BRIEF REPORT

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF CASES OF ACUTE CHAGAS DISEASE IN THE PERUVIAN AMAZON BASIN, 2009-2016

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ABSTRACT

Acute Chagas disease (ACD) cases are reported sporadically in Peru. In this report we describe the clinical and epidemiological characteristics of eight new ACD cases detected by the surveillance system in the Amazon basin, between 2009 and 2016. The average age was 12,7 ±13.7 years, range between 1 to 44 years and 4/8 cases were men. One case was associated with acute diarrheal disease, another with Leptospirosis, and two with urinary tract infection. The global case fatality-rate was 12.5% (1/8). Late detection is a frequent characteristic related with low diagnostic suspicion in patients with a history of fever. The TcI and TcIV lineage was identified as the etiological agent of Chagas disease. Eight new cases of ACDs are reported, of which seven were children.

Keywords: Vector Borne Diseases; Neglected Diseases; Chagas Disease; Case Reports; Leptospirosis; Fever; Coinfection; Peru (source: MeSH NLM).

Cite as: Vega S, Cabrera R, Álvarez CA, Uribe-Vilca I, Guerrero-Quincho S, Ancca-Juárez J, *et al.* Características clínicas y epidemiológicas de casos de enfermedad de Chagas aguda en la cuenca amazónica peruana, 2009-2016. Rev Peru Med Exp Salud Publica. 2021;38(1):70-6. doi: https://doi.org/10.17843/rpmesp.2021.381.6286.

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Received: 05/08/2020
Approved: 20/01/2021
Online: 16/02/2021

INTRODUCTION

Chagas disease is caused by *Trypanosoma cruzi*, which is transmitted by hematophagous triatomines infected with the parasite, blood transfusion, congenital route, contaminated food or beverages and by other means [1]. It has an acute and a chronic phase. People infected in the acute phase may present symptoms such as fever, inoculation chagoma, general malaise, hepatosplenomegaly and lymphadenopathy [1,2]; 95% are asymptomatic [2]. The diagnosis of this phase is based on the detection of the parasite by thick blood smear or microconcentration tests [1-2].

In Peru, between 2006 and 2010, seven cases of acute Chagas disease (ACD) had been reported, one in the district of Pevas and the other six in districts of the province of Datem del Marañón, department of Loreto [3,4]; two of them were related, so the infection could be due to a common source (outbreak) [4]. Reports of ACD in the last 20 years have been sporadic [4]. Despite being a notifiable disease [5], there are few clinical and epidemiological reports. One

https://doi.org/10.17843/rpmesp.2021.381.6286

of the objectives of surveillance is to detect acute cases and investigate the transmission method for timely treatment [5].

The aim of this report is to describe the clinical and epidemiological characteristics of eight cases of acute Chagas disease in the Peruvian Amazon, detected between 2009 and 2016.

KEY MESSAGES

Motivation for the study: There is limited information on the clinical and epidemiological characteristics of patients with acute Chagas disease (ACD) in Peru.

Main findings: Most cases were boys or girls under 11 years of age, three had, in addition, another disease. Diagnosis is late. Fever and general malaise showed in all patients. Three cases of ACD came from Loreto and two from Ayacucho. One four-month-old girl died due to the disease.

Implications: Chagas disease should be suspected in febrile patients, mainly in those from rural areas of the Peruvian Amazon.

THE STUDY

We carried out a case series study by reviewing medical records and clinical-epidemiological records of cases confirmed by the Instituto Nacional de Salud del Perú (INS) and reported to the epidemiological surveillance system, between 2009 and 2016. ACD cases were identified according to the definition of probable case —patient with fever as the main symptom, with at least one of the following symptoms: hepato-splenomegaly, adenomegaly, chagoma, Romaña sign, malaise, rash, meningoencephalitis, myocarditis, cardiomyopathy [5], coming from the Amazon basin— and were confirmed by the detection of the parasite.

Information was obtained by: a) interviewing cases or relatives [3,4,5]; b) parasitological studies: direct examination, microconcentration and blood culture [6]; c) identification of *Trypanosoma cruzi* by conventional PCR with amplification of a 188 bp nuclear satellite DNA sequence and a 330 bp fragment derived from the mini-exon variable region [7,8]; d) discrete typing units (DTU) were identified by amplification of three regions: intergenic of the mini-exon gene, of the variable domain of the gene coding for 18S rRNA and of the D7 divergent domain of the gene coding for 24S rRNA [9], the nomenclature followed the recommendation described by Zingales *et al* [10]; e) serological surveys of family or community collaterals for detection of IgG anti-*T. cruzi* antibodies by indirect immunofluorescence, ELISA and immunoblot [3,6] (Figure 1) and f) entomological investigation [3].

Demographic, clinical and epidemiological variables are shown in Table 1. The mean age (years) of the cases and the time between symptom onset and parasitological diagnosis in days were calculated with the MS-Excel program.

The data were collected during the epidemiological surveillance of ACD in Peru [5]. Patient data have been omitted to avoid identification.

FINDINGS

The mean age was 12.7 ± 13.7 years, range 1 to 44 years, and 4 cases were male. One case of ACD was associated with acute diarrheal disease (ADD), two with urinary tract infection (UTI) and a third with leptospirosis. The overall case fatality rate was 12.5% (1/8). Between 2009 to 2016, three cases of ACD came from Loreto, two from Ayacucho, one from Huánuco, one from San Martín and one from Amazonas. The predominant symptoms and/or signs were: fever and general malaise (8/8), inoculation chagoma (4/8) and Romaña sign (3/8). All were positive to parasitological tests (Table 1).

Case 1

A 15-year-old male, resident of a community one hour away by river from Puerto Inca (Table 1). On his first admission he was diagnosed with urinary tract infection (UTI) and febrile syndrome. Due to persistent symptoms, he was transferred to the Regional Hospital of Pucallpa, where a positive thick blood smear for *Plasmodium falciparum* was reported, as well as nausea, vomiting and two episodes of epistaxis. Urine examination showed turbidity, red blood cells (0 -1/field), leukocytes (2- 4/field) and bacteria. He started treatment for malaria and UTI. Parasitological controls were negative for the first three days. None of the family members were positive for *Plasmodium* and no *Anopheles* were found in the house. Quality control of thick blood smear at the Regional Referral Laboratory of Ucayali was positive for *T. cruzi*. The seroprevalence of *T. cruzi* infection among family and community collaterals was 1.5% (1/65) (Figure 1). He did not report blood transfusion. It was reported that the patient slept without a mosquito net in a palm leaf-roofed room tending a pig farm. Hematophagous triatomine nymphs were found in the poultry house (Figure 1). He was treated with nifurtimox (10 mg/kg body weight per day for 60 days) [11] (Table 1).

Table 1. Demographic, clinical and epidemiological characteristics of acute Chagas disease cases in Peru (2009-2016).

Variables	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Demographic, geographic and epidemiological								
Age (years)	15	0.3	1	44	6	5	8	10
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Location (probable place of infection)	San Antonio Village	Luisiana Union	Abeja Port	Winguilluycos Sector	Oropreto Community	Low Santa Rosa	Iquitos-Nauta Km 3.5	Yahuahua native community
District and department (probable place of infection)	Honoria, Huánuco	Santa Rosa, Ayacucho	Mazán, Loreto	Cacatachi, San Martín	Manseriche, Loreto	Sivia, Ayacucho	San Juan Bautista, Loreto	Nieva, Amazonas
Basin	Pachitea River	Santa Rosa river, tributary of the Apurimac river	Napo river	Cumbaza river	Marañón river	Apurimac river	Amazonas river	Marañón river
Employment	Student/ farmer	Pre-school student	Infant	Farmer	Student	Pre-school student	Student	Student
Previous history of triatomines at home	Unknown	Unknown	Unknown	Yes (November 2013)	Yes	Unknown	Didn't found	Unknown
Predominant housing material	Wooden wall, shevon palm ceiling	Wooden wall and roof palm trees	Unknown	Quincha and calamine roof	Palm leaf roof	Wooden wall and roof palm trees	Good-Quality Material	Wooden wall and roof palm trees
Collateral investigation	Yes	Yes	Yes	Yes	Yes	Unknown	Unknown	Yes
Clinical aspects								
Date of onset of symptoms	2/10/2009	14/04/2013	14/11/2013	4/02/2014	11/11/2014	6/03/2015	6/12/2015	26/12/2015
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Headache	Yes	No	No	Yes	No	Unknown	Unknown	Unknown
Abdominal pain	No	No	Yes	No		Unknown	Unknown	Unknown
Chills	Yes	No	No	Yes	No	Unknown	Unknown	Unknown
Myalgia	No	No	Unknown	Yes	Yes	Unknown	Unknown	Unknown
Hepatomegaly	Yes	Unknown	Unknown	Unknown	No	Unknown	Yes	No
Splenomegaly	Yes	Unknown	Unknown	Unknown	No	Unknown	No	No
Edema of lower extremities	No	Unknown	Unknown	Yes	No	Unknown	Unknown	Unknown
Inoculation chagoma	No	Yes	Unknown	Yes	No	Yes	No	Yes
Romaña sign	No	No	Unknown	Yes	No	Yes	Yes	No
General malaise	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Meningoencephalitis	No	Yes	No	No	No	Unknown	No	Unknown
Myocarditis	No	Yes	No	No	No	Unknown	No	Unknown
Death	No	Yes	No	No	No	No	No	No
Time between onset of symptoms and parasitological diagnosis (days)	47	9	70	23	21	Unknown	9	13
Date of confirmation	18/11/2009	23/04/2013	23/01/2014	27/02/2014	2/12/2014	7/03/2015	15/12/2015	8/01/2016
Finding of *T. cruzi* in thick blood smear	+	+	+	+	+	+	+	+
Microconcentration	+	Not performed	Not performed	+	Not performed	Not performed	+	+
Blood culture	+	Not performed	-	+	-	-	+	+
PCR for *T.cruzi* DNA detection	+	Not performed	Not performed	+	Not performed	+	+	+
T. cruzi DTU	Not identified	Not identified	Not identified	Tc I	Not identified	Tc I	Tc IV	Tc IV
Trypanosoma cruzi anti IgG ELISA	+	-	+	+	+	-	-	+
Indirect immunofluorescence (IFA) (+)	+	+	+	+	+	-	-	+
Immunoblot	+	Not performed	+	+	+	-	-	+
Treatment	Nifurtimox	Did not receive	Nifurtimox	Nifurtimox	Nifurtimox	Nifurtimox	Benznidazol	Benznidazol

DTU: Discrete Typing Units
PCR: Polymerase Chain Reaction

https://doi.org/10.17843/rpmesp.2021.381.6286

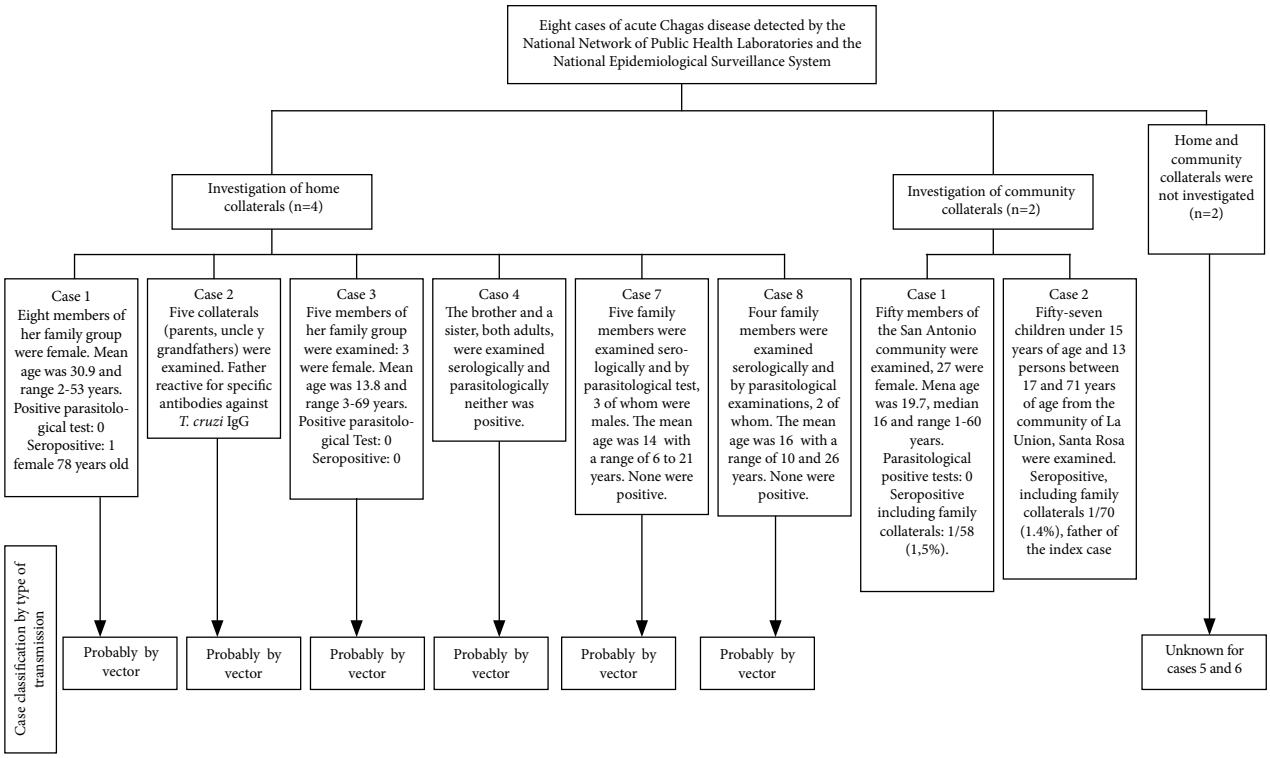

Figura 1. Flow chart of the detection of acute Chagas disease cases and epidemiological investigation (2009-2016).

Case 2

Infant aged 0.3 years, daughter of a 17-year-old adolescent who worked in a brick factory, living in a community at an altitude of 700 meters over sea level (high jungle) (Table 1). She was taken to the health center in Santa Rosa, department of Ayacucho for presenting fever a week ago. She was diagnosed with UTI and received treatment. Due to her irritable state and persistent fever, she was admitted for emergency care. The thick blood smear for *Plasmodium* and the smear for *Bartonella* were negative, but *Trypanosoma* trypomastigotes were found. She was referred to the San Francisco Support Hospital for confirmation and treatment. On admission she had a temperature of 37 ºC, pulse 122/min, respiratory rate of 60/min and oxygen saturation of 95%. In the anamnesis, she was in poor general condition, had subcostal tugging, nasal flaring, irritability, anuria, rapid breathing, dry skin, evidence of tachycardia and nuchal rigidity. Respiratory distress, distal cyanosis and a pupillary diameter of 1.5 cm on both sides were observed. Hemoglobin concentration was 8.9 mg/dL. Radiography showed cardiomegaly. Multiorgan failure occurred and she died. *T. cruzi* was confirmed in the thick blood smear. The diagnosis was meningoencephalitis due to Chagas disease. She had no history of blood transfusion. The mother reported an erythematous papular lesion (inoculation chagoma) measuring 2 x 2 cm^2 on the middle third of the medial side of the right thigh due to a possible triatomine bite, one week before the onset of symptoms. The father was seropositive for antibodies against *T. cruzi* (Figure 1).

Case 3

A one-year-old boy residing in a community three hours from the district of Mazán, department of Loreto. He went to the health facility for presenting episodes of watery ACD and fever (Table 1). The thick blood smear was negative for *Plasmodium* and quality control at the Loreto Regional Reference Laboratory reported the presence of *T. cruzi*. The parents reported a history of fever for almost three months since the onset of symptoms. They also reported that the child was taken to the farming field, and to logging and fishing activities within their community with his family. No family members were positive for *T. cruzi* (Figure 1). There was no history of transfusion. He was transferred to the Hospital de Apoyo de Iquitos, where the diagnosis was ACD and he received nifurtimox [11] (Table 1).

Case 4

A 44-year-old woman, resident of an urban area in the district of Morales, who was engaged in agricultural activi-

ties in Cacatachi, a rural area of the department of San Martín. She reported that when she fell asleep on a tree, she felt facial itching and the characteristic odor of a bedbug. After two days of fever (Table 1), she went to a private doctor's office. Due to persistent fever, she was admitted to the Tarapoto Hospital with a diagnosis of dengue fever, with no alarm signs. When the fever persisted, she went to a health center, where the rapid test for dengue was negative and the Widal test was reactive, and she was treated for typhoid fever. Due to the persistence of fever, she was treated at the Hospital Nacional Arzobispo Loayza in Lima with a diagnosis of febrile syndrome, associated in the last three days with facial and lower limb edema, rash on the face, chest and legs, cough with hemoptysis and epistaxis, for which she was hospitalized. The hemogram, liver profile, renal profile, chest X-ray and abdominal ultrasound were normal.

The INS reported thick blood smear positive for *T. cruzi*, and positive for detection of IgG antibodies against *T. cruzi* (Table 1). She also had positive IgM and microagglutination (MAT) against Leptospira and positive IgG for dengue virus. Cardiological evaluation revealed little pericardial effusion (100 mL) and the electrocardiogram and cardiac enzymes were normal, ruling out myocarditis. Family collaterals were seronegative for *T. cruzi* (Figure 1). It was reported that the patient raised animals at home and occasionally consumes juices. She received nifurtimox for Chagas disease (Table 1) and oral doxycycline for leptospirosis.

Cases 5, 6, 7 and 8

Case 5 (6-year-old male) was admitted to the Saramiriza Health Center (Loreto), with fever, cough, swollen glands and other symptoms (Table 1). In the thick blood smear taken for malaria, blood forms of *T. cruzi* were observed (Figure 2). He reported seeing "bedbugs" in his home. Case 6 (5-year-old female) came from a community 15 minutes from Sivia (Ayacucho). She went to the local hospital with fever, loss of appetite, among other symptoms. The blood count showed hemoglobin at 10.8 mg/dL and *T. cruzi* in the thick blood smear. Case 7 (8-year-old male) went to a health center with fever, decreased appetite, among other symptoms and signs (Table 1), with a suspected diagnosis of dengue fever without alarm signs. Due to the persistence of the symptoms, he was taken to an EsSalud (Social Security) hospital in Iquitos, with a temperature of 40 ºC, respiratory rate of 110/min and oxygen saturation of 98%. Physical examination revealed rhythmic heart sound without murmur, and evidence of hepatomegaly. In the neurological evaluation there was no evidence of meningeal signs. She had a normal hemogram and received treatment. Other data for cases 5, 6, 7 and 8 are shown in Table 1 and Figure 3.

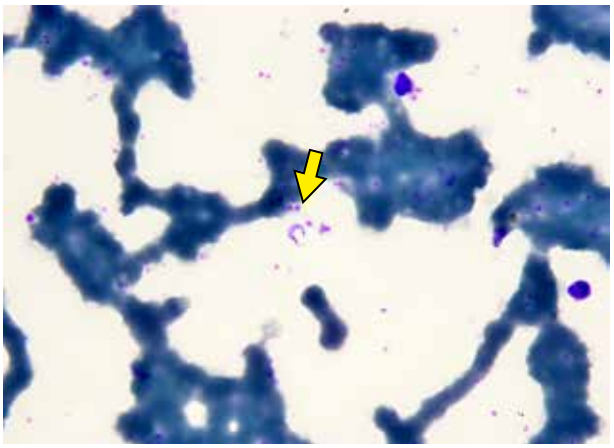

Figure 2. Blood Trypomastigote form of *Trypanosoma cruzi* in a thick blood smear slide stained with giemsa at 1000 X from case 5 (the yellow arrow indicates the parasite).

DISCUSSION

Eight cases of ACD were identified in this series, the most frequent symptoms and signs were fever and general malaise, the least frequent were inoculation chagoma, Romaña sign, myalgia, hepatomegaly, splenomegaly, meningoencephalitis, lower extremity edema and rash. These are similar to those found in a series in children from native communities of Loreto (Peru), as for fever, myalgia and splenomegaly, but differ in hepatomegaly, edema of lower extremities and general malaise [4]. These differences could be explained by the incomplete collection of data in this study and probably, by the lineage of the parasite. The Ro-

Figure 3. House of case 8 in the Yahuahua native community, Nieva, department of Amazonas.

https://doi.org/10.17843/rpmesp.2021.381.6286

maña sign and inoculation chagoma were also infrequent in the series of ACD in Brazil [12] and Colombia [13].

In six cases of this report fever was observed for more than one week, as previously described [3-5,14-16]. Only case 4, despite having coinfection with Leptospira, showed similarity with the clinical pattern of oral transmission of ACD because she was an adult. Generally, cases of ACD due to vector transmission are children [1]. In addition, we observed lower limb edema and rash, which is more frequent in oral transmission [12]. More than 57% and 27% of the 233 cases of ACD from the Brazilian Amazon had edema of limbs and face, and rash, respectively [12]. Edema and rash in leptospirosis are infrequent [17]. A characteristic of cases of ACD by oral transmission is the infrequent presentation of gateway signs [12] or the absence of gateway signs. In this series, 5 of 8 cases had gateway signs and in the epidemiological investigation of case 1, there was no evidence of oral transmission; therefore, 6 of 8 cases were probably vector-borne.

It is likely that the first case of ACD acquired dengue in Puerto Inca, since in addition to fever, the patient had alarm signs (vomiting and bleeding). At that time the incidence of dengue was more than 30 cases/100,000 inhabitants [18]. One of the cases of ACD was misdiagnosed as *P. falciparum* malaria, in two cases typhoid fever was suspected, one was associated with CDD and another was associated with leptospirosis. It is necessary to strengthen the recognition of *T. cruzi* against the misdiagnosis of *Plasmodium* at the local level [3,4,14], incorporating it into the Zero Malaria Plan [19]. Likewise, UTI was confirmed in two cases. The same was for a case of ACD reported in the central jungle that was initially suspected of UTI [15].

The time from symptom onset to parasitological confirmation averaged 27.4 days. This could be due to the fact that Chagas disease in areas of sporadic or active transmission is not a diagnostic first option in febrile patients; likewise, some microscopists at the first level of healthcare do not recognize the parasite in the thick blood smear slides. This could also be explained by the low sensitivity of the technique, which varies according to the date the sample is obtained in relation to the onset of infection and the delay in accessing health services, 2 to 23 days after the onset of symptoms. The mean time to diagnosis of ACD in this series is lower than that described in children from native communities in Peru [4].

In this series, the overall case fatality was 12.5%. The only death was probably associated with myocarditis by chagas and occurred rapidly —nine days from the onset of symptoms— as described [1]. Late detection could be a risk factor for death in native Amazonian children [4].

There is an increasing trend in the number of cases of ACD reported in Peru, similar to what has been described in other Amazonian countries [20]; besides to underreporting. The seroprevalence for IgG antibodies against *T. cruzi* among collaterals was higher than 1%; however, the sample is not reresentative and has not been randomly selected.

For two cases of ACD, the etiological agent was from the TcI lineage and for the other two it was from the TcIV lineage. The first is associated with wild reservoirs such as rodents, didelphids and wild vectors such as *Rhodnius*, *Panstrongylus* and the second is a sporadic agent of Chagas disease [21]. This is the first time that the *T. cruzi* lineage has been identified in cases of ACD in the Amazonian areas where they were detected. It has been described that TcI infects *P. geniculatus*, a wild vector, and TcIV circulates in *P. herrreri* [22].

Among the limitations of this study, we should mention that it was not possible to access all the clinical files and clinical-epidemiological records of the cases; in addition, the interviews with some patients were conducted several days/weeks after the date of symptom onset, which may have caused some memory bias. Also, the results of the vectors are not included in this series.

Eight new cases of ACD have been reported in the Peruvian Amazon, mostly associated with other co-infections. The districts of Santa Rosa and Sivia (Ayacucho), appear to be new areas of active transmission, in which wild vectors could be involved.

Acknowledgements: Dr. Mario Grandez-Armas of the Hospital Nacional Arzobispo Loayza and Dr. Omar Rodriguez for participating in the identification or follow-up of some of the cases presented in this series.

Author contributions: SV and RC conceived the idea of the article, participated in the investigation of the cases, data collection, writing and approval of the submission version. CAA, IUV and ACT participated in the research of the cases, data collection, writing and approval of the submission version. JAJ and BCC, participated in the identification of parasite lineages, drafting and approval of the submission version. SGQ, MZS, MBF and ROP participated in the investigation of the cases, drafting and approval of the submission version.

Funding: Instituto Nacional de Salud de Perú, Centro Nacional de Epidemiología, Prevención y Control de Enfermedades (CDC), Ministerio de Salud, Dirección Regional de Salud de Loreto, Dirección Regional de Salud de Huánuco, Dirección Regional de Salud de Ayacucho and Dirección Regional de Salud de Amazonas.

Conflict of interest: The authors declare that they have no conflict of interest.

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