

Identification of IgG antibodies against arenavirus, hepatitis E and HTLV in a municipality in the northeast of Colombia

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Abstract

Migration and urbanization processes contribute to the dispersion of pathogens, especially those with possible epizootic cycles, gaining importance in new populated areas. This panorama gets worst in developing countries like Colombia that still has limited capacity in strong epidemiological surveillance for noncommon microbial agents.

Aim: This study evaluated the presence of IgG antibodies against Mammarenavirus from Tacaribe complex, Hepatitis E virus and HTLV type I or II in patients who visited the health center of a Montelibano (Cordoba), a rural-urban municipality of Colombia.

Methods: ELISA's test carried out on 182 patients from Montelibano, Colombia in order to detect IgG antibodies. The sero-epidemiological study for IgG detection was conducted using Junin virus antigen, a HEV and an indirect HTLV-I/II Enzyme-Linked Immunosorbent Assay, respectively.

Results: Serological analysis allowed the detection of IgG antibodies for all the agents evaluated. Seroprevalence of IgG was 1.64% for the Mammarenavirus, 0,61% for the Hepatitis E and 0.61% for HTLV type 1 or 2.

Conclusions: The results indicate the circulation of Mammarenavirus, Hepatitis E and HTLV in Montelibano, a municipality in the northeast of Colombia. Current seropositive percentage is similar or lower in comparison to other studies nevertheless epidemiological conditions like the presence of rodents at home and poor garbage collection services underline the necessity of better surveillance of these neglected viruses of which sporadic outbreaks have been reported.

Introduction

Timely diagnosis of infectious diseases with complex clinical conditions confers an epidemiological advantage that prevents the advance, spread, and subsequent outbreak of infectious agents. Migration and urbanization processes contribute to the dispersion of pathogens, especially those with possible epizootic cycles, gaining importance in the global context.

However, the epidemiological surveillance infrastructure in developing countries like Colombia still has limited capacity, in consequence the presentation of some infections is not or misdiagnosed. Additionally, this problem is aggravated by the fact that many people live in small towns with agricultural and rainforest areas mixed with urban areas, accompanying by insufficient hospitals services, garbage collection systems, and aqueduct-sewerage, increasing the risk of being infected with this type of agents, and not be diagnosed.

Among the neglected viral agents that could be affecting the health in the Colombian towns, we can find the *Mammarenaviruses* genus. In this group of viruses there are etiological agents of hemorrhagic fevers in the American continent, such as the Junin virus in Argentina, Machupo and Chapare in Bolivia, Guranito in Venezuela, among others [1] are included. These viruses are mainly transmitted from rodents to humans through the inhalation of their excretory product. In Colombia, no cases of Mammarenavirus fever have been reported due the absence of appropriated diagnostic infrastructure and its symptoms could be confused with those produce by other endemic

agents as dengue, malaria, among others. However, previous studies have shown the circulation of Mammarenavirus in both rodents and humans [2–5]. Additionally, the high diversity of rodent species found in Colombia and the results of previous study that identified seropositive *Zygodontomys brevicauda* reservoirs of Guarinito virus, indicates the potential presentation of human cases in the territory [4,6].

Hepatitis E virus belongs to the genus *Orthohepevirus* (family *Hepeviridae*). This is enteric transmitted and is the most common cause of acute viral hepatitis in the world [7]. This virus causes a self-limited and generally asymptomatic infection that usually has an acute course, but in immunocompromised patients chronic disease could be seen [8]. Nowadays, the report of cases presentation in Colombia is not mandatory, it is not even very common for patients to be diagnosed, therefore, there is little knowledge about its epidemiology in the country. A recently published study with a bank of sera diagnosed with Hepatitis A, found that 25% of the patients were positive for IgG and

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5.6% for IgM for hepatitis E [9]. The circulation of this virus was also evidenced in waters source and pigs from the Antioquia department [10,11].

Although in Colombia it is mandatory to screen the blood donor for antibodies against Human immunodeficiency virus (HIV), hepatitis B surface antigen and human T-cell lymphotropic virus (HTLV) type I or II among others, the prevalence and epidemiology of the latter agent is poorly understood. Nevertheless, the importance of these viruses in human health is underrated, currently, there are approximately 10 million of infected people in the world [12]. The infection of HTLV type I and II is commonly asymptomatic, their transmission is mainly through sexual intercourse, vertically or by the use of intravenous drugs [12]. The individuals infected with HTLV-type 1 can develop lymphoproliferation called adult T-cell leukaemia/lymphoma (ATLL) and progressive chronic myelopathy called tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP / HAM). On the other hand, the HTLV type II has not been associated with any specific disease, but it has been described those previous infections produced by this agent predispose the host to undergo bacterial infections [13]. In Colombia there is limited information about HTLV epidemiology, however studies such as that of Cardona et al. [14] reported a seroprevalence of 0.1% in urban blood donors. In accordance with this panorama, this study evaluated the presence of IgG antibodies against Mammarenavirus from Tacaribe complex, hepatitis E virus and HTLV type I or II in patients who visited the health center of a Montelibano (Cordoba), a rural-urban municipality of northeast Colombia.

Materials and methods

Study design

This is a transversal study performed with patients that look for medical care in hospital "E.S.E Hospital Municipal de Montelibano" (Cordoba, Colombia). Montelibano is a town located the Northwest of Colombia into the Cordoba stated with average temperature of 32°C and relative humidity is between 78% and 81%, and average altitude of 44 m.a.m.s.l. It corresponds to a tropical forest area in which a good track of land dedicated to pig farming and livestock.

Sampling

Montelibano has 83.181 inhabitants, in according with that the sample size were calculated having in account a hypothetical seroprevalence of 3% for the three viruses and the precision of 3%, according to previous reports in other places of the country [5,14,15]. The calculated sample size was 129, however, 182 individuals were sampling between September 30 and October 2 of 2019. For each patient, 5 ml of blood was collected in tubes without anticoagulant. The tubes were centrifuge a 3000 r.p.m for 10 minutes and store in -at 20°C then the serum was separated into screw cap tubes. Finally, samples were transported in cold chain to "Laboratorio de Diagnóstico Molecular y Bioinformática" (Universidad de los Andes, Bogotá, Colombia) and stored at freezing temperature until processing for the detection of antibodies.

Antibody detection

IgG arenavirus antibodies were detected for all samples using the Junín virus ELISA tests donated by Instituto Nacional de Enfermedades Virales Humanas (INEVH) "Dr. Julio I. Maiztegui" (Pergamino, Argentina) [16]. The procedure briefly consisted in half of a 96-well plate which was coated with Junín antigens (infected-Vero cells lysate) and the other half with a negative antigen (uninfected-Vero cells lysate).

Then patient sera were prepared in a 1:100 dilution. Antibody detection was using anti human IgG conjugated with peroxidase (abm, British Columbia, Canada) and the substrate was TMB (KPL, Gaithersburg, United States). Three negative and one positive control were included in each plate. Samples with optical density (OD) higher than 0.2 at a dilution 1:400 were considered positive for both ELISA tests.

Only 164 patient samples were evaluated for IgG HEV and HTLV type I or II antibodies, since 18 patient samples were discarded by hemolyzed according to the recommendation of both commercial kits HEV IgG (DIA.PRO, Milano, Italy) and CelQuest HTLV-I/II (ATGen Diagnostica, Montevideo, Uruguay) respectively. The HEV IgG consist in an indirect ELISA with recombinant antigens for a conservative and immunodominant peptide for all the 4 subtypes. The CelQuest HTLV-I/II is an indirect ELISA too, with the recombinant antigen's gp 46-I, gp 46-II, gp 21-I, which has the capacity to detect antibodies against HTLV type I and II but not distinguish between them. For both commercial ELISAs, the samples were evaluated in a dilution 1/20. All the positive and the 10% of the negative samples were proceeded by duplicated to verify the results.

Data analysis

All the analysis were performed in RStudio Version 1.2.1335. The qualitative variables were described as percentage, they were evaluated whether fit the normal distribution using Cramer-von Mises normality test, the variables with normal distribution were described as median and standard deviations. The qualitative variables were expressed by absolute and relative frequencies. The confidence interval for the seropositive percentage were calculated with confidence level of 0.95.

Ethical consideration

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983. This research was approved by the ethic committee of the Universidad de los Andes (Bogota, Colombia), according to the resolutions 008430 of 1993 and 2378 of 2008. Each patient signed the informed consent and filled a survey about the epidemiological variables.

Results

The study population comprised 182 patients. 88.4% of them (161) belong to the urban area and 21(11.5%) to rural area. The demographic characteristic of the sample population was described in Table 1. It is noteworthy, that the lower percentage of people that have drinking water and garbage relocation services in the rural area, 42.86% and 33.3% respectively, emphasizing, the potential risks of the rural inhabitants to get infected with viruses presents in animals or contaminated water. The most common occupations are homemaker and farmers, 60.43% of the people have a least one animal at home and the more common animals inside homes are dogs, cats, or chickens. It is underlined that 2.74% of the people declared they have poultry at home (Table 1).

Five patients were positive for IgG Mammarenavirus antibodies in the title 1/100, however only three patients were also positive in the titer 1/400. According to criteria for considered a positive sample one with an optical density greater than 0.2 in a 1/400 dilution [16], the seropositive percentage was 1.64 CI (0.34-4.74). In this case the three seropositive patients were women with 30, 34 and 57 years old, and the antibodies title were 1/400, 1/1600, and 1/400, respectively. Two

Table 1. Description of the sample population

Variables	Sample population (n=182)
Age (mean ±SD)	41.25 ± 16.45
Number (Relative Frequency)	
Female	129 (70.87)
Race (mestizo/afrodescendant)	133 (70.08)/49 (26.92)
Intradomiciliary rodents	104(57.14)
Perydomiciliary rodents	102(56.04)
Drinking water	145 (79.67)
Sewer-aqueduct	145 (79.67)
Garbage collection system	159 (87.36)
Presence animals at home	
Dogs	73 (40.10)
Cats	56 (30.80)
Chickens	41(22.43)
Poultry	5(2.74)
Occupation	
Homemaker	63 (34.80)
Farmer	12 (6.62)
Hospital work staff	10 (5.49)
Others	97 (53.29)
Seropositivity	
Mammarenavirus IgG (positive/negative)	3/182(1.64)
Hepatitis E IgG	1/164 (0,61)
HTLV type 1 or 2	1/164 (0.61)
Hemolyzed sample	18 (9.89)
SD: standard deviation eighteen hemolyzed samples were discarded for the analysis	

of them were mestiza and the other one afro-descendant. One does not declare presence of rodent intra and peri-domiciliary but the other two declare the presence of rodent in both places, and two of them had garbage recollection service; their professions were homemaker, farmer and professional in human resources.

Only one patient was positive for IgG against hepatitis E virus and other for HTLV type I or II being the seropositive percentage 0,61 CI (0,15-3,35) in both cases, no patients sample were in the gray zone for both tests. The seropositive patient for hepatitis E was a mestizo 54-year-old man with drinking water service and garbage recollection service. His occupation was a politician, and he does not have animals in their home, neither presence of intra and peri-domiciliary rodents. The seropositive patients for HTLV Type I or II were a mestizo 40-year-old woman, she declared the absence the rodents intra and peri domiciliary and has also drinking water and garbage recollection service. Her occupation was a homemaker and does not have animals at home. Due to the characteristic of the test antigen was not able to distinguish between HTLV type I and II. The final titer for these patients was 1/20.

Statistic comparison to determine likely risk factors associated to these infections were not able to calculate due the low seropositive percentage obtained for all the viruses.

Discussion

In this study was found IgG seropositive individuals for the three agents evaluated (Mammarenavirus, hepatitis E and HTLV), indicating the circulation of these viruses in this geographical area and the exposure population. The situation suggests the necessity of better attention by the health personnel in the region for these agents. Since, it is possible that there are cases of disease due to these viruses that are not being diagnosed and consequently neglected.

The seroprevalence found for Mammarenavirus of the Tacaribe complex (1,64%) was comparable to that found in other studies carried out in human population in the country. Percentages between 0.5% and 3.1% had been described in indigenous populations and inhabitants of rural areas [3,5], indicating the circulation of these viruses between people who live in areas far from cities and who have contact with wild rodents such as those belonging to the *Cricetidae* family and the *Sigmodontine* subfamily. It should be clarified that although the seroprevalence values are low, they are similar to those found in other regions of the continent where important outbreaks of disease have occurred in humans [17].

Nonetheless, it is possible that the seropositivity found in this study for arenaviruses is underestimated due to two factors, the first is that the sampling was carried out for convenience, in individuals who attended the health center for any reason, favoring the selection of a larger population of inhabitants from urban areas 88.4%, in contrast to the total population of Montelibando where 78.9% live in urban areas [18]. The second factor is that the IgG antibodies detection were performed using the ELISA standardized by (INEVH, Perganimo, Argentina) with Junín virus antigen whose reservoir is *Calomys musculinus*, which circulates in southern Argentina [19]. Although this test is widely used in serological studies in South America, it is possible that this virus is very antigenically different from those that are circulating in the Colombian territory with respect to the Junin virus, a situation similar to that presented with the orthohantaviruses, when evaluating seropositivity in Colombia population with geographically distant virus antigens [20].

A previous report evaluated sera from patients from different regions of the country with a diagnosis of viral hepatitis, was found that 31.2% of the patients were positive for IgG and that 11.5% had antibodies IgM against the hepatitis E virus, additionally co-infections with other viral hepatitis were reported, suggesting that possibly the infections by this virus are more prevalent than what is currently considered [9]. In our study, a seropositivity for hepatitis E virus of 0.61% was found. Although it was lower than reported, it should be taken into consideration that the population we sampled was not people with specific symptoms, it was made up of volunteers who attended the medical center for any reason. However, it should be highlighted that finding seropositive individuals suggests the importance of having hepatitis E as a differential diagnosis when viral hepatitis is suspected, and even more in regions where there is a high proportion of the population without drinking water service. Another previous study evaluated the presence of hepatitis E genome in drinking water plants and in two municipalities of the state of Antioquia, finding a positivity of 23.3% of the samples [10].

In the case of HTLV, seropositive individuals were also found, unfortunately the nature of the ELISA used did not allow us to identify whether the infection was by HTLV-1 or 2. Although in Colombia in blood banks testing for the HTLV-virus of blood units is mandatory, the HTLV infection is considered neglected and underdiagnosed, with little information on its epidemiology available in Colombia [12]. This study found a seropositivity of 0.61% a little lower than that observed in the Quintana et al. [21] whose found in the department of Córdoba in 2004 a seropositivity percentage of 2.1% for HTLV-1 and 0.1% for HTLV-2. However, more than 15 years have passed between the two studies, which may suggest that the habits of the population are changing, and consequently a decrease the value. Unfortunately, the people in our study were not surveyed about risk factors associated with their sexual and reproductive health.

Although the seroprevalences found in this study were for the three agents evaluated (Mammarenavirus, hepatitis E and HTLV), it was expected to find seropositive individuals in all cases, due to the characteristics of the population, which presents a high vulnerability for the transmission of different infectious diseases, especially those with zoonotic nature or associated to polluted waters, due to the large number of people who have contact with a diversity of domestic and wild animals and do not have access to drinking water. In the case of infection by the HTLV-1 or 2 viruses, although its transmission is not zoonotic, it is also a neglected virus and a little forgotten in the Colombian panorama, therefore, few people are diagnosed and are aware of the risk of transmission, and even more so if these people live outside the big cities.

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