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GEOGRAPHIC DISTRIBUTION OF HBsAg SUBTYPES IN BRAZIL

ANA MARIA COIMBRA GASPAR & CLARA FUMIKO TACHIBANA YOSHIDA
Instituto Oswaldo Cruz, Departamento de Virologia, Centro de Referência Nacional para Hepatites Virais, Caixa Postal 926, 20001, Rio de Janeiro, RJ, Brasil

HBsAg positive serum samples (896) from five brazilian regions were analysed for HBsAg subty pes. The presence of five different subtypes (ayw_2, ayw_3, ayw_4, adw_2 and adw_4) was detected.

In Northern region subtypes adw_4 (41.2%) and adw_2 (37.2%) were predominant. In the North East only subtype adw_2 was encountered. In Central West, South-East and South, subtypes ayw_2, ayw_3, adw_2 and adw_4 were present, with predominance of adw_2 in Central West and South East (84.3% and 69.4% respectively) whereas in the South the predominant subtype was ayw_3 (41.9%) followed by ayw_2 (36.4%). Subtypes ayw_1, ayr and adr were not found among the samples studied.

These results show the difference in the incidence of HBsAg subtypes in the different regions of Brazil and their significance in relation to the colonization and migrations in this country.

Key words: HBsAg subtypes

The incidence of hepatitis B virus (HBV) infection varies according to the geographical region (Couroucé et al., 1976). In Brazil the prevalence of asymptomatic carriers, increases from South to North from 0,1% to 9-10%), respectively (Bensabath & Boshell, 1973; Bensabath et al., 1983; Yoshida & Schatzmayr, 1986).

The improvement of serological methods to differentiate antigenic subtypes of HBsAg led to the demonstration of unequal distribution of antigenic subtypes in different countries (Mazzur et al., 1973; Yamashita et al., 1975; Bancroft, 1976; Couroucé et al., 1983).

It seems that the HBV subtype found in an infected individual is more strongly related to the country of origin or residence in childhood than to the present residence. In some of the areas studied, the prevalence of one subtype reflects the geographic origin of the family, and tends to be maintained not only in immigrants but also in their first generation (Couroucé, 1976a; Couroucé et al., 1983 and Yoshida et al., 1979).

HBsAg subtypes have an important value as epidemiological markers; through their study sources of infection can be identified and migratory chains can be followed (Gaspar et al., 1984a; Mosley et al., 1972).

Brazil has an area of approximately 8.500.000 Km^2 and the number of inhabitants is around 130 millions. The country is divided in five regions where the climate variation is very wide. At colonization time, immigrants came from several countries and established themselves in different regions. The main colonizers were Portuguese and African which miscigenated with native aborigenes. Later, other europeans had an important role in the colonization of these same regions (Vianna, 1975).

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MATERIAL ALD METHODS

Serum samples from asymptomatic chronic carriers and acute cases of Hepatitis B were obtained from different regions: *North* – 22 samples from Belém, 15 from Altamira, Pará (1980) and 14 from the Amazonas State (1983); *North East* – 15 samples from Maceió, Alagoas, 10 from Recife, Pernambuco (1983); *Central West* – 20 samples from Brasília, Distrito Federal and 18 from Goiânia, Goiás (1983); *South East* – 32 samples from São Paulo, SP (1984), 589 from Rio de Janeiro, RJ (1979 to 1985) and 87 from a rural region of Espírito Santo (C. Itapemirim, 1984); *South* – 30 samples from Florianópolis, Santa Catarina and 44 from Curitiba, Paraná (1984).

All serum samples were initially tested by reverse passive hemmaglutination (R-PHA, Bio-Manguinhos, FIOCRUZ) and only titers $> 1/256$ were titrated by counter immuno electrophoresis (CIEP).

Samples with CIEP $> 1/16$ were subtyped by immunodifusion (I.D.).

Subtyping by I.D. – this technique was performed in microscope slide using agarose 0,9% (IBF A-45) in phosphate buffer (NaCl 0.1M, TRIS 0.01M, EDTA 0.001M, pH 7.6) by Ouchterlony's pattern for arrangement of wells for gel diffusion analysis (Soulier & Couroucé, 1973). Polyspecific anti-ad and anti-ay were produced in our laboratory immunizing rabbits with HBsAg/adw_2 and HBsAg/ayw_3 respectively (Gaspar, 1981).

These sera were evaluated with WHO standards for subtypes and their specificities were confirmed by Dr. A.M. Couroucé, CNTS, Paris.

These sera were used in the central well, and subtype was defined by spur formation between samples of standard subtypes and samples of HBsAg in test (Couroucé-Pauty & Soulier, 1974). Goat immune serum anti-ay + w_3 + w_4 for identification of w specificity was kindly supplied by Dr. A.M. Couroucé, CNTS-Paris.

Subtyping by CIEP – glass slides of 10x8cm were covered with agarose 0.4% (IBF-A-37) supplemented by agar 0.1% (Merck) diluted in Tris-acetate buffer (0.05M) pH 8.6 (Couroucé et al., 1984; Hatch, 1975; Holland et al., 1972). For subtype identification, monospecific antibodies anti-d and anti-y and other moroclonal antibody that only reacts with determinant d and other, to adw_2 subtype, were used (Gaspar et al., 1984b). Before subtyping, serum samples were titrated against goat immune serum.

RESULTS

The overall distribution of HBsAg subtypes is shown in Table I and Fig. 1. In the North (Table II), determinant d was predominant both in Pará and Amazonas and only subtypes adw_2 and adw_4 were found in the latter. In the North East, adw_2 was the only subtype encountered. In the Central West there was a difference in subtype distribution in the two localities studied. In Goiânia there was a predominance of adw_2 (94.5%), ayw_3 being found in only 5.5%. In Brasília four subtypes (ayw_2 – 5%, ayw_3 – 5%, adw_2 – 75% and adw_4 15%) were found.

In the South East, subtypes were equally distributed in São Paulo and Rio de Janeiro, but a marked difference was noticed in a rural zone of Espírito Santo (C. Itapemirim) where only two subtypes (adw_2 and ayw_2) where found in about the same proportions (Table III).

In the South, there was a higher incidence of determinant y (ayw_2 and ayw_3) in relation to d, in both States studied, and absence of adw_4 in Santa Catarina (Table IV).

There was a great variation of incidence of HBV subtypes in Rio de Janeiro in the last seven years (1979-1985). Increasing of determinant y is related to ayw_3 subtype and decreasing of determinant d, to adw_2. From 1979 to 1984, there was an increase in the incidence of subtype adw_4 and no change in ayw_2 incidence (Table V, Fig. 2).

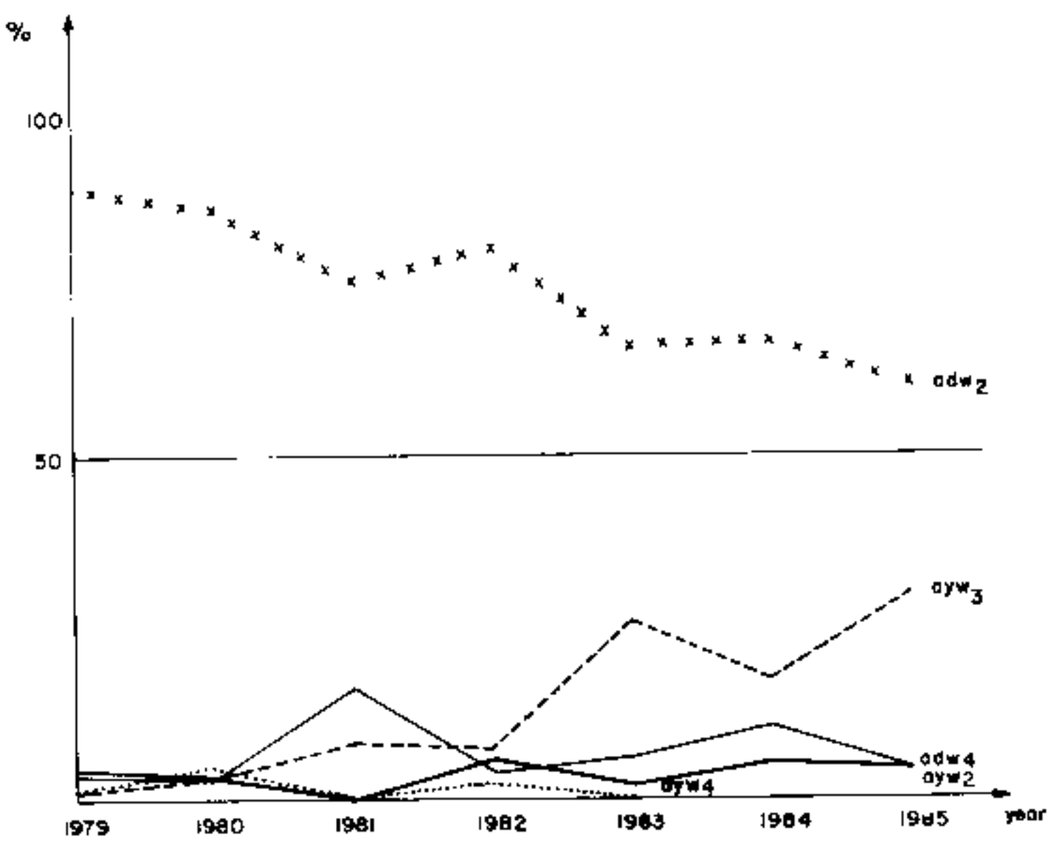

Fig. 1: HBsAg subtypes in regional areas of Brazil.

TABLE I

HBsAg subtypes in regional areas of Brazil

Subtypes	North		North East		Central West		South East		South	
	N	%	N	%	N	%	N	%	N	%
ayw	–	–	–	–	–	–	–	–	–	–
ayw^1	6	11,8	–	–	1	2,6	73	10,3	27	36,4
ayw^2	4	7,8	–	–	2	5,2	94	13,3	31	41,9
ayw^3	1	2,0	–	–	–	–	6	0,8	–	–
ayr^4	–	–	–	–	–	–	–	–	–	–
adw	19	37,2	25	100	32	84,3	491	69,4	11	14,8
adw^2	21	41,2	–	–	3	7,9	44	6,2	5	6,7
adr^4	–	–	–	–	–	–	–	–	–	–
Total	51	100	25	100	38	100	708	100	74	100

TABLE II

HBsAg subtypes in North of Brazil

Subtypes	Belém – PA		Altamira – PA		Amazonas	
	N	%	N	%	N	%
ayw	–	–	–	–	–	–
ayw[1]	2	9,0	4	26,6	–	–
ayw[2]	2	9,0	2	13,3	–	–
ayw[3]	1	4,5	–	–	–	–
ayr[4]	–	–	–	–	–	–
adw	9	40,9	8	53,3	2	14,3
adw[2]	8	36,3	1	6,6	12	85,7
adr[4]	–	–	–	–	–	–
Total	22	100	15	100	14	100

TABLE III

HBsAg subtypes in South East of Brazil

Subtypes	S. Paulo–SP		R. Janeiro–RJ		C. Itapemirim–ES	
	N	%	N	%	N	%
ayw	–	–	–	–	–	–
ayw[1]	1	3,1	23	3,9	49	56,3
ayw[2]	10	31,3	84	14,3	–	–
ayw[3]	–	–	6	1,0	–	–
ayr[4]	–	–	–	–	–	–
adw	16	50,0	437	74,2	38	43,7
adw[2]	5	15,6	39	6,6	–	–
adr[4]	–	–	–	–	–	–
Total	32	100	589	100	87	100

TABLE IV

HBsAg subtypes in South of Brazil

Subtypes	Florianópolis – SC		Curitiba – PR	
	N	%	N	%
ayw	–	–	–	–
ayw[1]	9	30,0	18	41,0
ayw[2]	19	63,4	12	27,3
ayw[3]	–	–	–	–
ayr[4]	–	–	–	–
adw	2	6,6	9	20,4
adw[2]	–	–	5	11,3
adr[4]	–	–	–	–
Total	30	100	44	100

TABLE V

Changes in the distribution of HBsAg subtypes in Rio de Janeiro, South East of Brazil

Subtypes	Years													
	1979		1980		1981		1982		1983		1984		1985	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
ayw	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ayw[1]	4	4,7	3	3,2	–	–	3	5,8	2	2	8	5,3	3	4,2
ayw[2]	1	1,1	3	3,2	3	8,1	4	7,7	26	26	26	17,3	21	30
ayw[3]	1	1,1	4	4,2	–	–	1	2,0	–	–	–	–	–	–
ayr[4]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
adw	77	89,5	8	86,2	28	75,7	42	80,7	66	66	100	66,7	43	61,4
adw[2]	3	3,6	3	3,2	6	16,2	2	3,8	6	6	16	10,7	3	4,2
adr[4]	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	86	100	94	100	37	100	52	100	100	100	150	100	70	100

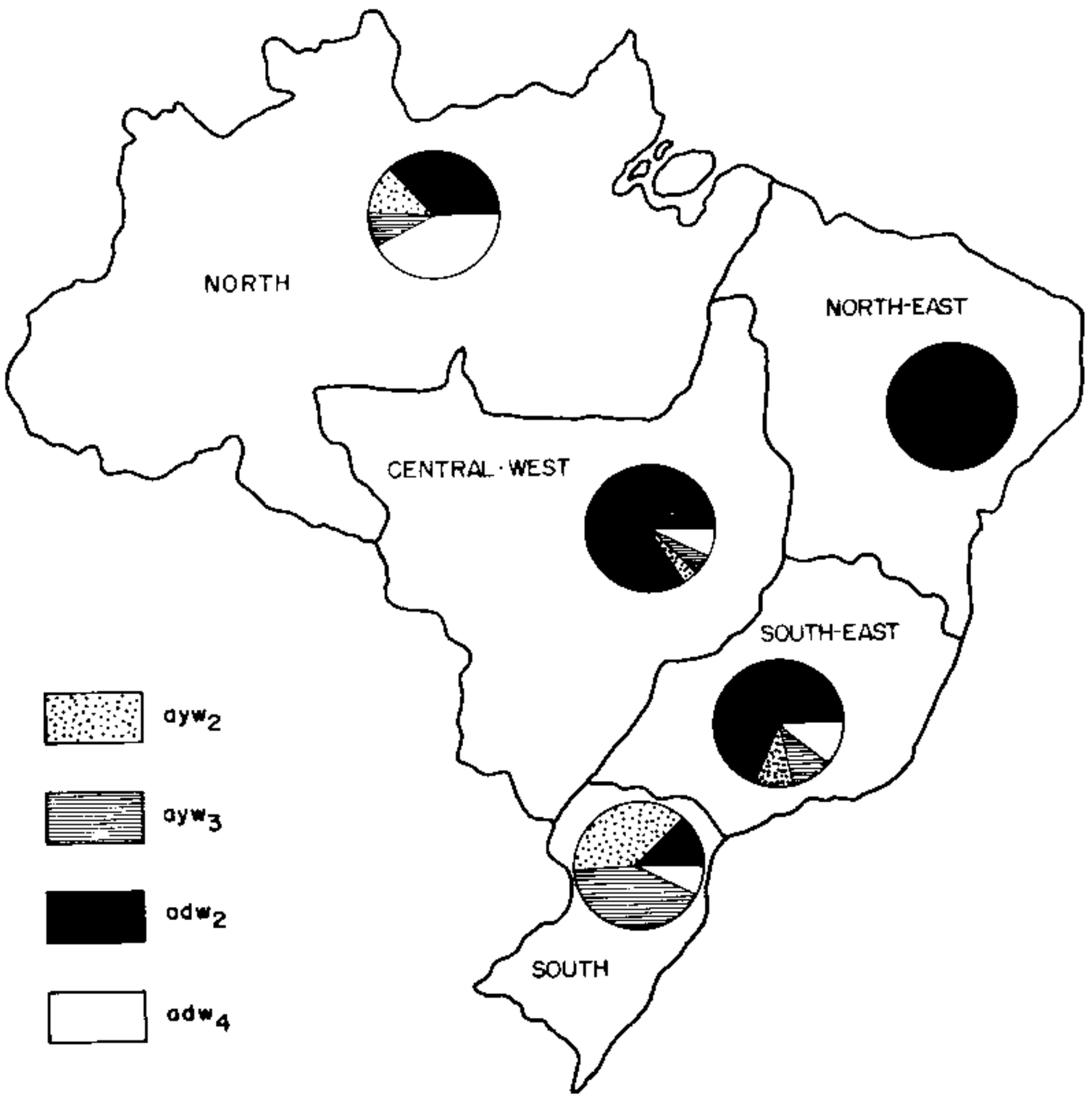

Fig. 2: temporal changes in the distribution of HBsAg subtypes in Rio de Janeiro, South East of Brasil.

DISCUSSION

Information on HBV subtypes circulating in a particular area is important for future investigations concerning the appearence of recombinants, identification of sources of infection, and also the production of a local vaccine which should contain the subtypes encountered in the country.

The Northern population of Brazil presents a high incidence of determinant d in Belém and in the Amazonas region, except in Altamira where the incidence of determinant y is higher.

Belém has a native population whereas Altamira was recently built in consequence of the interconnection of Central West and North routes which led to a great flow of immigrants from different parts of the country.

In the Amazonas State, where most of the population is descendent from aborigenes and the migratory flow is very low, an almost exclusive predominancy of subtype adw_4 was found.

In the North East 100% of HBV infections are caused by subtype adw_2. This region was colonized by Portuguese people and received a great flow of black slaves from the Southern region of Africa, where this subtype is prevalent (Couroucé, 1976b; Soulier & Couroucé-Pauty, 1977). Later it had some European intake, mainly French and Dutch.

The South East was mainly colonized by Portuguese people but also received a high number of immigrants coming from Italy, Spain, Lebanon and Japan. In the largest cities, São Paulo and Rio de Janeiro, there was considerable internal migration mainly from the North Eastern States and from the Central West of the State of Minas Gerais. It can be observed that subtype adw_2 has the highest incidence (50-60%) followed by ayw_3 (20-30%). However, due to the high migratory flow into this region, some changes of HBsAg subtypes were observed in the last seven years (Table V and Fig. 2). The percentage of subtype adw_2 dropped from 90% in 1979 to nearly 60% and subtype ayw_3 increased from 1.1% to 30%, probably due to an increase in migrations (Gust et al., 1980). The highest incidence of ayw_3 observed now, can be a consequence of increased sexual promiscuity and drug addiction considering that this subtype is the one most frequently spread by these routes (Couroucé, 1975; Couroucé-Pauty & Soulier, 1974).

Still in the South East, we studied a rural population of Italian descendants in Espírito Santo (C. Itapemirim), which showed a prevalence of HBsAg of about 7%. The subtypes adw_2 and ayw_2 were encountered in this region with about the same frequency. The high incidence of subtype ayw_2 in this community is closer to the one found in Italy (66.7%) (Couroucé et al. 1983), than to the one found in the other places studied in the South East region (Rio de Janeiro 5.7%, São Paulo 5.1%). The great number of infected children of the third generation of Italian immigrants demonstrates the intrafamiliar transmission of HBV and its maintenance through personal contact and vertical transmission (Stevens et al., 1979).

In the Central West, an incidence of 84.3% of subtype adw_2 was observed. In this region there was mainly native population, until the construction of Brasília, the capital of the country since 1960. There was a great migration flow from several regions to Brasília and we could observe the rise of other subtypes such as adw_4, which is predominant in the Northern region.

In the South, a high incidence of determinant y (ayw_2, ayw_3) was observed in two States studied (Santa Catarina and Paraná). The South has a population completely different from the North and North East concerning its ascendence, most of the people descending from Europeans, mainly Italians and Germans. Climate and cultural habits are also quite different and migration to other regions is not very common.

No ayw_1, ayr and adr were found in the present study. However subtype adr encountered in Asiatic population was already found in individuals of first generation of Japanese immigrants living in the South and South East regions (Yoshida et al., 1979).

The subtype ayw_4 which was rarely found in the South East until 1981, was not encountered any more after that year. Some doubtful samples were sent to Dr. A.M. Couroucé, CNTS (Paris-France) and it was verified that they belonged to an intermediate subtype between ayw_3 and ayw_4 recently incorporated to the Paris reference panel (Couroucé et al., 1984).

RESUMO

Distribuição geográfica dos subtipos de antígenos de HBsAg no Brasil – Foram analisadas 896 amostras de soros HBsAg positivos de cinco regiões brasileiras.

A presença de cinco diferentes subtipos (ayw_2, ayw_3, ayw_4, adw_2 e adw_4) foi determinada.

Na Região Norte, os subtipos adw_4 (41,2%) e adw_2 (37,2%) foram predominantes. No Nordeste somente o subtipo adw_2 foi encontrado. No Centro Oeste, Sudeste e Sul os subtipos ayw_2, ayw_3, adw_2 e adw_4 estão presentes, com predominância do adw_2 no Centro Oeste e Sudeste (84,3% e 69,4% respectivamente), enquanto que no Sul o subtipo predominante foi ayw_3 (41,9%) seguido pelo ayw_2 (36,4%). Os subtipos ayw_1 e ayr e adr não foram encontrados entre amostras estudadas.

Estes resultados mostram a diferença na incidência dos subtipos do HBsAg em diferentes regiões do Brasil e seu significado em relação à colonização e migrações neste país.

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