Emerging and reemerging arboviruses:
A threat of epidemic complications in the east of Peru

TESIS:
Para optar por el título de
MÉDICO CIRUJANO

AUTOR:
Carlos Alberto Alva Urcia

ASESORA
Juana Mercedes del Valle Mendoza

Lima, Perú
2017
DEDICATORY

To my parents, my adviser Juana del Valle and the UPC-IIN Research Laboratory team
ACKNOWLEDGEMENTS

We thank Brandy Russell at Centers for Disease Control and Prevention for providing the CHKV, ZIKV and MAY positive controls (CDC, Fort Collins, CO, USA).

INDEX

RESUMEN ........................................................................................................................................... 5

ABSTRACT ........................................................................................................................................... 6

THEORETICAL FRAMEWORK ........................................................................................................ 7

AIMS: ................................................................................................................................................ 9

MATERIALS AND METHODS ........................................................................................................... 10

PATIENTS AND SAMPLING ............................................................................................................. 10

ETHICS STATEMENT ....................................................................................................................... 10

SAMPLES ......................................................................................................................................... 11

RNA EXTRACTION ............................................................................................................................ 11

PCR AMPLIFICATION ....................................................................................................................... 11

Real-time RT-PCR assay for Detection DENV, CHKV and ZIKV with taqman probe ......................... 11

Detection MAYV and OROV by conventional PCR ........................................................................... 12

STATISTICAL ANALYSIS ................................................................................................................ 14

RESULTS .......................................................................................................................................... 15

DISCUSSION .................................................................................................................................... 19

LIMITATIONS ................................................................................................................................... 21

CONCLUSIONS ............................................................................................................................... 22

REFERENCES .................................................................................................................................... 23

ATTACHMENTS: ............................................................................................................................... 27
RESUMEN

Arbovirus emergentes y reemergentes: Una amenaza de complicaciones epidémicas en el este del Perú

Antecedentes: La infección por arbovirus es una de las causas más comunes de síndrome febril agudo y un problema de salud emergente en América del Sur. En el Perú, el número de casos de dengue se ha duplicado en el último año; sin embargo, menos del 50% de los síndromes febriles agudos fueron confirmados por laboratorio, lo que conduce a un diagnóstico limitado de otros arbovirus importantes.

Objetivo: Evaluar la frecuencia de Dengue (DENV), Oropuche (OROV), Chikungunya (CHIKV), Mayaro (MAYV) and Zika (ZIKV) en pacientes con síndrome febril agudo de Puerto Maldonado, Perú.

Metodología: Se obtuvieron muestras de pacientes con síndrome febril agudo entre enero de 2016 y marzo de 2016. Se recolectaron un total de 139 especímenes y se evaluó la presencia de DENV, OROV, CHIKV, MAYV y ZIKV con la técnica de RT-PCR.

Resultados: Los arbovirus de mayor frecuencia fueron CHIKV 9.35% (13/139) y OROV 8.63% (12/139), seguidos de DENV (6.47%) y ZIKV (5.04%). Entre todos los pacientes, los síntomas más comunes que acompañaron la fiebre fueron: Cefalea 79.86% (111/139), mialgias 65.47% (91/139) y artralgias 63.31% (88/139).

Conclusiones: CHIKV y OROV fueron los arbovirus más frecuentes en nuestro estudio. Es crucial mejorar la vigilancia de los arbovirus para poder entender el papel de estos patógenos en el Perú. La PCR representa una prueba confiable para la vigilancia de arbovirus y debe ser considerada como el método más adecuado para la confirmación por laboratorio en el Perú.

Palabras clave: Arbovirus, Dengue, Chikungunya, Zika, Oropuche, Peru, PCR
ABSTRACT

Background: Arboviruses are one of the most common causes of acute febrile illness and an emerging health problem in South America. In Peru, the number of Dengue cases have doubled in the last year; however, less than 50% of acute febrile illness are laboratory confirmed leading to an underdiagnoses of other important arboviruses.

Aim: To assess the frequency of Dengue (DENV), Oropuche (OROV), Chikungunya (CHIKV), Mayaro (MAYV) and Zika (ZIKV) in patients with acute febrile illness from Puerto Maldonado, Peru.

Methodology: Samples were obtained from patients with acute febrile illness during January 2016 to March 2016. A total of 139 specimens were collected and assessed for the presence of DENV, OROV, CHIKV, MAYV and ZIKV via RT-PCR.

Results: CHIKV in 9.35% (13/139) and OROV in 8.63% (12/139) were the most prevalent arboviruses, followed by DENV (6.47%) and ZIKV (5.04%). Among all patients, the most common symptoms accompanying fever were: Headache 79.86% (111/139), Muscle pain 65.47% (91/139) and Joint pain 63.31% (88/139).

Conclusions: CHIKV and OROV were the most common arboviruses in our study. To enhance arbovirus surveillance is crucial to understand the role of these pathogens in Peru. PCR represents a reliable test for arboviral surveillance and should be considered as the preferred method for laboratory confirmation in Peru.

Key words: Arbovirus, Dengue, Chikungunya, Zika, Oropuche, Peru, PCR
THEORETICAL FRAMEWORK

Acute febrile illness (AFI) is defined as fever of less than 1 week of duration, which has no identified source and may be accompanied with other symptoms. A wide variety of causes are described, the infectious cause being one of the most frequent and important. However, in many cases the etiologic agent causing the disease cannot be determined, especially in low and middle income countries, where limited resources impede laboratory support, which has become essential for the effective surveillance programs [1-2-3].

One of the most common agents responsible for acute febrile illness worldwide and an emerging concern over the recent decades are Arthropod-borne viruses, also known as “arboviruses” [1]. Arboviruses have a great capacity for evolution and adaptation which has allow them to produce outbreaks and become a major health problem in the world. [4-5]. Specially in tropical areas, with hot and humid conditions, well-suited for mosquitos and other arthropod-related arboviral spreads [1-5].

It is in this context that the introduction of new molecular techniques such as Polymerase Chain Reaction (PCR) have broadened the spectrum of etiologies and emphasized the role of Arthropod-borne viruses, present in 32.5% of overall febrile cases [1-3]. Arboviruses are a heterogeneous group; however, the most relevant belong to a few virus genus including: Flavivirus such as Dengue virus (DENV) and Zika virus (ZIKV); Alphavirus like Chikungunya (CHIKV) and Mayaro (MAYV); and Oropuche (OROV) one of the most common Orthobunyaviruses [1-5].

In the case of Flavivirus as in Dengue, Peru has reported an increase in the number of cases, which have doubled in one year, from 2014 (17234 cases) to 2015 (39440 cases) [6]. The most
affected regions are Ayacucho, Cuzco, Piura and Madre de Dios were 57.44% of cases have been registered [7]. However, despite of the National surveillance efforts to monitor dengue infections, in 2015 only 49.2% (19405/39440) of cases were laboratory-confirmed leading to the underdiagnoses of other important pathogens [7- 8]. Zika virus (ZIKV), on the other hand, have been an increasing concern in the last years, on February 2016 the World Health Organization (WHO) declared ZIKV a Public Health Emergency of international concern [9]. The last National reports from 2016 have not found any case of ZIKV since the epidemiologic alert for ZIKV surveillance was issued in October 2015 [10].

With respect to the Alphavirus genus in Perú, only 49.5% of the 208 native cases reported were confirmed as Chikungunya in 2015 [8]. The presence of the Mayaro virus (MAYV) has also been reported Amazon Basin Region between 2010 and 2013 reported a MAYV prevalence of 0.8% (16/2094) in febrile patients [11].

In 2011, the Oropuche virus (OROV), one of the most common members of the genus Orthobunyavirus, was responsible for 14.9% of the acute febrile infections of a population located in the Peruvian Amazon.[12] In January 2016, a OROV outbreak in the Madre de Dios region was confirmed, analyzing samples from patients who had been negative for DENV at first. [13]

To study the Arboviral epidemiology in Peru is essential to understand their real impact in our community. The aim of this study is to determine the frequency, epidemiological and clinical characteristics of DENV, ZIKV, CHIKV, MAYV and OROV in patients with Acute Febrile illness from Puerto Maldonado, Peru.
AIMS:

General objective:

- To assess the frequency of Dengue (DENV), Oropuche (OROV), Chikungunya (CHIKV), Mayaro (MAYV) and Zika (ZIKV) in patients with acute febrile illness from Puerto Maldonado, Peru.

Specific Objectives:

- To determine the frequency of DENV, CHIKV and ZIKV in serum samples from patients with febrile syndrome in the department of Puerto Maldonado-Peru using Real-time RT-PCR.
- To determine the frequency of MAYV and OROV in serum samples from patients with febrile syndrome in the department of Puerto Maldonado-Peru using conventional RT-PCR.
- To describe the clinical of patients with febrile syndromes and the molecular results positive for DENV, OROV, CHIKV, MAYV and ZIKV.
MATERIALS AND METHODS

Patients and Sampling

A consecutive cross-sectional study in was conducted in Puerto Maldonado in coordination with the “Dirección Regional de Salud Madre de Dios” using non-probability consecutive sampling. Puerto Maldonado is the capital of the Madre de Dios Region a city in Southeastern Peru in the Amazon rainforest 55 km west of the Bolivian border. Puerto Maldonado has an estimated population of 74,949 and has been extensively recognized as an endemic area for Dengue and other Arboviruses [6]. The totality of patients who fulfilled the selection criteria were studied from January to March 2016.

Inclusion criteria: Patients who presented in the outpatient clinics with acute, undifferentiated, febrile illness (greater than or equal to 38 C axillary temperature for 7 days of duration or less) along with one or more of the following symptoms: headache, muscle pain, ocular and/or joint pain, nausea, vomiting, sore throat, cough, rhinorrhea, difficulty breathing, diarrhea, jaundice, generalized fatigue, cough, among others.(ARI). Exclusion criteria was considered if an identifiable focus of infection was diagnosed, such as sinusitis, pneumonia, acute otitis media, acute tract infections, among others[23-24].

Ethics Statement

This study was approved by the Research Ethics Board of the Hospital Regional de Cajamarca, Peru. An informed consent was signed before enrollment.
Samples

One serum sample per patient was collected by using Vacuette® TUBE Serum Separator Clot Activator (Vacuette, Greiner Bio-One, Kremsmünster, Austria). After collection, the 139 samples were stored at -80°C. All samples were transported to Lima (Peru) under standardized frozen conditions to perform molecular assays.

Positive control material to CHKV, ZIKV and MAY were provided by the Centers for Disease Control and Prevention (CDC, Fort Collins, CO, USA).

RNA extraction

RNA extraction was performed from 200 μL of the serum samples with the High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany), according to the manufacturer’s instructions. Viral RNA obtained after extraction was eluted in 100 μl of nuclease free water and then processed or stored at -20°C until use.

PCR amplification

Real-time RT-PCR assay for Detection DENV, CHKV and ZIKV with Taqman probe

A one-step RT-PCR was performed using TaqMan with BHQ quencher probe at 125 nM and 250 nM of primers in final volume of 20μL. Five microliters of the extracted RNA was combined with 15 μl of the master mix and the reverse transcription step was performed 95°C for 15 minutes, 60 cycles of 15 seconds at 95°C and 45 seconds at 60°C. All the procedure was
performed in Light Cycler® 2.0 Instrument and data was analyzed in the LightCycler® Software 4.1 (Roche Diagnostic, Deutschland-Mannheim, Germany). The primers and the probe used are shown in Table 1[14-16-27].

**Detection MAYV and OROV by conventional PCR**

For the reverse transcription (RT), a 20 µl mixture was prepared containing 5 µl of RNA extracts, the Transcriptor Hight Fidelity cDNA SynthesisKit (Roche Applied Science, Mannheim, Germany) was used according to the manufacturer’s instructions.

A 166 bp and 300 bp fragments were amplified for Mayaro and Oropuche respectively. The Table 1 shows the primers used for amplification. The final volume of the PCR mixture was 50 µl, distributed as follows: 25 µl of enzyme mix (Taq polymerase, 2.5 mM MgCl₂, 15 mM Tris / HCl pH 8.3, 50 mM KCl, 200 µM of each deoxynucleotide), 20 pmol of each primer (Macrogen, Seoul, Korea), and 5 µl of DNA extraction. Thus, the PCR conditions were: 95°C for 10 min, followed by 55 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final elongation of 10 min at 72 °C. The amplified DNA products were analyzed by gel electrophoresis on 2% agarose (FMC, Rockland, ME) gel containing ethidium bromide (3 mg/L). Amplified products were gel recovered, purified (SpinPrep™ Gel DNA Kit, San Diego, USA) and sent to be sequenced (Macrogen, Seoul, Korea)[15-16].
Table 1: Primers and probe used in the RT-PCR assays.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5´-3´)</th>
<th>Amplicon (pb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DENV-F</strong></td>
<td>5´-AGG ACY AGA GGT TAG AGG AGA -3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DENV-R</strong></td>
<td>5´- CGY TCT GTG CCT GGA WTG AT - 3´</td>
<td>107</td>
<td>Leparc-Goffart et al., 2009</td>
</tr>
<tr>
<td><strong>DENV-</strong></td>
<td>5´-FAM-ACA GCA TAT TGA CGC TGG GAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Probe</strong></td>
<td>AGA CC-TAMRA-3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CHIKV-F</strong></td>
<td>5´- AAG CTY CGC GTC CTT TAC CAA G -3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CHIKV-R</strong></td>
<td>5´- CCA AAT TGT CCY GGT CTT CCT - 3´</td>
<td>209</td>
<td>Pastorino et al., 2005.</td>
</tr>
<tr>
<td><strong>CHIK</strong></td>
<td>5´-FAM-CCA ATG TCY TCM GCC TGG ACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Probe</strong></td>
<td>CCT TT-TAMRA-3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ZIKV-F</strong></td>
<td>5´- AAR TAC ACA TAC CAR AAC AAA GTG GT - 3´</td>
<td></td>
<td>Faye et al., 2013.</td>
</tr>
<tr>
<td><strong>ZIKV-R</strong></td>
<td>5´- TCC RCT CCC YCT YTG GTC TTG-3´</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td><strong>ZIKV-Probe</strong></td>
<td>5´- FAM-CTYAGACCAGCTGAAR-TAMRA-3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OROV-F</strong></td>
<td>5´- GTG GGG TCC AAT TTG C - 3´</td>
<td>300</td>
<td>Moreli et al., 2002.</td>
</tr>
<tr>
<td><strong>OROV-R</strong></td>
<td>5´- TGA ACC CTA TGC ATC T – 3´</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MAYV-F</strong></td>
<td>5´- TTC CRA AYC AAG TGG GAT TC – 3´</td>
<td></td>
<td>Llagonne-Barets et al., 2016</td>
</tr>
<tr>
<td><strong>MAYV-R</strong></td>
<td>5´- CAC TTT ACG TAY GGK GAT GG – 3´</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Statistical analysis

Qualitative variables were reported as frequencies and percentages. All analyses were processed with the IBM Statistical Package for the Social Sciences (SPSS) software version 21.0 (SPSS, Chicago, IL, USA).
RESULTS

A total of 139 patients with acute febrile illness (AFI) were studied from January to March 2016. Most of the patients were between 20 to 44 years old in 58.27% (81/139) followed by the group 5-19 years old in 21.58% (30/139) and patients older than 45 years old in 15.83% (22/139); a slightly predominance of male gender was observed in 52.98% (76/139) (Table 2).

CHIKV and OROV were the most prevalent arbovirus isolated in 9.35% (13/139) and 8.63% (12/139) respectively. DENV was observed in 6.47% (9/139) and ZIKV in 5.04% (7/139). (Table 2). Among all positive samples, patients between 20-44 years old were the most prevalent group and represented more than 50% of cases in each studied arbovirus: (5/9) of DENV, (7/12) of OROV, (4/7) of ZIKV and (7/13) in CHIKV. (Table 2) No cases of MAYV were detected in our 139 samples.

In our study population, the most common symptoms accompanying the AFI were: Headache in 79.86% (111/139), Muscle pain in 65.47% (91/139) and Joint pain in 63.31% (88/139); followed by low appetite (34.53%), Retroocular pain (33.81%) and Nauseas (28.78%) (Table 3).

In patients with DENV positive samples both Headaches and nauseas were frequently found in 44.44% (4/9). For OROV the most common symptoms were Headaches (66.67%), joint pain (58.33%) and muscle pain (50.00%).Among the 7 positive samples for ZIKV, headaches were reported in 57.14%, followed by nauseas, joint and muscle pain in 42.86% of cases. A similar situation was observed in CHIKV patients, were Headaches (92.31%), muscle pain (84.62%) and joint pain (76.92%) were the most predominant complains (Table 3).
Coinfections between DENV and other arboviruses were observed in 4 patients: OROV-DENV in 2 cases which presented with headaches, low appetite, nauseas and muscle pain; 1 case of ZIKV-DENV complaining only about fever with no other associated symptoms and 1 case of CHIKV-DENV who was hospitalized due to presenting fever, chills, dizziness, shortness of breath, nauseas, vomits, low appetite, abdominal pain and extreme sensitivity to light.
Table 2. Demographics in patients with arboviral infections from Puerto Maldonado, Peru.

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>TOTAL POPULATION</th>
<th>RT-PCR CONFIRMED ARBOVIRUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (N=139)</td>
<td>DENGUE</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>Positive cases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=9 (%)</td>
</tr>
<tr>
<td>00-04</td>
<td>6 (4.32)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>(5 - 19)</td>
<td>30 (21.58)</td>
<td>1 (11.11)</td>
</tr>
<tr>
<td>(20-44)</td>
<td>81 (58.27)</td>
<td>5 (55.55)</td>
</tr>
<tr>
<td>(45-+)</td>
<td>22 (15.83)</td>
<td>3 (33.33)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>76 (52.98%)</td>
<td>5(33.33)</td>
</tr>
<tr>
<td>Female</td>
<td>63 (47.02%)</td>
<td>4(66.67)</td>
</tr>
</tbody>
</table>
Table 3. Clinical symptoms in patients with arbovirus infection positive by PCR.

<table>
<thead>
<tr>
<th>Clinical Symptoms</th>
<th>Total population n= 139</th>
<th>Dengue Positive n=9 (%)</th>
<th>Oropouche Positive n=12 (%)</th>
<th>Zyka Positive n=7 (%)</th>
<th>Chikungunya Positive n=13 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>111</td>
<td>4 (44.44)</td>
<td>8 (66.67)</td>
<td>4 (57.14)</td>
<td>12 (92.31)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>91</td>
<td>3 (33.33)</td>
<td>6 (50.00)</td>
<td>3 (42.86)</td>
<td>11 (84.62)</td>
</tr>
<tr>
<td>Joint pain</td>
<td>88</td>
<td>3 (33.33)</td>
<td>7 (58.33)</td>
<td>3 (42.86)</td>
<td>10 (76.92)</td>
</tr>
<tr>
<td>Retroocular pain</td>
<td>47</td>
<td>1 (11.11)</td>
<td>4 (33.33)</td>
<td>1 (14.29)</td>
<td>6 (46.15)</td>
</tr>
<tr>
<td>Nauseas</td>
<td>40</td>
<td>4 (44.44)</td>
<td>3 (25.00)</td>
<td>3 (42.86)</td>
<td>3 (23.08)</td>
</tr>
<tr>
<td>Low appetite</td>
<td>48</td>
<td>3 (33.33)</td>
<td>4 (33.33)</td>
<td>3 (42.86)</td>
<td>6 (46.15)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>11</td>
<td>2 (22.22)</td>
<td>0 (00.00)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>9</td>
<td>1 (20.00)</td>
<td>1 (8.33)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>9</td>
<td>2 (22.22)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Chills</td>
<td>9</td>
<td>1 (11.11)</td>
<td>3 (25.00)</td>
<td>1 (14.29)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Rash</td>
<td>9</td>
<td>2 (22.22)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>2 (15.39)</td>
</tr>
<tr>
<td>Photophobia</td>
<td>7</td>
<td>1 (11.11)</td>
<td>1 (8.33)</td>
<td>1 (14.29)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Odynophobia</td>
<td>8</td>
<td>1 (11.11)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Cough</td>
<td>5</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Pallor</td>
<td>4</td>
<td>0 (0.00)</td>
<td>2 (25.00)</td>
<td>1 (14.29)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4</td>
<td>0 (0.00)</td>
<td>1 (8.33)</td>
<td>1 (14.29)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>3</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3</td>
<td>1 (11.11)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Conjunctival injection</td>
<td>3</td>
<td>1 (11.11)</td>
<td>0 (0.00)</td>
<td>1 (14.29)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Expectoration</td>
<td>3</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Dysuria</td>
<td>1</td>
<td>1 (11.11)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>1 (7.69)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>1</td>
<td>0 (0.00)</td>
<td>1 (8.33)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Convulsions</td>
<td>0</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>
DISCUSSION

Over the past decades there has been a resurgence of arthropod-borne viral pathogens in South America, especially in middle and low income countries [1-5]. In Peru, an increase of DENV and other arboviral infections have been observed in the last 5 years [6-7]. However, only DENV, CHIKV and ZIKV are pathogens for obligated reporting, and less than 50% of samples can be confirmed by the Instituto Nacional de Salud, the only National Laboratory designated for Epidemiological Surveillance in Peru [7-17-18]. This passive National surveillance strategy added to the diagnostic barriers in the rural areas leads to an underestimation in the impact of arboviruses in the Peruvian amazon basin [1-19].

Puerto Maldonado, located in the Department of Madre de Dios in Peru, is one of the most affected areas by arboviruses; and only DENV burden accounts for a total cost of more than $200 000 dollars every year with more than 900 cases reported in 2015 [6-20]. A study in 2010, have also demonstrated that Puerto Maldonado shows a higher prevalence of Alphavirus infection in comparison with other regions of the Amazon Basin [1]. Moreover, OROV have been recognized as endemic in Madre de Dios were outbreaks have been observed in the last years [21-22].

We studied 139 patients with AFI from Puerto Maldonado, Peru and in our study population most patients were males (52.98%) between 20 to 44 years old (58.27%), followed by the group 5-19 years old (21.58%). A similar demographic distribution was observed in a study conducted in South America from 2000-2007 were among 20 880 patients, 52.3% were males, the mean age was 24 years old with 89.5% of cases between the ages of 6 and 49 [1]. In this multinational study, in addition to fever, the most commonly reported symptoms were malaise (96.7%), headache (92.2%), chills (90.2%), myalgia (81.4%) and arthralgia (76.2%) [1]. This clinical
presentation is comparable to our patients were Headache (79.86%), Muscle pain (65.47%) and Joint pain (63.31%) were the most frequent complains.

Despite a previous study in 2010 were DENV was reported in 17.3% of patients with febrile illness in Puerto Maldonado, we found 9 cases of DENV positive samples, which only represented a 6.47% of our population [1]. This lower frequency of DENV can be related to the fact that we only used RT-PCR for case confirmation in comparison to the other study were RT-PCR and IgM seroconversion were used. Moreover, in this previous study, they reported a 32% of IgM reactive cross reactivity between DENV and Yellow fever virus (YFV) antigen in the acute or convalescent samples [1]. Therefore, the indirect diagnosis (IgM/IgG detection) limitations due to cross-reaction between similar viral families should be considered while implementing surveillance politics; even though is the most widespread test at public health facilities [1-4].

OROV were observed in 8.63% (12/139) of our patients, a smaller frequency than a previous study in Madre de Dios, were OROV was found in 24% of samples. However, both results should not be comparable since the previous study was conducted in DENV negative samples and again multiple laboratory test were used (RT-PCR, ELISA IgM and cellular culture) [13]. Moreover, it is difficult to compare our OROV frequency with previous reports, since OROV is not part of the National Surveillance program [23-24]. In our 12 patients with OROV positive samples the most common symptoms were Headaches (66.67%), joint pain (58.33%) and muscle pain (50.00%), which is consistent with previous studies in Iquitos and Madre de Dios were fever, headaches, joint and muscle pain were the most predominant symptoms [13-25].

In 2015, Peru reported 208 native cases of CHIKV in only 2 departments located in the northern coast: Tumbes were 91.3% (190/208) of cases were observed and Piura in 8.7% (18/208). We found 13 cases of CHIKV in Puerto Maldonado and one of this cases was a child younger than 4 years old. However, we cannot conclude if these 13 patients were native cases.
Limitations

Because we used a consecutive non-probability sampling the results may be not representative of the population.
CONCLUSIONS

This study is the first one to report 7 cases of ZIKV in Puerto Maldonado, and this finding raise our concern since no cases have been reported by the Peruvian National Surveillance program until January 2016 [7]. We strongly believe that our National Surveillance program should be strengthened, based on a reliable laboratory method. PCR have widely demonstrated to be a fast and effective diagnostic tool with high sensitivity for the detection of DENV, OROV, ZIKV, CHIKV, among other arboviruses [1-3-4-26]. Therefore, health politics in Peru should focus on increasing the number of PCR-confirmed arbovirus cases for surveillance. Further investigations should be conducted to have a better understanding of the impact of arboviruses in the Peruvian Amazon Basin.
REFERENCES


ATTACHMENTS:
Research Ethics approval

Hospital Regional de Cajamarca

COMITÉ DE ÉTICA EN INVESTIGACIÓN

Cajamarca, 16 de Octubre de 2015

Dra. Juana Del Valle Mendoza
Facultad de Ciencias de la Salud
Universidad Peruana de Ciencias Aplicadas

Ref. PI:

IMPLEMENTACIÓN DE UNA PLATAFORMA MULTIDISCIPLINAR PARA CONTENER EL IMPACTO DE SÍNDROMES FEBRILES EN ZONAS DE ALTO RIESGO

De mi especial consideración:

En atención a la remisión del Protocolo indicado, tengo a bien hacer de su conocimiento que el Comité de Ética en Investigación (CEI) de nuestra institución, ha determinado aprobar el estudio arriba mencionado, (Exp. N° 1958851) tal como ha sido planteado, con la única observación de fijar los plazos de entrega de los resultados confirmatorios (PCR).

Se le recuerda que el plazo de aprobación tiene una duración de 24 meses contados a partir de la fecha de esta carta, la que puede ser renovada luego de la presentación del informe anual de los avances.

Sin otro particular, le saludo atentamente.

[Signature]
Authorization of the “Dirección Regional de Salud Madre de Dios”

Puerto Maldonado, 02 de febrero 2017

Estimado Carlos Alva:
Egresado de la carrera de medicina
Universidad Peruana de Ciencias Aplicadas (UPC)
Lima.-

Reciba mis cordiales saludos. Agradecemos su interés por realizar su trabajo de investigación en Madre de Dios. Consideramos que es un tema de suma importancia teniendo en cuenta el impacto de las enfermedades metaxénicas virales en los últimos años en nuestro departamento. Por tal motivo, autorizamos el uso de la información de las fichas epidemiológicas que se ha venido trabajando en conjunto con el equipo de la UPC, para que usted pueda presentarlo como tesis para optar por el título de Médico Cirujano.

Sin otro particular me despido de usted.

Atentamente,

[Signature]

Dr. Carlos Hermógenes Manrique de Lara Estrada
Director Ejecutivo de Epidemiología, Prevención y Control de Emergencias y Desastres
DIRESA Madre de Dios
Data collection instrument:
Certificate of thesis defense:

ACTA DE SUSTENTACIÓN

En el día de hoy se reúne el jurado integrado por:

Presidente: Aldo Lucchetti Rodríguez
Jurado: Edward Mezones Holguín
Jurado: Jorge Maguña Quispe

para evaluar la sustentación de: [ ] Tesis [ ] Proyecto Profesional [ ] Expedientes

titulado: EMERGING AND REEMERGING ARBOVIRUSES: A THREAT OF EPIDEMIC COMPLICATIONS IN THE EAST OF PERÚ

desarrollado por: Carlos Alberto Alva Urcia

asesorado por: Juana del Valle Mendoza

para optar por el título: Médico Cirujano

Después de haber escuchado la exposición, así como las respuestas a las preguntas formuladas en la defensa, el jurado concluye que el/los graduado(s) ha(n) demostrado estar preparado(s) para iniciar el ejercicio profesional. Por lo tanto, teniendo en cuenta los rangos de calificación siguiente:

/ Aprobado / Notable / Sobresaliente / Summa Cum Laude / Desaprobado /

el jurado otorga el siguiente resultado a:

<table>
<thead>
<tr>
<th>Estudiante</th>
<th>Calificación</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlos Alberto Alva Urcia</td>
<td>APROBADO</td>
</tr>
</tbody>
</table>

Dado en la ciudad de Lima a los 14 días del mes de febrero de 2017.

[Signatures]

Presidente: Aldo Lucchetti Rodríguez
Jurado: Edward Mezones Holguín
Jurado: Jorge Maguña Quispe