

## STANDARDIZATION OF SEROLOGICAL TESTS FOR DETECTING ANTI-*TRYPANOSOMA CRUZI* ANTIBODIES IN DOGS

M. A. LAURICELLA; C. WISNIVESKY-COLLI\*; R. GÜRTLER\*; R. PETERSEN\*;  
M. BUJAS\*\* & E. L. SEGURA

Instituto Nacional de Chagas "Dr M. Fatała Chabén", Departamento de Investigación, Av. Paseo Colón 568, 1063, Buenos Aires, Argentina \*Unidad Ecología de Reservorios y Vectores de Parásitos, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires, Buenos Aires, Argentina \*\*Hospital Italiano, Servicio de Hemoterapia, Gascón 450, Buenos Aires, Argentina

*This paper reports on the standardization of four serological reactions currently used in human serodiagnosis for the detection of anti-*Trypanosoma cruzi* antibodies in naturally and experimentally infected dogs. Indirect immunofluorescence test (IFAT) and hemagglutination test (IHAT) were standardized, and complement fixation test (CFT) and direct agglutination test (DAT) were used for diagnostic confirmation.*

*Four hundred and eighty one mongrel dogs that were studied by xenodiagnosis were used: (1) parasitemic dogs of two localities of endemic area (EA) of Santiago del Estero province in Argentina (n = 134); (2) non-parasitemic dogs of the same area (n = 285); (3) dogs experimentally infected with *T. cruzi* in the patent period (n = 6); (4) non-infected dogs (n = 56) which were born in the city of Buenos Aires (BA), one non-EA for Chagas' disease.*

*For IFAT, parasitemic dogs EA showed 95% of reactive sera. Non parasitemic dogs EA showed 77% of non reactive sera. None sera from BA were reactive for dilutions higher than four.*

*For IHAT, 84% of sera of parasitemic dogs EA showed serological reactivity and among non parasitemic dogs BA, 61% were non reactive, while the remainder showed at most titres of 1/16.*

*The cut-off titres for IFAT and IHAT were 1/16 and 1/32 respectively, and for CFT and DAT 1/1 and 1/128 respectively.*

*Sensitivity for IFAT, IHAT, CF and DAT were 95%, 84%, 97% and 95% respectively.*

Key words: Chagas' disease - serology - dogs - experimental models

Dogs have been mentioned as important hosts of *Trypanosoma cruzi* in most American countries (reviewed by Barrett et al., 1979) and characterized as the main domestic reservoir of *T. cruzi* in Argentina (Wisnivesky-Colli et al., 1985) and as they are amplifying hosts of domestic transmission it has been proposed to use them as sentinels in controlled areas (Gürtler et al., 1987).

Standardized immunodiagnostic tests have

been developed for humans (Cerisola & Rosenbaum, 1958; Cerisola et al., 1962) however they can not be applied to animal sera as such. Due to the need to perform studies of canine populations in different fields - epidemiology, immunology, chemotherapy - adjustments in those serological techniques must be done.

Several workers have applied different serological techniques to dog sera (Lucena, 1957; Burkholder et al., 1980; Correa et al., 1982). The complement fixation test was the first one used for naturally (Freitas et al., 1952; Lucena, 1957; Serravalle, 1963) or experimentally in-

infected dogs (Andrade & Andrade, 1980). More recently, indirect hemagglutination (Burkholder et al., 1980), direct agglutination test (Tomlinson et al., 1981), indirect immunofluorescence and ELISA (Lana et al., 1992) were used in epidemiological surveys or with experimental models. In all these studies, however, no correlation of serological results with direct parasitological methods (xenodiagnosis or/and hemoculture) was made with only one exception (Freitas et al., 1952), and most of them did not include either negative or positive sera as control.

This paper reports on the standardization of tests to detect ant-*T. cruzi* antibodies in naturally and experimentally infected dogs with parasitemia detected by xenodiagnosis.

#### MATERIALS AND METHODS

Four hundred and eighty one mongrel dogs were used. Animals belonged to four groups: (1) 134 parasitemic dogs of two localities of endemic area (EA) of Santiago del Estero province in Argentina: namely, 67 parasitemic dogs from Amamá (Department of Moreno) and 67 parasitemic dogs from Termas (Department of Rio Hondo); (2) 285 non-parasitemic dogs, 21 of Amamá and 264 of Termas; (3) 6 dogs experimentally infected with *T. cruzi* in the patent period (Lauricella et al., 1986); (4) 56 non-infected dogs which had born to and were dwelling in the city of Buenos Aires (BA), one non-endemic area for Chagas' disease. All of them were studied by xenodiagnosis (Wisnivesky-Colli et al., 1985) to determine the presence of parasitemia.

Basing on clinical examination dogs from group 4 were classified in two sets: 52 apparently healthy ones and 4 diseased animals. These carried canine distemper (2), leptospirosis (1) and Carre's disease (1).

The general description of both endemic areas have already been reported (Gurtler et al., 1987; Lauricella et al., 1989).

Dog sera from groups 1 and 2 were collected at the field between 1982-1986, and they were used to determine diagnostic titre and sensitivity. Experimentally infected dogs and 28 of non parasitemic BA dogs (4) were used as positive and negative controls respectively

to determine sensitivity and cut off titre. The other 28 healthy non parasitemic dogs from BA were used to determine specificity of IHAT and IFAT.

Four serological reactions currently used in human serodiagnosis were adapted to dog sera: indirect immunofluorescence test (IFAT) (Alvarez et al., 1968), hemagglutination test (IHAT) (Cerisola et al., 1962), complement fixation test (CFT) (Cerisola & Rosenbaum, 1958) and direct agglutination test (DAT) (Vattuone & Yanovsky, 1971).

DAT and IHAT were performed using commercial kits (Polychaco SAIC, Buenos Aires, Argentina) following the procedures detailed by the maker. Rabbit anti dog gamma globuline labelled with fluoresceine (Biosys Compiegne, France) in 1:400 dilution and Evan's Blue dye in 10000 dilution in phosphate buffer (pH 7.2) were used.

Complement fixation test was carried out as described using complement in excess and 100% hemolysis (Lauricella et al., 1986).

Dog sera from Amamá were tested using four techniques, and those from Termas and Buenos Aires were studied by IFAT and IHAT. As for humans, a serum was considered positive when it was reactive in at least two techniques.

#### RESULTS

*Minimal diagnostic titre for serological tests in dogs* - Fig. 1 shows the frequency distribution of IFAT titres for non parasitemic dogs-BA (A), non parasitemic dogs-EA (B) and parasitemic dogs EA (C).

Parasitemic dogs EA showed 95% (127/134) of reactive sera and about 50% of samples ranged from 1/256 to 1/512.

Non parasitemic dogs EA showed 77% (219/285) of non reactive sera. The remainder was scattered between 8 and 2048 dilutions, being 64 the most frequently found.

About 93% (26/28) of non parasitemic dogs BA were non reactive, one healthy dog reacted at 1/2 dilution and another with distemper at 1/4.

**INDIRECT IMMUNOFLUORESCENCE TEST**

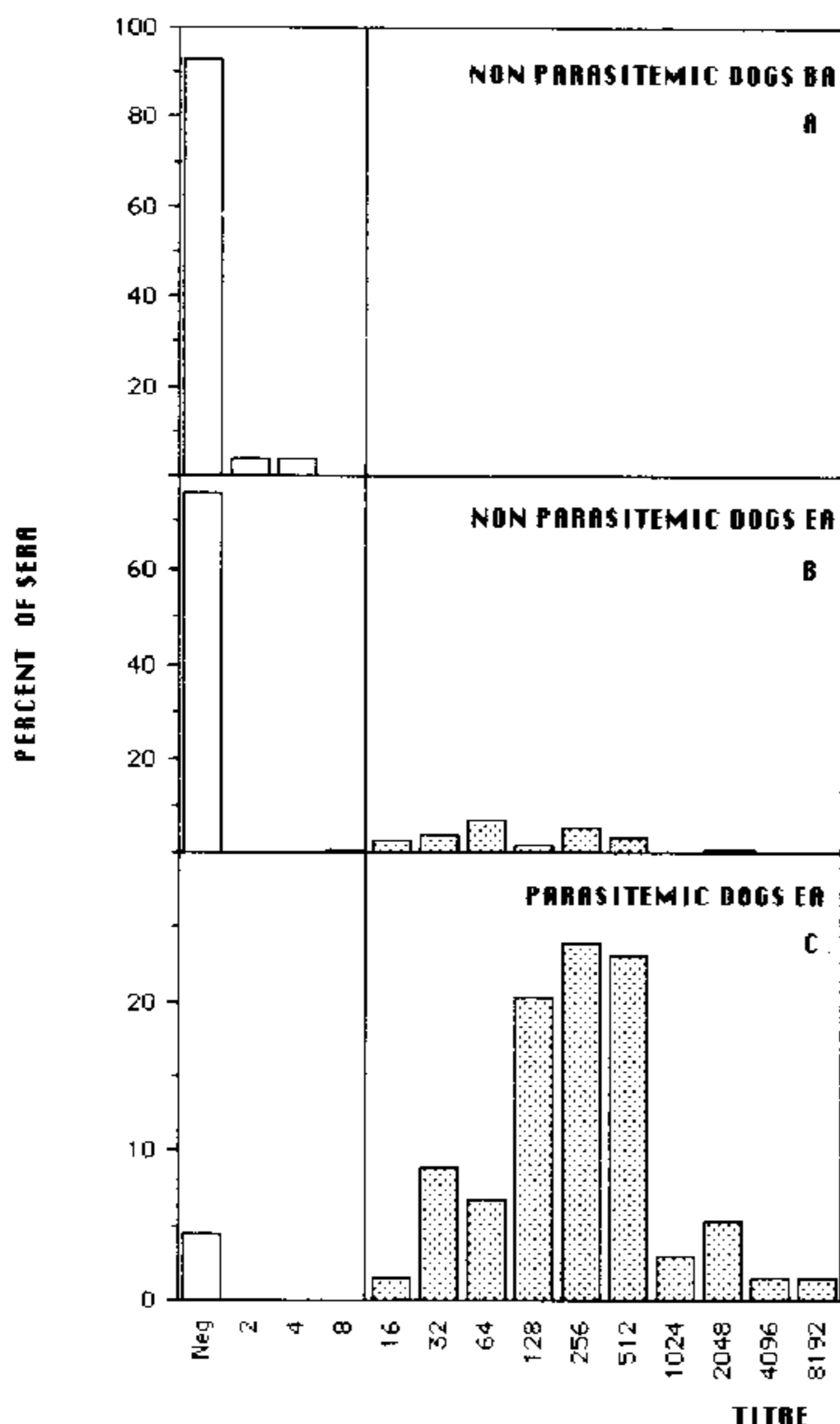


Fig. 1: frequency distribution of serological titres for indirect immunofluorescence (IFAT) of: (A) non-parasitemic dogs from Buenos Aires city (BA); (B) non-parasitemic dogs from endemic area (EA); (C) parasitemic dogs from endemic area (EA).

For IHAT (Fig. 2), most of the sera of parasitemic dogs EA (114/134, 85%) showed serological reactivity. The most frequent titre was 256. Seventy percent of non parasitemic dogs EA had non reactive sera, and the remainder showed titres up to 8192, being eight the most frequently found. Among non parasitemic dogs BA, 61% were non reactive and the remainder showed at most titres of 1/16.

Therefore, we chose 1/16 as the minimal reactive diagnostic titre for IFAT and 1/32 for IHAT.

The cut-off titres were 1/1 for CFT, according to previous results (Gürtler et al., 1987) and 128 for DAT since non parasitemic BA dogs negative for the other tests had maximum titre of 64 (Lauricella, unpublished data).

**INDIRECT HEMAGGLUTINATION TEST**

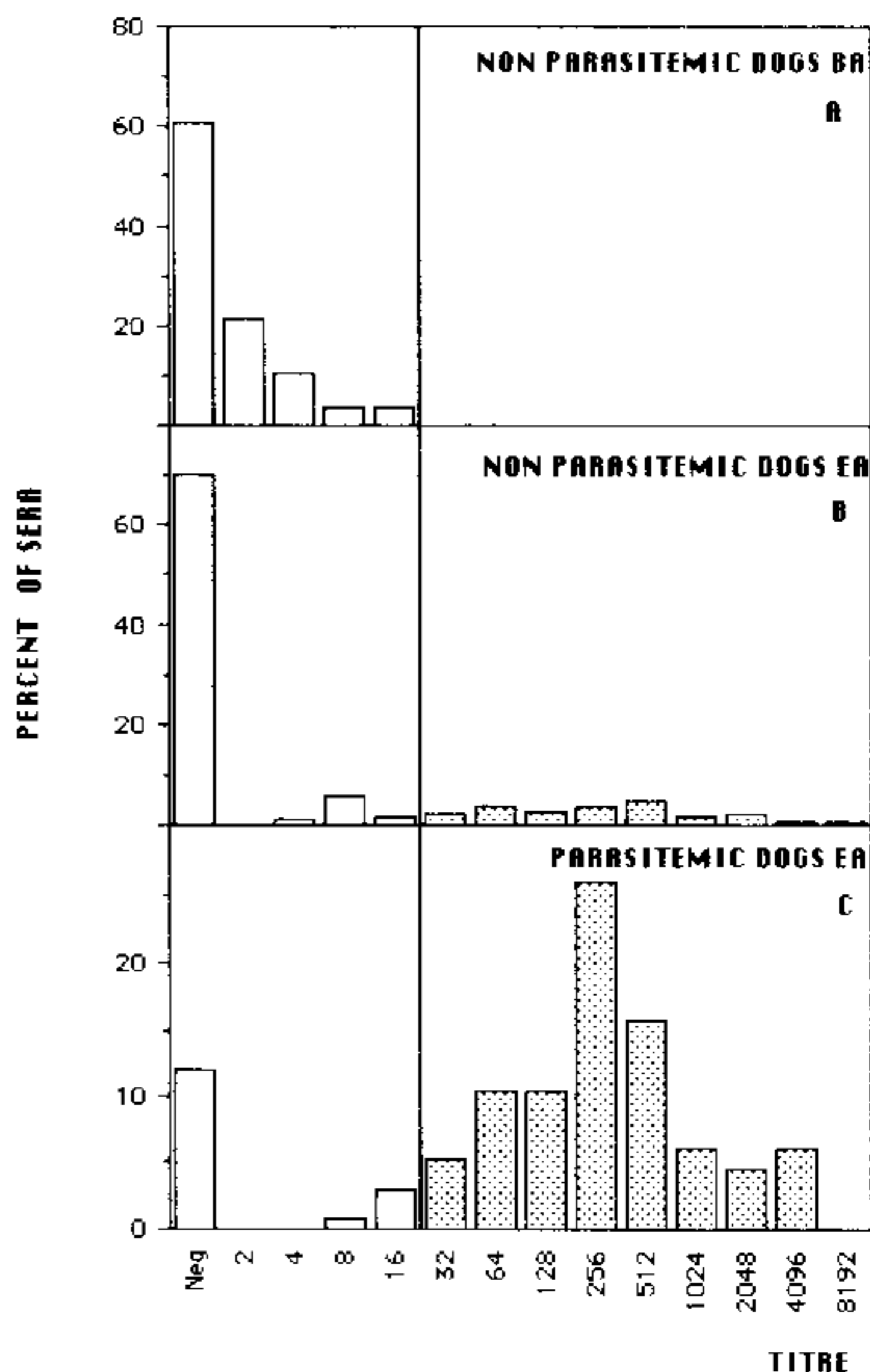


Fig. 2: frequency distribution of serological titres for indirect hemagglutination (HAT) of: (A) non-parasitemic dogs from Buenos Aires city (BA); (B) non-parasitemic dogs from endemic area (EA); (C) parasitemic dogs from endemic area (EA).

*Sensitivity and specificity of serological tests* – To evaluate sensitivity of chosen techniques, only parasitemic dogs were considered while in assessment of specificity, non parasitemic animals that did not react in at least two serological trials, were selected. Table I shows sensitivity and specificity of all studied assays.

*Correlation between IFAT and IHAT results in parasitemic dogs of endemic area* – Among parasitemic dogs EA sera, 84% (112/134) were seropositive for both assays and 12% were discordant (Table II). In order to evaluate discordant sera for IFAT and IHAT we analyzed those 16 sera that were only positive either for IFAT (15) or for IHAT (1). In all cases they were reactive for CFT and DAT. Therefore IFAT detected a higher number of



sera from parasitemic dogs EA than IHAT as it could be expected from the higher sensitivity previously showed (Table I). Among non parasitemic dogs EA 74% (210/285) were non reactive both for IFAT and IHAT and 8% (9 + 15) were discordant (Table III). About 16% (22/134) of parasitemic dogs were non reactive for either IFAT or IHAT but reactive for the other test (Table II), and nearly 18% (51/285) of non parasitemic dogs were reactive for both tests (Table III).

TABLE I

Sensitivity and specificity of indirect immunofluorescence (IFAT), indirect hemagglutination (IHAT), direct agglutination (DAT) and complement fixation (CFT) tests by using parasitemic infected dogs and non infected dogs

Serological test	Sensitivity	Specificity
	No. of reactive / No. of tested sera (%) from parasitemic dogs	No. of reactive / No. of tested sera (%) from non parasitemic dogs
CFT	38/39 (97)	Not determined
IHAT	113/134 (84)	27/28 (96)
DAT	38/40 (95)	Not determined
IFAT	127/134 (95)	28/28 (100)

Reactive/tested sera: reactive sera for each test respective to all tested sera.

TABLE II

Concordance between indirect immunofluorescence (IFAT) and indirect hemagglutination (IHAT) tests of 134 sera from parasitemic dogs from rural endemic areas of Santiago del Estero province

IFAT	IHAT		
	Positive (%)	Negative (%)	Total (%)
Positive	112 (83.6)	15 (11.2) <sup>c</sup>	127 (94.8) <sup>a</sup>
Negative	1 (0.7) <sup>c</sup>	6 (4.5)	7 (5.2)
Total	113 (84.3) <sup>b</sup>	21 (15.7)	134 (100.0)

a: IFAT sensitivity.

b: IHAT sensitivity.

c: sera reactive for direct agglutination and complement fixation tests.

TABLE III

Concordance between indirect immunofluorescence (IFAT) and hemagglutination (IHAT) of 285 sera from non parasitemic dogs from rural endemic areas of Santiago del Estero province

IFAT	IHAT		
	Positive (%)	Negative (%)	Total (%)
Positive	51 (17.9)	15 (5.2)	66 (23.1)
Negative	9 (3.1)	210 (73.8)	219 (76.9)
Total	60 (21.0)	225 (79.0)	285 (100.0)

## DISCUSSION

In the market, there are several available reactants to detect specific antibodies against *Leishmania*, *Entamoeba*, trypanosomes, *Schistosoma* and other parasites. Commercial kits to assess *T. cruzi* infection are used in human diagnosis and epidemiologic surveys. Standardized serological tests are used to detect human anti-*T. cruzi* immunoglobulins with complement fixation activity (Cerisola & Rosenbaum 1958), as well as agglutinating (Vattuone & Yanovsky, 1971), precipitating (Cerisola et al., 1962) and surface directed (Alvarez et al., 1968) antibodies. Those tests can not be directly applied to the study of animal infection, since for each species, sensitivity, specificity and diagnostic titres as well as appropriate controls should be established. Knowledge of those parameters for each test would insure confidence in the results obtained and allow to choose the appropriate test for each situation. Otherwise those are only loose indicators of presence of immunological response (Burkholder et al., 1980). Controls of serological reactivity are missed (Freitas et al., 1952) and specific sensitivity is frequently ignored (Freitas et al., 1952; Tomlinson et al., 1981) in most of the published surveys performed in animal populations.

In this study, the use of xenodiagnosis allowed us to determine sensitivity of the serological tests performed. The cut-off titres for IFAT and IHAT were obtained by intersecting the frequency distribution of titres of parasitemic and non parasitemic dogs from endemic area as well as dogs born and living in Buenos Aires. The selected cut-off titres were 1:16 for IFAT and 1:32 for IHAT. However the latter can be criticized on the following basis: (a) only one non infected dog from Buenos Aires showed a titre of 1:16 and (b) dogs from endemic area attaining 1:16 in IHAT were seroreactive for the other three tests, determining that sensitivity of IHAT were the lowest among all tests. However serological criteria are strict and many times involve to sacrifice sensitivity in order to insure specificity. In this work, the period of infection with *T. cruzi* in dogs was unknown, except for six experimentally infected animals. The sensitivity of parasitemic dogs of endemic area was the highest for IFAT (98%) as it has been recorded for subacute chagasic patients (Cerisola et al., 1969). Nevertheless, in our work, CFT showed a sensitivity of 97% and IHAT of 84% in sera

collected and stored either at the field or in the laboratory contrasting with results obtained in patients where both tests showed similar sensitivity (Cerisola et al., 1969). In this case, low sensitivity observed in IHAT is related to the selected cut-off titre.

The high correlation found between serological and parasitological results indicates that cross-reactivity with other parasites that could be present in dogs from endemic area are not showing at the diagnostic dilutions of sera. Though indirect hemagglutination showed the lowest sensitivity it met with the minimal requirements for a good test and it is suitable for field use since it does not require sophisticated laboratory infrastructure.

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