

HUMORAL IMMUNE RESPONSE IN DOGS EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI*

M. LANA; L. M. VIEIRA; G. L. L. MACHADO-COELHO*; E. CHIARI**;
V. M. VELOSO & W. L. TAFURI***

Departamento de Análises Clínicas, Escola de Farmácia, Universidade Federal de Ouro Preto,
Rua Costa Sena 171, 35400 Ouro Preto, MG, Brasil *Depto Biologia, ICB, UNIFENAS **Depto de Parasitologia,
ICB, UFMG ***Depto Ciências Biológicas, ICEB, UFOP, Ouro Preto, MG, Brasil

The present study shows for the first time the serological profile of IgM and IgG in dogs experimentally infected with *Trypanosoma cruzi* evaluated for 30 months. Eleven outbred young dogs, approximately two-month old, were inoculated by the conjunctival route with 2,000 metacyclic trypomastigotes obtained from triatomids (*Dipetalogaster maximus*) previously fed on infected albino mice. Seven dogs were infected with Be-62 *T. cruzi* strain isolated by J. A. Salgado (1962, *Rev. Inst. Med. trop. São Paulo*, 4: 330-337) and four with Be-78 *T. cruzi* strain studied by M. Lana & C. A. Chiari (1986, *Mem. Inst. Oswaldo Cruz*, 81: 247-253). Both strains were isolated from a patient considered to be the first identified human case of Chagas' disease described by C. Chagas (1909, *Mem. Inst. Oswaldo Cruz*, 1: 159-218). Sera were collected weekly during the first 70 days post-infection and every three months for 30 months, then stored at -20°C until tested. IgM and IgG levels were determined by indirect immunofluorescent (IIF) test and enzyme-linked immunosorbent assay (ELISA). Antigens were obtained from Y *T. cruzi* strain cultivated in LIT medium up to exponential phase of growth. The flagellates were treated with 2% formalin (IIF) or 0.15M NaOH (ELISA). Purified dog immunoglobulins (IgM and IgG classes) were purchased from CAPPEL laboratories, USA. The conjugates anti-dog-immunoglobulins were made in the Department of Parasitology, Federal University of Minas Gerais and labelled with fluorescein (Sigma Co.) for IIF and peroxidase (Sigma Co.) for ELISA test. IIF were performed in sera serially two-fold diluted starting at 1:10 dilution. ELISA tests were performed in sera using 1:320 dilutions and the quantification was done by

optical density at 492 nm. For statistical purposes serum titers (x) and absorbance (x) were transformed into $\log_{10} [(1000)(x) + 1]$ (Fig.). To compare the levels of IgM and IgG in each test, analysis of variance in split-plot design was used, where the immunoglobulins were considered main plot treatment, while the time of observation (weeks and months) were considered subplot treatments.

Significant increased levels of IgM and IgG ($p < 0.05$) were observed in IIF test from the beginning of infection until the third month. This phase of the infection is coincident with patent parasitemia, detected between 14 and 55 days of infection, electrocardiograph alterations and clinical signs commonly observed by F. S. Laranja et al. (1948, *Mem. Inst. Oswaldo Cruz*, 46: 473-529) in human Chagas' disease. IgM began to fall significantly ($p < 0.05$) after three months, but the opposite occurred with IgG which increased up to the 15th month (1:1076), then decreased progressively until two years and tended to stabilize with titres varying around 1:80. ELISA test was more sensitive than IIF in detecting higher levels of IgM and IgG up to two years of infection when only IgM begin to fall significantly ($p < 0.05$). During the period of study a correlation between antibody concentration, parasitemia, clinical signs and *T. cruzi* strain used was not observed.

Similar observations were reported in rabbits by L. E. Ramirez (1984, Thesis, Depto. Parasitologia, UFMG, 186 p.), in monkeys by S. K. K. Seah et al. (1974, *Trans. R. Soc. Trop. Med. Hyg.*, 68: 63-69) and in sporadic examination in human as described by G. A. Maekelt (1973, *Arch. Venez. Med. Trop.*, 5: 117-128) who also evaluated parasitological, clinical and serological aspects. The serological profile in dogs is similar to that observed by those authors

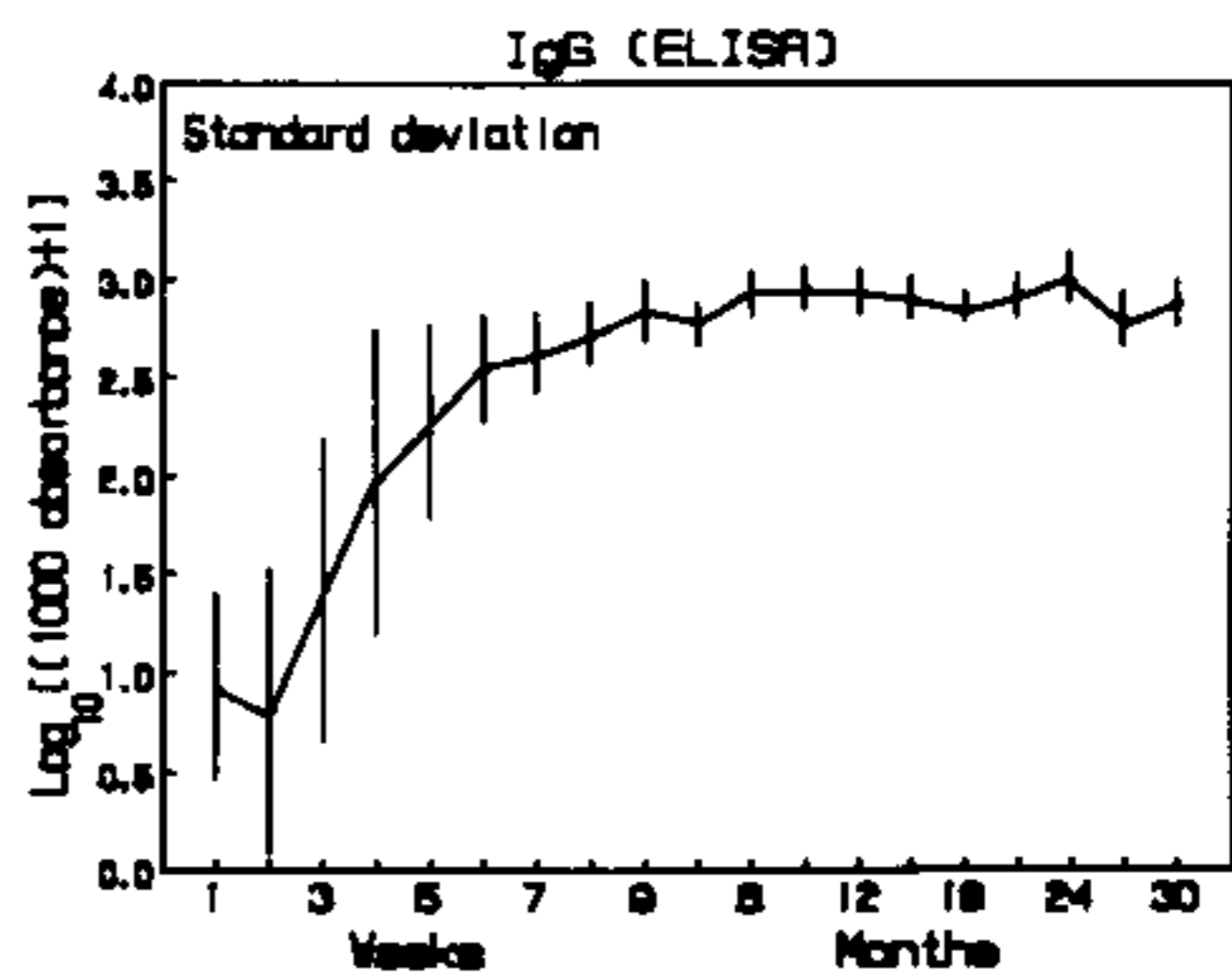
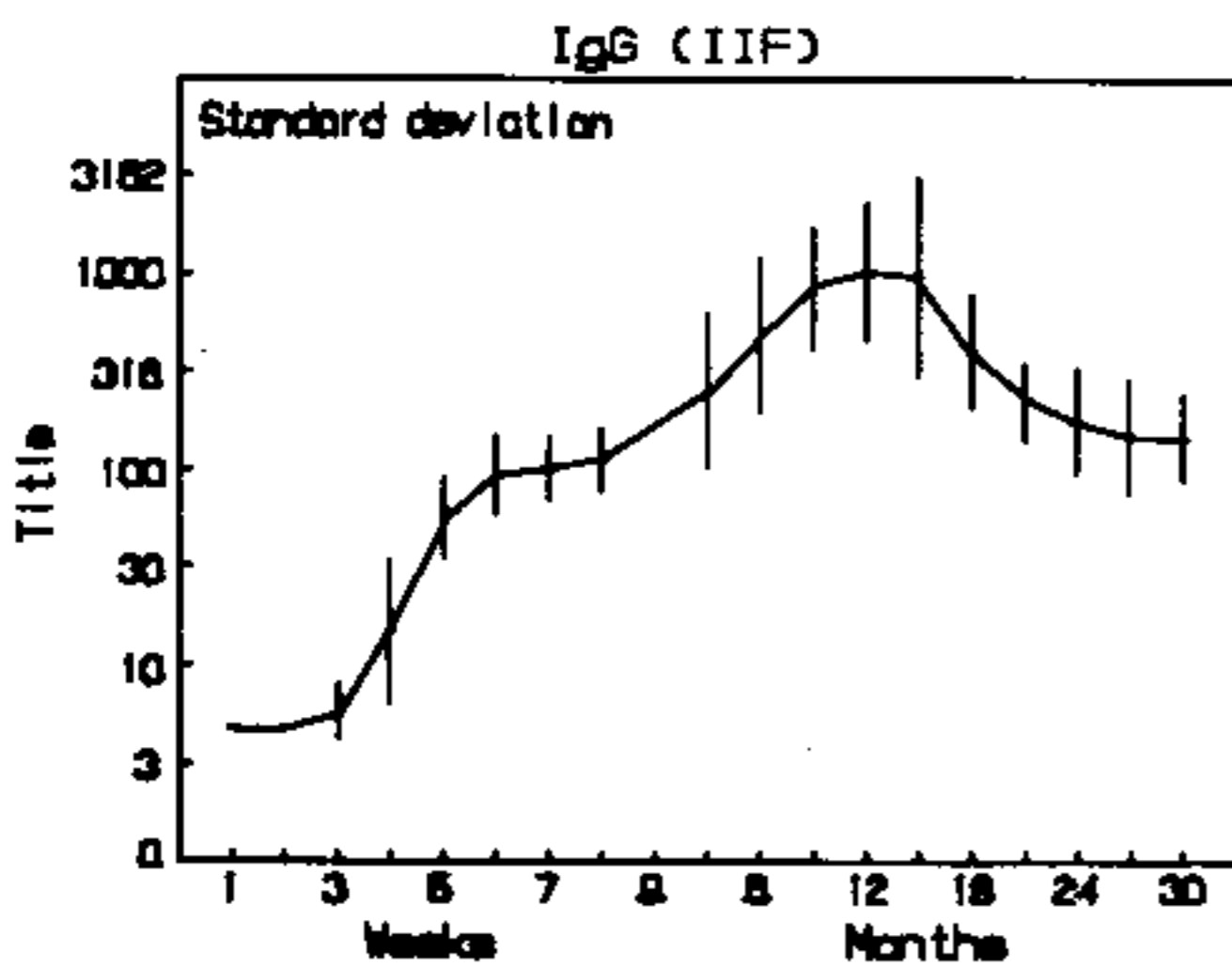
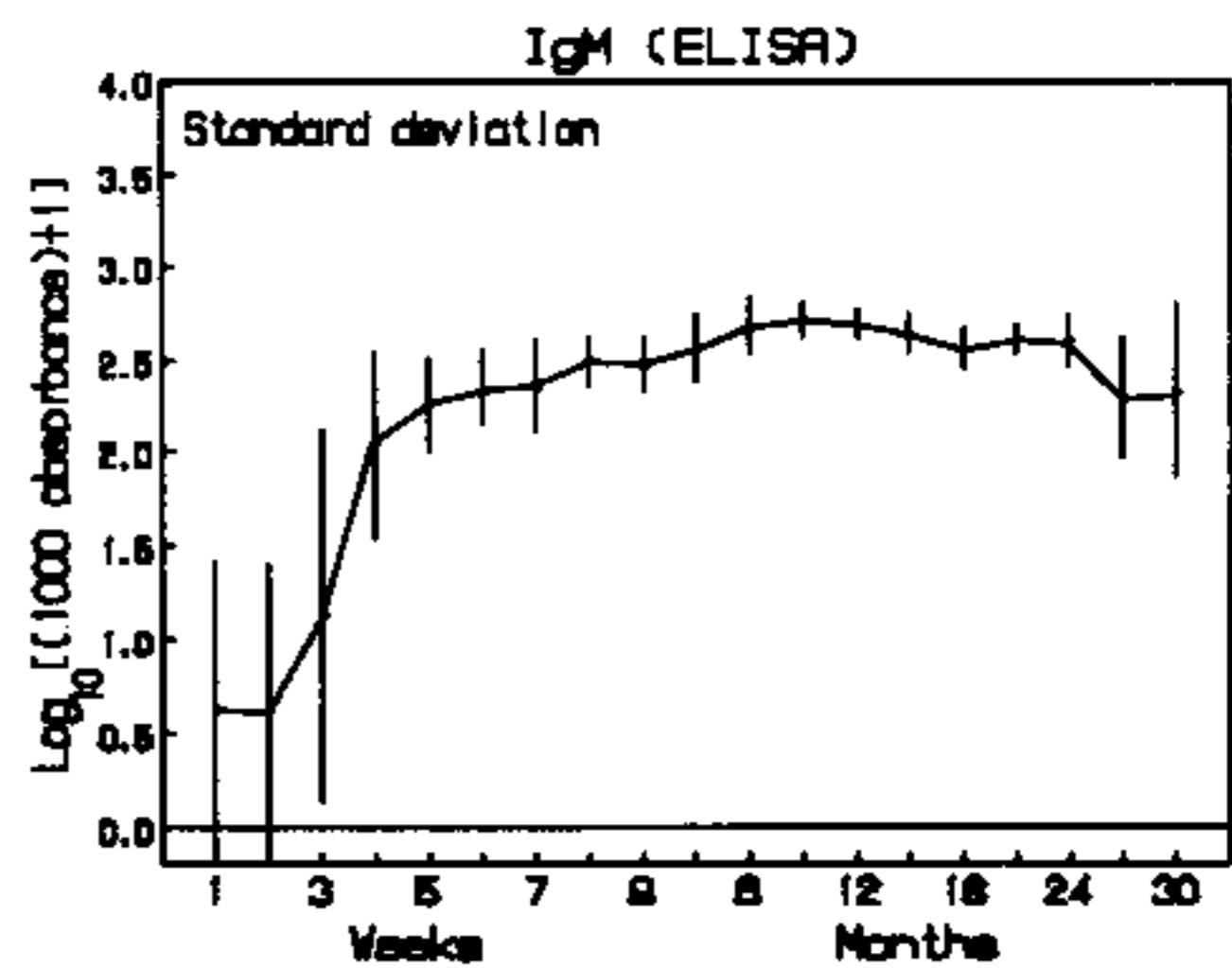
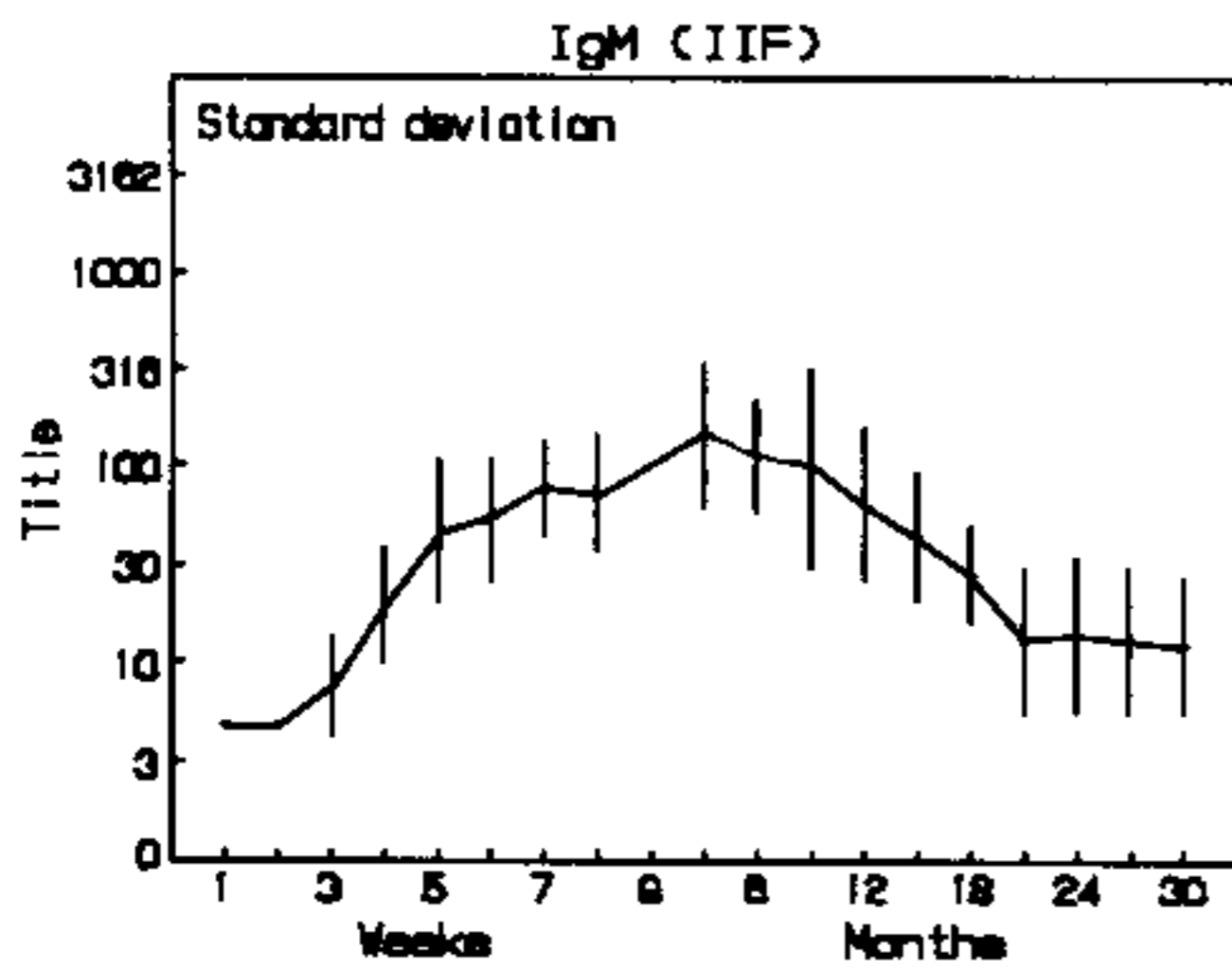
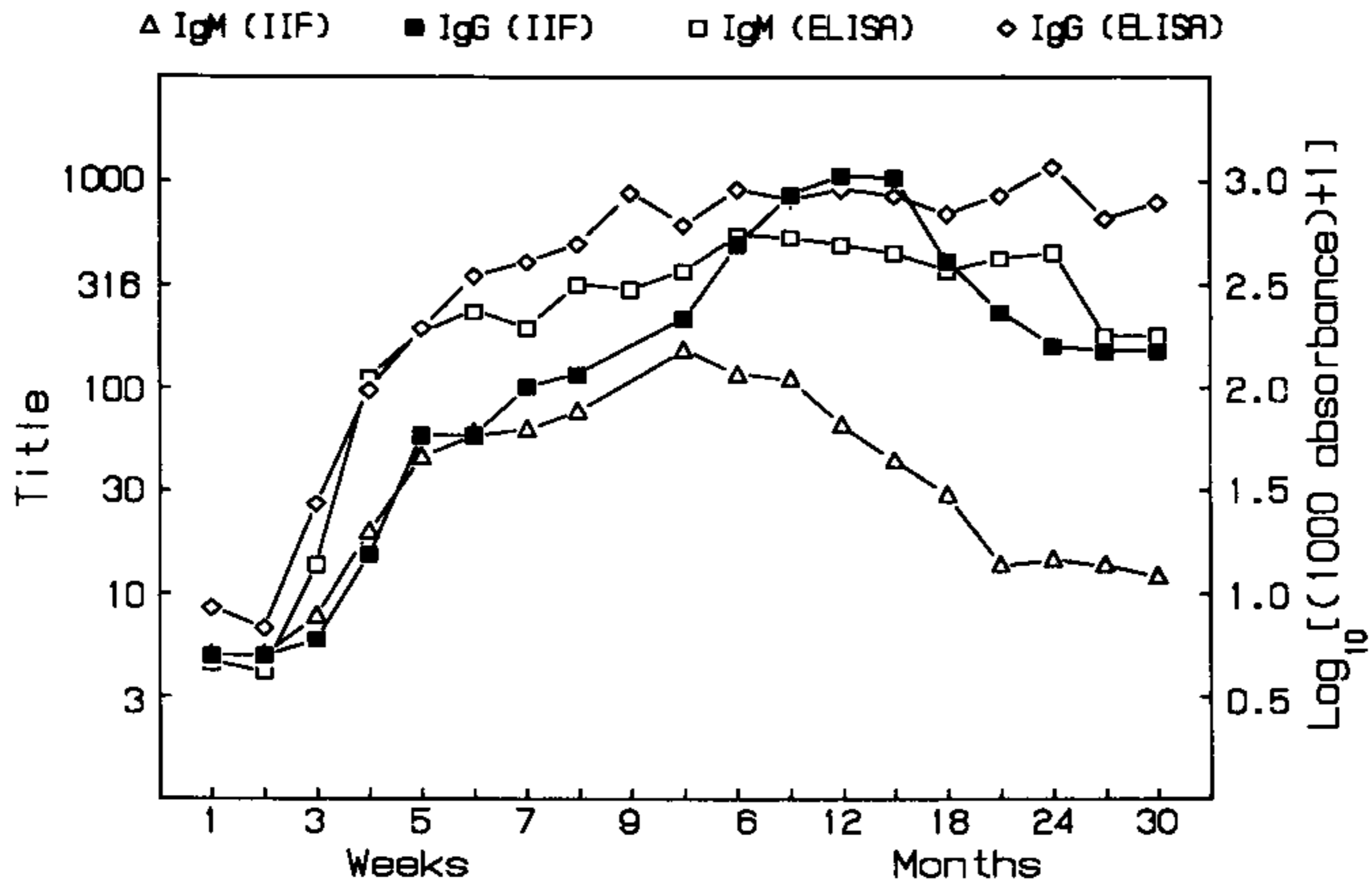


Fig. Immunoglobulin profiles in dogs experimentally infected with *Trypanosoma cruzi* and their respective standard deviations.

cited above using experimental models. The present study may contribute to a better comprehension of the immunological events during the infection with *T. cruzi* together with other clinical and pathological aspects of Chagas' disease in dogs. Although a pure and complete profile of human IgM and IgG without interference of treatment has not been well determined, authors agree that the concentration of IgM is higher in recent infections (acute phase) and absent or sporadically present in the later phases of the disease as described by

R. Lelchuck et al. (1970, *Clin. Exp. Immunol.*, 6: 547-555) and N. H. Vattuone et al. (1973, *J. Trop. Med. Hyg.*, 76: 45-47). IgG is always present, at higher levels in the first months of infection and at lower levels in the chronic phase of the disease as described by G. A. Maekelt (1973, *Arch. Venez. Med. Trop.*, 5: 117-128).

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