

## Arbovirus Antibody Seroprevalence in the Human Population from Cauca, Colombia

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**Abstract.** Several arboviruses have emerged or reemerged into the New World during the past several decades, causing outbreaks of significant proportion. In particular, the outbreaks of Dengue virus (DENV), Zika virus, and Chikungunya virus (CHIKV) have been explosive and unpredictable, and have led to significant adverse health effects. These viruses are considered the leading cause of acute undifferentiated febrile illnesses in Colombia. However, Venezuelan equine encephalitis virus (VEEV) is endemic in Colombia, and arboviruses such as the Mayaro virus (MAYV) and the Oropouche virus (OROV) cause febrile illnesses in neighboring countries. Yet, evidence of human exposure to MAYV and OROV in Colombia is scarce. In this study, we conducted a serosurvey study in healthy individuals from the Cauca Department in Colombia. We assessed the seroprevalence of antibodies against multiple arboviruses, including DENV serotype 2, CHIKV, VEEV, MAYV, and OROV. Based on serological analyses, we found that the overall seroprevalence for DENV serotype 2 was 30%, 1% for MAYV, 2.6% for CHIKV, 4.4% for VEEV, and 2% for OROV. This study provides evidence about the circulation of MAYV and OROV in Colombia, and suggests that they—along with VEEV and CHIKV—might be responsible for cases of acute undifferentiated febrile illnesses that remain undiagnosed in the region. The study results also highlight the need to strengthen surveillance programs to identify outbreaks caused by these and other vector-borne pathogens.

### INTRODUCTION

Arthropod-borne viruses (arboviruses) continue to be significant causes of mild to severe illness around the world. Among the arboviruses, Dengue virus (DENV), which belongs to the family *Flaviviridae*, is the most prevalent arbovirus affecting humans in the tropics.<sup>1</sup> However, as a result of the similarity of signs and symptoms with many other tropical infectious diseases, and the lack of affordable and available laboratory diagnostics in many locations, DENV has become an “umbrella” under which many other arboviral diseases remain hidden. Surveillance studies for febrile illness have shown that typically only about one third of presumptive dengue cases are caused by DENV, whereas the remainder is caused by a variety of other arboviral etiologies and other infectious agents.<sup>2,3</sup> Examples include emerging and reemerging arboviruses such as Zika virus (ZIKV; *Flaviviridae* family) and Chikungunya virus (CHIKV; *Togaviridae* family), which have caused explosive outbreaks resulting in a substantial economic impact and public health burden in recent years. There are several factors that are believed to be responsible for the emergence of these arboviruses, including deforestation, urbanization, climate change that causes the expansion of mosquito populations, lack of effective mosquito control strategies, globalization of air transport, and overall poor socioeconomic conditions in low-income countries.<sup>4,5</sup>

As a result of the extensive outbreaks caused by DENV,<sup>6,7</sup> ZIKV,<sup>8</sup> and CHIKV,<sup>9</sup> most acute undifferentiated febrile illnesses (AUFIs) in tropical regions are now being reported as

caused by these viruses.<sup>3</sup> Nevertheless, other arboviruses of significant importance in these areas include Venezuelan equine encephalitis virus (VEEV),<sup>10</sup> Mayaro virus (MAYV),<sup>11,12</sup> both from the *Togaviridae* family; and Oropouche virus (OROV), which belongs to the family *Peribunyaviridae*.<sup>13</sup> In the case of VEEV, it is estimated to cause tens of thousands of cases of febrile illness annually throughout Latin America from enzootic spillovers as well as during periodic equine-amplified outbreaks that can spread across continents.<sup>2,14,15</sup> Some of the most massive VEEV outbreaks on record occurred in Colombia in 1967 and 1968, when more than 200,000 human cases and more than 100,000 equine deaths were recorded.<sup>16</sup> Another arbovirus of significant importance is MAYV, which is considered the second most important alphavirus responsible for febrile illness in certain areas of South America.<sup>17,18</sup> A recent report<sup>17</sup> has shown that 54% of patients infected with MAYV develop persistent, debilitating arthralgia affecting the major joints, very similar to what is seen with CHIKV infection. Because surveillance activities in tropical regions are mainly based on symptoms, MAYV cases are likely misdiagnosed as CHIKV infections as a result of their similarities. It is also possible that the geographic range of MAYV circulation extends beyond the few countries that have reported cases. Last, OROV causes a significant portion of AUFIs in Latin America.<sup>2</sup> OROV is capable of infecting hundreds of thousands of people.<sup>2,19</sup> The segmented nature of its genome has allowed the emergence of new viruses capable of causing human illness, even in individuals infected previously with OROV.<sup>20,21</sup>

Colombia is a subtropical country in South America known for its biodiversity. It is also an endemic country for the transmission of arboviruses such as DENV, ZIKV, and CHIKV.<sup>22</sup> As in most low- and middle-income countries, only a handful of probable dengue, nonmalaria cases are confirmed by laboratory testing.<sup>23</sup> A past study<sup>24</sup> conducted in Colombia found that only 38% of the possible dengue, nonmalaria

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cases were confirmed as dengue by laboratory testing, whereas the agents responsible for 62% of the cases remained unknown. These findings highlight the need to intensify efforts to identify the pathogens responsible for AUFIs and to enhance the diagnostic capacity of researchers and health professionals working in the area.

In this study, we present the results of a population-based survey of neutralizing antibodies against MAYV, CHIKV, OROV, VEEV, and DENV serotype 2 (DENV-2) to understand more completely the exposure to these viruses in residents from the Cauca Department, Colombia. The main objective was to determine the circulation of these arboviruses in the human population from Colombia, and to identify their seroprevalence and eco-epidemiological risk factors associated with previous viral infections.

## MATERIALS AND METHODS

**Study site.** The study site was the Cauca Department, located in the southwestern region of Colombia, with ~1.24 million inhabitants in 2018.<sup>25</sup> It is located near the Pacific Ocean at an elevation of 1,738 m. The annual average temperature is ~19°C, with irregular periods of precipitation, and relative humidity that ranges between 80% and 85%. There were four municipalities chosen for this study: Caloto, with 17,642 habitants; Santander de Quilichao, with 96,500 habitants; El Tambo, with 53,769 habitants; and La Sierra, with 10,643 habitants (Figure 1). The sites were selected based on the information given by the Cauca Health Secretary regarding problems with arthropod vectors and accessibility to the armed conflict-affected area. Most people in those locations

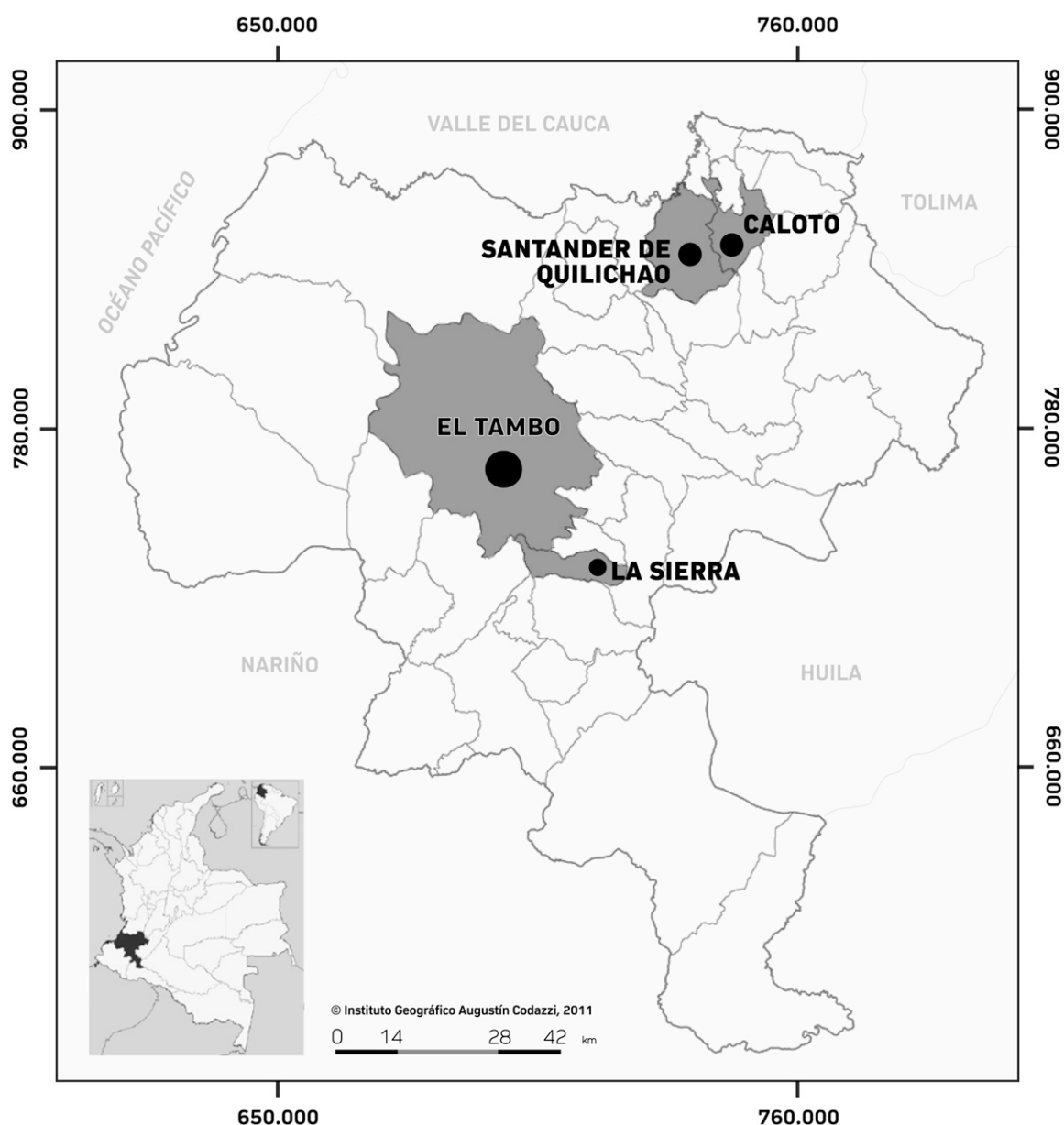


FIGURE 1. Location map of the Cauca department in which the municipalities included in the study are visible. Modified from the Instituto Geográfico Agustín Codazzi ([www.igac.gov.co](http://www.igac.gov.co)) and Wikimedia commons (Colombia departamentos [https://commons.wikimedia.org/wiki/File:Colombia\\_departamentos\\_otros.svg](https://commons.wikimedia.org/wiki/File:Colombia_departamentos_otros.svg)).

live in poverty and work primarily in agriculture and livestock production, forestry, mineral extraction, fishing, and trade.<sup>25</sup>

**Study population.** The study subjects were older than 18 years who lived in the chosen municipalities and agreed to participate in the study. Minors were not included in the study because of institutional review board regulations. Demographic and clinical information was obtained from each individual at the time of voluntary enrollment, along with signed consent. Demographic data included information about gender, age, occupation, housing material, period of residence in the area, episodes of fever recorded during the past year, and previous history of being diagnosed with DENV, ZIKV, CHIKV, or malaria. Blood samples were collected and transported to the Pontificia Universidad Javeriana (Bogota, Colombia). Serum samples were then aliquoted and stored at  $-20^{\circ}\text{C}$  before being transported to The Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, for further processing.

**Plaque reduction neutralization testing.** Plaque reduction neutralization testing (PRNT) was conducted as described previously.<sup>26-28</sup> Briefly, Vero cells were cultured in Minimal Essential Medium Eagle (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and maintained at  $37^{\circ}\text{C}$  under 5% carbon dioxide. The human serum samples collected previously were diluted in culture media and heat-inactivated at  $56^{\circ}\text{C}$  for 30 minutes to be tested for the detection of antibodies against the following viruses: MAYV (strain FPI00179), CHIKV (vaccine strain 181/clone 25), VEEV (vaccine strain

TC-83), OROV (FSE 0812), and DENV-2 (strain New Guinea C). All virus work was performed in a biosafety level 2 laboratory except for OROV, which was handled in a high-containment laboratory (biosafety level 3 laboratory). All samples were screened initially at 1:20 dilution, and positive samples were titrated further (except for DENV-2). The highest serum dilution reducing plaque numbers by 80% was determined.

**Data analysis.** Descriptive statistics counts and percentages are presented in Tables 1 through 4. Along with descriptive statistics we looked at running a generalized linear model for the binomial family model adjusted by municipality to find possible risk factors for neutralizing antibodies against MAYV, CHIKV, VEEV, and OROV considering the hypothesis that seroprevalence correlates with clinical and sociodemographic factors (e.g., gender, age, occupation) (Table 1). Because of the small number of individuals with neutralizing antibodies against MAYV, CHIKV, VEEV, and OROV, we combined the results, regardless of the pathogen evaluated, into one dependent variable. Our first outcome was having an infection with at least one virus, and we evaluated possible risk factors with crude and adjusted prevalence ratios (PRs) from all questions surveyed. We used a stepwise forward variable selection technique to find significant risk factors according to the researchers' criteria. Considering the exploratory nature of this study, we set the stopping rule to be a  $P$  value  $< 0.1$  to enter the model and a  $P$  value  $> 0.15$  to leave the model, and an alpha value of 0.05 for a 95% level of confidence. This analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC).

TABLE 1  
Demographic information of participants based on municipality

Demographic characteristic	Municipality				No. of participants (N = 505)
	El Tambo (n = 263)	Caloto (n = 50)	La Sierra (n = 40)	Santander de Quilichao (n = 152)	
Age group, years					
18-29	39	16	6	30	91 (18%)
30-64	147	32	30	100	309 (61%)
>65	77	2	4	22	105 (21%)
Gender					
Male	86	16	17	39	158 (31%)
Female	177	34	23	113	347 (69%)
Occupation					
Agricultural activities	74	21	30	35	160 (31.7%)
Homemakers	147	14	4	80	245 (48.5%)
Elementary activities	19	9	4	26	58 (11.5%)
Service workers and stores and market sellers	6	0	2	2	10 (2.0%)
Scientific and intellectual professionals	4	5	0	2	11 (2.2%)
Unemployed	13	1	0	7	21 (4.2%)
Wall household material					
Brick or block concrete	203	15	2	122	342 (68%)
Handcrafted (Bahereque)	42	29	38	21	130 (26%)
Plant materials (Guadua)	18	6	0	9	33 (6%)
Access to public services					
Water	260	48	40	151	499 (99%)
Electricity	261	49	40	150	500 (99%)
Additional information					
Report previous exposure to ZIKV, CHIKV, DENV, or malaria	17	3	3	24	47 (9.3%)
Do not report previous exposure to ZIKV, CHIKV, DENV, or malaria	243	46	37	126	452 (89.5%)
Does not know of previous exposure to ZIKV, CHIKV, DENV, or malaria	3	1	0	2	6 (1.2%)
Animals on property	239	47	34	133	453 (89.7%)

CHIKV = Chikungunya virus; DENV = Dengue virus; ZIKV = Zika virus.

TABLE 2  
Overall prevalence against MAYV, CHIKV, VEEV, OROV, and DENV-2 in Cauca by municipalities

Municipality	MAYV, n/N (%)	CHIKV, n/N (%)	VEEV, n/N (%)	OROV, n/N (%)	DENV-2, n/N (%)
Santander de Quilichao	1/152 (0.7)	7/152 (4.6)	12/152 (7.9)	1/152 (0.7)	87/150 (58)
El Tambo	3/263 (1.1)	3/263 (1.1)	5/263 (1.9)	8/263 (3)	45/262 (17.2)
La Sierra	1/40 (2.5)	1/40 (2.5)	4/40 (10)	1/40 (2.5)	10/39 (25.6)
Caloto	0/50 (0)	2/50 (4)	1/50 (2)	0/50 (0)	8/50 (16)
Total	5/505 (1)	13/505 (2.6)	22/505 (4.4)	10/505 (2)	150/501 (29.9)

CHIKV = Chikungunya virus; DENV-2 = Dengue virus serotype 2; MAYV = Mayaro virus; n = number of seropositive patients; N = total number of participants in each municipality; OROV = Oropouche virus; VEEV = Venezuelan equine encephalitis virus.

Prevalence was established based on an initial screening at a 1:20 dilution followed by an end point titration (except for DENV). The n indicates the number of seropositive participants, and N refers to the total number of participants in each municipality.

## RESULTS

**Study population.** Demographics of the study population are summarized in Table 1. A total of 505 participants were enrolled in this study and most of them resided in El Tambo ( $n = 263$ , 52.1%), followed by Santander de Quilichao ( $n = 152$ , 30.1%), Caloto ( $n = 50$ , 9.9%), and La Sierra ( $n = 40$ , 7.9%). The mean age of the participants was 48 years. There was a difference in gender distribution among the participants, with females represented more proportionally ( $n = 347$ , 69%) than males ( $n = 158$ , 31%). The principal occupations among the study population were homemaker ( $n = 245$ , 48.5%), and agricultural laborer ( $n = 160$ , 31.7%). The majority of the participants had access to water ( $n = 499$ , 99%) and electricity ( $n = 500$ , 99%), and lived with animals in their household ( $n = 453$ , 89.7%), including bovines ( $n = 52$ , 10.3%), horses ( $n = 79$ , 15.6%), dogs ( $n = 388$ , 76.8%), cats ( $n = 218$ , 43.2%), poultry ( $n = 155$ , 30.7%), wild birds ( $n = 4$ , 0.8%), pigs or sheep ( $n = 14$ , 2.8%), and rodents ( $n = 8$ , 1.6%). We also acquired information about past diagnoses of vector-borne diseases. Most individuals ( $n = 452$ , 89.5%) did not report a previous exposure to DENV, ZIKV, CHIKV, or malaria; only 9.3% ( $n = 47$ ) reported being diagnosed previously with DENV, ZIKV, CHIKV, or malaria. Only 1.2% ( $n = 6$ ) did not know about past exposure to these pathogens (Table 1).

**Alphavirus neutralizing antibody prevalence.** A total of 505 human sera samples were tested for the presence of VEEV, CHIKV, and MAYV neutralizing antibodies. Laboratory analysis showed an overall prevalence for these alphaviruses as follows: VEEV, 4.4% (22 of 505; 95% CI, 2.8–6.4); CHIKV, 2.6% (13 of 505; 95% CI, 1.4–4.2); and MAYV, 1% (5 of 505; 95% CI, 0.4–2.2) (Table 2). Antibody titers against VEEV and CHIKV ranged between 40 and  $\geq 640$ , and from 20 to  $\geq 640$  for MAYV (Table 3).

The VEEV PR across municipalities was 7.9% (12 of 152) in Santander de Quilichao, 1.9% (5 of 263) in El Tambo, 10% (4 of 40) in La Sierra, and 2% (1 of 50) in Caloto. The CHIKV PR was 4.6% (7 of 152) in Santander de Quilichao, 1.1% (3 of 263) in El Tambo, 2.5% (1 of 40) in La Sierra, and 4% (2 of 50) in Caloto. Last, MAYV seroprevalence was 0.7% (1 of 152) in Santander de Quilichao, 1.1% (3 of 263) in El Tambo and 2.5% (1 of 40) in La Sierra. Table 2 summarizes the alphavirus antibody seroprevalence in each municipality.

**OROV neutralizing antibody prevalence.** The overall OROV seroprevalence among the study population was 2% (10 of 505; 95% CI, 1.0–3.5; Table 2). End point neutralizing antibody titers for OROV ranged between 20 and  $\geq 640$  (Table 3). The OROV PR across municipalities was 0.7% (1 of 152) in Santander de Quilichao, 3% (8 of 263) in El Tambo, and 2.5% (1 of 40) in La Sierra (Table 2).

**DENV-2 neutralizing antibody prevalence.** Given the relatively low seroprevalence for MAYV, VEEV, CHIKV, and OROV, we proceeded to investigate the seroprevalence of DENV, which is the most prevalent arbovirus affecting humans in the tropics.<sup>1</sup> For the DENV seroprevalence studies, we selected DENV-2 for the analysis because this dengue serotype has been circulating in Colombia since at least the 1960s, and the virus is isolated yearly in Colombia from patients with febrile illness, representing the major DENV serotype in circulation in Colombia. The overall DENV-2 seroprevalence in the study population was 30% (150 of 501; 95% CI, 26–34). Santander de Quilichao had the highest seroprevalence, with 58% of the study population presenting neutralizing antibodies against DENV-2 (Table 2).

**Risk factors analysis.** The bivariate analyses for arbovirus seropositivity, excluding DENV-2, suggest an association with gender, age, bovines on the property, rats or mice near the property, outdoor occupations, not showering daily, infrequent handwashing, time of residence in the municipality, having a fever in the past year, and previous diagnoses of DENV, CHIKV, ZIKV, or malaria. In the multivariate analysis with a binomial model adjusted by municipality, seropositivity to MAYV, CHIKV, VEEV, and OROV was associated with gender, age, and presence of bovines and rats or mice in the property. Specifically, men (PR, 1.68) and participants older than 47 years (PR, 2.28) were at a greater risk of infection with MAYV, CHIKV, VEEV, and OROV. The presence of bovines (PR, 1.88) and rats or mice near the property (PR, 2.37), and the absence of canines in the property (PR, 1.63) were also associated significantly with MAYV, CHIKV, VEEV, and OROV seropositivity. Table 4 summarizes the results of the risk factor analyses.

## DISCUSSION

Colombia is one of the countries in South America with the highest level of AUFIs in the region, where the incidence of dengue-like illnesses has increased dramatically in recent years. In 2019, there were ~124,989 probable dengue cases reported to Colombia's National Health Institute, and 50.8% ( $n = 63,497$ ) presented with warning signs requiring hospitalization.<sup>29</sup> Because of limitations in diagnostic capacity, only a few cases were confirmed by laboratory testing as being caused by DENV, ZIKV, or CHIKV, and therefore most of the cases remain undiagnosed or diagnosed erroneously as "dengue." Thus, efforts to identify agents responsible for causing AUFIs are needed to develop proper public health control strategies.

In Colombia, no comprehensive studies on the circulation of arboviruses, other than DENV, ZIKV, and CHIKV, have

TABLE 3  
End point titers for neutralizing antibodies against MAYV, CHIKV, VEEV, and OROV

Sample	Report previous exposure to ZIKV, CHIKV, DENV, or malaria?	Neutralizing antibody titers (PRNT80)				PRNT positive
		MAYV	CHIKV	VEEV	OROV	
H017	No	–	–	> 640	–	VEEV
H019	No	320	> 640	–	–	MAYV, CHIKV
H025	No	–	–	40	–	VEEV
H027	No	–	–	> 640	–	VEEV
H032	No	–	–	> 640	–	VEEV
H038	No	–	–	–	> 640	OROV
H045	No	–	–	–	20	OROV
H048	No	–	–	–	40	OROV
H058	No	–	–	–	20	OROV
H074	No	–	–	–	20	OROV
H079	No	320	> 640	–	–	MAYV, CHIKV
H082	No	20	–	–	–	MAYV
H094	No	–	–	–	20	OROV
H107	No	–	–	320	–	VEEV
H127	No	–	–	> 640	–	VEEV
H131	CHIKV	40	–	–	–	MAYV
H132	No	–	–	> 640	–	VEEV
H192	No	–	40	–	160	CHIKV, OROV
H202	No	–	–	–	20	OROV
H245	Malaria	–	–	160	–	VEEV
H246	Malaria	–	–	160	> 640	VEEV, OROV
H272	CHIKV	–	> 640	–	–	CHIKV
H305	No	–	–	> 640	–	VEEV
H317	No	–	80	–	–	CHIKV
H319	DENV	–	> 640	–	–	CHIKV
H329	No	–	–	> 640	–	VEEV
H333	No	–	–	40	–	VEEV
H334	No	–	–	–	20	OROV
H347	No	–	–	> 640	–	VEEV
H363	No	–	–	80	–	VEEV
H384	No	–	–	> 640	–	VEEV
H387	CHIKV	–	80	–	–	CHIKV
H403	No	–	–	> 640	–	VEEV
H408	CHIKV	> 640	80	–	–	MAYV, CHIKV
H412	No	–	–	> 640	–	VEEV
H419	No	–	> 640	–	–	CHIKV
H425	DENV	–	–	640	–	VEEV
H429	Malaria	–	–	80	–	VEEV
H435	No	–	–	320	–	VEEV
H436	No	–	–	640	–	VEEV
H441	CHIKV	–	640	–	–	CHIKV
H450	CHIKV	–	640	–	–	CHIKV
H470	CHIKV	–	640	–	–	CHIKV
H474	DENV	–	–	640	–	VEEV
H486	CHIKV	–	640	–	–	CHIKV

CHIKV = Chikungunya virus; DENV = Dengue virus; MAYV = Mayaro virus; OROV = Oropouche virus; PRNT = plaque reduction neutralization test; PRNT80 = plaque reduction neutralization test 80; VEEV = Venezuelan equine encephalitis virus; ZIKV = Zika virus.

Responses regarding past exposure to ZIKV, CHIKV, DENV, or malaria are included as a reference.

been carried out. Therefore, the objective of our study was to determine the level of exposure to other arboviruses among the adult population living in the Cauca Department. This region was selected because it has a vast forest habitat in a subtropical climate, with a set of conditions that make this place ideal for the circulation of vector-borne pathogens.

In our study, 505 sera samples collected in 2017 from El Tambo, Santander de Quilichao, Caloto, and La Sierra were tested for the presence of neutralizing antibodies against VEEV, MAYV, CHIKV, OROV, and DENV-2. The overall seroprevalence for DENV-2 was 30%, which confirmed the relatively high exposure to DENV in the study population. We selected DENV-2 for the analyses because this serotype has been circulating in Colombia since the 1960s and cases are reported continuously on a yearly basis in this country.<sup>30</sup>

However, we acknowledge that because we only conducted PRNT using a DENV-2 strain, it is possible that the overall DENV seroprevalence is greater than 30%. In addition, given the substantial high level of PRNT cross-reactivity among the DENV serotypes and other flaviviruses, we also acknowledge that detailed serological analyses are needed to assess properly the DENV and flavivirus seroprevalence in the study population. However, we believe those investigations fall outside the scope of our study because our focus was VEEV, MAYV, CHIKV, and OROV.

In general, the results of our study show an overall low seroprevalence for VEEV, MAYV, OROV, and CHIKV. Nevertheless, our study confirmed human exposure to VEEV and CHIKV in the study population and, more importantly, it provides evidence of the circulation of as well as human exposure to MAYV and OROV in Cauca, Colombia. Our study

TABLE 4  
Description of the variables by outcome of MAYV, CHIKV, OROV, and VEEV, including the crude and adjusted PR from the multivariate analysis adjusted for municipality (N = 505)

Variable	n (%)	Crude PR (95% CI)	P value	Model 1		Null model
				Adjusted PR (95% CI)	P value	
Gender						
Female	24 (4.75)	Reference		Reference		–
Male	21 (4.16)	1.92 (1.27–2.90)	0.002	1.68 (1.08–2.61)	0.022	
Age, years						
18–46	13 (2.59)	Reference		Reference		
≥ 47	32 (6.37)	2.42 (1.70–3.46)	< 0.001	2.28 (1.65–3.14)	< 0.001	
Dogs on the property						
Yes	30 (5.94)	Reference		Reference		
No	15 (2.97)	1.66 (1.40–1.95)	< 0.001	1.63 (1.38–1.92)	< 0.001	
Bovines on the property						
No	37 (7.33)	Reference		Reference		
Yes	8 (1.58)	1.88 (1.05–3.36)	0.033	1.88 (1.25–2.82)	0.002	
Rats or mice on the property						
No	9 (1.78)	Reference		Reference		
Yes	36 (7.13)	2.21 (1.43–3.44)	< 0.001	2.37 (1.59–3.53)	< 0.001	
Occupation						
Indoors	22 (4.36)	Reference		–		
Outdoors	23 (4.55)	1.27 (0.96–1.69)	0.098			
Years in residence						
< 5	8 (1.58)	Reference				
≥ 5	37 (7.33)	1.44 (1.04–2.00)	0.03			
Frequency of bathing						
Daily	35 (6.93)	Reference				
Not daily	10 (1.98)	2.54 (1.55–4.18)	< 0.001			
Frequency of handwashing						
Often (> 4 times a day)	34 (6.73)	Reference				
Occasionally (< 3 times a day)	11 (2.18)	1.60 (1.51–1.69)	< 0.001			
Presence of fever in the past year						
No	27 (5.35)	Reference				
Yes	18 (3.56)	1.4 (0.97–2.0)	0.069			
Previous diagnosis of DENV, CHIKV, ZIKV, or malaria						
No	31 (6.14)	Reference				
Yes	14 (2.77)	4.3 (1.96–9.6)	< 0.001			
–	–	–	–	BIC*	297.74	BIC 309.7
–	–	–	–	AIC*	285.09	AIC 305.48

AIC = Akaike information criterion; BIC = Bayesian information criterion; CHIKV = Chikungunya virus; MAYV = Mayaro virus; OROV = Oropouche virus; PR = prevalence ratio; VEEV = Venezuelan equine encephalitis virus.

\* The smaller BIC of AIC value indicates the better model.

also identified risk factors associated with MAYV, CHIKV, VEEV, and OROV infection. Specifically, the multivariate model identified gender as a risk factor, with men as having a greater risk of infection than females, possibly because of their agricultural and livestock activities. Another risk factor is age, with individuals 47 years or older having significantly greater odds of infection compared with those younger than 47 years. The presence of bovines and rodents near homes is also a risk factor of infection, and may be related to occupational exposure and ecological conditions favoring arbovirus transmission. Overall, our findings are consistent with the results of prior studies conducted in other endemic areas in South America.<sup>20,31,32</sup> In Colombia, DENV, CHIKV, and VEEV are of mandatory notification, whereas MAYV and OROV are not considered as part of the routine diagnosis. Cases of CHIKV were first recognized after the introduction of CHIKV in South America in 2013, with ~106,592 clinical cases reported by Colombia's National Health Institute and only 1.3% cases confirmed by laboratory testing.<sup>33</sup> Interestingly, in our study, although the 13 individuals seropositive for CHIKV reported previous episodes of fever, only eight indicated having a previous diagnosis of DENV, ZIKV, or

CHIKV infection. The data indicate that cases of CHIKV are underreported in Colombia (Table 3).

In Colombia, VEEV is mainly considered a pathogen of veterinary importance, even though it is the second alphavirus responsible for AUFIs in South America.<sup>2</sup> Although VEEV is of mandatory notification, VEEV is mostly overlooked and is only considered as a potential cause of AUFIs when equine encephalitis cases are reported to the health authorities. For instance, in 2016, 86 clusters of equine encephalitis were recognized in the departments of Magdalena, Cauca, Meta, Santander, Casanare, and Cesar, among others. That same year, the NIH received a report of 20 possible VEEV cases in Santander, Cordoba, Cesar, and Antioquia, but no possible cases were reported from Cauca.<sup>34</sup> In our study, we found an overall VEEV seroprevalence of 4.4% (22 of 505), providing evidence of human exposure to VEEV in Cauca. Notably, 17 individuals did not report previous infection with DENV, ZIKV, CHIKV, or malaria whereas five indicated past infection with DENV or malaria. The data confirm VEEV is underreported in Colombia. Given the importance of VEEV as a veterinary and human pathogen, studies are needed to determine whether VEEV is a significant cause of AUFIs in

this area and to elucidate completely the public health impact of VEEV in Cauca and other endemic regions in Colombia.

Data concerning MAYV in Colombia was initially reported by Groot et al.<sup>35</sup> in 1959, when MAYV and other viruses were isolated from field-collected mosquitoes in San Vicente de Chucurí. Another study conducted in the population from this area found an overall MAYV positivity of 3.3% (3 of 90) based on the results of hemagglutination inhibition (HI) testing.<sup>36</sup> In another study from Colombia,<sup>37</sup> 159 sera samples obtained between 2001 and 2004 were tested for the presence of antibodies against several viruses by HI. Approximately 15.6% (10 of 64) of samples provided by the NIH were positive for MAYV.<sup>36,37</sup> Unfortunately, the high cross-reactivity among alphaviruses by HI testing made it difficult to confirm human exposure to MAYV in Colombia based on those studies. By using the gold standard confirmatory serological assay, our data provide confirmatory evidence about human exposure to MAYV in Colombia and highlight the need to intensify efforts to determine whether MAYV is a significant cause of AUFIs in Colombia. A previous study<sup>38</sup> suggested that individuals exposed to alphaviruses developed cross-reactive neutralizing antibody responses. Nevertheless, in our study we found individuals with specific neutralizing antibodies for CHIKV ( $n = 10$ ) or MAYV ( $n = 2$ ) as well as individuals with neutralizing antibodies against both pathogens ( $n = 3$ , Table 3). In addition, a recent study<sup>39</sup> conducted with samples collected from MAYV-infected individuals in Peru before the arrival of the CHIKV to the Americas did not find evidence supporting the development of cross-reactive neutralizing antibody responses between CHIKV and MAYV. Thus, PRNT continues to be the gold standard test to distinguish between human exposure to CHIKV or MAYV.<sup>40</sup> The evidence supports our conclusions about the circulation and human exposure to MAYV in Colombia.

Oropouche fever is a disease of public health concern in South America, and several outbreaks have occurred in Brazil, Panama, Ecuador, Peru, Trinidad, and Tobago since the 1960s. The circulation of OROV in Colombia has been poorly studied, although antibodies in humans were detected in a previous study,<sup>13</sup> and OROV was isolated from mosquitoes by Groot et al.<sup>35</sup> and from a patient with AUF1.<sup>41</sup> Our findings confirm that human exposure to OROV occurs in Colombia, and studies are needed to determine more fully the extent of OROV activity in this country.

One of the major limitations of this study is the relatively small sample size; however, the number of samples acquired are very valuable because of the challenges of having access to the municipalities in the Cauca Department, an armed-conflict area. In addition, our study population was not balanced with respect to gender, because females were more present at home during sampling and were more inclined to participate in the research. Thus, it is likely that the prevalence for these viruses might be greater than that reported in our study.

Nevertheless, our study provides evidence about the circulation of MAYV and OROV in this region, and confirms human exposure to these pathogens. Our study highlights the need to consider these pathogens as causes of AUFIs in Colombia and to determine further their impact on public health in Colombia. Altogether, our study highlights the need to strengthen surveillance to improve the diagnosis of AUFIs

in the region and to perform programs of vector control for the prevention of future arbovirus outbreaks.

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