HIGH INFECTION RATES OF _Triatoma dimidiata_ ARE ASSOCIATED WITH LOW LEVELS OF _Trypanosoma cruzi_ SEROPREVALENCE IN PEDRO CARBO, ECUADOR.

**Use of a tc24 gene-based PCR approach**

**GUEVARA A.G.***, GARZÓN E.***, BOWEN C.*, CÓRDOVA X.*, GÓMEZ E.** & OUAISSI A.***

**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 FRAÎCHE SÉROPRÉVALENCE DANS LE VILLAGE DE PEDRO CARBO (ÉQUATEUR). UTILISATION DE LA MÉTHODE DE POLYMÉRISATION EN CHAÎNE (PCR) POUR L’AMPLIFICATION DU GENE _tc24_.

Les programmes de contrôle de la transmission vectorielle de la maladie de Chagas recouvrent 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 contrastait fortement avec les très faibles niveaux de séroprévalence relevés (0,5 %). _T. dimidiata_ étant le seul vecteur de la maladie de Chagas rencontré à Pedro Carbo, nos données suggèrent qu’une faible séroprévalence 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.

**C**hagas’ disease is predominantly transmitted by domestic and/or peridomestic _Trypanosoma cruzi_ infected reduvids 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 triatominine 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_ (tc24) specific primers, for detecting _T. cruzi_ infection in _Triatoma dimidiata_, one of the principal Ecuadorian vectors of Chagas’ disease. For this purpose, triatominine 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 seroprevalence in Pedro Carbo.
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 Malária 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 burrows. 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, 10-20µl of triatomine feces individually collected in 300µl 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 tc24-specific primers T1 5’-GACGGCAA-GAACGCCAAGGAC-3’ and T2 5’-TCACGCGCTTCG-GCAGGTGTC-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 Tc24 protein. Finally, the commercial haemaglutination Serodia® test was used. 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 marsupialis 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 tc24 amplified gene in T. cruzi containing samples as well as in the positive control (Fig. 1).

<table>
<thead>
<tr>
<th>House</th>
<th>Habitat of collected vectors</th>
<th>n = 271</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Infested (%)</td>
<td>Domestic (%)</td>
</tr>
<tr>
<td>------</td>
<td>---------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>722</td>
<td>29 (4.0)</td>
<td>3 (1.1)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Microscopy</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>271</td>
<td>121 (44.65)</td>
<td>150 (55.35)</td>
</tr>
</tbody>
</table>

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%
of the samples examined. Such an agreement between the results obtained with both the microscopic and PCR-based techniques using the tc24-specific primers, indicates that not only PCR amplification of the tc24 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 tc24-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-

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pathology</th>
<th>Seropositivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>n</td>
<td>Age</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>15; 54</td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENTS

These investigations received financial support from FUNDACYT (Fundación para la Ciencia y Tecnología del Ecuador), project F-BID 422, Institut de Recherche pour le Développement (IRD) and Institut National de la Santé et de la Recherche Médicale (INSERM). E. Garzón is a recipient of a fellowship from Département Soutien Sante for the Communautés du Sud (IRD). We thank the collaboration of Galo Loor and Carlos Vega (SNEM) in the collection of vectors and that of Wilson Paredes for his technical assistance in the PCR assays. We are indebted to Dr. Keneth E. Voss and Dr. Ronald H. Gudertian for their comments and suggestions on the manuscript.

REFERENCES


Reçu le 28 octobre 2003
Accepté le 21 novembre 2004