

Serologic Evidence of Zoonotic Alphaviruses in Humans from an Indigenous Community in the Peruvian Amazon

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Abstract. Alphaviruses (Togaviridae, *Alphavirus*) are arthropod-borne single-stranded RNA pathogens that cause febrile and neurologic disease in much of Latin America. However, many features of Alphavirus epidemiology remain unknown. In 2011, we undertook a cross-sectional study in Nueva Esperanza, an indigenous community in the Peruvian Amazon. Here, we present the first serologic evidence of Mayaro (MAYV), Venezuelan equine encephalitis (VEE) complex alphavirus, Una (UNAV), and Madariaga (MADV) viruses reported in humans (24%, 16%, 13%, and 1.5%, respectively) from an Amazonian indigenous community in Peru. Hunting activity and cohabiting with hunters were the main risk factors for Mayaro seroconversion, but only hunting was associated with UNAV seropositivity. Our results suggest that alphavirus infection in this region is common, but we highlight the high UNAV seroprevalence found and corroborate the low MADV prevalence reported in this region. Furthermore, MAYV-neutralizing antibodies were also detected in stored samples from wild animals (18%) hunted by Nueva Esperanza inhabitants and another mestizo community located close to Iquitos. Further serological surveys of VEE complex alphaviruses, UNAV, and MADV in wild animals and assessing the ability of the MAYV seropositive species to transmit the virus will be relevant.

INTRODUCTION

Arthropod-borne zoonotic alphaviruses (*Togaviridae*, *Alphavirus*) are positive-sense single-strand RNA viruses that are recognized as an important cause of acute febrile illness in humans in Latin America. In the Peruvian Amazon, Mayaro virus (MAYV) accounts for nearly 1% of outpatient evaluations for febrile disease,¹ and cases have been reported in Latin American countries as well as in travelers returning from the Amazon Basin.^{2,3} Venezuelan equine encephalitis (VEE) complex alphaviruses, such as VEE virus (VEEV), have caused outbreak within the urban areas of Iquitos, the largest city in the Peruvian Amazon, but few neurological cases and deaths have been observed.^{4,5} Less is known about Madariaga virus (MADV) infection in this region. Despite the enzootic circulation and intensive surveillance, only few seropositive individuals and no neurologic disease or fatal cases have been reported.⁶ However, encephalitis cases and mild or asymptomatic cases mostly affecting children have been attributed to MADV recently in Venezuela, Haiti, and Panama.^{7–10} Finally, although Una virus (UNAV) has been isolated from mosquitos of the Peruvian Amazon, it has been rarely associated with human disease.^{11,12} Therefore, its distribution and the human disease risk in this region remain unknown.

Although these alphaviruses are a well-recognized cause of human disease in the Americas, prior research studies were only carried out in the peri-urban area of Iquitos. An important reason behind this is the challenge of conducting research in Amazonian communities because of different barriers (e.g., geographical, sociocultural, and health service access).¹³ The lack of surveillance studies in indigenous communities and the identification of potential wildlife hosts in the Amazon highlight the importance of

studying this region. Furthermore, the continuous ecological disturbance in the Amazon rainforest through human activities such as hunting, logging, and ecotourism may be significant risk factors for infection with these viruses.^{4,14} Therefore, we sought to investigate the epidemiology of these viruses through a cross-sectional serosurvey, involving humans and wildlife, in an indigenous community of the Peruvian Amazon.

MATERIALS AND METHODS

Human and animal survey. This study was conducted in Nueva Esperanza (S 04°19.53 W 71°57.33), 302 km northeast from Iquitos (Figure 1). This is an indigenous community of 297 inhabitants, whose main occupations are small-scale agriculture, fishing, logging, and subsistence hunting. In 2011, we collected human serum samples from 70 volunteers (ranged from age 5 to 81 years) of this community. Between 2007 and 2008, we also collected blood samples from 90 wild animals harvested by local hunters from Nueva Esperanza and Sol Naciente (S 03°38.26 W 73°12.57), a mestizo community located 26 km from Iquitos where agricultural land use has partially replaced the forest.

Laboratory testing. Human samples were first tested by plaque reduction neutralization tests (PRNT) against MAYV (strain TRVL15537), MADV (chimeric SIN/MADV derived from Brazilian MADV strain BeAn436087), and VEE complex alphavirus (vaccine strain TC-83).^{15,16} All samples with PRNT80 titers ≥ 20 were considered positive. To complement PRNT results and as a secondary goal because of the low serum volume available, the leftover sample was tested in triplicate for IgG antibodies to antigens of MAYV, MADV, and VEE complex viruses. We used a capture ELISA.¹⁷ For the ELISA, the TRVL15537 strain was used for MAYV and sucrose-acetone antigens were prepared from MADV- (prepared by Dr. Robert Shope at the Yale Arbovirus Research Unit in August 1989), and VEE complex alphaviruses (vaccine strain TC-83 and Mosso das Pedras 78V3531) infected mouse

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FIGURE 1. Nueva Esperanza and Sol Naciente communities in the Department of Loreto, Peru. This figure appears in color at www.ajtmh.org.

brain (provided by the US CDC and University of Texas Medical Branch (UTMB) World Reference Center for Emerging Viruses and Arboviruses, respectively). To discriminate MAYV positives from cross-reactivity with UNAV, the leftover from all the samples was first screened in 1:20 dilution of the UNAV strain BT-1495-3 and then tested by PRNT80. Chikungunya virus was not tested because these samples were collected in 2011, before chikungunya virus circulated in the Americas.

Wild animal samples were tested only by PRNT against MAYV (strain TRVL15537). Plaque reduction neutralization tests for VEE complex alphaviruses, MADV, and UNAV could not be performed because of insufficient samples.

Statistical analysis. We used a recursive modeling approach with classification trees to identify major risk factors linked to the presence of antibodies against these alphaviruses using the package “rpart” version 4.1–13 of the statistical software R version 3.4.2.^{18,19} Risk factors evaluated for prior human infection were gender, hunting activity, logging activity, and cohabitation with seropositive people. Model simplification takes into consideration the complexity parameter of the tree showing the minimum relative error after 10-fold cross-validations.

Ethics. Research protocols were approved by the Peruvian Forestry and Wildlife Agency (041-2007-DGFFS), the Institutional Animal Care and Use Committee of U.S. Naval Medical Research Unit No. 6, and Instituto Nacional de Salud del Perú (#08-982).

RESULTS

Characteristics of the human population. A total of 70 participants (23.6% of local inhabitants) who belong to 22 houses (48.9% of total houses) were surveyed. The age range was 5–81 years, with a median age of 20 ($Q_1 = 10$ and $Q_3 = 34.5$). The proportion of females was 58.8%. A total of 18.6% of participants declared to hunt, whereas 14.3% declared to participate in logging activities. The total number of persons per house ranged between one and seven (Table 1).

Human seroprevalence. Based on our PRNT results, the seroprevalence for MAYV, VEE complex alphavirus, UNAV, and MADV in people from Nueva Esperanza was 24% (17/70, 95% CI [16–35]), 16% (11/67, 95% CI [9–27]), 13% (6/48, 95% CI [6–25]), and 1.5% (1/67, 95% CI [0–8]), respectively (Supplemental Table 1). For MAYV, the most parsimonious tree model explained 27% of likelihood of showing antibodies against MAYV, including two risk factors: hunting activity and cohabitation with seropositive people (Figure 2). This means that being a hunter (75%, 95% CI [42.81–94.51]) or not being a hunter but sharing home with MAYV seropositive people (21%, 95% CI [9.5–37.3]) were the main risk factor associated with MAYV circulation. Individuals who were nonhunters and not living in a seropositive household were seronegative. The UNAV tree model also showed hunting activity as the main risk factor (80%, 95% CI [28.4–99.5]) for the virus infection, explaining 49% of the observed UNAV seropositive variability.

TABLE 1
Characteristics of PRNT-positive individuals (n = 40) and laboratory findings*

Sample ID	Gender	Age (years)	Hunter	Logger	House	Mayaro virus			Madariaga virus			Venezuelan equine encephalitis complex virus			Una virus		
						PRNT	PRNT titer	ELISA	PRNT	PRNT titer	ELISA	PRNT	PRNT titer	ELISA	Screen PRNT	PRNT	PRNT titer
MIS0963	F	21	No	No	A	–	–	–	–	–	+	+	20	+	–	–	–
MIS0965	F	35	No	No	A	+	20	+	NP	NP	NP	NP	NP	NP	NP	NP	NP
MIS0967	M	41	Yes	Yes	A	–	–	+	–	–	+	–	–	–	+	+	20
MIS0968	F	33	No	No	A	+	40	+	–	–	NP	–	–	NP	NP	NP	NP
MIS0969	M	8	No	No	A	+	40	+	–	–	NP	–	–	NP	NP	NP	NP
MIS0972	M	7	No	No	D	–	–	+	–	–	+	+	80	–	–	–	–
MIS0974	M	67	Yes	No	E	–	–	+	–	–	–	–	–	–	+	+	80
MIS0975	F	64	No	No	E	+	160	+	–	–	+	–	–	–	NP	NP	NP
MIS0977	M	29	No	Yes	F	–	–	+	–	–	NP	+	80	NP	NP	NP	NP
MIS0980	M	81	Yes	No	F	+	160	+	–	–	–	–	–	–	+	+	80
MIS0983	M	70	Yes	No	G	+	40	+	–	–	–	–	–	–	+	+	40
MIS0985	M	33	No	Yes	H	+	20	+	–	–	+	–	–	–	NP	NP	NP
MIS0986	M	30	Yes	No	H	+	40	+	NP	NP	NP	NP	NP	NP	NP	NP	NP
MIS0988	M	39	Yes	Yes	I	+	20	+	–	–	NP	–	–	NP	NP	NP	NP
MIS0990	F	32	No	No	J	–	–	–	–	–	–	–	–	–	+	+	40
MIS0991	F	26	No	No	K	–	–	+	–	–	+	+	120	–	NP	NP	NP
MIS0992	M	10	No	No	K	+	40	+	–	–	NP	–	–	NP	NP	NP	NP
MIS0994	F	20	No	No	L	–	–	+	–	–	–	–	–	–	–	–	–
MIS0995	M	45	Yes	Yes	L	+	40	+	+	40	NP	–	–	NP	NP	NP	NP
MIS1000	F	31	No	No	M	–	–	+	–	–	+	+	40	+	+	+	80
MIS1001	F	6	No	No	M	–	–	+	–	–	–	–	–	–	–	–	–
MIS1004	F	25	No	No	N	–	–	+	–	–	–	–	–	–	–	–	–
MIS1006	M	52	Yes	Yes	N	+	40	+	–	–	NP	–	–	NP	NP	NP	NP
MIS1007	M	46	Yes	Yes	N	+	20	+	–	–	NP	–	–	NP	NP	NP	NP
MIS1008	F	13	No	No	O	–	–	+	–	–	–	+	160	+	–	–	–
MIS1011	M	30	Yes	Yes	Q	–	–	+	–	–	+	–	–	–	NP	NP	NP
MIS1013	M	13	No	No	R	–	–	+	–	–	–	–	–	–	–	–	–
MIS1014	M	10	No	No	S	–	–	+	–	–	+	+	40	+	–	–	–
MIS1018	F	7	No	No	T	–	–	+	–	–	–	–	–	–	–	–	–
MIS1021	M	18	Yes	No	U	–	–	+	NP	NP	NP	NP	NP	NP	NP	NP	NP
MIS1022	M	47	No	Yes	U	+	20	+	–	–	NP	–	–	NP	NP	NP	NP
MIS1023	M	74	Yes	No	U	+	20	+	–	–	NP	–	–	NP	NP	NP	NP
MIS1024	M	67	Yes	No	Z	+	40	+	–	–	–	+	40	+	–	–	–
MIS1025	F	8	No	No	Z	–	–	+	–	–	+	+	80	+	–	–	–
MIS1026	F	20	No	No	Z	–	–	+	–	–	+	+	80	+	NP	NP	NP
MIS1028	M	20	No	No	Z	–	–	+	–	–	+	–	–	–	–	–	–
MIS1029	F	17	No	No	NA	+	20	+	–	–	NP	–	–	NP	NP	NP	NP
MIS1030	F	6	No	No	NA	–	–	+	–	–	–	–	–	–	–	–	–
MIS1031	F	12	No	No	NA	–	–	+	–	–	–	–	–	–	–	–	–
MIS1032	F	35	No	No	NA	–	–	+	–	–	+	+	40	–	NP	NP	NP

M = males; F = females; NA = data not available; NP = not performed because of insufficient sample; PRNT = plaque reduction neutralization test.

* Each letter represents a house.

People who did not report hunting had a lower UNAV seroprevalence of 4–7% (95% CI [0.6–16.2]). No other variables were associated. Una virus seropositives were also exposed to MAYV or other alphavirus, but some individuals only showed exposure to UNAV. Despite the high seroprevalence (16%) of VEE complex virus, the tree models showed that none of the studied variables were related to the positive cases. Risk factors to MADV infection were not evaluated because of concerns about the representativeness of the sample because only one sample was found seropositive.

Animal seroprevalence. Anti-MAYV-neutralizing antibodies were detected in wild animals (18%, 95% CI [11–27%]) collected near Nueva Esperanza and Sol Naciente communities. The highest MAYV seroprevalence in wild animals was observed in armadillos (40%), followed by nonhuman primates (33%), peccaries (11%), and rodents (10%) (Table 2).

DISCUSSION

Recent epidemiological and surveillance studies have shown that human alphavirus infections in Latin America are

common.^{2,16,20} Herein, we demonstrate endemic infection with new world alphaviruses in the Amazon region of Peru. In addition, we provide serologic results of potential wildlife hosts of MAYV.

Madariaga virus is the new designation for the South American isolates of eastern equine encephalitis virus.²¹ In South America, this virus has caused equine encephalitis outbreaks, but only two human cases were detected to date (in Brazil and Trinidad).⁶ The low MADV seroprevalence observed in this study corroborates a previous study in Iquitos that showed a seroprevalence of 3% (9/359).⁶ This result is in contrast to the high human seroprevalence and cases reported in Panama.⁸ Cross-protective antibodies may be one of the factors limiting the infection and disease in South America.⁶ Although the virus was isolated from *Culex* mosquitoes in areas near Iquitos, our study found only one seropositive individual, a 45-year-old male hunter and logger who also showed exposure to MAYV.¹¹ Evidence of exposure to only MADV is unusual: most seropositive individuals also show exposure to MAYV, VEEV, or UNAV.⁶

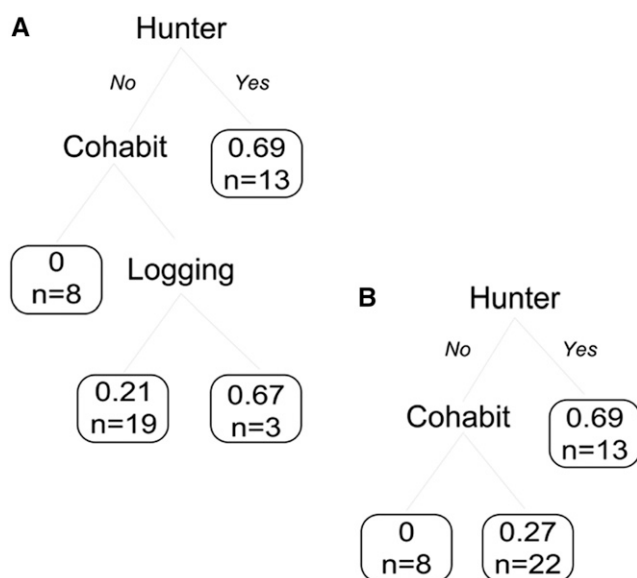


FIGURE 2. Complete (A) and final (B) classification trees exploring the influence of gender and hunting or logging behaviors on the probability of showing antibodies against Mayaro virus in the Peruvian Amazon forest. The factor “cohabit” indicates people who share home with a Mayaro seropositive person. Note that gender was initially retained but later excluded after model simplification. Left branches indicate no hunters or no cohabitation, whereas the right branches summarize the probability for male hunters or cohabiting people.

Venezuelan equine encephalitis complex viruses such as VEEV are known to cause human disease in the Peruvian Amazon. The most recent VEEV outbreak caused by the enzootic Panama/Peru ID genotype in Iquitos, in early 2006,

accounted for 63 cases and one fatality.^{4,5} In contrast, a higher fatality rate was reported in Panama (5%, two deaths from 39 cases studied). Different factors may be associated with this difference: a more active surveillance in Panama during outbreaks, underreporting of VEEV cases in Peru, or other factors involved in the emergence of the enzootic VEEV (virus adaptation to mosquito species or mosquito abundance).²² In this study, the vaccine strain TC-83, closely related to subtype ID, was used for the PRNT. Unfortunately, PRNTs were not performed using other VEE complex viruses that are geographically relevant, limiting our ability to definitively state which VEE complex virus was involved in these cases. Interestingly, the VEE complex virus titers are low, presumably because of the infection with sympatric alphaviruses inducing PRNT cross-reaction. However, antibody titers waning over time is also a possibility, as most of the VEE complex virus positives are in older age.

Mayaro infections have been principally recognized during outbreaks in South America; however, risk factors for the infection and the clinical spectrum of MAYV infections are poorly understood.^{1,23–26} The high seroprevalence (24%) found in our study corroborates previous studies in indigenous groups of the Brazilian Amazon, where MAYV seroprevalence ranged from 20% to 47%, with an overall of 42% for all the groups together.²⁷ A greater seroprevalence was also found in ethnic groups of French Guiana living in contact with the forest compared with those who do not.²⁸ Despite the growing concern of MAYV as an emerging virus that may potentially spread and cause human outbreaks in the Western Hemisphere, less is known about the virus ecology.²⁹ Most evidence comes from Brazilian studies, which proposed

TABLE 2

Mayaro virus seroprevalence in wild animals hunted by Nueva Esperanza and Sol Naciente communities in the northeastern Peruvian Amazon

Group	Common name	Scientific name	No. PRNT positive (%)*
Mammals	Edentate	Nine-banded armadillo	2/4 (50.0)
		Three-toed sloth	0/1 (0.0)
	Primate	Subtotal	2/5 (40)
		Poepig's woolly monkey	6/11 (54.5)
		Red howler monkey	1/1 (100.0)
		Red uakari	1/3 (33.3)
		Large-headed capuchin	1/6 (16.7)
		Squirrel monkey	0/3 (0.0)
		White-fronted capuchin	0/2 (0.0)
		Monk saki	0/1 (0.0)
		Subtotal	9/27 (33)
	Artiodactyla	Collared peccary	1/6 (16.7)
		Red brocket deer	0/3 (0.0)
	Rodent	Subtotal	1/9 (11)
		Wild black agouti	3/27 (11.1)
		Lowland paca	1/10 (10.0)
		Northern Amazon red squirrel	0/1 (0.0)
		Spiny rat	0/1 (0.0)
		Subtotal	4/39 (10)
	Carnivora	South American coati	0/4 (0.0)
		Subtotal	0/4 (0)
	Subtotal		16/84 (19)
Birds		Blue-and-yellow macaw	0/2 (0.0)
		Great tinamou	0/2 (0.0)
		Rufescent tiger heron	0/1 (0.0)
		Speckled chachalaca	0/1 (0.0)
Total	Subtotal		0/6 (0)
			16/90 (18)

PRNT = plaque reduction neutralization test.

* Samples were first tested with an ELISA for the presence of Mayaro virus antibodies, and in those screening positive, a confirmatory PRNT was performed.

nonhuman primates as the most important amplifying hosts.²³ The virus was isolated from a marmoset (*Callithrix* sp.) captured during an MAYV outbreak in Brazil, and experimental infections in these animals confirmed the occurrence of viremia post-MAYV infection.²³ The virus was also isolated from only one migrating bird (*Icterus spurius*) from a total of 1,300 birds studied in the United States in 1967.³⁰ Serologic studies found MAYV antibodies in other sylvatic vertebrates and birds, but their exact role in the transmission of the virus is not determined.^{31–33} This study detected anti-MAYV-neutralizing antibodies in wild animal species, which are consistent with previously described epidemiologic patterns of the sylvatic cycle, where arboreal and diurnal wild animals were more likely to be infected.³² Nevertheless, other terrestrial and nocturnal species were also seropositive in our study. Similar to our findings, previous studies indicated that nonhuman primates had high MAYV seropositive rates, presumably reflecting these animals' preference for a lower to mid-canopy habitat, where potential vectors are often present.^{23,32} We also found seropositive rodents and marsupials, possibly infected by *Haemagogus janthinomys* mosquitoes, known enzootic vector of MAYV that reside in upper and lower canopy in the Peruvian Amazon.³⁴ These mosquitoes are daytime feeders, which may explain why hunting was a risk factor. This result corroborates a previous study in the Amazon rainforest of Ecuador, where the high seroprevalence detected in the Amazon-native personnel of the Ecuadorian armed forces was associated with hunting.¹⁴ The higher MAYV risk due to cohabitation may suggest the presence of infected dwelling species, opening up the possibility for a human–mosquito–human transmission cycle. Although *H. janthinomys* is reported to have a low activity near houses in the Amazon rainforest, the lack of MAYV isolation from other mosquito species and vector competence studies are unable to confirm a human-mosquito-human transmission.^{23,35} Mayaro virus has been isolated from *Aedes* (*Ochlerotatus*) *serratus*, *Psorophora ferox*, *Psorophora albipes*, and *Culex* spp. captured indoor and outdoor in a village in the Iquitos region and at the forest area.³⁶ Evaluating the capacity of these species to transmit the virus is highly relevant. Urban-adapted species such as *Aedes aegypti* and *Aedes albopictus*, known vectors of other human arboviruses (e.g., dengue and chikungunya) in Iquitos, are proved competent MAYV vectors in laboratory conditions. However, other species, for example, *Anopheles* spp., may also be important vectors that sustain the transmission of MAYV.^{35,37}

Here, we also demonstrate the highest observed human prevalence of UNAV reported to date in Latin America. Low UNAV prevalence was previously reported in Iquitos and peri-urban and urban areas in Argentina and Paraguay where it is less likely to be in contact with the enzootic cycle.^{6,12,38} Our results show that hunting was a main risk factor for UNAV infection, and this suggests that introduction into the forest potentially increases the contact with humans and the transmission cycle. Ecological evidence suggests that *Ae.* (*Ochlerotatus*) *fulvus*, *P. ferox*, and *Psorophora albigena* might be the mosquito species involved in the enzootic circulation of UNAV, which is principally distributed in sylvatic areas of Latin American countries.¹¹

Although disease severity varies between individuals, the chronic debilitating joint pain associated with Mayaro fever may severely impair the livelihoods of MAYV-infected people,

whereas VEEV may cause fatal encephalitis.¹ Unfortunately, no medical history was available to associate the seropositivity with the presence of chronic arthralgia or neurologic disease.

Although wild animals may have contributed to the amplification of MAYV during past Amazonian outbreaks, reservoir hosts for MADV, UNAV, and VEE complex alphaviruses in the Peruvian Amazon remain unknown.²³ We also do not rule out the possibility that some MAYV seropositive animal samples could be due to a cross-reaction with UNAV; however, this hypothesis could not be tested because of insufficient samples. In humans, we found antibodies against both MAYV and UNAV in two individuals, but most MAYV seropositives were not tested for UNAV. The sample limitation did not allow us to indicate a reliable proportion of MAYV/UNAV cross-reactions. It is unclear whether the cross-neutralizing patterns shown in these two individuals are due to cross-reaction among these viruses or real independent infections. Future studies should evaluate this possibility in a prospective follow-up of patients.

The cross-sectional nature of the study design does not allow us to draw a conclusion of temporal association between the exposure to the virus and infection. However, our study was designed to explore potential factors of alphavirus infection and not to test hypothesis-driven questions. As sympatric alphaviruses circulate in the Amazon region of Peru, it is possible to observe cross-reaction among members of the genus *Alphavirus*.^{8,39} However, we used PRNT, which is the gold standard for alphavirus diagnosis and provides a highly accurate classification among members of the same antigenic complex.

In summary, we provide the first evidence of human alphavirus infection in an indigenous community from the Peruvian Amazon. Our results also suggest that sympatric human alphavirus infections are common in the Amazon region of Peru.

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