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DISTRIBUTION OF HEPATITIS B VIRUS (HBV) MARKERS IN BLOOD DONORS OF 13 WESTERN HEMISPHERE COUNTRIES: PROCEEDINGS OF THE RED CROSS LATIN AMERICAN HEPATITIS B WORKSHOP

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Although hepatitis B is a significant health problem in the Americas, data about it in many parts of the Hemisphere have tended to be scanty. To help define the situation, a study was made of hepatitis B viral markers in blood samples from 13 Hemisphere countries. This report presents the results of that study.

Introduction

The Red Cross Latin American Hepatitis B Workshop was sponsored by the Puerto Rican chapter of the American Red Cross and the American Red Cross Blood Services Laboratories on 19-20 May 1977. The workshop, which was held at the University of Puerto Rico, fulfilled two primary objectives. The first was that of demonstrating and defining various methods used to detect hepatitis B surface antigen (HBsAg) in blood. The second was that of defining the magnitude of the hepatitis B problem among donor populations of the various participating countries and comparing different serologic tests' efficacy in detecting both HBsAg carriers and previous hepatitis B virus (HBV) infections. In connection with this work, each participating country submitted about 500 or about 1,000 donor specimens (see Table 1) to the Red Cross Blood Services Laboratories in Bethesda, Maryland. These specimens were tested for HBsAg, antibody to surface antigen (anti-HBs), antibody to core antigen (anti-HBc), HBeAg, antibody to e (anti-HBe), and subtypes of HBsAg.

Previous reports concerning the prevalence of hepatitis B in Latin America had been sporadic, and their findings were not comparable because of differences in the methods used. Since all of the testing described in this article was done in the

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Hepatitis Research and Testing Section of the American Red Cross Blood Services Laboratories in Bethesda, the results obtained with specimens from different countries are strictly comparable. These results, presented in this communication, thus provide a unified picture of the distribution of HBsAg and other HBV markers in a wide range of Latin American blood donor populations.

Materials and Methods

Blood Samples

A total of 7,487 blood samples were obtained from donors in 13 Western Hemisphere countries (Argentina, Barbados, Brazil, Chile, Colombia, Costa Rica, the Dominican Republic, Ecuador, Mexico, Peru, Puerto Rico, Suriname, and Venezuela). Aliquots of serum in one-dram vials containing 200 μg sodium azide were shipped by air at ambient temperatures to the American Red Cross Blood Services Laboratories in Bethesda, Maryland.

HBsAg

All samples were tested for HBsAg by means of solid phase radioimmunoassay employing a double antibody sandwich technique (*1*) and commercially available kits (AUSRIA II®, Abbott Laboratories, North Chicago, Illinois). Those samples found reactive for HBsAg on screening were tested for specificity by inhibition, following the manufacturer's instructions. Human serum containing high titers of antibody to HBsAg (anti-HBs) was used for this purpose. Only those samples that were specifically inhibited by preincubation with anti-HBs were considered positive for HBsAg. All samples positive for HBsAg were retested in three ways—by immunodiffusion (ID) using the technique described by Mazzur (*2*); by counterelectrophoresis (CEP) using Hapindex® kits supplied by Ortho Diagnostics (Raritan, New Jersey); and by reverse passive hemagglutination (RPHA) using AUSCELL® kits produced by Abbott Laboratories.

Antibodies to HBsAg

All samples were tested for the presence of anti-HBs with the passive hemagglutination (PHA) microtiter technique (*3*), using human erythrocytes coated with HBsAg (supplied by Electro-Nucleonics in Bethesda, Maryland). Samples were screened at 1:4, 1:8, and 1:16 dilutions. Uncoated cells were included to determine nonspecific reactions.

Antibodies to HBcAg

All samples were tested for anti-HBc using the RIA test and CORAB® kits donated by Abbott Laboratories.

HBeAg and anti-HBe

All samples found reactive to HBsAg were screened for the presence of HBeAg/anti-HBe with a rheophoresis technique. Rheophoresis plates were obtained from Abbott Laboratories. Reagents for testing, i.e., sera positive for anti-HBe and HBeAg, were selected from the American Red Cross collection of HBsAg samples. The specificity of these samples was confirmed using reagents supplied by Dr. L. O. Magnius of the Department of Virology, Statens Bakteriologiska Laboratory, Stockholm, Sweden.

Subtyping of HBsAg

Samples positive for HBsAg were tested for their subtype specificity by immunodiffusion (*2*) and passive hemagglutination-inhibition (*4*). The type-specific antisera used for subtyping were produced in rabbits by injecting HBsAg with the specific sub-

type ad or ay that had been purified by a previously described method (*5*). Monospecific sera used in the passive hemagglutination-inhibition tests were obtained by heterologous absorption in the liquid phase.

Results

HBsAg Testing

All 7,487 serum samples were tested for HBsAg by radioimmunoassay (RIA), the most sensitive technique. By this method, 121 samples (1.6 per cent) were found to contain HBsAg. Those samples found HBsAg-positive by RIA were subsequently retested by immunodiffusion (ID), counterelectrophoresis (CEP), and reverse passive hemagglutination (RPHA). As indicated in Table 1, RPHA, the most sensitive of these three tests, detected 90.9 per cent of those samples shown by RIA screening to contain HBsAg. Only 11 of the 121 samples found positive (with low levels of HBsAg) by RIA yielded negative RPHA results. CEP detected 67.2 per cent of the positive samples, while ID, the least sensitive method, detected only 62.0 per cent. The HBsAg titers determined by RPHA ranged from 1:8 to at least 1:25,600, with the mode occurring at 1:6,400. As indicated in Figure 1, ID identified all samples with RPHA titers equalling or exceeding 1:25,600, as well as some samples with RPHA titers between 1:16 and 1:12,800. None of the 10 negative controls was judged positive. Similarly, CEP detected HBsAg in all samples with RPHA titers above 1:3,200, and also in some of the samples with titers ranging from 1:8 to 1:1,600.

Anti-HBs and Anti-HBc Testing

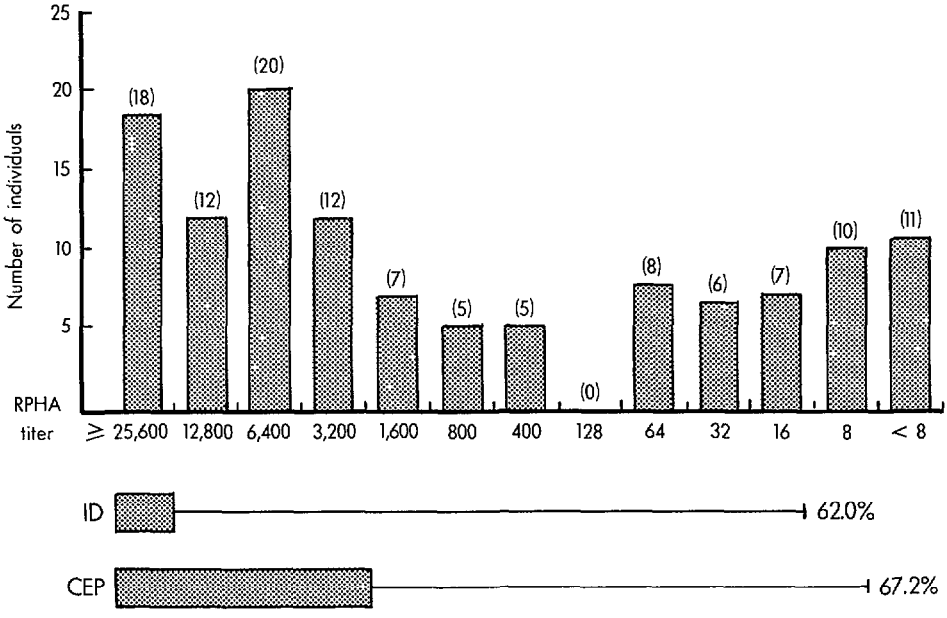
As Table 2 shows, the prevalence of antibody to HBsAg was found to range from 3.8 per cent in the Chilean samples to 55.3 per cent in the samples from the Dominican Republic. This prevalence generally in-

Table 1. Comparison of methods used to detect HBsAg.

Country providing samples	No. of samples tested	Samples positive by RIA		% of RIA-positive samples found positive by RPHA, CEP, and ID		
		No.	%	Positive by RPHA	Positive by CEP	Positive by ID
Argentina	1,013	8	0.8	87.5	62.5	50.0
Barbados	500	7	1.4	85.7	85.7	85.7
Brazil	1,022	21	2.1	81.0	23.8	42.9
Chile	500	2	0.4	100.0	100.0	50.0
Colombia	499	5	1.0	80.0	80.0	80.0
Costa Rica	480	3	0.6	100.0	100.0	100.0
Dominican Republic	489	20	4.1	100.0	75.0[a]	40.0
Ecuador	500	10	2.0	90.0	80.0	80.0
Mexico	500	8	1.6	100.0	100.0	87.5
Peru	500	11	2.2	100.0	100.0[b]	100.0
Puerto Rico	499	1	0.2	100.0	100.0	100.0
Suriname	488	11	2.3	90.9	63.6	45.5
Venezuela	497	14	2.8	85.7	50.0	57.1
Total	7,487	121	1.6	90.9	67.2	62.0

[a] 4 samples insufficient for CEP.
[b] 2 samples insufficient for CEP.

Figure 1. A total of 121 HBsAg-positive samples detected by radioimmunoassay (RIA) were retested by reverse passive hemagglutination (RPHA), counterelectrophoresis (CEP), and immunodiffusion (ID).



Vertical bars show the number of samples yielding the indicated titers in RPHA tests. The two horizontal bars, marked "ID" and CEP," pass below certain RPHA titers. Within these ranges (for HBsAg-positive samples with these RPHA titers) ID and CEP tests yielded positive results. Some positive ID and CEP results were also obtained with samples whose RPHA titers fell within the lower ranges indicated by the two horizontal lines.

Table 2. Hepatitis B virus markers found in serum samples from 13 Western Hemisphere countries.

Countries, by order of prevalence of HBV markers	% of hepatitis B infection (at least one HBV markers)	X^2 test[a] applied to % of hepatitis B infection	% positive for HBsAg	% positive for anti-HBs	% positive for anti-HBc
Chile	6.7	110.56	0.4	3.8	5.3
Puerto Rico	11.1	66.68	0.2[b]	9.2	10.1
Barbados	13.1	52.12	1.4	9.0	11.9
Mexico	16.8	28.34	1.6	11.6	9.0
Venezuela	17.9	22.38	2.8	11.6	15.5
Argentina	18.6	41.80	0.8	14.7	9.4
Costa Rica	20.6	10.21	0.6	17.3	16.7
Peru	27.3	0.02	2.2	20.2	20.4
Colombia	29.3	1 44	1.0	25.1	18.1
Brazil	33.9	28.21	2.1	26.7	27.6
Ecuador	35.3	18.20	2.0	29.4	21.9
Suriname	40.9	51.27	2.3	28.1	37.9
Dominican Republic	82.8	805.38	4.1	55.3	81.1
Total	27.0		1.6	20.2	21.3

[a]Chi-square calculated by comparing positive and negative results for one country with the total positive and negative results for the remaining countries.

[b]Donor population known to have been prescreened by RIA.

creased in proportion to the number of HBsAg carriers in the population. Testing for anti-HBc showed its prevalence to range from 5.3 per cent in the Chilean samples to 81.1 per cent in the Dominican samples. The prevalence of anti-HBc was shown to increase as the prevalence of other hepatitis B markers rose.

Subtyping

Serum samples known to contain HBsAg were tested for antigen subtypes by the passive hemagglutination inhibition reaction and by immunodiffusion. The former test, which is more sensitive than immunodiffusion, succeeded in identifying 66 of the 121 samples (54.5 per cent) as HBsAg/ad[17] and 5 of the samples (4.1 per cent) as HBsAg/ay. These ay samples came from Argentina, the Dominican Republic, and Suriname. Subtyping by immunodiffusion confirmed the subtypes of those samples that had sufficiently high titers to permit detection by this method; it also further identified the samples involved as HBsAg/adw and HBsAg/ayw. None of these samples contained the r determinant.

HBeAg and Anti-HBe

As previously noted, the 121 HBsAg-positive samples were tested by rheophoresis for HBeAg and anti-HBe. Of these, seven samples (5.8 per cent) were found to contain HBeAg. One of these came from Argentina, one from Barbados, one from Suriname, and four from Mexico. The presence of HBeAg in blood donor samples did not correlate with the prevalence of HBsAg in the populations involved.

Antibody to HBeAg was found in 30 of the 121 samples (24.8 per cent); these 30 samples came from Argentina, Brazil, the Dominican Republic, Ecuador, Peru, Puerto Rico, and Venezuela.

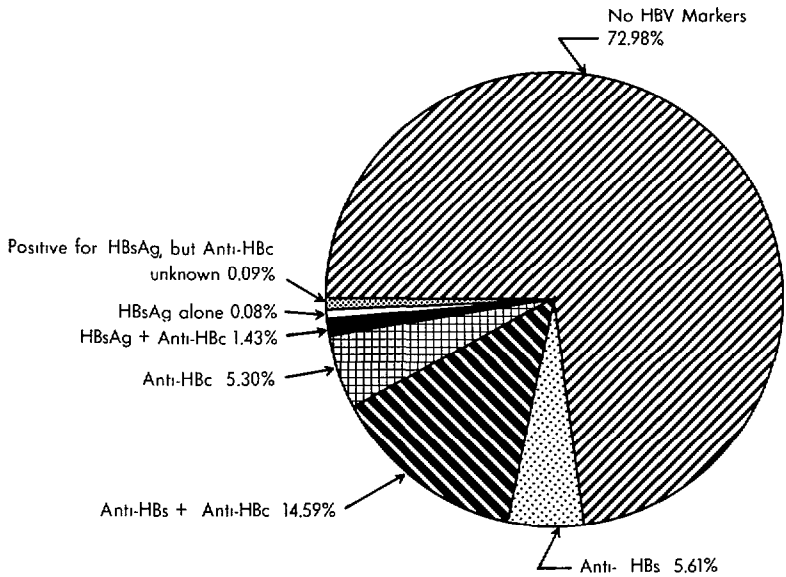
Overall Test Results

Testing of all the samples from participating Latin American countries showed 1.6 per cent positive for HBsAg, 20.2 per cent positive for anti-HBs, and 21.3 per cent positive for anti-HBc. HBsAg was found to occur alone without any of the other HBV markers on occasion, although 94.6 per cent of the HBsAg-positive samples were also found to contain anti-HBc (Figure 2). Anti-HBc was also found in 72.2 per cent of the samples containing anti-HBs, and was detected in 5.3 per cent of the total samples unaccompanied by any other HBV serum marker. These anti-BHc figures cannot be confirmed because no method for confirming the CORAB® kit RIA test results obtained is available at the present time.

The prevalences of HBsAg, anti-HBs, anti-HBc, and HBV infection in the blood donor populations of each of the 13 countries were compared with the prevalences of the indicated markers in all of the countries combined. This was done by applying a chi-square test with one degree of freedom (see Table 2). The results indicated that blood donors of Argentina, Chile, and Puerto Rico had significantly lower HBsAg prevalences than the overall population tested ($p < 0.05$), while those of the Dominican Republic and Venezuela had significantly higher HBsAg prevalences ($p < 0.005$). The prevalence of HBsAg in Costa Rica (0.6 per cent) was not significantly lower ($p < 0.1$) as it was in Argentina (0.8 per cent). The difference may be due to the different number of samples tested. Similarly, the donors in Argentina, Barbados, Chile, Mexico, Puerto Rico, and Venezuela had significantly lower anti-HBs prevalences ($p < 0.005$), while those in Brazil, Colombia, the Dominican Republic, Ecuador, and Suriname had significantly higher anti-HBs

[17]The major antigenic determinants of HBsAg are a, d, y, w, and γ. Various combinations of these occur in nature, adw, ayw, and adr being the most common. The antigenic subtypes are currently used as an important marker in epidemiologic studies.

Figure 2. The distribution of HBV serum markers in samples from 7,487 Latin American blood donors.



All the samples were tested for HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe. A few (0.09 per cent) of the samples could not be tested for anti-HBc because of insufficient sample material.

prevalences ($p < 0.005$). Observed anti-HBs prevalences were significantly lower among donors in Argentina, Barbados, Chile, Costa Rica, Mexico, Puerto Rico, and Venezuela ($p < 0.025$), but were significantly higher ($p < 0.005$) among donors in Brazil, the Dominican Republic, and Suriname.

Prevalences of hepatitis B infection, as indicated by the presence of at least one hepatitis B virus marker, ranged from 6.7 per cent in Chile to 82.8 per cent in the Dominican Republic. The average prevalence found in the samples from all the participating Latin American countries was 27.0 per cent. Countries that had prevalences of infection significantly below this average ($p < 0.005$), as judged by the presence of at least one HBV marker, were Argentina, Barbados, Chile, Costa Rica, Mexico, Puerto Rico, and Venezuela. In contrast, prevalences significantly above the average ($p < 0.005$) were found for Brazil, the Dominican Republic, Ecuador, and Suriname.

Discussion

This study constitutes the first unified investigation of HBV markers in blood donor populations of 13 Western Hemisphere countries. The overall prevalence of HBsAg carriers among 7,487 blood donors in these countries was found to be 1.6 per cent. Respective overall prevalences of anti-HBs and anti-HBc were 20.2 and 21.3 per cent.

Evidence of infection, as indicated by the presence of one or more HBV markers, was found in only 27.0 per cent of the sample tested. On balance this points to a dangerous situation, since 1.6 per cent of the donors were HBsAg carriers, while about 73 per cent of the overall population was apparently susceptible to infection. The presence of anti-HBc was strongly associated with the presence of HBsAg ($p < 0.005$) or with high titers ($\geq 1{:}16$) of anti-HBs ($p < 0.005$); samples showing no HBsAg or having low anti-HBs titers ($\leq 1{:}8$) tended not to contain anti-HBc.

A striking feature of the data was the extreme variation in the prevalence of HBV markers found in different populations (see Table 2), a prevalence ranging from 6.7 per cent in Chile to 82.8 per cent in the Dominican Republic. This high Dominican Republic figure confirms earlier work done in that country. That work found HBsAg prevalences of 5.3 per cent by RIA (*6*) and 2.0 per cent by the immunodiffusion method (*7*). In addition, a 41 per cent prevalence of anti-HBs, detected by passive hemagglutination, has been reported among a series of women giving birth at the Salvador B. Gautier Hospital in Santo Domingo (*8*). Thus the level of HBV infection appears to have been much higher in the Dominican population than among other populations studied.

The contrast between the lowest prevalence of HBV infection found (6.7 per cent in Chile) and the highest (82.8 per cent in the Dominican Republic) was very dramatic. However, prevalence levels could not be explained on the basis of geographic contiguity. For example, low-prevalence Chile is bordered by Argentina and Peru, where the respective prevalences were 18.6 and 27.3 per cent. Likewise, the prevalences of HBV markers found for the nearby island populations of Barbados, the Dominican Republic, and Puerto Rico were quite dissimilar.

It is difficult to account for these significant variations in HBV prevalence among the various blood donor populations studied; however, some of the variations could be due to local variables—such as whether paid or volunteer blood donors were used and the relative socioeconomic status of those donors in the community. Factors such as enhanced virulence of certain HBV strains, amplification of an existing transmission route, or development of more effective routes could also play some part in these variations.

It was interesting to note that of five HBsAg/ay subtypes identified, three came from the Dominican Republic and one came from Suriname. The Dominican Republic and Suriname populations studied had the two highest prevalences of HBV. It has been suggested that HBsAg/ay is more infectious than other subtypes (*9*).

Comparative testing of samples reactive for HBsAg by RIA showed that reverse passive hemagglutination (RPHA) detected HBsAg in 90.9 per cent of the RIA-positive samples, while counterelectrophoresis (CEP) and immunodiffusion (ID) respectively detected HBsAg in 67.2 and 62.0 per cent of the RIA-positive samples. These data are similar to those obtained by many other investigators (*10*). Although CEP and ID are much less sensitive than RIA and RPHA, more than half of the HBsAg carriers have sufficient antigen to be detected by these methods; so, while not optimal tests, CEP and ID will detect a significant number of HBsAg carriers and will permit them to be eliminated from the donor pool. Thus CEP or ID would perhaps be appropriate methods for use by blood programs lacking access to more sensitive technology.

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SUMMARY

A total of 7,487 donor blood samples from 13 Western Hemisphere countries (Argentina, Barbados, Brazil, Chile, Colombia, Costa Rica, the Dominican Republic, Ecuador, Mexico, Peru, Puerto Rico, Suriname, and Venezuela) were tested for various markers of hepatitis B virus (HBV) infection with several different techniques. HBsAg was detected in 1.6 per cent of the samples, anti-HBs in 20.2 per cent, and anti-HBs in 21.3 per cent. The incidence of HBsAg varied from 0.2 per cent (in the Puerto Rican samples) to 4.1 per cent (in the samples from the Dominican Republic). Overall, 5.8 per cent of the samples found to contain HBsAg also contained HBeAg, while 24.8 per cent had detectable anti-HBe.

Sixty-six (54.5 per cent) of the 121 HBsAg-positive samples were found to contain subtype HBsAg/ad, and 5 (4.1 per cent) were found to contain subtype HBsAg/ay. Subtypes of the remaining samples could not be determined because of insufficient antigen. In a comparative study, reverse passive hemagglutination (RPHA), counterelectrophoresis (CEP), and immunodiffusion (ID) detected HBsAg in 90.9, 67.2, and 62.0 per cent of the samples previously found positive by radioimmunoassay (RIA) screening.

Considerable variation was observed in the prevalence of HBV markers in samples from different countries, the highest prevalence being 82.8 per cent in samples from the Dominican Republic. Overall, the findings suggest that exposure to HBV is quite extensive in some Latin American populations.

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