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HIGH INFECTION RATES OF *TRIATOMA DIMIDIATA* ARE ASSOCIATED WITH LOW LEVELS OF *TRYPANOSOMA CRUZI* SEROPREVALENCE IN PEDRO CARBO, ECUADOR. USE OF A *TC*24 GENE-BASED PCR APPROACH

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Summary:

In control programs for vectorial transmission of Chagas' disease, conventional microscopic procedures are generally performed to determine baseline levels of infectivity of vectors. Reported here are data using Polymerase Chain Reaction in the detection of *Trypanosoma cruzi* in *Triatoma dimidiata*, one of the principal vectors of Chagas' disease in Ecuador. The microscopy and PCR techniques showed a high percentage of vector infection in Pedro Carbo, province of Guayas (Ecuador), with 44.16 % and 46.13 % positive insects, respectively. This contrasted with the very low Chagas seropositivity recorded (0.5 %). Since *T. dimidiata* was the only vector of the Chagas' disease found in Pedro Carbo and looking at the vector behavior, our data suggest that despite the high *T. dimidiata* infection, the low Chagas seropositivity detected is closely associated with the epidemiological and ecological context of *T. dimidiata* in Pedro Carbo.

KEY WORDS : Chagas' disease, *Trypanosoma cruzi*, *Triatoma dimidiata*, vectorial capacity, polymerase chain reaction, Ecuador.

Résumé : DES TAUX D'INFECTION ÉLEVÉS DE *TRIATOMA DIMIDIATA* PAR *TRYPANOSOMA CRUZI* SONT ACCOMPAGNÉS D'UNE FAIBLE SÉROPRÉVALENCE DANS LE VILLAGE DE PEDRO CARBO (ÉQUATEUR). UTILISATION DE LA MÉTHODE DE POLYMÉRISATION EN CHAÎNE (PCR) POUR L'AMPLIFICATION DU GENE *TC*24.

Les programmes de contrôle de la transmission vectorielle de la maladie de Chagas recourent généralement à des techniques d'observation microscopique pour déterminer le niveau d'infection des vecteurs. Nous rapportons ici l'utilisation de l'amplification par polymérisation en chaîne (PCR) pour la détection de Trypanosoma cruzi *chez* Triatoma dimidiata, *l'un des vecteurs principaux de la maladie de Chagas en Équateur. Les techniques de microscopie et de PCR ont révélé un haut pourcentage d'infection vectorielle à Pedro Carbo, province de Guayas (Équateur), avec respectivement 44,16 % et 46,13 % d'insectes positifs. Ceci contraste fortement avec les très faibles niveaux de séropositivité relevés (0.5 %).* T. dimidiata *étant le seul vecteur de la maladie de Chagas rencontré à Pedro Carbo, nos données suggèrent qu'une faible séropositivité peut être associée au contexte épidémiologique et écologique de* T. dimidiata *dans la ville de Pedro Carbo.*

MOTS CLÉS : *Maladie de Chagas,* Trypanosoma cruzi, Triatoma dimidiata, *capacité vectorielle, amplification par réaction en chaîne, Équateur.*

Chagas' disease is predominantly transmitted by domestic and/or peridomestic *Trypanosoma cruzi* infected reduviids within homes of people living in endemic areas. The use of residual pyrethroid insecticides spraying has been proposed as an efficient method of vector control. Appropriate spraying in the endemic regions of Argentina, Brazil, Chile and Uruguay has stopped the vectorial transmission due to *Triatoma infestans*. In fact, one of the major objectives of the World Health Organization (WHO, 1998) is the interruption of vectorial transmission for Chagas' disease. However, it is not clear if this approach is appropriate for non-domestic vectors. Therefore, the investigation for non-domestic or peridomestic vectors is still of interest. The detection of an infected triatomine bug is classically determined by the microscopic examination of insects fecal samples. The microscopy procedure is specific but time consuming since each insect must be individually dissected and analyzed. Molecular tools such as the Polymerase Chain Reaction (PCR) allow the analysis of multiple samples with high specificity and sensitivity. Previous reports have shown the applicability of PCR for epidemiological studies of *T. cruzi* (Brenière *et al.*, 1995; Dorn *et al.*, 1999; Shikanai *et al.*, 1996).

In this study, we compare the microscopic and PCR method, using *T. cruzi* (*tc*24) specific primers, for detecting *T. cruzi* infection in *Triatoma dimidiata*, one of the principal Ecuadorian vectors of Chagas' disease. For this purpose, triatomine vectors were collected in the rural town of Pedro Carbo located in the province of Guayas, Ecuador. This town is known to be an endemic area for Chagas' disease (Defranc, 1987). The data are compared to the Chagas' disease seropositivity in Pedro Carbo.

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MATERIALS AND METHODS

Vector insects and human blood were collected from the endemic area of Pedro Carbo, Ecuador, in August 1998. Informed consent from individuals involved in the study was obtained. All the triatomine bugs were collected alive from within homes and surroundings areas by trained workers from the SNEM (Servicio Nacional de Erradicación de la Malaria del Ecuador). A total of 722 non selected houses were inspected for one hour searching for the presence of triatomines, the search was performed in domestic (inside houses) and peridomestic habitats (yards around houses), sylvatic habitats were not included. Domestic searches included beds, walls, floors, roofs, furniture; peridomestic searches were focused on spaces beneath the floors, rocks and wood piles, hollow trees, animal housing or nests (dogs, chicken, pigs) and animal borrows. The bugs were transported to the laboratory in plastic vials containing folded filter paper. At the laboratory, individual drops of the bug's feces were mixed with phosphate-buffered saline and examined under a light microscope using a 400 × magnification searching for flagellated parasites. In order to document the parasites, positive samples were fixed on a glass slide, stained with Giemsa and observed at 100 × magnification. In parallel, 10-20 µl of triatomine feces individually collected in 300 µl of TE buffer (10 mM Tris-HCl pH 8.0, 10 mM EDTA), were placed in cryotubes and stored at – 20° C until further PCR analysis. The PCR was performed as described previously (Guevara *et al.*, 1996) with the *T. cruzi tc*24-specific primers T1 5´-GACGGCAA-GAACGCCAAGGAC-3´ and T2 5´-TCACGCGCTCTCCG-GCACGTTGTC-3´. After 35 cycles at 94° C (1 min), 60° C (1 min) and 72° C (2 min), the amplified products were analyzed by 1 % agarose gel electrophoresis and stained with ethidium bromide.

Individual samples of human blood were withdrawn from Pedro Carbo inhabitants and treated as follows. Blood cells were removed by centrifugation (20 min, 1,000 × g, 4° C), and serum was analyzed by microELISA for Chagas seropositivity. Antigens were *T. cruzi* total lysate, 2DE synthetic peptides, as well as the recombinant *Tc*24 protein. Finally, the commercial haemaglutination Serodia® probe was used. In seropositive individuals, clinical diagnosis was performed based on complete clinical history, clinical examination, electrocardiogram and thoracic X-ray examination.

RESULTS AND DISCUSSION

Although the main objective of our study was the comparison between two completely different techniques, namely microscopic observation and PCR, to detect *T. cruzi* in vectors' feces samples, and since the type of construction of houses is considered as a risk factor for *T. cruzi* infestation, it is of interest to mention that of the 722 houses inspected 390 (54 %) were made of bricks, 55 (7.6 %) of cement, 111 (15.4 %) were made of mixed materials and 166 (23 %) were made of bamboo walls and thatch roofs. All houses had yards and dogs, some of them have free-ranging chickens, dogs and bird nests, one had a *Didelphis marsuapialis* burrow. In addition, all houses had rocks and wood piles in their yards. Concerning the 722 houses inspected, 29 (4.0 %) were shown infested with triatomine insects, a total of 271 vectors were collected, 268 (98.9 %) in peridomestic and only three (1.1 %) in domestic areas (Table I). Over the 271 insects collected, only *Triatoma dimidiata* were found. Insect feces were analyzed for the presence of *T. cruzi* using both light microscopy and PCR. The light microscopy analysis of the *Triatoma dimidiata* insect feces showed the presence of metacyclic parasite forms identified as *T. cruzi* using Giemsa stain in 121 out of 271 samples examined (44.16 %) (Table II). The percentage of positive samples increased to 46.13 % (125 positive samples out of 274 analyzed) when using the PCR approach (Table II), as indicated by the presence of a 600 bp DNA fragment corresponding to the *tc*24 amplified gene in *T. cruzi* containing samples as well as in the positive control (Fig. 1).

House		**Habitat of collected vectors n = 271**	
Total 722	Infested (%) 29 (4.0)	Domestic (%) 3 (1.1)	Peridomestic (%) 268 (98.9)

Table I. – Houses evaluated for the presence of *T. dimidiata* vectors in Pedro Carbo, Ecuador.

Samples	**Microscopy**		**PCR**	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
271	121 (44.65)	150 (55.35)	125 (46.13)	146 (53.87)

Table II. – *T. cruzi* infection of the *T. dimidiata* vectors collected in Pedro Carbo, Ecuador.

Using the microscopy method as a "gold standard" in detecting infected bugs, the PCR procedure identified 111 positive samples out of the 121 found positive by microscopic observation (data not shown), thus raising a number of 10 false negative PCR results (3.69 %). This represents a 92 % sensitivity for the PCR procedure. Conversely, 14 samples (5.17 %) were positive for PCR while they were negative by microscopy (data not shown). Both the microscopic and the PCR procedures agreed for 247 of the 271 samples i.e. 91.1 %

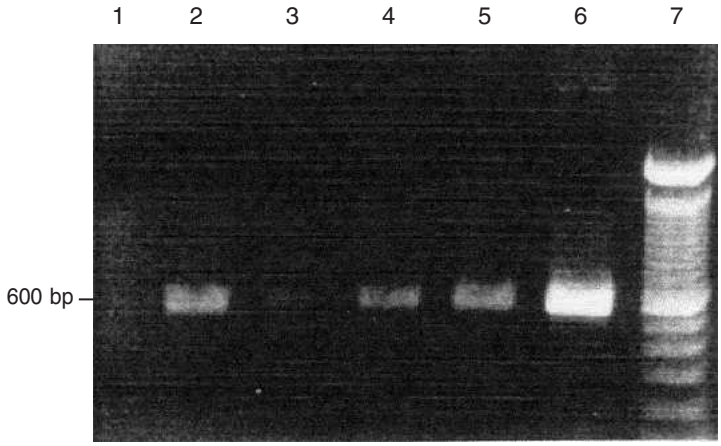

Fig 1. – Detection of *T. cruzi*-infected *T. dimidiata* by the *tc*24 gene-based Polymerase Chain Reaction. DNA amplification was performed on DNA samples extracted from triatomine feces. Positive reactions present a 600 bp amplified DNA fragment.
Lane 1: negative control (uninfected insect). Lanes 2, 3, 4 and 5: positive samples (infected insects) showing various intensity of *tc*24-gene amplification. Lane 6: positive control (*tc*24 plasmid). Lane 7: molecular weight DNA markers.

of the samples examined. Such an agreement between the results obtained with both the microscopic and PCR-based techniques using the *tc*24-specific primers, indicates that not only PCR amplification of the *tc*24 gene can be used for the detection of *T. cruzi* in blood samples, as previously shown in Guevara *et al.* (1996), but also in *T. dimidiata* feces. In a similar epidemiological survey in Guatemala, *T. dimidiata* feces were subjected to PCR with primers hybridizing to the conserved regions of the *T. cruzi* kinetoplast minicircles. However, the authors reported a higher discrepancy between the microscopic and PCR techniques with a number of positive insects 1.5 times higher when using PCR-detection (Dorn *et al.*, 1999). Therefore, we believe that the *tc*24-based PCR is a reliable technique that could be a useful tool for Chagas' disease control based in the detection of infected vectors.
From our study, the infection rate of the *T. dimidiata* living in the domestic and peridomestic area of Pedro Carbo (44 to 46 %) is rather high when compared to that found in other regions of Latin America. For comparison, in Guatemala, where *T. dimidiata* is one of the three main vectors of Chagas' disease, the percentage of infected *T. dimidiata* was reported to vary from 2.5 % to 29.1 % depending on the region considered (Monroy *et al.*, 2003).

T. dimidiata is known as strongly synantropic in coastal Ecuador where it is considered an important Chagas' disease vector (Aguilar *et al.*, 1999; Lazo, 1985) that can invade and sometimes colonize houses or peridomestic structures. Surprisingly, the high infection rate of *T. dimidiata* in Pedro Carbo contrasts with the low seropositivity of the inhabitants, as only two out of 377 blood samples collected (i.e. 0.5 %) were found positive for *T. cruzi* in four different assays (Table III). Since *T. dimidiata* was the only vector of *T. cruzi* found in Pedro Carbo, *T. dimidiata* can be associated with the low levels of seroprevalence in this region of Ecuador. In contrast, in a previous study, we showed that high levels of Chagas seropositivity are found in El Oro (16.4 %) (Garzon *et al.*, 2002), a province of Ecuador where *Rhodnius ecuadoriensis* is known as the principal vector of the Chagas' disease (Abad-Franch *et al.*, 2001). From these results, it is tempting to hypothesize that *T. dimidiata* displays a poor vectorial capacity in Ecuador. Similar observations have also been reported in Guatemala and Honduras where *Rhodnius prolixus* was found a much better vector than *T. dimidiata* (Paz-Bailey *et al.*, 2002; Ponce *et al.*, 1995). However, since our study included only data concerning the presence, distribution (domestic or peridomestic), infestation and infection rates of *T. dimidiata*, we cannot conclude that low seroprevalence of *T. cruzi* found in human population of Pedro Carbo is directly related to poor vectorial capacity of *T. dimidiata* but to the epidemiology of this particular vector. In fact, detailed data related to mobility and dispersion index, source and availability of food for the vector are presently lacking in our study. However, during the course of our study some interesting findings such as high number of parasite-positive *T. dimidiata* found in habitats of various domestic (dogs, cats, birds) and wild animals (marsupials) known as reservoirs for *T. cruzi* support the hypothesis that the proximity between reservoir ani-

Samples			**Pathology**	**Seropositivity (%)**			
Sex	n	Age		Total lysate	Peptides	*rTc*24	Serodia®
F	2	15; 54	A; C	2 (0.5 %)	2 (0.5 %)	2 (0.5 %)	2 (0.5 %)
M	0	–	–	–	–	–	–

F, female; M, male; A, asymptomatic; C, cardiac pathology; n, number of positive samples.

Table III. – Chagas seropositivity among the 377 blood samples collected in Pedro Carbo, Ecuador.

mals and vector insects provides a natural barrier for the transmission to humans. Further studies are required to confirm the low vectorial capacity of *T. dimidiata* and/or the influence of reservoirs, mainly marsupials and dogs (Montenegro *et al.*, 2002) for *T. cruzi* in the low prevalence of *T. cruzi* human infection in Pedro Carbo.

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