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Entomological aspects of Chagas' disease transmission in the domestic habitat, Argentina

Aspectos entomológicos de la transmisión de la Enfermedad de Chagas en Argentina

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Keywords
Chagas' disease. Transmission. *Trypanosoma cruzi*. *Triatoma*. Risk factors. Household.

Abstract

Objective
To study the risk of *Trypanosoma cruzi* domestic transmission using an entomological index and to explore its relationship with household's characteristics and cultural aspects.
Methods
There were studied 158 households in an endemic area in Argentina. Each household was classified according to an entomological risk indicator (number of risky bites/human). A questionnaire was administered to evaluate risk factors among householders.
Results
Infested households showed a wide range of risk values (0 to 5 risky bites/human) with skewed distribution, a high frequency of lower values and few very high risk households. Of all collected *Triatoma infestans*, 44% had had human blood meals whereas 27% had had dogs or chickens blood meals. Having dogs and birds sharing room with humans increased the risk values. Tidy clean households had contributed significantly to lower risk values as a result of low vector density. The infested households showed a 24.3% correlation between time after insecticide application and the number of vectors. But there was no correlation between the time after insecticide application and *T. infestans'* infectivity. The statistical analysis showed a high correlation between current values of the entomological risk indicator and *Trypanosoma cruzi* seroprevalence in children.
Conclusions
The risk of *T. cruzi* domestic transmission assessed using an entomological index show a correlation with children seroprevalence for Chagas' disease and householders' habits.

Descriptores
Enfermedad de chagas. Transmisión. Trypanosoma cruzi. Triatoma. *Factores de riesgo. Vivienda.*

Resumen

Objetivo
Estudiar el riesgo doméstico de transmisión de Trypanosoma cruzi *por medio de un indicador entomológico y analizar su relación con características culturales y de las viviendas.*
Métodos
Se estudiaron 158 casas en el área endémica argentina. Cada vivienda infestada se clasificó de acuerdo con un indicador entomológico de riesgo (número de picadas riesgosas/ humano). Mediante encuestas se evaluaron factores de riesgo asociados a la vivienda y habitos de los moradores.

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Resultados
Las casas infestadas mostraron un rango amplio de valores de riesgo (0 a 5 picadas riesgosas/ humano). Se observó un gran número de viviendas con bajos valores de riesgo y pocas viviendas con valores elevados. El 44% de las Triatoma infestans *colectadas estaban alimentadas sobre ser humano y el 27% sobre perro o gallina. Las viviendas donde perros y/o gallinas compartían la habitación humana, tuvieron valores de riesgo más elevados. Las viviendas ordenadas y limpias exhibieron bajos valores, debido a un escaso número de* T. infestans. *Se comprobó un 24.3% de correlación entre el tiempo transcurrido luego de aplicar insecticidas y el número de vectores en la vivienda. Sin embargo, no hubo correlación entre el tiempo post-rociado y la infectividad de* T. infestans. *Se observó correlación entre los valores del indicador entomológico y la seroprevalencia en niños.*
Conclusiones
El riesgo de transmisión de T. cruzi *en cada vivienda, medido a través de un indicador entomológico, se correlaciona positivamente con la prevalencia de seropositividad en niños y con hábitos de los moradores.*

INTRODUCTION

Chagas disease is an endemic disease in Latin America. The ethiological agent, *Trypanosoma cruzi*, is transmitted by the faeces of haematophagous insects of the subfamily Triatominae (Hemiptera, Reduviidae). More than 120 species of Triatominae live in the most diverse habitats and some are well adapted to the human houses and constitute a serious problem of public health. The parasite can also be transmitted by blood transfusion or organ transplant from infected donors and, occasionally, through the oral route by ingesting contaminated material. Congenital transmission causes about 2% of cases but vector-borne transmission still accounts for over 80% of all cases of Chagas disease.[18]

Although *T. cruzi* is not introduced by the bite itself, it is during the bite that *T. cruzi* contaminated feces are deposited on the skin of the host. Thus, the number of bites received by a mammal from *T. cruzi* infected vectors is one of the main determinants of the transmission risks. The parasite transmission via the bug feces is relatively inefficient. Rabinovich et al[17] estimated that the average probability that a contact with an infected bug would lead to a new infection is about 1 in 1,000. But, how often an infected bug is in contact with a human being? How many bites a human being received each night? Which factors modify the biting rate? Are all the Triatominae species similar in their biting rate? The host-vector contact rate, an important measure of vectorial capacity for haematophagous insects, is almost unknown for Triatominae, even in the best known species as *Triatoma infestans* and *Rhodnius prolixus*.

On the other hand, the official health programs of the endemic countries, has been using several indicators to estimate the transmission risk for Chagas disease.[19] Most of them are based on the number of vectors found in houses and/or peridomestic habitats (eg. Density Index, Crowding Index) or in their infestation by *T. cruzi* (Natural Infection Index). However, none of these index group together vector density, infection and antropophily as a more adjusted indicator of the transmission risk for Chagas disease.[15] The most demonstrative index currently in use, is the seroprevalence in children. Actually, it expresses the vectorial capacity of insects reflecting the risk during the years previous to the evaluation date.

The human-*T. infestans* contact rate had been estimated few years ago. *T. cruzi* Transmission Risk Index (TcTRI) was proposed by Catalá et al[4] as an entomological indicator of Chagas disease transmission. This index estimates the number of infected-vector bites per night, considering the *T. infestans* density, *T. cruzi* infection and human biting rate of vectors. Here, the TcTRI was estimated in several domiciles of the endemic area of Argentina in order to explore its relationship with variables such as insecticide spraying, blood meal source, cultural customs and seroprevalence in children.

METHODS

The study areas were placed within the Argentinean Central and Northwest endemic region (Salta, Jujuy, La Rioja, Santa Fé, Córdoba and Santiago del Estero provinces) (Table 1).

Houses were not randomly selected. In order to check the entomological characteristics of Chagas disease transmission, 158 poorly build rural houses were visited. Most of them were typical “ranchos” with adobe walls and thatched roofs or houses with brick walls and roofs of corrugated metal sheeting.

The walls were partially plastered or non-plastered and many crevices were apparent. Most houses had been sprayed during the last five years but the 25 houses from Atamisqui (Santiago del Estero) had not been treated (official campaigns) since 1980.

The TcTRI is the estimation of the number of risky bites that a human receives per night. Risky bites are defined as those bites produced by infective bugs. The analysis of the insects allows the estimation of the TcTRI for each house, as follows.[4]

$$\text{TcTRI for each house} = \frac{\text{Bites on humans}^{a} * \text{Proportion of infective bugs}^{b}}{\text{Number of humans}^{c}}$$

Where:

a: number of bugs with colorless urine and human blood in the promesenteron (see below).

b: proportion of bugs with metaciclyc tripomastigotes in the rectum.

c: People sleeping in the house the last night.

The methodology used to obtain the Index has been described in detail by Catalá et al.[4] Briefly, each house was closed and each room was treated with fumigant canisters (Musal or Agufog, based on DDVP in combination with one or more synthetic pyrethroids, 1 canister/ 30 m^3). After two hours, the knocked down triatomines were collected and stored at 4ºC. The three youngest nymphal stages were not considered in the study because their small size complicates capture, increasing error probability. A fingerprick blood sample was obtained from people living in the houses (N=528), in order to determine seropositivity for *T. cruzi* infection. The blood samples were collected with a commercial kit (Serokit, Polychaco) and analyzed in order to detect the presence of anti-*T. cruzi* antibodies using ELISA and indirect hemaglutination. These studies were carried out by the National laboratory for Chagas disease (Córdoba).

A questionnaire was performed to the head of the family, at each house. This questionnaire was designed to gather information related with variables of interest, in order to detect association with TcTRI. Those variables were: type of roof, walls material, age of the house, number of inhabitants, sex, age, number of beds, cleanliness and order of house, number of cats, dogs, chickens and other domestic animals, number of peridomestic structures, domestic animals sleeping within the house, activity of the family head, education level of the family head, number of children going to school, knowledge about Chagas disease, vector control actions by the family and time since the last official intervention against vectors in the house.

The insects collected from each house were analyzed in the laboratory. The presence of colorless urine in their rectum was used as an indicator of feeding during the last night.[4] Then, the number of insects displaying colorless urine represented the number of bug's bites in the house. These insects were separated and the blood within the promesenteron was used for blood meal identification by double gel diffusion (four antibodies: human, dog, cat and chicken).[4] The number of insects with human blood in their promesenteron represented the number of bug's bites on humans. The same estimation was done for the number of bites on cats, dogs and chickens. The presence of metacyclic trypomastigotes was checked by microscopical analysis of the rectum contents (400 xs).

The comparisons among regions or provinces were not done providing that houses were not randomly selected.

RESULTS

The TcTRI followed a skewed distribution with a high proportion of houses with the lowest values of

Table 1 - Localities and provinces studied, Argentinean Central Northest endemic region.

Localities	Province	Latitude	Longitude	Sample/date	N households
Tasigasta, Bajadita, Boquerón	Santiago del Estero	28.4	64.2	November/94 February/95	25
La Tosca, Quilino	Córdoba	30.2	64.6	November/95	8
El Puestito, Las Ollas	Córdoba	30.2	64.6	December/95	8
Paraje San Miguel, San Carlos, Santa Lucía	Córdoba	30.2	64.6	March/96	8
Guanaco Muerto	Córdoba	30.5	65.1	April/96	6
El Guanaco, La Penca	Córdoba	29.8	63.3	December/96	9
Hacheral	Jujuy	24.3	64.8	March/96	8
El Perchel, Huancalera, Caleta, Humahuaca	Jujuy	23.4	65.3	March/96	8
La Unión, Sector 5	Salta	23.9	63.2	April/96	5
La Banda, San Agustín, La Florida	Salta	26.1	65.8	April/96	8
Tobas	Santa Fé	29.2	60.2	November/95	18
Villa Mineti	Santa Fé	28.6	61.6	December/95 March/96	36
Chepes	La Rioja	31.2	66.24	October/97	11
Total					158

TcTRI and only few houses with high or very high Risk Index. Sixteen percent of houses showed 0.5 to 6 bites/person/night while, 84% of domiciles showed a TcTRI under 0.5 bites/person/night.

Ninety one houses (57.6%) were found infested by *T. infestans* but only 50% of these bugs were positive for *T. cruzi*. Thus, many houses displayed TcTRI=0 as a consequence of the lack of infection in bugs. The *T. infestans* number within each infested house, showed a skewed distribution (Figure 1A). If we exclude houses whith zero natural infection, the frequency distribution of *T.cruzi* infection in bugs showed a clear bimodality (Figure 1B).

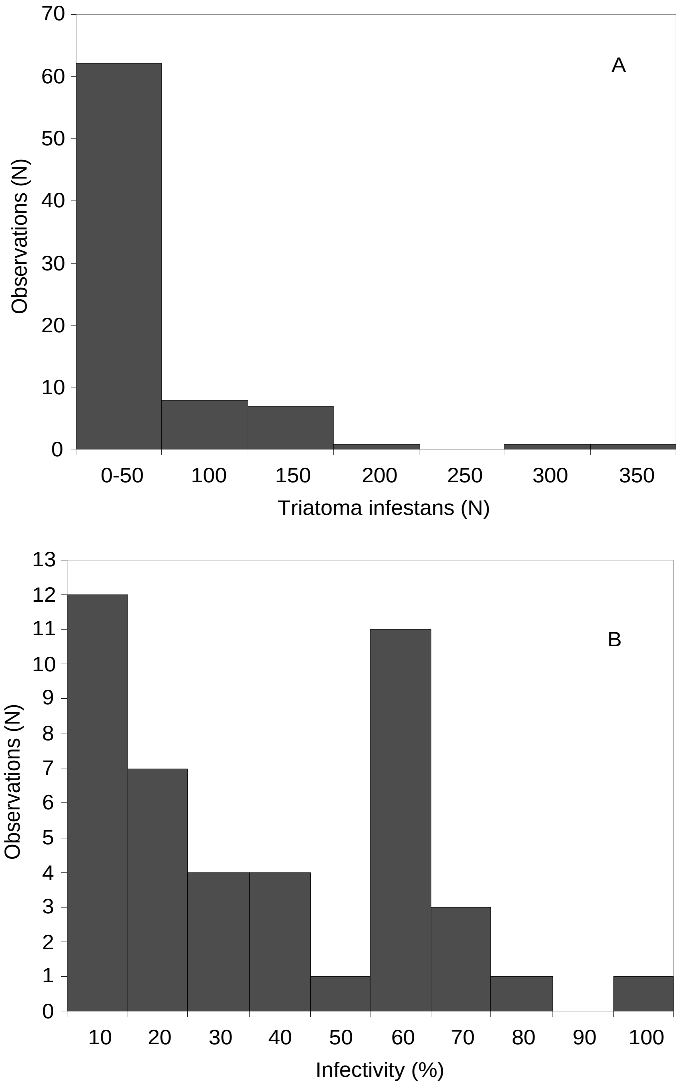

Figure 1 - Frequency distribution of *T. infestans* A) and infectivity; B) by households.

Analysis of factors associated to TcTRI

Data from the questionnaires were primarily analyzed by cluster analysis, in order to evaluate possible factors linked to the Chagas transmission risk. The houses with the highest TcTRI (mean: 2.26) appeared associated to the presence of dogs and chickens sleeping in the house. The lowest TcTRI (mean: 0.004) were related to tidy houses and dogs sleeping out of the bedrooms. Other variables were not significantly associated to TcTRI.

Bivariate test (Kolmogorov-Smirnov) were performed in order to explore how the presence of dogs and birds in bedrooms (mainly chickens), affects variables related to TcTRI. The presence of birds within houses seems to favor the *T. infestans* population increase (birds within: *T. infestans* =86, N=24; birds out: *T. infestans* =15, N=49; p<0.001). On the other hand, the infection of bugs was higher in houses where dogs were permitted indoor (23.6%, N=42), than in houses where dogs did not (8.36%, N=46, p<0.001). Nonetheless, owners of several houses (Santa Fé province) did not allow dogs indoor but showed the highest infection in bugs. Notoriously, these houses registered seropositive children inside.

The analysis of the blood in the promesenteron of recently fed bugs, confirmed the importance of domestic animals as blood source for *T. infestans* living in the human domicile. Host identification was possible on 78% of all the samples with the four antibodies used: chicken, human, cat and dog. The human being was the preferred host (44%), although the analysis revealed differences among localities. Remarkably, houses from Tobas and Villa Minetti (Santa Fé province) showed 74% of blood meals on humans, which represent twice the mean values obtained from other localities. Dog (10%) and chicken (17%) was the most representative host, following humans. Cats were not important as host of *T. infestans* (0-1%). Six percent of bugs showed mixed meals. Twenty two percent of the samples could not be associated to one of these four hosts. As revealed by questionnaires, other domestic animals as goats, ducks and turkeys, shared the bedrooms with humans and may be the source of these no-identified meals.

Tidy houses showed a significant decrease on the risk values as a result of the low vector density. They displayed significantly less *T. infestans* than disordered domiciles (ordered: 14 *T. infestans;* disordered: 70 *T. infestans,* Kolmogorov-Smirnov p<0.001).

In order to know wether the infested houses showed correlation between time after insecticide treatment

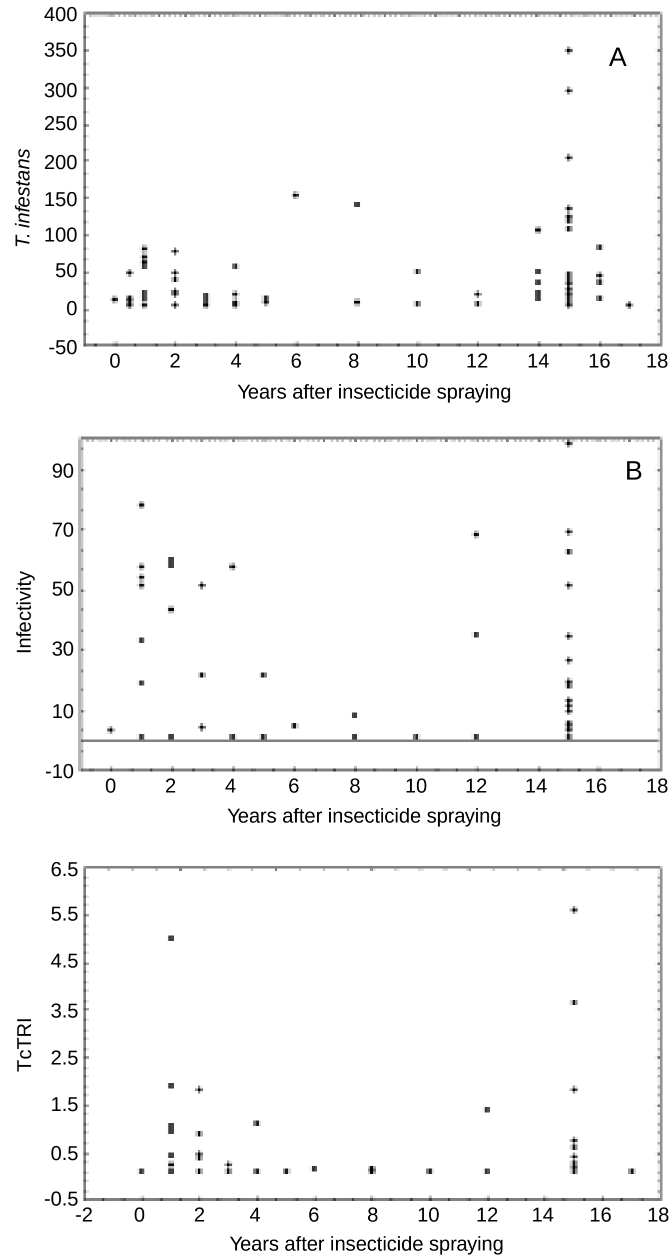

Figure 2 - *T. infestans* A) infectivity; B) and TcTRI; C) at different time after insecticide application. Each point represents a household.

and the number of *T. infestans* hold, a Spearman Rank order correlation was performed. The analysis showed that a 24.3% of the variability on bugs number is explained by the time elapsed since the insecticide spraying (t=2.29, p=0.024, N=86). Houses with 10 or more years after the last official campaign (eg. Atamisqui region), exhibited the largest populations of *T. infestans*. However, within the same region we also found houses (ranchos) with very low populations of the vector (Figure 2A). On the other hand, the variability in the natural *T. cruzi* infection of *T. infestans* and in TcTRI, were not correlated with the time after insecticide treatment (Figure 2B, C).

Table 2 shows the seroprevalence for 436 adults and 212 children from the studied areas.

In order to study the relationship between TcTRI and *T. cruzi* seroprevalence, the data were extracted from the 81 houses holding children under 15 years old. These houses were grouped into 5 levels of increasing risk. Those domiciles with TcTRI=0 (zero) were subdivided in two risk groups according to the presence or absence of *T. infestans*.

- Risk 0. TcTRI= 0 bites /night/person, houses without *T. infestans*
- Risk 1. TcTRI= 0 bites /night/person, houses with *T. infestans*
- Risk 2. TcTRI= 0.01 to 0.2 bites/night/person
- Risk 3. TcTRI= 0.21 to 0.5 bites/night/person
- Risk 4. TcTRI more than 0.5 bites/night/ person

A pooled seroprevalence was calculated for children living in houses within the same risk level. Two different statistical analyses were performed: a Chi-Square test among seroprevalence values at each risk level and, a correlation analysis.

Houses where TcTRI was zero (Risk 0 and 1) had the lowest prevalence (0.02-0.03). These two groups showed no significant difference in children seroprevalence. The houses with the highest levels of risk (Risk 3 and 4) showed a significant increase of the seroprevalence, differing with the two first groups (Table 3).

DISCUSSION

There is ample evidence that vectorial transmission of *T. cruzi* is controlled by multiple factors that favour the vector multiplication and the parasite cycling between vectors and humans.[1,2,5,6,8-12,16,18] Within the general characteristics of an endemic region, each domicile has particular sociocultural patterns, economic level and family structuring, that results in a particular way of life. Under our hypothesis, the ecological, physiological and parasitological characteristics of a vector population living in a house, is the expression of that way of life. Thus, if we check a *T. infestans* population during the hot season, when it is expressing its maximum reproductive potential[3,4,8] natural infection[7] and capacity for *T. cruzi* transmission to humans,[13] we should be able to estimate the risk.

Current vigilance strategies give the same priority to all houses, no matter how many *T. infestans* hold or what

Table 2 - Seroprevalence for Chagas disease on adults and children (under 15 years old) of the studied areas.

Localities from	Adults N	Positives N (%)	Children N	Positives N (%)
Santiago del Estero	81	26 (32)	37	7 (19)
Córdoba	95	15 (16)	36	7 (19)
Santa Fé	136	40 (29)	77	16 (21)
La Rioja	27	4 (15)	8	1 (12)
Salta-Jujuy	97	6 (6)	54	1 (2)
Total	436	91 (20.87)	212	32 (15.1)

Despite the data were organized by province it must not be considered as representative values for the whole province.

Table 3 - TcTRI relationship with children seroprevalence.

Risk level	TcTRI	Houses	Children	Sero-positives	Prevalence (%)	Chi sq test
0	0 (-)	39	163	4	0.0331	
1	0 (+)	19	42	1	0.0238	
2	0.01-0.20	11	18	2	0.1110	
3	0.2-0.5	4	11	3	0.2730	*
4	>0.5	8	23	12	0.5220	**

TcTRI – *T. cruzi* Transmission Risk Index.
(-): houses without *T. infestans*; (+): houses with *T. infestans*.
*Differs with the two first values;
**Differs with the three first values.

natural infection they have. It is considered that the presence of this triatomine, by itself, already demands immediate control actions. But, in the practice this is not allways possible to carry out. It is very well known that economic and politic problems, very common in endemic countries, causes severe delays in control actions as there are not enough resources. To give the same effort of vigilance to all houses infested by *T. infestans*, means to assume that the risk is the same for all houses. Our results (Table 3) shows that in the studied area, there are a small proportion of houses (12/91=13.2%) where the vector's population has a very high transmission potential (Risk level 3 and 4). In these houses, 44.1% of children were infected by the parasite. Then, these houses need a special priority and an special effort from Health Programs. Moreover, after insecticide spraying, these domiciles should be carefully observed in order to avoid a fast recovery of the risk.

The highest natural infection of bugs in houses where humans shared the bedrooms with dogs has been widely demonstrated by Gutler et al.[10-12] On the other hand the presence of birds, mainly chicken, was also suggested as a risk factor.[5,6,14] Our results show that the highest values of TcTRI are associated to domestic animals sharing the bedrooms with humans. Dogs inside the house were responsible for a higher infection of bugs and chickens inside were responsible for the highest triatomine population. The analysis of blood meal source revealed that, in general, more than 50% of bugs fed on a non-human host, but cultural differences could produce regional differences as shown in domiciles from Santa Fé. Almost all people living in that region declared that domestic animals were not allowed inside. Correspondingly, bugs bites on human beings showed a significant increase. In houses where this factor was combined with the presence of infected children, the TcTRI increased notoriously.

The effect of keeping tidy and ordered domicile had an important impact over the *T. infestans* density inside bedrooms. For example, cardboard boxes plenty of clothes; papers and others non-used things constitute appropriated refuges for triatomine, near the human's resting sites. Frequently, these untidy houses were associated with a complex peridomicile holding many *T. infestans*.[14] Changes in cultural patterns may drastically modify the bug's infection, the vector population density or the antropophily, diminishing the risk. These very important factors will only be modified by education.

Chemical control of triatomine had demonstrated a notorious reduction in Chagas transmission.[17] However, results from Gorla[9] on experimental populations of *Triatoma infestans*, showed that populations treated with γHCH recovered to untreated levels within 1-3 years, depending on the season when the insecticide was applied. Our study involving domestic populations showed that one year after official campaigns, some houses displayed low or intermediate densities of bugs. In houses without treatment for many years (Atamisqui, Santiago del Estero province) large populations of *T. infestans* were occasionally found. Although the time elapsed since the last insecticide spraying explained 24% of variability in vector number, the infection and the risk of transmission had no correlation with the time since insecticide treatment. The recovery of risk was faster than the recovery of *T. infestans* populations. Factors mentioned above, as dogs resting inside the house and even the sero-positive children may contribute to recover the risk in those domiciles.

The number of contacts required to produce a new case of Chagas in humans, had been estimated as 1,000 bites by Rabinovich.[17] On the other hand, Catalá et al[3] estimated that 1,462 bites from infected bugs were necessary to produce a new case in a guinea pig population under field conditions. Following Rabinovich[17] and considering a house where each human receives 0.5 bites/night (Risk 3), a new case may be expected each 2,000 days (5.5 years). But considering that triatomine bites diminish drastically during the cold season,[2] it would takes 7 or more years to produce a new case in humans. This is very well related with the age of maximum incidence in humans. Houses with higher transmission risk would accelerate the process and children would be infected early. These houses require urgent attention from health authorities.

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