Leishmaniasis is a disease that affects people in tropical and subtropical regions of the world. In Brazil dogs are considered the main domestic reservoir of the protozoa *Leishmania infantum chagasi*, agent of visceral leishmaniasis (VL) in new World. The euthanasia of dogs positive for Leishmaniasis is one of the methods utilized to stop the dissemination of the parasite in Brazil. Ecto-nucleotidases from E-NTPDase family are ecto-enzymes that act as facilitators of infection and virulence factor in leishmania infection. *L. infantum chagasi* E-NTPDase-2, purified after heterologous expression in *E. coli* cells showed potential to be used in immunodiagnosis of canine VL (unpublished data). In the present study, we evaluated the use of anti-NTPDase-2 antiserum in the immunodiagnosis by immunohistochemistry as a method to confirm canine VL diagnosis. In this work we used lymph nodes from 48 dogs from the endemic region of Governador Valadares, MG, Brazil. This group of dogs was previously diagnosed as positive for leishmaniasis (indirect immunofluorescence-Biomanguinhos Kit). The lymph node samples were included in paraffin and processed by indirect immunoperoxidase technique (IIP). A polyclonal and mono specific antiserum produced in rabbit after immunization with the recombinant E-NTPDase-2 was used for recognition of parasite. The results showed the presence of E-NTPDase-2 in free and intracellular amastigote forms in the lymph nodes, and confirm for the first time the expression of E-NTPDase-2 in this parasite form. In conclusion, these data corroborate with the potential use of this protein as target in the diagnosis of Leishmaniasis.