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Association between nutritional indicators and infectivity of dogs seroreactive for *Trypanosoma cruzi* in a rural area of northwestern Argentina

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Abstract The association between the nutritional state of mongrel dogs naturally infected with *Trypanosoma cruzi* and their infectivity to *Triatoma infestans* bugs and immune response to *Trypanosoma cruzi* were studied in the rural village of Amamá, northwestern Argentina. All of the 97 evaluated dogs were classified into one of three categories of external clinical aspect (ECA) based on the degree of muscle development, external evidence of bone structures, state of the hair of the coat, existence of fatty deposits, and facial expression. ECA was significantly associated with two nutritional indicators, hematocrit and skin-fold thickness, but not with total serum proteins. For all dogs, hematocrit was significantly correlated with skin-fold thickness. The 2-year survival probability decreased significantly from 60.7% for dogs with good ECA to 45.9% and 31.2% for those with regular and bad ECA, respectively. The age-adjusted relative odds of infection for *Triatoma infestans* xenodiagnosis nymphs that fed once on a dog seroreactive for *Trypanosoma cruzi* decreased significantly as ECA improved, when tested by multiple logistic regression analysis. A delayed hypersensitivity reaction was observed in all of the seroreactive dogs with good ECA but only in 45–50% of those with regular or bad ECA. Dogs with bad ECA had a 2.6 and 6.3 times greater probability of infecting triatomines after a single full blood meal than dogs with regular or good ECA, respectively. Our study shows that the reservoir competence of dogs for *Trypanosoma cruzi* was associated with ECA, which is a surrogate and valid index of nutritional state.

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Introduction

Host nutritional status plays a very important role in the dynamic interaction between host and parasite populations (Solomons and Scott 1994). Interactions between malnutrition and infection are usually synergistic, with infection aggravating nutritional deficiencies and malnutrition aggravating infections, or they may be antagonistic, with increasing degrees of malnutrition leading to reduced levels of infection (Scrimshaw and San Giovanni 1997). Malnutrition causes alterations to the infected host's immune response (Keusch et al. 1983).

Dogs are important domestic reservoirs of *Trypanosoma cruzi* (Minter 1976). In highly infested rural areas of northwestern Argentina, the prevalence rates of *T. cruzi* infection in dogs were as high as 84% as determined by serological procedures or xenodiagnosis (Gürtler et al. 1990, 1996). Dogs play an outstanding role in domestic transmission cycles in rural areas because: (1) they are abundant and live in close association with their owner's habitat; (2) they are one of the most frequent blood meal sources of domestic *Triatoma infestans* and other triatomines (Gürtler et al. 1997); and (3) the infectivity to triatomine bugs of dogs seroreactive for *Trypanosoma cruzi*, as measured by the proportion of laboratory-reared, uninfected *Triatoma infestans* bugs that acquire the infection after a full blood meal, is greater and more persistent than that of seroreactive humans (Gürtler et al. 1992, 1996). Dogs are also sensitive sentinels of the domestic transmission of *Trypanosoma cruzi* after insecticidal campaigns (Castañera et al. 1998).

In field studies, the infectivity to bugs of seroreactive dogs was significantly associated with the density of *Trypanosoma cruzi*-infected *Triatoma infestans* in bedroom areas, but not with age, sex and titers of antibodies

to *Trypanosoma cruzi* (Gürtler et al. 1992, 1996). Although the nutritional state of dogs was suggested as a possible determinant of infectivity, no study has yet examined this association. In mice experimentally infected with *T. cruzi*, malnutrition increased parasitemia, and reduced host immune response and survival (Carlomagno et al. 1987, 1996). Calorie malnutrition interacted synergistically with *T. cruzi* infection, and such effects reverted upon renutrition (Carlomagno et al. 1991). In addition, Andrade and Zicker (1995) reported a significant association between chronic malnutrition and seroreactivity for *T. cruzi* in Brazilian children. Therefore, one of our objectives was to test whether the nutritional state of dogs naturally infected with *T. cruzi* was associated with their infectivity to bugs and immune response to *T. cruzi* antigens. As laboratory methods employed to evaluate nutritional state are frequently difficult to use under field conditions, we also developed a practical index of nutritional state based on the dogs' external clinical aspect (ECA).

Materials and methods

Study area

The study was carried out in the rural village of Amamá (27°S, 63°W), Province of Santiago del Estero, Argentina. The study area and the dog population were previously described by Gürtler et al. (1990). After being sprayed with deltamethrin for the first time in 1985, Amamá became increasingly reinfested by *Triatoma infestans*, and evidence of renewed transmission of *Trypanosoma cruzi* to resident children was obtained in 1989 (Gürtler et al. 1994). All houses were re-sprayed with deltamethrin in 1992.

Study design

A cross-sectional, house-to-house survey of all existing dogs was done in March 1990. On the first round, each animal was identified by its name, sex, owner's name and house number, and then classified independently by three observers as having a good, regular, or bad ECA (Fig. 1). These categories were based on the external appearance of the dog: the degree of muscle development, external evidence of bone structure, state of the hair of the coat, existence of fatty deposits, and facial expression (Table 1). The classification of dogs according to ECA category was agreed upon by the observers, who did not consider any prior information on the dogs' seroreactivity for *Trypanosoma cruzi* at this stage of the study. A new census of the dogs, carried out in March 1992 for other purposes, was used to estimate the 2-year survival probabilities of each ECA category.

On the second round, a blood sample for serological and microhematocrit determinations was obtained by antebrachial venipuncture from all available dogs aged approximately 1 year or more. Skin-fold thickness was measured with a pocket gauge at the level of the rib cage. Blood samples were allowed to clot and the sera were then separated and preserved in buffered neutral glycerine (Serokit, Polychaco, Buenos Aires) at room temperature. An aliquot of each sample was stored at 4 °C until testing for serum proteins by Lowry's method at Buenos Aires. Within 6 h of sample collection, two heparinized capillary tubes of each blood sample were centrifuged for 5 min at 10,000 rpm (9,000*g*) in a microhematocrit centrifuge, and the hematocrit was measured with a rule. During fieldwork, all sera were tested by indirect hemagglutination test (IHAT) to assess seroreactivity for *T. cruzi* (see below) and to determine whether each dog would be tested by xenodiagnosis and the delayed hypersensitivity skin test (DTH).

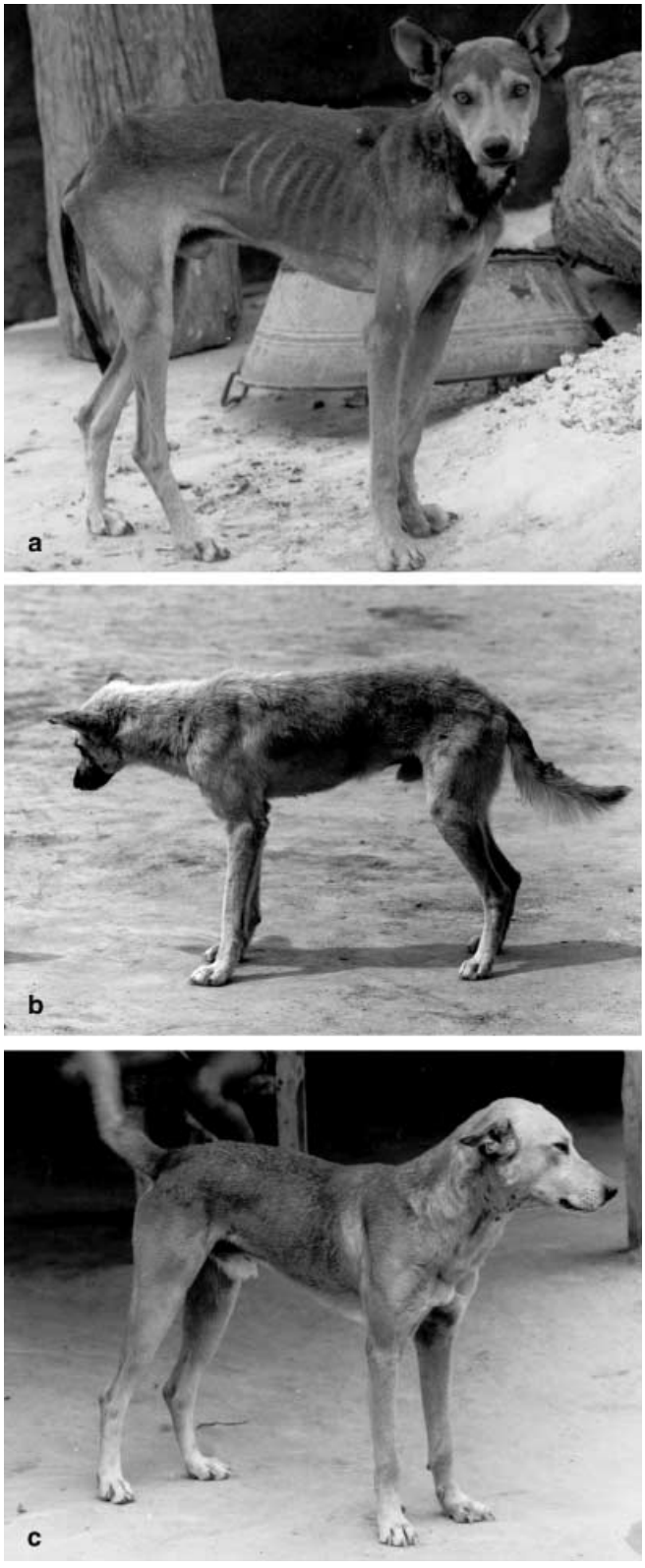

Fig. 1 External clinical aspect (ECA) of dogs in bad (**a**), regular (**b**), and good state (**c**)

Table 1 Characteristics of external clinical aspect (ECA) categories

External appearance	External clinical aspect		
	Good	Regular	Bad
Muscular development	Good	Regular	Poor
Osseous apophysis and ribs	Nearly imperceptible	Apparent	Very apparent[a]
Hair coat aspect	Smooth and shiny	Rough and opaque	Hirsute and dry
Fatty deposit in the base of the tail	Evident	Scarce	Absent
Expression of face	Vivacious	Passive	Apathetic

[a] Frequent xiphosis

On the third round, infectivity to bugs of dogs seroreactive for *T. cruzi* was determined by xenodiagnosis. For the present purposes, xenodiagnosis of seronegative dogs was considered irrelevant. Two boxes, each containing ten uninfected third instar nymphs of *Triatoma infestans*, were placed on the belly or inner thigh of each seroreactive dog for 25 min. After exposure, each box was inspected to ensure that the bugs were fully engorged; in the few cases in which they were not, a new exposure period followed. The boxes were then transported to Buenos Aires and kept at the insectary at ambient temperature until examination. Feces from each individual bug were examined for *Trypanosoma cruzi* infection at ×400 at 30 and 60 days after feeding; microscopists were not aware of the identity or ECA status of the dog on which the bugs had fed. For each seroreactive dog, an overall proportion of infected bugs was calculated among those examined for infection at least once (i.e., infectivity to bugs). Bugs dead before the first examination were not examined, and were excluded from the calculations of infectivity to bugs.

The cellular immune response of *T. cruzi*-seroreactive dogs was measured by DTH. A total of 0.1 ml physiological solution containing 250 μg of a whole *T. cruzi* homogenate (Segura et al. 1974) was inoculated intradermally in the internal area of one of the thighs. In addition, 0.1 ml physiological solution was inoculated in the other thigh as a control. The presence of induration was observed 24 and 48 h later, but in a few cases the dog could not be relocated on the second occasion. When the induration was present, the diameter was measured with a gauge. Dogs with a specific induration on either occasion were considered DTH-positive.

Detection of specific antibodies to *T. cruzi* was carried out by titered IHAT (Polychaco, Buenos Aires, Argentina) and enzyme-linked immunosorbent assay (ELISA), as described by Lauricella et al. (1998), using a blind procedure at Buenos Aires. Minimum diagnostic titers of seroreactivity were 1/16 for IHAT and an absorbance of 0.2 for ELISA. Dogs positive by both techniques were considered seroreactive for *T. cruzi*.

Criteria for data analysis

To establish an association between nutritional state and infectivity, only dogs aged 1 year or more that had been seroreactive for *T. cruzi* by IHAT and ELISA in September 1989 (unpublished data) were included in the study. Younger dogs were excluded to avoid potential confoundment due to growth effects and acute infections with *T. cruzi*, which in most cases occurred below 1 year of age. Therefore, the current study focused on adult dogs undergoing the chronic phase of *T. cruzi* infection.

The relationship between bug infection with *T. cruzi* (INFEC, the dependent variable) and its determinants was studied by maximum likelihood logistic multiple regression analysis. Logistic regression analysis was preferred to standard linear regression because the data for *T. cruzi* infection are binary for an individual bug, and for a sample of bugs yield fractions between 0 and 1 that tend to have a binomial distribution. As the bugs used in xenodiagnosis were clustered on individual dogs, the data might violate the standard regression assumption of independent response probabilities across observations, then possibly leading to the underestimation of standard errors. To avoid this, the logistic-binomial random effects model for distinguishable data implemented in EGRET software was used (Egret 1993); this model includes a random effects parameter that measures a residual effect (e.g., due to subject) on the probability of infection. The dependent variable was the status of *T. cruzi*-infection of each bug; uninfected was indexed as 0, and infected as 1. The explanatory variables were age of the dog (in years), good ECA (ECAGOOD, indexed as 1, regular or bad state indexed as 0), and regular ECA (ECAREG, indexed as 1, bad or good state indexed as 0).

Results

The ECA of 97 (96%) of the 101 dogs identified in Amamá was evaluated (Table 2). Approximately one-third of the population fitted into each category. ECA categories did not differ significantly in mean age (range, 2.3–2.6 years; ANOVA, $F = 0.73$; $P > 0.1$; $n = 97$) or in sex ratio (range, 14–30%) ($\chi^2 = 2.53$; $df = 2$; $P = 0.28$). The percentage of dogs that survived from March 1990 to March 1992 decreased significantly from 60.7% for those in good ECA to 31.2% for dogs in bad ECA (χ^2 for trend $= 5.13$; $df = 2$; $P = 0.0235$). The prevalence of seroreactivity for *Trypanosoma cruzi*

Table 2 Frequency distribution of ECA, age, sex ratio, and survival probability from 1990 to 1992 of the dog population. Amamá, March 1990

External clinical aspect	No. of dogs (%)[a]	Mean age (standard deviation, in years)	Percentage of all dogs that were female	Percentage of dogs surviving 2 years	No. of dogs tested by serology[b]
Good	28 (29)	2.6 (1.9)	14	60.7	18
Regular	37 (38)	2.3 (2.1)	30	45.9	21
Bad	32 (33)	2.5 (2.5)	30[c]	31.2	20
Total	97 (100)	2.5 (2.2)	25	45.4	59

[a] All ages
[b] Only includes dogs of 1 year of age or more. Five dogs below 1 year of age were found infected
[c] Calculated on 30 dogs of known sex

among dogs aged 1 year or more steadily increased from 22.2% in good ECA dogs to 42.9 and 60.0% in regular or bad ECA dogs, respectively. All dogs had serologically concordant results by ELISA and IHAT.

Hematocrit values increased from 27.6% (range, 20.2–29.6%) in dogs with bad ECA to 41.2% (range, 38.5–43.1%) in those with good ECA (Fig. 2A). Using multi-way analysis of variance, hematocrit values differed significantly among ECA categories ($F = 20.0$, $df = 2$ and 56, $P < 0.0001$), but not between *T. cruzi*-seroreactive and seronegative dogs ($F = 0.62$; $df = 1$ and 56; $P = 0.43$). However, the interaction between ECA and seroreactivity for *T. cruzi* was statistically significant ($F = 3.83$; $df = 2$ and 56; $P = 0.028$).

Skin-fold thickness also differed significantly among ECA categories ($F = 9.50$; $df = 2$ and 43; $P < 0.001$), but not between *T. cruzi*-seroreactive and -seronegative dogs ($F = 0.15$; $df = 1$ and 43; $P = 0.70$) (Fig. 2B). The interaction between ECA and seroreactivity was statistically significant ($F = 3.37$; $df = 2$ and 43; $P = 0.044$). Serum protein values did not differ significantly among ECA categories ($F = 0.53$; $df = 2$ and 37; $P = 0.59$) or between *T. cruzi*-seroreactive and -seronegative dogs ($F = 2.42$; $df = 1$ and 37; $P = 0.133$) (Fig. 2C). For all dogs, hematocrit values were significantly correlated with skin-fold thickness ($r = 0.38$; $P < 0.05$; $n = 47$; Fig. 3), but neither was correlated significantly with total serum proteins ($P > 0.5$). The number of dogs tested by the different methods (range, 41–59) differed because of difficulties in handling the animal or because the blood sample was faulty.

The percentage of seroreactive dogs infectious to *Triatoma infestans*, as determined by xenodiagnosis, decreased from 80% in those with a bad or regular ECA, to 45% for those with a good ECA (Table 3), the differences being marginally significant by the chi-square test ($\chi^2 = 3.67$; $df = 1$, $P = 0.056$). The median infectivity to bugs of seroreactive dogs also decreased from 26–31% among those with a regular or bad ECA (with a median age of 3 and 4 years, respectively) to 0% among dogs with a good ECA (with a median age of 4.5 years). Mean and median percentages of infected bugs differed so much because the frequency distribution was overdispersed; therefore, the median was a better statistic of central tendency than the mean. Using multiple logistic regression analysis, the relative odds of bug infection adjusted for dog age decreased significantly in dogs with a regular (odds ratio, OR = 0.60; confidence interval, CI = 0.25–1.45) and a good (OR = 0.25, CI = 0.08–0.80) ECA compared to dogs with a bad ECA. Addition of the random effects parameter significantly improved the fit of the model, thus indicating a significant source of heterogeneity due to subject.

A delayed hypersensitivity reaction to *T. cruzi* was observed in all three seroreactive dogs with good ECA but only in 45–50% of those with regular or bad ECA (Fisher test, $df = 1$, $P = 0.22$) (Table 4). The size of the reaction did not differ among groups. The log-transformed titer of specific antibodies to *T. cruzi* by IHAT

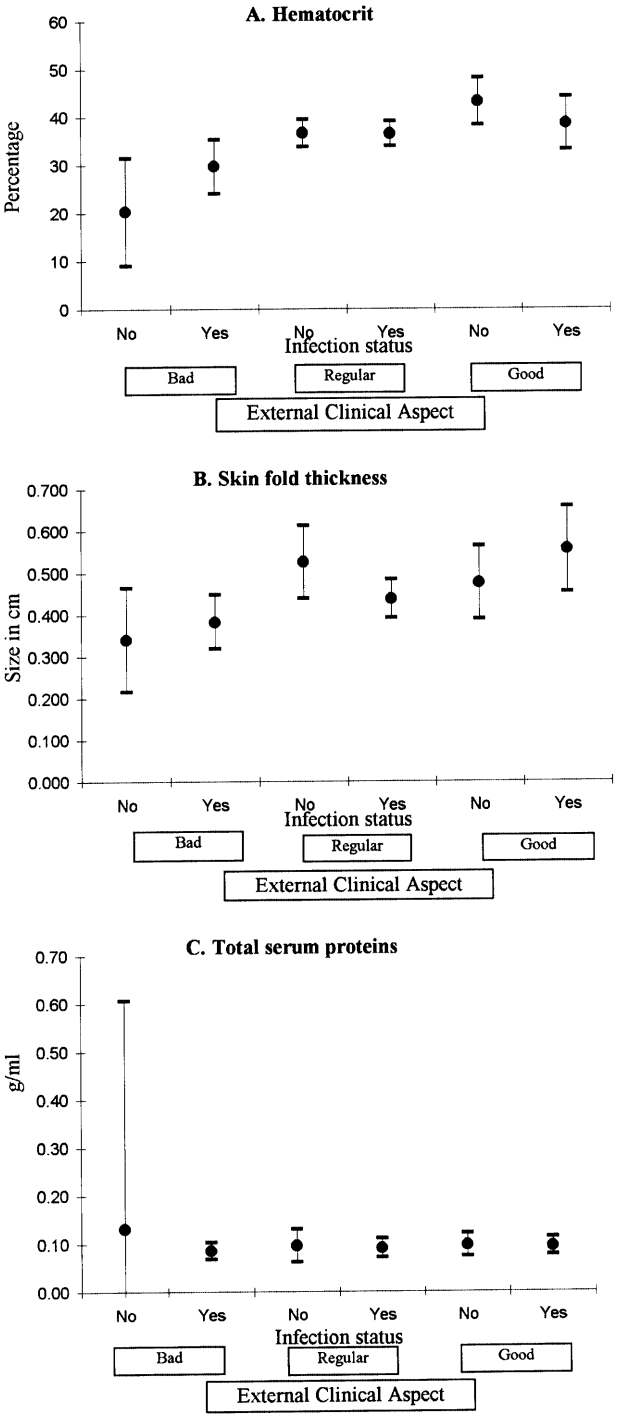

Fig. 2 Hematocrit (**A**), skin-fold thickness (**B**), and total serum protein (**C**) values of the dog population according to their ECA and seroreactivity for *Trypanosoma cruzi*. Amamá, March 1990. Mean and 95% confidence intervals for hematocrit, skin-fold thickness and total serum proteins

($F = 0.08$; $df = 33, 2$; $P = 0.93$) and optical absorbances by ELISA ($F = 1.01$; $df = 33, 2$; $P = 0.37$) did not differ significantly among ECA categories (Table 4).

To reflect the potential capacity of a dog in a given ECA category to infect triatomines after a single full

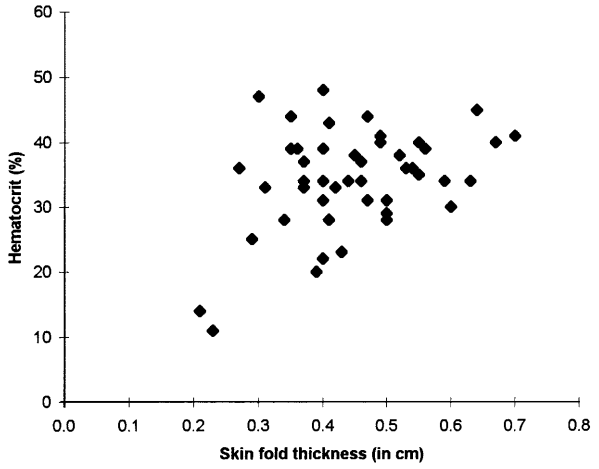

Fig. 3 Correlation between hematocrit and skin-fold thickness in dogs from Amamá, March 1990

Table 3 Infectivity to *Triatoma infestans* of dogs seroreactive for *Trypanosoma cruzi* of 1 year of age or more according to their ECA. Amamá, March 1990. Fitted model (deviance = 215; *df* = 368): Logit (INFEC) = −4.149–ECAREG × 0.5069–ECAGOOD × 1.367–AGE × 0.0339 + 1.515[c]

External clinical aspect	No. of seroreactive dogs tested	Percentage of dogs with a positive xenodiagnosis	Proportion of infected bugs (median percentage)[a,b]
Good	9	45	25/124 (0)
Regular	10	80	33/122 (26)
Bad	10	80	45/125 (31)
Total	29	69	103/371 (19)

[a] No. of infected bugs/no. of bugs examined for infection
[b] The percentage of infected bugs from seroreactive dogs with a positive xenodiagnosis was 50%, 37.5%, and 65.5%, for those with good, regular and bad ECA, respectively
[c] Random effects parameter

blood meal, we calculated an infective potential index (IPI). For each ECA category, the IPI was calculated as the product of the proportion of seroreactive dogs, the proportion of seroreactive dogs with a positive xenodiagnosis (from the third column of Table 3), and the median proportion of infected bugs in positive xenodiagnoses (from footnote b in Table 3). In Amamá, the infectivity to *Triatoma infestans* bugs of dogs seroreactive for *Trypanosoma cruzi* 1 year of age or more with a bad ECA (IPI = 0.3144) was 2.6 and 6.3 times greater than that of dogs with a regular (IPI = 0.1287) or good ECA (IPI = 0.04995), respectively.

Discussion

This is the first field study that measures nutritional parameters in animal reservoirs of *Trypanosoma cruzi*. The statistically significant associations between hematocrit and skin-fold thickness, and between both of them and ECA suggest that this index may be a helpful and valid tool for assessing the nutritional state of rural dog populations. Conversely, total serum protein levels were not related to ECA and other nutritional indicators. In children, total serum proteins have not been considered a reliable nutritional indicator because they fluctuate due to concomitant infections, the type of nutritional deficiency involved, and other factors (Suskind et al. 1977; Tshikuka et al. 1997). Under controlled laboratory conditions, serum protein levels differed significantly between malnourished and control mice, but all values fell within the normal range (Carlomagno et al. 1987). Measurement of chronic malnutrition by indices of height or weight-for-age in rural mongrel dogs is particularly difficult because of great variations in race, size, and weight. The positive association between ECA and survival probability over 2 years gives strong support to the validity of ECA as a measurement of the health status of individual dogs.

The ECA index provides a subjective classification of the dogs' nutritional or clinical state that likely depends on the ability of the examiner. Not surprisingly, subjects that were borderline between categories were common. The ECA index could be useful to stratify field dog populations for vaccine (Basombrío et al. 1993) or drug field trials, in which the outcome may be greatly affected by the animals' clinical or nutritional state (Keusch et al. 1983). In addition, indicators of health, nutritional state, and productivity, such as ECA, have been considered necessary to assess the welfare of animal populations in relation to habitat conditions (Kirkpatrick 1987). Although the classification of ECA in this study specifically refers to rural dog populations under ownership, it could

Table 4 Delayed type hypersensitivity (*DTH*) reaction and titers of indirect hemagglutination test (*IHAT*) and enzyme-linked immunosorbent assay (*ELISA*) of dogs seroreactive for *Trypanosoma cruzi* according to their ECA. Amamá, March 1990 (*n* number of dogs studied; *SD* standard deviation of the mean)

External clinical aspect	DTH		ELISA			IHAT[a]		
	n	% reactives	*n*	Mean	SD	*n*	Mean	SD
Good	3	100	10	1.07	0.225	10	5.2	1.65
Regular	6	50	12	0.91	0.306	12	5.3	2.22
Bad	11	45	14	1.01	0.251	14	5.0	1.81
Total	20	70	36	0.94	0.272	36	4.9	1.91

[a] Data were transformed to $\log_2$ 1/titer

possibly be extended to other canids and animal reservoirs after giving due consideration to their particular characteristics.

Nearly half (47%) of the dogs had hematocrit values below those considered the lowest normal value (37%) by Kirk (1974), thus indicating that the study population was malnourished. Iron deficiencies result in early alterations of muscle metabolism and immune defenses, which occur long before the red blood cell mass is compromised (Solomons and Scott 1994). When the latter occurs major clinical manifestations of deficiency become detectable. Malnutrition was likely associated with several factors observed in the area: (1) calorie- and protein-deficient diets; (2) apparent infestations by ticks and frequent bites by *Triatoma infestans* (Gürtler et al. 1997), especially during the warm season, which, combined with untreated, prevalent enteroparasitic infections, likely produced strong debilitating effects; and (3) the stress caused by high spring-summer temperatures, which frequently rose to 45 °C, combined with regular working or hunting efforts. Nearly all the study dogs were mongrels without regular veterinary supervision or vaccination. Most of the dogs were employed for hunting or herding goats and very few were considered pets.

Seroreactivity for *Trypanosoma cruzi* infection alone did not modify any of the nutritional indicators, but there were statistically significant interaction effects between seroreactivity and ECA on hematocrit or skinfold thickness. Due to the small number of dogs tested for some of the categories, the validity of these interactions requires confirmation. For example, seroreactive dogs with a bad ECA had a higher hematocrit than those seronegative for *T. cruzi*, which might be explained by effects of dehydration. In mice experimentally infected with *T. cruzi*, body weight gain and serum protein or albumin levels did not differ significantly between infected and uninfected mice (Carlomagno et al. 1987), suggesting that *T. cruzi* infection did not cause malnutrition, at least in these animals. Although a significant association between child chronic malnutrition (measured by height or weight-for-age) and seroreactivity for *T. cruzi* has been observed in a case-control study (Andrade and Zicker 1995), such an association may not necessarily be causal.

Natural *T. cruzi* infection in dogs has been characterized by a high and persistent infectivity to bugs (Gürtler et al. 1992, 1996). However, the proportion of seroreactive dogs with a positive xenodiagnosis and their infectivity to bugs decreased significantly as ECA improved, suggesting that dogs in a good nutritional condition controlled parasitemia more effectively than those in a worse condition. These results cannot be explained by: (1) a ''false positive'' diagnosis due to serologic cross-reactions with *Leishmania* sp., the likelihood of which was negligible in the study area at the time of our survey (Lauricella et al. 1998); and (2) an age-related effect, which was allowed for in the multiple logistic regression analysis. Moreover, the median ages of seroreactive dogs did not differ among ECA categories. Because these data emerged from a cross-sectional survey, a double-blind, refeeding cohort study would be required to establish a causal link between host nutritional plane and infectivity to the vector. A possible implication of the inverse relationship between ECA and infectivity to bugs is that detectability of *T. cruzi* infection by xenodiagnosis in seroreactive dogs might be affected by the dog's nutritional state.

Unlike malnourished mice experimentally infected with *T. cruzi* (Carlomagno et al. 1987), the specific humoral immune response of naturally infected dogs measured by ELISA and IHAT did not vary significantly with ECA. These results agree with those obtained in other animal models in which a moderate malnutrition did not affect, and sometimes even enhanced, immune responsiveness (Keusch et al. 1983). In contrast to xenodiagnosis, therefore, the dogs' nutritional state indexed by ECA did not modify the level of antibodies for *T. cruzi* determined by ELISA and IHAT.

Host nutrition and cellular immune response measured by DTH were associated positively in experimental animals (McMurray and Yetley 1982; Nohr et al. 1985; Carlomagno et al. 1987). In our study, there was some indication that DTH was associated positively with ECA, but the final number of study dogs was too small to detect statistically significant differences. The control of *T. cruzi* parasitemia is exerted by reactive oxygen species of phagocytic cells regulated by interleukin IL-10, IL-12, and γ-interferon (Abrahamsohn and Coffman 1996; Cardoni et al. 1997). Therefore, the reduced DTH response could reflect a negative effect of protein deficiency upon T_1-helper cells and, as a consequence, an increase in the susceptibility to *T. cruzi* or increased parasite survival in the bloodstream. DTH proved to be a poor diagnostic tool of *T. cruzi* infection in the study population.

The reservoir competence of individual dogs was associated with their nutritional state. Dogs with a bad ECA had a 6.3 times greater probability of infecting triatomines that ingested a single full blood meal than those with a good ECA. Although dogs with a bad ECA accounted for nearly one-third of the population and had a greater rate of turnover than other ECA categories, their potential contribution to transmission of *T. cruzi* may be disproportionately greater in the face of their relative infectivity to bugs. Because dogs are the principal domestic reservoirs of *T. cruzi* in northwestern Argentina and possibly elsewhere, control programs with an integrated approach should orient efforts to inform the affected human populations of the risks due to close cohabitation with dogs (and cats), and promote responsible dog ownership through maintenance of fewer dogs in a better state, in adequate animal housing separated from human sleeping quarters.

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