Evaluation of Vector-Enabled Xenosurveillance in Rural Guatemala

Rebekah J. McMinn,1 Andrea Chacon,2 Claudia Rückert,1,3 Valeria Scorca,1 Michael C. Young,1 Delaney Worthington,1 Molly M. Lamb,1 Ramon E. Medrano,5 Emma K. Harris,1 Kareen Arias,6 Maria Renee Lopez,5 Edwin J. Asturias,3,4,7 Brian D. Foy,1 Mark D. Stenglein,1 Daniel Olson,4,6,7* and Gregory D. Ebel1*

1Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado; 2Fundacion para la Salud Integral de los Guatemaltecos, Retalhuleu, Guatemala; 3Department of Biochemistry and Molecular Biology, University of Nevada, Reno, Nevada; 4Colorado School of Public Health, Aurora, Colorado; 5Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala City, Guatemala; 6Center for Human Development, Retalhuleu, Guatemala; 7Department of Pediatrics, Section of Infectious Diseases, University of Colorado School of Medicine, Aurora, Colorado

Abstract. Surveillance methods that permit rapid detection of circulating pathogens in low-resource settings are desperately needed. In this study, we evaluated a mosquito bloodmeal-based surveillance method (“xenosurveillance”) in rural Guatemala. Twenty households from two villages (Los Encuentros and Chiquirines) in rural southwest Guatemala were enrolled and underwent weekly prospective surveillance from August 2019 to December 2019 (16 weeks). When febrile illness was reported in a household, recently blood-fed mosquitoes were collected from within dwellings and blood samples taken from each member of the household. Mosquitoes were identified to species and blood sources identified by sequencing. Shotgun metagenomic sequencing was used to identify circulating viruses. Culex pipiens (60.9%) and Aedes aegypti (18.6%) were the most abundant mosquitoes collected. Bloodmeal sources were most commonly human (32.6%) and chicken (31.6%), with various other mammal and avian hosts detected. Several mosquito-specific viruses were detected, including Culex orthophasma virus. Human pathogens were not detected. Therefore, xenosurveillance may require more intensive sampling to detect human pathogens in Guatemala and ecologically similar localities in Central America.

INTRODUCTION

The ability to detect circulating pathogens rapidly in remote, resource-limited areas is inhibited by a lack of a surveillance infrastructure. Rural populations with limited access to healthcare and biomedical support are frequently excluded from traditional surveillance methods due to extreme logistical complexities.1,2 Noninvasive surveillance in these settings could have a significant impact by enabling early detection of viruses. Xenosurveillance is a novel surveillance approach that takes advantage of mosquito feeding behavior to identify bloodborne pathogens that may be circulating in human and animal hosts. This approach circumvents invasive blood sampling of individuals and results in an abundant sample source derived from both humans and animals. We therefore have proposed that xenosurveillance may be a useful method for disease surveillance in rural, resource-limited areas.

The detection of bacteria, viruses, and parasites from mosquito bloodmeals via membrane and animal blood feedings has been previously demonstrated.3–5 Using field-caught blood-fed Anopheles mosquitoes from Liberia and Senegal, we previously detected non–mosquito-borne viruses such as canine distemper virus, Epstein–Barr virus, GB virus C, and hepatitis B virus.3,6 The utility of this approach in the American tropics, however, where the main human-biting mosquitoes are Aedes and Culex, not Anopheles, has not been addressed.

Accordingly, in the present study, we evaluated the utility of xenosurveillance in rural southwest Guatemala, where Culex and Aedes mosquitoes are highly abundant. We conducted xenosurveillance and human-directed biosurveillance in parallel to evaluate the logistical and technical feasibility to detect circulating bloodborne pathogens in humans over the course of a 16-week study. We also determined the species composition of mosquitoes collected from within Guatemalan dwellings and their host feeding preferences.

MATERIALS AND METHODS

Study site. This study was performed through the Center for Human Development located in the coastal lowlands of southwestern Guatemala, approximately 20 km from the border with Chiapas, Mexico.7 The population in the region suffers from high levels of food insecurity, poverty, and poor access to healthcare; diarrheal, respiratory, and other communicable diseases are frequent, especially in children.7,8 Many households use pit latrines, which can flood during heavy rain and create ideal environments for mosquito breeding. Chickens, ducks, pigs, rats, dogs, and cats are common in households and have close contact with families.

Study design/enrollment. Twenty households from two villages, Los Encuentros and Chiquirines, adjacent to the Center for Human Development, were enrolled in August 2019. Requirements for enrollment included having at least three animals in the household and ability and willingness to consent to participate. Once consent was obtained, capillary blood samples of all enrolled humans in addition to mosquito bloodmeals (described subsequently) were collected at enrollment. We also collected epidemiologic data, including demographics, animals (indoor, outdoor, grazing), and risk factors for infection (water features, septic system, mosquito exposure, contact with animals, etc.) through participant interviews. The study was approved by the Colorado Multiple Institutional Review Board (COMIRB) and the University del Valle de Guatemala (UVG) Ethics Committee. The local Community Advisory Board for Research agreed to the study. The protocols for blood extraction and animal handling were approved by the Colorado State University Institutional Animal
Sciencies, Chicago, IL) and soaked in 70% using a Harris 3-mm micro-puncher (GE Healthcare Life Sciences) extract solution for 8 to 18 hours at 4°C. Mosquito samples were collected at enrollment and during symptomatic illness as described earlier. The study team was prepared to provide supportive medications to symptomatic household members and triage individuals to higher levels of care if they exhibited WHO danger signs. However, no such danger signs were observed over the course of the study.

Sample collection and pre-processing. Human and mosquito samples were collected at enrollment and during symptomatic illness as described earlier. Mosquito bloodmeals. Local technicians were trained on mosquito collection, identification, and sample processing methods before the start of the study. Mosquitoes were collected using an InsectaZooka aspirator (BioQuip Products, Rancho Dominguez, CA) from the indoor areas of enrolled households, specifically targeting resting blood-fed mosquitoes on the walls and surfaces. Attempts to collect mosquitoes were usually made in the morning and placed in a −20°C freezer for at least 2 hours or overnight to ensure death and processed within 24 hours of collection. Blood-fed mosquitoes (female) were identified according to a simplified version of a previously published key for mosquitoes in Guatemala and individual mosquito bloodmeals were expressed onto FTA cards (Whatman, Maidstone, United Kingdom). Forceps were dipped in ethanol and wiped clean between each mosquito. Fifty microliters of RNA later was added to each mosquito dried blood spot (M-DBS) at the end of each processing session.

Human capillary blood. Blood was collected from all members of the household during enrollment and when a febrile illness occurred in the household. The subject’s index finger was disinfected with an alcohol swab before using a sterile lancet to prick the finger. Blood was expressed from the finger and dabbed onto a labeled FTA card, and 100 μL of RNA was added to each human dried blood spot (H-DBS) by the end of the day. FTA cards were stored at −80°C until they were shipped (at room temperature) to Colorado State University, where they were again stored at −80°C until further processing.

RNA/DNA extraction. H-DBS and M-DBS were removed using a Harris 3-mm micro-puncher (GE Healthcare Life Sciences, Chicago, IL) and soaked in 70% RNA rapid extract solution for 8 to 18 hours at 4°C. Fifty microliters was subsequently used for nucleic acid extraction using the Mag-Bind Viral DNA/RNA kit (Omega Bio-Tek, Norcross, GA) with the King Fisher Flex Magnetic Particle Processor (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s protocol. Nucleic acid was eluted in 50 μL nuclease-free water and stored at −80°C until further processing.

Mosquito speciation and bloodmeal identification. Mosquito species was determined for all M-DBS by polymerase chain reaction (PCR) and Sanger sequencing using primers directed against the cytochrome oxidase I (COI) gene as described previously. To identify the mosquito bloodmeal host source, COI DNA was PCR amplified with M13-tagged primers as previously described. PCR products were separated on an agarose gel, and the corresponding ~800-bp piece was excised. DNA was purified using the NucleoSpin Gel and PCR Clean-Up kit (Machery-Nagel, Allentown, PA) and Sanger sequenced with M13 primers. Obtained sequences were trimmed for quality and blasted against the Barcode of Life COI database to identify the bloodmeal source. Samples with no visible band or poor-quality sequencing were rerun before being designated as “No Match.”

Shotgun metagenomic sequencing library preparation. A subset of H-DBS and M-DBS from seven symptomatic episodes, comprising 43.6% of all samples, were used to identify circulating viruses using shotgun metagenomic sequencing. Sample sets (all DBS from a household collection event) were chosen based on the week in study, number of symptomatic individuals, number of mosquito samples, and recurrent sampling events. DBS nucleic acid samples were pooled by volume according to time of collection and household. A total of seven human and seven mosquito libraries were generated and sequenced along with water and a blank extracted FTA card as negative controls (Supplemental Table 1). RNA extracted from HeLa cells was used as a positive control for virus detection. Solid phase reversible immobilization (SPRI) bead clean-up was used between each step in the following library preparation protocol. Samples were DNAse-treated and rRNA-depleted using methods described previously. Previously designed mosquito probes were used on M-DBS and human probes (NEB) were used on H-DBS and HeLa cell RNA control. Water and FTA-negative controls received both human and mosquito probes. The sequence-independent, single primer amplification (SISPA) was used to generate and amplify cDNA as described previously. Briefly, cDNA was created via the Superscript IV First-Strand Synthesis System (Invitrogen, Waltham, MA) following the standard protocol, except for the use of tagged random primers: CATAGTCGTAGCTACATC-(Nx12). Second strand synthesis was performed using a Klenow DNA polymerase I fragment (NEB). Primers aligning to the above tag were used to amplify dsDNA fragments and increase sensitivity of detection. Libraries approximately 300 bp in length were prepared using the Nextera XT DNA Sample Preparation kit (Illumina, San Diego, CA). Dual-indexed libraries were pooled together using DNA concentration measured with a Quibit Fluorometer (Thermo Fisher Scientific, Waltham, MA) and normalized for the number of individual samples included in each library. Size and quality of the pooled libraries were determined using the TapeStation high sensitivity DNA system (Agilent, Santa Clara, CA). A final concentration was determined via library quantification quantitative PCR (qPCR; New England Biolabs, Ipswich, MA) and the pooled library was sent to Genomics and Microarray Core at the University of Colorado—Denver Cancer Center for 2 × 150 paired-end sequencing on an Illumina NovaSeq6000 platform.

Shotgun metagenomic sequencing analysis. Shotgun metagenomic sequencing datasets were processed to identify and tally viral reads. An existing taxonomic identification pipeline was modified for use in this study. Reads were quality assessed using FastQC. Low-quality, adapter, and SISPA sequences were removed using cutadapt tool version 1.14. The CD-HIT-EST tool version 4.6.8 was used to
remove PCR duplicates. FastQC was again used to assess sequence quality post trimming and filtering. Human and mosquito bowtie indices were created to remove host reads using Bowtie version 2.2.9. The human index (GRCh38.p3) was used on H-DBS and HeLa cell libraries. The mosquito index was created using four mosquito genomes (Ae. aegypti GCF_002204515.2, Ae. albopictus GCA_006496715.1, Anopheles darlingi GCA_002114553.3, C. pipiens GCF_000209185.1) and was used to filter M-DBS libraries. No host filtering was done on water and FTA negative control libraries. The remaining reads were assembled using SPAdes genome assembler version 3.6.1. Contigs and nonassembled reads were taxonomically assigned using the BLASTn alignment tool version 2.9.0. Taxonomic assignment was based on the highest alignment score and an E value $< 10^{-8}$. If not able to be taxonomically assigned based on nucleotide sequence, DIAMOND version 0.9.30 was used to search reads against the NCBI nr database. Identified viral sequences (excluding phage) were tallied according to NCBI taxonomic ID with a minimum cutoff of 30 hits. Taxonomic identifications were validated by reblasting contigs (NCBI BLAST nucleotide) and mapping reads to indexed reference genomes using Bowtie versus 2.2.9 (Supplemental Table 2).

Sample screening by quantitative PCR. The H-DBS and M-DBS nucleic acid samples were pooled by volume ($n = 8$ per pool) before screening. Quantitative PCR (qPCR) was run using the Express SuperMix Universal One Step system (Invitrogen, Waltham, MA) on a Quantt Studio machine (Applied Biosystems, Waltham, MA) according to the manufacturer’s instructions. The following primers and probes were used: dengue virus (DENV)-2 primers as previously described, DENV-3 forward TGGCAACAGGTCCCTTTCTG, DENV-3 reverse TTGCGATTGGAGCTATGCTAAGA, COPV forward TCCAATCAAGACCGCATACAGCT, COPV reverse TCGTCACACTGTACCACCA. DENV-3 primer sequences were kindly provided by Dr. Laura St. Clair and Dr. Rushika Perera (Colorado State University, Ft. Collins, CO). DENV-2 and DENV-3 positive controls were provided by Dr. Irina Sanchez (Colorado State University, Fort Collins, CO). Melt curves were used to determine the validity of all amplified products.

RESULTS

Study demographics and household features. Two communities in rural southwest Guatemala were targeted for participation in this study: Los Encuentros and Chiquirines. Of 37 households screened for participation by convenience, 32 (86.5%) were eligible, 20 (62.5%) were enrolled (10 from each village), and 19 (59.7%) completed the study. Households that were not enrolled either did not meet the enrollment requirements (13.5%) or declined to participate (32.4%) largely due to daytime availability. Of enrolled households, nearly all dwellings consisted of cement block structures without screened windows or doors. All households had open water wells or tanks on the property, and many had additional sources of standing water (potted plants, tires, natural water); 50.0% of households did not have a septic system (Supplemental Table 3).

Households had a median of 4.5 people enrolled; 46.7% were under age 18, and 60.2% were female (Table 1). Animals were abundant in households (interquartile range: 16–34 per household) and often found in and around the home. The most abundant animal was chickens (55% of all animals, excluding humans), although pigs, dogs, cats, and horses were also present (Supplemental Table 4).

Symptomatic episodes and samples collected. During 16 weeks of observation, there were 15 symptomatic episodes, all of which included at least one report of fever, rash, or body aches. Seven of 15 episodes reported more than one symptom, and symptoms were reported in multiple household members for four episodes. Two households reported illness in two separate episodes, and a single household reported three instances of febrile illness. Most of the reported illness (15 of 20 reported episodes) occurred in Los Encuentros (Tables 1 and 2). Including enrollment, 1,200 mosquitoes were collected during the study, 507 (42.3%; Figure 1 and Table 2), of which appeared to have taken a bloodmeal in the past 36 hours observed by a dark red/black bloated abdomen.26,27 Indoor mosquitoes were most abundantly collected at the end of the rainy season (September–October) (Figure 1); 488 of 517 (94.4%) blood-fed mosquito samples were speciated by PCR. Culex, Aedes, Mansonia, Anopheles, and Psorophora mosquitoes were collected, although Culex pipiens was by far the most abundant (60.9%) (Table 3).

Mosquito abundance and bloodmeal identification. Five genera of mosquitoes were identified: Culex, Aedes, Mansonia, Anopheles, and Psorophora. Culex mosquitoes were most abundantly identified as Cx. pipiens though Cx. nigripalpus also were collected. Aedes mosquitoes included Ae. aegypti, Ae. taeniorhynchus, and Ae. albopictus, from highest to least abundant (Supplemental Table 5). Cx. pippis and Ae. aegypti were the most frequently collected species.
accounting for 60.9% and 18.6% of identified mosquitoes respectively (Table 3). To identify mosquito bloodmeal sources, we used a previously described protocol to amplify and sequence vertebrate cytochrome oxidase 1 (CO1) sequences. We successfully amplified 464 of 488 (95.1%) total bloodmeal samples, indicated by the presence of a band corresponding to 2400 base pairs on an agarose gel. In total, 343 of 488 (70.3%) amplified samples were able to be host-identified. Unidentified samples were a result of poor sequencing results, indicating potential mixed hosts (i.e., mosquito feeding on multiple species). Of the 343 identified mosquito bloodmeal samples, our results demonstrate that humans and chickens were the most common sources of blood, accounting for 32.6% and 31.8% of identified bloodmeals respectively. All mosquito genera (excluding Psorophora, which had no identified bloodmeals) fed on humans. Cx. pipiens fed most abundantly on chickens and other avian hosts (63.6%), whereas Ae. aegypti fed mainly upon humans (91.7%) (Figure 2 and Supplemental Table 5).

Virus identification in human and mosquito dried blood spots. Human and mosquito dried blood spots (H-DBS, M-DBS) collected during seven symptomatic episodes were used to identify viral agents using a shotgun metagenomic sequencing approach (Supplemental Table 1). Retrospective bloodmeal analysis of the mosquitoes used for sequencing revealed 33.0% M-DBS derived from human hosts. To increase our sensitivity of detection using the SISPA method, all libraries underwent a high degree of amplification, thus even FTA and water control libraries produced reads from spurious sequence contaminants (Figure 3A). No human or animal viruses were detected in any library except in HeLa cells, which are stably infected with human papilloma virus and act as a control for virus detection. Insect-specific viruses were detected in H-DBS, water, and FTA control libraries though did not exceed 27 identified reads. Thus, a threshold of 30 reads was used to identify viruses that were present above background. Many insect-specific viruses were detected at high levels in M-DBS (Figure 3B and C). The most abundantly identified insect-specific viruses (ISVs) were Culex orthophasma virus (COPV; Table 3).

### Table 2

<table>
<thead>
<tr>
<th>Week</th>
<th>Village</th>
<th>Household ID</th>
<th>Subjects with fever</th>
<th>Subjects with rash</th>
<th>Subjects with body aches</th>
<th>H-DBS obtained</th>
<th>M-DBS obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Los Encuentros</td>
<td>All</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>49</td>
<td>105</td>
</tr>
<tr>
<td>2</td>
<td>Chiquirines</td>
<td>All</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>49</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>Los Encuentros</td>
<td>1007</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Chiquirines</td>
<td>2013</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Los Encuentros</td>
<td>1001</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>Chiquirines</td>
<td>2002</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Los Encuentros</td>
<td>1016</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>Chiquirines</td>
<td>2019</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>9</td>
<td>Los Encuentros</td>
<td>2003</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>Chiquirines</td>
<td>2002</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>11</td>
<td>Los Encuentros</td>
<td>1019</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>12</td>
<td>Chiquirines</td>
<td>2001</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>13</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

H-DBS = human dried blood spots; M-DBS = mosquito dried blood spots; NR = none reported; NS = not surveyed.

### Table 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Los Encuentros</th>
<th>Chiquirines</th>
<th>Sum (% of identified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes aegypti</td>
<td>61</td>
<td>30</td>
<td>91 (18.6)</td>
</tr>
<tr>
<td>Aedes albopictus</td>
<td>2</td>
<td>1</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Aedes taeniorhynchus</td>
<td>0</td>
<td>3</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Anopheles sp.</td>
<td>0</td>
<td>2</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Anopheles albimanus</td>
<td>3</td>
<td>5</td>
<td>8 (1.6)</td>
</tr>
<tr>
<td>Culex sp.</td>
<td>36</td>
<td>14</td>
<td>50 (10.2)</td>
</tr>
<tr>
<td>Culex nigripalpus</td>
<td>9</td>
<td>1</td>
<td>10 (2.0)</td>
</tr>
<tr>
<td>Culex pipiens</td>
<td>194</td>
<td>103</td>
<td>297 (60.9)</td>
</tr>
<tr>
<td>Mansonia dyari</td>
<td>11</td>
<td>9</td>
<td>20 (4.1)</td>
</tr>
<tr>
<td>Mansonia titilans</td>
<td>1</td>
<td>1</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Psorophora ferox</td>
<td>2</td>
<td>0</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Total identified</td>
<td>319</td>
<td>169</td>
<td>488</td>
</tr>
</tbody>
</table>

Figure 1. Total and blood-fed mosquitoes collected increases over rainy season. Total mosquitoes collected per household during symptomatic episodes over the 16-week study (gray dashed line). Blood-fed mosquitoes collected per household (black solid line). Rainy season indicated by shaded background.
The rise of global pandemics, coupled with the complexity and uncertainty associated with predicting pathogen emergence, emphasizes the need for minimally invasive, accessible, low-cost surveillance methods that are applicable under field conditions. We therefore evaluated xenosurveillance in rural Guatemala, a region that is of interest for three principal reasons. First, it is a region that has a high burden of infectious diseases with significant needs for and barriers to effective health surveillance. Second, there is a high rate of human migration within and through the region, providing an important sentinel population for emerging infectious disease surveillance. Finally, and perhaps most important, the region is quite different from those where we have previously conducted pilot studies of xenosurveillance, in that Culex and Aedes mosquitoes, which tend to be less anthropophilic compared with Anopheles gambiae, are the main mosquitoes that fed on humans indoors. Therefore, we sought to assess the extent to which xenosurveillance may be effective in this type of environment.

We collected and identified several mosquito genera, including Culex, Aedes, Mansonia, Anopheles, and Psorophora. Culex pipiens (60.9%) were most abundant and fed mainly on birds, whereas Ae. aegypti (18.6%) fed mostly on humans. Thus, the feeding patterns of Cx. pipiens and Ae. aegypti at our site are typical for these species. The disproportionate number of Culex samples skewed our blood samples toward those derived from birds, in contrast to our prior study in which An. gambiae accounted for 80% of the collection and strongly prefer to feed on humans. These observations present challenges and opportunities for implementation of xenosurveillance in Latin America; more intensive sampling may be required to capture human infection adequately, but surveillance of domestic and peridomestic animals may be enhanced.

Analysis of M-DBS and H-DBS revealed no human viruses present, in contrast to prior detection of human viruses (GB virus C, Epstein–Barr virus, and hepatitis B virus) from Anopheles bloodmeals in Liberia. Many factors likely contributed to the lack of human virus detection in the present study. First, the number of blood-fed mosquitoes was not sufficient for virus detection in humans. As previously described, the abundance of Cx. pipiens in this study limited the retrieval of human-sourced mosquito bloodmeals because the majority were derived from chickens and other birds. In addition, multiple samples are likely needed for adequate detection of bloodborne viruses from any one individual, although the absence of viruses detected in H-DBS suggests circulating bloodborne viruses were not present at the time of collection. Second, symptom surveillance was done once weekly, resulting in samples that were not collected at the time of illness. We may therefore have missed critical windows where mosquitoes may have sampled circulating viremia. Finally, virus discovery was performed on a chosen subset of samples and thus could result in missed human viruses. Collectively, these results suggest that efficient detection of human pathogens in this setting may require more frequent and abundant sampling, or that human pathogens were not circulating at detectable levels.

Although no human viruses were detected, analysis of M-DBS revealed the presence of ISVs. The detection of ISVs was expected because excretion of the mosquito bloodmeal onto FTA cards is accompanied by mosquito tissues (e.g., the midgut) that may be infected by ISVs. ISVs have been described in xenosurveillance-based studies published previously and provide an additional internal control for our ability to detect viral RNA by our sample processing and analysis procedures. We found several ISVs that were highly abundant in M-DBS, including COPV and HRLV7. TV was also identified, although on further analysis, we found reads mapped solely to regions that were highly identical to COPV; thus, we reattributed TV-identified reads as COPV. COPV has been identified in a number of Culex mosquito collections in Australia, Brazil, and Grenada. Similarly, HRLV7 has been described in both Aedes and Culex mosquitoes on three continents, as well as in this study. There have been a few recent studies showing decreased vector competence and replication of pathogenic flaviviruses...
after cells or mosquitoes undergo primary infection with ISVs.\textsuperscript{39,40} Despite the clear abundance of these ISVs around the world, there is currently comparatively little information on how they affect mosquito biology, arthropod-borne virus evolution, and pathogen transmission.

During this study, we identified several benefits and challenges in conducting xenosurveillance in Guatemala. We were successful in enrolling and maintaining a household cohort for this 16-week study. This was facilitated by The University of Colorado Center for Human Development, which has developed a good relationship with the surrounding communities. Notably, all household enrollments, interviews, sampling of blood and mosquitoes, and mosquito identification was performed by locally trained Guatemalan study nurses, highlighting the importance of developing local connections and expertise to conduct surveillance efficiently. We collected numerous blood-fed mosquitoes for this study using the Insectazooka, although mosquitoes were somewhat damaged, which makes species identification difficult and error-prone without extensive training and processing time. We were successful in detecting...
viral RNA from M-DBS; however, more frequent sampling or targeted detection of human pathogens, is needed.

In summary, xenosurveillance has some potential as a useful surveillance strategy in low- to middle-income countries; however, mitigating the described challenges and maximizing the unique opportunities presented by the lack of host-specificity of most human-biting mosquitoes in Guatemala requires further work and development. In particular, the frequency of mosquito collection and feeding behavior (including the peak feeding times and host preferences) should be carefully considered. Building local capacity for beginning to end sample processing and analysis would ultimately benefit the application of this surveillance method. Technologies such as nanopore sequencing (Oxford Nanopore Technologies, Oxford, United Kingdom) and BioFire (BioFire Diagnostics, Salt Lake City, UT) could be useful tools for on-site virus identification. Our evidence suggests that abundant and frequent sample sources are essential for early detection of circulating pathogens. We therefore foresee xenosurveillance being useful as a targeted approach in regions where zoonotic outbreaks are inevitable.

Received December 14, 2022. Accepted for publication June 2, 2023.

Published online October 16, 2023.

Note: Supplemental material appears at www.ajtmh.org.

Acknowledgment: We thank the CU Trifinio Research Team, the Trifinio community research subjects, and Jose R. C. Cifuentes for their contributions to this research.

Financial support: This work was supported by a seed grant from the Colorado State University One Health Institute and by Colorado State University’s Office of the Vice President for Research. V. S. was partially supported by Colorado State University’s Office of the Vice President for Research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Office of the Vice President for Research. D. O. is supported by NIH/NCATS Colorado CTSI grant no. UL1 TR001082 and NIH/NIAID 1K23AI143967. R. J. M. was supported by NIH project no. 5F31AI154737-02. This project is continued with the support of NIH project no. 5R21AI154011-02.

Authors’ addresses: Rebekah J. McMinn, Valeria Scorza, Michael C. Young, Delaney Worthington, Emma K. Harris, Brian D. Foy, Mark D. Stenglein, and Gregory D. Ebel, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, E-mails: rebekah.mcminn@colostate.edu, andrea.scorza@colostate.edu, emceeyoung@gmail.com, delaney.worthington208@alumni.colostate.edu, emkate.harris@colostate.edu, brian.foy@colostate.edu, mark.stenglein@colostate.edu, and gregory.ebel@colostate.edu. Andrea Chacon, Fundacion para la Salud Integral de los Guatemaltecos, Retalhuleu, Guatemala, E-mail: chaconjuanjorge@gmail.com. Claudia Rücker, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, and Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV, E-mail: crueckett1@unr.edu. Molly M. Lamb, Colorado School of Public Health, Aurora, CO, E-mail: molly.lamb@cuanschutz.edu. Ramon E. Medrano and Maria Renee Lopez, Centro de Estudios en Salud, Universidad del Valle de Guatemala, Guatemala City, Guatemala, E-mails: estuardomedrano@gmail.com and mlopez@cces.uvg.edu.gt. Kareen Arias, Center for Human Development, Retalhuleu, Guatemala, E-mail: kareen.arias@gmail.com. Edwin J. Asturias and Daniel Olson, Colorado School of Public Health, Aurora, CO, Center for Human Development, Retalhuleu, Guatemala, and Department of Pediatrics, Section of Infectious Diseases, University of Colorado School of Medicine, Aurora, CO, E-mails: edwin.asturias@childrensalute.org and daniel.olson@childrenscolorado.org.

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES


