RESEARCH NOTE

Number of Vector Bites Determining the Infection of Guinea Pigs with *Trypanosoma cruzi*

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The number of bites from *Trypanosoma cruzi* infected *Triatoma infestans* received by a mammal is one of the main determinants of its risk of becoming infected by this parasite. Although *T. cruzi* is not introduced by the bite itself, it is mostly during the bite that *T. cruzi* contaminated feces are deposited on the skin (C Chagas 1909 *Mem Inst Oswaldo Cruz 1*: 159-218, E Brumpt 1912 B*ull Soc Path Exot 5:* 723-724).

Domestic guinea pig corrals or "cuyeras" are known as foci of *T. cruzi* spread (RA Torrico 1950 *Bol of Sanit Panam 29*: 827-840) and have also been used for experimental purposes (MA Basombrío et al. 1987 *Am J Trop Med Hyg 37:* 57-62). When guinea pigs are placed in *T. infestans*-colonized corrals, the number of bites necessary for infecting a guinea pig (NBNI) should be equal to the number of bites taking place in the corral during the time of exposure (A), divided by the number of infected guinea pigs (B):

$$NBNI = \frac{A}{B}$$

Since often not all guinea pigs are infected, factor B can be expressed as the number of animals in the corral (N) multiplied by the proportion of animals infected (I), so that:

$$NBNI = \frac{A}{N \cdot I} \quad (\text{for } I > 0)$$

(formula 1)

After the first animal becomes infected, some of the bites are "wasted" on infected animals and should be substracted from NBNI. Since I increases with time, these bites are estimated as one half of those received by the animals presenting infection at the end of the exposure period. This total number of redundant bites is then divided by N, to estimate those corresponding to one guinea pig, so that

$$NBNI = \frac{A}{N.I} - \frac{\frac{1}{2}\ A.\ I}{N}$$

and using a common denominator:

$$NBNI = \frac{A - \frac{1}{2}\ AI^2}{NI}$$

(formula 2)

Regarding factor A, several measurements can allow its estimation with increased (although not complete) accuracy. Taking into account the number of insects in the corral at the time of censing (n), the number of days vectors and hosts have lived together (d) and the daily proportion of fed vectors (PFV), factor A could be estimated as n x PFV x d. Furthermore, not all bugs are infected and it is possible to examine them and determine the proportion of vectors infected (PVI). Thus, it was possible to dissect formula 2, incorporating all the determinants just mentioned:

$$A = \frac{PFV \cdot n \cdot PVI \cdot d}{N \cdot I}$$

Measurements for each of these factors could be obtained in the field post of our laboratory. After an initial, pilot determination previously reported within an ecologic study (S Catalá et al. 1992 *Am J Trop Med Hyg 47*: 20-26), we present here the results of 11 sets of data from 4 separate experiments performed in uniform, comparable conditions. The post is placed in Cobos (24'47 S 65'06 W), Province of Salta, Argentina, a Chagas'disease endemic rural area, now under insecticide control. Average temperatures range from 9°C in winter to 30° C in summer and humidity from 60 to 100%. A system based on standardized guinea pig

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corrals, made of loose brick, measuring 1 m^2 x 40 cm height, and isolated by two layers of mosquito nets (Fig.) was used.

Corrals designed to perform entomological studies of *Triatoma infestans* populations and to obtain vectorial transmission of *Trypanosoma cruzi* to guinea pigs under natural climatic conditions.

In experiments 1, 2 and 3, five guinea pigs and an original seeding population 696 *T. cruzi* bearing *T. infestans* bugs were initially placed in a corral. During 4 to 7 months, this population was censused at intervals, but care was taken not to introduce changes in either bugs or guinea pigs. The original bug seed consisted of 42 adult (12 male and 30 female) and 654 nymphal stages (57N5, 72N4, 290N3, 191N2 and 44N1). In experiment 4, the number of guinea pigs was eight and the vector population started with 696, mostly adult, non stratified *T. infestans* bugs. In all experiments, initial PVI exceeded 95% in samples and was assumed to be 100% in populations.

n: the number of bugs was determined by periodic censuses. The yards were completely disassembled and all bugs were collected and taken to the laboratory. Developmental stages and sexes were recorded but only the total number of bugs was considered in this work.

PFV: the method used to estimate this factor (S Catalá et al. 1991 *Med Vet Entomol 5:* 325-333) was based on the finding of transparent urine in the rectal ampoule of bugs fed during the last 24 hr. Since PFV is temperature-dependent (Catalá et al. *loc. cit*.), the average temperature recorded during each period between censuses was used to adjust PFV in the following census.

PVI: feces from bugs were examined under the microscope for the presence of *T. cruzi*. For both PFV and PVI determinations, samples of 140 bugs (20 for each developmental stage) were used.

N and I: the number and proportion of guinea pigs carrying *T. cruzi* infection was recorded. Infection was determined by the microhematocrit method (H Freilij et al. 1986 *J Clin Microbiol 18:* 327-330) using three capillaries per animal, followed by xenodiagnosis with 20 nymphs and a serologic test (direct agglutination or ELISA).

The Table presents data of 11 censuses from 4

TABLE

Field samples for estimation of the number of *Triatoma infestans* bites necessary for the infection of one guinea pig with *Trypanosoma cruzi*

Experiment	Census No.	Date of census	PFV[a,c]	n[b]	PVI[b]	d	I	N	ENB[c]
1	1	07-11-91	0.22	653	0.516	7	0.00	5	≤519
1	2	04-12-91	0.22	463	0.486	34	0.20	5	1650
1	3	05-03-92	0.24	965	0.850	121	0.40	5	10957
2	4	03-09-92	0.16	696	0.651	7	0.00	5	>508
2	5	05-11-92	0.17	470	0.792	70	0.20	5	4341
2	6	21-12-92	0.21	708	0.770	114	0.60	5	3567
3	7	05-08-93	0.02	545	0.770	35	0.00	5	>294
3	8	16-12-93	0.10	1050	0.850	166	0.60	5	4049
4	9	10-11-94	0.20	696	0.685	70	0.37	8	2071
4	10	23-12-94	0.26	2483	0.633	111	0.50	8	9922
4	11	30-03-95	0.27	2793	0.705	205	1.00	4	≥13624

a: PFV proportion of fed vectors per day, n: number of bugs, PVI: proportion of vectors infected, d: days of exposure, I: proportion of infected guinea pigs, N: number of guinea pigs, NBNI: number of bites necessary for one infection (calculated with formula 2); *b*: the values for n and PVI are the average of values obtained from the initial (seeding) bug population, of previous censuses and of last census; *c*: ENB (estimated number of bites) is equal to NBNI for all cases where I>0. In censuses 1, 4, and 7 I=0 and NBNI= ∞. ENB represents in these cases the number of bites per guinea pig, which is less than NBNI. The values for PFV are the average of the values obtained in previous and last censuses.

separate experiments. It can be seen that, in 3 censuses (No. 1, 4 and 7), the number of bites (≤519) was too small to produce any infection (I=0). In census No. 11, the number of bites was 13624, a number equal or higher than necessary to infect all animals in the yard (I=1). In 7 censes, the number of bites that produced the infection of one guinea pig fell between 1650 and 10957 ($\overline{X}$ = 5222; SE = 1402). This range is slightly higher, but not substantially distinct from a previous, preliminary determination by our group for a larger, partially sampled guinea pig corral (NBNI = 1461; Catalá et al. *loc. cit.*) or to estimates for human infection by *T. cruzi* in triatomine infested human dwellings (NBNI = 1000 to 2500, JE Rabinovich et al. 1990 *Bull WHO 68:* 737-746).

Some possible confounding factors have to be considered, which possibly reduce the accuracy of these estimates. Underestimation of NBNI may stem from infections not related to bites, such as may occur by contamination of drinking water or food with *T. infestans* feces or by direct ingestion of infected bugs by the guinea pigs. This alternative was suspected in this system by a rare episode of sudden infection of all animals in a corral with very high density of bugs and an open drinking water dish. In this study, we have attempted to reduce this possibility by using improved water dispensers (Fig.) and avoiding an excessive number of bugs. Insects failing to collect transparent urine after unsuccessful bites may also result in underestimation of NBNI. On the other hand, overestimation of NBNI can result from bug populations with an excess of younger developmental stages with low vectorial capacity, such as occurs early in the summer. Finally, a crucial and highly variable factor in transmission, e.g., the concentration of infective, metacyclic *T. cruzi* stages in bug's feces, has not been considered as such in these estimates. Bug infectivity has instead been approached in this study by PVI, a parameter which is less influential in transmission. In spite of these possible inaccuracies, this system allows the measurement of factors which decisively correlate the behaviour of *T. infestans* and the transmission of *T. cruzi.*

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