



## Case Report/Relato de Caso

### Notification of the first isolation of Cacipacore virus in a human in the State of Rondônia, Brazil

Notificação do primeiro isolamento do vírus Cacipacoré em um ser humano, no Estado de Rondônia, Brasil

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#### ABSTRACT

Flavivirus is a genus of arthropod-transmitted viruses of the family Flaviviridae, and in Brazil, up to eleven different Flavivirus have been isolated. We collected blood from farmers in the municipality of Theobroma, which is located 320km from the City of Porto Velho, the former capital of the Brazilian State of Rondônia. For viral isolation, we used newborn mouse brain, followed by RT-PCR with specific universal Flavivirus primers. We obtained fragments 958bp and 800bp in length. Based on BLAST, these sequences were 91% similar to a sequence of Cacipacore virus.

**Keywords:** Cacipacore virus. RT-PCR. Flavivirus.

#### RESUMO

*Flavivirus* é um gênero dos vírus transmitidos por artrópode da família *Flaviviridae* e, no Brasil, são isolados onze *Flavivirus* diferentes. Foi coletado o sangue de um agricultor, no município de Theobroma situado a 320km de distância da Cidade de Porto Velho, capital do Estado Brasileiro, Rondônia. Para isolamento viral, foi usado cérebro de camundongos recém-nascido, seguido por RT-PCR com *primers* universais específicos de *Flavivirus*. Nós obtivemos fragmentos com 958bp e 800bp de comprimento. Ao Blast das sequências obtivemos 91% de similaridade com uma sequência do vírus de Cacipacoré.

**Palavras-chaves:** Vírus Cacipacoré. RT-PCR. *Flavivirus*.

#### INTRODUCTION

The genus *Flavivirus* is composed of arthropod-transmitted viruses belonging to the *Flaviviridae* family<sup>1</sup>. *Flavivirus* comprises more than 70 viruses sharing common antigenic determinants and contains the first human virus to be isolated, yellow fever virus, which is also the prototype virus of the genus. Eleven flaviviruses are known to occur in Brazil: Bussuquara virus (BSGV), Cacipacore virus (CPCV), dengue virus (DENV-1, 2, 3, and 4), Iguape virus (IGUV), Ilheus virus (ILHV), Rocio virus (ROCV), Saint Louis encephalitis virus (SLEV) and yellow fever virus (YFV). Most of the Brazilian flaviviruses are maintained in nature as sylvatic zoonoses that occasionally infect humans and domestic animals in rural and

periurban areas<sup>2</sup>. The differential clinical diagnosis among arboviruses can be difficult, principally in the acute phase of the infection where disease symptoms are similar. Traditionally, the diagnosis of *Flavivirus* infection has been done by virus isolation or serological testing. To overcome such problems, reverse transcription-polymerase chain reaction (RT-PCR) methods using universal primers have been described for certain flaviviruses<sup>3-7</sup>.

The patient under study was a farmworker thirty three years of age, originating from the municipality of Theobroma, which is located 320km from the City of Porto Velho, the former capital of the Brazilian State of Rondônia. He was admitted to the Intensive Treatment Unit of the Dr. Ary Pinheiro Hospital with suspected diagnoses of both leptospirosis and yellow fever.

Blood samples were collected in the intensive treatment unit and sent to Institute for Research in Tropical Diseases to be used in viral isolation using newborn mouse brain. After isolation RT-PCR with specific universal *Flavivirus* primers would be conducted for the identification of the viruses via the electrophoretic migration pattern of the amplicons, nucleotide sequencing and comparison with published sequences.

#### CASE REPORT

##### Viral isolation

Five microliters of the patient's serum was inoculated into the brains of six newborn mice. The mice were observed for approximately seven days for signs of encephalitis. Diseased mice were stored at -80°C. The brain was eventually macerated and diluted into Leibovitz's-15 media in a 1/20 dilution. Next, 300µL of this mixture was inoculated in a monolayer of C6/36 cultures of *Aedes albopictus* cells<sup>8</sup>. Uninfected cell culture supernatant was used as a negative control. Strains of yellow fever virus and dengue virus type 3 were used as positive controls to test the specificity of the assays and were grown and maintained with Leibovitz's-15 medium (Leibovitz) containing 10% fetal bovine serum and 100mg/ml of antibiotic-antimycotic. The infected cultures were observed for seven days, and 500µl of supernatant was collected for viral RNA extraction. Cultures were maintained in a humidity controlled atmosphere at 28°C until the supernatants were collected.

##### Viral ribonucleic acid (RNA) extraction

RNA was extracted from the supernatants of infected C6/36 cultures using TRIzol (Life Technologies, USA) with modifications to the original protocol<sup>9</sup>.

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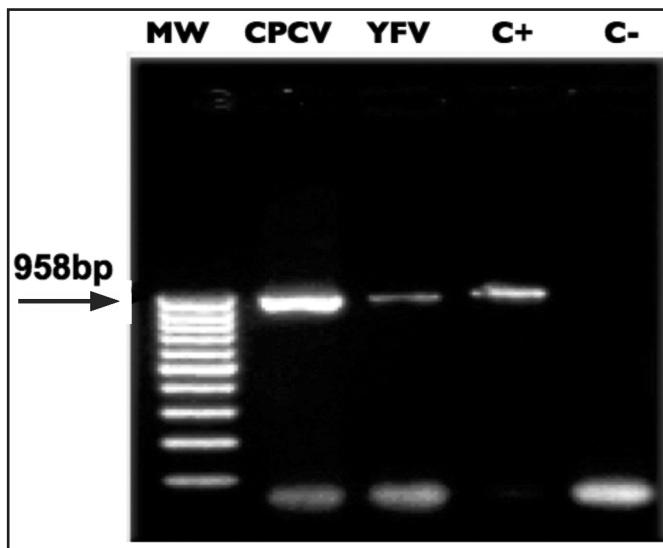
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**FIGURE 1** - Image of a 2% agarose gel stained with ethidium bromide showing the NS5 gene amplicon with universal primers for Flavivirus, FG1/FG2.

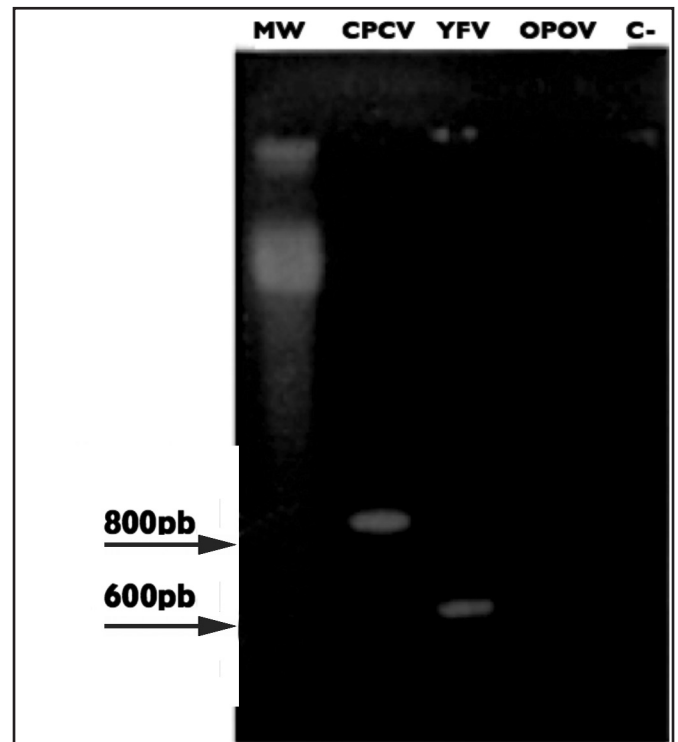
Line 1: MW: 100bp molecular weight marker. Line 2: 958bp fragment corresponding to amplification of the Cacipacore virus (CPCV) genome. Line 3: C+ positive control fragment of 958bp corresponding to amplification of the yellow fever 17DD vaccinal strain genome. Line 4: C+: positive control using dengue virus 3 genome isolated in Porto Velho. Line 5: C-: negative control with non-infected monolayer of C6/36 tissue.

#### Reverse transcription - polymerase chain reaction

As previously described by De Moraes Bronzoni et al.<sup>10</sup>, PCR was performed using genus-specific rapid detection of the Flavivirus genus, which amplifies the NS5 gene, and using the mosquito-borne Flavivirus universal primer pair, selected by Tanaka et al.<sup>7</sup>, for maximum homology with six species of non-American flaviviruses based on the original sequence of the yellow fever virus (17D vaccine).

#### Purification, cloning and nucleotide sequencing

Amplicons were purified from a 2% agarose gel using Perfectprep® Gel cleanup Kit (Eppendorf, USA). The DNA fragments were cloned using the pGEM T Easy cloning kit (Promega-USA). The cloned DNA fragment was sequenced using the DYEnamic ET DYE



**FIGURE 2** - Image of a 2% agarose gel stained with ethidium bromide showing the NS5 and 3'NCR amplicons using universal primers for Flavivirus, FLAV1/FLAV2.

Line 1: MW: 50bp molecular weight marker of. Line 2: 800bp fragment corresponding to amplification of the Cacipacore virus (CPCV) genome. Line 3: 600bp positive control fragment corresponding to amplification of the Yellow Fever virus (YFV) 17DD vaccinal strain genome. Line 4: C- negative control with Oropouche virus (OPOV-Orthobunyavirus). Line 5: C-: negative control with non-infected monolayer of C6/36 tissue.

terminator cycle sequencing kit in a mega BACE 1000 sequencer (Amersham Pharmacia Biotech, USA).

Amplicons of 958bp and 800bp were obtained by RT-PCR using the genus-specific, Flavivirus primer pairs FG1/FG2 and (Figure 1) FLAV1/FLAV2, respectively (Figure 2). Results revealed that the virus isolated from the patient showed 91% sequence similarity to other isolates of Cacipacore BeAn 4073 when using BLAST and the sequences available in GenBank (Figure 3).

Cacipacore virus BeAn 4073 NS5 gene, partial cds					
Length=274					
Score = 272 bits (137), Expect = 1e-69					
Identities = 242/264 (91%), Gaps = 11/264 (4%)					
Strand=Plus/Plus					
Query	378	GGAATATGGTAGTATTAAGTTAAGCAAATAGATAAGAAATTGTTAGAATAT-ATATGTT	436		
CACVP	18	GGAATATGGTAGTATTAAGTTA-GCAA-TAGATAAGGAAAT-GTTAGAATATTATATGTT	74		
Query	437	AGTTAAGAAGGTATTTG-TAGATTATATAGTATGGTTAAGTTAAGAATAAGGGATTGTA	495		
CACVP	75	AGTTAAGAAG-TATTTGGTAGATTATATAGTATGGTTA--TTACGTATTAGGGTTTGTGA	131		
Query	496	TAAATCCAAATGTAAATAAGGGACAATCTTATATTTAGTGGTTTAGAGACTTATTAGTC	555		
CACVP	132	-AAATC-AAATGTAAATAAAGGAAAATTTT-TATTTAGTGGTTTAGAGACTTATTAGTC	188		
Query	556	AGGCCAGAACTGCCACCGGATGCTGGGTAAACGGTGCTGACTGTAACCTAGCCCCAGGAG	615		
CACVP	189	AGGCCAGAAATGCCACCGGATGCTGGGTAAACGGTGCTGCCTGTAACCTAGCCCCAGGAG	248		
Query	616	GACTGTGTGAACAACTTACGTAA	639		
CACVP	249	GACTGGGTGAACAACTTCCGTAA	272		

**FIGURE 3** - Alignment of nucleotide sequence of Cacipacore virus (CACV) isolated in Theobroma City, in the State of Rondônia, with strain Cacipacore BeAn 4073 virus obtained by BLAST.

## DISCUSSION

In Brazil, arboviruses (arthropod born viruses) have a wide geographic distribution and cause several diseases, thus constituting an important health problem. Efficient and continued epidemiological surveillance programs are necessary to monitor viral activity in regions where they are endemic or enzootic<sup>10</sup>. These programs should help to restrict viral spreading and reduce the socioeconomic impact caused by these diseases. To achieve this, fast, sensitive, specific, and low-cost methods of diagnosing arboviral diseases are essential. The region in which the state of Rondônia is located is favorable to the dissemination of arboviruses because it possesses a large border with Bolivia and because the State is crossed by the BR 364 road, which links southern Brazil to the State of Acre, to Peru via the Inter-ocean road, and to the southern state of Amazonas (and thus to newly expanded agricultural frontiers). The totality of these conditions is favorable for the dissemination of arboviruses in the State of Rondônia.

The use of molecular methodologies, such as RT-PCR with universal Flavivirus primers, is very important because it allows rapid diagnosis of viral infections, including outbreaks of emergent and reemergent of Flaviviruses in Rondônia.

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