INTRODUCTION. Leishmaniasis are zoonotic diseases caused by a protozoa of the genus *Leishmania*. Visceral leishmaniasis, caused by *L. chagasi* in the Americas, is the most severe form of the disease, according to the World Health Organization, and it is considered one of the seven greatest epidemics worldwide in terms of impact, affecting more than 68 countries and four continents, with estimated incidence of 500,000 new cases per year. Brazil is part of the group of countries with the highest prevalence of this disease, concentrating 90% of the cases registered in Latin America. In the state of São Paulo, more than 90% of autochthonous cases are concentrated in the Midwest region. Taking this into account, the regions of Marília, Bauru and Adamantina have been selected as the focus of this work, which aimed to develop a new molecular diagnostic method, based on the detection of a parasite single-copy gene by polymerase chain reaction (PCR), sensitive and specific for detection of *Leishmania* species in clinical samples from humans and dogs. MATERIAL AND METHODS. The primers have been designed for detection of *L. chagasi* and *L. braziliensis* and tested in clinical samples of human blood and bone marrow. All PCR amplified fragments were cloned in *E. coli* SURE strain and sequenced. RESULTS AND DISCUSSION. The developed molecular diagnostic method showed high specificity and sensitivity in clinical samples, not showing any amplification of other Trypanosomatidae not belonged to the target genus. Furthermore, it has been possible to perform a retrospective genomic-epidemiological study about visceral leishmaniasis through molecular characterization of the V7-V8 SSU rDNA gene region from some of human clinical samples from symptomatic patients. CONCLUSIONS. With these results, we intend to generate subsidies for epidemiological surveillance programs of visceral leishmaniasis in the Midwest region, as well as in the state of São Paulo.

Keywords: Leishmaniasis, *L. chagasi* and *L. braziliensis*