



## Seroprevalence of hepatitis B infection and active immunization in Ecuadorian Amazonian indigenous people: A cross-sectional study

Soroprevalência da infecção por hepatite B e imunização ativa em indígenas da Amazônia Equatoriana: Um estudo transversal

Seroprevalencia de infección por hepatitis B e inmunización activa en indígenas de la Amazonía ecuatoriana: Un estudio transversal

Natalia Romero-Sandoval<sup>1,2</sup> , Andrés Flores<sup>2,3</sup> , Nicole Mora-Bowen<sup>1</sup> , Lino Arisqueta<sup>4</sup> , Cristina Moreno<sup>1</sup> , Richard Douce<sup>5</sup> , Miguel Martín<sup>2,3</sup> 

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<sup>1</sup> School of Medicine, Universidad Internacional del Ecuador, Quito, Ecuador.

<sup>2</sup> Red GRAAL, Barcelona, Spain.

<sup>3</sup> Faculty of Medicine, Universidad Autónoma de Barcelona, Barcelona, Catalunya, Spain.

<sup>4</sup> Facultad de Ciencias de la Salud, Universidad Internacional SEK Ecuador, Quito, Pichincha, Ecuador.

<sup>5</sup> Spectrum/Lakeland Hospital, Saint Joseph, Michigan, United State of America.

### ABSTRACT

**Introduction:** Hepatitis B surveillance is crucial. **Aim:** We proposed to describe the seroprevalence of hepatitis B infection and the associated factors among Ecuadorian Amazonian indigenous people. **Outlining:** A serologic cross-sectional study was carried out in subjects over two years old. We classified seropositivity according to the Centers of Diseases Control criteria and recorded sex, age, temporary migration, sometime hepatitis B in their life, and previous vaccination. **Results:** Out of 215 participants, 50.0% were susceptible (2-11 years 68.1%), 8.8% immune due to natural infection, 1.9% chronic infection, and 1.4% early acute HBV infection. Eight people reported having ever been diagnosed with hepatitis B, none of them showed chronic or acute infection and one was positive for immunity due to the vaccine. HBsAg was found in 3.3% (95% CI 0.9-7.0) and was higher among those who migrate 7.7% (p-value 0.04). 16.3% (95% CI 11.6-21.4) were immune due to HBV vaccination. Among the 58 participants who declared having received the vaccine, 20.7% presented post-vaccine immunity. **Implications:** Participants who should have benefited from vaccination are children. Opportunistic assessing the burden of viral hepatitis B in Amazonian indigenous people and guide preventive measures is mandatory.

### DESCRIPTORS

Hepatitis B antibodies; Hepatitis B antigens; Health of Indigenous Peoples; Epidemiology.

### Corresponding author:

Natalia Romero-Sandoval  
Address: Av. Jorge Fernández s/n y Simón Bolívar  
ZIP-Code 170113 – Quito, Ecuador  
Telephone: +59 322985600  
E-mail: [nromero@uide.edu.ec](mailto:nromero@uide.edu.ec)

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## INTRODUCTION

The hepatitis B (HBV) epidemiological surveillance regarding South America has shown high endemicity zones, being one of them the western Amazon.<sup>1</sup> In 1996, the prevalence of HBV was higher in natives (64.3%) than in mestizos (50.6%) of the different basins of the Peruvian Amazon, and between 1989 to 2003, in 2700 individuals from different indigenous ethnical groups from Brazilian Amazon almost 10% were chronic carriers, with an average age of 19 years.<sup>2</sup> A review systematic (1972-2008) revealed that surface antigen (HBsAg) prevalence remains four times higher among Australian indigenous people compared with non-indigenous people.<sup>3</sup>

The Amazonian indigenous people are under vulnerability conditions such as temporary migration due to the lack of paid employment, promotion of sex tourism, migrant smuggling, human trafficking, forced displacement due to armed conflicts, drug smuggling,<sup>4</sup> poor hygienic conditions, high fertility rates, poor health situation, and environmental degradation.<sup>5</sup> 96% of people live in rural communities without a resident physician and travel, on average, 3 hours to reach the closest health care centre to receive primary care.<sup>6</sup>

The Centers for Disease Control and Prevention (CDC) places Ecuador in the low-intermediate prevalence (2% - 4%) group for chronic HBV infection and defines the Amazon region as an area of high endemicity.<sup>7</sup> In Ecuador, since 2003, the pentavalent vaccine has been administered as part of a cost-free and mandatory immunization program held by the Ecuadorian Ministry of Health; the HepB vaccination birth dose (active immunization) was introduced under the same program in 2012.<sup>8</sup> In 2015, there were 219,000 hepatitis B surface antigen (HBsAg) carriers in Ecuador.<sup>9</sup>

Although exact vaccination rates are unknown, a report from 2006 states that only 28% of children from Amazonian indigenous ethnic groups (Shuar and Achuar) under five years old had received at least

one documented vaccination, and only 11% of them had completed a full vaccination regimen.<sup>10</sup>

Against this unknown context, the objective of this study was to estimate the HBV antigens and antibodies seroprevalence and active immunization coverage among indigenous people of the southern Amazon region of Ecuador and to explore associated factors.

## METHOD

### Study Design

A cross-sectional study was conducted in two Amazonian indigenous communities (Shuar ethnic). The study was approved by the International University of Ecuador Ethics Committee. We followed the Strobe initiative.

### Setting

The studied population settles in the southeastern Ecuador, and their territory extends to Amazon in northern Peru. The period of recruitment was during June 2015, previously two Community Assembly were realized to explain the aim and the logistic of this study.

### Participants

Those invited to participate were all persons who were at least two years old. Only persons who signed the written consent form and in the case of children and adolescents who were authorized by their parents and witnesses were recruited.

### Variables

Two main outcomes were established, HBV antigens and antibodies seroprevalence and active immunization coverage. The serological status was defined according to the CDC criteria into five categories (Table 1).<sup>11</sup> We also include the category "Early acute HBV infection" established following the World Health Organization (WHO) criteria: positive

for HBsAg and negative for IgM anti-HBc and hepatitis B surface antibody (anti-HBs).<sup>12</sup>

**Table 1** – Definitions of HBV serological status.

	Hepatitis B surface Antigen (HBsAg)	Total hepatitis B core Antibody (anti-HBc)	Hepatitis B surface Antibody (anti-HBs)	IgM antibody to hepatitis B core antigen (IgM anti-HBc)
Susceptible <sup>1</sup>	×	×	×	–
Immune due to natural infection <sup>1</sup>	×	✓	✓	–
Immune due to vaccination <sup>1</sup>	×	×	✓	–
Chronically infected <sup>1</sup>	✓	✓	×	×
Unclear interpretation <sup>1</sup>	×	✓	×	–
Early acute HBV infection <sup>2</sup>	✓	×	×	–

<sup>1</sup> Based on Centers of Diseases Control criteria.

<sup>2</sup> Based on World Health Organization criteria.

The analysed factors were sex, age divided into three groups (2-11 years old, 12-19 years old, and 20 years old or more) according to the scholar and working stage, temporary migration defined as 7-30 days of absence from the community due to work, health, or family reasons. Also, we asked if a physician had ever communicated the diagnosis of hepatitis B to a participant (“Hepatitis B at any time”) and if they were ever immunized against the virus (“Having received the vaccine against HBV”). The surveys were conducted in Spanish and with a native translator. We consider as a confounding factor the unknowledge of the number of vaccine doses that the participants may have received or if any serology had been done prior to vaccination. To address potential source of bias, we invited the whole population of the communities to participate.

### Study Size

215 participants were included in this study. The sampling was a convenience sample. The participants, or their legal representatives, after signed an informed consent form, and the research team obtained prior approval from the indigenous leaders, the questionnaire was administered, and blood samples were collected.

### Data Sources and Measurement

An *in-situ* laboratory was assembled for blood extraction and serum separation. The serum samples were separated in aliquots, at least one per each test, to further avoid repeated thawing and refreezing, and stored in CO<sub>2</sub> refrigerated containers. Samples were then transported to the central laboratory located in Quito, 600 km away from the *in-situ* lab, in which the Enzyme-Linked ImmunoSorbent Assay (ELISA) were conducted.

Serum samples were screened in the Biomedical Research Laboratory at Universidad Internacional del Ecuador for HBsAg, anti-HBs, and IgM anti-HBc, using a commercial enzyme-linked immunosorbent assay kit (Human Gesellschaft für Biochemica und Diagnostica mbH®) following the manufacturer's instructions. For anti-HBc, a competitive enzyme immunoassay kit (Diagnostic Bioprobes Srl®) was used. Outcomes were informed to participants through a new visit to the communities.

### Statistical Analysis

The serological status was analysed by sex, age, and migration status with Likelihood Ratio test (LR). The same analysis was conducted for HBsAg alone. A p-value less than 0.05 was considered significant. The 95% Confidence Interval was used to estimate the prevalence of immune status due to hepatitis B vaccination as well as HBsAg. Data were

analysed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

The study comprised 215 participants, of whom 94 (43.7%) were between 2 and 11 years old, 39 (18.1%) were from 12 to 19 years old, and 82 (38.1%) were 20 years old or more. The male population represented 41.9% (90). 71 (34.1%) participants answered affirmatively to having migrated, of whom 32 (45.0%) were men and 39 (55.0%) women.

“Hepatitis B at any time” was declared by 8 (3.7%), five of whom were males. From the 8 participants, 7 belonged to the age group 20 years old or more, and the remaining participant diagnosed with hepatitis belonged to the group of 2 to 11 years old.

“Having received the vaccine against HBV” was declared by 58 people (27.0%). Among those who claimed vaccination, the female-male ratio was 1.15. Thirty (51.7%) were from ages 2-11; eleven (19.0%)

were from ages 12-19; and seventeen (29.3%) participants claiming vaccination were 20 or more years old.

Moreover, 116 (54.0%) participants were susceptible, and 19 (8.8%) participants were immune due to natural infection. The immune status due to hepatitis B vaccination was found in 35 (16.3%; 95% CI 11.6-21.4), but only 12 (34.3%) reported having received the vaccine. Among 58 participants who declared that they had been vaccinated, 20.7% were positive for immunity due to vaccination.

Unclear interpretation was found in 38 (17.7%) participants. Chronically infected were 4 (1.9%) and early acute HBV infection 3 (1.4%). From the 8 people who declared having ever been diagnosed with HBV, none had a chronic, acute, or immune/natural infection, one was positive for immunity due to the vaccine, two were susceptible and the rest had unclear interpretation. The distribution of the serological status by the studied factors is shown in table 2.

**Table 2** – Distribution of HBV serological status by the studied factors.

	Susceptible (n= 116)	Immune/ Natural Infection (n= 19)	Chronically Infected (n= 4)	Early Acute (n= 3)	Unclear Interpretatio n (n= 38)	Immune due to vaccination (n= 35)	Likelihood ratio (p-value)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
<b>Sex</b>							9.7 (0.08)
Male	55 (61.1)	4 (4.4)	1 (1.1)	1 (1.1)	11 (12.2)	18 (20.0)	
Female	61 (48.8)	15 (12.0)	3 (2.4)	2 (1.6)	27 (21.6)	17 (13.6)	
<b>Age</b>							87.8 (0.04)
2–11	64 (68.1)	2 (2.1)	1 (1.1)	3 (3.2)	2 (2.1)	22 (23.4)	
12–19	29 (74.4)	2 (5.1)	1 (2.6)	0	2 (5.1)	5 (12.8)	
≥ 20	23 (28.0)	15 (18.3)	2 (2.4)	0	34 (41.5)	8 (9.8)	
<b>Temporary migration</b>							7.5 (0.19)
No	80 (58.4)	9 (6.6)	1 (0.7)	1 (0.7)	25 (18.2)	21 (15.3)	
Yes	32 (45.1)	8 (11.3)	3 (4.2)	2 (2.8)	12 (16.9)	14 (19.7)	
<b>Received an HBV Vaccination</b>							7.05 (0.72)

Yes	25 (43.1)	6 (10.3)	1 (1.7)	1 (1.7)	13 (22.4)	12 (20.7)
No	73 (57.5)	11 (8.7)	3 (2.4)	2 (1.6)	21 (16.5)	17 (13.4)
Unknown	18 (60.0)	2 (6.7)	0	0	4 (13.3)	6 (20.0)

The HBsAg was positive in seven (3.3%; 95% IC 0.9-7.0). In women the prevalence was 4.0% (5/125) and in men 2.2% (2/90), LR 0.55, p-value 0.46. In the three age groups, 2.9% (1/34), 4.0% (4/99), and 2.4% (2/82) were found respectively, LR 0.39, p-value 0.83. In those who migrated it was prevalent in 7.0% (5/71), LR 4.20, p-value 0.04.

## DISCUSSION

Reports like the one presented in Colombia and Brazil exist, but as far as we know, this is the first study on hepatitis B seroprevalence carried out in indigenous people in the Ecuadorian Amazon. In our study we found that 1.9% were serologically compatible with chronic HBV infection and is consistent with the CDC estimation for low-intermediate chronic HBV infection prevalence countries.<sup>7</sup>

Latin American countries started universal hepatitis B vaccination during the mid-1990s. Nevertheless, there are still knowledge gaps in relation to the quality of the vaccination process, its coverage, and its effectiveness with birth dose scheme and three doses scheme in high endemicity areas as the Amazon region.<sup>8,10</sup>

The Ecuadorian government provides the HepB vaccine three doses short scheme (at the time of birth, second and third doses 2 months afterward), the registered coverage in 2017 was 57.8% for the birth dose.<sup>7</sup> In our study, the vaccinated matched results show that it is doubtful that immunization have reached this population. “Susceptible” status was present in 54.0% of the studied sample while the Pan American Health Organization indicates that vaccination against hepatitis B should reach coverage levels of 95.0%.

The fact that in the age group of 2 to 11 years one of four shows immune response due to vaccination (23.4%) is of concern because this is the group that should have already benefited from immunization campaigns. Likewise, the age group of 12 to 19 years shows the highest serological proportion of “Susceptibility” cases, 74.4%.

The worldwide seroprevalence of HBsAg is 3.61%.<sup>13</sup> The total HBsAg prevalence found in our study was 7% (95% CI 1.0-6.0). Other studies conducted in the Amazon region are inconsistent with each other regarding HBsAg prevalence due to its wide ranges, between less than 1% and more than 8%.<sup>14-15</sup>

In our study, 2.4% of participants from 2 to 11 years old were carriers of HBsAg. In the Colombian Amazon region, Garcia *et al.* reported in 2018 that less than 1% of children were found to be HBsAg carriers compared to 2% found in a study conducted in 1999 under similar settings, 8 years after vaccine introduction in that country.<sup>16</sup> The WHO Global Hepatitis Report (2017) points it out, the first five years of life constitute a critical time for the prevention of HBV infection and its consequences.<sup>1</sup>

The regional situation of hepatitis B is complex, and the results must be interpreted depending on factors such as whether the studies occur in urban vs. rural settings as well as the volume of the studied sample.<sup>17</sup>

## LIMITATIONS

The diminished male participation, in some way, reduces the representativeness of the sample. 2. A confounding factor could be that participants do not know the number of doses of hepatitis B vaccination and whether they had any serology prior to this vaccination. 3. This study does not include the

quantification of HBV infection associated or disease development/progression risk factors, as others such as F. De La Hoz *et al.*<sup>18</sup> or Garcia *et al.*<sup>16</sup> included in their studies.

## CONCLUSION

Hepatitis B seroprevalence in the Ecuadorian Amazonian indigenous people showed that who should have benefited from the vaccination are precisely those with the lowest immune response due to vaccination. The serological status “Susceptible” was present in one of two participants. Hepatitis B surveillance in the Ecuadorian Amazon needs to be strengthened.

## RESUMO

**Introdução:** A vigilância da hepatite é crucial. **Objetivo:** Propusemos a descrição da soroprevalência da infecção por hepatite B e dos fatores associados entre indígenas da Amazônia Equatoriana. **Delineamento:** Fez-se um estudo sorológico transversal em indivíduos ao longo de dois anos. Classificou-se a soropositividade de acordo com os critérios do Centros de Controle de Doenças e registraram-se sexo, idade, migração temporária, se contraiu hepatite B alguma vez na vida e vacinação prévia. **Resultados:** De 215 participantes, 50,0% eram susceptíveis (2-11 anos 68,1%), 8,8% imunes devido à infecção natural, 1,9% infecção crônica e 1,4% infecção aguda precoce pelo VHB. Oito pessoas relataram já ter sido diagnosticadas com hepatite B, nenhuma delas demonstrou infecção aguda crônica, e uma era positiva para imunidade devido à vacina. O HBsAg foi encontrado em 3,3% (95% IC 0,9-7,0) e era maior entre aqueles que migraram 7,7% (valor-p 0,04). 16,3% (95% IC 11,6-21,4) eram imunes devido à vacinação contra o VHB. Entre os 58 participantes que declararam ter sido vacinados, 20,7% apresentaram imunidade pós-vacina. **Implicações:** Participantes que deveriam ser beneficiados pela vacina são crianças. É obrigatória a avaliação oportunista da carga viral da hepatite B em indígenas da Amazônia e a orientação de medidas preventivas.

## DESCRITORES

Anticorpos Anti-Hepatite B; Anticorpos Anti-Hepatite B; Saúde de Populações Indígenas; Epidemiologia.

## RESUMEN

**Introducción:** La vigilancia de la hepatitis es crucial. **Objetivo:** Nos propusimos describir la seroprevalencia de infección por hepatitis B y factores asociados entre los pueblos indígenas de la Amazonía ecuatoriana. **Diseño:** Se realizó un estudio serológico transversal en individuos durante dos años. La seropositividad se clasificó según los criterios de los Centros para el Control de Enfermedades y se registró sexo, edad, migración temporal, haber contraído hepatitis B en algún momento de la vida y vacunación previa. **Resultados:** De 215 participantes, 50,0% eran susceptibles (2-11 años 68,1%), 8,8% inmunes por infección natural, 1,9% infección crónica y 1,4% infección aguda temprana por VHB. Ocho personas reportaron haber sido diagnosticadas ya con hepatitis B, ninguna de ellas demostró infección crónica aguda y una resultó positiva para inmunidad por la vacuna. HBsAg se encontró en 3,3% (95% CI 0,9-7,0) y fue mayor entre los que migraron 7,7% (p-valor 0,04). El 16,3 % (IC 95 % 11,6-21,4) eran inmunes debido a la vacunación contra el VHB. Entre los 58 participantes que declararon haber sido vacunados, el 20,7% tenía inmunidad posvacunal. **Implicaciones:** Los participantes que deberían beneficiarse de la vacuna son los niños. Es obligatoria la evaluación oportunista de la carga viral de la hepatitis B en pueblos indígenas de la Amazonía y orientación sobre medidas preventivas.

## DESCRIPTORES

Anticuerpos contra a Hepatite B; Anticuerpos contra la Hepatitis B; Salud de Poblaciones Indígenas; Epidemiología.

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## COLLABORATIONS

NRS: Substantial contributions to work conception or outline; to data collection, analysis and interpretation; manuscript's writing. AF: Substantial contributions to data analysis and interpretation; manuscript's writing, critical review, and formatting of the manuscript. LA: Substantial contributions to work conception or outline; to data collection, laboratory analysis and interpretation. NMB: Substantial contributions to data collection, laboratory analysis and interpretation. CM: Substantial contributions to work conception or outline; data interpretation. RD: Substantial contributions to data interpretation; to writing the article; and to a critical review in the final version to be published. MM: Substantial contributions to work outline; to data collection and analysis; to writing the article. All the authors agree and take responsibility for the content of this manuscript version to be published.

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## AVAILABILITY OF DATA

The data that support the findings of this study are available from the corresponding author, NRS, upon reasonable request.

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## CONFLICTS OF INTEREST

There are no conflicts of interest to declare.