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BRIEF COMMUNICATION

EPIDEMIOLOGICAL INVESTIGATION OF AN ACUTE CASE OF CHAGAS DISEASE IN AN AREA OF ACTIVE TRANSMISSION IN PERUVIAN AMAZON REGION

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SUMMARY

The study objective was to investigate an acute case of Chagas disease in the San Pedro de Shishita community, Pebas District, in the Peruvian Amazon basin, a non-endemic area. Both parents of the index case (acute case) were thoroughly interviewed, a seroepidemiological survey was carried out in the community, parasitological exams were carried out only in relatives of the index case, and triatomine bugs were searched for inside houses, peridomiciliary, and in wild environments. Seroprevalence for IgG anti-*T. cruzi* antibodies was 1/104 (0.96%), using an ELISA test and an indirect immunofluorescence assay. *Panstrongylus geniculatus* and *Rhodnius pictipes* adults were found. The index case is autochthonous from San Pedro de Shishita, but the source of transmission is unknown.

KEYWORDS: Chagas disease; Seroepidemiologic studies; Triatominae; Amazonian Ecosystem; Peru.

Chagas disease is caused by the protozoan *Trypanosoma cruzi*. The main routes of transmission are through vectors, blood transfusion or contaminated blood products, vertical transmission and through an oral route by the ingestion of contaminated food. There are an estimated 300 000 new cases per year in the American continent and 23 000 deaths due to Chagas disease[15]. Most cases reported to the Ministry of Health in Perú come from the department of Arequipa and the remaining cases from the north-west of the country (Amazonas, Cajamarca and Piura departments)[3]. In the last three years, seven acute cases have been notified in the Peruvian Amazon region, an area previously not considered to be endemic to Chagas disease. One case was from the department of Pasco, province of Oxapampa, district of Pozuzo[19] and six cases were from the department of Loreto, five from the province of Datem del Marañón[4] and the other one, an eight year old girl, from the province of Mariscal Ramón Castilla, district of Pebas. She presented with fever, headache and abdominal discomfort seven days before seeking medical attention, and was originally misdiagnosed with malaria[2]. This is the first acute case of Chagas disease reported in this area. Because it was a new area for the surveillance system, it was important to determine if the acute case was autochthonous from Pebas, if it was an isolated case and to investigate the transmission focus in this part of the Amazon basin. The objective of the study was to perform the epidemiologic investigation of the acute case of Chagas disease in the community of San Pedro de Shishita in Pebas district.

Study area: The district of Pebas, is located 101 meters above sea level, is a wet forest area in the Amazon region of Perú, with an average temperature of 25-26 ºC and annual precipitation ranges between 2000 to 3400 mm. It is located in the province of Mariscal Ramón Castilla, in the department of Loreto. Pebas district belongs to the humid forest eco-region[17]. The population consists of settlers and indigenous groups distributed in more than 60 rural communities (Fig. 1). San Pedro de Shishita consists of 45 houses[11] and along with the nearby Nuevo Pebas and Nuevo Tarma are settlements situated on the banks of the Amazon River[16]. Their most important economic activities are agriculture, fishing and commerce. Typical house construction is characterized by two to three small rooms, wooden walls and floor, and roofs made from palm leaves. The area is endemic for *Plasmodium vivax* and *P. falciparum* malaria and cutaneous leishmaniasis. The house of the index case is situated about 1 km from the community of San Pedro de Shishita, with trees and palms nearby.

Study design. The epidemiologic investigation included: a) an in-depth interview, b) a seroprevalence survey, c) a parasitological

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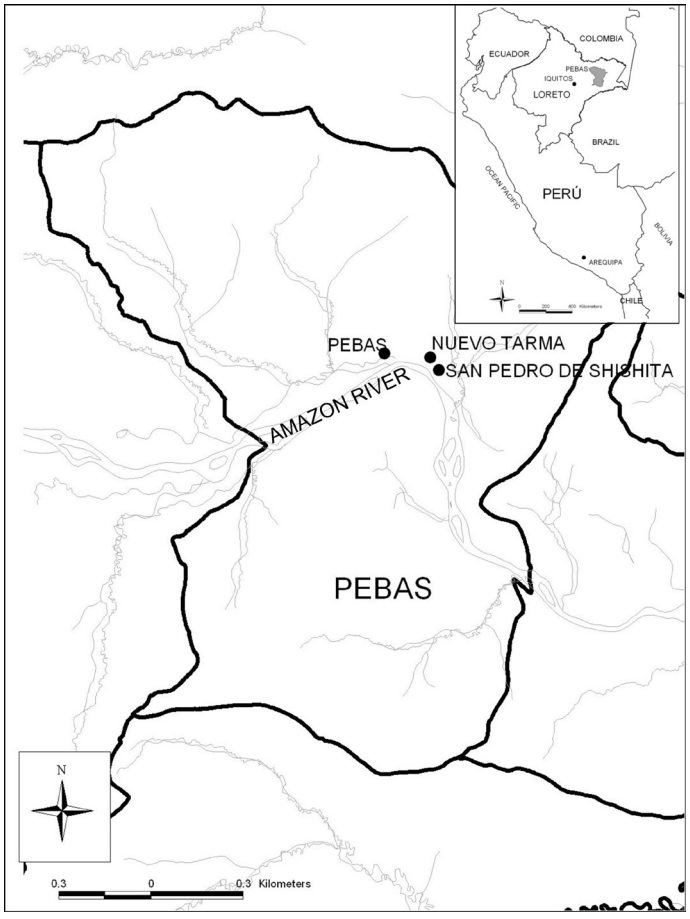

Fig. 1 - Location of the communities studied, Pebas District, Mariscal Ramón Castilla province, Loreto, Peruvian Amazon Basin.

evaluation, d) experimental inoculation e) triatomines searching, and f) domestic reservoir search.

In-depth interview: The acute case reported in Pebas[2] was considered as the index case, according to the definition elaborated by LAST[12]. A semi-structured in-depth interview was conducted with the parents of the index case to identify disease signs and symptoms, and other known epidemiological factors as food ingested in the last two weeks, sleeping outdoors, travel history 30 days before the disease onset, presence of triatomines and collateral cases (who shared the family home of the index case). The data were obtained by the investigators, recorded in a notebook, and compared with the data obtained with the questionnaire of the seroprevalence survey.

Seroprevalence survey: Included an epidemiologic structured questionnaire, conducted by the research team to a non-random sample of the inhabitants, to obtain socio-demographic data (gender, age, education, religion, occupation). Epidemiologic antecedents such as the presence of disease vectors and the identification of triatomine bites were determined using dead triatomine samples in Petri plates for comparison. Information regarding animal breeding, blood transfusions and migration was also obtained. The questionnaire was given to the head of the households and data on children was gathered with the permission and help of their parents. Blood samples were obtained from consenting people present at the community at the time of the field investigation. Five mL of venous blood from adults was collected into vacuum tubes without anticoagulant, and from children blood was passed into four capillary tubes with heparin by finger prick. Serum samples were tested to detect anti-*T. cruzi* antibodies using an ELISA test (Chagatest ELISA recombinant v.30 Wiener Lab®) which uses recombinant antigens of specific proteins from epimastigotes and trypomastigotes of *T. cruzi*, and by indirect immunofluorescence (IIF) with a diagnostic titer value of 1:32 according to the methods described elsewhere[18]. A participant was considered seropositive when both tests gave a reactive result. The questionnaire and laboratory results were entered into a database using Epi-Info version 6.4 (Centers for Disease Control and Prevention, Atlanta, USA), and a descriptive analysis was performed. This study was not submitted to an ethics committee because it was part of an outbreak investigation.

Parasitological study: Blood samples were processed by micro concentration technique for *Trypanosoma* only to identify collateral cases.

Three sisters of the index case were also evaluated by xenodiagnosis with laboratory-bred third and fourth instars of *Triatoma infestans*. Triatomines were kept between 25 to 30 °C at 70% relative humidity, and fed on chickens every 15 days. After 30 days a sample of triatomine feces were microscopically examined and after 60 days the digestive tract homogenate was also examined. As this is an endemic zone for malaria, we prepared a thick smear for each participant, which were stained with Giemsa and interpreted in a field laboratory. Those positive for *P. vivax* started treatment with chloroquine and primaquine the same day according to the protocol of the Ministry of Health.

Experimental inoculation: To isolate and characterize the *Trypanosoma* spp. strains, 0.1 mL of blood from the index case was inoculated intraperitoneally in a Balb/c mouse. Every seven days a blood sample of the experimental infected mouse was examined by micro concentration, thick smear and was stained with Giemsa searching for trypomastigotes. The mouse was sacrificed after two months and sections of the heart, gut and brain were histologically examined.

The entomological evaluation: Triatomines were collected between 8:00 am and 7:00 pm by manual search, using flashlights and 30 cm wires. The collection was performed in: a) intradomiciliary environment, mainly in the bedroom, kitchen and guinea pigs breeding room; b) peridomiciliary environment, 20 meters around the house, especially in wood stockpiles and animal breeding places and, c) extradomiciliary, in the crop fields that the index case visited occasionally before the disease onset, as well as around fallen trees, wood stockpiles and rustic houses.

Adult triatomines were transported alive to the laboratory for natural infection evaluation. Feces were obtained by abdominal pressure, homogenated with saline solution and observed with microscope at 10X and 40 X[5]. Triatomines were identified according to the methods described elsewhere[13].

Domestic reservoir investigation: Only one dog, from the house of the index case, was examined by xenodiagnosis with four third instar nymphs of *T. infestans*. Triatomine feces examination has been described elsewhere[5].

In-depth interview: In the 30 days before the onset of signs and symptoms, the index case had no history of travel, blood transfusions nor consumed fruit juice or other potentially contaminated foods. The patient presented with headache, malaise, foot swelling, and hyporexia, and was negative for the inoculation chagoma. On the third day of treatment with nifurtimox, she presented retroocular pain, arthralgias and nausea without vomiting. The fever disappeared on the fifth day. In addition, she had clinical symptoms of gastritis at the beginning and end of treatment.

Seroepidemological survey: Information was collected from 98 (92.5%) inhabitants from San Pedro de Shishita, six (5.7%) from Nuevo Tarma and two (1.9%) from Nuevo Pebas. Average age was 18 years (SD 17.43, range 0-82, median 11), fifty (47%) were men, only 29 (27%) had completed elementary school, 48 (45%) were illiterate, 38 (36%) were students, 28 (26%) were without occupation, 20 (19%) were farmers, 17 (16%) were housewives and three (3%) performed other activities (logger, teacher). Triatomines were known as "*chinches*" by 38 (36.5%) participants and by five (5%) as insect or *pelón*. Forty-three inhabitants recognized the triatomines, and seven of them reported having being bitten at least once in their lifetime. None had received a blood transfusion. Participants who migrated from other departments were 30 (28.3%) and 34 (32.2%) bred some animals like birds, dogs, guinea pigs, cats, monkeys, marsupials and others.

Of the 106 persons interviewed, 104 blood samples were obtained. The overall seroprevalence for anti-*T. cruzi* was 0.96% (1/104) as determined by ELISA and IIF (1/32). The positive case (IIF 1/256) was a 26 year old woman, born in the province of Huancabamba, department of Piura. She knew the vector as a "*chinche*". She lived for nine years in Huancabamba and eight years in Tornavista, in the province of Puerto Inca (Huánuco department), before settling in San Pedro de Shishita. The clinical evaluation showed no clinical signs compatible with Chagas disease.

Parasitological study and experimental inoculation: 14 of the 16 possible collateral cases were examined by microconcentration, and no *T. cruzi* was found. The xenodiagnosis in three sisters of the index case was negative after 60 days. *Trypanosoma* isolated from the index case were identified as *T. cruzi-like* trypomastigote by the morphologic characteristics in thick smears, and the demonstration of amastigote nests in the histological sections of heart muscle of Balb/c mice experimentally infected with blood from the index case.

Thick smear slides were obtained only from 100 participants, six were positive for *P. vivax* and three were positive for microfilariae, the species of which were not determined. None was positive for *Trypanosoma*.

Entomological findings: A *P. geniculatus* female deteriorated (dead) was found on a shelf in the house of the index case. Another *P. geniculatus* female was found 20 meters from the house in a henhouse built with banana leaves. A *Rhodnius pictipes* male was captured in the area used for public meetings.

Domestic reservoir investigation: The only dog examined by xenodiagnosis from the house of the index case, was negative.

The detection of an acute autochthonous case of Chagas disease[2], indicates that the community of San Pedro de Shishita (Pebas) is an active transmission area with sporadic acute cases presentation, similar to Pozuzo in the *Selva Central* of Perú[19]. The two foci differ from inter-Andean valley of Marañón in the Amazonas department, because acute cases have been frequently reported in this area[3] since 1950s[10], where, *P. herreri/lignarius*, of domestic habitats is the main vector. It also differs from Datem del Marañón because more acute cases have been reported in the last three years in this last area[4]. Nevertheless, vectors involved in disease transmission are unknown. There are reports of sylvatic triatomines sporadically invading houses[1]. *P. geniculatus* adults have been observed getting inside dwellings during the night and are associated to an acute case of Chagas disease in *Selva Central* area[19]. Usually acute cases vectored by sylvatic triatomines in Amazonia present as isolated cases[1].

The infection source of index case is unknown. Vector transmission cannot be ruled out because the unique specimen found, a *P. geniculatus,* was dead and we could not rule out *Trypanosoma* infection from it. Neither can we rule out oral transmission. Vertical transmission can be ruled out because the negative results of the patient's mother.

The absence of natural infection by *T. cruzi* in the triatomines found could be explained by the small number of specimens collected. *P. geniculatus* is likely to be a competent vector due to its capacity to colonize dwellings and the varied food sources it has, including human blood[14].

The finding of sylvatic triatomines in Pebas district as *P. geniculatus* and *R. pictipes* and the prior reports of *R. robustus, Eratyrus mucronatus*[9] and *Cavernicola pilosa*[6] presence in the Loreto department, indicate a risk transmission area for *T. cruzi*.

The antecedent of a foot swelling occurred two weeks before signs and symptoms began, while she was in the outskirts[2]. It was probably an allergic reaction produced by the bite of a non hematophagous insect, because it lasted for only three days. She did not sleep in that area and there were no triatomines in the entomologic investigation performed in the area where she was probably bitten. This case was similar to other cases infected in the Amazon by vector transmission[1].

Besides the acute case, the only seropositive person was a woman that migrated into the area. She was an asymptomatic isolated case that could either have been infected in San Pedro de Shishita (Pebas), in Huancabamba - where there is presence of *R. ecuadoriensis* and *P. chinai*[6] - or other areas where she had lived before. At the same time, and given similar precedents in similar Amazonian communities, 'isolated' cases may add up to a hypo-endemic pattern, as has been suggested[1]. It has been postulated that people could migrate with the disease and carry the vectors[1]. The infection risk by *T. cruzi* in people from the rural area of the Amazon region from Ecuador is four times higher than in migrants, and the prevalence increases with age suggesting that the time of residence in the Amazon is a risk factor for Chagas disease[8].

Epidemiologic and demographic characteristics of the surveyed people were similar to what has been described in Ecuador's Amazonia[8]; nevertheless, the number of triatomines found, and the bit antecedents are lower in Pebas.

This study has some limitations. The sample number is low and the subjects have not been randomly selected. In addition, the number of

bugs used in xenodiagnosis was scarce. This investigation was conducted as part of the field investigation of a confirmed Chagas disease case, in order to rule out an oral transmission outbreak. It is necessary to plan and conduct cross sectional studies with appropriate design and sampling to determine the epidemiologic pattern of Chagas disease in these settings.

In the Amazon region of Brazil, malaria laboratories have been trained to recognize *T. cruzi*[1], allowing the identification of new areas of transmission. This strategy should be implemented in Perú, prioritizing areas of active transmission to improve the detection and early treatment of cases. Chagas disease remains a neglected disease in Perú, nearly 90 years after the detection of the first case, in Madre de Dios[7]. Widely scattered villages, poverty, poor education and limited accessibility to healthcare, with malaria, pneumonia, malnutrition as main morbidity causes, mean that other strategies of surveillance need to be implemented.

In summary, the acute case reported in Loreto[2] is autochthonous from Pebas; nevertheless, it has not been possible to determine the source of infection. San Pedro de Shishita community is an active transmission area for Chagas disease in the Peruvian Amazon region with sporadic case presentation, probably due to sporadic invasion of sylvatic triatomines.

RESUMO

Investigação epidemiológica de caso agudo da doença de Chagas em área de transmissão ativa na região da Amazônia peruana

O objetivo deste trabalho foi estudar caso da doença de Chagas aguda na comunidade indígena de San Pedro de Shishita, sem conhecimento da origem da transmissão. San Pedro de Shishita, distrito Pebas, região da Amazônia peruana é uma área não endêmica. Foram entrevistados os pais do paciente e feito inquérito soroepidemiológico dos participantes e estudos parasitológicos da família e procurou-se também triatomíneos no ambiente doméstico, peridomiciliar e silvestre. A soroprevalência de anticorpos IgG anti-*T. cruzi* foi 1/ 104 (0,96%) por ELISA e imunofluorescência indireta. Foram encontrados adultos de *Panstrongylus geniculatus* e *Rhodnius pictipes*.

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