



Metropolitan threat: cities like São Paulo may suffer outbreaks in upcoming years

A pre-announced disease

Lethal infection caused by a single cell parasite, visceral leishmaniasis spreads throughout Brazilian cities

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A highly lethal disease that attacks some 3,100 people a year in the country and kills in over 90% of the cases, if not properly treated, is reaching large Brazilian cities: visceral leishmaniasis. Caused by a single-cell parasite, the protozoan *Leishmania chagasi*, it settles within the body's defense cells and damages the spleen, the liver and the bone marrow. For a long time, visceral leishmaniasis was considered a solely forest-related issue or a disease restricted to Brazil's rural areas. Not anymore.

In the last three decades, health authorities have been identifying the first cases contracted in the cities, initially in the northeast. Since then, for reasons that are yet to be properly understood, visceral leishmaniasis has become urban and national, reaching cities in the North, Midwest and Southeast. It has already spread across medium-sized and large cities in 20 of Brazil's 26 states; only the South is free of the disease, as it risks encroaching upon cities such as Rio de Janeiro and São Paulo, which, much like fortified medieval towns, may be unable to contain the spread of leishmaniasis within the walls of buildings and houses.

Seventy years after being first described by physician Evandro Chagas, in a *Science* magazine article, as the cause of the new form of visceral leishmaniasis, different from the strain found in Europe and India, the parasite *Leishmania chagasi* and the insect that transmits it to humans in Brazil continue to challenge researchers and public health authorities. Throughout this period, the Brazilian population became urban – it was essentially rural until the beginning of the last century and migrated from one region of the country to the other in an ongoing quest for work. Today, eight out of every ten Brazilians are city dwellers. Thirty percent of the country's forests were consumed in order to harbor these cities. Forests are the natural habitat of the leishmaniasis parasite, found in animals such as the crab-eating fox (*Cerdocyon thous*) and the hoary fox (*Lycalopex vetulus*), as well as of its carrier, the insect *Lutzomyia longipalpis*.



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As a result, the disease spread and the number of cases increased. In 1985, Pará parasitologist Leônidas Deane, who was part of the commission headed by Chagas, counted 8,959 visceral leishmaniasis cases in Brazil since the first cases were identified by Henrique Penna in 1932. This situation has deteriorated. The Ministry of Health reported 53,480 cases from 1990 to 2007 – and 1,750 deaths. Visceral leishmaniasis is also more aggressive. In 2000, it killed three out of every one hundred people who contracted it. Now, seven out of one hundred die.

“There may be an epidemic outbreak in the city of São Paulo in the next five years,” says public health physician Carlos Henrique Nery Costa, from the Federal University of Piauí (UFPI). Costa states this based on his experience of almost 20 years in the study of visceral leishmaniasis transmission. He has thoroughly investigated the causes of the outbreak of the epidemic that has underscored the disease’s recent urbanization: the one thousand cases recorded in the city of Teresina between 1981 and 1985. This outburst was followed by another almost ten years later, with over 1,200 cases.

As the capital city of Pará was treating its ill and trying to understand the causes of the problem, distant cities such as São Luís, in Maranhão, Santarém, in Pará, Montes Claros, in Minas Gerais and Corumbá, in Mato Grosso do Sul also witnessed the rise of visceral leishmaniasis. “The disease appeared in these places as if out of nowhere, with no defined patterns,” says Costa.

The situation was different in the country’s Center-South region. Soon after the number of urban cases of visceral leishmaniasis increased in Corumbá, in the western portion of the Pantanal swamplands, in the state of Mato Grosso do Sul, on the border with Bolivia, the disease rapidly spread across the State towards the east. According to epidemiologist Suely Antonioli’s team, from the Jorge David



Lunch time: *Lutzomyia* feeds on blood

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Nasser Public Health School in Campo Grande, in an article published in 2007 in the *Journal of Infection*, by the end of the 1990s, the disease had already arrived in the capital city of Campo Grande and reached Três Lagoas, on the border with the State of São Paulo. Its advance followed the path of the Brazil-Bolivia pipeline, which follows the course of the Tietê river towards the city of São Paulo and highway BR-262. This links Corumbá to the State of Espírito Santo.

From Três Lagoas, it did not take long for the disease to cross the Paraná river and spread through the northwest of the State of São Paulo and finally, to the capital of the State. Since the identification of the insect in 1997, of the disease in dogs in 1998 and the first human case in the city of Araçatuba in 1999, visceral leishmaniasis has established itself in the State and has been silently spreading across it, along the Marechal Rondon highway (SP-300), the main connection between Mato Grosso do Sul and the city of São Paulo. For almost ten years, the Epidemic Outbreak Surveillance Center (CVE) of São Paulo reported 1,258 cases in 49 cities in the State of São Paulo – and 112 deaths.

“The disease has spread from the western region to the eastern region of

the State of São Paulo and may reach the capital,” says epidemiologist Vera Lucia Camargo-Neves, CVE researcher. In her assessment of the dispersion of visceral leishmaniasis, Vera Camargo found that the parasite migrates 30 kilometers nearer to São Paulo every year, transported by an insect only three millimeters long, with hairy legs and wings: the *Lutzomyia longipalpis*, commonly known as a sandfly.

Based on this information, the prediction that sooner or later the disease will reach the largest South American metropolis, where 19 million people live, is not as absurd as it may seem. Two years ago, the surveillance system identified a child with visceral leishmaniasis in the neighborhood of Vila Prudente. The case is still under investigation, its causes still being unknown, and it has not been broadly divulged by the Health Bureau.

It was not the first case. Two other cases were detected 30 years ago by Lygia Iversson, then a researcher from the Public Health School of the University of São Paulo (USP). In 1979, Lygia identified a person with visceral leishmaniasis in Diadema, in the Greater São Paulo. Two years before she had reported another infection, this time in a two-year old boy who had never left the city.

To date, all three cases remain unexplained, since the transmitting insect was not found in the 39 towns and cities of Greater São Paulo. However, in 2002, a transmission of *Leishmania chagasi* between dogs was recorded in the towns of Cotia and Embu. In these cases, other insect species were captured, of the *Lutzomyia* genera, hosts of the *Leishmania braziliensis* parasite, which causes the commonest and most mild form of the disease - cutaneous leishmaniasis, which leaves lesions and nasty-looking ulcers on the skin. “We suspect that the insect captured in the Greater São Paulo area is a species that only transmits leishmaniasis between dogs,” says epidemiologist Luiz Jacintho da Silva, Sucep superintendent when the first cases were detected in the State. He has

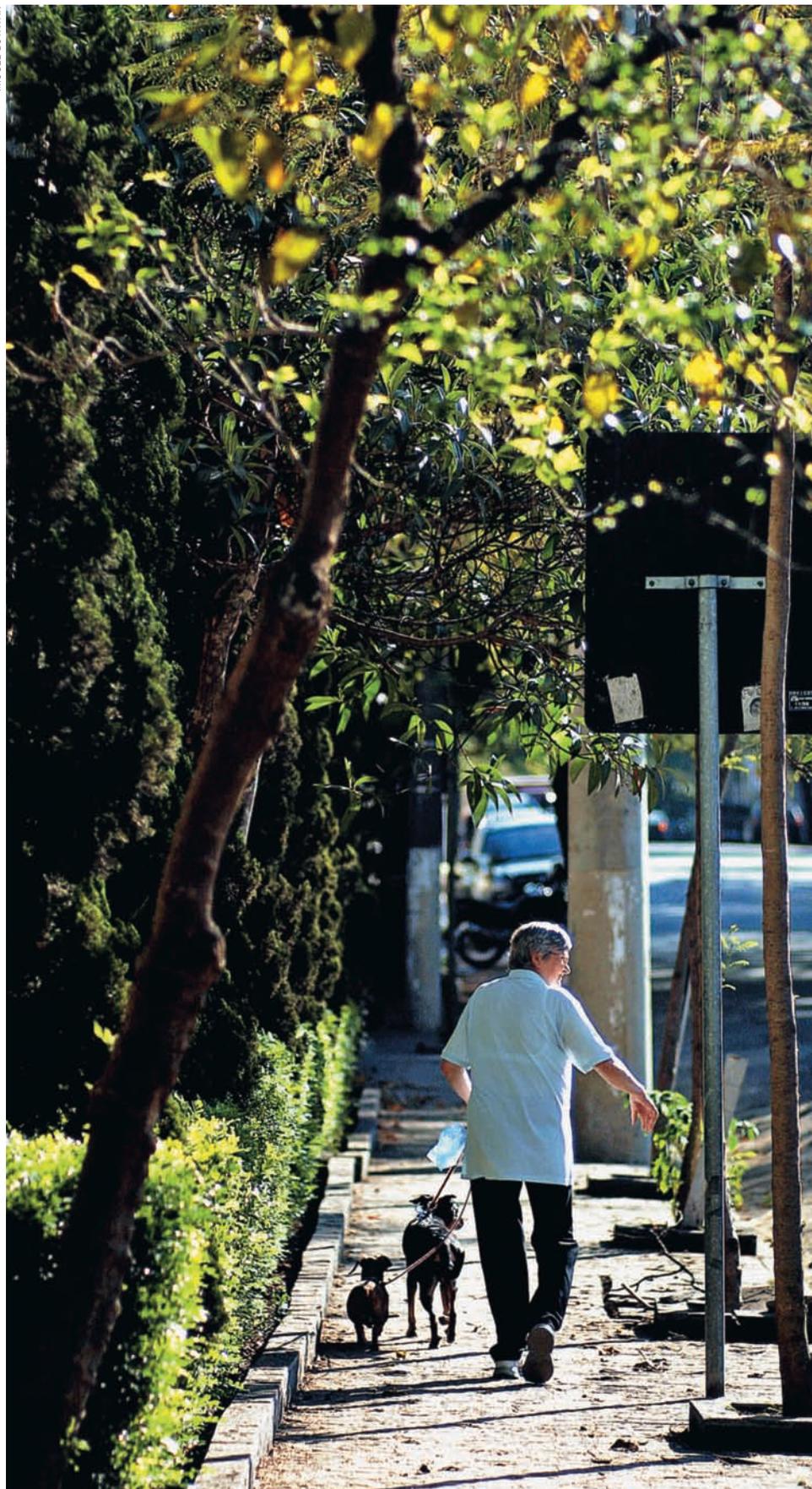
tracked the issue since. “We are unsure whether visceral leishmaniasis will get to the city of São Paulo,” he says.

Even if it does not reach the capital, the health authorities are concerned about the spread of the disease in medium-sized and large cities such as Bauru, in inner-state São Paulo, and Belo Horizonte, in Minas Gerais. What gives rise to such concern is that the larger the number of people in the region in which the parasite and its carrier are present, the larger the risk of contracting the disease. The three most important control methods adopted in the past fifty years – pesticides, the elimination of ill dogs or those suspected of being contaminated, and the treatment of human cases – have proven to be ineffective in containing the spread of the disease. “Visceral leishmaniasis kills about 200 people every year, more than malaria and dengue fever together, and it’s harder to control it than we’d imagined,” says Costa, of UFPI.

Researchers suspect that internal migrations, especially from the Northeast to the Southeast, have fuelled the spread of visceral leishmaniasis in the country. However, other factors may have contributed to this as well. British parasitologist Jeffrey Jon Shaw has lived in Brazil for the past 43 years and studies the life cycle of protozoa of the *Leishmania* genera and their transmitters. He believes that the insect carrying visceral leishmaniasis has adapted very well to cities. “We are creating favorable environments for the proliferation of the carrier, such as humidity and lots of food,” says Shaw, USP professor emeritus of and currently a researcher at the Fundação Tropical de Pesquisas e Tecnologia André Tosello (André Tosello Research and Technology Tropical Foundation), in Campinas.

It is still impossible to identify a spreading pattern for all the areas in the country. No one knows whether the insect populations that currently live in the outskirts of many cities already existed in these areas or whether they migrated from regions with better preserved vegetation. Shaw believes in both possibilities. “In Belo Horizonte, it is almost certain that there was a mosquito invasion in the suburbs; however, in

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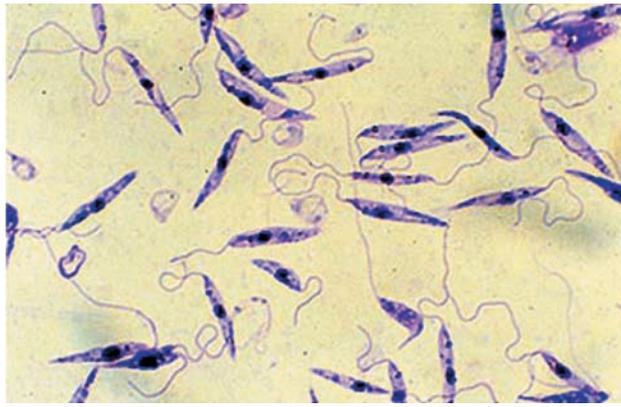
Close to danger: high dog population increases transmission risk

other states, it may be a result of the expansion of the populations that lived in the woods along the rivers,” says the parasitologist, who studies the population dynamics of *Lutzomyia* in São Paulo, Mato Grosso do Sul and Pernambuco.

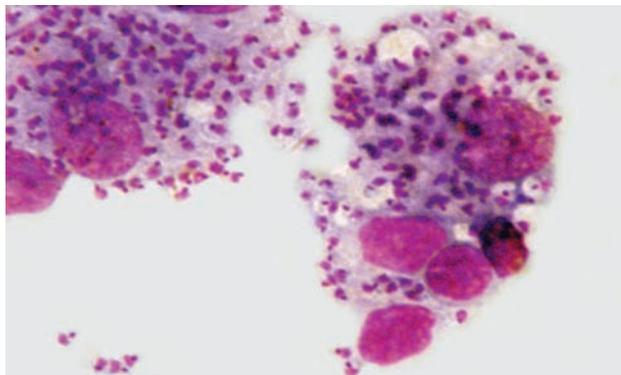
Costa, from UFPI, has a different suggestion. He believes the spread of the disease-carrying insect is linked to the use of exotic trees such as acacias, with their small leaves and yellow flowers, in the urban planning projects of the cities. There are reasons that support this suspicion. Acacias had been planted in Teresina at the time of the first outbreak, back in the 1980s. At that time, another devastating outbreak left 100 thousand dead in the Sudan, and affected especially the families living near acacias, possibly a source of nectar for the insects. There are also indications that the nectar of certain plants favors the proliferation of the parasites in the insects’ intestines.

It is still necessary to prove whether this in fact takes place in Brazil. However, it is certain that with the reduction of the natural vegetation areas, insects have adapted to parks and backyards, common in the inner-state areas. Unlike the dengue-transmitting mosquito (*Aedes aegypti*), which needs water in order to reproduce, the *Lutzomyia longipalpis* female lays its eggs on moist surfaces, such as rocks and leaves in contact with the ground. Once the eggs hatch, the larvae feed from organic material in the soil until they develop into adult insects. Once they have wings and the rest of their body is formed, adults feed on the nectar of plants and always land with open wings in moist and shady areas. In the early evening, the females seek the blood they need to lay their eggs. They engage in short flights, land, and bite uncovered body parts.

The bite is painful; the female makes a small incision in the skin and injects saliva and other substances that increase blood vessel diameter and that keep the blood from clotting. During its meal, the insect regurgitates forms



CÉLIA GONTIJO/CPQRR/FIOCRUZ



RAFAEL TEIXEIRA NETO/CPQRR/FIOCRUZ

Leishmania chagasi: form found in the insect (at the top); above form (dark dots) that attaches to macrophages

of the parasite that only reproduce in its digestive system. Once in the blood, the parasite profits from the defense system mechanism and hides before invading other cells and reproducing, according to recent findings.

David Sacks’ team, from the United States National Health Institutes, placed females of the *Phlebotomus duboscqi* insects hosting *Leishmania major*, capable of infecting laboratory animals, to feed on the ears of mice. The team followed the struggle against the parasites with a microscope that enables seeing images of live animal tissues. As soon as the mice’s immune system identified the invasion, the defense cells (neutrophils) moved towards the bite area. In just over one half-hour, the neutrophils had already involved most of the parasites and were trying to destroy them with digestive enzymes. Because they live for only a couple of hours, the neutrophils are then digested by a second group of defense cells, the macrophages – a sort of cleaning-up team.

Researchers observed that after the neutrophils died, live parasites approached the macrophages, cells to which they attach and in which they reproduce. In an article published on August 15 in *Science*, Sacks’ team referred to the strategy as a Trojan horse, in reference to the tactic of the Greeks to enter the walls of Troy, in the war narrated by Homer. Probably, this same disguise enables *Leishmania chagasi* to penetrate human and other mammal macrophages, hindering the defense system and causing the typical visceral leishmaniasis symptoms – recurrent fever that lasts for weeks, swollen liver and spleen, loss of appetite and weakness. “Physicians must be aware of these symptoms all over the country,” says Costa. “If the patient runs a fever for a long time for no apparent reason, is pale and the spleen is enlarged, a bone marrow

exam should be requested to eliminate the suspicion of leishmaniasis.”

From the public health point of view, the solution is to try to control the sandfly population with the deltamethrin pesticide, applying it to leishmaniasis focal points. However, this measure, currently under the responsibility of city councils, is not always effective. The pesticide lasts for three months and has to be applied to every single house wall, and the insects do not always die. Sometimes they only drop on the floor but are able to fly again later on. “We know of no application method for the pesticide to kill a larger number of the insects,” says Vera Camargo, of CVE.

The arrival of the sandfly in large cities involves yet another complication. The insect found an excellent source of blood that people like to keep by their side, plus the shade and fresh soil in backyards: dogs, which easily contract the infection and become as debilitated as their owners.

The Ministry of Health determines the elimination of infected dogs in order to contain the spread of leishmaniasis.

It is a controversial measure that is not sufficient if carried out alone. In several states, the dog population is high – there is one dog for every four people in São Paulo, while the World Health Organization suggests the ideal ratio is of one to ten, and the infection rate is as much as 20% of the animals in some cities. Moreover, owners are not prone to give up their dogs to be put down. “People only give up their dogs when they find out someone in the neighborhood died from visceral leishmaniasis,” says veterinarian Maria Cecília Luvizotto, from the State University of São Paulo (Unesp) in Araçatuba, who identified the first infected dog case in 1998.

Studies carried out in different cities indicate that about half of the dogs identified with leishmaniasis are eliminated. Veterinarians and animal protection groups criticize the strategy for diagnosis as exams can fail under certain circumstances. “We are unable to distinguish visceral leishmaniasis from cutaneous leishmaniasis if the dog has already been given the vaccine against the disease,” says parasitologist Célia Gontijo, from Fiocruz (the Oswaldo Cruz Foundation) in Belo Horizonte. “The test can still suggest the animal has leishmaniasis when, in fact, it may have other curable diseases, such as babesiosis.”

In an attempt to reduce the errors, Olindo Martins Filho and Renata Andrada, from the Fiocruz in Minas Gerais, developed a test that distinguishes the positive result caused by the infection from that caused by the vaccine, described in 2007 in *Veterinary Immunology and Immunopathology*. They are currently trying to use it to distinguish the visceral form from the cutaneous form. Even Célia obtained more precise results than the traditional tests by using the polymerase chain reaction (PCR), which identifies the parasite’s DNA.

Other groups test the use of dog collars with deltamethrin, which would keep the insects far from the dogs for months. The collar costs about R\$ 60 and has to be changed from time to time. In 2004, Richard Reithinger, from Fiocruz in Minas Gerais, compared the use of the collar with euthanasia. He showed that the collars provide a feasible alternative, if people use them correctly.

At the Federal University of Rio de Janeiro (UFRJ), Clarisa Palatnik de Sousa’s team developed a vaccine based on the parasite’s antigens and that has only been used in private clinics. In 2003, the vaccine was authorized by the Ministry of Agriculture – the Ministry of Health, responsible for leishmaniasis control, is yet to authorize its use as a mass protection method. The chief criticism of this vaccine is that it has only been tested in small groups of animals. The health authorities’ decision may now change with the publication of the most recent tests in the August issue of *Vaccine*. Clarisa monitored two groups of dogs for two years (550 had received the vaccine, and 588 had not) in Andradina, a town in inner-state São Paulo, where visceral leishmaniasis is endemic. The vaccine protected the animals in 99% of the cases.

Some experts regard the preventive vaccine as a measure to protect dogs, since the Ministry of Health prohibited the use of human medication to treat canine leishmaniasis in July. There are reasons for caution. Although they do improve clinically, dogs are not cured and can continue to transmit the parasite to insects that bite them. There is also the risk that the treatment promotes the selection of *Leishmania chagasi* strains resistant to human drugs, such as pentavalent antimonials, amphotericin B and pentamycin.

After decades with no new compounds for the treatment of humans, a study published in June in *Plos Neglected*

Tropical Diseases shows an important advance. At USP, parasitologists Silvia Uliana and Danilo Miguel proved that tamoxifen, a drug used to treat and prevent breast cancer, is effective against *Leishmania amazonensis* infection in rats. They are now getting ready to repeat the tests against *Leishmania chagasi* in hamsters, prior to evaluating the effects in a small number of patients. The advantage of tamoxifen over new drugs is that its action mechanism is already known and it has proved safe. “Still,” says Silvia, “three years of study are necessary”.

Among the compounds currently being tested against leishmaniasis, at least one was entirely developed in Brazil by the research network Farmabrasilis. The compound is P-MAPA, an acronym for protein magnesium ammonium phospholipoleate anhydride polymer. In tests conducted in Brazil and in the United States, it has proven to be effective against *Listeria monocytogenes* bacteria, whose survival mechanism in the organism is similar to that of the protozoa of the *Leishmania* genera. ■

➤ Scientific articles

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Evandro Chagas: case investigation in 1936, in the State of Pará

ARCHIVE OF CASA DE OSWALDO CRUZ/ARCHIVE AND DOCUMENTS DEPARTMENT /IMAGE: IOC (P) CHAGAS, E.S.