restriction enzyme analysis of HDV and HBV genotypes was done after PCR amplification. A 2-μL aliquot of the PCR product was radiolabeled by a single cycle reaction containing 10 μCi of [α-^{32}P]dCTP (3000 Ci/mmol; Amersham, Amersham, UK), 0.01 m*M* dNTPs, 0.5 U of Taq polymerase (Boehringer Mannheim), primers, and buffer supplied by the manufacturer. A 5-μL aliquot of the labeled PCR product was incubated for 2 h at 30°C with 5 U of restriction enzyme *Sma*I (Life Technologies); digested products were analyzed by electrophoresis through 6% acrylamide gels followed by autoradiography.

PCR-amplified viral genomes were either sequenced directly or cloned and then sequenced. For direct sequencing, PCR products were electrophoresed through 1.2% agarose gels, purified using the GeneClean kit (Bio101, La Jolla, CA), and sequenced with the dsDNA cycle sequencing kit (Life Technologies) using primers 5414, 5415, 8180, and 1835 for HDV and 1754 and 1755 for HBV (figure 1). Alternatively, PCR products were first cloned, using the CloneAmp system (Life Technologies), then sequenced by the same method after purification of cloned products with ion-exchange resin columns (Qiagen, Chatsworth, CA). Sequences were analyzed with the Wisconsin Genetics Computer Group programs PILEUP and DISTANCES [19].

Results

Markers of HBV and HDV infection. Serologic markers indicated a very high percentage of acute HBV infection among 88 patients from all four sites in the jungle (table 1), with total occurrences of 84 (95%) patients positive for IgM anti-HBc and 77 (88%) positive for HBsAg. A single patient each from sites A, B, and C had serologic results consistent with chronic HBV infection (HBsAg-positive, anti-HBc–positive, IgM anti-HBc–negative), and 1 patient from site B had serology consistent with a previous exposure to HBV (HBsAg-negative, anti-HBc–positive, IgM anti-HBc–negative). Thus, all patients were positive for at least one HBV marker, indicating that all were or had been infected by HBV at some time, and 87 (99%)

Table 1. Summary of HBV infection among Peruvian military troops stationed in the Amazon jungle of Peru and hospitalized with acute hepatitis.

Site	No. of patients	Acute	Chronic	Previously exposed	Not exposed
A	7	6 (86)	1	0	0
B	35	33 (94)	1	1	0
C	40	39 (98)	1	0	0
D	6	6 (100)	0	0	0
Total	88	84 (95)	3 (3)	1	0

NOTE. HBV status was assessed based on analysis of serum samples obtained at time of admission to hospital. Acute = positive for IgM antibody to hepatitis B core antigen (anti-HBc); chronic = positive for anti-HBc and hepatitis B surface antigen (HBsAg), negative for IgM anti-HBc; previously exposed = anti-HBc–positive, HBsAg- and IgM anti-HBc–negative; not exposed = negative for all HBV markers. Data are no. of patients (%).

had evidence of primary HBV infection at the time of hospital admission.

The possibility that coinfection with HBV and HDV could be responsible for the outbreaks of acute hepatitis was considered by testing sera from the acute stage of disease for both HDV RNA and anti-HD and convalescent sera for anti-HD [20]. The serologic results indicated a high proportion of acute HDV infection (table 2). At the time of admission to the hospital, 41% of HBV-positive patients had detectable HDV RNA in the serum, and 22% had anti-HD. All HDV RNA–positive patients were also positive for HBsAg. Sera obtained from 3 patients at the time of admission appeared to correspond to the late postacute phase of infection, as indicated by negative assay results for HBsAg and HDV RNA but positive results for IgM anti-HBc and anti-HD. Before release from the hospital, anti-HD was found in the sera of 41% of HBV-positive patients. Overall, 66% of HBV-positive patients were positive for at least one marker of HDV infection. Because of the transient and variable nature of HDV viremia and antibody response in acute coinfections [20], and the limited number of serum samples available for analysis, it is possible that the 66% rate of positive HDV serologic markers is an underestimate of HDV infection in this group.

Table 2. Summary of HDV serology among HBV-positive patients.

HDV marker	No. positive/ no. tested (%)
Anti-HD (acute)	19/87 (22)
HDV RNA (acute)	36/87 (41)
Anti-HD (convalescent)	29/71 (41)
HDV RNA + anti-HD (acute)	7/87 (8)
HDV RNA (acute) + anti-HD (convalescent)	12/71 (17)
Anti-HD (acute + convalescent)	12/71 (17)
HDV RNA (acute) + anti-HD (acute + convalescent)	4/71 (6)
Any HDV marker	57/87 (66)

NOTE. HBV-positive = positive for hepatitis B surface antigen or IgM antibody to hepatitis B core. Anti-HD, antibody to hepatitis delta antigen.

Table 3. Frequency of HDV infection among HBV-infected troops stationed at different jungle outposts.

Outpost	No. (%) of patients positive for HDV infection among those with: Acute HBV	Chronic HBV	Acute or chronic HBV
A	3/6 (50)	1/1	4/7 (57)
B	23/33 (70)	1/1	24/34 (71)
C	28/39 (72)	1/1	29/40 (72)
D	0/6	—	0/6
Total	54/84 (64)	3/3	57/87 (66)

Of the three methods used for detecting HDV infection, analysis of acute sera for HDV RNA by PCR and convalescent sera for anti-HD yielded similar rates of 41% positive (table 2). However, some patients were positive for only one of these two markers; thus, the use of both methods together gave a more accurate assessment of HDV infection than either alone. In acute sera, analysis of anti-HD was less effective, as only 22% of patients were positive. However, at least 2 and possibly 2 additional cases of HDV infection were diagnosed that were not detected by the other two methods (for 2 of these 4, convalescent sera were not available). Thus, a combination of analysis for HDV RNA in acute sera and analysis for anti-HD in convalescent (postacute) sera provided the most effective approach to detecting acute HDV infection superimposed on acute HBV infection.

HDV infection among the military patients apparently was confined to three of the four remote jungle outposts (table 3). Acute HBV coinfection was accompanied by HDV infection in at least 50% of hospitalized patients from sites A, B, and C, and all 3 persons (1 from each of these sites) with chronic HBV infection were also infected with HDV. Thus, the total rates of HDV infection, based on the detection of at least one serologic marker, were 57% (4/7), 71% (24/34), and 72% (29/40) at outposts A, B, and C, respectively. Although the percentage of acute HBV infection was also very high among hospitalized patients from site D, the number of cases was lower, and no HDV was found in this group of 6 patients (tables 1 and 3).

Genotype analysis of HDV. The results of a previous study indicated that a unique genotype of HDV, genotype III, was associated with severe hepatitis among patients in Peru and Colombia [13]. Examination of both published and unpublished sequences has indicated that the three HDV genotypes can be rapidly distinguished by digestion of a 357-nt cDNA segment of the 1680-nt HDV RNA genome with the restriction enzyme *Sma*I (figure 2). Application of this *Sma*I digestion assay to the 37 HDV RNA–positive samples from the 1992–1993 outbreaks among Peruvian military personnel revealed that all were infected with HDV genotype III (figure 2). The finding

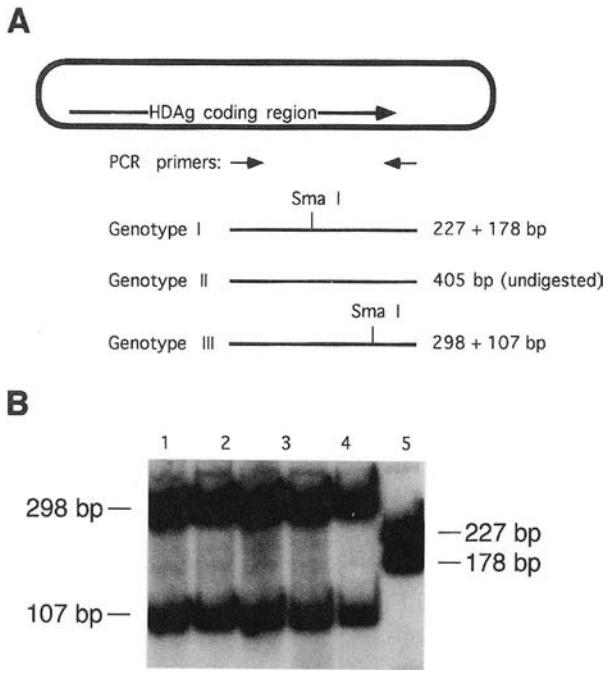

Figure 2. *Sma*I digestion of HDV polymerase chain reaction (PCR) products identifies Peruvian HDV isolates as genotype III. **A**, Schematic diagram indicating region of HDV genome amplified and location of *Sma*I restriction sites in 3 known genotypes of HDV. Thick closed line indicates genomic HDV RNA; short horizontal arrows indicate positions of PCR primers; large arrow indicates location of hepatitis delta antigen coding region. Horizontal lines indicate expected PCR amplification products and location of *Sma*I restriction site, with sizes of *Sma*I digestion products indicated at right. **B**, Digestion of representative PCR products with *Sma*I. Lanes 1–4, *Sma*I digestion products from 3 representative Peruvian isolates; lane 5, *Sma*I digestion of PCR product derived from animal 57 [21], experimentally infected with HDV genotype I.

of HDV genotype III in all 37 RNA-positive samples, obtained from three different sites between 1992 and 1993, further confirmed the role of HDV genotype III as the cause of acute hepatitis in this region.

Sequence analysis of HDV isolates. Sequence analysis of 14 of the HDV RNA–positive samples from outposts A, B, and C further confirmed that these isolates were HDV genotype III. Overall sequence identities ranged from 92% to 100%, with 16 sequence pairs differing by ≤1% and 4 pairs of sequences being identical (figure 3). This pattern of nearly identical HDV sequences differed from the divergence observed among published HDV genotype I sequences, for which the sequence identity in the region analyzed was between 88% and 96%, with no two human HDV isolates being identical [13].

Analysis of HDV sequences by outpost indicated that all identical sets of sequences were obtained from patients who were stationed at the same location: isolates 1, 2, and 4 from outpost A and isolates 6 and 7 from outpost B (figure 3). Moreover, in all but 1 case (isolate 10), the most closely related sequences were obtained from patients stationed at the same outpost (figure 3). These patterns of sequence identity likely reflected a unique setting with focal sources of HDV infection. Isolate 10, for which the HDV sequence was not closely related to others from the same outpost, was obtained from a patient with chronic HBV infection; thus, it is possible that this patient acquired HDV infection before assignment to the jungle outpost. He was apparently not a source of infection for other military personnel at outpost B, as no other isolates contained a closely related sequence.

Comparison of the HDV sequences in isolates obtained during 1992 and 1993 with those of isolates obtained during outbreaks in 1986 in the same region of Peru [13] indicated that the sequence relatedness extended to isolates obtained during the previous outbreaks (figure 3). Indeed, the sequence of isolate 12, from outpost C, was identical to that of 2 isolates obtained from this site in 1986: Peru-2, as indicated in figure 3, and another isolate, 1986-3 (not shown). A third isolate obtained from site C in 1986, 1986-4, was 99% identical to others obtained from this site in 1986 and 1992–1993 (not shown). Isolate 3, from outpost A, differed at only one position from the Peru-1 (figure 3) and 1986-5 (not shown) isolates obtained from this site in 1986. These data suggested that these patients were exposed to limited, localized sources of HDV infection that have persisted for at least 7 years.

Genotype analysis of HBV. Analysis of published sequences [16–18] of the HBV surface antigen gene indicated that the restriction enzyme *Sma*I digests only sequences of genotype F (figure 4A), which has been found elsewhere in South America. Fifteen isolates from the Peruvian acute hepatitis outbreak were examined by *Sma*I digestion of HBV PCR products; all exhibited the HBV genotype F digestion pattern (figure 4B). The analysis included at least 3 isolates from each of the four outposts, and both HDV-positive and HDV-negative sera, indicating that HBV genotype F was the predominant form of this virus in the area. Partial sequence analysis confirmed the genotype identity and indicated a high degree of similarity among isolates obtained from the same site, as was found for the HDV RNA sequences (not shown).

Discussion

Acute HBV and HDV coinfection was the primary cause of severe acute hepatitis during 1992–1993 among Peruvian military personnel deployed to remote outposts in the Amazon jungle. Among hospitalized troops, the proportions with antibody and virus markers for recent HBV infection (99%) and HDV infection (66%) were very high. Other known potential etiologic agents for acute hepatitis seem unlikely with these levels of acute HBV and HDV infection. All patients were negative for hepatitis A virus infection, and no outbreaks of yellow fever have been documented in the region of the outposts. In addition, efforts to detect hepatitis E virus infection among cases of acute hepatitis involving military patients at these outposts during 1994 were unsuccessful.

This high incidence of HBV and HDV infection differed substantially from that of the general population from which

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Peru-1	Peru-2	Colombia	Italy (I)
1				A														
2	0																	
3	1.5	1.5																
4	0	0	1.5															
5	6.8	6.8	5.8	6.8						B								
6	7.4	7.4	6.5	7.4	0.6													
7	7.4	7.4	6.5	7.4	0.6	0												
8	7.7	7.7	6.8	7.7	0.9	0.3	0.3											
9	7.1	7.1	6.1	7.1	0.3	0.9	0.9	1.2										
10	3	3	1.5	3	6.1	6.8	6.8	7.1	6.5									
11	3.6	3.6	2.7	3.6	8.1	8.1	8.1	8.4	7.7	4.2				C				
12	3.3	3.3	2.4	3.3	7.7	7.7	7.7	8.1	7.4	3.9	0.9							
13	3.6	3.6	2.7	3.6	8.1	8.1	8.1	8.4	7.7	3.6	1.2	0.9						
14	3.6	3.6	2.7	3.6	8.1	8.1	8.1	8.4	7.7	3.6	1.2	0.9	0.6					
Peru-1	1.2	1.2	0.3	1.2	6.1	6.8	6.8	7.1	6.5	1.8	3	2.7	3	3				
Peru-2	3.3	3.3	2.4	3.3	7.7	7.7	7.7	8.1	7.4	3.9	0.9	0	0.9	0.9	2.7			
Colombia	4.2	4.2	4.5	4.2	7.1	7.7	7.7	8.1	7.4	6.1	6.1	5.8	6.1	6.1	4.9	5.8		
Italy (I)	31.2	31.2	30.3	31.2	32.6	31	31	31.2	30.7	30.6	32.6	32.1	32.6	32.3	30.6	32.1	30.3	
Japan-2 (II)	32.6	32.6	32	32.6	32.3	31.2	31.2	31	32.3	31	32.6	32	32.1	32.6	32.3	32	33.2	27.9

Figure 3. Genetic distances between different Peruvian HDV isolates for sequences between 927 and 1266 [13, 22]. Column and row numbers indicate different isolates in study. Numbers within table indicate % difference between pairs of isolates. Peru-1 and Peru-2 were obtained from outbreak of acute hepatitis in same region in 1986–1987 [13]. Also included for comparison are genotype I isolate [22] and genotype II isolate [23]. Comparisons between isolates from same outpost are included inside boxes A, B, and C. A distance of 0 (underlined) indicates that sequence of 2 isolates is identical in region analyzed.

the troops had been recruited. Typically, between 10% and 30% of nonmilitary persons exhibit serologic evidence of previous HBV infection and <10% are chronically infected [10, 12, 24]; <20% of HBsAg-positive Peruvians are also positive for HDV infection [10, 12]. In addition to the high rate of HBV and HDV coinfection found in cases of acute hepatitis during 1992–1993, acute HBV and HDV coinfection, with closely related or identical HDV sequences, was also detected in patients from a similar outbreak of acute hepatitis that occurred at two of the same outposts 7 years previously, in 1986 [13]. Taken together, the data suggested that the outbreaks of severe acute hepatitis were due to the continual transmission of HDV and HBV among troops newly deployed to the jungle outposts. The association of particular HDV sequences with each of the outposts (figure 3) suggested focal sources of HDV infection at each site, either within the outpost itself or in the surrounding environment.

Our results are consistent with other studies that suggested that HBV and HDV were widespread in the Amazon jungle [9–12] and further confirm the unique association of HDV genotype III with severe liver disease in northern South America. However, the extent to which the typically very severe disease associated with HDV infection in other areas of northern South America also occurs among the Peruvian troops in this study could not be determined because of limited clinical data. Nevertheless, we consider the requirements for helicopter evacuation, which is costly, to be an indication that the disease was severe enough to require prompt medical attention. It is worth noting that while most studies of severe HDV disease in northern South America have involved HDV superinfection of HBV carriers [5–7, 9], the military personnel examined in this study experienced predominantly HBV and HDV coinfection, which has a different disease course. Furthermore, the epidemiologic setting, mode of virus transmission, and medical facilities in jungle outposts are substantially different from those of developed countries, where most clinical studies of less severe HDV infection have been done. Further investigation of HDV genotype III infection in other settings will be required to fully assess the pathogenic role of this genotype.

Although diagnosis of acute HDV infection can be difficult [20], it appears that HBV infection alone, without HDV infection, was responsible for a substantial number of cases of acute hepatitis at all four jungle outposts. Indeed, HDV infection was not detected among any of the 6 HBV-infected patients from site D. The absence of detectable HDV infection at this outpost could reflect the focal distribution pattern of HDV in the region, a milder form of disease, or inadequate sampling.

It is not clear to what extent interactions between specific HDV and HBV genotypes are important. The observations that the genotypes of both viruses common in northern South America were distantly related to those found in other areas of the world suggested that the colocalization of the two genotypes could be more than coincidental. Variations in the antigen-coding region of the different HDV genotypes

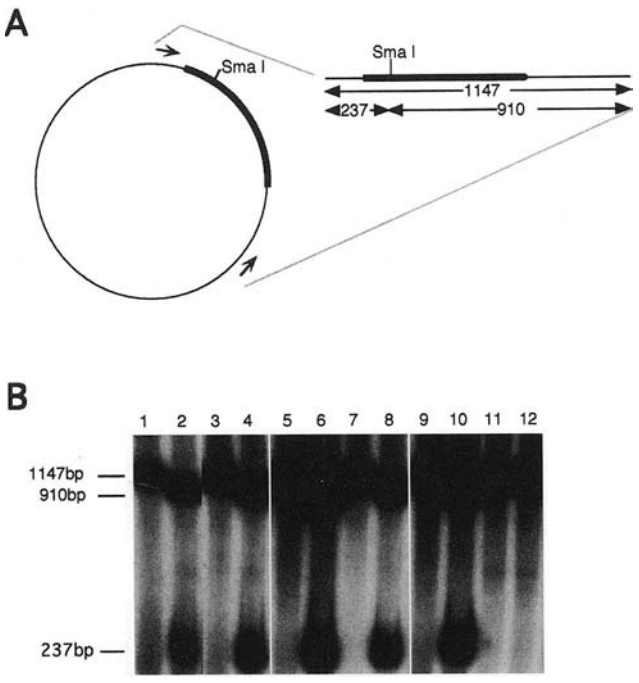

Figure 4. *Sma*I digestion of HBV polymerase chain reaction (PCR) products identifies Peruvian HBV isolates as genotype F. **A**, Schematic diagram indicating region of HBV genome amplified and location of *Sma*I restriction site in hepatitis B surface antigen–coding region. Circle indicates HBV genome, with surface antigen–coding region indicated by thicker line width; arrows indicate positions of PCR primers. Horizontal line indicates expected PCR amplification product and location of *Sma*I restriction site. **B**, Digestion of representative PCR products with *Sma*I. Lanes 1–10, 5 representative Peruvian isolates; lanes 11 and 12, non–genotype F (serotype *adw*) control, experimentally infected animal 57 [21]. Even-numbered lanes, *Sma*I-digested; odd-numbered lanes, undigested.

substantially alter amino acids that interact with HBsAg to create virus particles and could possibly affect packaging efficiency or infectivity (or both), depending on the HBsAg genotypes. Molecular studies and animal models of HDV and HBV coinfection might be able to provide answers to these questions.

Our results indicated that infection with HBV and HDV continues to be a serious health problem in some jungle areas of northern South America. Outbreaks of acute hepatitis due to HBV and HDV coinfection have been occurring for at least 7 years among troops deployed to remote jungle outposts [10, 13]. These outbreaks adversely affect morale and readiness and have been very expensive, requiring evacuation by helicopter, lengthy hospitalization, and loss of service. Most of the patients examined in this study experienced acute HBV and HDV coinfection that is likely to be self-limiting. However, some patients (the majority of the chronic HBV carriers superinfected with HDV and a small percentage of those experiencing acute coinfection) were likely to have developed chronic infection and disease and may pose an additional threat for transmission in their own communities after discharge from the military.

Because of the requirement of HBV for productive HDV infection, vaccination against HBV infection before assignment to jungle outposts would appear to be the most effective preventive measure. However, the efficacy of such vaccination for the prevention of HDV infection and disease has not been studied. In addition, the presence of a different HBV genotype in the region, although possessing the protective *a* determinant, raises potential concerns about the short- and long-term efficacy of vaccination with HBsAg derived from other genotypes.

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