

## DYNAMICS OF TRANSMISSION OF *TRYPANOSOMA CRUZI* IN A RURAL AREA OF ARGENTINA

I — The dog reservoir: an epidemiological profile (\*)

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### S U M M A R Y

A community-based survey of household-associated mongrel dogs was undertaken in a rural area of Argentina to investigate pet population dynamics and the behavior of natural *Trypanosoma cruzi* infection in the reservoir by xenodiagnosis and serologic techniques. Age structure of dog population suggested that individuals participate in the domestic cycle of transmission for a mean period of 4 years; age-specific sex ratios indicated differential survival of female dogs in all age groups, especially among pups. An overall prevalence rate of 84%, increasing from 69% below age 1 to 100% in dogs older than 3 years, strongly support a highly efficient *T. cruzi* transmission to dogs, where congenital/lactogenic transmission might be involved. A 98% of concordance was found between serologic results and parasitologically confirmed infections. Seroreactive dogs below age 10, unlike humans, showed an age-independent persistence of parasitemia. At least 50% of dogs tested were closely associated with sleeping quarters of people. A highly efficient *T. cruzi* transmission, close trophic association with *Triatoma infestans* bugs, age-independent persistence of parasitemia, suitable exposure patterns and high recruitment of susceptible individuals, qualify dogs as amplifying hosts of Chagas' disease in rural communities of central and northern Argentina. Control measures directed to reduce dog-vector contact should be encouraged by primary healthcare programmes, and performed in connection with chemical control of vector bugs.

**KEY WORDS:** Chagas' Disease — Xenodiagnosis — Reservoirs — Dogs — Rural Area in Argentina — Transmission of the Disease from animal to man — Serodiagnosis.

### I N T R O D U C T I O N

As it has been said, "... seldom in studies of zoonoses are the normal hosts dealt with beyond being mentioned as the sources of the pathogens or parasites. Little or no information is recorded regarding their ecology, ..." <sup>15</sup>. Undoubtedly, this argument applies to Chagas'

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disease, where demographic analysis of domestic reservoirs has been a neglected topic for a long time, despite their recognition as important hosts in domestic transmission cycles of *T. cruzi*<sup>15,17,25</sup>. As far as we know, only one attempt has been made previously to assess age-specific infection rates of pet populations<sup>12</sup> and where additional ecologic data can be drawn. On the other hand, the behavior of natural *T. cruzi* infection in dogs, which is fundamental for our understanding of their role in the epidemiology of Chagas disease<sup>17</sup>, has not been studied yet. Therefore, a community-based survey of household-associated dogs was undertaken in a rural endemic area of Argentina to investigate: a) the age structure and behavior patterns of the canine population; b) the relationship to *T. cruzi* infection and age in these hosts, evidenced by xenodiagnosis and serological techniques.

## MATERIALS AND METHODS

The study was done in the locality of Amamá located at latitude 27° N and longitude 63° W, Departamento of Moreno, Province of Santiago del Estero, República Argentina, in november 1982. Human population density of the Departamento according to the 1980 census is very low: 2.4 inhabitants per km<sup>2</sup>. The area is semiarid, with a mean annual rainfall of 740 mm mainly in summer, and a mean annual temperature of 22°C. Vegetation is dense and xerophytic, consisting primarily of a thorn forest of "quebracho" (*Schinopsis lorentzii*) selectively exploited in the last 50 years and therefore, with little modification of original natural landscape. This characteristic, coupled with that the Departamento had never been sprayed by the official executor of the vector control campaign, were the main criteria used to select the survey area, where a longitudinal study on the dynamics of transmission of *T. cruzi* is being carried out.

Twenty households of neighboring settlements within 400 km<sup>2</sup> around Amamá were chosen for epidemiologic studies. Each settlement consisted of a cluster of not more than 5 houses isolated in the forest. Residences followed similar construction patterns as those already described<sup>23</sup>, with a front porch where house-

holders sleep almost all the year when the weather is not extremely cold or rainy.

Heads of each family were informed of the contents of the survey, and consents were signed by all of them. Householders were canvassed to obtain the following data: name, sex and age of resident people, dogs and cats; residence period of the family; date of house building and of recent improvements; sleeping places of people and animals; domestic use of insecticides; family income and educational level. *Triatoma infestans* captures were carried out at each household, and shipped to the laboratory where fecal examinations for trypanosomes and blood meal studies were performed (to be published separately).

Blood samples were obtained from dogs and cats by venipuncture. The serum was kept one week in the refrigerator while in the field, and then frozen and stored at -70°C in the laboratory until serological examination.

Xenodiagnosis was performed on dogs and cats by using 20 3rd or 4th instar *T. infestans* nymphs placed in boxes, each containing 10 insects. The rectal contents of bugs mixed with physiological saline solution were searched for trypanosomes 30 and 60 days after feeding<sup>3</sup>. Two wet preparations were made from each box and microscopically examined at 400 X.

Serologic studies performed on domestic animals sera were done by one of us (M. L.) and included: a) Direct agglutination test (DA) (Polychaco SAIC, Argentina); b) Indirect hemagglutination test (IHA) (Polychaco); c) Quantitative Complement Fixation test (CF) in microplate<sup>1</sup>. Six sera from laboratory infected dogs<sup>7</sup> and an equal number from dogs of non endemic areas were used as positive and negative controls.

## RESULTS

### Age structure of dog population

A complete census of pets was undertaken in all the households visited. Fifty-six of 73 mongrel dogs (77%) as well as 43% of 14 cats were examined. Cats, found in a small number and considered to be differently involved in transmission than dogs, are included elsewhere

re<sup>25</sup>. Six sheep-dogs living in pens 50-100 m apart from the main house were not examined. On the average, there were 3.6 dogs per household.

The population structure of dogs was skewed toward younger age groups, showing only

10% of individuals older than 6 years (Fig. 1). Mean age of dogs was 3.3 years (SD = 2.97); median age was 3 years. Overall sex ratio (2.5M:1F) was significantly biased toward males ( $G_{adj} = 11.564, p < 0.001$ )<sup>19</sup>. Age-specific sex ratios showed an age-related trend ranging from 2.2:1 below age 1 to 5:1 in dogs older than 7 years.

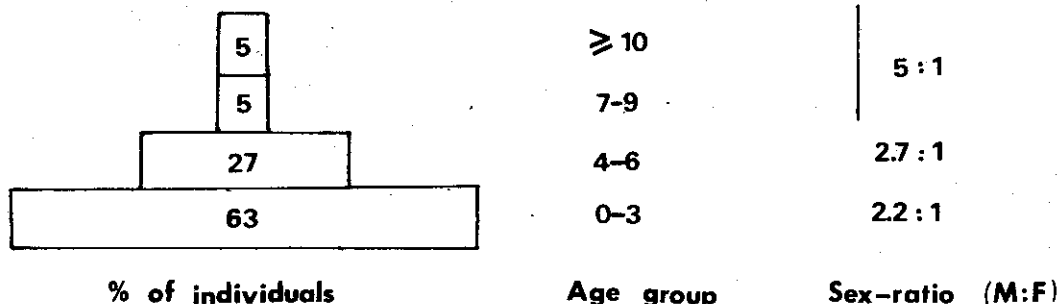


Fig 1 - Age pyramid and age-specific sex ratios of dog population from Amama

A new visit to 8 of the 20 houses previously surveyed was accomplished 1 year later. Thirty per cent of 24 dogs censused previously have disappeared for different reasons: among them, 3 pups have been slaughtered by the owners when informed that they were infected. On the other hand, 5 new pups were found, and one 2-years-old female dog had been adopted by a different household. One family moved to another house in the area, carrying their dogs to the new place. Three litters (5-6 individuals each) were produced in the intervening period; in one of them all pups were born dead.

#### Seroreactivity and xenodiagnosis results

The overall prevalence of *T. cruzi* infection considering the composite results of serology

and xenodiagnosis was 83.9% among 56 dogs (Table I). The proportion of parasitemic individuals (= parasite rate) (75%) did not differ from the prevalence rate of seropositivity (83.3%) by the G-test ( $p > 0.25$ ). No significant differences were found between sex-specific parasite or seropositivity rates ( $p > 0.25$ ).

The age-specific prevalence rate of infection increased in successively older age groups from 68.7% below age 1 to 100% in dogs older than 3 years, remaining stable at older ages (Table II). After a steep rise below age 1, infection rates seemed to follow a linear increasing trend up to age group 4-6, suggesting a constant force of infection acting on them over this period.

A 98% of concordance was found between positive xenodiagnoses and serologic results:

T A B L E I

Sex-specific prevalence rates of *T. cruzi* infection determined by xenodiagnosis and serologic procedures in dogs from Amama

Sex	Parasitemia (*)			Seroreactivity (**)		Prevalence rate of infection (***)
	No. tested	No. pos.	(%)	No. tested	No. pos. (%)	
Males	40	30	(75.0)	38	33 (86.8)	87.5
Females	16	12	(75.0)	16	12 (75.0)	75.0
Total	56	42	(75.0)	54	45 (83.3)	83.9

(\*) Parasitemia detected by xenodiagnosis with 20 3rd or 4th instar *T. infestans* nymphs.

(\*\*) Individuals showing at least two positive techniques were considered seropositive. Positive titers for dog sera: 1:16 for IHA; 1:128 for DA and 1:8 for CF.

(\*\*\*) Determined using composite results of xenodiagnosis and serology. Infected individual: seropositive and/or with parasitemia. Total number of tested individuals = 56.

only one 2-months-old pup did not show anti-*T. cruzi* antibodies among parasitemic dogs. On the other hand, only 5 among 44 (11.4%) seropositive dogs were negative by xenodiagnosis. The ranked ages of seroreactive dogs up to age 10 with parasitemia did not differ from those

without parasitemia by the Wilcoxon test corrected for tied values<sup>19</sup> ( $U=74.5$ ;  $0.20 < p < 0.50$ ), although when 12 and 14-years-old seroreactive dogs were considered, a borderline level of significance was attained ( $U=154.4$ ; one-tailed  $p=0.033$ ; two-tailed  $p=0.067$ ).

T A B L E II

Age-specific prevalence rates of *T. cruzi* infection determined by xenodiagnosis and serologic procedures in dogs from Amama

Age group	with parasitemia		without parasitemia		Prevalence rate of infection (**)
	No. tested	No. seropos. (*)	No. tested	No. seropos. (*)	
<1	11 (***)	9	5	0	68.7
1-3	14	14	5 (***)	1	78.9
4-6	13	13	2	2	100
7-9	3	3	0	0	100
≥10	1	1	2	2	100
Total	42	40	14	5	83.9

(\*) Individuals showing at least two positive techniques were considered seropositive. Positive titers for dog sera: 1:16 for IHA, 1:128 for DA and 1:8 for CF

(\*\*) Determined using composite results of xenodiagnosis and serology. Infected individual: seropositive and/or with parasitemia

(\*\*\*) Includes one dog not studied by serology

T A B L E III

Comparison of complement fixation (CF), indirect hemagglutination (IHA) and direct agglutination (DA) results in dogs with and without *T. cruzi* parasitemia

Age group	with parasitemia					with parasitemia				
	No. tested	CF		IHA	DA	No. tested	CF		IHA	DA
		Pos. (*)	Ac. (**)	Pos.	Pos.		Pos.	Ac.	Pos.	Pos.
<1	10 (***)	8	1	5	5	5	0	2	0	0
1-3	14	13	1	12	14	4	0	2	1	2
4-6	13 (***)	10	3	10	12	2	2	0	2	2
7-9	3	3	0	3	3	0	—	—	—	—
≥10	1	1	0	1	1	2	2	0	2	2
Total	41	35	5	31	39	13	4	4	5	6

(\*) Positive titers for dog sera: 1:16 for IHA, 1:128 for DA and 1:8 for CF

(\*\*) Anticomplementary serum

(\*\*\*) One sera not tested by DA

Comparison of serological techniques results in dogs with and without parasitemia is shown in Table III. The DA test showed a 100% degree of sensitivity in detecting dogs with parasitemia, followed very closely by the CF test. On the other hand, the IHA test lost almost 25% of patent infections, especially among pups where "false negatives" rate rose to 50% (Fisher test:  $p=0.106$ , two-tailed). Among individuals without parasitemia, a full agreement was observed between techniques, except in one case where DA gave a discordant result.

### Exposure patterns

Dog infection rates were analyzed in relation to exposure patterns of animals, that is, to their resting place at night. As all dogs were reported to sleep outdoors, categories chosen were: a) in peridomestic sites (kitchen, store-rooms, etc); b) in the porch or variable. All cases where the exposure type of pets could not be asserted by the owner were eliminated from the comparison.

Householder's reports showed that almost 50% of dogs slept in the porch, the rest in peridomestic sites. No differences were found between infection rates of both groups, yielding 90.5% and 85.7%, respectively.

## DISCUSSION

The age structure and sex ratio of the dog population reported in this paper clearly resembles previous findings in an area of similar cultural patterns as those described here<sup>12</sup>. Mean age of household-associated mongrel dogs suggests that individuals participate in the domestic cycle of transmission for a mean period of 4 years. On the other hand, biased sex ratios toward males showing an age-related trend suggest differential survival of females in all age groups. Slaughtering of female pups, a widespread rural practice to prevent the increase of dog population size at each household, may account for the overwhelmingly higher proportion of males in the 0-1 age group. From then onwards, mortality seems to affect female dogs in a fairly constant way. Although reports of acute, fatal cases of naturally-acquired canine American Trypanosomiasis indicate that there may exist a great mortality pressure on infected pups<sup>13,22</sup>, additional data on mortality due to natural *T. cruzi* infections are needed.

Since lifetime residence is the usual feature of household-associated rural dogs of this long-established human population, canine age may be considered as a measure of time of exposure to infection associated with length of residence in a specific focus. The extremely high incidence of *T. cruzi* infection among dogs below age 1 and the successive steady increase of infection rates until attaining 100% at age 4, not only exceeds previous reports<sup>12</sup> but strongly support a high efficiency of transmission of the parasite to the reservoir. Three factors already documented may be summing up to depict this situation: a) a high degree of host-vector contact; b) congenital or lactogenic infection; c) a high susceptibility to the parasite (reviewed in<sup>20</sup>). Although *T. infestans* feeding profile of this area yielded almost 60% of 470 bugs feeding on dogs<sup>26</sup>, congenital transmission or during nursing may be co-responsible for the enhanced incidence recorded. Reports on intrauterine *T. cruzi* transmission in experimen-

tally infected female dogs<sup>21</sup>, and of suckling puppies presumably infected via maternal milk<sup>14</sup>, support that point of view. The present finding of 3 parasitemic 3-months-old pups from neighboring households delivered by an infected dam, and similar previous reports from Mazza<sup>13</sup>, reinforce the need of more studies on congenital/lactogenic transmission in dogs. On the other hand, transmission via the oral route (e.g.: licking of contaminated furs, ingestion of infected bugs or rodents) coupled with *T. cruzi* infection among peridomestic and sylvatic rodents of the cricetid family in our country<sup>2</sup>, may provide an alternative pathway of arrival of parasites to dogs.

The fact that parasitemia in dogs with serologic evidence of exposure to infection, unlike humans<sup>6,11</sup>, does not decline with age not only illustrates the importance of the dog reservoir but suggests that the immune response may play a different function role in the control of parasitemia than in humans. The existence of 2 seropositive dogs older than age 10 with negative xenodiagnosis suggests that mechanisms operating on the control of *T. cruzi* blood forms could be more effective in long-term infections. However, since life expectancy of rural dogs is far below age 10, persistence of parasitemia through life can be considered characteristic of natural *T. cruzi* infection among rural dogs. This feature surely accounts for the high detection rate of parasites in rural dogs submitted to serial xenodiagnosis over a 6-10 months period<sup>9</sup>, as well as for the high infection rates of bugs fed on dogs either from xenodiagnosis<sup>5</sup> or from *T. infestans* field populations<sup>23</sup>. Additionally, persistence of parasitemia should be considered together with the existence of potential reinfections, in the light of the positive association observed between both events in a human population from Brazil<sup>11</sup>.

The existence of only one discrepancy between serological results and parasitologically confirmed infections among dogs reinforces the value of the tests performed as epidemiologic tools in canine Chagas' disease surveys, contrasting with the scarce previous attempts made already discussed<sup>18</sup>. In relation to sensitivity of tests utilized, our data are in agreement with previous experience on the subject<sup>8</sup>: CF and DA tests show higher sensitivity than

IHA test. On the other hand, specificity studies, not available in the literature, are presently being undertaken<sup>8</sup>.

No previous attempts have been made to differentiate exposure patterns determined by specific resting sites of household associated dogs in relation to bug-mediated *T. cruzi* transmission. The fact that nearly 50% of dogs were found closely associated with sleeping quarters or even specific beds is undoubtedly a key factor in their epidemiologic role as has been already suggested<sup>13,25</sup>. On the other hand, that no correlation was found between infection rates of animals and exposure types may seem an unexpected result. However, resting sites of dogs may not be constant, through life, and moreover, infection seems to take place at an early age (Table II), therefore rendering overall infection rates deceiving. Additionally, peridomestically-acquired infections either through the vector or by ingestion of infected rodents, as above-mentioned, might be potentially involved.

The magnitude of the dog reservoir must be viewed in the light of complex host vector-parasite interactions as well as on pet population dynamics. Dogs as a species show a high reproductive capacity, reflected in fast annual turnover rates attaining 68%<sup>10</sup>, which imply high recruitment rates of susceptible individuals ready to enter the disease transmission cycle. Present data show that a highly efficient *T. cruzi* transmission to dogs, involving among other factors congenital/lactogenic infections, renders the population rapidly infected, maintaining then persistent parasitemias through life. During a mean period of 4 years, exposure patterns of dogs and their close trophic association with *T. infestans* bugs determinate a constant input of parasites into the domestic transmission cycle, thus qualifying dogs as amplifying hosts of Chagas disease in this area. Interchange of pups after weaning between households (or even of adult dogs as was recorded in this survey) assures the spread of infected reservoirs to the community.

Several control measures have been suggested to face the problem of dogs as disease reservoirs, including slaughtering<sup>4</sup> and chemosterilization<sup>10</sup>. Without deepening into the viability of these methods but focusing on the search of alternative ecological strategies to face di-

sease transmission<sup>10</sup>, it is quite straightforward that the more householders reduce dog-vector contact by not letting pets rest during the night indoors or in the porch, the lower *T. infestans* bugs will become infected. Control measures directed to reduce dog-vector contact should be encouraged by primary health-care rural programmes, and performed in connection with chemical control of vector bugs in rural areas of Argentina.

## RESUMO

### Dinâmica da transmissão do *Trypanosoma cruzi* numa área rural da Argentina. I — Os cães como reservatórios: um perfil epidemiológico.

Levou-se a cabo numa área rural da Argentina uma pesquisa epidemiológica dos cães associados ao domicílio, com vistas a caracterizar sua dinâmica populacional as infecções naturais pelo *T. cruzi* empregando técnicas sorológicas e xenodiagnóstico. A estrutura de idades da população mostrou que os indivíduos participam no ciclo doméstico da transmissão uma média de 4 anos; a proporção de sexos por grupos etários sugeriu uma mortalidade diferencial para as fêmeas, especialmente nas cadelas. Achou-se uma prevalência geral de infecção de 84%, crescendo desde 69% em cães 1 ano até 100% nos maiores de 3 anos, indicando alta eficiência de transmissão do *T. cruzi* aos cães na qual a infecção congênita ou pelo leite pode estar implicada. A sorologia mostrou concordância de 98% com os xenodiagnósticos positivos. A parasitemia não diminuiu com a idade em cães seropositivos  $\leq 10$  anos. Ao menos os 50% dos reservatórios achavam-se estreitamente associados as moradas da gente. A estreita associação trófica entre os cães e *T. infestans*; a persistência da parasitemia nos reservatórios; a alta eficiência de transmissão do parasito a este; padrões de exposição do hospedeiro adequados; e altas taxas de recrutamento de indivíduos suscetíveis qualificam o cão como hospedeiro amplificador da doença de Chagas nas áreas rurais do centro e norte da Argentina. Os programas rurais de atenção primária da saúde vieram estimular a introdução de medidas dirigidas a diminuir o contacto cão-barbeiro para ser executadas em conexão à luta química contra os vetores na Argentina.

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