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Endemic and Epidemic Human Alphavirus Infections in Eastern Panama: An Analysis of Population-Based Cross-Sectional Surveys

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Abstract. Madariaga virus (MADV) has recently been associated with severe human disease in Panama, where the closely related Venezuelan equine encephalitis virus (VEEV) also circulates. In June 2017, a fatal MADV infection was confirmed in a community of Darien Province. We conducted a cross-sectional outbreak investigation with human and mosquito collections in July 2017, where sera were tested for alphavirus antibodies and viral RNA. In addition, by applying a catalytic, force-of-infection (FOI) statistical model to two serosurveys from Darien Province in 2012 and 2017, we investigated whether endemic or epidemic alphavirus transmission occurred historically. In 2017, MADV and VEEV IgM seroprevalences were 1.6% and 4.4%, respectively; IgG antibody prevalences were MADV: 13.2%, VEEV: 16.8%, Una virus (UNAV): 16.0%, and Mayaro virus: 1.1%. Active viral circulation was not detected. Evidence of MADV and UNAV infection was found near households, raising questions about its vectors and enzootic transmission cycles. Insomnia was associated with MADV and VEEV infections, depression symptoms were associated with MADV, and dizziness with VEEV and UNAV. Force-of-infection analyses suggest endemic alphavirus transmission historically, with recent increased human exposure to MADV and VEEV in Aruza and Mercadeo, respectively. The lack of additional neurological cases suggests that severe MADV and VEEV infections occur only rarely. Our results indicate that over the past five decades, alphavirus infections have occurred at low levels in eastern Panama, but that MADV and VEEV infections have recently increased—potentially during the past decade. Endemic infections and outbreaks of MADV and VEEV appear to differ spatially in some locations of eastern Panama.

INTRODUCTION

Alphaviruses (*Togaviridae: Alphavirus*) are important zoonotic, single-stranded RNA arthropod-borne viruses. Clinically, alphaviruses are associated with febrile, severe and sometimes fatal disease in the Americas.[1] Among the most important alphaviruses are eastern equine encephalitis virus (EEEV), Venezuelan equine encephalitis virus (VEEV), and members of the Semliki Forest antigenic complex. These viruses have caused explosive epidemics of human encephalitis and arthritogenic disease in Latin American tropical regions.[2,3]

Eastern equine encephalitis virus has recently been reclassified as two different species: EEEV in North America and Madariaga virus (MADV) in other parts of Latin America[4]—each with different predispositions to cause human disease.[5] In 2010, we reported severe neurologic diseases in humans associated with MADV infection in Panama.[6] The mechanism underlying this outbreak remains unknown, but age-specific seroprevalence data obtained during the 2010 and 2012 studies suggest recent MADV emergence in Panama.[7,8] Venezuelan equine encephalitis virus epizootic subtypes IAB and IC are associated with explosive human and equine epidemics/epizootics, which occur chiefly in South and Central America.[2] Those epizootic subtypes emerge from enzootic ID subtype ancestors because of viral adaptations for infection of equids and mosquitoes that allow it to spread rapidly among human and animal populations.[9] In Panama, enzootic subtypes ID and IE circulate in eastern-central and western Panama, respectively, where the natural cycle occurs in mosquitoes (subgenus *Melanoconion*) and sylvatic rodents.[10]

The Semliki Forest alphavirus complex includes Mayaro virus (MAYV) and Una virus (UNAV) that are mostly found in the Amazon region of Peru, Brazil, and Venezuela. Mayaro virus is characterized by fever and arthralgia, which can persist for years.[11] However, UNAV has not been associated with human disease. In the Americas, sizeable human MAYV outbreaks have most often been reported in the Amazon Basin, although recently, this virus was isolated from a febrile child in Haiti, suggesting it may be moving beyond its established territory.[12] Una virus has been detected at low levels during epidemiological studies and surveillance,[13,14] but because this virus has rarely been associated with human disease, the risk

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to people living in endemic Latin America remains unclear.[15] Both MAYV and UNAV are vectored by forest mosquitoes: *Haemagogus janthinomys* mosquitoes are the primary vectors of MAYV,[15] whereas *Psorophora ferox* and *Psorophora albipes* mosquitoes are thought to be the main vectors of UNAV.[16,17] The MAYV enzootic cycle is also known to involve nonhuman primates as amplification hosts.[15,18]

In June 2017, a fatal MADV infection was confirmed in the Mogue community in Darien, the most eastern province of Panama, prompting field investigations. Here, we use seroprevalence data collected during this survey to determine population exposure and to characterize factors associated with seroprevalence for MADV and other alphaviruses. By combining seroprevalence survey data from 2012 with that from the recent survey, we also attempted to determine whether alphaviruses emerged recently or were present historically.

MATERIALS AND METHODS

We reconstructed the epidemiological dynamics of MADV and VEEV using data from cross-sectional surveys undertaken in 2012 and 2017 in Darien Province villages (Figure 1). We also identified factors associated with alphavirus exposure, measured as IgG seroprevalence. Maps were constructed using the GPS coordinates collected during the investigation using ArcGIS package online version (Argis Solutions, Inc., Denver, CO). Land use shapes were validated by the Ministry of Environment (https://www.miambiente.gob.pa).

2012 serosurvey. The original 2012 study was conducted by the Gorgas Memorial Institute of Health Studies (GMI) to estimate prevalence and to identify risk factors for zoonotic diseases in Panama.[8] The study included five villages (Figure 1). A total of 897 participants were surveyed, but only 774 sera were available for laboratory testing. In Tamarindo, 176 participants were surveyed, 167 in Aruza, 250 in El Real, 130 in Mercadeo, and 174 in Pijibasal/Pirre 1-2. All available samples were tested to detect neutralizing antibodies against MADV and VEEV using a plaque reduction neutralization test (PRNT). Non-antibody detection against UNAV was addressed during this study. Details of this survey have been described previously.[8] Specific characteristics of the study sites are given in the Supplemental Materials.

2017 serosurvey. On June 30, 2017, a fatal human MADV case was confirmed with viral isolation in Mogue village (Figure 1). This was followed by a collaborative initiative between the Panamanian Ministry of Health and the GMI for outbreak investigation and response. From July 18 to 22, 2017, 83.3% of inhabitants (250 of 300) were surveyed, including members from all households. Each participant was interviewed using a standardized epidemiological form to record occupation, activities, livestock, and crop holdings. Other details are given in the Supplemental Materials and Figure S1. Human sera collected in 2017 were tested using alphavirus genus-specific real-time reverse transcription polymerase chain reaction (RT-PCR)[19] and by ELISAs to detect IgM and IgG antibodies against MADV and VEEV. Positive sera were then confirmed using the PRNT with the same method as in the 2012 serosurvey.[8] ELISA antigens were prepared from EEEV (prepared by Robert Shope at the Yale Arbovirus Research Unit in August 1989)- and VEE complex virus (strain 78V-3531)-infected mouse brain. For the PRNT, we used chimeric Sindbis virus (SINV)/MADV—shown to be an accurate surrogate for MADV in these assays[20]—and VEEV vaccine

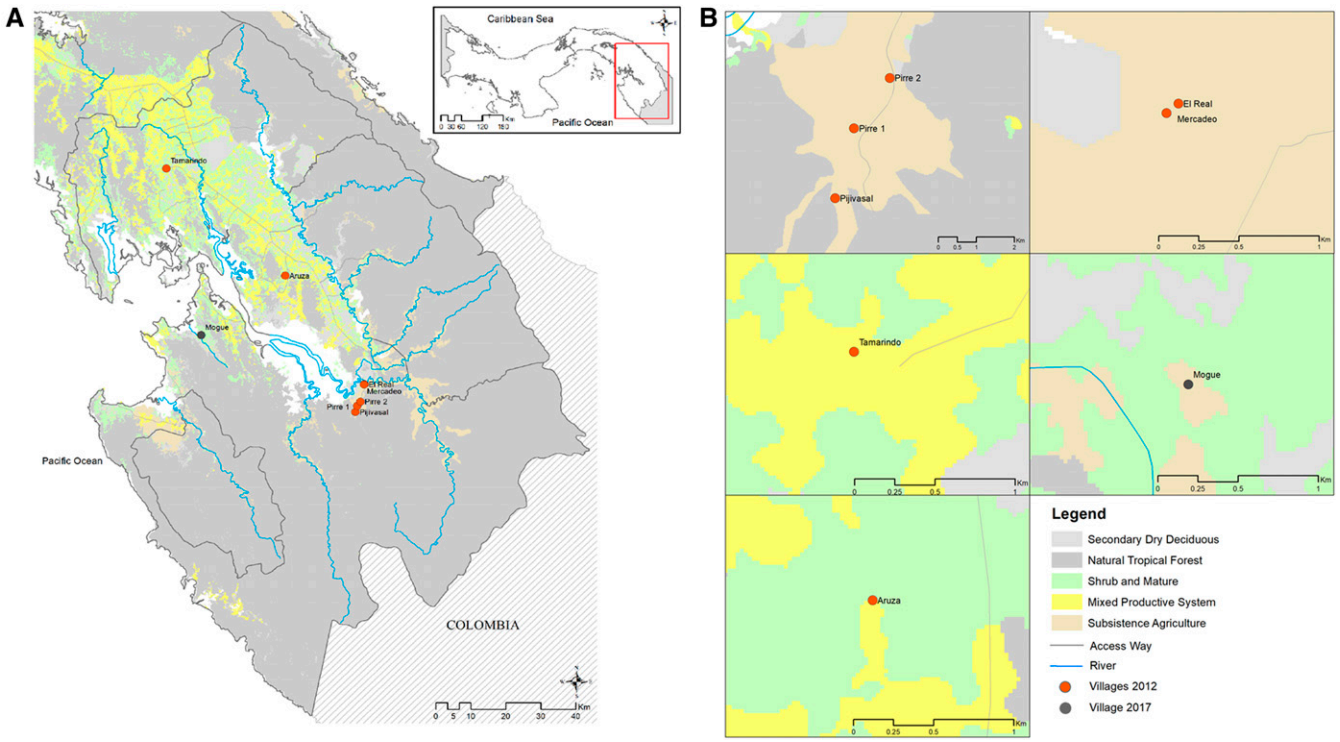

FIGURE 1. Map of the study sites in eastern Panama: (**A**) Sampling sites in the Darien Province in eastern Panama. (**B**) Zoom-in projection of sampling sites on a land-use layer. This figure appears in color at www.ajtmh.org.

strain TC83. In addition, sera were tested for MAYV, UNAV, and Chikungunya virus (CHIKV) by using the PRNT using wild-type strains (MAYV-ARV-0565, UNAV-BT-1495-3, and CHIKV-256899). "Plaque reduction neutralization test$_{80}$" was positive to more than one virus at a titer of ≥ 1:20, and there was less than a 4-fold difference in titers.

Mosquito collection and testing in 2017. Mosquitoes were collected during 2 consecutive days in Mogue from July 19 to 21 using 10 traps: five CDC light traps were baited with octanol, and five Trinidad traps were baited with laboratory mice. Traps were placed outdoors in peridomestic areas at the edge of the vegetation, from 18:00 to 06:00. Trapped mosquitoes were collected early in the morning and placed in cryovials for storage in liquid nitrogen and transportation to the GMI. Mosquitoes were maintained cold, sorted to species level using taxonomic keys,[21] and grouped into pools of 20 individuals.

Mosquito pools were homogenized in 2 mL of minimum essential medium supplemented with penicillin and streptomycin and 20% fetal bovine serum using a TissueLyser (Qiagen, Hidden, Germany). After centrifugation at 12,000 rpm for 10 minutes, 200 µL of the supernatant was inoculated in each of two 12.5-cm^2 flasks of vero cells. Samples were passaged twice for cytopathic effect confirmation. The original mosquito suspensions were used for RNA extraction and tested using alphavirus genus–specific RT-PCR.[19]

Statistical methods. *Associated symptoms and risk factors analysis.* We conducted separate analyses for MADV, VEEV, and UNAV; in each case, the outcome variable was the presence/absence of antibodies against the virus, as determined by a PRNT$_{80}$ titer ≥ 1:20. The associations between each outcome and self-reported symptoms in the last 2 weeks were tested using chi-squared and Fisher exact tests; $P < 0.05$ was considered significant. The associations between each outcome and independent variables were estimated using generalized estimating equations for logistic regression models[22] and were expressed as odds ratios (ORs). The most parsimonious model was obtained with the log likelihood ratio test variable selection.[23] Univariable and multivariable ORs were calculated with 95% CIs.

Force-of-infection (FOI) analysis. To investigate the endemicity and/or recent emergence of three alphaviruses (VEEV, MADV, and UNAV), we combined age-structured seroprevalence data from both surveys (i.e., from 2012[8] to 2017), which encompassed seven sites (Pirre 1-2, Pijibasal, Mercadeo, Tamarindo, El Real, Aruza, and Mogue) where either human or equine cases of VEEV or MADV have occasionally been reported. See Figure 1 and Supplemental Materials for a detailed description of these sites.

The historical FOI was estimated using a catalytic model,[24] where the number of seropositive individuals in each sample was modeled using a binomial distribution,

$$n(a,t) \sim B(N, P[a,t]).$$

Here, $n(a,t)$ is the number of seropositive individuals and $p(a,t)$ is the underlying seroprevalence; in both cases, a denotes age and t denotes time; N is sample size. By making assumptions about $p(a,t)$ (described in the following), we tested whether MADV, VEEV, and UNAV transmission rates have historically been constant over time ("constant FOI" model) or have varied—for example, because of recent introduction of these viruses ("time-varying FOI" model).

For a constant FOI (λ), we modeled seroprevalence for age a in year t (i.e., the time when the serosurvey occurred) as,

$$p(a,t) = 1 - \exp(-\lambda a).$$

For a time-varying FOI (λ_t), we modeled seroprevalence for age a as,

$$p(a,t) = 1 - \exp\left(-\sum_{i=t-a+1}^{t} \lambda_i\right).$$

In this framework, we assume no seroreversion (loss of antibodies over time), no age dependence in susceptibility or exposure,[25] and the mortality rate of infected individuals is the same as for susceptible individuals. The models were estimated in a Bayesian framework using Stan's no-U-turn sampler.[26,27] Details of priors and model simulations and

TABLE 1
Characteristics of the 243 study participants with complete data from the 2017 survey

Characteristic	*N* (%)
Gender	
Male	120 (49.4)
Female	123 (50.6)
Ages (years)	
2–11	80 (32.9)
12–30	82 (33.7)
≥ 31	81 (33.3)
House members*	4 (2–6)
Activities	
Main occupation	
Student	122 (50.2)
Farmer/rancher	48 (19.8)
Homemaker/occupation at home	73 (30.0)
Breeding poultry	45 (18.5)
Fishing for consumption	7 (2.9)
Contact with pastures	78 (32.1)
Contact with crops	123 (50.6)
Clearing vegetation	80 (32.9)
Working in agriculture	86 (35.4)
Working in pastures	24 (9.9)
Working in grain deposits	21 (8.6)
Working in sawmills/forest	33 (13.6)
Working in chicken coops	58 (23.9)
Working in pigsties	44 (18.1)
Washing clothes in ravines or rivers	111 (45.7)
Taking bath in natural water source	211 (86.8)
House-level features	
Total houses	59
House floor material	
Wood	55 (93.2)
Other	4 (6.8)
House with walls	29 (49.2)
House window material	
Concrete (ornamental blocks)	42 (71.2)
Wood	17 (28.8)
Roof material of house	
Tin roof	28 (47.5)
Straw thatched	31 (52.5)
Vegetation around the house	25 (42.4)
Rice cultivation around the house	4 (6.8)
Corn cultivation around the house	3 (5.1)
Waste disposal methods	
Burying	5 (8.5)
Burning	43 (72.9)
Other	11 (18.6)
Rain water	57 (96.6)

* Range.

packages used are provided in Supplemental Materials and Figures S1–S7. Median of the posterior distribution of the parameters and their corresponding 95% credible intervals (95% CrIs) are presented.

Ethics. The outbreak investigation was undertaken during a public health outbreak response, and ethical approval for use of surveillance data and cross-sectional surveys was given by the GMI Ethics Committee (IRB #0277/CBI/ICGES/15 and IRB #047/CNBI/ICGES/11). The written informed consent of participants was obtained. All identifying information of participants was removed, and confidentiality was strictly respected. The animal component of this study was approved by the GMI Committee of Care and Use of Animals (001/05 CIUCAL/ICGES, July 4, 2005) and conducted in accordance with law number 23 of January 15, 1997 (Animal Welfare Guarantee) of the Republic of Panama.

RESULTS

Characteristics of the study population. In 2017, 250 participants belonging to 59 houses were surveyed, with complete risk factor data available for only 243 individuals (97.2%). Ages ranged from 1 to 97 years, and females comprised 51% of surveyed individuals. Further characteristics of the surveyed population are given in Table 1.

In 2012, a total of 826 participants were surveyed, but only 774 sera were available for laboratory testing. The risk factors determined from this serosurvey have previously been published.[8]

Alphavirus detection and seroprevalence in 2012 and 2017. In 2012, the overall neutralizing antibody seroprevalence was 4.8% (95% CI: 3.4–6.5) for MADV and 31.6% (95% CI: 28.3–35.0) for VEEV.

In 2017, the overall neutralizing antibody seroprevalence was MADV: 13.2% (95% CI: 9.2–18.0), VEEV: 16.8% (95% CI: 12.4–22.0), UNAV: 16.0% (95% CI: 11.7–21.1), and MAYV: 1.2% (95% CI: 0.3–3.5). No evidence of CHIKV infection was found. Neutralizing antibody seroprevalence to more than one virus was observed in 3.6% (95% CI: 1.6–6.7) of participants. The proportion of subjects with both MADV and VEEV antibodies was 3.7% (df = 1; Pearson chi-square = 3.43; test for independence $P = 0.064$), both UNAV and VEEV antibodies 3.7% (df = 1; Pearson chi-square = 0.91; test for independence $P = 0.340$), and both MADV and UNAV antibodies 2.9% (df = 1; Pearson chi-square = 0.97; test for independence $P = 0.325$). Only one subject presented antibodies against these three viruses. IgM prevalence was: MADV 1.6% (95% CI: 0.4–4.2) and VEEV 4.4% (95% CI: 2.2–7.8). Concurrent MADV and VEEV IgM were observed in 0.8% of individuals (95% CI: 0.1–2.9). Viral RNA was not detected in sera.

Associated symptoms and risk factors. Exposure to MADV was significantly associated with self-reported dizziness, fatigue, depression, and difficulty cooking. Having VEEV neutralizing antibodies was associated with dizziness and insomnia (Table 2). Participants older than 11 years were more likely to test positive for UNAV antibodies, with those older than 30 years being the most likely (Tables 3 and 4). Having a house with walls reduced the risk of testing positive for UNAV antibodies (Tables 3 and 4). The most parsimonious multivariable model revealed that being older and having vegetation around the house were positively associated with MADV antibody prevalence (Table 4). Washing clothes in ravines or rivers was also positively associated with VEEV antibodies in the multivariable model (Table 4).

Enzootic vectors. In 2017, a total of 113 mosquitoes across 10 species were collected: *Culex* (*Culex*) *coronator* (36.3%), *Cx.* (*Melanoconion*) *pedroi* (14.2%), *Cx.* (*Mel.*) *spissipes* (10.6%), *Cx.* (*Cx.*) *nigripalpus* (10.6%), *Cx.* (*Mel.*) *vomerifer* (8.8%), *Cx.* (*Cx.*) *declarator* (5.3%), *Cx.* (*Mel.*) *adamesi* (2.7%), and *Cx.* (*Mel.*) *dunni* (2.7%). The overall mean number

TABLE 2
Symptoms and signs associated with UNAV, MADV, and VEEV exposure (neutralizing antibodies)

Symptom		UNAV*		MADV*		VEEV*	
	N (%)§	*n* (%)†	*P*-value‡	*n* (%)†	*P*-value‡	*n* (%)	*P*-value‡
Fatigue	85 (35.0)	14 (35.0)	0.998	15 (48.4)	0.094	19 (45.4)	0.125
Difficulty with concentration	60 (24.7)	13 (32.5)	0.210	10 (32.3)	0.296	11 (26.2)	0.804
Memory loss	58 (23.9)	12 (30.0)	0.320	11 (35.5)	0.104	13 (31.0)	0.236
Confusion	41 (16.9)	10 (25.0)	0.133	6 (19.4)	0.693	11 (26.2)	0.076
Dizziness	72 (29.6)	**18 (45.0)**	**0.020**	12 (38.7)	0.236	**18 (42.9)**	**0.039**
Seizures	5 (2.1)	2 (5.0)	0.191	2 (6.5)	0.123	2 (4.8)	0.207
General weakness	65 (26.7)	15 (37.5)	0.093	**13 (41.9)**	**0.041**	13 (31.0)	0.499
Paralysis	11 (4.5)	3 (7.5)	0.396	1 (3.2)	1.000	4 (36.4)	0.102
Difficulty ambulating	29 (11.9)	5 (12.5)	0.540	5 (16.1)	0.302	8 (19.1)	0.118
Headache	110 (45.3)	22 (55.0)	0.176	15 (48.4)	0.709	21 (50.0)	0.498
Insomnia	33 (13.6)	3 (7.5)	0.313	**9 (29.0)**	**0.012**	**12 (28.6)**	**0.002**
Depression	22 (9.1)	5 (12.5)	0.285	**6 (19.4)**	**0.044**	2 (4.8)	0.228
Irritability	16 (6.6)	3 (7.5)	0.732	2 (6.5)	1.000	4 (9.5)	0.490
Difficulty cooking	23 (9.5)	5 (12.5)	0.473	**6 (19.4)**	**0.044**	6 (14.3)	0.241
Difficulty cleaning	28 (11.5)	5 (12.5)	0.832	6 (19.4)	0.144	5 (11.9)	0.932
Difficulty working	25 (10.3)	3 (7.5)	0.776	6 (19.4)	0.075	6 (14.3)	0.348
Fever	6 (2.5)	1 (2.5)	1.000	0 (0.0)	1.000	1 (2.4)	0.173
Chills	2 (0.8)	1 (2.5)	0.303	0 (0.0)	1.000	0 (0.0)	1.000
Emesis	1 (0.4)	0 (0.0)	1.000	0 (0.0)	1.000	1 (2.4)	0.173
Diarrhea	1 (0.4)	0 (0.0)	1.000	0 (0.0)	1.000	1 (2.4)	0.173

MADV = Madariaga virus; UNAV = Una virus; VEEV = Venezuelan equine encephalitis virus. $n = 40$ with UNAV antibodies; $n = 31$ with MADV antibodies; $n = 42$ with VEEV antibodies; $n = 243$ participants in total.

* Based on plaque reduction neutralization test results.

† Proportion of those with antibodies that reported symptoms.

‡ Results with $P < 0.05$ are shown in boldface type.

§ Overall proportion of participants with symptoms.

TABLE 3

Independent factors associated with the seroprevalence of UNAV, MADV, and VEEV neutralizing antibodies in univariate generalized estimating equations for logistic regression models (n = 243)

	UNAV*			MADV*			VEEV*		
	Univariate analysis			Univariate analysis			Univariate analysis		
Factor	OR	95% CI	P-value†	OR	95% CI	P-value†	OR	95% CI	P-value†
Gender									
Male	Ref.	–	–	Ref.	–	–	Ref.	–	–
Female	0.76	0.39–1.51	0.436	0.92	0.44–1.92	0.817	1.77	0.92–3.42	0.087
Age-group (years)									
2–11	Ref.	–	–	Ref.	–	–	Ref.	–	–
12–30	2.35	0.69–8.00	0.170	**6.50**	**2.49–31.89**	***0.021***	2.68	0.93–7.73	0.067
31–97	**9.59**	**3.15–29.17**	< ***0.001***	**12.00**	**2.49–57.75**	***0.002***	**5.91**	**2.16–16.20**	***0.001***
Activities									
Main occupation									
Student	Ref.	–	–	Ref.	–	–	Ref.	–	–
Farmer/rancher	**8.24**	**3.38–20.11**	< ***0.001***	2.43	0.94–6.31	0.068	**3.40**	**1.36–8.47**	***0.009***
Housewife/at home	2.48	0.92–5.04	0.053	1.98	0.82–4.82	0.198	**4.21**	**1.86–9.52**	***0.001***
Breeding poultry	0.92	0.38–2.24	0.858	**2.35**	**1.01–5.50**	***0.048***	1.45	0.65–3.25	0.366
Walking/playing through pastures	0.77	0.36–1.64	0.499	1.36	0.61–3.01	0.451	1.34	0.66–2.73	0.418
Walking/playing through crops	1.23	0.62–2.44	0.546	**2.64**	**1.15–6.03**	***0.021***	1.75	0.87–3.41	0.117
Clearing vegetation	1.85	0.93–3.71	0.080	**2.25**	**1.05–4.84**	***0.037***	1.76	0.90–3.46	0.100
Working in agriculture	**3.02**	**1.51–6.04**	***0.002***	1.83	0.86–3.90	0.114	**2.56**	**1.31–4.98**	***0.006***
Working in sawmills/forest	2.19	0.93–5.17	0.073	1.25	0.44–3.56	0.664	2.07	0.89–4.52	0.092
Working in chicken coops	1.27	0.58–2.73	0.545	2.20	0.98–4.93	0.054	1.13	0.53–2.44	0.750
Working in pigsties	1.40	0.61–3.19	0.422	0.63	0.20–1.94	0.420	2.08	0.94–4.58	*0.069*
Washing clothes in ravines or rivers	1.40	0.75–2.32	0.337	1.74	0.81–3.75	0.152	**3.11**	**1.53–6.33**	***0.002***
Taking bath in natural water source	1.08	0.39–3.04	0.871	2.34	0.53–10.25	0.259	1.95	0.60–6.42	0.269
House level									
House with walls	**0.47**	**0.39–3.04**	***0.042***	1.83	0.83–4.02	0.133	0.78	0.37–1.64	0.515
House window material									
Concrete‡	Ref.	–	–	Ref.	–	–	Ref.	–	–
Wood	0.68	0.28–1.66	0.397	0.59	0.20–1.74	0.341	0.89	0.37–2.15	0.799
Roof material house									
Tin roof	Ref.	–	–	Ref.	–	–	Ref.	–	–
Straw thatched	0.93	0.47–1.86	0.853	1.61	0.72–3.63	0.249	1.42	0.68–2.59	0.349
Vegetation around the house	0.64	0.31–1.35	0.245	**2.94**	**1.24–5.26**	***0.006***	1.18	0.56–2.49	0.653
Waste disposal methods									
Burying	Ref.	–	–	Ref.	Ref.	–	Ref.	–	–
Burning	1.21	0.42–3.54	0.721	0.23	0.03–2.02	0.189	1.20	0.37–3.87	0.755
Other	1.28	0.48–3.44	0.616	0.89	0.28–2.84	0.846	0.89	0.29–269	0.846

MADV = Madariaga virus; OR = odds ratio; UNAV = Una virus; VEEV = Venezuelan equine encephalitis virus. Results with $P < 0.05$ are shown boldface and bold-italic type.
* Based on plaque reduction neutralization test results.
† Results with $P < 0.05$ are shown in boldface type.
‡ Ornamental blocks.

of females per trap night was 6.7 in the Trinidad traps compared with 4.6 in the CDC traps. No viruses were detected in samples from mosquitoes.

Alphavirus FOI. For each virus, we fit both constant and time-varying FOI models to the seroprevalence data (see Methods) to describe the per capita rate at which susceptible individuals become infected per year. Because the constant FOI model is effectively nested within the time-varying FOI model, we report on whether the latter model improved the fit relative to the former.

TABLE 4

Independent factors associated with the seroprevalence of UNAV, MADV, and VEEV neutralizing antibodies in multivariable generalized estimating equations for logistic regression models (n = 243)

	UNAV*			MADV*			VEEV*		
	Multiple regression			Multiple regression			Multiple regression		
Factor	OR	95% CI	P-value†	OR	95% CI	P-value†	OR	95% CI	P-value†
Age-group (years)									
2–11	Ref.	–	–	Ref.	–	–	Ref.	–	–
12–30	2.39	0.70–8.15	0.164	**6.28**	**1.27–31.00**	**0.024**	1.83	0.61–5.53	0.279
≥ 31	**9.98**	**3.27–30.48**	**< 0.001**	**12.64**	**2.61–60.19**	**0.002**	**4.53**	**1.61–12.74**	**0.004**
House with walls	**0.43**	**0.20–0.94**	**0.035**	–	–	–	–	–	–
Washing clothes in ravines or rivers	–	–	–	–	–	–	**2.65**	**1.24–5.63**	**0.011**
Vegetation around the house	–	–	–	**2.96**	**1.25–6.98**	**0.013**	–	–	–

MADV = Madariaga virus; OR = odds ratio; UNAV = Una virus; VEEV = Venezuelan equine encephalitis virus.
* Based on plaque reduction neutralization test results.
† Results with $P < 0.05$ are shown in boldface type.

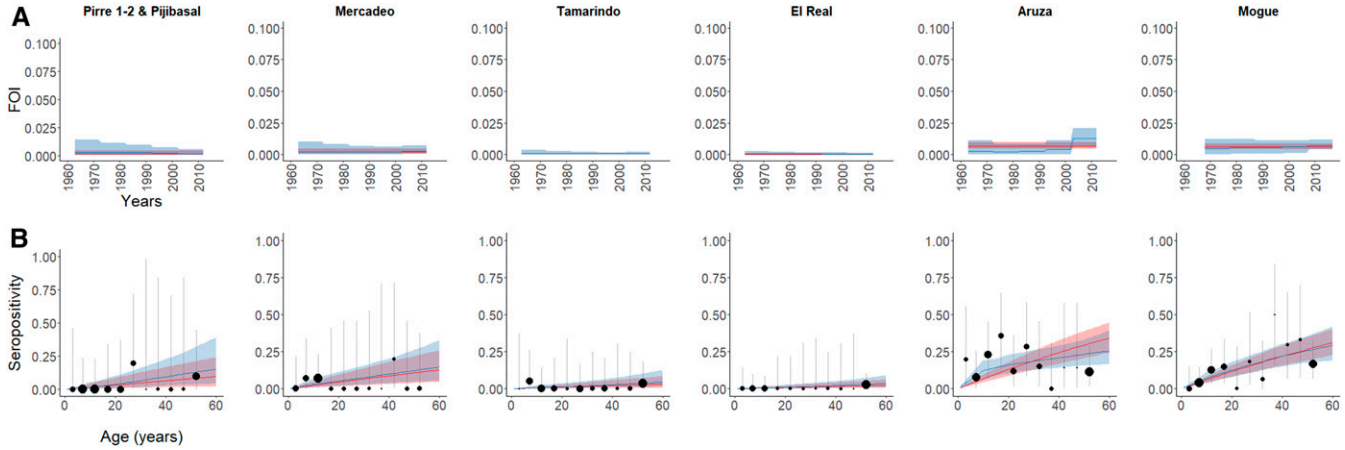

FIGURE 2. Force-of-infection (FOI) models fitted to Madariaga virus (MADV) seroprevalence data. (**A**) (Top panels) estimated constant (red) vs. time-varying FOI (blue) for MADV in eastern Panama over 50 years and (**B**) (bottom panels) fitted and observed seroprevalence. Red lines represent the estimated constant FOI and blue lines the estimated time-varying FOI. In each case, the shading represents 95% credible intervals from the model. The circles' radii in the lower panels indicate sample size in each 5-year age-group, and the vertical lines represent 95% CIs for observed seroprevalence. This figure appears in color at www.ajtmh.org.

Our results indicate temporal and geographic heterogeneity in the human population's exposure to MADV (Figure 2), VEEV (Figure 3), and UNAV (Figure 4). The highest estimated seroprevalence of each of the three viruses in younger than 10-year-olds (an indirect metric of recent transmission) was estimated for VEEV in Pirre 1-2 and Pijibasal at a posterior median of 44.8% (95% CrI: 34.9–55.0), followed by UNAV in Mogue at 5.6% (95% CrI: 4.1–7.5) and by MADV in Aruza at 4.7% (95% CrI: 3.2–6.7).

For MADV, in six of the seven locations, there was no evidence of time-varying transmission (Table 5); but in one location, Aruza, FOI was estimated as 0.012 (95% CrI: 0.006–0.021) (Figure 2A) in the latest decade analyzed (2002–2012)—a multiple of 4.6 and 5.3 times (ratio of posterior medians) the values estimated for 1992–2012 and 1982–1992, respectively (Figure 2B).

For VEEV, in six of the seven locations, there was no statistical support for time-varying transmission (Table 5). For the constant model, we estimated an annual FOI of 0.08 (95% CrI: 0.06–0.11) for VEEV in Pirre 1-2 and Pijibasal, corresponding to seroprevalence reaching 75% in 15-year-olds and almost 100% by 60-year-olds (Figure 3A). However, from the relatively small sample (only 75 subjects), it is unclear whether these results are due to consistently high endemic transmission or recent introductions and/or recent outbreaks. For one location, Mercadeo, a time-varying FOI model fit the data best. In this case, FOI in the most recently analyzed decade (2002–2012) was estimated at 0.04 (95% CrI: 0.03–0.06)—an increase of 1.5 times (ratio of posterior medians) over the previous decade (1992–2012) and 3.1 times compared with that of 1972–1992 (Figure 3B).

For UNAV, only tested in Mogue, a constant model fit the data best with an FOI estimated at 0.008 (95% CrI: 0.006–0.011) (Figure 4). No changes or increases in the incidence of UNAV associated with epidemics were observed, and infections occurred constantly during the analyzed period of time.

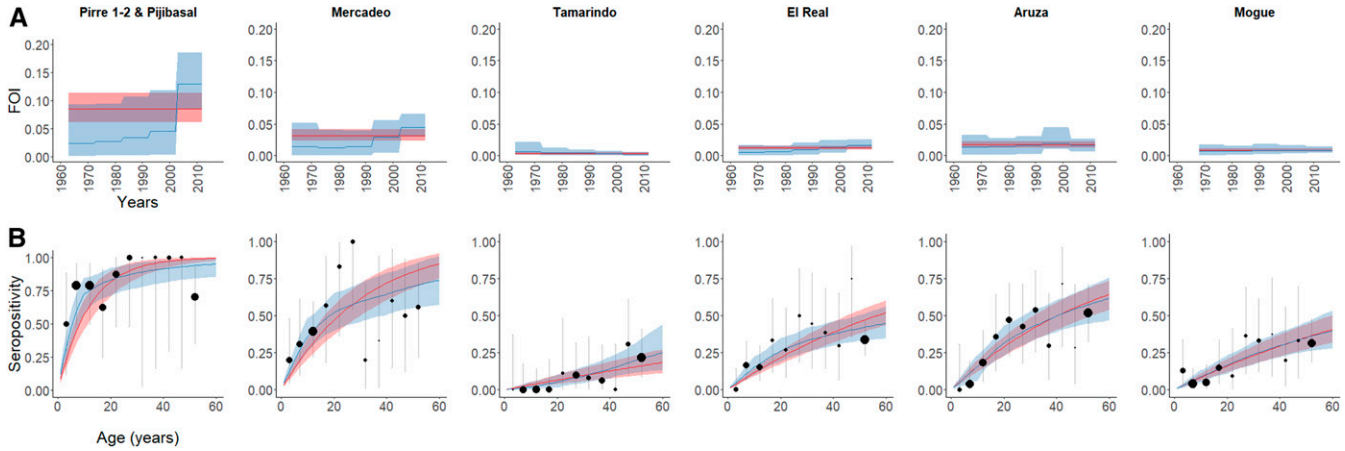

FIGURE 3. Force-of-infection (FOI) models fitted to Venezuelan equine encephalitis virus (VEEV) seroprevalence data. (**A**) (Top panels) estimated constant (red) vs. time-varying FOI (blue) for VEEV in eastern Panama over 50 years and (**B**) (bottom panels) fitted and observed seroprevalence. Red lines represent the estimated constant FOI and blue lines the estimated time-varying FOI. In each case, the shading represents 95% credible intervals from the model. The circles' radii in the lower panels indicate sample size in each 5-year age-group, and the vertical lines represent 95% CIs for observed seroprevalence. This figure appears in color at www.ajtmh.org.

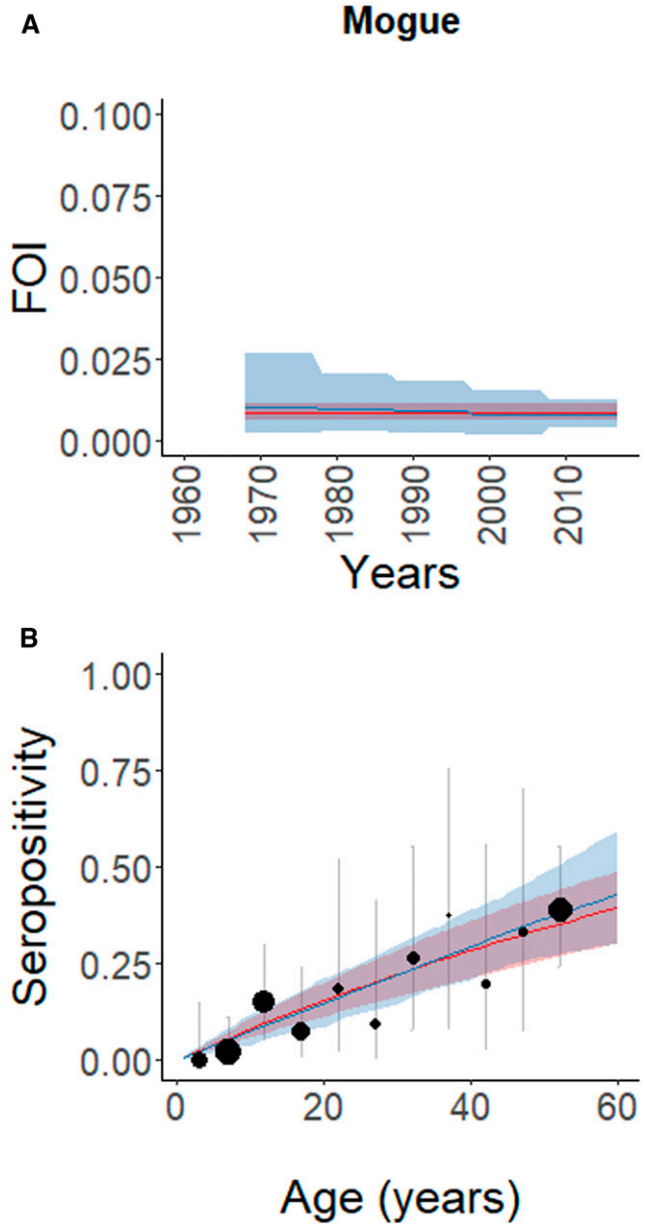

FIGURE 4. Force-of-infection (FOI) models fitted to Una virus (UNAV) seroprevalence data. (**A**) (Top panels) estimated constant (red) vs. time-varying FOI (blue) for UNAV in eastern Panama over 50 years and (**B**) (bottom panels) fitted and observed seroprevalence. Red lines represent the estimated constant FOI and blue lines the estimated time-varying FOI. In each case, the shading represents 95% credible intervals from the model. The circles' radii in the lower panels indicate sample size in each 5-year age-group, and the vertical lines represent 95% CIs for observed seroprevalence. This figure appears in color at www.ajtmh.org.

DISCUSSION

By analyzing data from recent cross-sectional seroprevalence studies, we reconstructed alphavirus transmission in eastern Panama. Historical transmission rates indicated endemic transmission of VEEV, MADV, and UNAV in humans with increased human exposure during the past decade. Here, we show evidence of acute IgM antibody responses against MADV and VEEV in people without signs of neurologic disease, suggesting asymptomatic infections or mild disease. To our knowledge, this is the first evidence of human infection with UNAV in Panama, although its circulation was reported during the 1960s in mosquitoes (*Ps. ferox* and *Ps. albipes*) collected in western Panama.[16] To our knowledge, our results demonstrate the highest seroprevalence of UNAV reported in the literature as for July 2020.[14,28]

Using catalytic FOI model fit to age-stratified seroprevalence data, we reconstructed 50 years of historical transmission rates for VEEV and MADV for seven locations in Darien Province. In most locations, the data indicated consistent endemic transmission of these viruses. In two locations—Mercadeo (for VEEV) and Aruza (for MADV), there was evidence of a recent increase in human exposure. These results suggest that MADV and VEEV incidences differ geographically. The observed FOI profile suggests that VEEV infections increased in Pirre 1-2 and Pijibasal and Mercadeo, locations surrounded by tropical forest, whereas MADV infections increased mostly in Aruza, a formerly forested area converted to agricultural land over 30 years ago.[29] Although ecological changes could be associated with the increased exposure to MADV in Aruza, it is unclear which drivers could also explain the simultaneous increase in VEEV we estimated.

Only 3.6% of participants had antibodies to more than one alphavirus. Mixed alphavirus antibody responses in Peru[5] and Panama[8] suggest cross-protective immunity. However, the mechanism of cross-protection and whether some alphaviruses induce a stronger heterologous response than others remain unclear.

The MADV seroprevalence in 2017 was greater for those living with vegetation around the house, contrasting with previous evidence in 2012, suggesting possible change in exposure risk.[8] However, characteristics of houses in Mogue in 2017 may differ from areas that were surveyed in 2012.[8] Potential MADV vectors within the *Culex* (*Melanoconion*) subgenus[30] were found during our peri-domestic investigation in Mogue. This finding of vectors near houses with surrounding vegetation as a risk factor supports the hypothesis that MADV infections can occur near houses. This contrasts with VEEV risk factors, which include washing clothes in ravines or rivers, suggesting that VEEV seropositivity is associated with human incursion into the gallery forest, a potential natural habitat for development of larvae of the main vectors *Culex* (*Melanoconion*) spp.[30]

Having a house with walls was associated with lower UNAV seroprevalence in Mogue. This suggests that UNAV infections can also occur outside the forest, where the main vector *Ps. ferox* and nonhuman primates are believed to maintain the enzootic cycle.[15,16,18] *Psorophora* spp. have been also found in disturbed areas of Panama,[31] indicating potential changes in the vector habitat usage.

Alphaviral exposure was associated with several self-reported neurological and constitutional sequelae. Specifically, weakness, insomnia, depression, and dizziness were commonly associated with prior MADV, VEEV, and UNAV exposure. Depression and other neurological symptoms have also been observed after neurotropic flavivirus infections in North America.[32] However, the role of several alphaviruses in long-term neurological impairment is still unknown. This highlights the need to further investigate the long-term ramifications of alphaviral infection with objective testing (e.g., neuropsychological testing and imaging).

TABLE 5
Comparison of constant vs. time-varying FOI for UNAV, MADV, and VEEV in 2012 and 2017

Place	Virus*	Sample size	Age classes	Constant FOI model		Time-varying FOI model		Comparison		
				elpd	se	elpd	se	$elpd_{diff}$	se	*P*-value†
Pirre and Pijibasal	MADV	74	11	−4.98	1.92	−5.43	1.62	−0.45	0.46	0.835
Mercadeo	MADV	103	11	−9.19	2.40	−9.36	2.09	−0.17	0.50	0.634
Tamarindo	MADV	176	11	−6.33	2.85	−6.78	2.57	−0.45	0.33	0.916
El Real	MADV	251	11	−3.48	1.90	−3.55	1.59	−0.06	0.33	0.577
Aruza	MADV	167	11	−30.12	5.23	**−24.27**	**3.32**	5.86	2.10	0.003
Mogue	MADV	243	11	−20.92	3.16	−21.26	3.14	−0.35	0.29	0.880
Pirre and Pijibasal	VEEV	73	11	−25.15	11.38	−18.58	7.08	6.56	4.56	0.075
Mercadeo	VEEV	103	11	−26.07	2.32	**−22.26**	**2.31**	3.81	0.87	**< 0.001**
Tamarindo	VEEV	176	11	−14.01	2.54	−13.18	2.37	0.83	0.71	0.120
El Real	VEEV	251	11	−27.68	5.89	−25.34	4.23	2.35	1.94	0.112
Aruza	VEEV	167	11	−20.98	1.87	−20.98	1.79	0.00	0.40	0.503
Mogue	VEEV	243	11	−21.53	2.68	−22.24	2.80	−0.70	0.36	0.976
Mogue	UNAV	243	11	−17.84	1.78	−18.63	2.22	−0.78	0.67	0.880

elpd = expected log predictive density for an out-of-sample data point; $elpd_{diff}$ = difference in elpd between the two models; FOI = force-of-infection; MADV = Madariaga virus; UNAV = Una virus; VEEV = Venezuelan equine encephalitis virus; se = standard error.
* Based on plaque reduction neutralization test results.
† Based on comparing *z* statistics with standard normal quantiles; results with $P < 0.05$ (shown in boldface) indicate the time-varying FOI model significantly outperformed the constant FOI model.

Alphaviral RNA was not detected in samples from either humans or mosquitoes, although field surveys and collection were performed soon after the confirmation of a fatal MADV infection in the community. Although sample size is always a limiting factor in attempts to identify ongoing infections, these results suggest that these alphaviruses may be short-lived peripherally, or produce low viremia.[7] Low MAYV seroprevalence was also detected in our earlier research,[7] indicating little human exposure to this virus in Panama.

Our study has several limitations. Clinical outcomes statistically associated with exposure to these alphaviruses represent exploratory and causal inference studies that should be followed up with more comprehensive assessments. Our study only obtained preliminary data during an outbreak response to generate hypotheses. However, mosquito collections were only performed over 2 days, and the number of collected mosquitos does not allow us to draw conclusions about active viral circulation. The collection of few mosquito vectors near houses suggests close contact between vectors and humans. The use of both CDC traps baited with octanol and Trinidad traps enhanced our ability to capture alphavirus enzootic vectors.[33] The sample size used in these serosurveys only allowed us to describe general trends in the FOI over time. Also, we cannot exclude cross-reactivity or age-dependency in exposure or susceptibility. More precise estimates would require an increased sample size and, ideally, longitudinal data collection.

In summary, we investigated alphavirus transmission in Panama using age-specific seroprevalence data to look back over five decades. Our results suggest that human alphavirus infections may have gone undetected by the Panamanian surveillance system, and hint that the MADV and VEEV outbreaks in 2010 may have been due to a common increase in enzootic circulation. The antibody seroprevalence we determined for UNAV is the highest reported in Latin America. Taken together, these results coupled with potential symptoms of MADV and VEEV infection underscore the importance of developing comprehensive arboviral surveillance in Latin American enzootic regions.

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