



First outbreak of Oropouche Fever reported in a non-endemic western region of the Peruvian Amazon: Molecular diagnosis and clinical characteristics

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ABSTRACT

Introduction: Oropouche fever is an under-reported and emerging infectious disease caused by Oropouche virus (OROV). Its incidence is under-estimated mainly due to clinical similarities with other endemic arboviral diseases and the lack of specific diagnostic tests. We report the first outbreak of Oropouche fever in a western region of the Peruvian Amazon in Huanuco, Peru.

Methods: A transversal study was carried out during an outbreak in the western Region of Huanuco, Peru between January and July of 2016. Blood samples of 268 patients with acute febrile syndrome were collected and analyzed for OROV via RT-PCR and genetic sequencing.

Results: Of all 268 patients, 46 (17%) cases tested positive for OROV. The most common symptoms reported were headache with a frequency of 87% (n = 40) followed by myalgia with 76% (n = 35), arthralgia with 65.2% (n = 30), retro-ocular pain 60.8% (n = 28) and hyporexia with 50% (n = 23). Some patients showed a clinical presentation suggestive of severe OROV infection, of which 4.3% (n = 2) had low platelet count, 8.6% (n = 4) had intense abdominal pain, and 2.1% (n = 1) had a presentation with thoracic pain.

Conclusion: This study reports an outbreak of OROV in a region where this virus was not previously identified. The disease caused by OROV is an emerging, underdiagnosed infection that requires further research to determine its virulence, pathogenesis, host range and vectors involved in the urban and sylvatic cycles as well as identifying new genotypes to implement sensitive and specific diagnostic tools that can be applied to endemic regions.

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Introduction

The Oropouche virus (OROV) is an arbovirus member of the *Peribunyaviridae* family and the *Orthobunyavirus* genus (Briese et al., 2016), responsible for a febrile syndrome that has affected

people since its first discovery in 1955 in Trinidad and Tobago (Anderson et al., 1961). The etiological diagnosis of OROV infection is challenging due to the clinical similarities with other arboviral diseases including dengue, chikungunya, zika, and mayaro viruses, in addition to other pathogens such as Malaria (da Costa et al., 2017; Alvarez-Falconi and Ríos, 2010; Travassos da Rosa et al., 2017), and the lack of molecular differential diagnostic methods in endemic regions. Consequently, the infection caused by OROV is a disease with an underestimated incidence.

During the past decade, four different genotypes have been described in more than 30 outbreaks within Central and South

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America (Travassos da Rosa et al., 2017). Co-existence of some genotypes has been evidenced and suggested to be due to the geographic migration of the virus as shown in previous outbreak reports following the eastern migration of OROV Genotype II virus (Travassos da Rosa et al., 2017).

Two biological cycles have been described for OROV: sylvatic and urban. There are reports that pale-throated three-toed sloths (*Bradypus tridactylus*) and some primates act as vertebrate hosts in the rainforest (Pinheiro et al., 1981; Tilston-Lunel et al., 2015; Nunes et al., 2005). The main vector for sylvatic OROV transmission has not been identified yet. However, previous studies describe the isolation of OROV in the vectors *Ochlerotatus serratus* and *Coquilletidia venezuelensis* (Pinheiro et al., 1981; Pinheiro et al., 2004). The primary vector of OROV in the urban cycle is *Culicoides paraensis* but other vectors have also been described, including *Culex p. quinquefasciatus* (Travassos da Rosa et al., 2017). Interestingly, with the geographic extension of the virus, as evidenced in recent outbreaks, possible vectors should not be ruled out.

The febrile syndrome caused by OROV is reported to have an incubation period of four to eight days (Travassos da Rosa et al., 2017). The most common symptoms include fever, headache, myalgia, and arthralgia (Alvarez-Falconi and Ríos, 2010). Additionally some patients may experience gastrointestinal symptoms such as anorexia, nausea, emesis, diarrhea and epigastric pain (Alvarez-Falconi and Ríos, 2010). A Rubella-like rash has also been reported in some outbreaks. Neurological symptoms are uncommon, but patients with cerebrospinal fluid (CSF) isolation of OROV have shown symptoms similar to meningitis. Other signs and symptoms include dizziness, photophobia, retro-orbital pain, (da Costa et al., 2017; Travassos da Rosa et al., 2017) and gum and vaginal bleeding in some cases (Alvarez-Falconi and Ríos, 2010). No deaths have been attributed to OROV infection.

The OROV has characteristics that allow for sustained transmission in endemic areas (Travassos da Rosa et al., 2017),

and silent circulation has been evidenced in some Amazonian regions (Briese et al., 2016). Different OROV outbreaks have been reported in Peru, including San Martin, Cajamarca and Cusco (Alvarez-Falconi and Ríos, 2010). The virus presents differences in the replication in vertebrate and invertebrate cells and highlights the phenomenon of genetic rearrangement (Elliott and Blakqori, 2011). In addition, two variants of the genetically rearranged virus were identified in Iquitos and Madre de Dios, respectively (Aguilar et al., 2011; Ladner et al., 2014). These conditions make the spread of OROV a potential public health issue in the future (Travassos da Rosa et al., 2017; Weaver and Reisen, 2010).

We report the first outbreak of OROV in Huanuco, which is located in the western region of the Peruvian Amazon.

Methods

Place of study

This study is descriptive and cross sectional, and was carried out in the region of Huanuco between January and July 2016. The work was performed at the primary health care centers that were part of the Leoncio Prado Health Network of the Ministry of Health of Peru and in the “Tingo María Contingency Hospital” (Figure 1).

Leoncio Prado province is located in the natural jungle region, which is part of the Upper Huallaga basin. The relief in this province is characterized by the presence of physiographic units of tectonic, orogenic, lithological and climatic factors.

The present physiographic units such as a fluvial valley, hills, an alluvial valley, mountainous landscape, and a synclinal valley. It also has a certain ecological homogeneity with diverse tropical characteristics, predominating the humid montane humid forest (bh-MBT), tropical humid forest (bh-T) and very humid tropical premontane (bhm-PT) forest. The annual average of minimum and maximum temperature is 18.7 °C and 30.5 °C, respectively The



Figure 1. Location map of the health facilities.

average annual accumulated precipitation is approximately 3472.8 mm with a relative humidity of 77.5% (Gobierno Regional de Huánuco, 2014).

Study subjects

The inclusion criteria comprised a diagnosis of acute febrile syndrome, defined as fever higher than 38 °C for less than or equal to 7 days, without an identifiable source of infection and associated with one or more of the following signs and symptoms: headache, myalgias, arthralgia, retro-ocular pain, lower back pain, cutaneous rash, hyporexia, odynophagia, nausea, emesis, abdominal pain, asthenia, syncope, hypothermia, jaundice. Also, signs that may suggest severe disease such as epistaxis, bleeding gums, petechiae, ecchymosis, bloody sputum, hematemesis, thrombocytopenia, neck rigidity and altered mental state were considered. We included patients of both genders and without any age restriction. The exclusion criteria were patients with an incomplete record of their medical data, patients with an identifiable source of infection, such as acute upper respiratory tract infections, pneumonia, urinary tract infections, among others.

Ethics statement

This study was approved by the Research Ethics Board of the Hospital Regional de Cajamarca, Peru. In addition, we have the approval of the participating facilities, the Leoncio Prado Health Network and the Tingo María Hospital. All samples were analyzed after all participants provided their informed consent before enrollment or by the parents or children's caregivers in the case of underage patients below 18 years of age.

Samples

A total of 268 samples was collected by using Vacuette® TUBE Serum Separator Clot Activator (Vacuette, Greiner Bio-One, Kremsmünster, Austria). The amount of blood extracted was 3 ml. All the samples were stored at –80 °C and transported to Lima (Peru) under standardized frozen conditions for further molecular analysis.

RNA extraction

RNA extraction was performed following the manufacturer's instruction of High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany) using 200 µl of the samples. Viral RNA obtained after extraction was eluted in 100 µl of nuclease free water and then processed or stored at –20 °C until use.

RT-PCR amplification for detection of OROV

For the reverse transcription (RT), a 20 µl mixture was prepared to containing 5 µl of RNA extracts. The transcript High Fidelity cDNA Synthesis Kit (Roche Applied Science, Mannheim, Germany) was used according to the manufacturer's instructions.

300 bp fragments of the S segment of OROV were amplified, the primers BUN-S 5'-AGT AGT GTG CTC CAC-3' and BUN-C 5'-AGT AGT ATA CTC CAC AGT AGT ATA CTC CAC -3', BS-S 5'-AGT AGT ATA CTC CAC-3' and BS-C 5'-TGA ACC CTA TGC ATC T-3' were described by Moreli et al. (2002). Polymerase chain reaction was performed with 5 µL of template DNA and FastStar TAq DNA Polymerase dNTPack (Roche Applied Science, Mannheim, Germany). Amplifications started with an initial incubation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 45 s, with a final extension at 72 °C for 5 min. Amplicons were detected as 300 bp bands after gel electrophoresis and nucleic acid

staining (SyberGreen, Promega). PCR products were purified using SpinPrep™ Gel DNA Kit, San Diego, USA and sequenced by Sanger method (Macrogen, Seoul, South-Korea).

Statistical analysis

Qualitative variables were reported as frequencies in percentage. Fisher test (F-test) was used to determine the significative differences with a p -value ≤ 0.05 . The correlation matrix was made to analyze the association of signs and symptoms. All analyses were processed with the Minitab Inc. software version 18.1 (USA). The graphic representation of the data was made with the OriginPro v10 software (OriginLab Corp., USA).

Results

Demographic characteristics

A total of 46 out of 268 samples was positive for OROV. Hence, the rate of OROV infection was 17.2% (Figure 2a). This frequency is significantly greater ($p < 0.004$, F-test) than the hypothetical frequency established at 8.6% in studies conducted in the endemic area of OROV (Alva-Urcia et al., 2017). The diagnosis of OROV was not influenced by the gender of the patients (Figure 2b). The ages in the studied population were adjusted to a Gaussian model with an average age of 20.8 ± 15.7 years (average \pm standard deviation), and similarly the population with OROV- diagnosis showed an average of 22.3 ± 15.6 years. However, the population with the OROV+ diagnosis showed a clear deviation towards younger ages, with an average of 15.2 ± 9.8 years (Figure 3). For a more detailed

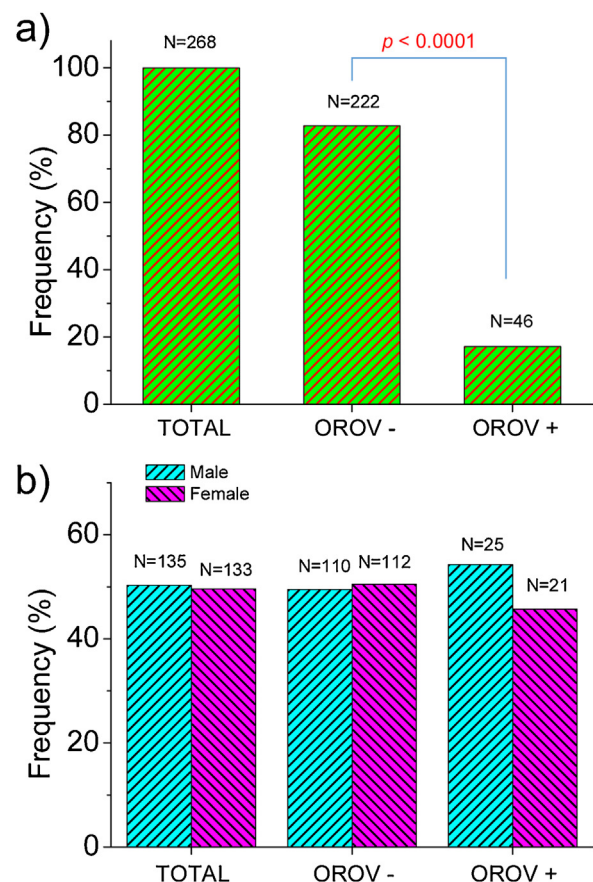


Figure 2. Frequency of patients diagnosed with OROV+ (a). Distribution by gender of patients (b).

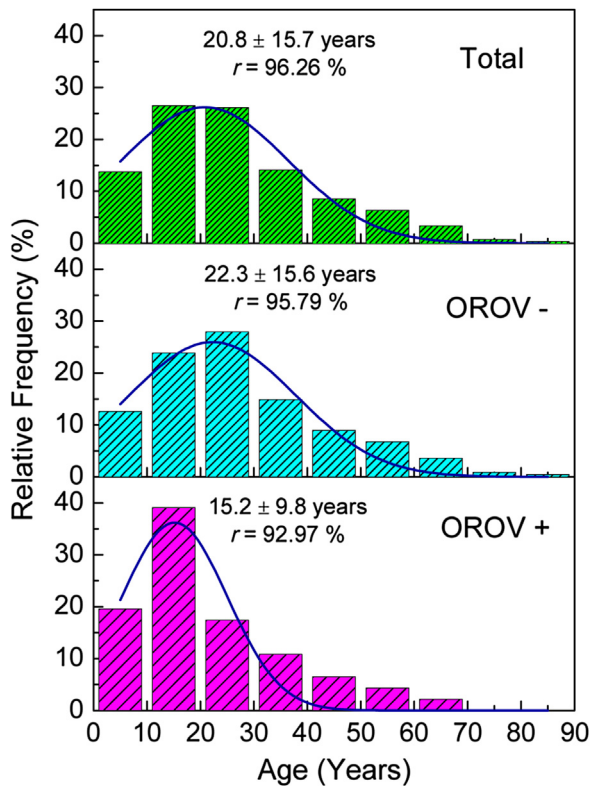


Figure 3. Distribution of the ages of patients.

Table 1
Demographic characteristics of the studied population ORO+ and OROV-.

Age (Years)	Total of cases		ORO+ve		ORO-ve		F-test (p-Value)
	(N)	(%)	(N)	(%)	(N)	(%)	
<5	15	5.6	4	8.7	11	5.0	0.0270
5–11	38	14.2	8	17.3	30	13.5	<0.0001
12–17	40	14.9	13	28.2	27	12.2	0.0030
18–39	123	45.9	15	32.6	108	48.6	<0.0001
40–59	40	14.9	5	10.9	35	15.8	<0.0001
>60	12	4.5	1	2.1	11	5.0	<0.0001
Total	268	100.0	46	100.0	222	100.0	

analysis the ages were grouped into classes as shown in Table 1. The higher frequencies of positive cases were found in the age groups of 18–39 and 12–17 with 32.6%, 28.2% respectively. Significant differences were found between the frequencies of the positive and negative cases in all age groups (Table 1).

Clinical presentation

Among patients molecularly diagnosed with OROV infection, the most common symptom reported was headache with a frequency of 87.0% (n = 40) followed by myalgia with 76.1% (n = 35), arthralgia with 65.2% (n = 30), retro-ocular pain 60.9% (n = 28) and hypoxemia with 50.0% (n = 23) of the patients (Table 2).

Some patients showed a clinical presentation suggestive of severe OROV infection, of which 4.3% (n = 2) had low platelet count, 8.6% (n = 4) had intense abdominal pain, 2.2% (n = 1) had thoracic pain, 2.2% (n = 1) showed gynecological bleeding and 2.2% (n = 1) had hematemesis. None of the patients in this outbreak reported neurologic symptoms (Table 2).

A correlation analysis of the clinical signs and symptoms of patients with OROV+ diagnoses was made according to the age of the patients

(Figure 4). A close association between febrile syndrome and the diagnosis of OROV+ was observed (r = 100.0 %). This association is clearly detected in patients aged 5–59 years. However, the groups of extreme ages, less than 5 years and older than 60 years show varied signs and symptoms that are not clearly associated with febrile syndrome (r with values of 78.8% and 86.1%, respectively); this may correspond to an ambiguity of the patient's age.

Discussion

We report the first OROV outbreak registered in the Province of Leoncio Prado. We were able to confirm 46 positive cases of Oropouche virus infection using molecular diagnostic methods.

Along with other recently reported outbreaks in Cusco, Madre de Dios (World Health Organization, 2016), Venezuela (Navarro et al., 2016) and the first reported case of OROV outside the Amazonian region (de Souza Luna et al., 2017), this report may indicate that the virus is re-emerging and expanding. This expansion may be due to recent local ecosystem changes that could affect the known transmission cycle of the disease by favoring the migration of the vectors and involved hosts (Khalil et al., 2016). A similar hypothesis was proposed recently in an outbreak in the region of San Martín due to the construction of a highway (Alvarez-Falconi and Ríos, 2010) and in Cusco due to vegetation loss (Romero-Alvarez and Escobar, 2017). In the location of this study, there is evidence of an increase in agricultural activities, highway and road development, as well as bridge constructions, that may affect the ecosystem either due to population migration, vegetation loss, or redistribution of wild reservoirs and vectors (Khalil et al., 2016).

The geographic expansion of OROV could be explained by climate change as temperature and precipitation anomalies that have been described to alter patterns of arboviral transmission cycles (Gould et al., 2017; Agarwal et al., 2017). In the case of *Culicoides*; increasing temperatures favor oogenesis and shorten periods of mature development (Purse et al., 2015). In this context, during the study period of this outbreak, the National Service of Meteorology and Hydrology of Peru (SENHAMI) reported a temperature anomaly of 2.0 °C above the maximum temperature and 0.4 °C above the minimum temperature in the high and low altitude Amazonian regions of Huanuco (Boletín Hidrometeorológico Regional de Huánuco, 2017).

Several studies consider arboviruses as a serious threat to world public health, especially in tropical and subtropical countries in which factors such as deforestation, migration, lack of urban planning and poor sanitation in the context of change global climate, facilitate the expansion of vectors into new areas and exponentially increase the population at risk (Mota et al., 2016).

The epidemic potential of arboviruses has been reported by the World Health Organization. This potential is considered both endemic and Dengue virus (DENV), in addition to recently expanded viruses or those recently introduced in Latin American countries (Brazil and Peru among others), such as the Mayaro virus (MAYV), the virus Chikungunya (CHIKV) and the Zika virus (ZIKV) (Mota et al., 2016; Figueiredo, 2015). Our study reinforces the idea of considering arboviruses as a threat while we report the presence of OROV in an area where it has not been previously described. This future description may imply the appearance of OROV epidemics both in regions where it is already present and in regions where it can be expanded.

Patients may be asymptomatic, develop an isolated febrile episode, or develop a febrile illness associated with skeletal, dermatological and neurological or hematological complications in severe cases (Donalisio et al., 2017). As previously mentioned, the most common signs or symptoms in the clinical presentation of OROV infection include headache, myalgia, and arthralgias

Table 2
Clinical presentation of patients with Oropouche virus infection.

Sign or symptom	Total cases n=268 (%)	OROV+ N=46 (%)	Positive cases for OROV					
			<5 years n=4 (%)	5–11 years n=8 (%)	12–17 years n=13 (%)	18–39 years n=15 (%)	40–59 years n=5 (%)	≥60 years n=1 (%)
Fever	268 (100.0)	46 (100.0)	4 (100.0)	8 (100.0)	13 (100.0)	15 (100.0)	5 (100.0)	1 (100.0)
Headache	249 (92.9)	40 (86.9)	3 (75.0)	6 (75.0)	12 (92.3)	13 (86.7)	5 (100.0)	1 (100.0)
Myalgias	231 (86.2)	35 (76.1)	1 (25.0)	8 (100.0)	9 (69.2)	12 (80.0)	4 (80.0)	1 (100.0)
Arthralgias	228 (85.1)	30 (65.2)	2 (50.0)	5 (62.5)	6 (46.2)	12 (80.0)	4 (80.0)	1 (100.0)
Retro-ocular pain	174 (64.9)	28 (60.9)	2 (50.0)	5 (62.5)	6 (46.2)	11 (73.3)	3 (60.0)	1 (100.0)
Hyporexia	145 (50.1)	23 (50.0)	4 (100.0)	2 (25.0)	6 (46.2)	7 (46.7)	3 (60.0)	1 (100.0)
Nausea/emesis	132 (49.3)	22 (47.8)	3 (75.0)	3 (37.5)	7 (53.8)	5 (33.3)	4 (80.0)	0 (0.0)
Lumbar pain	127 (47.4)	16 (34.8)	0 (0.0)	2 (25.0)	5 (38.5)	5 (33.3)	3 (60.0)	1 (100.0)
Odinophagia	80 (29.9)	16 (34.8)	3 (75.0)	3 (37.5)	5 (38.5)	4 (26.7)	0 (0.0)	1 (100.0)
Cutaneous rash	71 (26.5)	15 (32.6)	2 (50.0)	2 (25.0)	5 (38.5)	4 (26.7)	1 (20.0)	1 (100.0)
Abdominal pain	12 (4.5)	4 (8.7)	1 (25.0)	0 (0.0)	1 (7.7)	1 (6.7)	1 (20.0)	0 (0.0)
Platelet decrease	8 (3.0)	2 (4.3)	0 (0.0)	0 (0.0)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)
Hematemesis	6 (2.2)	1 (2.2)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)
Epistaxis	4 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Petechiae	4 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bleeding gums	3 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bloody sputum	3 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Thoracic pain	3 (1.1)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)
Melena	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gynecological bleeding	2 (0.7)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)
Diuresis reduction	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Jaundice	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hematocrit increase	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Altered mental state	2 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ecchymosis	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Persistent vomiting	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypothermia	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lipothymia	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypotension	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

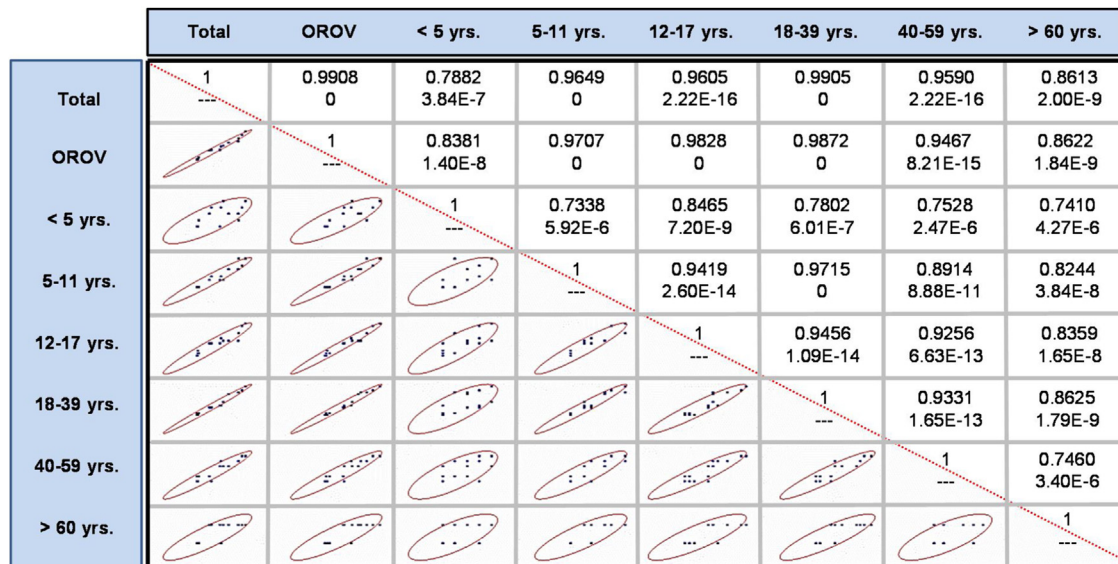


Figure 4. Pearson correlation of the clinical signs and symptoms of patients with OROV + diagnosis was made according to the age. The dispersion matrix presents ellipses at 95% confidence in the lower symmetric part that represent the values of the Pearson correlation coefficient (r) and its associated p value in the upper symmetric part of the matrix.

(Alvarez-Falconi and Ríos, 2010). Our findings show that during this outbreak, the most common symptoms were fever at presentation with 100.0%, followed by headache in 92.9%, myalgia in 86.2%, arthralgias in 85.1%, retro-ocular pain in 64.9% and hyporexia in 50.1% of patients. However, this study did not consider

recurrent symptoms because the patients were not followed up, a finding similar to the outbreak registered in Begazan, San Martin (Alvarez-Falconi and Ríos, 2010).

As a member of Orthobunyavirus, OROV is able to interchange segments of RNA with other strains through genetic reassortment,

particularly with segments S and L, allowing the incorporation of genes that may develop novel virulent viral strains that may lead to new outbreaks, such as the recently reported IQTV and MDDV (Aguilar et al., 2011; Ladner et al., 2014). This could imply that OROV increases its virulence and pathogenicity, increasing its capacity to generate epidemics and severe disease. These relevant strains were shown to spread to brain and the liver, which may explain the more severe clinical presentation seen in some patients (Travassos da Rosa et al., 2017). Central nervous system symptoms have also been reported among patients with cerebrospinal fluid (CSF) isolates of OROV (Alvarez-Falconi and Ríos, 2010; Travassos da Rosa et al., 2017). During this outbreak, 4.3% had low platelet count, 8.6% had abdominal pain, one patient had hematemesis, one patient had gynecological bleeding, and none had symptomatology that suggested central nervous system involvement.

Conclusions

The disease caused by the OROV is an emerging, under-diagnosed infection that requires ongoing research to determine its virulence, pathogenesis, host range and most common vectors. Furthermore, the identification of new genotypes could aid the development of more sensitive diagnostic tools that could be used in endemic regions. This study reports an outbreak of OROV in Huanuco for the first time. Even though there have not been any mortalities reported since the initial identification of OROV, recent outbreaks show a pattern that highlights the emerging potential of the virus and demonstrates the need to implement new public health policies prioritizing local epidemiological surveillance.

Limitations

On the basis of genetic and antigenic analyses, other studies have detected several reordered viruses involving OROV and other unknown Simbu serogroup viruses, which for the time being constitutes a limitation in our work and encourages us to carry out further investigations.

Conflict of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest or funding related to this study.

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Ethical approval

This study has been approved by Ethic Committee from Hospital Regional Docente de Cajamarca. All samples were analyzed after the participant gave his informed consent.

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