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CASE REPORT

Epidemiology of human immunodeficiency virus-visceral leishmaniasis-co-infection

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In Brazil, the rates of mother-to-child-transmission (MTCT) of human immunodeficiency virus (HIV) decreased from 20% to 1—2% in some regions. However, the country contains 90% of individuals infected with visceral leishmaniasis (VL) in Latin America, and the west region of São Paulo state faces an alarming expansion of the disease. We describe the epidemiological aspects of the expanding infection of VL and a case report of an HIV-VL-co-infected child from the west region of São Paulo state. The patient was an AIDS-C3 with low levels of CD4, high viral load, severe diarrhea, oral and perineal candidiasis, severe thrombocytopenia, and protein-caloric malnourishment. She evolved with sepsis, renal and cardiac failure. An rK rapid diagnosis test, indirect fluorescent antibody test (IFAT), and bone marrow aspirate were performed for VL. Her symptoms improved significantly after liposomal amphotericin B administration. From the 45 municipalities that compose the Regional Health Department of Presidente Prudente, *Lutzomyia longipalpis* vectors were found in 58% of them. VL infected dogs were found in 33% of those municipalities, infected dogs and humans were found in 29%, 20% are starting and 33% of the municipalities are preparing VL investigation. It is likely, in this patient, that VL advanced the clinical progression of the HIV disease and the development of AIDS severity. Supported by favorable conditions, the region becomes a new frontier of VL in Brazil.

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Introduction

Brazil was one of the first developing countries to adopt measures against mother-to-child-transmission (MTCT) of human immunodeficiency virus (HIV) and in some regions rates decreased from 20% to 1—2%.[1] Despite all the efforts made by public and private organizations, MTCT still arises, mainly from drug users and women living in poor settings.[2] HIV-infected children with irregular use of antiretroviral therapy (ART) are predisposed to opportunistic and pathogenic agents, such as tuberculosis and leishmaniasis. Visceral leishmaniasis (VL) is a chronic and frequently lethal disease distributed worldwide. Brazil contains about 90% of individuals infected with VL in Latin America.[3] In 2003, the sandfly was found in Dracena, the west region of São Paulo state and in 2004, the Adolfo Lutz Institute diagnosed infected dogs with *Leishmania (Leishmania) infantum chagasi* by polymerase chain reaction (PCR). In 2005, the first human case with VL was found in Dracena.[4,5]

The HIV-infection increases the risk of developing VL by 100—2320 times in endemic areas.[3] VL associated HIV-AIDS is becoming an important public health problem, however, few reports of HIV-VL co-infection are described in São Paulo state. Our aim was to analyze the epidemiological aspects of the expanding infection of VL and to describe a case report of an HIV-VL-co-infected child from the west region of São Paulo state.

Case report

Written consent was obtained from the family for publication of this case report. On November 1, 2011, an 11 year-old girl, native of Pilar county, Alagoas state, northeast of Brazil and residing in Paulicéia county, the west region of São Paulo state [Fig. 1A and C, on the border with Mato Grosso do Sul (MS), circled in blue] was referred to the Regional Hospital (RH) of Presidente Prudente, São Paulo state. The city harbors the Regional Health Department (DRS XI). In the preceding 2 months, she lived with her grandparents. The girl's mother, an HIV-seropositive, chronic alcoholic, and street-living woman, remained in Alagoas state. Contrary to the hospitalization, the girl's grandparents were convinced of her condition, due to the seriousness of her health status. However, the high level of fear, stigma, discrimination, and negative aspect of the disease expressed by them was clear. The child was an MTCT-HIV-AIDS-C3-exposed patient with irregular use of ART, presenting with diarrhea for several days characterized by being liquid, with no blood or pus. On physical examination, she was uncommunicative, hypoactive, severely malnourished, pale, eupneic, afebrile, showing moderate dehydration with pasty turgor and petechiae distributed in the trunk and face. With a painless excavated abdomen, a mild hepatomegaly was present and extending to 5 cm below the costal margin. Lymphadenopathy and splenomegaly were not detected. She presented perineal and oral candidiasis, difficulties in eating and walking and precarious teething, weighing 15 kg. Laboratory investigations showed leukocytes 4,69 $\times$ 10^3/mL, 84% neutrophils, 6% lymphocytes, and 10% monocytes. The hemoglobin level was 9.7 g/L. Severe thrombocytopenia (18,000/mL) and hypoalbuminemia (1.98 g/mL) were found. A urine sample showed 5 $\times$ 10^3 of leukocytes/mL and 5.0 $\times$ 10^3 of erythrocytes/mL. Serum creatinine was at 0.21 mg/dL. Hepatic enzymes alanine/aspartate transaminases resulted in 30 U/L and 15 U/L, respectively. Serum sodium, potassium, and phosphorus electrolytes resulted in 113 mEq/L, 4.8 mEq/L, and 3.68 mEq/L, respectively. HIV-viral load resulted in 45,089 copies/mL. CD4 and CD8 levels resulted in 12 (2.2%) cells/mm^3 and 371 (68.71%) cells/mm^3, respectively. An abdominal ultrasound revealed hepatomegaly with spleen in the normal range; an echocardiogram resulted in normal values. After clinical and laboratory assessments, her initial diagnosis was AIDS/C3 with irregular lamivudine + tenofovir + lopina/ritonavir treatment (ART), plus severe protein-caloric malnourishment, electrolyte disturbance (hyponatremia), oral and perineal candidiasis, chronic diarrhea, precarious teething, and depression. The patient was treated by a multidisciplinary staff and evolved with persistent diarrhea, decreased consciousness level, fluctuating in periods of drowsiness and stupor. A mild hepatic enlargement was detected, secondary to congestive cardiac insufficiency. On the 17th day of hospitalization, she was sent to the pediatric intensive care unit (ICU), with a sepsis of unknown origin, remaining 4 days and receiving antibiotic therapy (vancomycin 40 mg/kg/day and meropenem 120 mg/kg/day) during 21 days; methylprednisolone (1 mg/kg/day) during 5 days and human intravenous IgG immunoglobulins (400 mg/kg/day) during 5 days. On the 23rd day of hospitalization, she had not improved and developed severe fever, a marked decrease of diuresis, moderate tachydyspnea and tachycardia with presence of bilateral stertor in the base of the lung, cardiac and renal failure. The patient was admitted again into the pediatric ICU, with a diagnosis of septic shock of the blood of fungal origin.

The origin and destination of the patient from the endemic area of Alagoas state to another emerging area of VL, the west region of São Paulo, was considered. Furthermore, due to her clinical symptoms (hepatomegaly, petechiae, renal failure, and sepsis of fungal origin) and laboratorial results of thrombocytopenia, liposomal amphotericin B deoxycholate (ampho B, 5 mg/kg/day) was introduced. Simultaneously, an rK rapid diagnosis test (Kalazar detect™, InBios, Seattle, Washington, USA), indirect fluorescent antibody test (IFAT; Bio-Manguinhos/FIOCRUZ, Rio de Janeiro, Brazil) and bone marrow aspirate by direct parasitological (DP) were performed for VL. Even though the rapid diagnosis test and IFAT results were negative, liposomal ampho B (2 mg/kg/day) was maintained. No pathogens were isolated from different culture samples. Later, DP bone marrow aspirate showed macrophages filled with intracytoplasmatic *Leishmania* spp. amastigotes parasites (Leishman-Donovan bodies) on Giemsa staining.

The patient's clinical symptoms gradually improved, with moderate weight gain, and she was interacting with other children of the pediatric ward, with an easy smile, active, collaborative, and communicative. After the 47th day of hospitalization, she relapsed with thrombocytopenia and a retreatment with ampho B and intravenous human IgG immunoglobulin was applied, but was not sufficient for platelets normalization. On admission, she weighed 15 kg; after 71 days of hospitalization, she weighed 23.8 kg, with a gain of 8.8 kg in the period. On

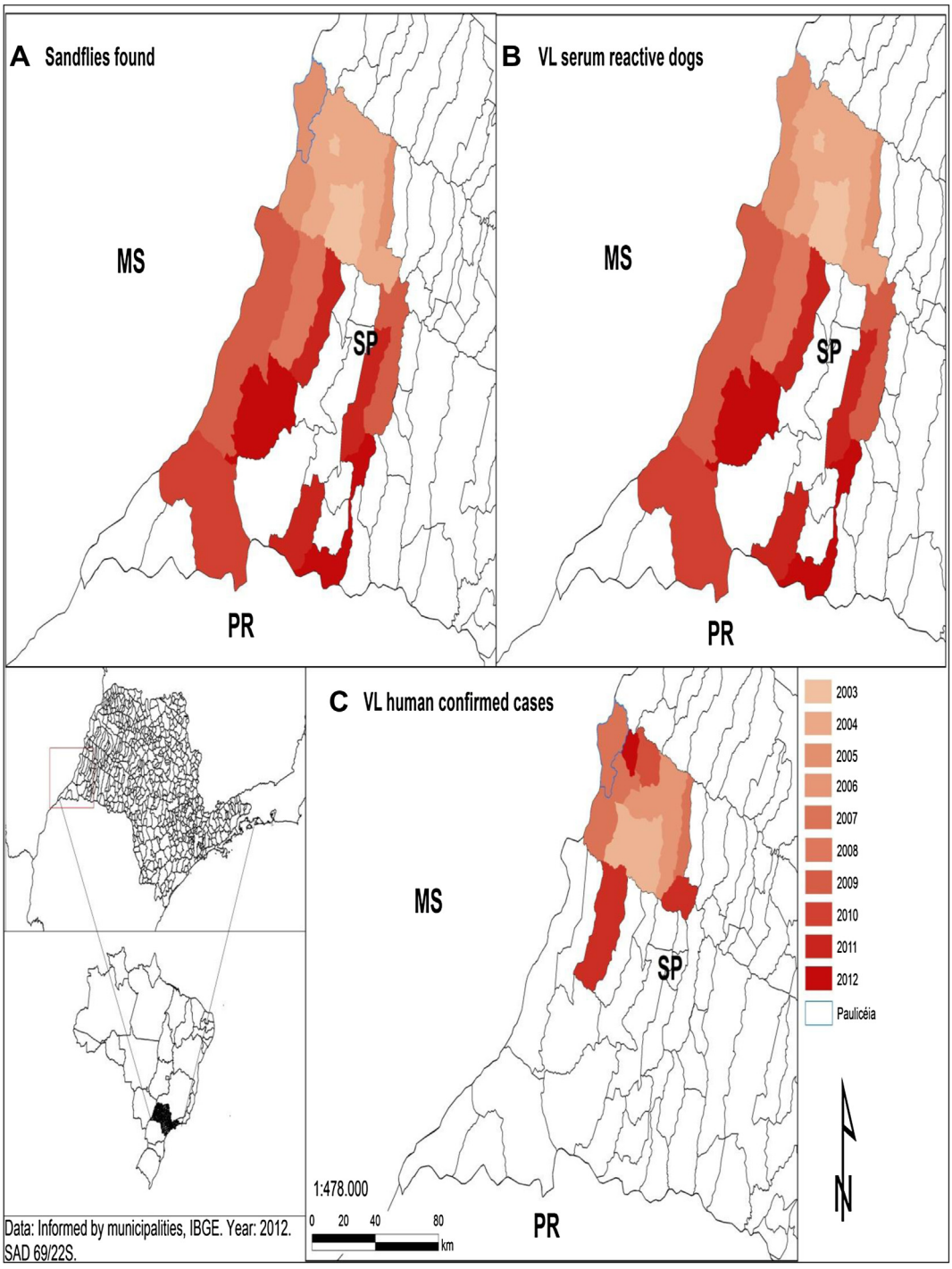

Figure 1. Western São Paulo state. Spatial distribution of (A) counties of sand flies vectors, Paulicéia is circled in blue; (B) counties with VL serum-reactive dogs; and (C) counties with visceral leishmaniasis (VL) human confirmed cases. The colors represent the evolution of the VL events linked to the year. *Note*. Data were obtained from: Brazilian Institute of Geography and Statistics (IBGE), available from: ftp://geoftp.ibge.gov.br/mapas_tematicos/fisico/regionais/centro_oeste_fisico.pdf, Accessed January, 05, 2013; Supervision and Control of Epidemic (SUCEM), available from: saude.sp.gov.br/resources/sucen/programas/leishmanioses-tegumentar-e-americana/bepa9611.pdf. Accessed December, 20, 2012; and Nucleus of Biomedical Sciences, Regional Laboratories Center, Adolfo Lutz Institute, Presidente Prudente, São Paulo.

January 10, 2012, the girl's family communicated to the RH that they would return to Alagoas state and they signed a document, removing the patient. She was referred to the public government agencies of Pilar (Sexual Diseases Transmission-DST-AIDS program and Children Protection Agency). The patient segment was lost to follow-up.

Discussion

The present case shows an HIV-AIDS-C3 child, conduced to a referred hospital with an acute diarrhea, severe malnourishment, electrolyte disturbance, and depression, irregular use of ART, low levels of CD4, and a high level of plasmatic virus. Over 25 years, Brazil was one of the first developing countries to guarantee universal and free access to ART to people living with HIV and to develop a national plan for reduction of MTCT.[1,2] Nowadays, infected drug users, seropositive adolescents, limited knowledge about how to prevent AIDS, poor and less education, and lack of HIV testing and treatment during pregnancy, are considered the main sources of HIV-MTCT-spreading in Brazil.[1,2,6] Our patient is a representative model of the current HIV-exposed children in the country, due to her young alcoholic drug using and homeless mother. In recent studies conducted in the west region of São Paulo state, the maternal age of HIV-infected mothers varied from 15 years to 40 years and 50% of mothers that transmitted the virus to their newborns were diagnosed during or after delivery.[6]

After the 17th and 23rd days of hospitalization, respectively, the patient evolved with a sepsis of unknown origin and septic shock. Treatment with ampho B was introduced, resulting in a gradual improvement of the health status of the patient. HIV-AIDS and VL co-infection have been reported in 35 countries worldwide, becoming a health public problem in southern Europe, especially in injecting drug users.[3] In Brazil, most of the cases originate from adults of northeast states and few co-infections are described in infants.[7] The chronic co-infection of both pathogens in macrophage host cells, as well as in other cells, increases the activation and cytokines production not only in macrophages, but also in HIV-infected CD4-T cells. Chronic activation of the immune system is considered an important mechanism of disease progression, linked to the decrease in number and function of T lymphocytes, evidenced by the proportion of $CD38^{+}$ and HLA-DR expression. In HIV-VL-infected patients, an impaired immune response with high levels of Th2-associated CD4+ T cell cytokines subsets [interleukin (IL)-4, IL-5, and IL-10], CD4+ T cell depletion and Th1-type cytokines inhibition has been demonstrated. Taken together, these factors increase the susceptibility to infection, accelerating HIV replication, Leishmania growth, and progression toward AIDS.[3,8]

Ampho B was introduced although the rK39 and IFAT screening test results were negative. In immunosuppressed individuals, serological tests have limited diagnostic value, due to a significant number of co-infected individuals who have no detectable antibodies levels against VL by standard techniques. A possible mechanism is the oligoclonal B-cell activation, due to an alteration in antigen presentation by macrophages or in T and B lymphocytes cooperation and recognition of specific *Leishmania* spp. antigens.[3,8] The sensitivity of IFAT for the diagnosis of co-infected patients is about 11–65% and 74–85% for rK39 immunoblotting, respectively.[3] All of the studies were performed in adult co-infected patients and no data are available in juveniles.

The origin of the infection is difficult to establish, because the patient lived in Pilar county Alagoas state, a known endemic area of VL in Brazil and in Paulicéia county (Fig. 1A and C, on the border of Mato Grosso do Sul, circled in blue), the west region of São Paulo state, also considered an emerging region of VL. The incubation period of VL generally varies from 3 months to 8 months, but it could be as short as 2 weeks or longer than 1 year.[3] Despite measures adopted to control VL, the disease is spreading in a fast and worrying way throughout the west region, becoming a new endemic area and changing the scenario of the disease in São Paulo state.[4,5] The region harbors several conditions that can lead to a rapid spread of the infection. It is in close proximity to the state of Mato Grosso do Sul (Fig. 1A–C) and Dracena in São Paulo state, two well known endemic regions. The presence of hundreds of fragments of rain forests distributed in peri-urban areas is due to the destruction of primary forests and the higher number of rural settlements of the country. It is well known that establishment of human settlements in modified environments is crucial for the spreading of infectious diseases.[9] However, one of the most important factors, is the overlapping of VL and cutaneous-mucosal leishmaniasis (CML). The first case of CML in São Paulo state was reported in 1909, among workers building a railway in a region known as Pontal of Paranapanema located in the west region of São Paulo state. Outbreaks were described in the 1950s, in an intense deforestation for agricultural expansion. A recent outbreak of CML was detected in a settlement of the region.[10]

Until 1997, VL was known in the state of São Paulo only by imported cases, however, in 1997, *Lutzomyia longipalpis* infected sandflies were found in Araçatuba, a surrounding area of the west region of São Paulo state. In 1998, infected dogs were identified and since 1999, human cases are continuously noted. In the west region, *L. longipalpis* was detected in Dracena in 2003 and canine and human cases have been detected since 2005.[4,5] The city of Dracena is considered by the Health Ministry to be an area with a high transmission rate.[5] The first patients attended the pediatric ward unit of RH in 2006 and since then, approximately 100 individuals, <18 years of age, with VL, were identified. From the 45 municipalities that compose the Regional Health Department of Presidente Prudente (DRS XI), *L. longipalpis* vectors were found in 26 (58%) municipalities (Fig. 1A), VL infected dogs were found in 15 (33%) municipalities (Fig. 1B), infected dogs and humans were found in 13 (29%; Fig. 1C) municipalities and 9 (20%) municipalities are starting or are preparing VL investigation (Fig. 1). A scenario of the evolution of sandflies found, VL serum reactive dogs, and confirmed VL cases in the period 2003–2012 in the west region of São Paulo state is shown in Fig. 1.

In conclusion, in areas where VL was not a frequent disease, clinicians should be aware of leishmaniasis, mainly in primary or acquired immunosuppressed, such as HIV-infected, individuals. It is likely in this patient, that VL advanced the clinical progression and severity of the HIV disease. To the best of our knowledge, there was no case of

HIV-AIDS-VL-co-infection described in the west region of São Paulo state. Supported by favorable conditions, the region becomes an emerging new frontier of VL in Brazil.

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