Oropouche Virus: More Questions than Answers

Eduardo Jurado-Cobena

Abstract

Oropouche virus (genus Orthobunyavirus, family Peribunyaviridae) is an arthropod-borne virus that infects several species of animals and humans, primarily in South America. Despite being described as a human pathogen >60 years ago, little progress has been made towards describing the ecologic and pathologic characteristics of this pathogen. However, with recent viral spread northward reaching Haiti and Cuba, oropouche virus has been receiving more attention, as evidenced by the growing number of relevant research articles. This commentary provides a summary of the potential natural reservoirs and expansion of endemic regions within the context of One Health. The clinical aspects of the human infection are revisited and discussed based on the latest evidence. Moreover, research on the molecular virology and pathology is briefly reviewed, highlighting unanswered questions crucial for a comprehensive understanding of this viral disease, which imposes a significant burden on affected populations.

Keywords: OROV, Orthobunyavirus, One-Health, vectors, host-pathogen interactions, vertebrate hosts

INTRODUCTION

Oropouche virus (OROV) is the causative agent of Oropouche fever (ORO), a debilitating febrile illness that affects humans in South America [1–3] and North America [4, 5] (Fig 1). The virus triggers “explosive outbreaks of acute febrile illness” [9] with a high percentage of convalescent individuals experiencing symptom recurrence [10], as well as circulating silently in human populations [3]. Although no fatalities attributed to ORO have been reported to date, the incapacitating febrile illness, occasionally accompanied by meningitis or meningoencephalitis, poses a significant public health concern in endemic countries. OROV is transmitted by arthropods, such as biting midges (Culicoides paraensis) or mosquitoes. OROV belongs to the Orthobunyavirus genus, family Peribunyaviridae. Serologically, OROV is classified within the Simbu serogroup, which contains viruses of human and veterinary importance ([1] and references within). The tripartite negative-sense RNA genome comprises small (S), medium (M), and large (L) segments. The L segment encodes an RNA-dependent RNA polymerase (L protein). The M segment encodes a polyprotein containing two envelope glycoproteins (Gn and Gc), as well as a non-structural protein (NSm). The S segment encodes a nucleocapsid (N) protein and a small non-structural protein [S] (NSs), both of which are within overlapping reading frames. In other Bunyavirales known to cause...
human diseases, the non-structural NSs protein from the S segment has been identified as a major virulence factor [11].

OROV infection was detected in patient sera or monocytes, lymphocytes, and dendritic cells within human peripheral blood mononuclear cells (PBMCs) [12, 13]. However, the mechanisms underlying the viral pathogenesis that triggers febrile illness or meningoencephalitis and the mode of viral transmission in arthropod vectors have not been established. There are no licensed antivirals or therapeutic interventions to support ORO patient recovery. Although OROV has not reached the US, the geographic expansion of OROV into Haiti and Cuba [4, 5] has raised concerns about potential viral spread into the continental US due to the presence of the vector midges in the southern to central US [14]. For a visual representation of the geographic distribution of Culicoides paraensis, the review article by Files et al. [1] provides a compelling map.

Facilitating OROV surveillance through the One Health approach

The initial human OROV case was documented in 1955. The same study identified neutralizing antibodies in the sera of monkeys, including cebus (Cebus trinitatis) and howler monkeys (Alouatta seniculus) [15]. Twenty years later, reports from outbreaks in Brazil revealed not only several thousand infected humans but also the involvement of various other animal species, including a rodent of the genus Proechimys and various wild (families Cuculidae, Dendrocolaptidae, Formicariidae, Fringillidae, Pipridae, Thraupidae, Troglodytidae, Vitreomax, and Tyrannidae) and domestic birds (Gallus gallus domesticus and Anas platyrhynchos domesticus) [10]. It is worth noting that species within the families Fringillidae, Tyrannidae, and Troglodytidae are also distributed throughout the Americas and other regions of the world [16]. OROV was first isolated from a three-toed sloth (Bradypus tridactylus) in Brazil in 1960. These animals are considered possible wild reservoirs for
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Oropouche Virus (OROV) has been detected across various geographic locations in North and South America. More recently, marmosets (Callithrix spp.) [2] have been implicated as a potential host for OROV. Notably, a reasortant containing an OROV-like S segment and a dissimilar M segment (referred to as Jatobal virus) was isolated from a South American coati (Nasua nasua) in 1985. Jatobal virus belongs to the Simbu serogroup, similar to OROV [17].

Table 1 summarizes the most recent reports (2009–2018) of OROV in animals. For previous years, the review by Romero-Alvarez and Escobar has a complete table [2].

Table 2 summarizes the reports in which arthropods have been designated as OROV vectors. The main vectors implicated for urban infections are Culicoides paraensis and Culex quinquefasciatus [29]. Reinforcing this epidemiology-led entomologic finding, another study reported the identification of OROV genetic material in Culex quinquefasciatus as well as human patients during one outbreak investigation in Brazil [25]. Interestingly, genomic material from OROV has been identified in male mosquitoes (Culex quinquefasciatus-Gen Bank accession MT247713 and Aedes aegypti-Gen Bank accession MT247714) during a vertical transmission study of arboviruses in vector mosquitoes [27]. Whether these findings explain the lack of C. paraensis in an Ecuadorian city where positive human cases of OROV infection were identified [30] remains to be confirmed. Also, Culicoides sonorensis, a North American midge, has shown high infection and dissemination rates for OROV under experimental conditions [28]. For a visual distribution of the

**TABLE 1** | Recent evidence of Oropouche virus infection in vertebrates.

<table>
<thead>
<tr>
<th>Animal identified</th>
<th>Type of viral identification</th>
<th>Viral transmission cycle</th>
<th>Year of report</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse (Equus caballus)</td>
<td>Antibody (PRNT&lt;sub&gt;90&lt;/sub&gt;)</td>
<td>Sylvatic/rural</td>
<td>2009-2011</td>
<td>[18]</td>
</tr>
<tr>
<td>Sheep (Ovis aries)</td>
<td>Antibody (PRNT&lt;sub&gt;90&lt;/sub&gt;)</td>
<td>Sylvatic/rural</td>
<td>2009-2011</td>
<td>[18]</td>
</tr>
<tr>
<td>Water buffalo (Bubalus bubalis)</td>
<td>Antibody (HI)</td>
<td>Rural/Urban</td>
<td>2009</td>
<td>[19]</td>
</tr>
<tr>
<td>Cattle (Bos taurus/Bos indicus)</td>
<td>Antibody (PRNT&lt;sub&gt;90&lt;/sub&gt;)</td>
<td>Rural/Urban</td>
<td>2016-2018</td>
<td>[20]</td>
</tr>
</tbody>
</table>

PRNT<sub>90</sub> = 90% plaque reduction neutralization test; HI = hemagglutination inhibition test.

**TABLE 2** | Oropouche virus vectors reported in the literature.

<table>
<thead>
<tr>
<th>Arthropod identified</th>
<th>Type of viral identification</th>
<th>Viral transmission cycle</th>
<th>Year of report</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mansonia venezuelensis (Theobald)</td>
<td>Viral isolation</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[21]</td>
</tr>
<tr>
<td>Aedes scapularis</td>
<td>Experimental (viral isolation)</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[21]</td>
</tr>
<tr>
<td>Aedes serratus</td>
<td>Experimental (viral isolation)</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[21]</td>
</tr>
<tr>
<td>Culex fatigans</td>
<td>Experimental (viral isolation)</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[21]</td>
</tr>
<tr>
<td>Psorophora ferox</td>
<td>Experimental (viral isolation)</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[21]</td>
</tr>
<tr>
<td>Aedes (Ochlerotatus) serratus</td>
<td>Viral isolation</td>
<td>Sylvatic</td>
<td>1960</td>
<td>[22]</td>
</tr>
<tr>
<td>Culicoides paraensis</td>
<td>Viral isolation</td>
<td>Urban</td>
<td>1975</td>
<td>[10]</td>
</tr>
<tr>
<td>Culicoides paraensis</td>
<td>Experimental (viremic man to hamster)</td>
<td>Urban</td>
<td>1982</td>
<td>[23]</td>
</tr>
<tr>
<td>Culex quinquefasciatus</td>
<td>Experimental (viremic hamster to hamster)</td>
<td>n/a</td>
<td>1987</td>
<td>[24]</td>
</tr>
<tr>
<td>Culex quinquefasciatus</td>
<td>S segment</td>
<td>Urban*</td>
<td>2013</td>
<td>[25]</td>
</tr>
<tr>
<td>Psorophora cingulata</td>
<td>S segment (RT-qPCR)</td>
<td>Sylvatic</td>
<td>2016</td>
<td>[26]</td>
</tr>
<tr>
<td>Haemagogus tropicalis</td>
<td>S segment (RT-qPCR)</td>
<td>Sylvatic</td>
<td>2016</td>
<td>[26]</td>
</tr>
<tr>
<td>Aedes (Ochlerotatus) serratus</td>
<td>S segment (RT-qPCR)</td>
<td>Sylvatic</td>
<td>2016</td>
<td>[26]</td>
</tr>
<tr>
<td>Culex quinquefasciatus**</td>
<td>S segment</td>
<td>Urban</td>
<td>2017/2018</td>
<td>[27]</td>
</tr>
<tr>
<td>Aedes aegypti**</td>
<td>S segment</td>
<td>Urban</td>
<td>2017/2018</td>
<td>[27]</td>
</tr>
<tr>
<td>Culicoides sonorensis</td>
<td>Experimental</td>
<td>Urban</td>
<td>2021</td>
<td>[28]</td>
</tr>
</tbody>
</table>

*In this report, C. paraensis was not present on the surrounding areas using the collection methods described within the article.

**Male mosquitoes.
Culex quinquefasciatus vector in North America, the reader is directed to the article by Gorris et al. [6]. Caution is advised to interpret the species names because the Pipiens Assemblage includes Cx. pipiens, Cx. Quinquefasciatus [32], adding an extra layer of biological complexity. For a visual distribution of Culicoides sonorensis, the article by Shults et al. [7] is recommended.

All these observations indicate that the natural cycle of OROV is likely a time-related evolution in terms of potential reservoir and vector species and adaptability to new species within the currently recognized transmission cycles (Fig 2). Such observations underscore the need of an integrated One Health approach for further identification of new reservoirs and the potential consequence for expanding the geographic range. Moreover, there is a need to monitor potential viral reassortment events [33] through active surveillance programs that incorporate wildlife. This approach is crucial because several OROV isolates encode the M segment of unknown origin (M segment reassortant), such as Jatobal virus (Brazil), Iquitos virus (Peru), Perdões virus (Brazil), or Madre de Dios virus (Venezuela or Peru).

Gaps in understanding the pathogenesis of human Oropouche fever

Cases of meningitis or meningoencephalitis following OROV infection have been reported in a small number of patients. Consequently, few studies have addressed the pathogenesis of OROV-induced meningoencephalitis in patients. One study reported that OROV infects microglia and neurons within human brain slice cultures, while astrocytes remain unaffected. Moreover, a significant increase in TNF-α levels was detected in brain slice tissues infected with OROV [34], suggesting that OROV infection within the central nervous system may induce proinflammatory reactions. Nevertheless, further characterization is required to fully elucidate the pathogenesis of OROV infection in the human brain. A “Trojan horse” mechanism has been proposed as the primary means of viral dissemination within OROV-infected humans, suggesting that the virus transverses the blood-brain barrier inside infected PBMCs. Nonetheless, the dynamics of viral dissemination, as well as the viral components facilitating the viral persistence within PBMCs, are still not fully understood, which warrants further investigation.

While limited pathologic findings of OROV infections in humans exist, several animal models have been developed to characterize OROV infections in vivo. For example, subcutaneous inoculation of the OROV BeAn19991 strain in 3-week-old Syrian hamsters (Mesocricetus auratus) has been shown to induce meningoencephalitis with viral antigens detected in neurons and hepatocytes [35]. The median lethal dose 50 (LD₅₀) was shown to be 10⁵.6 TCID₅₀/ml. Additionally, subcutaneous inoculation of the OROV BeAn19991 strain in 1-day-old BALB/c mice induced mild meningitis and viral infection in neurons, but not in hepatocytes [36]. These studies suggested that OROV demonstrates potent neurotropism in rodent models, mirroring the infection pattern in humans. Furthermore, research has demonstrated that 6-week-old

![FIGURE 2 | Oropouche virus transmission cycle.](image-url)
C57BL/6 mice exhibit resistance to OROV BeAn19991 strain infection via the subcutaneous route. In contrast, Ifnar−/− C57BL/6 mice inoculated with the OROV BeAn19991 strain uniformly succumb to infection, with a mean survival time of 5 days [37]. Infected Ifnar−/− C57BL/6 mice displayed OROV-infected hepatocytes, focal hepatocytic necrosis, and infiltration of mononuclear cells. While there is limited evidence of hepatitis associated with OROV infection in humans, hepatocytes can serve as a potential viral target with influence by the competency of innate immunity.

Hemorrhagic symptoms, including petechial rashes, epistaxis, gingival bleeding, and menorrhagia, have been documented during ORO outbreaks in Peru and Brazil [9, 38, 39]. In Brazil, up to 15.5% of patients reported hemorrhagic manifestations during an outbreak [9]. Additionally, two women infected in a laboratory setting experienced continued and profuse menses [39]. Chemokines are known to play a role in directing the migration of white blood cells from the bloodstream to infected tissue sites. A recent study demonstrated that patients with an acute OROV infection exhibit elevated levels of CCL2, CXCL8, CXCL10, IL–6, IL–10, IL–17A, TNF–α, and IFN–α [40]. However, whether cytokines and chemokines contribute to the hemorrhagic manifestations in OROV infection remains to be elucidated.

Furthermore, an early report highlighted miscarriages in two of nine pregnant women in the second month of pregnancy [41]. Although the number of reported cases is limited, viral tropism to the placenta and fetus should be characterized in corollary studies.

Viral virulence factors associated with OROV remain largely unknown. While NSs proteins from other bunyaviruses, including the families Peribunyaviridae, Nairoviridae, Hantaviridae, and Phenuiviridae, have been shown to be a major virulence factor [8], the role of OROV NSs in pathogenicity remains incompletely characterized. It has been demonstrated that OROV NSs protein serves as a type-I IFN antagonist, as evidenced by experiments with the recombinant OROV prototype, Brazilian BeAn19991 strain, and a strain lacking the NSs gene [42]. Another non-structural protein, NSm, has a crucial role in the assembly and morphogenesis of Bunyamwera virus [43], which is another member of the genus Orthobunyavirus. It has been shown that NSm has implications on the arthropod vector infection cycle in the case of Phlebovirus–Rift Valley Fever Virus (RVFV) [44]. The NSm gene in OROV is situated between Gn and Gc and can undergo co-translational cleavage by signal peptidase [45]. However, limited information is available regarding the virologic functions of the NSm protein in OROV despite the adapted evolution of this protein, as evidenced on evolutionary studies [46].

Regarding the cellular receptor, Schwarz et al. [47] reported that Lrp1 KO cell lines exhibited reduced, but not abolished OROV infection. In addition, upon intracranial (IC) inoculation with OROV BeAn19991 strain (100 PFU) with or without a purified domain from receptor-associated protein (RAP, a ligand for Lrp1) in female mice (3–4-wk-old C57BL/6J), animals inoculated with the OROV+RAP purified domain had 90% survival in contrast to the mice in the control group that succumbed [47], suggesting that Lrp1 is important in the context of neural infection on youth mice. With respect to host factors, the endosomal sorting complex required for transport (ESCRT) has been identified as a target during OROV replication using HeLa cells [48]. ESCRT is sequestered to Golgi membranes during OROV assembly, requiring the interaction with charged multivesicular body protein 6 (CHMP6), which is part of the ESCRT III machinery complex, as identified using recombinant expression of the OROV M polyprotein or its components (Gn/Gc) transfected on either HeLa or HEK293 cells [49]. Importantly, the co-expression of OROV Gn and Gc proteins was necessary for progression into the Golgi.

**DISCUSSION**

During one of the initial outbreaks of Oropouche fever in Brazil [10], chickens and ducks were shown to have antibodies against OROV. This led to suggestions that wild and domestic birds could act as amplifiers, but solid evidence to support this hypothesis is lacking [2] and references within). However, this finding represents a significant knowledge gap considering that many of the wild birds reported to harbor antibodies against OROV have geographic ranges covering the entire American continent from south-to-north. Furthermore, there is an increased risk that poultry farm workers may face if OROV can indeed be amplified in domestic birds. A review attempted to pinpoint the geographic places where OROV has been identified and correlate the areas with the geographic places where known vectors had been reported, finding a “lack of significant relationships” [50]. This emphasizes the need to improve collaborations between epidemiologic and entomologic expertise under the One Health approach to clearly identify the vectors responsible for OROV transmission during outbreaks given the new evidence available [25–28, 31]. Additionally, on a recent OROV outbreak in French Guyana, just one specimen of C. paraensis was identified, with a large percentage of arthropods being Culex quinquefasciatus, indicative of an important vector in urban and rural transmission [51].

Characterizing the proinflammatory responses during OROV infection and understanding the molecular functions of OROV NSs and NSm proteins are crucial for gaining insight into OROV pathogenesis in humans. It is concerning that meningocerebralitis in human patients has not been thoroughly characterized, despite resolving without apparent sequelae in most cases. A recent study analyzing two different human biobanks reported that patients exposed to viruses causing encephalitis had a higher risk of developing neurodegenerative diseases,
with the most significant association observed between viral encephalitis exposure and Alzheimer’s disease [52]. Although not lethal, hemorrhagic symptoms (petechial rashes, epistaxis, gingival bleeding, and menorrhagia) are potential clinical concerns, but the pathogenesis lacks scientific evidence. As suggested in a previous study [38], a larger number of exposed and infected individuals increases the likelihood of symptoms, thereby making less common symptoms more apparent than in smaller outbreaks. An intriguing hypothesis proposed by the authors of the Peru outbreak [38] suggests that the absence of hemorrhagic manifestations and the “explosive outbreak” were not observed during another OROV outbreak in Iquitos, Peru in 1992. The authors speculated that the prevalence of dengue in that region might have overshadowed the effects of OROV on the population. This notion is supported by a report from Brazil [9], which noted that all patients exhibiting hemorrhagic symptoms tested negative for dengue IgM antibodies. Similarly, a report from Colombia indicated no occurrence of hemorrhagic symptoms despite identifying co-infections of OROV and dengue virus in 10 patients residing in a hyperendemic dengue area during efforts to identify the causative agent of acute febrile illnesses [53]. A previous proof-of-concept study reported the protective effects of a non-related virus (virus-like particles of polio virus) on SARS-CoV-2 and other respiratory viruses by means of innate immunity stimulation using mice [54]. Whether this is the case within the context of OROV infection in dengue endemic areas needs to be explored, especially since the human immune response to dengue is not fully understood. If this hypothesis holds true, it could be promising news for much of the endemic regions where OROV is silently circulating. However, such a hypothesis would be concerning for North America, potentially impacting the estimates of a recent study that used spatial epidemiology models based on human outbreaks and concluded that up to 5 million people are at risk [55].

It was reported that LD$_{50}$ of the OROV MD023 strain is 45 PFU in 3–4-week-old hamsters [56], which indicates that the MD023 strain is more pathogenic than BeAn19991 strain (LD$_{50}$ of $10^{3.6}$ TCID$_{50}$/ml). Although BeAn19991 and MD023 strains are similarly pathogenic in humans, hamsters show a distinct susceptibility to these two OROV strains. However, the pathologic changes induced by the MD023 strain have not been analyzed compared to the changes caused by the BeAn19991 strain. This comparative model will be valuable for elucidating host and viral factors that may influence the outcome of OROV infection as well as potential use in testing antiviral treatments.

**CONCLUSIONS**

The questions involving vectors on different viral transmission cycles, the lack of consistent results between vector presence and outbreaks on recent reports, a poor understanding of natural reservoirs, an incomplete understanding of OROV pathogenic factors linked to the pathogenesis in humans and the human susceptibility to develop hemorrhagic symptoms, the replication compartments within the human body, and if viral tropism to placenta and fetus exists remains to be elucidated. The characterization of certain viral strains on animal models and its use on the search for identifying host factors affected and potential treatments is slowly developing. The recent reports from outbreaks across several Latin-American countries and the recent detection in Cuba are clear indicators of the geographic expansion of this neglected tropical disease, which has some potential fatalities under investigation as the number of susceptible individuals increased [57].

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**CONFLICT OF INTEREST**

The author declares no conflicts of interest.

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