

Testing of tissues from vacuum aspiration and from chorionic villi sampling revealed that placenta and chorion contained Zika virus RNA. Isolation of Zika virus from the karyotype cell culture confirmed active viral replication in embryonic cells. All the tests performed suggest that the spontaneous abortion in this woman was likely associated with a symptomatic Zika virus infection occurring early in pregnancy. These findings provide further evidence of the association between Zika virus infection early in pregnancy and transplacental infection, as well as embryonic damage, leading to poor pregnancy outcomes (2). Given that embryo loss had probably occurred days before maternal-related symptoms, we hypothesize that spontaneous abortion happened early during maternal viremia. The prolonged viremia in the mother beyond the first week after symptom onset concurs with other recent reports (1,5). However, persistent viremia 3 weeks after pregnancy outcome has not been described previously and underscores the current lack of knowledge regarding the persistence of Zika virus infection. Because we identified Zika virus RNA in placental tissues, our findings reinforce the evidence for early gestational placental tissue as the preferred target for viral tropism (2,4). Finally, although laboratory tests were performed to dismiss other maternal infections (see online Technical Appendix), the attribution of Zika virus as the cause of the spontaneous abortion must be interpreted with caution, because a non-Zika-related etiology cannot be entirely ruled out. Further studies are warranted to investigate the natural history of Zika virus infection in pregnant women.

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### References

- van der Eijk AA, van Genderen PJ, Verdijk RM, Reusken CB, M gling R, van Kampen JJ, et al. Miscarriage associated with Zika virus infection. *N Engl J Med*. 2016;375:1002–4. <http://dx.doi.org/10.1056/NEJMc1605898>
- Martines RB, Bhatnagar J, de Oliveira Ramos AM, Davi HP, Iglezias SD, Kanamura CT, et al. Pathology of congenital Zika syndrome in Brazil: a case series. *Lancet*. 2016;388:898–904. [http://dx.doi.org/10.1016/S0140-6736\(16\)30883-2](http://dx.doi.org/10.1016/S0140-6736(16)30883-2)
- Schaub B, Monthieux A, Najioullah F, Harte C, C saire R, Jolivet E, et al. Late miscarriage: another Zika concern? *Eur J Obstet Gynecol Reprod Biol*. 2016;207:240–1. <http://dx.doi.org/10.1016/j.ejogrb.2016.10.041>
- Bhatnagar J, Rabeneck DB, Martines RB, Reagan-Steiner S, Ermias Y, Estetter LB, et al. Zika virus RNA replication and persistence in brain and placental tissue. *Emerg Infect Dis*. 2017;23:405–14. <http://dx.doi.org/10.3201/eid2303.161499>
- Driggers RW, Ho CY, Korhonen EM, Kuivanen S, J askel inen AJ, Smura T, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N Engl J Med*. 2016;374:2142–51. <http://dx.doi.org/10.1056/NEJMoal601824>

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## Isolation of Oropouche Virus from Febrile Patient, Ecuador

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We report identification of an Oropouche virus strain in a febrile patient from Ecuador by using metagenomic sequencing and real-time reverse transcription PCR. Virus was isolated from patient serum by using Vero cells. Phylogenetic analysis of the whole-genome sequence showed the virus to be similar to a strain from Peru.

Oropouche virus (OROV) is a negative-sense, single-stranded RNA virus (family *Bunyaviridae*, genus *Orthobunyaviridae*) with a tripartite genome consisting of large (L), medium (M), and small (S) segments. OROV causes a self-limiting acute febrile illness, Oropouche fever (1). Since its discovery in Trinidad in 1955 (2), >30 outbreaks of OROV have been reported from Brazil, Panama, and Peru, demonstrating the ability of this midgeborne virus to cause epidemics. Approximately 500,000 cases of Oropouche fever have been reported, making OROV one of the most clinically significant orthobunyaviruses (1). Two previous studies reported unconfirmed infections in Ecuador by using

serologic or antigenic evidence (3,4). We describe whole-genome sequencing and virus isolation of OROV in Ecuador.

We collected a blood sample from a consenting 41-year-old male patient in Esmeraldas, Ecuador, who sought treatment in April 2016 after 7 days of fever, headache, joint pain, muscle pain, and nausea. The patient reported that he had been in Esmeraldas for  $\geq 3$  months and had not traveled outside the province during that time. RNA was extracted from plasma of the blood sample and tested at Universidad San Francisco de Quito, Ecuador, and Public Health England, UK, for dengue virus (DENV), chikungunya virus (CHIKV), Zika virus, yellow fever virus, Mayaro virus, *Plasmodium* spp., *Leptospira* spp., and *Rickettsia* spp. by using real-time reverse transcription PCR (rRT-PCR) and conventional RT-PCR assays developed in-house or acquired commercially (Genesig, Primerdesign Ltd., Cambridge, UK). The sample gave borderline results for DENV (quantitation cycle [Cq] 35.3) and CHIKV (Cq 36.6; reference ranges  $\leq 35$  positive, 35–40 borderline,  $>40$  negative) and negative results for the other pathogens.

As an initial screen for other pathogens, we applied unbiased metagenomic sequencing. Analysis of sequencing reads by using Kraken, a system for assigning taxonomic labels to individual reads (5), identified 1% reads (5,016 of 464,444) as specific to OROV. We generated an OROV consensus sequence by mapping reads to a reference sequence, which resulted in coverage of 69% for S, 76% for M, and 79% for L OROV viral RNA segments (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/24/2/17-1569-Techapp1.pdf>). We classified 1,228 reads as DENV serotype 1, all of which mapped to a single 732-nt region of the DENV-1 reference genome. No reads mapped to CHIKV.

After confirmation of the presence of OROV by using a validated rRT-PCR (6), we attempted to isolate OROV by using Vero and C6/36 cell lines inoculated with the patient's plasma. We confirmed virus replication by detecting increasing OROV RNA over time by using rRT-PCR. We obtained whole-genome sequences by sequencing viral RNA from harvested OROV supernatant; each genome segment was sequenced at average depths of coverage of 55,532 $\times$  for S, 4,954 $\times$  for M, and 5,674 $\times$  for L segments. We deposited sequences in GenBank (online Technical Appendix). Genetic organization was similar to that of other OROV strains: segment lengths 952 nt for S, 4,387 nt for M, and 6,852 nt for L.

Phylogenetic analysis (online Technical Appendix) showed that the virus we isolated, OROV/EC/Esmeraldas/087/2016, was most closely related to a strain isolated from a patient in Peru during 2008 and excluded the possibility of the virus being a reassortant orthobunyavirus, such as Iquitos virus. This finding suggests a potential introduction across the Peru–Ecuador border; however, further investigation is required to understand the origin and incidence of OROV in Ecuador. The known urban OROV

vector, the *Culicoides paraensis* midge, is absent in the Pacific Coast region, including Esmeraldas (S. Zapata, pers. comm., 2017 Aug 31), which raises the question of alternative insect vectors in OROV transmission. *Culex* mosquitoes have previously been implicated as vectors in the OROV urban cycle, notably *C. quinquefasciatus* (1), a species that is widespread throughout South America (7).

DENV and CHIKV rRT-PCR results for this patient were inconclusive. The small proportion of DENV reads in the metagenomic data suggests DENV-1 infection is possible. Using ELISA to detect DENV and CHIKV-specific antibodies may help clarify the results.

It is likely that cases of Oropouche fever go unreported or misdiagnosed. Clinical features of the disease are similar to those of other viral, protozoan, and bacterial diseases previously reported in Ecuador (1,4,8,9). OROV might spread unnoticed across a wide geographic area, as suggested by this unexpected detection. Several studies have successfully documented the use of metagenomic sequencing for virus identification in febrile patients (10); this approach is becoming more practicable as costs decrease, the major benefit being the ability to detect unexpected or novel viral sequences, as evidenced by this detection of OROV.

This work highlights the need for increased surveillance of OROV in Ecuador and effective differential diagnostic assays to distinguish between emerging pathogens sharing common clinical descriptions to those already circulating. To clarify the true prevalence of this disease in Ecuador, the OROV rRT-PCR assay will be used to screen archived and newly collected samples from a cohort of patients seeking treatment for acute undifferentiated febrile illness during 2016–2017.

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This study was approved by the bioethics committee of Universidad San Francisco de Quito. The patients provided written consent indicating that they agreed for their samples to be tested for additional pathogens.

Ms. Wise is a PhD student registered with Plymouth University and funded by Public Health England. Her research interests are emerging viral infections and viral immunology.

### References

1. Travassos da Rosa JF, de Souza WM, Pinheiro FP, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected orthobunyavirus. *Am J Trop Med Hyg.* 2017;96:1019–30.
2. Anderson CR, Spence L, Downs WG, Aitken THG. Oropouche virus: a new human disease agent from Trinidad, West Indies. *Am J Trop Med Hyg.* 1961;10:574–8. <http://dx.doi.org/10.4269/ajtmh.1961.10.574>

3. Forshey BM, Guevara C, Laguna-Torres VA, Cespedes M, Vargas J, Gianella A, et al. Arboviral etiologies of acute febrile illnesses in Western South America, 2000–2007. *PLoS Negl Trop Dis*. 2010;10:4:e787. PubMed
4. Manock SR, Jacobsen KH, de Bravo NB, Russell KL, Negrete M, Olson JG, et al. Etiology of acute undifferentiated febrile illness in the Amazon basin of Ecuador. *Am J Trop Med Hyg*. 2009;81:146–51.
5. Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol*. 2014;15:R46. <http://dx.doi.org/10.1186/gb-2014-15-3-r46>
6. Weidmann M, Rudaz V, Nunes MRT, Vasconcelos PFC, Hufert FT. Rapid detection of human pathogenic orthobunyaviruses. *J Clin Microbiol*. 2003;41:3299–305. <http://dx.doi.org/10.1128/JCM.41.7.3299-3305.2003>
7. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. “Bird biting” mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect Genet Evol*. 2011;11:1577–85. <http://dx.doi.org/10.1016/j.meegid.2011.08.013>
8. Márquez S, Carrera J, Pullan ST, Lewandowski K, Paz V, Loman N, et al. First complete genome sequences of Zika virus isolated from febrile patient sera in Ecuador. *Genome Announc*. 2017;5:e01673–16. <http://dx.doi.org/10.1128/genomeA.01673-16>
9. Zambrano H, Waggoner JJ, Almeida C, Rivera L, Benjamin JQ, Pinsky BA. Zika virus and Chikungunya virus coInfections: a series of three cases from a single center in Ecuador. *Am J Trop Med Hyg*. 2016;95:894–6. <http://dx.doi.org/10.4269/ajtmh.16-0323>
10. Robinson ML, Manabe YC. Reducing uncertainty for acute febrile illness in resource-limited settings: the current diagnostic landscape. *Am J Trop Med Hyg*. 2017;96:1285–95. <http://dx.doi.org/10.4269/ajtmh.16-0667>

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## etymologia

### Oropouche [o'ro-poo"che] Virus

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In September 1955, a virus was isolated from a 24-year-old forest worker from the community of Vega de Oropouche, near the town of Sangre Grande, on the island of Trinidad (country: Trinidad and Tobago), who presented with fever, backache, and cough, which resolved spontaneously after 3 days. The virus was isolated from the patient’s blood by intracranial inoculation of suckling mice at the Trinidad Regional Virus Laboratory. Five years later, the virus was isolated from *Coquillettidia venezuelensis* (Theobald) mosquitoes collected ~30 miles away in the Bush Bush Forest. The urban vector was later identified as the midge *Culicoides paraensis*, but the sylvatic vector remains unknown. Virus has been isolated from the three-toed sloth, which is believed to be involved in the sylvatic transmission cycle.

The virus was shown to be unique but antigenically related to Simbu virus, which had recently been described from South Africa. It therefore became a member of the large family of bunyaviruses.<sup>1</sup>

The virus, Oropouche virus, named in keeping with the tradition of designating arboviruses by using local geographic names, stems from the name of the village, a nearby swamp (wetland), and river. It derives from an Amerindian word, but the ancient meaning of the word is not clear.

Oropouche virus has since proven to be one of the most common arthropodborne viruses infecting humans in the tropics of the Western Hemisphere. Clinical signs of infection include headache, myalgia, arthralgia, and chills; no deaths have been reported. It has been estimated to have infected more than half a million persons in Brazil alone, and there have also been large outbreaks in Panama and Peru. In keeping with the recent emergence of other arboviruses such as West Nile, chikungunya, and Zika viruses, Oropouche virus is a candidate for possible further urban spread and therefore warrants increased surveillance and diagnostics.

<sup>1</sup>In 2017, the International Committee on Taxonomy of Viruses (ICTV) reclassified the family *Bunyaviridae*, elevating the family to an order. The formal taxonomic placement of Oropouche virus is order *Bunyavirales*; family *Peribunyaviridae*; genus *Orthobunyavirus*; species; *Oropouche orthobunyavirus*.

#### Sources

1. Anderson CR, Spence L, Downs WG, Aitken TH. Oropouche virus: a new human disease agent from Trinidad, West Indies. *Am J Trop Med Hyg*. 1961;10:574–8. <http://dx.doi.org/10.4269/ajtmh.1961.10.574>
2. Travassos da Rosa JF, de Souza WM, Pinheiro FP, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche virus: clinical, epidemiological, and molecular aspects of a neglected orthobunyavirus. *Am J Trop Med Hyg*. 2017;96:1019–30.
3. Virus Taxonomy. V. The Classification and Nomenclature of Viruses. The Online (10th) Report of the International Committee on Taxonomy of Viruses (ICTV) [cited 2018 Mar 12]. <https://talk.ictvonline.org/taxonomy/>

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# Isolation of Oropouche Virus from Febrile Patient, Ecuador

## Technical Appendix

### RT-PCR testing

RNA was extracted from plasma by using the QIAmp viral RNA mini kit (Qiagen, Manchester, UK) and tested for 8 pathogens; Dengue virus (DENV), Chikungunya virus (CHIKV), Zika virus, Yellow fever virus, Mayaro virus, *Plasmodium*, *Leptospira* and *Rickettsia*, by using real-time and conventional RT-PCR assays developed or optimised at PHE, in addition to a commercial multiplex real-time RT-PCR kit (Genesig).

### Metagenomic Sequencing

RNA was DNase treated, before cDNA preparation and random amplification by SISPA (Sequence-independent Single Primer Amplification) and Illumina sequencing as described previously (1,2). An OROV consensus sequence was generated from patient plasma RNA using Quasibam (3), following mapping to reference sequences (S segment Genbank accession KP691632, M: KP052851, L: KP691612) using BWA MEM (4).

RNA extracted from harvested OROV supernatant was sequenced and analyzed as before. Kraken identified 31% of the reads as specific to OROV. Consensus sequences are deposited in Genbank, strain name 'OROV/EC/Esmeraldas/087/2016', S: MF926352, M: MF926353 and L: MF926354. The S segment contains 2 overlapping open reading frames (ORFs) encoding the nucleoprotein (696 nt) and non-structural protein (276 nt). The M segment contains a single ORF encoding a polyprotein (4,263 nt). The L segment contains a single ORF encoding the RNA-dependant RNA polymerase (6,759 nt). 3' terminal sequences were confirmed by random amplification of cDNA ends and sanger sequencing (5).

OROV/EC/Esmeraldas/087/2016 consensus sequences were used to re-map reads from the patient sample. This improved coverage of the M segment to 98.7%. Sequences from patient

plasma and cultured virus were compared, all segments are >99.8% identical. Eleven positions show single nucleotide polymorphisms, of which 3 are non-synonymous.

## **Phylogenetic Analysis**

For each genome segment, maximum-likelihood phylogenetic trees were generated in MEGA7 by using all complete coding sequences in Genbank. Analysis of S, M, and L segments showed that the most closely related strain was TVP-19256/IQE-7894 from Peru, 2008 (KP795086). Additionally, the Ecuador strain N gene is 97% identical to that of 2 Iquitos virus strains isolated in Peru.

## **Virus Isolation**

A 1:10 dilution of patient plasma was inoculated into Vero and C6/36 cell lines, incubated for 14 days or until cytopathic effect (CPE) was observed. CPE was observed in Vero cells at day 7, no CPE was observed in C6/36 cells. After 1 passage, virus was harvested by low speed centrifugation and filtration of cell-free supernatants.

RNA from supernatants taken at days 5, 7, 11, and 14 was tested by using a modified OROV specific rRT-PCR (6). OROV RNA was detected in supernatants from both cell lines and relative quantity of viral RNA increased over time, demonstrating that OROV was replicating within the cultures. Supernatants were also tested for DENV and CHIKV by using virus specific RT-PCR assays. No DENV or CHIKV RNA were detected.

## **References**

1. Atkinson B, Graham V, Miles RW, Lewandowski K, Dowall SD, Pullan ST, et al. Complete genome sequence of Zika virus isolated from semen. *Genome Announc.* 2016;4:e01116–16. [PubMed](https://pubmed.ncbi.nlm.nih.gov/26711116/) <http://dx.doi.org/10.1128/genomeA.01116-16>
2. Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, et al. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis. *Genome Med.* 2015;7:99. [PubMed](https://pubmed.ncbi.nlm.nih.gov/25711116/) <http://dx.doi.org/10.1186/s13073-015-0220-9>

3. Penedos AR, Myers R, Hadeb B, Aladin F, Brown KE. Assessment of the utility of whole genome sequencing of measles virus in the characterisation of outbreaks. PLoS One. 2015;10:e0143081. [PubMed http://dx.doi.org/10.1371/journal.pone.0143081](http://dx.doi.org/10.1371/journal.pone.0143081)
4. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26:589–95. [PubMed http://dx.doi.org/10.1093/bioinformatics/btp698](http://dx.doi.org/10.1093/bioinformatics/btp698)
5. Acrani GO, Tilston-Lunel NL, Spiegel M, Weidmann M, Dilcher M, Andrade da Silva DE, et al. Establishment of a minigenome system for Oropouche virus reveals the S genome segment to be significantly longer than reported previously. J Gen Virol. 2015;96:513–23. [PubMed http://dx.doi.org/10.1099/jgv.0.000005](http://dx.doi.org/10.1099/jgv.0.000005)
6. Weidmann M, Rudaz V, Nunes MRT, Vasconcelos PFC, Hufert FT. Rapid detection of human pathogenic orthobunyaviruses. J Clin Microbiol. 2003;41:3299–305. [PubMed http://dx.doi.org/10.1128/JCM.41.7.3299-3305.2003](http://dx.doi.org/10.1128/JCM.41.7.3299-3305.2003)