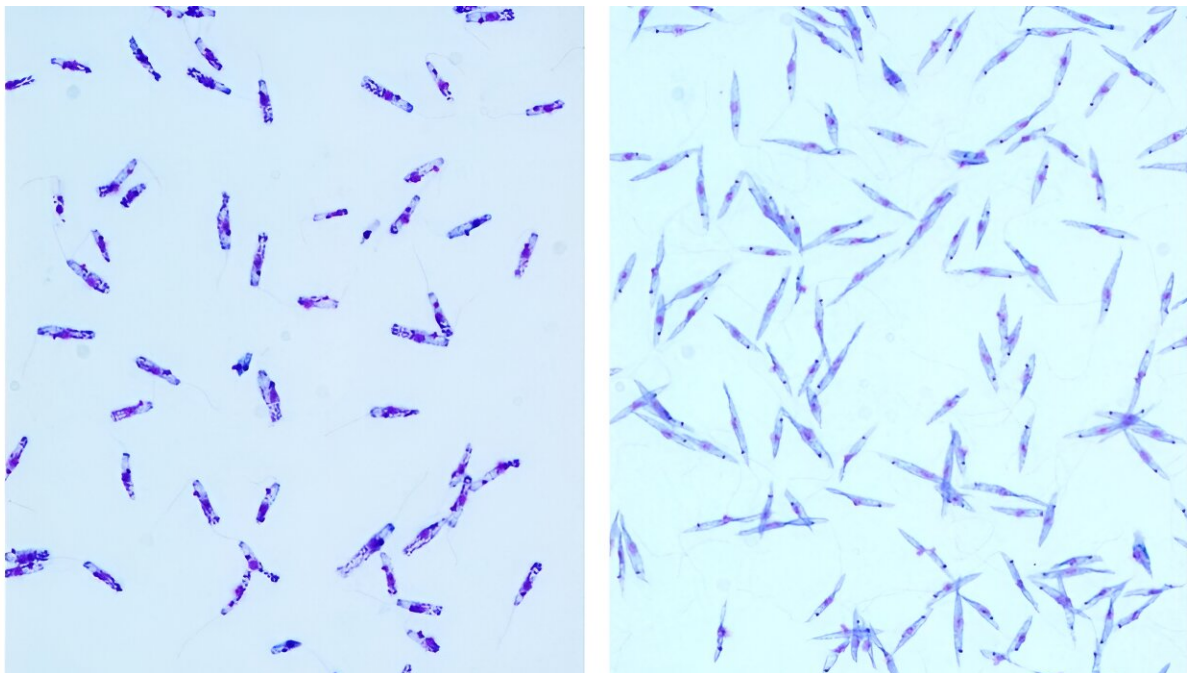


# Co-infection by novel species of parasite is confirmed in visceral leishmaniasis patient

August 8 2023, by Julia Moióli

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Left, culture of the Crithidia strain LVH60A; right, culture of Leishmania infantum. Credit: Luana Rogerio, UFSCar

The first case of concomitant visceral leishmaniasis and non-ulcerated cutaneous leishmaniasis in the same patient has been reported recently by researchers at the Federal University of São Carlos (UFSCar) in São Paulo state, Brazil, in an article published in the *International Journal of Infectious Diseases*.

The patient, a nine-year-old boy admitted to the hospital run by the Federal University of Sergipe (UFS), was infected by *Leishmania infantum*, a [protozoan parasite](#) that causes visceral leishmaniasis, and by another parasite of an as-yet unconfirmed species belonging to the genus *Crithidia* that causes symptoms similar to those of leishmaniasis, or even more severe symptoms in some cases.

In the Americas, Brazil is the country most affected by [visceral leishmaniasis](#) (VL), the most serious form of the disease, which can be lethal if it is not correctly treated or is misdiagnosed. The main agent of VL is *L. infantum*. Typical symptoms include weakness, weight loss, fever, an enlarged spleen and liver, and pancytopenia, i.e. low levels of all three blood cell types (red blood cells, white blood cells and platelets).

The advance of genomics in recent years has enabled researchers to find out that leishmaniasis patients can be co-infected with other genera of trypanosomatids in addition to *Leishmania*, such as *Leptomonas* and *Crithidia*, which in principle are not pathogenic to humans, or *Trypanosoma*, a genus of protozoan parasites that includes the agent of Chagas disease. Cases like these used to be associated with immunosuppression but are now seen in people with normal immune systems.

In 2019, the same group of researchers described a fatal case of a disease that was similar to VL but which they believed to be caused by a novel species of *Crithidia*, whereas *C. fasciculata* is known to infect only insects.

The report aroused controversy in the [scientific community](#). Species of *Leishmania* are dixenous: their life cycle unfolds in two hosts, first in the vector (sandflies) and then in a human or other mammal. *Crithidia* are considered monoxenous, surviving only in one kind of host—typically mosquitoes or other insects.

The main alternative explanation presented at the time was that it could be a case of co-infection. The patient might have been infected by both *Leishmania infantum* and *Crithidia*, which is easily cultured in the laboratory and, if grown together with *L. infantum*, eliminates it from the culture.

Co-infection was demonstrated in this latest study. Genome sequencing of bone marrow and spleen samples from the child hospitalized in Sergipe revealed the presence of both *L. infantum* and *Crithidia*. Furthermore, the child had a clinical manifestation not typical of VL, described as "non-ulcerated cutaneous leishmaniasis," with nodular skin lesions from which *L. infantum* parasites were isolated.

The patient, who was monitored from 2016 to 2020 at the university hospital in Sergipe, had several VL relapses that did not respond to the available treatments, with clinical complications and a rare cutaneous manifestation concomitant with the visceral disease. During the fourth period of hospitalization, he underwent removal of his spleen and of a probable parasite reservoir.

Genomic sequencing of the parasites isolated from the patient suggested they were not in fact *C. fasciculata*, but another species that has yet to be named. "These results point to a series of novel hypotheses regarding not only the genus *Crithidia*, but also leishmaniasis itself," said Sandra Regina Costa Maruyama, last author of the article.

Maruyama is a professor in UFSCar's program of graduate studies in evolutionary genetics and molecular biology, and a researcher in its young investigator program.

"Which parasite caused the first infection? Did the patient already have severe leishmaniasis, so that he was more susceptible to infection by *Crithidia*? Or was *Crithidia* responsible for the primary infection,

making the host more susceptible to infection by *L. infantum*?" Maruyama wondered.

"What about the vector for transmission of *Crithidia* to humans? Can the vector itself be co-infected by both parasites? We have many more questions than answers. It's important to study infections by *Crithidia* in vertebrates and identify the possible vectors."

Recent research in metagenomics of vector insects has shown that phlebotomine sandflies (the insects that transmit leishmaniasis) carry several microorganisms, such as viruses, bacteria and trypanosomatids, including *Crithidia*, Maruyama added.

## **Novel diagnostic target**

VL is usually diagnosed on the basis of clinical symptoms, although several of its symptoms are common to other infectious diseases. Epidemiological features also contribute to the diagnosis. The main strategy currently used by [public health services](#) in the SUS (Sistema Único de Saúde, Brazil's national health network) is a quick test to detect antibodies against *Leishmania* rather than the parasite itself.

This is practical but not as highly specific as molecular methods that directly detect the presence of the protozoan in the sample. More precise differentiation of *L. infantum* and the species of *Crithidia* during the study was therefore crucial. The researchers developed a molecular method specifically to detect these species. The recent article outlines the method, and more details will be provided in a forthcoming article.

"We managed to find the same species of *Crithidia* in samples from several other VL patients," Maruyama said.

## Public health

Besides investigating the possible new *Crithidia* species to confirm whether it is indeed an emerging parasite capable of having an impact in Brazil, the next step for the researchers will be to analyze the effects of co-infection, especially whether it aggravates VL.

"We know only a small proportion of patients who test positive for infection by *Leishmania* develop the disease. However, the tests are serological and the two types of parasite have very similar proteins, so it's impossible to distinguish between them in order to be sure which is the infecting species," Maruyama said.

The importance of finding out more and understanding the novel component is even greater in view of the advance of VL in Brazil. "VL used to be concentrated in the Northeast [the region where Sergipe is located], but cases of the disease are now reported even in the South. The situation is critical in Minas Gerais state. Experts have warned us about the advance of the disease in São Paulo state," she said.

**More information:** Luana Aparecida Rogerio et al, Co-infection of *Leishmania infantum* and a *Crithidia*-related species in a case of refractory relapsed visceral leishmaniasis with non-ulcerated cutaneous manifestation in Brazil, *International Journal of Infectious Diseases* (2023). [DOI: 10.1016/j.ijid.2023.05.012](https://doi.org/10.1016/j.ijid.2023.05.012)

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