Isolation of a protozoan parasite genetically related to the insect trypanosomatid *Herpetomonas samuelpessoa* from an HIV-positive patient

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ABSTRACT

Severely immunocompromised HIV-patients can develop various opportunistic infections due to bacteria, viruses, fungi, or protozoa. Here, we report the first isolation of a flagellated protozoan genetically closely related to Herpetomonas samuelpessoai, which is usually a parasite of insects, from the blood of a HIV-infected patient.
CASE REPORT

In November 2003, a 42-year-old man living in the south of France with no history of recent travel outside France was admitted to Montpellier University Hospital with a fever (38.9 °C) and left-side continual frontal headache that had persisted for 15 days. The patient was working as a florist, was homosexual, and had no history of drug abuse. He had been HIV-positive since 1991 and had experienced multiple antiretroviral therapy failures. His CD4 cell count had been below 20 cells/mm$^3$ for seven years. He had presented several opportunistic infections such as anal condyloma, zoster, and multiple episodes of oral candidiasis. At the time of admission, he was treated with abacavir, lamivudine, ritonavir, amprenavir, and cotrimoxazole.

Upon admission, physical examination was normal. No neurological signs nor primary infection site were noted. Cerebral tomodensitometry showed no abnormality. Laboratory findings revealed a moderate inflammatory syndrome (C-reactive protein, 18.8 mg/L) and pancytopenia: anemia (Hb=10.7 g/dL), neutropenia (White Blood Cell 0.9 x 10$^3$/mm$^3$ with 65 % neutrophils, 19 % lymphocytes, 11 % monocytes, and 3 % eosinophils), and thrombocytopenia (83 x 10$^3$/mm$^3$). Bone marrow biopsy performed on day four after admission showed a regenerative process. Taking into account the fever and pancytopenia, bacterial, viral, fungal, and parasitological investigations (Toxoplasma detection by PCR) were performed on the sputum, blood, cerebrospinal fluid, and bone marrow. All of these investigations were negative. Because leishmaniasis is endemic in the South of France, a blood sample was inoculated on Novy-MacNeal-Nicolle medium (NNN), a specific blood-enriched culture medium (6) at 27°C and checked weekly for the presence of Leishmania parasites. Four weeks after seeding, a flagellated protozoan was isolated and the Giemsa-stained preparations from this culture showed a cell body mean size of 10 µm X 2.5 µm and a flagellum mean size around 12 µm. The kinetoplast was generally located close to the nucleus in a para- or pro-mastigote position. The global morphology of the cells was clearly different from that of the Leishmania promastigotes (Figure 1).
Therefore, molecular identification was performed based on nucleotide sequence analysis of 5S and 18S ribosomal DNA regions. Amplification of the 5S region was performed using the previously described primer pair 5S-L and 5S-R, whereas amplification of the 18S region was performed using two primers designed for this study, SSUeuglenD (5’-GCGTGCGGTTAATTTGACT-3’) and SSUeuglenR (5’-GGACGTAATCGGCACAGTTT-3’) (12). PCR products were sequenced at MWG Biotech (Hedersberg, Germany). Comparison of the 5S rRNA amplified region (442 bp) to the GenBank database revealed a 92 % similarity to *Herpetomonas samuelpessoai* (X62331) and a 99.8 % similarity to *Herpetomonas* sp. Tom. Sp 3.13 (DQ441589). Comparison of the 18S rRNA amplified region (490 bp) revealed 99.8 % similarity to *H. samuelpessoai* (U0101) as shown in Figure 2. These molecular results suggest that the protozoan belongs to the genus *Herpetomonas* and is genetically close to *H. samuelpessoai*. Nucleotide sequences have been deposited in GenBank under accession numbers EU555432 and EU555433.

From a clinical point of view, the general condition of the patient improved spontaneously and rapidly during the weeks following admission, and he could be discharged from hospital. No antiparasitic treatment was initiated due to the late isolation of the parasite from the blood. Until now, no relapse has been noted.

Immunodeficiency resulting from HIV infection allows for the emergence of various opportunistic infections due to bacteria, viruses, fungi, or protozoa. During advanced stages of HIV-infection, due to the huge deficit in cellular immunity, human host immune responses are not sufficient to prevent the development of organisms usually considered to be non-pathogenic. Excluding human pathogens from the *Leishmania* genus, which are largely investigated during HIV infection, and the *Trypanosoma* genus, the
majority of trypanosomatid protozoa are parasites of plants or insects and are considered to be non-pathogenic for humans (1). So far, the potential implications of these currently non-pathogenic trypanosomatids in human infections remain unclear. Nevertheless, a few potential cases have been described in the literature, but questions remain due to the absence of an exact identification of the causative agents. Interestingly, most of these cases, excluding one, were described in HIV-infected patients (5, 7, 9, 11, 13). In this article, we describe the first isolation of a protozoan that is genetically closely related to a parasite of insects, *H. samuelpessoaai*, in an HIV-infected patient.

Considering that the patient’s condition improved spontaneously without antiparasitic treatment and the parasite was not isolated again during the months following discharge from hospital either from the bone marrow or blood cultured on NNN medium, one should consider the possibility of laboratory contamination of the sample. Such contamination could occur, for example, through deposition of feces by infected Hemiptera or Diptera insects, both of which are natural hosts of *Herpetomonas* parasites. However, this hypothesis is unlikely for several reasons: i) the sample was analyzed in mid-November when the population density of insects is strongly reduced; ii) samples were handled in a Biosafety Level 3 Lab (i.e. restricted access with airlock, directional airflow, biological safety cabinets, etc.). Hence, we assume that the recovery of the protozoa from the blood of our patient was not due to contamination, but indicates a true infection.

Additionally, we verified that the parasites were able to grow at 37°C, which is a requirement to develop in the human body. This surprising potential of these insect parasites to grow at 37°C without previous adaptation has been previously described, and suggests its ability to exist in these conditions (9).

To date, transmission routes to humans remain largely speculative. Most likely, humans could be infected through contact with feces of parasitized insects, as observed for the human pathogen *Trypanosoma cruzi* (3, 9, 11). In the present report, it could not be determined how our patient was infected, but, as he had no history of intravenous drug abuse, the hypothesis of contamination through needle sharing, as demonstrated for *Leishmania* parasites, is unlikely (4, 7). Finally, considering that *Herpetomonas* spp.
have also been isolated from plants, a possible explanation is that our patient could have been contaminated during his work as a florist (2, 8).

The prevalence of lower trypanosomatid infections in human is difficult to determine. Asymptomatic infections in immunocompromised patients or even healthy subjects could be more frequent than previously considered, mostly because recovery of trypanosomatids requires specific techniques that render their isolation difficult. Indeed, these parasites cannot be cultured using the traditional blood culture systems used for bacteria but require specific media such as the NNN medium. Moreover, when the parasite is isolated, identification at the genus/species level can be difficult using classical microscopic examination. Hence, molecular methods using pan-specific ribosomal RNAs targets are of particular interest.

To conclude, there is no doubt that the immunodeficiency resulting from HIV infection leads to the recovery of various eukaryotic microorganisms usually considered to be non-pathogenic in healthy individuals. This case highlights the need for physicians and microbiologists to be aware of these opportunistic pathogens that could infect immunocompromised subjects. We hope that further studies will help us to gain a better understanding of transmission routes, pathogenicity, and the clinical significance of these protozoa in humans.

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REFERENCES


**Figure 1:** Comparative microscopical morphology between W313 strain and Leishmania promastigotes (May-Grünwald Giemsa staining, X1000 magnification). W313 strain and *Leishmania infantum* reference strain (MHOM/FR/78/LEM75) were cultivated on Novy-MacNeal-Nicolle medium, mixed to a fresh blood sample and then spread on slides. Even though a morphological polymorphism is observed in culture, W313 strain promastigotes appear to be less slender, with the kinetoplast close to the nucleus compared to *Leishmania* parasites. Arrows are indicating the nucleus (N) and the kinetoplast (K). The scale bar represents 10µm.

**Figure 2:** Maximum likelihood tree showing the phylogenetic placement of the strain W313 near *Herpetomonas samuelpessoai*. Thirty-one kinetoplastid protozoa 18S rDNA sequences from a previously published alignment where aligned with that of strain W313 using Multalin software (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html) (10). The tree was built using PhyML software, under the GTR model of nucleotide substitution. Numbers given near each node represent the bootstrap values based on 100 replicates. The scale bar indicates 0.02 substitutions per nucleotide position.
Figure 1

Strain W313

L. infantum