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Clinical profile of Oropouche Fever in Bahia, Brazil: unexpected fatal cases

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ABSTRACT

Oropouche virus (OROV) is an arbovirus transmitted to humans by mosquitoes, with the *Culicoides paraensis* mosquito species as its primary vector, causing Oropouche fever. Records of an outbreak in Brazil have so far been restricted to Central-North region of the country. However, an increase in the occurrence of cases of this disease has been observed in the state of Bahia, where the rapid spread of the OROV virus is configured as an outbreak in the South and East macro-regions of great concern for public health. This is a case-based study of acute OROV infection that led to the death of two young women without comorbidities amid an outbreak of the disease. The patient's biological samples were subjected to routine real-time PCR assays for the diagnosis of Oropouche fever and other pathologies. In addition, serological tests and metagenomics were performed during the laboratory investigation. This study shows the need for an active and efficient surveillance system to control the spread of this virus, as well as the importance of carrying out prospective studies to better clarify the natural history of this disease.

Keywords: Oropouche Fever, Outbreak, Bahia.

INTRODUCTION

Oropouche virus (OROV), the etiological agent of Oropouche fever, is an arbovirus that belongs to the Orthobunyavirus, of the Peribunyaviridae family (1, 2, 3). Discovered in 1955 in Trinidad and Tobago, OROV has been detected with an increasing incidence and geographic spread over time (4, 5). In Brazil, the virus was initially isolated in 1960, from a sloth (*Bradypus tridactylus*) (2,6,7). Subsequently, human infections have been predominantly observed in the Northern region of the country, with sporadic cases documented in the Central, Northeast and Southeast

regions (8,9). Transmission to humans occurs mainly through the bite of the *Culicoides paraensis* mosquito, known as “maruim” (in Portuguese) (8,9,10).

The Oropouche Fever is considered one of the most important vectors transmitting diseases in Latin America, requiring special attention from public health authorities (4). Preliminary estimates indicate that more than half a million people have already been infected by this virus in Brazil (11). However, its incidence is underestimate, primarily due to clinical similarities with other arboviruses, such as Dengue Fever, Chikungunya and Zika, in addition to unavailability of specific diagnosis tests (2,3,8).

The state of Bahia is in the Northeast Region of Brazil and has 417 municipalities that is divided in 28 regions and nine Health macro-regions. It is the fourth largest state in the country in terms of population, estimated in 14,141,626 inhabitants. The distribution of this population in the territory is heterogeneous, with highly populated areas such as the Eastern macro-regions, which encompasses the Metropolitan Region of Salvador (the capital of the state) and concentrates 31.6% of the population, and others where only 5.5% of the population lives (Extreme South macro-region).

In 2020, the Oropouche virus was detected for the first time in the Metropolitan Region of Salvador, Bahia (8). From October 2023, the Central Public Health Laboratory (LACEN-BA) began a diagnostic expansion in samples suspected of the urban arboviruses and which were negative in the molecular biology method (Rt-PCR) for Dengue, Chikungunya and Zika, expanding the differential diagnosis to Oropouche and Mayaro viruses. In March 2024, Lacen identified the presence of OROV in notified cases in state of Bahia. Since then, the spread of the disease has been monitored by state epidemiological surveillance, and recently, an increase in the occurrence of cases has been observed in the state, where the rapid spread of the OROV virus already represents an outbreak of great concern for public health (12). Until epidemiological week 25 of the year 2024, 748 confirmed cases of Oropouche Fever were recorded and distributed across seven health macro-regions: Center-East, Center-North, Extreme-South, East, North-East, Southwest and South. In this context, surveillance laboratory stands out as a crucial tool for monitoring the spread of the virus, contributing to the implementation of more effective prevention and control measures (13).

With the purpose of fulfilling this objective of monitoring the cases, since October 2023, the Health Department of the State of Bahia (SESAB) has begun the epidemiological investigation process, with monitoring and following up to improve the knowledge and surveillance of Oropouche Fever in the State. During this screening process, two patients with a laboratory diagnosis of OROV infection died.

METHODS

Type of study. This is a case-based study involving patients who were infected by the Oropouche virus and died as a result. Clinical information was collected

retrospectively, through analysis of digital records and an epidemiological investigation was conducted to collect clinical and laboratory data. In addition, interviews were conducted with the medical teams who cared for the patients, and residents living in the same household as the cases were investigated. In addition, an extensive literature review was carried out on the topic.

Ethical Procedures. The study was approved by the Research Ethics Committee (CEP) under the number CAAE 81053724.6.0000.0052.

Laboratorial Procedures.

1. Isolation of genetic material and diagnosis by qPCR

The genetic material (DNA/RNA) was extracted from 200 µL of clinical samples using the EXTRACTA KIT- DNA and RNA OF PATHOGENS – MDx (Loccus, São Paulo, Brazil), following the manufacturer's instructions. Subsequently, real-time PCR reactions were carried out for the diagnosis of different diseases.

The diagnosis of Oropouche virus (OROV) infection was established by real-time polymerase chain reaction with reverse transcription (RT-qPCR) using an own methodology with inputs produced by the Institute of Molecular Biology of Paraná (IBMP). All reaction conditions were based on the assay by Naveca et al described previously (14). The following pairs of primers and probe were used: Forward Primer - OROV_FNF (5' TCCGGAGGCAGCATATGTG 3'), Reverse Primer - OROV_FNR (5' ACAACACCAGCATTGAGCACTT 3') and the Probe - OROV_FNP (5'(FAM) CATTGAAGCTAGATACGG 3'). Thermal cycler conditions consisted of reverse transcription at 50°C per 15 minutes, RT inactivation/initial denaturation by 45 cycles of denaturation at 95°C per 15 seconds and annealing and extension at 60°C per 45 seconds.

The diagnosis of other diseases was also carried out using the real-time PCR-technique, as described below: virus Mayaro - *in-house* methodology, IBMP, Paraná, Brazil; **Leptospirosis** - *in-house* methodology for detecting the *lipL32* target gene; **Zika, Chikungunya and Dengue Fever viruses** – ZC D-Typing Molecular Kit, Bio-Manguinhos, Rio de Janeiro, Brazil, The Brazilian Health Regulatory Agency (**ANVISA**) 80142170061 – detection limits of 200 copies/mL for Zika and of 100 copies/mL for Chikungunya and the for the 4 serotypes from Dengue; **Haemophilus influenzae, Neisseria meningitidis and Streptococcus pneumoniae** – VIASURE PCR Detection Kit real-time PCR Detection Kit, Certest Biotec, San Mateo de Gallego, Zaragoza, Spain.

Metagenomics: cDNA synthesis, PCR and Nanopore sequencing

The samples were sequenced using the viral metagenomics approach, according to the SMART-9N protocol, described by Claro et al. (15). Initially, the samples were subjected to nucleic acid extraction (DNA and RNA) and concentrated to 10µL with the Zymo RNA clean & concentrator-5 (Zymo Research, USA). Next, cDNA synthesis was performed using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, USA) and random primers (RLB RT 9N and RLB TSO). Subsequently, the

PCR reaction was conducted using Q5 Hot Start High-Fidelity DNA Polymerase (New England BioLabs, USA) and another primer described in the SMART-9N protocol (RLB PCR). The incubation conditions for both reactions were carried out following the manufacturers' recommendations. The PCR products were purified with AMPure XP beads (Beckman Coulter, UK), quantified using the Qubit dsDNA High Sensitivity Assay kit (Life Technologies, USA) on the Qubit 3.0 equipment (Life Technologies, USA) and normalized to 300ng. The sequencing library was prepared using the Ligation Sequencing Kit (SQK-LSK109) and Native Barcoding (EXP-NBD114). The final library (60ng) was loaded into an R9.4.1 flow cell (FLO-MIN106) and sequenced on the MinION device (Oxford Nanopore Technologies, UK) for 24h. All procedures described above were performed for the negative control. The sequencing library was demultiplexed and the reads had their barcodes removed using Guppy software (v6.5.7) (16). Classification of reads was performed using the online tool Genome Detective (17).

2. Serological Tests

Serological tests were performed for **Dengue Fever, IgM** (Immunoenzymatic Capture Reaction (MAC-ELISA), Panbio ELISA, Abbott Diagnostics Korea Inc, Korea); **Chikungunya, IgM** (Ezyme Immunoassay, Euroimmun, Lubeck, Germany); **Leptospirosis, IgM** (Ezyme immunoassay, Panbio ELISA, Abbott Diagnostics Korea Inc, Korea); **Hepatitis B, HBsAG** – Electrochemiluminescence Immunoassay, Elecsys HBsAG II, Roche Diagnostics, Germany); **Hepatitis B, Total Anti-HBc** – Electrochemiluminescence Immunoassay, Elecsys Anti-HBc II, Roche Diagnostics, Germany); **Hepatitis C, Anti-HCV** - Electrochemiluminescence Immunoassay, Elecsys Anti-HCV II, Roche Diagnostics, Germany). **Hepatitis A, Anti-HAV Total** (Electroluminescence Immunoassay, Elecsys Anti-HAV II, Roche Diagnostics, Germany); **Hepatitis A, Anti-HAV IgM** (Electroluminescence Immunoassay, Elecsys Anti-HAV IgM, Roche Diagnostics, Germany). The kits were used in accordance with the manufacturers' guidelines.

CLINICAL DISCUSSION

Patient #1, female, 24 years old, from Serra Grande district of the municipality of Valença, South Bahia macro-region.

Symptoms began on March 23th, 2024, with reports of fever lasting one day, headache, retroorbital pain, myalgia, severe abdominal pain, diarrhea, nausea and vomiting. No comorbidities.

She was admitted to Matuípe Municipal Hospital on March 25th, 2024, and discharged on the same day. On March 23rd, 2024, she sought another Family Health Unit in the district of Serra Grande and discharged. Later that same day, she went to the reference hospital in the municipality of Valença, at night, and discharged on the morning of March 26th, 2024, returning to the same unit with a

worse condition at night. Vital data upon admission and during hospitalization in the health care unit are shown in Table 1.

During hospitalization at Valença hospital, dipyrone, 1000mL crystalloid solution, ceftriaxone 2g, dexamethasone 4mg, ondansetron and, B complex were administered. She then reported blurred vision, reposting difficulty in seeing. Seven hours after the admission, the patient continued to have severe abdominal pain, being hypoactive, with ocular edema.

After 10 hours, the patient developed psychomotor agitation and in the subsequent two hours she began to experience hypotension and desaturation. A Venturi mask was introduced at eight liters/min, followed by orotracheal intubation, with a moderate amount of blood in the tube. After an hour the case progressed to cardiorespiratory arrest (CRA) and death on March 27th, 2024, 13 hours after admission to the hospital unit.

Table 2 presents the laboratory test results at two different time points, indicating hemoconcentration, leukocytosis, and thrombocytopenia, with hepatic and renal dysfunction initially and a sharp drop in hemoglobin and hematocrit with deterioration in coagulation parameters and liver function in the 2nd measurement, already close to the moment of death. A tourniquet test was performed with a negative result.

After an epidemiological investigation, it was found that three more people who live in the same household fell in the period with similar clinical symptoms.

Table 1. Vital signs for patient #1, in hours, after admission

Variable	Admission	Time after the admission		
		+ 7 hours	+ 10 hours	+ 12 hours
BP*	90/60	80/60	90/60	80/40
PS**	100	100	101	61
SatO2***	99	99	96	92
Temp****	36,0	np [@]	np	np

* Blood pressure (mmHg) ** Pulse (beats per minute) ***Oxygen saturation (%) ****Temperature (armpit, in Celsius)) [@]not performed.

Table 2. Laboratory test results for patient #1 after admission

Variable	6 hours after admission	13 hours after admission
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Ht, Hb, MCV, MCH*	50.3%, 16.7g%, 82, 27	20.9%, 7.00g%, 88, 29
Leukocytes (per mm³)	44,700	24,500
Neutrophils (%)	71%	80%
Band forms (%)	8%	10%
Metamyelocytes (%)	1%	1%
Lymphocytes (%)	16%	6%
Platelets (per mm³)	125,000	43,000
Bleeding time		1 minute
Clotting time		Over 30 minutes
Clot retraction		complete
AST (U/L)**		970
ALT (U/L)***	7	404
GGT****	559	144
TB/DB (mg/dL)*****	2,78/1,52	
Creatinine (mg/dL)	4,1	2,3

*ht=hematocrit, hb=hemoglobin, mcv=mean corpuscular volume, mch=mean corpuscular hemoglobin; **=aspartate aminotransferase; ***=alanine aminotransferase; ****gamma-glutamyl transferase; *****=total/direct bilirubin.)

The results of the molecular biology and serological tests are presented in Table 3, which confirms the OROV. Sequencing the complete genome of the pathogen made it possible to generate three complete segments: the S segment, with 99.7% coverage, the M segment, with 99.7% coverage, and the L segment, with 98.9% coverage.

Table 3. Results of molecular biology and serological tests for patient # 1*

Diagnosis method	Result
RT-qPCR for Dengue	Not detectable
RT-qPCR for Chikungunya	Not detectable

RT-qPCR for Zika	Not detectable
RT-qPCR for Virus Mayaro	Not detectable
RT-qPCR for Virus Oropouche	Detectable (Ct=16)
Serology for Dengue (IgM)	Non-reactive
Serology for Leptospirosis (IgM)	Non-reactive
Serology for Chikungunya (IgM)	Non-reactive
qPCR for <i>Neisseria meningitidis</i>	Not detectable
qPCR for <i>Streptococcus pneumoniae</i>	Not detectable
qPCR for <i>Haemophilus influenzae</i>	Not detectable

*All performed in serum sample

Patient # 2, female, 21 years, from Camamu, South Bahia macro-region.

Symptoms began on June 5th, 2024, with fever, myalgia, headache, retroorbital pain, pain in the lower limbs, asthenia, joint pain and she remained at home using dipyrone. After four days, she developed a reddish rash and purple spots on her body as well as nose, gum and vaginal bleeding. She also complained of weakness, drowsiness and vomiting. She had no comorbidities and denied pregnancy and/or previous miscarriage.

She was then admitted to a health unit at Camamu Municipal Hospital, and after nine hours she was transferred to a hospital in Itabuna. She appeared drowsy, with cyanosis of the extremities, reporting persistent vomiting, and prolonged fasting. On examination, she had bleeding gums and epistaxis, vaginal bleeding, cold and clammy skin, in addition to widespread petechia, and died two hours after admission. She used 1500mL of crystalloid solution and glucose, ondansetron, dipyrone and K vitamin, omeprazole, and dipyrone/scopolamine. Her vital signs are depicted in Table 4.

After an epidemiological investigation, it was found that the patient's grandfather presented with an acute febrile illness 20 days before the patient started experiencing symptoms and serological tests, carried out 17 days after the patient's death, showed that he had evidence of recent infection by OROV, with negative IgM for Dengue Fever, negative IgM for Chikungunya and, positive IgM for OROV.

Laboratory findings for patient #2 is presented in Table 5.

Table 4. Vital signs for patient #2, in hours, after admission.

Variable	Time after admission		
	Admission	+ 5 hours	+ 9 hours
BP*	120/70	120/80	110/77
PS**	54	56	93
SatO2***	98	99	98
Temp****	36.1	36.1	np [@]

Blood pressure (mmHg) ** Pulse (beats per minute) *Oxygen saturation (%)
 ****Temperature (armpit, in Celsius) [@]not performed

Table 5. Laboratory test results for patient #2 after admission.

Variable	On admission	10 hours from admission
Ht, hb, MCV, MCH*	38.7%, 13.5g%, 86, 30	43.7%, 14.0g%, 82, 26
Leukocytes (per mm3)	9,500	19,400
Neutrophils (%)	59%	58%
Band forms (%)	0%	0%
Metamyelocytes (%)	0%	0%
Lymphocytes (%)	34%	36%
Platelets (per mm3)	147,000	91,000
Prothrombin time (seconds)		Above 120 seconds
Partial thromboplastin time (seconds)		Above 120 seconds
Bleeding time		5 minutes
Clotting time		Above 30 minutes

TB/DB (mg/dL)**	2,71/1,54
Creatinine (mg/dL)	3.6
NS1***	Non-reactive

*ht=hematocrit, hb=hemoglobin, mcv=mean corpuscular volume, mch=mean corpuscular hemoglobin; **=total/direct bilirubin; ***rapid immunochromatographic test for Dengue.

The results of molecular biology and serological tests are presented in Table 6, confirming OROV infection. Sequencing the complete genome of the pathogen made it possible to generate three complete segments: the S segment, with 99.9% coverage, the M segment, with 99.7% coverage, and the L segment, with 99.2% coverage.

Table 6. Results of molecular biology and serological tests for patient # 2*

Diagnosis method	Result
RT-qPCR for Dengue	Not detectable
RT-qPCR for Chikungunya	Not detectable
RT-qPCR for Zika	Not detectable
qPCR for Leptospirosis	Not detectable
RT-qPCR for Mayaro Virus	Not detectable
RT-qPCR for Oropouche Virus	Detectable (Ct=8)
Serology for Dengue (IgM)	Non-reactive
Serology for Leptospirosis (IgM)	Non-reactive
Serology for Chikungunya (IgM)	Non-reactive
Serology for Hepatitis C (IgG/IgM)	Non-reactive
Anti-Hbc (IgG/IgM) / AgHbs	Non-reactive/Non-reactive
Serology for Hepatitis A (IgG/IgM)	Non-reactive
qPCR for <i>Neisseria meningitidis</i>	Not detectable

qPCR for <i>Streptococcus pneumoniae</i>	Not detectable
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qPCR for <i>Haemophilus influenzae</i>	Not detectable
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*Performed in serum samples, with the exception from qPCR to Leptospirosis (total blood).

DISCUSSION

This is the first record of acute OROV infection that led to the death of two women. In both clinical cases presented in this study lived in areas of active transmission of OROV, amid an ongoing outbreak at the south coast in the state of Bahia. Additionally, they were young and did not have chronic diseases or any other comorbidities.

Also, an extensive investigation for both cases showed high viral loads for OROV (Ct of 16 and 8, respectively) and negative results for Dengue Fever, Chikungunya, Zika, Mayaro, Leptospirosis, meningococcal and pneumococcal disease, and *Haemophilus influenzae*. So, for both cases, epidemiological and clinical findings were confirmed with laboratory results for OROV infection with absence of co-infection with the other pathogens tested.

The clinical course of these two cases highlights four important points to discuss. Firstly, the rapid evolution from the onset of symptoms to death; secondly, the outcome of the two cases in a disease where there were no reports of deaths; the third one, severe coagulopathy as the probable mechanism that led to death; and, finally, the important liver involvement that may have contributed to the coagulopathy itself and, consequently, to death.

OROV infection is described as having an incubation period of four to eight days with viremia detected within the first four days after symptom onset, and symptoms are characterized as an acute febrile illness, usually accompanied by headache, myalgia, arthralgia, anorexia, dizziness, chills, and photophobia. (18).

These authors discuss that the disease may present a bimodal behavior, and that after the initial febrile episode, symptoms may recur, generally with less intensity, in approximately 60% of cases, without reporting clinical worsening or progression to a serious hemorrhagic disease (18).

Due to the rapid evolution between symptom onset and death, neither of the two cases showed a bimodal pattern of disease.

Studies show that aseptic meningitis and meningoencephalitis are associated with OROV infection, but no sequelae or death have been reported (19,20,21). In the

present study, the patients did not present neurological findings compatible with meningitis or encephalitis.

Mourão et al studying an outbreak of OROV in Manaus, state of Amazonas, Brazil, in 2007-2008, observed hemorrhagic phenomena (petechiae, epistaxis and bleeding gums) in 20 patients (representing 15.5% of the positive sample), all patients recovered without sequelae (22). Hemorrhagic phenomena were present in the cases here, but with a much more dramatic evolution.

Another study conducted in Peru described an outbreak of OROV infection in 2010, with 108 confirmed cases, with epistaxis, gingival and vaginal bleeding observed in some cases, with one case presenting petechiae, but no deaths reported. (23).

In 1978, Araújo et al demonstrated that OROV could be detected in the liver six hours after OROV was inoculated intracerebrally into three-week-old hamsters, suggesting hematogenous transmission of the virus from the brain to liver lesions, with significant necrosis of hepatocytes (24).

Thus, it is clear that OROV infection can lead to hemorrhagic phenomena, as previous studies have demonstrated, and liver involvement can also be expected in this infection. However, how can both phenomena interact in a given patient and lead to a severe form of the disease?

Both patients in the present clinical study had a bleeding diathesis – with normal bleeding time and prolongation of clotting time for the first case, and prolongation of clotting time together with important elevations of prothrombin time (PT) and partial thromboplastin time (PTT) for the second.

Bleeding time is an exam to evaluate platelet function, it is evident that the first patient presented mild thrombocytopenia (125,000 per mm³) which reached the lowest level of 43,000 per mm³ few minutes before death, corroborating a mild to moderate consumption of platelets. In contrast, her clotting time was very high (beyond 30 minutes) pointing to a significant deficiency in clotting factors (25).

Elevated liver enzyme levels may signal that severe liver involvement may be behind the coagulopathy observed in this patient. And, combined with a marked drop in hematocrit (50.3% to 20.9%), this points to a possible internal bleeding, further explaining the unfavorable outcome of this case.

The second patient had highly elevated PT and PTT times. PT is the time for plasma to clot after the addition of thromboplastin and evaluates extrinsic and common coagulation pathways, which helps detect deficiencies of factors II, V, VII, X and low fibrinogen (26). PTT measures the time it takes a patient's blood to form a clot and is a measure of the activity of the intrinsic pathway of the coagulation cascade (27).

Therefore, both coagulation pathways were severely affected in this second patient, in parallel with an almost normal level of platelets (147,000 per mm³) and, also as it happened with the first patient, a high prolongation of the clotting time. Once

again, this combination of laboratory results points to a severe coagulopathy, explaining the many sites of hemorrhage observed and acute kidney failure.

Other possible mechanisms involved could be a cytokine storm syndrome secondary to infection, leading to consumptive coagulopathy and platelet dysfunction in parallel to the hemodynamic malfunction of target organs, including kidney and liver. This is a possibility that needs further investigation.

In both cases, the clinical course was remarkably similar to a severe Dengue Fever virus infection, with shock, bleeding, and extensive coagulopathy. If it were not for the extensive laboratory evaluation associated with an ongoing OROV outbreak in the region, both cases would likely be inappropriately classified as deaths from Dengue Fever rather than OROV.

The pathogenesis of this neglected disease must be better studied to explain the rapid evolution observed in the present study, compatible with a severe hemorrhagic fever. Furthermore, a surveillance network must be quickly established to monitor patients with Oropouche and prospective studies must be carried out to better clarify the natural history of this disease.

CONCLUSION

The clinical course of the patients with Oropouche Fever in the present clinical study was very similar to that of a severe hemorrhagic fever, commonly seen in Dengue cases. Therefore, it is extremely important to implement active epidemiological surveillance, with case definition and laboratory markers that can quickly differentiate currently circulating arboviruses and guarantee the collection of sufficient samples to monitor other diseases, in addition to carrying out surveillance genomics.

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