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Case report

Epidemiological aspects of the first human autochthonous visceral leishmaniosis cases in Porto Alegre, Brazil

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ABSTRACT

Human visceral leishmaniasis is a growing anthropozoonosis in Brazil, and particularly in the southern region of the country. It is an infectious disease transmitted to humans, dogs and other animals in urban and rural areas of the Americas, mainly due to the bite of *Lutzomya longipalpis* infected with *Leishmania infantum*. This article aims to portray the current epidemiological situation of the human visceral leishmaniasis arrival in Porto Alegre city, located in the southern region of Brazil. It is a descriptive study, a case series and a critical review. Six human cases with human visceral leishmaniasis were notified by the date of conclusion of the study, all human visceral leishmaniasis cases were diagnosed at late stage, leading to four deaths.

Introduction

Human visceral leishmaniasis (HVL) is a growing anthropozoonosis in Brazil and in Rio Grande do Sul (state located in southern Brazil). According to the *Handbook on Surveillance and Control of Visceral Leishmaniasis*,[1] in Brazil, HVL use to have an eminently rural character, but has reached the population of urban areas due to intense migratory flow. Some large urban areas, such as Rio de Janeiro (RJ), Belo Horizonte (MG), Santarém (PA), Fortaleza (CE), and Campo Grande (MT) have suffered this urbanization process of the disease, highlighting several outbreaks that occurred in the region.[2,3]

In the southern region, the states of Paraná and Santa Catarina did not report cases of HVL, but Rio Grande do Sul (RS) is not in this situation, since the municipalities of São Borja, Uruguaiana and Itaqui, bordering Argentina, presented autochthonous cases in 2008 and 2009. It is believed that this

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situation expanded due to the transit of people with their pets coming from Argentina, as cases of HVL were recorded in Posadas-Misiones (2006) and Virasoro-Corrientes (2008). During this period, research in the area verified, by the year 2010, five cases of confirmed HVL in São Tomé (border with São Borja).[4–6] In Porto Alegre, capital of RS, the first autochthonous case registered in September 2016 was a patient aged 1 year and 7 months who ended up dying four months after the onset of symptoms. This case led to great mobilization of the government towards the qualification of the professionals and implementation of epidemiological control measures.

Methodology

This was a descriptive study, a series of cases and critical review. Data were collected as follows: hospital records with prior authorization, including the ERC (only the fourth case), and secondary public data of the General Coordination of Health Surveillance (CGVS) (other cases reported). This study was approved by the Ethics and Research Committee of the Santo Antônio da Criança Hospital – Santa Casa/RS under no. 2,657,622.

Results

Cases of human visceral leishmaniasis

Porto Alegre is a city located to the east of the state of RS, being the most southern capital of Brazil, belongs to the metropolitan mesoregion and has a very diversified geography, with hills, lowlands and a great lake and presents a humid subtropical climate. According to the 2010 census, the population is approximately 1 million and 500 thousand inhabitants.

Until the last epidemiological bulletin of the CGVS, six cases of HVL had been diagnosed and of those four people died.[7] Of the six cases, five were diagnosed in the emergency room of the hospitals with diverse manifestations that led to suspicion of HVL. In all cases, the disease was confirmed by bone marrow aspiration for investigation of cytopenias. The sixth case was diagnosed in a primary health unit (UBS).

The first case of autochthonous HVL was a 1 year and 7 month-old female patient with no comorbidities. In September 2016, the patient with severe anemia and thrombocytopenia was seen at an emergency room with a history of episodes of intermittent fever that had started four months before without resolution. As the patient was suspected to have leukemia, she was referred to a hospital for further investigation. Based on the result bone marrow aspiration the patient was diagnosed with HVL and began treatment with liposomal amphotericin B, but died after two days. This situation led the Health Department of Porto Alegre to declare an epidemiological alert situation, and established a "Situation Room" with the mandate of analyzing the region where the patient lived, qualifying professionals and capturing vectors.

The second case had investigation initiated in February 2017, when the hospital notified the case after the result of bone marrow aspirate. The patient, a 43 year-old male, had a history of tuberculosis treatment and relapses, so it was believed that he was having another recurrence. After evidencing the parasites in the aspirate, treatment with liposomal amphotericin B was initiated, but the patient died within two days.

The third case of HVL occurred in May 2017. The patient was 82 year-old female, hypertensive and diabetic. She presented with a history of dizziness and inappetence 15 days before being admitted to the hospital with abdominal pain and a weight loss complaint. Leukemia was also suspected, leading to bone marrow aspiration, which confirmed the diagnosis of HVL. After the start of treatment, the patient died.

The fourth case was diagnosed in August 2017. The patient was a 1 year and 5 month-old female with no comorbidities. She had a history of 15 days of irritation, inappetence and intermittent fever of 39 °C, and was started on amoxicillin-clavulanate at the primary health center due to radiological diagnosis of bronchopneumonia. As the patient showed no improvement of his clinical condition and complained of abdominal pain, she was admitted to a secondary hospital in the city, where hepatosplenomegaly and severe pancytopenia (hemoglobin 6.8 g/dL, leucocytes 2690/mm^3, platelets 50,000/L). As a result, she was referred to a pediatric tertiary hospital for further investigation. A bone marrow aspirate was performed, which showed intracellular parasites characteristic of leishmania. The patient was treated with liposomal amphotericin B 4 mg/kg/day for five days, cefepime 150 mg/kg/day for pneumonia, and blood transfusion. The patient was discharged in better clinical condition nine days after hospitalization. Ambulatory follow-up showed blood count normalization 60 days after the end of treatment and normalization of hepatosplenomegaly four months after the end of treatment. The patient had no complication of the described infectious disease 10 months after hospital discharge and was in excellent general condition. In this case, the patient did not dye, emphasizing that after five days of treatment there was clinical and laboratory improvement.

It should be pointed out that, with the exception of the elderly patient, all had intermittent fever with for more than seven days. Hepatosplenomegaly and cytopenias were observed in all cases with ensuing severe opportunistic infections leading to death of these three patients. It is important to clarify that the elderly patient probably did not present the classic picture due to the physiological immunosenescence state due to aging, thus making diagnosis difficult.

The fifth case was two-year and eight months old girl who was admitted to a city hospital for cancer treatment. In her MO aspirate the amastigotes were found, and she died in October 2017.

Finally, the most recent case was a young adult who started with fever and looked for care in an emergency room where he received treatment for dengue and discharged. Nonetheless, his clinical condition deteriorated (tiredness, pallor, dizziness) and patient was examined in other emergency rooms in the region, where laboratory tests were performed that did not led to the correct diagnosis or suspicion of the disease. On the 13th day of fever, he was seen at a primary health center (UBS), where the disease was ultimately diagnosed and the patient transferred to a referral hospital, where he received specific treatment and had a favorable outcome.

Entomological study

It is known that HVL is an infectious disease transmitted to humans, to dogs and to other animals, both in urban and rural areas of the Americas, mainly by the bite of *Lutzomya longipalpis* females infected with *Leishmania infantum*. This disease was first diagnosed in dogs of the region in the year 2010, triggering an intense investigation by the vectors. According to data published in the Epidemiological Bulletin of the CGVS, in 2014, after evidence of another sick animal, new investigation was started in search of the vectors.[7] The sandflies identified were *Psathyromya lanei*, *Brumptomya* sp., *Pintomyia fischeri*, *Migonemya migonei*, and *Nyssomyia neivai*. After sending the samples to a reference research center, *Le. infantum* (PCR technique) was found in the females of *Pi. fischeri*, thus evidencing the importance of this phlebotomine in the transmission of the disease.

Thus, with the confirmation of the first case of LV in humans in September 2016, a new phase began aiming at capturing vectors involved in the transmission of LV, generating an intense collection of sandflies in the regions where the patients lived, within a radius of 50 m, using light baited traps (CDC type) in three consecutive nights per month between October 2016 and May 2017.

During this period, 437 phlebotomines of different species were found, and in the internal environment of the residences three species were captured: *Pi. Fischeri*, *Mg. migonei* and *Lu. gaminarai*, the latter reached 90.4% of the specimens detected in houses. In the peridomiciliary environment, there was a predominance of *Mg. migonei* (45.79%), followed by *Pi. Fischeri* (26.01%) and *Lu. gaminaria* (17.58%). It should be emphasized that in *Pi. Fischeri* and *Mg. migonei* species *Le. infantum* were found by PCR-multiplex technique.

Discussion

Diagnosis, symptomatology and warning signs

HVL is a serious and lethal zoonosis in 90% of cases. After being infected by the vector, the individual undergoes an incubation period that varies according to his/her immune status, between 10 days and 24 months. It should be underscored that not all infected patients will develop the disease. The Brazilian Ministry of Health recommends that the available diagnostic methods be used only in cases with compatible symptomatology, since persons residing in high prevalence areas may be asymptomatic despite being infected and do not require any treatment.[1,8]

According to the Ministry of Health[1] and the GCVS diagnostic flowchart,[9] every patient with a prolonged fever of at least seven days, and presence of hepatomegaly and/or splenomegaly should be notified and investigated if coming from an endemic area. There must be a previous discussion with the professional of the CGVS for further serological examination.

In the city of Porto Alegre, the rapid test is found in specific health units of each district health management, and patients are screened with the rapid immunochromatographic test rK39. In 2003, the Ministry of Health funded a multicenter study to validate the DiaMed-IT (IT-Leish) test that presented promising results. However, in 2009 the Ministry of Health acquired the rK39 test of Kalazar Detect®.[10,11]

In 2015, Kalazar Detect® was replaced by IT-Leish®, which was widely used by the Ministry of Health. It has as the advantage of using whole blood, unlike rK39 that uses the blood serum.[12] According to the Brazilian Ministry of Health,[12] studies conducted for evaluating IT-Leish® in Brazil showed 92–93% sensitivity and 95.6–97% specificity, better results than those obtained with Kalazar Detect®, 84.7–88.1% sensitivity and 90.6–96.8% specificity. It is emphasized in the flowchart[9] that the patient must be previously submitted to the rapid test to rule out infection by the Human Immunodeficiency Virus (HIV).[12,13]

The patient should be referred to a referral hospital, if possible, to perform bone marrow aspiration, which will evidence the presence or absence of the parasite. In the four cases reported only spinal aspirate was obtained, since there was no suspicion of HVL, and thus the rapid test rK39 was not performed. That means the flowchart was not "followed".

The symptomatology varies quite considerably, but as shown in the flowchart,[9] patients will have prolonged fever, increased liver and/or spleen, and cytopenias are common. Anemia is the most frequent and translated by a pallor appearance, fatigue, adynamia, dyspnea, and general malaise. Neutropenia can occur and lead to infections which should be treated early to avoid sepsis and, potentially, death. Other associated symptoms are: weight loss in recent months, chronic cough, diarrhea, abdominal pain, and edema (on lower limbs).

The cases presented herein were diagnosed with severe cytopenias, but five out of six had classic symptoms that should have raised the suspicion of leishmaniasis. We emphasize the clinical picture of the elderly patient, who, due to physiological immunosenescence, did not develop fever and hepatosplenomegaly, thus making diagnosis more challenging.

The fourth and sixth cases had a rapid evolution and presented all classic symptoms of the disease, but nonetheless there was a delay in suspecting HVL. It should be noted that the patients had a favorable prognosis as they had been diagnosed at an early stage of the disease, even after a long febrile period.

Alarm or severity signals are associated with increased lethality of the disease, which call for more aggressive therapy from the start. The Ministry of Health[12] carried out a systematic review in which the factors associated with higher risk of death in patients with HVL are categorized according to the level of importance as A, B, C and D, where A and B are evidence based on observational and experimental studies. Risk factors stratified as A and B are: age less than one year and over 40 years, associated bacterial infection, presence of diarrhea or vomiting, edema, fever for more than 60 days, jaundice, hemorrhagic episodes, signs of toxemia, comorbidities, platelet count $\leq$50,000/mm^3, hemoglobin level $\leq$ 6 g/dL and bilirubin above the reference values. The more criteria present, the worse the patient's prognosis.[12]

Treatment of human visceral leishmaniasis

Currently, few drugs are available for treating HVL. In Brazil, the available drugs are pentavalent antimoniate and amphotericin B (deoxycholate and liposomal). The choice of drug should take into account the age range, pregnancy, and presence of comorbidities. Pentavalent antimonial (Glucantime®) is the most used drug, since it has the advantage of being administered in the clinic, thus reducing the risks associated with hospitalization. However, this treatment has several contraindications and adverse effects, besides lasting for 30 days.

Amphotericin B is the most potent leishmanicidal drug available commercially, acting against promastigote and amastigote forms of the parasite. Currently, the Ministry of Health offers two presentations, amphotericin B deoxycholate and liposomal amphotericin B, the latter being less toxic.

According to the Ministry of Health guidelines,[14] liposomal amphotericin B has specific indications and should be requested in a separate form, as it is restricted only for patients with renal impairment or renal transplantation, over 50 years of age, or presenting either cardiac or hepatic abnormalities.[14]

The use of liposomal amphotericin B can be administered for a period of only 5–7 days according to the dose used and provides a faster response compared to other drugs. Thus, hospital length of stay is much shorter when compared to amphotericin B deoxycholate regimen, which should be administered for a period of 14–20 days.

The treatment of HVL is based not only on specific management with leishmanicidal drugs, but often the patient will require red blood cells transfusions and even platelets. In addition, the management of neutropenia and infectious foci is important for the patient's prognosis, with sepsis and febrile neutropenia being very frequent in this group of patients. In the case reported, the patient required red blood cell concentrate and antibiotic therapy due to respiratory infection, as well as the use of liposomal amphotericin B for five days, achieving a rapid improvement in clinical status.

After ending treatment and improvement of the clinical picture, the patient is discharged, but should be followed up, since relapses occur in 5% of cases in the first year after treatment, being more frequent in the first three months. The clinical picture of the patient improves progressively and slowly, cure criteria are essentially clinical, and early signs of response are usually nonspecific, such as improvement in appetite and overall health. Defervescence of fever occurs between the second and fifth day of treatment. Weight gain and reduction of spleen and liver volume can be seen in the first few weeks, although full regression may take a few months. Hematological parameters improve after the second week. Normalization of serum proteins is slow and may last for months.[14]

The Ministry of Health[1] recommends consultations for clinical control of the patient at 3, 6 and 12 months post-treatment. Only after this period without symptoms and laboratory abnormalities the patient is considered to be clinically cured. Survivors of HVL episode should be followed up for 12 months after their episode.

Etiologic agent, vectors, reservoirs and their implications

The etiological agents of HVL are trypanosomatid protozoans of the genus *Leishmania*, which are obligate intracellular parasites of cells of the mononuclear phagocytic system and can have two forms: flagellate or promastigote. The first can be found in the digestive tract of the insect vector, and the second, non-flagellate or amastigote, in tissues of vertebrate animals. The life cycle of the parasite in the insect takes around 72 h.[1]

The vectors of HVL are insects classified as sandflies, popularly known as mosquito-straw, birigui, tatuquiras etc. They are very small dipterans, measuring 4–5 mm, have composite eyes that takes a large part of the head, absent ocelli, long antennae, wings with lancearian shape and with parallel longitudinal veins, abdomen with 10 segments – that from the eighth onwards are modified to form the parts of the genital tract – and have sucking mouthparts, and thus are considered hematophagous. The biological cycle presents four stages of development: egg, larva (four stages), pupa, and adult. After copulation, the female lays eggs on humid substrates in soil with presence of a lot of organic matter, which under optimal conditions of temperature and humidity develop in approximately a week, resulting in larval hatching. The larva are vermiform, apodic and, after ecdysis, only move after the hardening of the chitin. Its development occurs around 20–30 days in decaying vegetable matter, tree holes filled with rotting leaves and in excrement found with vegetable remains and food in animal dens. The pupa phase takes 10–15 days, does not feed and can breathe. The whole cycle lasts around 30–40 days, resulting in hematophagous females and males that feed on vegetable sap. The activity of these insects is twilight and nocturnal. They have little ability to fly and do not seek food more than 200 m away. During the day, they are sheltered from the wind and from natural predators, at rest, in shady and humid places. Phlebotomines are found peri and in-house, close to food sources. The longevity of adult insects is 27 days.

From February to May 2017, the most prevalent species in Porto Alegre were *Mg. migonei*, *Lu. gaminarai* and *Pi. fischeri*, which present a high degree of anthropophilia and can be captured in the residual forests of the marginal areas of the cities, in shelters of domestic animals and internal walls of human households. The rampant urbanization, with residences near areas of forests, makes possible the development of these insects in these places.[7]

The reservoirs of VL in the urban area are dogs (*canis familiaris*), even though cases of cats and poultry infected with parasites have been detected. The high reproductive rate of these animals and the increasing abandonment facilitates the dispersion of the vector in the urban environment, generating ideal conditions for an epidemic of the disease. In the wild, the main reservoirs are foxes (*Dusicyon vetulus* and *Cerdocyon thous*) and marsupials (*Didelphis albiventris*).[13] The infected foxes were found in the Northeast, Southeast and Amazon regions, and marsupials were found in Brazil and Colombia.[1]

HVL is an immune-mediated disease, but when dogs and humans are compared, dogs present higher rates of morbidity

and mortality, and this can be explained by the greater parasite load in the skin, favoring vector infestation. The immune response of humans is greater and more effective when compared to that of dogs.[14]

Methods of prevention and control of the vector

The geographical distribution of Porto Alegre assists in the entomological and epidemiological study of VL, since it is possible to verify the endemic areas of disease transmission, to observe the type of vegetation, and the socioeconomic conditions of the population. In the six cases described in the municipality in 2017, the patients' residences were always in the last blocks of the streets, located in the middle of native forest, with sandy and humid soil, presence of several dogs and chickens, and garbage accumulation. The high social vulnerability of this population was also noted. Adding all these factors, the environment is optimal for proliferation and reproduction of the sand-fly. Based on these data, it is important to chart a series of vector prevention and control actions to reduce disease transmission.

The Municipal Health Department of Porto Alegre created a Municipal Plan for Intensification of the Actions of Surveillance and Control of Visceral Leishmaniasis, in which, firstly, the city was stratified, highlighting the risk areas for the disease, using as a parameter infected dogs and the presence of vectors, which were collected with the help of specific traps. The health teams had to learn how to approach and host the suspect patient, as well as to educate the population on ways to decrease vector spread.

Community agents and endemic disease control agents carried out active search visits to homes near the confirmed cases, to search for possible symptomatic patients. In addition, based on actions of the Sanitary Surveillance, at the time of blood collection (rapid test) of dogs residing 50 m of the patient's area the agents instructed the residents to always keep their patio clean, collecting garbage and animal waste. In the 200-m radius of confirmed cases, alpha-cypermethrin insecticide was applied in the peridomicile, in the external walls of the houses and in other external areas, and in the in-dwelling for the residents who accepted this measure, since they should return to their residences only 24 h after application.[7]

According to the Ministry of Health,[1] the population needs to follow individual protection methods, such as the use of thin mesh nets, door and window sizing, repellent use and no exposure during vector activity times. Environmental management issues such as yard hygiene, public lands and squares, proper disposal of organic solid waste, elimination of sources of moisture, and avoiding domestic animals are required.

Another important issue is preventive measures and care of domestic animals, as they are carriers of the disease. Treatment of positive dogs and the preventive vaccination are not recommended by the Ministry of Health because they do not make full coverage and the animal remains susceptible to contracting leishmaniasis. The best alternative is having dogs to wear collars, a simple action that can repel the phlebotomine.[15,16]

Final considerations

Health teams of the city of Porto Alegre should be trained considering that professionals are not aware of HVL. In the majority of the cases, professionals did not suspect of HVL in the differential diagnoses in febrile patients. It is also worth noting the significant lethality rate of HVL in our patients which deserves further investigation.

According to the diagnosed cases of HVL, it was possible to draw a profile of vector behavior, which was rural, but is becoming urban because people increasingly invade these regions of native forest to build their homes. Phlebotomine and the HVL parasite do not have a predilection for the human species for its development, but the lack of other hosts and the proximity of the population with forest environments end up facilitating the spread of the disease. Regarding dogs, the best form of prevention is still the nesting; and for humans, is the use of repellents, window-sizing and shrinkage of the vector breeding grounds.

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