PRESENCE OF *TRYPANOSOMA CRUZI* IN THE ANAL GLANDS OF NATURALLY INFECTED OPOSSUM (*DIDELPHIS MARSUPIALIS*) IN THE STATE OF SANTA CATARINA, BRAZIL

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Opossums (*Didelphis* sp) are sylvatic reservoirs of *T. cruzi* normally presenting high infection rates. Although parasites can be easily detected in the blood of naturally infected opossums through repeated fresh blood examinations, xenodiagnosis and haemoculture, intracellular forms (amastigotes) in tissues are extremely difficult to find (Barretto et al., 1964, *Rev. Brasil. Biol.*, 24: 298-300).

However, abundant parasites were found in the lumen of the anal glands of a high proportion of laboratory reared opossums subcutaneously inoculated with *T. cruzi* strains G-N and G-49 of opossum origin; the parasites were epimastigotes and metacyclic trypomastigotes and the material proved to be infective to mice and opossums by the subcutaneous and oral routes (Deane et al., 1984, *Mem. Inst. Oswaldo Cruz*, 79: 513-515; Deane et al., 1986, *Parasitology Today*, 12: 146-147 and Lenzi et al., 1984. XI Reunião Anual Sobre Pesquisa Básica em Doença de Doença de Chagas, Caxambu, BI:44). Later (Deane & Jansen, 1986a, *Mem. Inst. Oswaldo Cruz*, 81: 131-132; 1986b, *Mem. Inst. Oswaldo Cruz*, 81 Suppl. BI:26) another trypansoma – *T. (Megatrypanum) freitasi*, was reported multiplying as epimastigotes in the anal glands of an opossum with natural infection by this trypansome. The anal glands were negative among a number of opossums with a natural infection by *T. cruzi* (Deane, personal communication).

These findings prompted us to investigate the presence of *T. cruzi* in the anal glands of opossums. In Santa Catarina Island parasites were not found in the anal glands of 13 opossums naturally infected with *T. cruzi* (Steindel et al., 1986, *Mem. Inst. Oswaldo Cruz*, 81 Suppl. Bi:28).

Two previously negative opossums were subcutaneously inoculated with metacyclic triatomine forms of *T. cruzi* SC-42 strain originated from *D. marsupialis*; infection was demonstrated by fresh blood examination, xenodiagnosis and haemoculture. Repeated fresh examination of anal glands secretion obtained by manual compression were always negative, but one animal killed on the 70th day after infection, presented epimastigotes and trypomastigotes in the lumen of those glands while the other one killed on the 120th day, was negative.

Afterwards 13 *D. marsupialis* from Arvoredo Island about 11 km from Santa Catarina coast were examined and 9 of them had the parasites in the peripheral blood; in one of these the parasite was also detected in the anal glands. Thus 4.5% out of a total 22 opossums naturally infected with *T. cruzi* had the parasite in the anal glands. The opossum with positive glands, a female weighing 1320 g, was maintained for 5 days in captivity and submitted to blood examinations, xenodiagnosis and haemoculture, all positive for *T. cruzi*. Twice the material obtained by manual compression of the glands was negative in fresh preparations. The animal was killed and in the secretion obtained by punction large numbers of epimastigotes and metacyclic trypomastigotes free in the gland's lumen were observed in fresh and Giemsa stained preparations (Fig.). The parasites were similar to the forms found in triatomine feces. This material was cultivated in LIT (Liver Infusion Triptose) medium and inoculated in six albine mice. The culture was positive and the mice fresh blood examination carried out up to 60 days after inoculation, were negative;

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however the xenodiagnosis was positive for *T. cruzi* for all mice. Reinoculation of the mice with the virulent Y strain on the 65th day indicated that a high degree of protection had been conferred by the first inoculation.

Histological sections of the anal glands stained by the hematoxylin and eosin revealed the presence of a large number of epimastigotes and metacyclic forms only in the gland lumen and in the intervillosity spaces. No amastigotes nests were found in sections of the heart, liver, spleen, oesophagus, intestine and skeletal muscle.

Our results showed, for the first time, that *T. cruzi* parasitism of opossum anal glands may occur in naturally infected animals. These findings support previous suggestions (Deane et al., 1986. *Parasitology Today*, 12: 146-147) that this may be a new mechanism of transmission of *T. cruzi* to man and other mammals, without interference of insect vector, and that this mechanism may be responsible for epi-
demic outbreaks of Chagas' disease in non-endemic areas, such as occurred in Nova Teotônia, Rio Grande do Sul State (Silva et al., 1968, Rev. Inst. Med. Trop. São Paulo, 10: 256-276) where no triatominine bugs were detected and simultaneous infection of several people by the oral route was suspected. Infection of mice by ingestion of food contaminated with parasites from opossum's anal glands has been proved (Jansen & Deane, 1985, XII Reunião Anual Sobre Pesquisa Básica em Doença de Chagas, Caxambu, BI:09). In areas where triatominine bugs are sparse this mechanism may be particularly important in the maintenance of high infection rates by T. cruzi among opossums.

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