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DYNAMICS OF TRANSMISSION OF TRYPANOSOMA CRUZI IN A RURAL AREA OF ARGENTINA. III. PERSISTENCE OF T. CRUZI PARASITEMIA AMONG CANINE RESERVOIRS IN A TWO-YEAR FOLLOW-UP *

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SUMMARY

A new cross-sectional survey of household-associated mongrel dogs as well as follow-up of previously parasitemic individuals was carried out in 1984 by means of xenodiagnosis and serologic techniques to get a deeper insight into the relationship of **T. cruzi** parasitemia and age among canine hosts in a rural area of Argentina. Persistence of detectable parasitemia was age-independent, or at most, loosely related to age, confirming the pattern observed in 1982. Similarly no significant age-decreasing effect was recorded among seropositive dogs in: a) the probability of detecting parasites in a 2-year follow-up; b) their intensity of infectiousness (=infective force) for **T. infestans** 3rd-4th instar nymphs, as measured by the percentage of infected bugs observed in each dog xenodiagnosis. Moreover, not only was the infective force of seropositive dogs for bugs approximately constant through lifetime, but it was significantly higher than the one recorded for children in the present survey, and for human people by other researchers. Therefore, and since **T. infestans** field populations show high feeding frequencies on dogs, the latter are expected to make the greatest contribution to the pool of infected vectors in the rural household of Argentina. This characteristic should be sufficient to involve canine reservoirs definitely as a risk factor for human people residing in the same house. The increased severity of parasitemia observed among dogs in this survey may be related to the acute undernutrition characteristic of canine populations of poor rural areas in our country, which is expected to affect the ability of the host to manage the infection.

KEY WORDS: Chaga's disease — *Trypanosoma cruzi* — Canine reservoir in Argentina

INTRODUCTION

Canine reservoirs of Chagas' disease have been widely involved in domestic transmission cycles of **T. cruzi** throughout Latin America [10], especially in Argentina [18], where blood meal studies of **T. infestans** have revealed high percentages of infected bugs among those fed upon dogs [17]. Thus, it was considered of utmost importance to characterize the natural behavior

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of **T. cruzi** infection in these reservoirs from a parasitological standpoint. A cross-sectional survey of household-associated dogs in 1982 showed that persistence of detectable parasitemia by xenodiagnosis was age-independent among seropositive dogs up to age 10, but with an ambiguous pattern when the few dogs available beyond age 10 were included [5]. Therefore, a new and wider survey in the same areas was designed to get a deeper insight into the relationship between parasitemia and age among naturally-infected dogs, trying to: a) confirm or reject the age-independent pattern previously observed; b) gather new evidence through follow-up of previously parasitemic dogs; c) quantify the intensity of infectiousness (= "infective force") of seroreactive dogs, as estimated by the percentage of bugs that become infected by xenodiagnosis, and establish its relationship with age. Additionally, estimates of the infective force of goatpen-associated dogs, cats and children below age 13 showing a positive xenodiagnosis in this survey are given.

MATERIALS AND METHODS

The study was done in Amamá, Department of Moreno, Province of Santiago del Estero, Argentina, in november 1984. A general description of the study area and of the survey design have been previously published [5].

All dogs existing in 29 households were censused, recording name, sex, age, color and weight, which may permit a further individual identification. Based in these characteristics, dogs censused in 1982 and still alive were reidentified, and its identity was later confirmed by the head of the family. Information on dogs that were not present as well as behavioral and reproductive aspects of each dog was required at each house by means of a questionnaire (to be published separately).

Xenodiagnosis on dogs, cats and children below age 13 were performed by using 20 3rd or 4th-instar **T. infestans** nymphs placed in two boxes, excepting pups (4) and kittens (5) under 3 months old and children up to 2 years old (2) where 10 insects were used. Each box was observed by the pool technique at about 30 and 60 days post-feeding [5]. Search for trypanosomes was made without exceptions at 400 X under a "blind" procedure. Bugs from individuals with a positive xenodiagnosis, and from seroreactive dogs which were still negative after the second fecal examination, were all dissected and examined individually.

Serologic studies of dog sera included indirect hemagglutination test (IHA) [5] and immunofluorescence antibody test (IFAT) (M. LAURICELLA, unpubl.). Positive titer for IHA was 32 and for IFAT 16.

Statistical procedures. Equality of differences between rates or percentages were analyzed in 2 x 2 contingency tables by G-test or Fisher test according to sample size [15]. The relationship between parasitemia and age among seropositive dogs was analyzed during the Wilcoxon test corrected for tied values [14]. The effect of age on the intensity of infectiousness of seropositive dogs for bugs was studied by means of Kruskal-Wallis test [14]. Additionally, differences in the distribution of percentages between age groups under 1 and each one of the rest was analyzed by means of Kolmogorov-Smirnov two-sample test [15].

RESULTS

The relationship between age-specific **T. cruzi** parasitemia and seropositivity in household-associated dogs in 1982 and 1984 is shown in Table I. The 1984 survey included 10 new households and almost 60% of new dogs (= not tested in 1982). Overall prevalence rates of seropositivity and detection rates of parasites did not significantly differ from each other as well as within each category in both surveys ($p > 0.05$), meanly around 85%. Similarly, no significant differences were observed between age-specific rates of both years, either of seropositivity or parasitemia.

A slightly decreasing trend in the percentages of seropositive dogs with detectable **T. cruzi** in relation to age of the host was observed in both surveys. In 1984, the ranked ages of seropositive dogs with parasitemia did not differ from those without parasitemia ($p > 0.2$), agreeing with the overall pattern observed in 1982 in dogs up to age 10. The latter showed a borderline level of significance (two-tailed $p=0.067$) when 2 unusually old seroreactive dogs without

T A B L E I

Relationship between age-specific **T. cruzi** parasitemia and seropositivity in household-associated dogs from Amamá in a 2-years follow-up

Age (years)	1982 No. exam.	1982 % sero+**	1982 % sero+ indiv. with parasitemia***		1984* No. exam.	1984* % sero+**	1984* % sero+ indiv. with paristemia***	
< 1	14	64.3	100		11	63.6	100	
1-3	19	78.9	93.3		22	81.8	83.3	
4-6	15	100	86.7		13	100	76.9	
7-9	3 } 6	100	100 }	66.7	6 } 7	100	100 }	85.7
≥ 10	3 }	100	33.3 }		1 }	100	0 }	
Total	54	83.3	88.9		53	84.9	84.4	

* Are not included 10 dogs examined by xenodiagnosis by not by serology, all under age 6. Almost 60% of tested dogs were not present in 1982.

** Seropositive individuals showed at least two seroreactive tests in 1982 (Direct Agglutination, IHA or CF), or both in 1984 (IHA and IFAT).

*** Parasitemia demonstrated by xenodiagnosis using 20 3rd or 4th instar **T. infestans** nymps, except for pups where 10 insects were used.

parasitemia and residing in the same house were included [5]. To increase sample size, data from 1982 and 1984 were pooled together and seropositive dogs present in both surveys considered once, yielding again a minor level of significance ($N = 78$, $0{,}1 > p > 0.05$).

A 2 year follow-up of parasitemia among 15 seroreactive dogs detected in 1982 is presented in Table II. Two-thirds of dogs had a positive xenodiagnosis in both surveys. Persistence of parasitemia in these dogs was age-independent ($p > 0.05$). Parasites were not detected in either occasion in only one dog among 15 examined (7%). This dog was of a breed type (boxer) and very well-looked after. Bugs captured in the kitchen where it slept at night showed blood meals on dog and were **T. cruzi**-infected.

T A B L E II

Persistence of **T. cruzi** parasitemia' over a 2-year period in household-associated dogs from Amamá, Province of Santiago del Estero

Age in years in 1982	No. individuals seropositive in 1982 and 1984	No. of seropositive individuals in 1982 and 1984 in whom parasitemia was detected (%)		
		Both in 1982 and 1984	Either in 1982 or 1984	Neither in 1982 nor 1984
≤ 2	5	4 (80.0)**	1 (20.0)	0
≥ 3	10	6 (60.0)**	3 (30.0)*	1 (10.0)
Total	15	10 (66.7)	4 (26.7)	1 (6.7)

* Does not include one dog in which bugs were not fed at repletion.

** Statistical difference between percentages tested by Fisher test (one-tailed): p = non significant.

Figure I shows the age-specific intensity of infectiousness of seroreactive dogs for **T. infestans** 3rd or 4th-instar nymphs, as measured by the percentage of infected bugs from each dog xenodiagnosis. An overdispersed pattern of individual values at each age class and skewed toward high percentages led us to employ as statistics medians and quartiles using the schematic box-plots of TUKEY [16]. Overall median percentage of infected bugs was 60, and first and third quartiles were respectively 44 and 81. Median percentages ranged from 77 under age 1 to 56 in age-group 7-10. However, no significant age-related decrease in percentages could be detected ($p > 0.05$). Additionally, no differences in the distribution of percentages between the more divergent age groups could be recorded ($p > 0.05$).

The comparison of the overall percentages of infected bugs detected in positive xenodiag

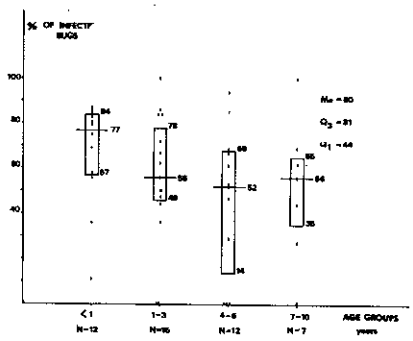

Fig. 1 — Intensity of infectiousness (= infective force) of seroreactive dogs for **T. infestans** bugs according to age of the host. Each individual value is represented by a point. Values at the extremes of boxes represent the first (Q_1) third (Q_3) quartiles, and the bar within the median (Me). N = number of dogs examined in each age group

noses from household and goatpen-associated dogs, cats and children below age 13 is shown in Table III. The percentage of infected bugs

T A B L E III

Comparison between the proportion of infected **T. infestans** 3rd-4th instar nymphs in household and goatpen-associated dogs, cats and children with a positive xenodiagnosis

Host	No. individuals examined	Proportion of infected bug examined (%)
Household-associated dogs (H)	40	378/583 (64.8)
Goatpen-associated dogs (G)	6	49/102 (48.0)
Cats (C)	5	19/31 (61.3)
Children < age 13 (CH)	6	23/82 (28.1)

* Significance levels of differences (Two-tailed probabilities):

H vs G:	p =	0.002
H vs CH:	p =	0.001
H vs C:	n.s.	
G vs CH:	p =	0.001
C vs CH:	p =	0.001
C vs G:	n.s.	

fed upon household dogs (65%) was significantly different from that of goatpen dogs (48%) ($p < 0.002$). However, when individual percentages were considered, a borderline level of significance is shown by the Wilcoxon test ($p=0.052$). On the other hand, while no differences were detected between both dog categories and cats (61%), children's percentage of infected bugs was significantly different from that of the rest of host-species ($p < 0.001$).

DISCUSSION

The present survey strongly confirms previous data obtained by us in 1982 [5], showing that **T. cruzi** parasitemia among household-associated seropositive dogs is an age-independent event, or at most, that it is loosely related to age. Moreover, no significant age-decreasing effect was observed among seropositive dogs in: a) the probability of detecting parasites over a 2 year follow-up; b) their intensity of infectiousness for **T. infestans** bugs, as estimated by the percentage of infected vectors observed in each dog xenodiagnosis. However, it should be mentioned that in both surveys, parasitemia could be demonstrated in all seropositive dogs under age 1 whereas few cases of non-patent infections were detected among older ones, and that the infective force of the former was slightly higher that of older age-groups. While recent primary infection may be a common explanation for both features, the fact that parasites were not recovered in 3 out of 4 seropositive dogs aged 10 or more suggests that a long-term effect of age or other concomitant variable on parasitemia pattern cannot be definitely excluded. If the main goal were to uncover significant differences among age-groups, a greater number of subjects should be examined. On the other hand, not only was the age-related decline recorded in parasite rates and infective force gradual, but its range was small and occurred mainly on dogs aged above the mean life expectancy of the population (= 4 years).

The observed phenomena among dogs clearly contrast with the abrupt age-related decline in parasite rates as well as in the levels of circulating tripomastigotes reported for seropositive human beings in Brazil, either in cross-sectional [7] or longitudinal studies [9]. On the other hand, the high detection rate of **T. cruzi** observed among dogs in the present and in previous studies made by us [18] agrees with data of other researchers obtained in different experimental settings and with different parasite strains [8] (M. LAURICELLA, unpubl. res.).

The main consequence arising from persisting levels of circulating parasites in canine

hosts is that their infective force for vector bugs in approximately constant during lifetime. This oustanding feature, coupled with high **T. infestans** feeding frequencies upon dogs in Argentina [17,19], reinforces previous suggestions made upon the amplifying role dogs possess in transmission cycles from this area [5]. Consequently, a synergistic effect traduced in an increased number of infected vectors in the household may be expected from domestic infestation plus the presence of infected dogs, which gives even more relevance to the epidemiological role appointed for canine reservoirs [5,18]. In relation to cats, although its infective force is similar to that of dogs, exposure to **T. infestans** bites seems not to be suitable, as indicated by blood meal studies and already discussed [18].

Not only was the infective force of canine reservoirs independent of age, but it was significantly higher, on the average, than the one recorded for children in the present survey, and for human people by other researchers. While this study and a pioneering one of FREITAS [4] have shown overwhelmingly similar percentages of infected bugs fed upon parasitemic dogs (65 vs 71%), wider cross-sectional surveys of human populations have shown that people with parasitemia infect on the average from 20-30% of **T. infestans** 5th instar nymps [7,9] to 60% [4]. Interestingly enough, during a parasitological follow-up of a seroreactive family, only 5% of infected xenodiagnosis bugs were obtained [3]. On the other hand, presumably chronic human subjects selected for xenodiagnosis trials have been informed to infect variable percentages of **T. infestans** nymphs, ranging from 3% (3rd instar) [2] to 26-44% (5th instar) [11]. Contrasting with this picture, acute individuals [11] and infected children [9] have been reported to yield the highest percentages of infected bugs.

The increased severity of parasitemia presently observed in natural **T. cruzi** infections of dogs may arise from the acute undernutrition characteristic of canine populations from poor rural areas of Argentina. In general, nutritional deficiencies of the host are known to impair humoral and cell-mediated immune responses of the infected individual, thus facilitating invasion and proliferation of the parasite [13]. Furthermore, infection "per se" has a deleterious effect on nutritional conditions, therefore interacting synergistically with inadequate diets. In the case of the present canine population, dense ectoparasitic infestations and helminthic diseases are very common. In this context, it is noteworthy that well-cared dogs showing a healthy aspect, namely goatpen-associated dogs and the boxer mentioned in Results, yielded a significantly lower percentage of infected xenodiagnosis bugs than household ones. On the other hand, exposure to reinfections may be reasonably expected to be much lower for goat-dogs than for household ones, since the former sleep at goatpens far from sleeping quarters and only one pen in this area was found infested with triatomine bugs. Whether this trait may affect the course of **T. cruzi infection** in dogs has to be worked out.

Besides nutritional aspects, the influence of the parasite strain on parasitemia pattern should be properly considered, as indicated by experimental dog infections [1]. However, it should be noted that no differences can be appreciated between the infective force of the present dog population and a brazilian one [4], despite geographical distance and potentially different parasite strains.

In a previous report, much discussion has been devoted to the subject of **T. cruzi**-infected dogs as a risk factor for people inhabiting the same household [6]. Evidences produced in this paper show that the greatest contribution to the pool of infected vectors in the rural house of Argentina is expected to be made mainly by dogs. This sole characteristic should be sufficient to involve canine reservoirs definitely as a risk factor for the human population. A similar conclusion reached by other researchers in a **Panstrongylus megistus**-infested area [12] may rest on the underlying mechanism described above.

RESUMO

Dinâmica da transmissão do Trypanosoma cruzi numa área rural da Argentina. III. Persistência da parasitemia pelo T. cruzi em reservatórios caninos durante um seguimento de dois anos.

Foi realizada em 1984 uma nova pesquisa epidemiológica dos cães domésticos e seguimento dos indivíduos que apresentaram parasitemia

dois anos antes a fim de compreender a relação entre a parasitemia e a idade dos cães, empregando para isso técnicas sorológicas e xenodiagnóstico. A persistência da parasitemia foi independente ou pouco relacionada à idade, confirmando assim o padrão observado em 1982. Da mesma maneira, nos cães soropositivos não se registrou nenhuma diminuição significativa com respeito a idade em: a) a probabilidade de descobrir parasitas por meio de um seguimento durante 2 anos; b) sua capacidade de infecção para as ninfas de 3.ª 4.ª fase de **T. infestans**, medido pela porcentagem de barbeiros infectados que se observou em cada um dos xenodiagnósticos dos cães. A capacidade infectante dos cães soropositivos foi aproximadamente constante ao longo da vida, e significativamente maior que a registrada para as crianças no presente estudo, e para os agrupamentos humanos pesquisados por outros investigadores.

Assim sendo e considerando as altas freqüências de alimentação no cão que são observados nas populações pelo **T. infestans**, espera-se que o cão contribua para o total de barbeiros infectados existentes nas áreas rurais da Argentina. Esta característica deveria ser suficiente para incriminar definitivamente os reservatórios caninos como um fator de risco para as pessoas residentes na mesma casa.

A alta intensidade da parasitemia registrada entre os cães na presente pesquisa poderia estar relacionada com a acentuada desnutrição característica dos cães nas áreas rurais pobres da Argentina, o que poderia afetar a capacidade do hospedeiro para controlar a infecção.

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