RESEARCH ARTICLE

Enzootic mosquito vector species at equine encephalitis transmission foci in the República de Panamá

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Abstract

The identification of mosquito vector species present at arboviral enzootic transmission foci is important to understand transmission eco-epidemiology and to propose and implement prevention and control strategies that reduce vector-borne equine encephalitis transmission. The goal of this study was to identify mosquito species potentially involved in the transmission of enzootic equine encephalitis, in relation to their abundance and diversity at three endemic regions in the República de Panamá. We sampled adult mosquitoes during the dry and rainy season of Panamá. We employed CDC light traps with octanol, EV traps with CO2 and Trinidad 17 traps baited with live hamsters. Traps were deployed in the peridomicile and extradomicile of houses from 18:00 to 6:00 h. We estimated the abundance and diversity of sampled species. We collected a total of 4868 mosquitoes, belonging to 45 species and 11 genera, over 216 sampling nights. Culex (Melanoconion) pedroi, a major Venezuelan equine encephalitis vector was relatively rare (<2.0% of all sampled mosquitoes). We also found Cx. (Mel) adamesi, Cx. (Mel) crybda, Cx. (Mel) ocossa, Cx. (Mel) spissipes, Cx. (Mel) taeniopus, Cx. (Mel) vomerifer, Aedes scapularis, Ae. angustivittatus, Coquillettidia venezuelensis, Cx. nigripalpus, Cx. declarator, Mansonia titillans, M. pseudotitillans and Psorophora ferox all species known to be vectorially competent for the transmission of arboviruses. Abundance and diversity of mosquitoes in the sampled locations was high, when compared with similar surveys in temperate areas. Information from previous reports about vectorial competence / capacity of the sampled mosquito species suggest that sampled locations have all the elements to support enzootic outbreaks of Venezuelan and Eastern equine encephalitides.
Introduction

New World alphaviruses, like Venezuelan (VEEV) Eastern (EEEV) and Western equine encephalitis virus (WEEV), are etiologic agents of major zoonotic diseases transmitted by mosquitoes that affect humans and equines [1]. The equine encephalitides are often lethal or leave severe neurological sequelae following periodic epizootics and epidemics. Therefore, these diseases have mandatory reporting to the World Organisation for Animal Health, OIE [2]. To date there are no safe and efficient vaccines against an alphavirus [2–4]. VEE is considered the most important re-emerging zoonosis affecting hundreds of thousands of equines and humans through the Americas [4–6]. Meanwhile several EEE outbreaks have affected equines and humans, with a high mortality rate and significant neurologic damage in surviving individuals [7].

VEEV is a RNA virus belonging to the Togaviridae family and the Alphavirus genus [8]. VEEV is a diverse virus where specific subtypes have been associated with the epidemic/epizootic cycle, IAB and IC, which have been frequently isolated in human and equine epidemics, associated with zoophilic vectors [9]. It remains an open question how these subtypes are maintained during the inter-epizootic periods and the role of vectors in such periods, highlighting the need for a better understanding of vector species diversity in transmission areas [10–12]. By contrast, subtypes ID, IE, IF and II-VI have been associated with enzootic and enzootic transmission in tropical and subtropical sylvatic areas. These subtypes can be easily isolated from mosquito vectors and small vertebrate reservoir hosts [13] and are becoming increasingly associated with human cases [14]. For example, subtype ID is very common across the República de Panamá and all over Central America, Colombia, Venezuela, Mexico and the USA [8,15–18]. In Panamá subtype IE, following its 1962 isolation from Almirante in Bocas del Toro province [19], has never been isolated again.

EEE epizootics have been recorded in Panamá since 1936 [20]. In 1986 a well-documented EEE epizootic outbreak occurred in Panamá, mainly affecting horses, during the rainy season. This outbreak was simultaneous with bird migrations from North to South America [21]. The most recent well documented EEE outbreak in Panamá occurred in 2010 in Darién, where VEEV was also being transmitted [6]. In this outbreak, there were 19 human encephalitis cases of which 7 were infections by EEEV, 3 by VEEV, one case was a co-infection by VEEV and EEEV, while 3 patients died [4].

Dominant VEEV vectors include Aedes taeniorhynchus (Wiedemann, 1821) and Psorophora confinis (Lynch & Arribalzaga, 1821), which have been associated with epizootic VEEV transmission [22,23]. Enzootic transmission is believed to be almost exclusively carried out by the Spissipes section of the Melanoconion subgenus of the Culex genus [15,24]. VEEV has been isolated from Cx. (Mel) portesi (Sevenet and Abonnenc, 1941) which transmits Mucambo virus (VEEV subtype IIIA) in Trinidad; Cx. (Mel) cedeci (Stone & Hair, 1968) which transmits Everglades virus (VEEV subtype II) in Southern Florida, USA; Cx. (Mel) aikenii (Aiken & Rowland, 1906) sensu lato ocossa and panocossa which transmits subtype ID in Panamá and Cx. (Mel) taeniopus (Dyar & Knab, 1907) which transmits subtype IE and is the main VEEV vector in Guatemala [8,9,11,23,25].

In Panamá, from the time of the first enzootic VEEV isolation (subtype ID a.k.a., strain 3880) from a fatal human case [26], frequent endemic and enzootic outbreaks have been described via virus isolation from mosquito vectors, rodent reservoirs, equines and humans [15]. The recent cyclic and explosive enzootic and epizootic VEE outbreaks in countries neighboring Panamá, in addition to the frequent isolation of enzootic subtype ID VEEV from the Darién province and other regions in Panamá [4,6] calls for a better knowledge of the mosquito fauna, especially the identification of potential VEEV vectors. Here, we will define a potential vector as a species which has been found infected by a pathogen without a bloodmeal
in a previous field study elsewhere or which has been experimentally shown as competent to transmit the pathogen in the laboratory [13]. In this study we present results from a series of mosquito surveys in three regions with a history of equine encephalitis transmission, placing an emphasis on the diversity and abundance of potential VEEV and EEEV vectors.

Materials and methods

Study site

We designed this study to compare mosquito species composition from three enzootic arbovirus (VEEV and EEEV) transmission foci in the Panamá and Darién provinces and the autonomous indigenous Comarca (territorial political division assigned to indigenous groups) Ngäbe Buglé. In the selected study areas infections in humans, horses and/or wildlife animals have been reported [4,6], or in mosquito pools identified to the genus level [27]. Darién is the easternmost province in Panamá, bordering Colombia. The natural landscape is dominated by tropical rainforest and the climate is tropical with an extended dry season. Total annual rainfall is over 2500 mm, with one or two dry months with less than 60 mm. Temperature ranges between 18 and 23°C around the year [28]. In this province we selected the following locations for mosquito sampling: Mercadeo with 36 households and 206 inhabitants, Santa Librada with 170 households and 300 inhabitants and Los Pavitos with 30 houses and 95 inhabitants. Western Panamá Province has a warm pre-mountain humid tropical rainforest. Annual rainfall adds to 1571 mm, with a mean annual temperature of 26.5°C [29]. Here, we selected El Cacao and Ciri Grande as sampling locations. Ngäbe Buglé is also covered by premountain tropical rainforest and has an annual rainfall around 400 mm and mean temperatures around 25°C year-round. Here, we collected mosquitoes at Pumona. In all the sampling locations it is worth highlighting that the landscape is very homogeneous from the standpoint of ecological disturbances, since at the local scale of our sampling locations there was a similar mix of forest and cattle farming grounds near households. In all studied locations the main economic activities are cattle farming, wood extraction and subsistence agriculture. Fig 1 is a map showing the sampling locations.

Mosquito sampling

At each sampling location we put three kinds of traps over three consecutive nights (18:00 to 6:00) at 1.5 m above the ground in peridomiciliary areas and forests near to the houses, hereafter referred as extradomicile [30,31]. In each locality we employed 10 CDC light traps baited with octanol (Fig 2A), eight modified Trinidad 17 (TT-17) traps (Fig 2B), baited with one live hamster and eight EVS traps (Fig 2C) baited with CO₂ [25]. Sampling was done during February-March (dry season) and September-October (rainy season) of 2011 and 2012, trying to sample species from both the dry and rainy season. Collected mosquitoes were killed, by flash-freezing, soon after collection and identified at the genus level in the field. Samples were then placed in plastic vials by trap type and sampling date and stored in liquid nitrogen before transportation to the Departamento de Entomología Médica at the Instituto Conmemorativo Gorgas de Estudios de la Salud, where identification at the species level, whenever possible, was performed using taxonomic keys [24,32,33] and the reference collection at the Institute.

Data analysis

We used mosquito species abundance data to estimate mosquito species relative abundance and diversity using the software EstimateS, 8.2.0” [34]. We specifically estimated the Simpson and Shannon-Wiener diversity indices to compare patterns of diversity at each study site. The choice of these two indices was done given the emphasis of the former on dominant species, as
opposed to the latter which focuses on the whole community [35]. We also estimated species richness by counting the number of species at each site and by estimating the Margalef index. Finally, we estimated species similarity between sampling locations using the Sorensen index. We also estimated the median abundance and its SE for females of all collected taxonomic units. For the analyses we used the additive mosquito counts, from all three types of traps, for each mosquito taxonomic unit. In all the analyses we considered taxonomic units identified at the genus level as a distinct species, since they likely included, in all cases, individuals belonging to species not identifiable with morphological keys.

Ethical clearance

No permits were required since humans were not involved in the study. Use of hamsters was approved by the “Comité Institucional de Uso y Cuidado de Animales de Laboratorio” (CIU-CAL) of Instituto Conmemorativo Gorgas de Estudios de la Salud, in accordance with law No. 23 of January 15 1997 (Animal Welfare Assurance) of República de Panamá, as presented within the research protocol of project “Estudio del subtipo ID del complejo de Encefalitis Equina Venezolana en Panamá”.

Materials and data availability

All data analyzed in the results section are presented within the text of this article. Voucher specimens of collected mosquito species are available at the Colección de Insectos, Departamento de Entomología Medica, Instituto Conmemorativo Gorgas de Estudios de la Salud.

Results

For each of the six study locations we sampled a total of 36 nights, totaling 216 sampling nights across all study sites. We collected a total of 4868 mosquitoes belonging to the following 11
genera: Aedes, Anopheles, Aedeomyia, Coquilletidia, Culex, Deinocerites, Haemagogus, Mansonia, Psorophora, Uranotaenia and Wyeomyia. From these 11 genera we were able to identify 45 mosquito species, and a total of 61 taxonomic units (Table 1). The most abundant species was C. venezuelensis (Theobald, 1912) 22.2%, followed by Cx. (Mel) dunnii (Dyar, 1918) 4.0%, Ae. angustivitatus (Dyar & Knab, 1907) 2.6%, Ps. cingulata (Fabricius, 1805) 2.5%, Cx. (Mel) pedroi

Fig 2. Mosquito TRAPS. (A) CDC light with Octanol, (B) Modified Trinidad 17, baited with one hamster and (C) EVS baited with CO2.

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Table 1. Mosquito species composition and abundance in three regions with equine encephalitis transmission in the República de Panamá. Data are presented as total by region. The sampling trap-nights effort is indicated by \( n \). Please note that sampling effort was the same at the location level, and differences in \( n \) reflect differences in the number of sampled locations by region.

| Species | Darién | Panamá | Ngābe Buglé |
|---------|--------|--------|--------------|
|         | Median Abundance ± SE | Median Abundance ± SE | Median Abundance ± SE |
|         | \( n = 108 \) | \( n = 72 \) | \( n = 36 \) |
| Ae. angustivittatus | 42.00 ± 0.00 | 51.70 ± 0.00 | 5.00 ± 0.00 |
| Ae. fulvus | 221.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Ae. scapularis | 12.70 ± 0.00 | 16.50 ± 0.00 | 0.00 ± 0.00 |
| Ae. serratus | 10.70 ± 0.00 | 11.50 ± 0.00 | 2.00 ± 0.00 |
| Aedes spp. | 7.00 ± 0.00 | 0.00 ± 0.00 | 6.00 ± 0.00 |
| Aedes (Ochlerotatus) spp. | 26.00 ± 0.00 | 31.10 ± 0.00 | 6.00 ± 0.00 |
| Aedes (Finlaya) spp. | 1.33 ± 0.00 | 1.87 ± 0.00 | 7.00 ± 0.00 |
| Aedesomyia squamipennis | 101.00 ± 0.00 | 99.00 ± 0.00 | 0.00 ± 0.00 |
| An. albimanus | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.00 ± 0.00 |
| An. apicimacula | 6.00 ± 3.00 | 3.00 ± 2.00 | 4.50 ± 2.50 |
| An. malefactor | 5.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| An. neomaculipalpus | 3.00 ± 0.00 | 2.00 ± 0.00 | 47.50 ± 4.95 |
| An. oswaldoi | 3.00 ± 0.00 | 0.00 ± 0.00 | 7.00 ± 0.00 |
| An. pseudopunctipennis | 4.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| An. punctimacula | 7.50 ± 1.00 | 2.50 ± 1.00 | 0.00 ± 0.00 |
| An. strodei | 0.00 ± 0.00 | 0.00 ± 0.00 | 8.50 ± 0.71 |
| An. triannulatus | 4.50 ± 0.00 | 2.50 ± 0.00 | 0.00 ± 0.00 |
| An. (Anopheles) spp. | 0.11 ± 0.00 | 0.33 ± 0.00 | 0.00 ± 0.00 |
| An. (Nyssorhinus) spp. | 0.00 ± 0.00 | 2.50 ± 0.71 | 8.50 ± 2.12 |
| Anopheles albimanus | 0.00 ± 0.00 | 0.00 ± 0.00 | 5.00 ± 2.83 |
| Coquillettidia venezuelensis | 267.30 ± 3.50 | 360.40 ± 2.50 | 481.00 ± 8.49 |
| Coquillettidia spp. | 4.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Culex (Cx) coronator | 0.00 ± 0.00 | 0.00 ± 1.00 | 0.00 ± 0.00 |
| Cx. (Cx) declarator | 18.00 ± 3.00 | 3.50 ± 1.00 | 0.00 ± 3.00 |
| Cx. (Cx) interrogator | 15.50 ± 0.00 | 14.50 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Cx) nigripalpus | 15.00 ± 0.00 | 14.30 ± 0.00 | 0.00 ± 0.00 |
| Culex (Cx) spp. | 198.70 ± 9.50 | 102.40 ± 3.50 | 28.00 ± 0.00 |
| Culex spp. | 0.67 ± 0.00 | 67.00 ± 2.00 | 3.00 ± 0.00 |
| Cx. (Anoediporpa) spp. | 0.67 ± 1.32 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Culex (Aedimus) spp. | 6.50 ± 3.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Lutzia) alllostigma | 0.00 ± 0.00 | 0.00 ± 0.00 | 3.00 ± 2.83 |
| Cx. (Mel) adamesi | 5.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) crybda | 2.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) dunni | 72.30 ± 53.90 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) ocosaa | 2.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) pedroi | 24.00 ± 23.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) spissipes | 23.50 ± 2.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) taeniopus | 15.40 ± 11.50 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) vomenifer | 5.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Cx. (Mel) spp. | 177.70 ± 200.30 | 6.00 ± 5.00 | 36.00 ± 4.24 |
| Cx. (Mel) spp. Secc Mel | 40.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Deinocerites dyari | 12.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Haemagogus lucifer | 1.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

(Continued)
(Sirivanakam & Belkin, 1980) 2.0%, *Ps. confinis* (Theobald, 1887) 2.0% and *U. lowii* (Theobald, 1901) 1.8%, *Cx. declarator* (Dyar and Knab, 1906) 1.2%, *Cx. (Mel) spissipes* (Theobald, 1903) 1.1%, *An. neomaculipalpus* (Curry, 1933) 1.1% and *Ae. fulvus* 1.0%. All other species accounted for less than 1.0% of the total sample. The most species rich region was Darién with 52 taxonomic units, followed by Ngäbe Buglé with 23 taxonomic units and Panamá with 21 taxonomic units.

*C. venezuelensis* (Table 1) was captured in all sampling localities, being the most abundant in Mercadeo (54.9%) and Pumona (42.3%), and less abundant in the other localities: Santa Librada (1.6%), Los Pavitos (0.6%), El Cacao (0.5%) and Ciri Grande (0.1%). In contrast, *Cx. (Lutzia) allostigma* (Howard, Dyar & Knab, 1915), *Ps. ferox* (Humboldt 1819), *Wyeomyia chalcoccephala* (Dyar & Knab, 1906) and *W. hosauto* (Dyar & Knab. 1907) were only collected at single locations.

The number of mosquito species and their abundance was variable according to the sampling locality (Table 2 and Table 3). The highest abundance and richness of species was found in Darién, Republica de Panamá.

Table 2. Mosquito species diversity indices for sampling locations in Darién, República de Panamá.

| Locations/Index | Mercadeo | Santa Librada | Los Pavitos |
|-----------------|----------|---------------|-------------|
| Taxa S          | 45       | 29            | 23          |
| Individuals     | 2919     | 749           | 218         |
| Simpson 1-D     | 0.879    | 0.772         | 0.797       |
| Shannon-Wiener H| 2.636    | 2.188         | 2.185       |
| Margalef        | 5.264    | 4.079         | 3.714       |

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at Mercadeo where we collected 2787 mosquitoes from 45 species (86.5% of all collected species). This site was followed by Santa Librada (762; 15.1%), Pumona (718; 14.2%), and the lowest mosquito abundance was at Los Pavitos (294; 5.8%), Ciri Grande (281; 5.5%) and El Cacao (215; 4.3%). Alpha diversity, when measured using species richness, decreased in the following order: Mercadeo, Santa Librada, Los Pavitos, Pumona, El Cacao and Ciri Grande. Nevertheless, when the ranking was based on the Margalef index, El Cacao had a larger alpha diversity than Pumona, the rest of locations keeping the same rank (Tables 2 and 3).

Regarding mosquito species diversity equity (Tables 2 and 3) we have that according to the Simpson index Mercadeo (0.879), Los Pavitos (0.797) and El Cacao (0.773) were the most diverse. When considering the Shannon-Wiener index sites were ranked as follows: Mercadeo (2.640), Santa Librada (2.188) and Los Pavitos (2.185). The Sorensen similarity index (Table 4) showed that Mercadeo and Santa Librada, in Darién, shared 70% of the mosquito species, the highest species similarity observed in this study. The extent of species similarity was also high between Santa Librada and Los Pavitos (65% of species shared), both located in Darién. Although with a lower species richness, El Cacao and Ciri Grande, both in Panamá, had high mosquito species similarity (58% of species shared), similar to what was observed for Los Pavitos and Pumona (58% of species shared). Interestingly, Pumona shared over half of the species with all other sampling locations but Ciri Grande (40%) (Table 4).

**Discussion**

Knowledge about mosquito species diversity in transmission areas is fundamental to understand the entomological risk of vector-borne disease transmission, given that slight bionomic differences between species can lead to significant differences in transmission, the persistence of a disease, or the ability of a vector-borne disease to spread into new host species [36–40]. The mosquito diversity patterns we observed are within what is normally expected for ecological communities of mosquitoes and other diptera species, where local environmental factors are

| Province | Panama | Ngäbe Buglé |
| --- | --- | --- |
| Locations/Index | El Cacao | Ciri Grande | Pumona |
| **Taxa S** | 18 | 14 | 20 |
| **Individuals** | 107 | 146 | 729 |
| **Simpson 1-D** | 0.773 | 0.622 | 0.565 |
| **Shannon-Wiener H** | 2.08 | 1.551 | 1.485 |
| **Margalef** | 3.638 | 2.408 | 3.186 |

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Table 3. Mosquito species diversity indices for sampling locations in Panamá and Ngäbe Buglé, República de Panamá.

| Province | Mercadeo | Santa Librada | Los Pavitos | El Cacao | Ciri Grande | Pumona |
| --- | --- | --- | --- | --- | --- | --- |
| **Sampling Sites** | 100 | 70 | 56 | 29 | 25 | 52 |
| **Mercadeo** | 100 | 70 | 56 | 29 | 25 | 52 |
| **Santa Librada** | 70 | 100 | 65 | 30 | 39 | 56 |
| **Los Pavitos** | 56 | 65 | 100 | 31 | 35 | 56 |
| **El Cacao** | 29 | 30 | 31 | 100 | 58 | 50 |
| **Ciri Grande** | 25 | 39 | 35 | 58 | 100 | 40 |
| **Pumona** | 52 | 56 | 56 | 50 | 40 | 100 |

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Table 4. Mosquito species pairwise Sorensen similarity index for sampling locations from three regions with VEE transmission in the República de Panamá.
similar [40–43]. Mosquito communities from places geographically close (Fig 1) had more similar faunas, as inferred from the higher Sorensen similarity (Table 4). The most species rich region was Darién, followed by Ngäbe Buglé and then by Panamá. Here, it is important to highlight this result likely not only reflects the larger sampling effort at Darién, but also that individual Darién sampling locations had a higher species richness when compared to locations in the other two studied regions. Mercadeo was the sampling site with the highest mosquito abundance and species richness, including most of the Culex (Melanoconion) spp, which include many major equine encephalitiides vector species [9,44], in contrast with sites from Panamá and Ngäbe Buglé where species from this subgenus were either absent or not identifiable at the species level. This result is very important since it implies a potentially higher entomological risk for enzootic VEEV transmission in Darién, something that could explain the common occurrence of VEE and EEE outbreaks in this region over recent years [4,45]. By contrast Ciri Grande had the lowest species richness, a high abundance of Culex spp. (64.2%).

A detailed examination of the species we collected reveals that from the 45 species (out of a total of 52 taxonomic units) we collected, at least 22 species have been reported as VEEV vectors in Panamá or elsewhere in the New World [3,9,26]. The species previously identified as VEEV vectors include 10 Culex spp., eight belonging to the Melanoconion subgenus, Spissipes section: Cx. (Mel) dunnii (Dyar, 1918), Cx. (Mel) pedroii (Sirivanakam & Belkin, 1980), Cx. (Mel) spissipes (Theobald, 1903), Cx. (Mel) adamesi (Sirivanakam & Galindo, 1980), Cx. (Mel) crybda (Dyar, 1924), Cx. (Mel) vomerifer (Komp, 1932), Cx. (Mel) occosa (Dyar & Knab, 1919) and Cx. (Mel) taeniopus (Dyar & Knab, 1907); two belonging to the subgenus Culex: Cx. nigripalpus (Theobald, 1901), Cx. declarator (Dyar & Knab, 1906). Four species belong to the genus Aedes subgenus Ochlerotatus: Ae. scapularis (Rondani, 1848), Ae. angustivittatus (Dyar & Knab, 1907), Ae. serratus (Theobald, 1901) and Ae. fulvus (Wiedemann, 1828). Other species of importance for VEEV transmission included: Coquillettidia venezuelensis, Psorophora ferox (Humboldt, 1819), Ps. albipes (Theobald, 1907), Ps. confinis (Theobald, 1887), Mansonia indubitans (Dyar & Shannon, 1925), M. titillans (Walker, 1848), M. dyari (Belkin, Heinemann & Page 1970) and An. pseudopunctipennis (Theobald 1901). Several of these species are known to have catholic bloodfeeding habits in the República de Panamá [46], an essential condition to facilitate the transmission of enzootic arboviruses [47], and, more generally, a common pattern observed in mosquito communities studied elsewhere [7,48].

The widespread importance of Culex (Melanoconion) spp for the transmission of VEEV has been well documented all over Latin America. Specifically, Cx. (Mel.) vomerifer, Cx. (Mel.) pedroii and Cx. (Mel.) adamesi have been found infected with subtype ID in the Magdalena Valley, Colombia [3,47,49,50]. Cx. (Mel.) pedroii has also been found infected with VEEV in Puerto Almendras, Perú [51,52]. Cx. (Mel) taeniopus is a vector of VEEV subtype IE in México and Central America [9,53]. Similarly, EEEV has been isolated from Cx. (Mel.) pedroii and Cx. (Mel) taeniopus [54]. Cx. (Mel) vomerifer from Iquitos, Perú is also susceptible to VEEV [9] and Caraparu virus infection [55,56]. In the República de Panamá Cx. (Mel) aikeni s. l., Cx. (Mel) taeniopus and Cx. (Mel) vomerifer have been found infected with VEEV subtype ID [10,53,57]. VEEV has been isolated from Cx. (Mel) erraticus, Cx. (Mel) occosa and M. dyari in Lake Bayano, Panamá [12,58–60]. C. venezuelensis is associated with permanent water bodies with floating vegetation [61]. It is a vector of Mayaro, Oropuche, VEE and SLE viruses [33,62,63] and West Nile virus [64]. VEEV has also been isolated from Ps. ferox and Ps. albipes [65]. VEEV subtypes IC and IAB have been isolated from M. indubitans, M. titillans, M. dyari, Ps. confinis and An. pseudopunctipennis [13,66,67]. Ps. albipes, Ae. serratus and Ae. fulvus are susceptible to the infection with VEE [13,68]. Ae. angustivittatus has been found infected with Ilheus virus in Panamá and VEEV in Colombia [69–71]. Ae. scapularis has been incriminated as VEEV vector in epizootic and enzootic outbreaks[70,72,73]. Cx. nigripalpus was collected at
the three sites in Darién. This species is able to colonize urban and rural landscapes and exhibits a catholic bloodfeeding [74,75]. This species is a major SLE virus vector in the USA [76], but also in Central America, Ecuador and Trinidad and Tobago [77]. Cx. coronator, also collected in this study at Darién, has an ecology similar to that of Cx. nigripalpus and has been found infected with SLE virus [71] and Mucambo virus in the Brazilian Amazon [78].

Co-occurring with the VEEV vectors we also found the two most important malaria vector species in the República de Panamá [79,80]: An. (Nys) albimanus (Wiedemann, 1820), An. (An) punctimacula (Dyar & Knab, 1906). We also were able to identify several secondary malaria vectors, including: An. pseudopunctipennis, An. malefactor (Dyar & Knab, 1907), An. neomaculipalpus (Curry, 1930), An. apicimacula (Theobald, 1901), An. oswaldoi (Peryassú, 1922) and An. triannulatus (Neiva & Pinto, 1922) [80–82]. In general, these malaria vectors were less common than VEEV vectors (Table 1).

A major limitation of our study was our inability to identify a large proportion of Culex spp. mosquitoes and other specimens that we were only able to identify at the genus level (29.8%; 1453/4868). This was mostly due to poor specimen conditions, but also to some mosquitoes having distinctive features from those of species described in taxonomic keys for mosquito species of the New World. In that sense it would be desirable to develop a barcoding library to molecularly identify all mosquitoes present in the República de Panamá, as has been done elsewhere [83]. This can help to both aid the description of new species and with the identification of morphologically damaged specimens. A second limitation was the sampling during night time, which could have limited the possibility of sampling Haemagogus spp, of which we only found one mosquito, and Sabethes spp, which we did not collect. Both Haemagogus and Sabethes are genera with species known to be active during daytime, and which include some species that are medically important, given their role in the transmission of yellow fever virus, another major arbovirus [84,85]. Similarly, the study would have greatly benefited by sampling mosquitoes in areas where no alphavirus transmission has been detected, in order to better understand the role of dominant vector species on disease transmission [38,86] or mosquito diversity on infection [41], while also looking at domestic and wildlife reservoirs, as done for other zoonotic vector borne diseases, for example Leishmaniasis [87–90], in the República de Panamá and for alphaviruses in other regions of Latin America [9].

Finally, we would like to highlight this report is the first to describe the mosquito fauna of locations that have frequently reported VEE outbreaks in the República de Panamá. We were able to identify 22 species that are vectorially competent for VEEV transmission, and other species that also transmit medically important arboviruses and parasites across the New World [9,23,44]. This result is very important as it is a first step for further research looking at the ecology of VEEV-mosquito interactions in order to better understand the enzootic transmission of this and related viruses, especially the invasion of new areas by VEEV [51,91], as well as, transmission during the inter-epizootic periods in the República de Panamá. Further research is needed to better understand why, even though all our study sites had a similar environment, where primary and secondary forest were mixed with cattle farming and agricultural land, in places like the sites in Panamá province there were very few Culex (Melanoconion) spp, even though they have been found previously in this region [10,92], and they were common in the two other study regions.

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References
1. Atasheva S, Kim DY, Akhrymuk M, Morgan DG, Frolova EI, Frolov I (2012) Pseudoinfectious Venezuelan Equine Encephalitis Virus: a New Means of Alphavirus Attenuation. Journal of Virology 87: 2023–2035. https://doi.org/10.1128/JVI.02881-12 PMID: 23221545
2. Arechiga Ceballos N, Aguilar Setien A (2015) Alphaviral equine encephalomyelitis (Eastern, Western and Venezuelan). Revue Scientifique et Technique de l’OIE 34: 491–501. PMID: 26601451
3. Atasheva S, Kim DY, Frolova EI, Frolov I (2014) Venezuelan Equine Encephalitis Virus Variants Lacking Transcription Inhibitory Functions Demonstrate Highly Attenuated Phenotype. Journal of Virology 89: 71–82. https://doi.org/10.1128/JVI.02252-14 PMID: 25320296
4. Carrera J-P, Forrestner N, Wang E, Vittor AY, Haddow AD, López-Vergès S, et al. (2013) Eastern Equine Encephalitis in Latin America. New England Journal of Medicine 369: 732–744. https://doi.org/10.1056/NEJMoa1212628 PMID: 23964935
5. Barrera R, Liria J, Salas R, Boshell J, Vasquez C, Ahumada M, et al. (2002) Contrasting sylvatic foci of Venezuelan equine encephalitis virus in northern South America. The American Journal of Tropical Medicine and Hygiene 67: 324–334. PMID: 12408676
6. Vittor AY, Armien B, Gonzalez P, Carrera J-P, Dominguez C, Valderrama A, et al. (2016) Epidemiology of Emergent Madariaga Encephalitis in a Region with Endemic Venezuelan Equine Encephalitis: Initial Host Studies and Human Cross-Sectional Study in Darien, Panama. PLOS Neglected Tropical Diseases 10: e0004554. https://doi.org/10.1371/journal.pntd.0004554 PMID: 27101567
7. Molaei G, Thomas MC, Muller T, Medlock J, Shepard JJ, Armstrong PM, et al. (2016) Dynamics of Vector-Host Interactions in Avian Communities in Four Eastern Equine Encephalitis Virus Foci in the Northeastern U.S. PLOS Neglected Tropical Diseases 10: e0004347. https://doi.org/10.1371/journal.pntd.0004347 PMID: 26751704
8. Aguilar PV, Estrada-Franco JG, Navarro-Lopez R, Ferro C, Haddow AD, Weaver SC (2011) Endemic Venezuelan equine encephalitis in the Americas: hidden under the dengue umbrella. Future virology 6: 721–740. https://doi.org/10.2217/FVL.11.5 PMID: 21765860
9. Weaver SC, Ferro C, Barrera R, Boshell J, Navarro J-C (2004) Venezuelan equine encephalitis. Annual Reviews in Entomology 49: 141–174.
10. Galindo P, Grayson MA (1971) Culex (Melanoconion) aikenii: Natural Vector in Panama of Endemic Venezuelan Encephalitis. Science 172: 594–595. PMID: 5555082
11. Cupp EW, Ordonez JV, Scherer WF (1979) Transmission of Venezuelan Encephalitis Virus by Naturally Infected Culex (Melanoconion) Opisthopus. The American Journal of Tropical Medicine and Hygiene 28: 1060–1063. PMID: 5072883
12. Grayson MA, Galindo P (1968) Epidemiologic studies of Venezuelan Equine Encephalitis Virus in Almirante, Panama. American Journal of Epidemiology 88: 80–96. PMID: 5690806

13. Turell MJ, Jones JW, Sardelis MR, Dohm DJ, Coleman RE, Watts DM, et al. (2000) Vector Competence of Peruvian Mosquitoes (Diptera: Culicidae) for Epizootic and Enzootic Strains of Venezuelan Equine Encephalomyelitis Virus. Journal of Medical Entomology 37: 835–839. PMID: 11265377

14. Petrov A, Lebedev V, Kulish V, Pyshnaya N, Stovba L, Borisevich S (2014) Epidemiologic analysis of outbreaks of diseases caused by American equine encephalitis causative agents in endemic regions. Zhurnal mikrobiologii, epidemiologii, i immunobiologii: 103–110.

15. Galindo P (1978) Los arbovirus de Panamá. Revista Médica de Panamá 3: 1–41.

16. Powers AM, Oberste MS, Brault AC, Rico-Hesse R, Schmura SM, Smith JF, et al. (1997) Repeated emergence of epidemic/epizootic Venezuelan equine encephalitis from a single genotype of enzootic subtype ID virus. Journal of virology 71: 6697–6705. PMID: 9261393

17. Wang E, Barrera R, Boshell J, Ferro C, Freier JE, Navarro JC, et al. (1999) Genetic and phenotypic changes accompanying the emergence of epizootic subtype IC Venezuelan equine encephalomyelitis viruses from an enzootic subtype ID progenitor. Journal of virology 73: 4266–4271. PMID: 10196323

18. Brault AC, Powers AM, Holmes EC, Woelk CH, Weaver SC (2002) Positively Charged Amino Acid Substitutions in the E2 Envelope Glycoprotein Are Associated with the Emergence of Venezuelan Equine Encephalitis Virus. Journal of Virology 76: 1718–1730. https://doi.org/10.1128/JVI.76.4.1718-1730.2002 PMID: 11799167

19. Galindo P, Srihongse S, De Rodaniche E, Grayson MA (1966) An Ecological Survey for Arboviruses in Almirante, Panama, 1959–1962. The American Journal of Tropical Medicine and Hygiene 15: 385–400. PMID: 4380043

20. Kesler RA (1937) Equine encephalomyelitis in Panama. Vet Bull 31: 19–21.

21. Obaldia N, Dutary B, Clavel F (1991) Encefalomielitis equina del este, epizootia de 1986 en Panamá. Notas Veterinarias Assoc Panameña de Médicos Veterinarios 1: 4–7.

22. Solomon T (2004) Flavivirus Encephalitis. New England Journal of Medicine 351: 370–378. https://doi.org/10.1056/NEJMra03076 PMID: 15269317

23. Ferro MC, Olanov VA, Ahumada M, Weaver S (2008) Mosquitoes (Diptera: Culicidae) in the caserio of Chingalé, Santander, donde se registró un caso humano de encefalitis equina venezolana. Biomédica 28: 234. PMID: 18719725

24. Sallum M, Forattini OP (1996) Revision of the Spissipes Section of Culex (Melanoconion) (Diptera: Culicidae). Journal of the American Mosquito Control Association 12: 517–600. PMID: 8887711

25. Ferro C, Boshell J, Moncayo AC, Gonzalez M, Ahumada ML, Kang W, et al. (2003) Natural Enzootic Vectors of Venezuelan equine encephalitis virus in the Magdalena Valley, Colombia. Emerging Infections Diseases 9: 49–54. https://doi.org/10.3201/eid0901.020136 PMID: 12533281

26. Johnson KM, Young NA, Dammin GJ, Peralta PH, Shelokov A (1968) Recovery of Venezuelan Equine Encephalomyelitis Virus in Panama. The American Journal of Tropical Medicine and Hygiene 17: 432–440. PMID: 5690051

27. Carrera J-P, Guzman H, Beltrán D, Díaz Y, López-Vergés S, Torres-Cosme R, et al. (2015) Mercadeo Virus: A Novel Mosquito-Specific Flavivirus from Panama. The American Journal of Tropical Medicine and Hygiene 93: 1014–1019. https://doi.org/10.4269/ajtmh.15-0117 PMID: 26304915

28. Autoridad Nacional del Ambiente (2010) Atlas Ambiental de la República de Panamá. Ciudad de Panamá: Editora Novo Art. 187 p.

29. Hidrometeorología Dd (2014) Estadística Panameña. Situación física. Panamá: ETESA. pp. 38.

30. Chamberlain R, Sudia D (1967) Methods for study of mosquitoes as virus hosts and vectors. Methods Virol 1: 63–103.

31. Silver JB (2008) Mosquito ecology: field sampling methods. New York: Springer. 1498 p.

32. Lane J (1953) Neotropical culicidae, vol. 2. University of Sao Paulo, Sao Paulo, Brazil.

33. Forattini O (2002) Culicidologia médica: identificación, biología e epidemiología, Vol. II. EDUSP, São Paulo.

34. Colwell RK, Ebersohn JE (2014) EstimateS turns 20: statistical estimation of species richness and shared species from samples, with non-parametric extrapolation. Ecology 37: 609–613.

35. Krebs CJ (1998) Ecological Methodology: Benjamin Cummings. 624 p.

36. Reisen WK (2014) Medical entomology—Back to the future? Infection, Genetics and Evolution 28: 573–582. https://doi.org/10.1016/j.meegid.2013.11.025 PMID: 24316291
Enzootic mosquito vector species of equine encephalitis

37. Reisen WK (2012) The contrasting bionomics of Culex mosquitoes in western North America. Journal of the American Mosquito Control Association 28: 82–91. https://doi.org/10.2987/8756-971X-28.4.82 PMID: 23401947

38. Chaves LF (2017) Climate change and the biology of insect vectors of human pathogens. In: Johnson S, Jones H, editors. Invertebrates and Global Climate Change. Chichester, UK: Wiley. pp. 126–147.

39. Edman JD (1988) Disease control through manipulation of vector-host interaction: some historical and evolutionary perspectives. In: Scott TW, Grumstrup-Scott J, editors. Proceedings of a Symposium: The Role of vector-host interactions in disease transmission. Washington, D.C.: Entomological Society of America. pp. 43–50.

40. Hoshi T, Imanishi N, Higa Y, Chaves LF (2014) Mosquito Biodiversity Patterns Around Urban Environments in South-Central Okinawa Island, Japan. Journal of the American Mosquito Control Association 30: 260–267. https://doi.org/10.2987/14-6432R.1 PMID: 25843131

41. Chaves LF, Hamer GL, Walker ED, Brown WM, Ruiz MO, Kitron UD (2011) Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. Ecosphere 2: art70.

42. Chaves LF, Añez N (2016) Nestedness patterns of sand fly (Diptera: Psychodidae) species in a neotropical semi-arid environment. Acta Tropica 153: 7–13. https://doi.org/10.1016/j.actatropica.2015.10.001 PMID: 26456179

43. Chaves LF, Añez N (2004) Species co-occurrence and feeding behavior in sand fly transmission of American cutaneous leishmaniasis in western Venezuela. Acta Tropica 92: 219–224. https://doi.org/10.1016/S0001-706X(04)00047-2 PMID: 15039320

44. Weaver SC, Barrett ADT (2004) Transmission cycles, host range, evolution and emergence of arboviral diseases. Nat Rev Micro 2: 789–801.

45. Quiroz E, Aguilar PV, Cisneros J, Tesh RB, Weaver SC (2009) Venezuelan Equine Encephalitis in Panama: Fatal Enzootic Disease and Genetic Diversity of Etiologic Viral Strains. PLoS Neglected Tropical Diseases 3: e472. https://doi.org/10.1371/journal.pntd.0000472 PMID: 19564908

46. Navia-Gine WG, Loaiza JR, Miller MJ (2013) Mosquito-Host Interactions during and after an Outbreak of Equine Viral Encephalitis in Eastern Panama. PLOS ONE 8: e81788. https://doi.org/10.1371/journal.pone.0081788 PMID: 24339965

47. Weaver SC, Reisen WK (2010) Present and future arboviral threats. Antiviral Research 85: 328–345. https://doi.org/10.1016/j.antiviral.2009.10.008 PMID: 19857523

48. Chaves LF, Harrington LC, Keogh CL, Nguyen AM, Kitron UD (2010) Blood feeding patterns of mosquitoes: random or structured? Frontiers in Zoology 7: 3. https://doi.org/10.1186/1742-9994-7-3 PMID: 20205866

49. Groot H, Morales A, Romero M, Ferro C, Prias E, Vidalles H, et al. (1996) Estudios de arbovirosis en Colombia en la década de 1970. Biomédica 16: 331.

50. Dickerman RW, Cupp EW, Groot H, Morales Alarcon A, Cura EN, Dickerman A, et al. (1986) Venezuelan equine encephalitis virus activity in northern Colombia during April and May 1983. PMID: 2879583

51. Turell MJ, O’guinn ML, Jones JW, Sardelis MR, Dohm DJ, Watts DM, et al. (2005) Isolation of Viruses from Mosquitoes (Diptera: Culicidae) Collected in the Amazon Basin Region of Peru. Journal of Medical Entomology 42: 891–898. https://doi.org/10.1603/0022-2585(2005)042[0891:IOVFM]2.0.CO;2 PMID: 16366001

52. O’guinn ML, Lee JS, Kondig JP, Fernandez R, Carabajal F (2004) Field detection of eastern equine encephalitis virus in the Amazon Basin region of Peru using reverse transcription-polymerase chain reaction adapted for field identification of arthropod-borne pathogens. The American Journal of tropical medicine and hygiene 70: 164–171. PMID: 14993628

53. Deardorf ER, Weaver SC (2010) Vector Competence of Culex (Melanoconion) taeniopus for Equine-Virulent Subtype IE Strains of Venezuelan Equine Encephalitis Virus. The American Journal of Tropical Medicine and Hygiene 82: 1047–1052. https://doi.org/10.4269/ajtmh.2010.09-0556 PMID: 20519599

54. Srihongs S, Galindo P (1967) The isolation of eastern equine encephalitis virus from Culex (Melanoco- nion) taeniopus Dyar and Knab in Panama. Mosq News 27: 74–76.

55. Causey OR, Causey CE, Maroja OM, Macedo DG (1961) The Isolation of Arthropod-Borne Viruses, including Members of Two Hitherto Undescribed Serological Groups, in the Amazon Region of Brazil. The American Journal of Tropical Medicine and Hygiene 10: 227–249. PMID: 13691675

56. Coimbra TLM, Nassar ES, Nagamori AH, Ferreira IE, Pereira LE, Rocco IM, et al. (1993) Iguape: A Newly Recognized Flavivirus from São Paulo State, Brazil. Intervirology 36: 144–152. PMID: 8150595

57. Galindo P (1972) Endemic vectors of Venezuelan encephalitis. PAHO Sc Publ 243: 249–253.

58. Galindo P, Adames A, Peralta P, Johnson C, Read R (1983) Impacto de la hidroeléctrica de Bayano en la transmisión de arbovirus. Revista médica de Panamá 8: 89–134. PMID: 6878761
59. Adames A, Peralta P, Saenz R, Johnson C, Read R (1979) Brote de Encefalomielitis Equina Venezolana (VEE) durante la formación del lago Bayano en Panamá. 1977. Revista Médica Panamá 4: 246–257.

60. Dutary BE, Peralta PH, Petersen JL (1984) Estudios biológicos del virus de la encefalitis de San Luis en Mage, Bayano. Rev méd Panamá 9: 200–211. PMID: 6149603

61. Lopes J, Lozovei AL (1995) Ecologia de mosquitos (Diptera: Culicidae) em criadouros naturais e artificiais de área rural do Norte do Estado do Paraná, Brasil: I—Coletas ao longo do leito de ribeirão. Revista de Saúde Pública 29: 183–191. PMID: 8539529

62. Aitken THG, Downs WG, Anderson CR, Spence L, Casals J (1960) Mayaro Virus Isolated from a Trinidian Mosquito, Mansonia venezuelensis. Science 131: 986–986.

63. Hervé JP, Déglainier N, Rosa A, Pinheiro FdP, Sa Filho GC (1986) Aspectos ecológicos. Instituto Evandro Chagas: 50 anos de contribuição às ciências biológicas e à medicina tropical: Fundação Serviços de Saúde Pública. pp. 409–437.

64. Velásquez G, Ulloa A, Montañez H, Guimarães A, Maldonado A, Bastardo J (2013) Evidence of the presence of West Nile Virus in mosquito pools in North Eastern Region of Venezuela.

65. Groot H, Sanmartín C, Vidal H, Kerr JA (1959) Antibodies to Yellow Fever and Other Arthropod-Borne Viruses in Human Residents of San Vicente de Chucuri, Santander, Colombia *. The American Journal of Tropical Medicine and Hygiene 8: 175–189. PMID: 13637316

66. Smith DR, Adams AP, Kenney JL, Wang E, Weaver SC (2008) Venezuelan equine encephalitis virus in the mosquito vector Aedes taeniorhynchus: Infection initiated by a small number of susceptible epithelial cells and a population bottleneck. Virology 372: 176–186. https://doi.org/10.1016/j.virol.2007.10.011 PMID: 18023837

67. Sudia W, Newhouse V (1971) Venezuelan equine encephalitis in Texas, 1971. Informational report. Mosquito News 31: 350–351.

68. Méndez W, Liria J, Navarro J-C, García CZ, Freier JE, Salas R, et al. (2001) Spatial Dispersion of Adult Mosquitoes (Diptera: Culicidae) in a Sylvatic Focus of Venezuelan Equine Encephalitis Virus. Journal of Medical Entomology 38: 813–821. PMID: 11761379

69. Parra-Henao G, Suárez L (2012) Mosquitos (Diptera: Culicidae) vectores potenciales de arbovirus en la región de Urabá, noroccidente de Colombia. Biomédica 32.

70. Forattini OP, Kakitani I, Massad E, Marucci D (1995) Studies on mosquitoes (Diptera: Culicidae) and anthropic environment: 9- Synanthropy and epidemiological vector role of Aedes scapularis in South-Eastern Brazil. Revista de Saúde Pública 29: 199–207. PMID: 8539531

71. Sanmartín C (1973) Venezuelan equine encephalitis in Colombia, 1967. Boletín de la Oficina Sanitaria Panamericana 74: 109–137. PMID: 4265714

72. Forattini OP, Gomes AD, Natal D, Kakitani I, Marucci D (1995) Preferências alimentares e domiciliação de mosquitos Culicidae no Vale do Ribeira, São Paulo, Brasil, com especial referência a Aedes scapularis e a Culex (Melanoconion). Revista de Saúde Pública 23: 9–19.

73. Forattini OP, Gomes AD (1988) Biting activity of Aedes scapularis (Rondani) and Haemagogus mosquitoes in Southern Brazil (Diptera: Culicidae). Revista de Saúde Pública 22: 84–93. PMID: 2905827

74. Adames A, Dutary B, Tejera H, Adames E, Galindo P (1979) Brote de Encefalomielitis Equina Venezolana (VEE) durante la formación del lago Bayano en Panamá. Malaria Journal 14: 459. https://doi.org/10.1186/s12936-015-0987-6 PMID: 26578076

75. Guimarães AÉ, Gentile C, Lopes CM, Sant’Anna A, Jovita AM (2000) Ecologia de mosquitos (Diptera: Culicidae) em áreas do Parque Nacional da Serra da Bocaina, Brasil.I.—Distribuição por habitat. Revista de Saúde Pública 34: 243–250. PMID: 10920446

76. Day JF, Curtis GA (1999) Blood Feeding and Oviposition by Culex nigripalpus (Diptera: Culicidae) Before, During, and After a Widespread St. Louis Encephalitis Virus Epidemic in Florida. Journal of Medical Entomology 36: 176–181. PMID: 10083754

77. Kopp A, Gillespie TR, Hobelsberger D, Estrada A, Harper JM, Miller RA, et al. (2013) Provenance and Geographic Spread of St. Louis Encephalitis Virus. mBio 4.

78. Vasconcelos PFdC, Travassos da Rosa JFS, Travassos da Rosa APdA, Déglainier N, Pinheiro FdP, Sá Filho GC (1991) Epidemiologia das encefalites por arbovírus na amazônia brasileira. Revista do Instituto de Medicina Tropical de São Paulo 33: 465–476. PMID: 1844977

79. Calzada JE, Marquez R, Rigg C, Victoria C, De La Cruz M, Chaves LF, et al. (2015) Characterization of a recent malaria outbreak in the autonomous indigenous region of Guna Yala, Panama. Malaria Journal 14: 459. https://doi.org/10.1186/s12936-015-0987-6 PMID: 26578076

80. Loaiza JR, Bermingham E, Scott ME, Rovira JR, Conn JE (2008) Species Composition and Distribution of Adult Anopheles (Diptera: Culicidae) in Panama. Journal of Medical Entomology 45: 841–851. PMID: 18826029
81. Rubio-Palis Y, Bevilacqua M, Medina DA, Moreno JE, Cárdenas L, Sánchez V, et al. (2013) Malaria entomological risk factors in relation to land cover in the Lower Caura River Basin, Venezuela. Memórias do Instituto Oswaldo Cruz 108: 220–228. https://doi.org/10.1590/0074-0276108022013015 PMID: 23579603

82. Rubio-Palis Y, Moreno JE, Bevilacqua M, Medina D, Martínez Á, Cardenas L, et al. (2010) Caracterización ecológica de los anofelinos y otros culicidos en territorio indígena del Bajo Caura, Estado Bolívar, Venezuela. Boletín de Malariología y Salud Ambiental 50: 95–107.

83. Taira K, Toma T, Tamashiro M, Miyagi I (2012) DNA barcoding for identification of mosquitoes (Diptera: Culicidae) from the Ryukyu Archipelago, Japan. Medical Entomology and Zoology 63: 289–306.

84. de Rodaniche E, Galindo P, Trapido H (1956) Experimental transmission of yellow fever by Central American species of Haemagogus and Sabethes chloropterus. American Journal of Tropical Medicine and Hygiene 5: 1022–1031. PMID: 13381877

85. Galindo P, Carpenter SJ, Trapido H (1951) Ecological observations on forest mosquitoes of an endemic yellow fever area in Panama. American Journal of Tropical Medicine 31: 98–137.

86. Chaves LF (2016) Globally invasive, withdrawing at home: *Aedes albopictus* and *Aedes japonicus* facing the rise of *Aedes flavopictus*. International Journal of Biometeorology 60: 1727–1738. https://doi.org/10.1007/s00484-016-1162-7 PMID: 27039106

87. González K, Calzada JE, Saldaña A, Rigg C, Alvarado G, Rodríguez-Herrera B, et al. (2015) Survey of Wild Mammal Hosts of Cutaneous Leishmaniasis Parasites in Panamá and Costa Rica. Tropical Medicine and Health 43: 75–78. https://doi.org/10.2149/tmh.2014-30 PMID: 25859156

88. Calzada JE, Saldaña A, González K, Rigg C, Pineda V, Santamaría AM, et al. (2015) Cutaneous Leishmaniasis in dogs: is high seroprevalence indicative of a reservoir role? Parasitology 142: 1202–1214. https://doi.org/10.1017/S0031182015000475 PMID: 25990429

89. Calzada JE, Saldaña A, Rigg C, Valderrama A, Romero L, Chaves LF (2013) Changes in phlebotomine sand fly species composition following insecticide thermal fogging in a rural setting of western Panamá. PLoS One 8: e53289. https://doi.org/10.1371/journal.pone.0053289 PMID: 23536748

90. Chaves L, Calzada J, Rigg C, Valderrama A, Gottdenker N, Saldaña A (2013) Leishmaniasis sand fly vector density reduction is less marked in destitute housing after insecticide thermal fogging. Parasites & Vectors 6: 164.

91. Salas RA, García CZ, Liria J, Barrera R, Navarro JC, Medina G, et al. (2001) Ecological studies of enzootic Venezuelan equine encephalitis in north-central Venezuela, 1997–1998. The American Journal of Tropical Medicine and Hygiene 64: 84–92. PMID: 11425168

92. Eastwood G, Loaiza JR, Pongsiri MJ, Sanjur OI, Pecor JE, Auguste AJ, et al. (2016) Enzootic Arbovirus Surveillance in Forest Habitat and Phylogenetic Characterization of Novel Isolates of Gamboa Virus in Panama. The American Journal of Tropical Medicine and Hygiene 94: 786–793. https://doi.org/10.4269/ajtmh.15-0445 PMID: 26834200