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Virological findings in a case of travel-associated Oropouche virus (OROV) infection imported to Italy, June 2024

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ABSTRACT

Here we report the acute and post-acute virological findings in a OROV infected traveller returning to Italy from Cuba. Testing multiple specimen types and the prolonged detection of OROV RNA in whole blood and urine samples extend the possibility of cases confirmation through direct diagnosis even in convalescence-phase of infection.

1. Introduction

Oropouche virus (OROV) is an emerging and poorly identified arbovirus endemic in many South American countries and over the past decade, outbreaks have mainly occurred in the Amazon region [1]. Since the onset of 2024, an unprecedented spread of OROV has been documented in South America and the Caribbean, expanding into previously nonendemic areas [2]. OROV is a member of the genus *Orthobunyavirus*, Peribunyaviridae family. The virus is transmitted to humans in urban settings via the bites of infected *Culicoides paraensis* midges. However, it is noteworthy that the virus can also be transmitted by certain species of mosquito. Replication-competent OROV was detected in the semen of an infected patient, a finding that prompts concerns regarding the potential for sexual transmission [3]. The potential for vertical transmission of OROV, resulting in adverse pregnancy outcomes, including foetal death and congenital abnormalities, is currently under evaluation [4].

OROV disease is characterized by the onset of an acute febrile illness accompanied by symptoms such as headache, nausea, vomiting, and muscle and joint pain. It is important to note the similarity in the clinical presentation of OROV disease and dengue fever that can result in the difficulty of distinguishing between the two diseases clinically, and consequently, many OROV infections have been under-reported or misdiagnosed as cases of dengue fever. In the course of the 2024 OROV outbreak, a number of severe clinical consequences have been documented. These include reports of fatalities in adults afflicted with acute neurological diseases, as well as cases of progression to haemorrhagic manifestations [2,5,6].

Several travel-associated cases, mostly from Cuba and Brazil, have been reported in the United States, Canada and Europe [7,8]. Acute OROV infection can be confirmed by identification of viral RNA on different specimen types, though serum and cerebrospinal fluid (CSF) are used most often as suggested on international guidelines [9]. Limited data on the kinetic, persistence and detection rate of OROV RNA in different biological matrices are available from reports on imported cases ([3,10–15]).

2. The study

This report presents the virological findings in a traveller who has been infected with OROV and who is returning to Italy from Cuba. As the Regional Reference Laboratory for Arbovirus infections in Emilia-Romagna region, following the international alerts regarding the ongoing outbreak of OROV in Cuba, we introduced OROV into our diagnostic algorithm for travellers returning from South and Central America and the Caribbean presenting with clinical illness indicative of an arbovirus infection. Following the implementation of the updated diagnostic workflow in our Center, 16 patients were tested for OROV infection between mid-June and the end of October 2024. In mid-June, a case of OROV infection was identified in a traveller returning to Italy from a 3-week journey in Cienfuegos, a province in Cuba, which was one

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of the most affected areas during the Cuban outbreak of OROV [16].

The patient, a 48-year-old immunocompetent male, upon returning to Italy on 15 June, presented with fever, nausea, headache, asthenia lipotimia, malaise, fatigue, retro-orbital pain, arthralgia and myalgia, the onset of which had been on 13 June. On 16 June, the patient attended the Center for Care and Urgency (CAU) in Piacenza, Emilia-Romagna. On 16 June, the Regional Reference Center for Microbiological Emergencies (CRREM), Unit of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, conducted molecular and serological diagnostic for the identification of dengue, chikungunya and Zika. Dengue NS1 antigen (Standard F Dengue NS1 Ag FIA, SD Biosensor) on serum and RT-PCR for Dengue, Zika and Chikungunya viruses (RealStar Chikungunya RT-PCR Kit 2.0, RealStar Dengue RT-PCR Kit 2.0 and RealStar Zika virus RT-PCR Kit 1.0, altona Diagnostics GmbH) on whole blood, serum, plasma and urine samples were negative as well as IgM and IgG antibodies for Dengue, Chikungunya and Zika (Dengue VirClia IgG Monotest, Dengue VirClia IgM, Chikungunya VirClia IgG Monotest, Chikungunya VirClia IgM Monotest, Zika VirClia IgG Monotest and Zika VirClia IgM Monotest, VirCell Microbiologists, Spain). The patient was diagnosed with OROV infection by detection of OROV RNA in serum (Ct, 22), plasma (Ct, 22), whole blood (Ct, 20) and urine (Ct, 33) samples by a specific OROV real time RT-PCR protocol [17] (Table 1) and virus was successfully isolated on Vero E6 cells from serum samples collected at 3 days post onset. Cytopathic effect (CPE) was observed by light microscopy after 4 days (Fig. 1) and viral replication was confirmed by an increased OROV-RNA load (Ct-value 13.0) in cell growth supernatant.

The patient was not admitted to hospital, and active surveillance was established for a period of seven days (involving daily telephone communication) by Public Health authorities in order to gather information regarding the patient's health conditions. Within eight days of the clinical onset, the patient exhibited a full recovery, with all symptoms resolved. Follow-up samples (serum, plasma, whole blood and urine) were longitudinally collected at 22 days after the initial symptom's onset and OROV RNA was detected in whole blood (Ct, 36) and urine (Ct, 26) samples while serum and plasma samples tested negative (Table 1).

3. Conclusions

Travellers returning from regions where arbovirus outbreaks are ongoing, usually represent important sentinels for international surveillance and often offer the opportunity to know more about epidemiology, clinical features of infections and diagnostic and to study the pathogenesis and the genomics of viruses. The number of OROV cases in travellers is most probably underestimated due to mild and self-limiting clinical course of the infection and to a potential underdiagnosis caused by limited diagnostic availability. In this context, the capability to detect OROV RNA is of crucial importance for cases confirmation. The present study reports that the presence of OROV RNA in urine and whole blood samples may offer enhanced sensitivity compared to testing serum and/or plasma, particularly in the post-acute phase of infection. This finding aligns with previous reports on the detection of various arboviruses, including West Nile virus (WNV) and Zika virus (ZIKV), where similar observations have been made [18–20].

Table 1
Virological findings in a OROV infected patient during a 22-days follow-up.

Days post symptoms onset	Specimen type	OROV RNA (cycle threshold, Ct)
3 days	Serum	Positive (22.0)
	Plasma	Positive (22.0)
	Whole blood	Positive (20,0)
	Urine	Positive (33.0)
22 days	Serum	Negative
	Plasma	Negative
	Whole blood	Positive (36.0)
	Urine	Positive (26.0)

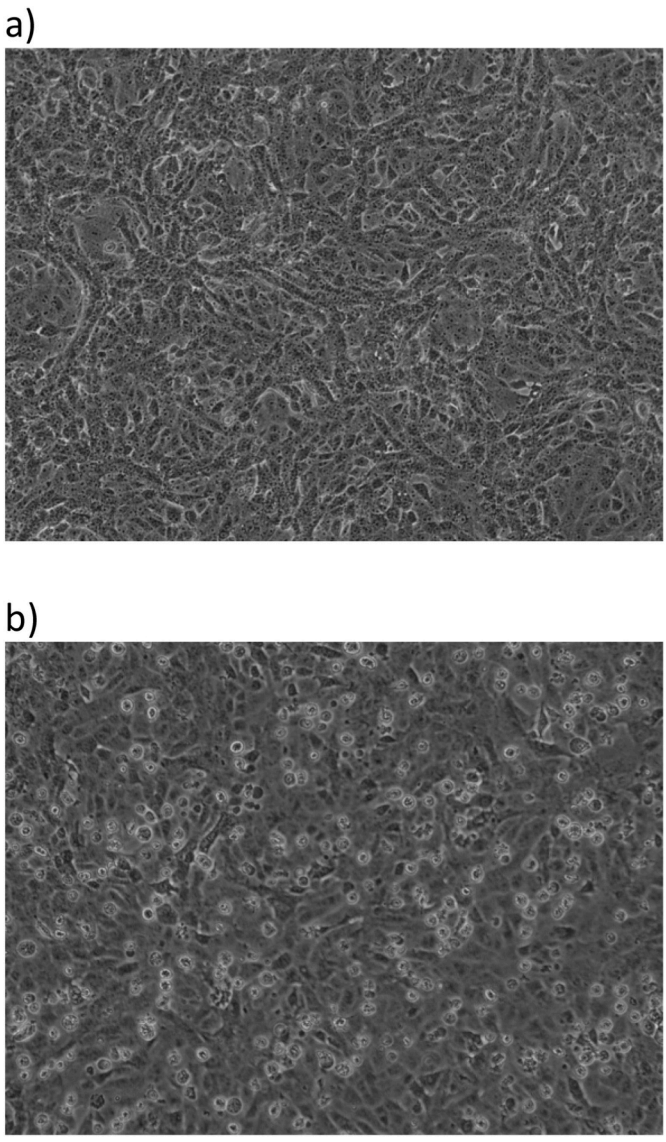

Fig. 1. Cytopathic effect (CPE) associated with OROV virus infection. Serum samples from an Italian traveller who visited Cienfuegos province in Cuba during July 2024, was inoculated onto Vero E6 cells monolayer and after a period of 4 days of incubation, the presence of a CPE was observed under light microscopy. Original magnification was set at × 10
a) Uninfected Vero E6 cells.
b) Vero E6 cells inoculated with serum sample from patient collected 3 days after the onset of symptoms.

The simultaneous testing of multiple specimen types (serum, plasma, whole blood and urine) for the presence of OROV RNA, particularly when samples are collected more than one week after the onset of symptoms, has been shown to greatly extend the rate and the time frame of OROV RNA detection increasing the possibility of cases confirmation through direct diagnosis even in the convalescence-phase of infection when the detection rate of viral RNA in plasma specimens is reduced [11–14]. In one patient, the presence of OROV RNA was identified in whole blood and urine samples up to 58 days and 32 days after the onset of symptoms, respectively [11]. As reported by Barbiero et al., two patients exhibited the presence of OROV RNA in whole blood at 68 and 82 days following the onset of symptoms [13]. In two other patients, OROV RNA was detected in whole blood samples collected at 90 and 95 days after the onset of symptoms, respectively [14]. Although, viraemia can

be persistently detectable in whole blood samples from individuals with OROV infection, no replication-competent virus has been recovered at several delayed timepoints [13,14]. The data presented here, albeit limited to a single case of OROV infection, contributes to the advancement of knowledge regarding viral kinetics and the diagnostic opportunities for OROV infection.

Overall, our case report emphasizes how crucial is the promptness of reference laboratories in extending their diagnostic algorithms to other neglected arbovirus infections following the rapidly changing international epidemiological scenario, thus supporting continuous surveillance for the identification of imported and autochthonous cases, in order to undertake effective measures to reduce the risk of transmission and potentially the risk of epidemics.

CRediT authorship contribution statement

Giada Rossini: Writing – review & editing, Writing – original draft, Conceptualization. **Beatrice Mola:** Writing – review & editing, Formal analysis, Data curation. **Alessandra Rampini:** Writing – review & editing, Formal analysis, Data curation. **Margherita Ortalli:** Writing – review & editing, Writing – original draft. **Giovanna Mattei:** Writing – review & editing, Formal analysis, Data curation. **Tiziana Lazzarotto:** Writing – review & editing, Writing – original draft.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Prof Tiziana Lazzarotto reports financial support was provided by MUR-PNRR Extended Partnership Initiative on Emerging Infectious Diseases. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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