USEFULNESS OF SEROLOGY FOR THE EVALUATION OF TRYPANOSOMA CRUZI TRANSMISSION IN ENDEMIC AREAS OF CHAGAS’ DISEASE

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Thirteen communities from 7 Argentinian provinces were selected for the evaluation of serology as an indicator of transmission of Chagas disease. Of the communities appraised, 6 did not have a history of previous treatment with insecticides and 7 had received sporadic or continuous insecticide treatment. The inhabitants of 20% of the houses of each locality were studied by serology. The samples were obtained by finger pricking and 50 μl of blood were mixed with 150 μl of 50% glycerine solution in tissue culture media to be assayed by Indirect Hemagglutination and Indirect Immunofluorescence tests. In untreated areas, the prevalence of infection in infants 0-4 years old was 17.5%, reaching to over 22% for the 5-9 year old group, and to 33.3% in 10-14 year old individuals. The prevalence in treated and surveyed areas was 2.6% in 0-4 year old children, 5.4% in 5-9 year old and 6.2% in 10-14 year old youngsters. The differences between both areas were statistically significant (p < 0.005). This study favors serology as a valid indicator for the evaluation of transmission of Chagas disease in rural areas.


Chagas’ disease produced by infection with T. cruzi, is widely disseminated through the Americas17. The triatomine vector of T. cruzi may lodge in human dwellings, and can be found from Rio Colorado, in Northern Mexico, to the Parallel 42° South in Argentina and Chile27.

The suggestion of controlling domestic triatomine by spraying houses with insecticides as a control measure of disease transmission, was advanced at the beginning of the forties in the Continent10 20. In Argentina, the first steps were undertaken around 1940 and later on, in the mid fifties, control campaigns were initiated in some provinces4 24. In 1962 the programme extended over 9 provinces, and in the seventies the whole country was under control. Activities involved insecticide application in dwellings and serological control of blood to be transfused23. On that occasion, the National Control Agency, the Servicio Nacional de Chagas (ChCA) and the Instituto Nacional de Diagnóstico e Investigación de la Enfermedad de Chagas (INDIECH) were created. The first to care for the control of the insect population and the second, to control the inter-human transmission of Chagas’ disease23.

The activity carried out for the control of vectors consists mainly of insecticide spraying of domiciles during the chemical attack phase13 19. After the attack phase, periodic entomological evaluations are recommended, to be carried out by means of the man/hour method, as part of the so called “classical surveillance” method in treated houses19. These actions, performed by trained personnel of the Control Agency are costly as they demand 60 minutes of work of skilled men per house to be completed. Houses found infested, are sprayed with insecticide13 19.

Serology has been one of the powerful tools to study Chagas’ disease, and to develop control strategies, at first by the use of Complement Fixation and nowadays by the use of Hemagglutination techniques among others, which has permitted is to define endemic areas in the Continent9 12. Serology has been also used by several groups as an alternative method of retrospective evaluation for the status of Chagas’ disease transmission in populations of endemic areas, such as in studies carried out in Argentina and the rest of the Continent8 23. It was demonstrated that the
prevalence of Chagas' disease in 18 year old men in Argentina decreased from 10.3% in 1968 to 5.9% in 1981. Such a decrease included 18 year old men born in 1963, and showed the effect that the control Program may have had on transmission after 19 years of application.

One of the limitations of serology for field work is related to blood sampling. At present, a new procedure is being applied, which enables collection of capillary blood samples by finger puncture, rendering unnecessary, for the next 30 days after collection, any special laboratory conditions and allowing the storage of samples at room temperature and humidity.

The serological study of children is relevant because it would permit the detection over short periods of the effects of the control actions.

This paper outlines a proposed scheme for serological studies in rural communities, which could be used as a prospective tool for the evaluation and planning of control campaigns. For that purpose all the inhabitants of each house selected in thirteen areas from seven Argentinian Provinces, were serologically studied. Serological results were correlated with the activities carried out by the Argentinian Chagas disease Control Agency (ChCA).

MATERIALS AND METHODS

This study was performed from December, 1983, to May, 1985 and involved thirteen rural villages from thirteen counties of seven Argentinian Provinces (Figure 1). These selected localities were similar in the number and type of population, housing, geographical distribution and socio-economical conditions.

The areas were selected according to the below-mentioned criteria, and with data obtained from records of the ChCA.

Category 1) Areas without chemical treatment. Areas which had not received insecticidal treatment up to the date when the study began.

Category 2) Areas chemically treated
a) Surveyed: areas chemically treated in the attack phase and surveyed by the Control Programme more than once in a determined period.

b) Recently treated, or areas where no surveillance activities were made for a long period of time (Table 1).

Sampling procedures: The houses selected were considered as the sampling unit, studying all their occupants. According to cartographic information obtained from the Primary Health Care Programme (PHC), an average of 15% to 20% of the houses

inhabited in the whole area were selected representing one of five of the houses existing in the area.

The first house to be surveyed was selected at random and then a systematic sampling procedure was used. Those people absent at the beginning of the study were included upon their return.

**Sample collection and serological study:** Blood samples were collected by pricking a fingertip with a disposable sterile blade, collecting 50μl with a gauged capillary tube, and mixing the sample with 150 μl of the preservative contained in a polypropylene tube with an hermetic rubber cap.

These tubes were placed in 60-tube trays each one contained in a woodenbox suitable for handling. Blood samples collected in the field were sent by mail to INDIECH to be analysed. Indirect

### Table 1 - Distribution of serological prevalence for T. cruzi infection grouped by area, age-group and category of insecticide treatment – Argentina 1983-1985.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>City</th>
<th>Country-Prov</th>
<th>% (Pos/Np)</th>
<th>% (Pos/Np)</th>
<th>% (Pos/Np)</th>
<th>% (Pos/Np)</th>
<th>% (Pos/Np)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>Caden - Retamo</td>
<td>Ayacucho - San Luis</td>
<td>9.5 (2/21)</td>
<td>22.2 (4/18)</td>
<td>33.3 (5/15)</td>
<td>33.3 (4/12)</td>
<td>29.9 (26/87)</td>
<td>1</td>
</tr>
<tr>
<td>5-9</td>
<td>Amama</td>
<td>Moreno - Sgo. del Estero</td>
<td>13.0 (3/23)</td>
<td>24.2 (8/33)</td>
<td>47.7 (21/44)</td>
<td>48.5 (16/33)</td>
<td>44.4 (20/45)</td>
<td>1</td>
</tr>
<tr>
<td>10-14</td>
<td>San Martin N°</td>
<td>Patiño - Formosa</td>
<td>22.9 (14/61)</td>
<td>40.0 (48/120)</td>
<td>55.9 (33/59)</td>
<td>24.1 (13/54)</td>
<td>42.8 (36/84)</td>
<td>1</td>
</tr>
<tr>
<td>15-19</td>
<td>Los Telares Quebrachos - Sgo. del Estero</td>
<td>10.0 (2/20)</td>
<td>34.8 (8/23)</td>
<td>41.2 (7/17)</td>
<td>38.5 (5/13)</td>
<td>60.0 (36/60)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>+ 20</td>
<td>Santa Victoria E.</td>
<td>San Martin – Salta</td>
<td>26.7 (4/15)</td>
<td>46.0 (58/126)</td>
<td>48.5 (65/134)</td>
<td>35.0 (7/20)</td>
<td>N.D.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sotelos</td>
<td>Río Hondo - Sgo. del Estero</td>
<td>22.2 (2/9)</td>
<td>42.1 (8/19)</td>
<td>44.4 (4/9)</td>
<td>(1/3)</td>
<td>54.5 (18/33)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Yavi Chico Yavi - Jujuy</td>
<td>0.0 (0/12)</td>
<td>0.0 (0/11)</td>
<td>0.0 (0/8)</td>
<td>0.0 (0/10)</td>
<td>19.0 (4/21)</td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lobaton - Acheral San Pedro - Jujuy</td>
<td>2.8 (2/72)</td>
<td>5.5 (4/73)</td>
<td>6.2 (1/16)</td>
<td>17.6 (3/17)</td>
<td>54.5 (30/55)</td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paclín El Alto - Catamarca</td>
<td>0.0 (0/32)</td>
<td>0.0 (0/21)</td>
<td>0.0 (0/24)</td>
<td>0.0 (0/26)</td>
<td>37.5 (18/48)</td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chancani Pocho - Córdoba</td>
<td>2.8 (1/35)</td>
<td>23.8 (15/63)</td>
<td>45.8 (11/24)</td>
<td>52.2 (12/23)</td>
<td>56.4 (35/62)</td>
<td>2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pirquitas Huaycamo Ambato – Catamarca</td>
<td>5.0 (1/20)</td>
<td>19.2 (5/26)</td>
<td>20.0 (3/15)</td>
<td>21.4 (3/14)</td>
<td>16.0 (4/25)</td>
<td>2b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>San Martin N° 1 Patiño – Formosa</td>
<td>0.0 (0/10)</td>
<td>20.8 (5/24)</td>
<td>21.4 (3/14)</td>
<td>23.0 (3/13)</td>
<td>18.4 (7/38)</td>
<td>2b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pajonal Poman Catamarca</td>
<td>5.7 (2/35)</td>
<td>12.0 (7/58)</td>
<td>8.8 (3/34)</td>
<td>N.D.</td>
<td>27.0 (10/37)</td>
<td>2b</td>
<td></td>
</tr>
</tbody>
</table>

N.D. Not Done: 1 = without treatment 2a = with treatment and continuous surveillance 2b = with treatment and no regular surveillance.

Hemagglutination (IHA), and Indirect Immunofluorescence (IFI) reactions, specific for anti T. cruzi antibodies were used as the serologic tests and non coincident results between both reactions were verified by the Enzyme Immunoassay (ELISA) using T. cruzi epimastigote membrane antigens. IHA and IFI provided a 99.5% of specificity when performed together at the minimum reactive dilution of 1:325. The initial serum dilution was 1:16 in all cases due to the 1:4 dilution of the blood in the preserver. Reactivity of blood samples with the preservative had up to a 97% correlation with identical samples of serum obtained from venopuncture as was previously described.

RESULTS

A total of 2226 blood samples were collected from patients of both sexes with ages ranging from 6 months to 93 years of age. Serological analysis showed 670 positive results (30.09%).

Serological results of the studied samples are presented in Table 1, grouped by the origin of the area: city, county or province; by the age groups of the population; and by the modality of application of the chemical treatments performed in the houses.

All areas which had not received insecticidal treatment (Category 1) showed similar results in terms of prevalence: in children aging from 0 to 4 years, higher than 10%; an increasing prevalence of more than 22.2% in children from 5 to 9 years of age, reaching its maximum level in children aging from 10 to 14 years. No significant differences were found among prevalence rates shown by the 10 to 14 years old group with respect to children 15 to 19 years of age or 20 years or older (\( p > 0.1 \)). For instance, the results for the San Martin 2 population, in Patino, in the province of Formosa, a locality of this category, are presented in Figure 2.

The prevalence rates observed in areas treated and continuously surveyed for household reinfestations by triatomines (Category 2a) are similar to those observed in the cities of Lobaton and Acheral, San Pedro, in the Province of Jujuy (Figure 2). In these areas, children aged from 0 to 4 years, showed a prevalence of 2.6% while those from 5 to 9 years of age had a prevalence rate of 5.4%; those from 10 to 14 years showed a rate of 6.2%, which rose to 17.6% in children from 15 to 19 years of age and to 54.5% in people older than 19 years of age.

Within Category 2b in the locality of Pajonal, in the Province of Catamarca, where control activities were not continuous, children 5-9 years of age showed an increased prevalence rate of 12% compared to children 10 to 14 years of age (8.8%), but these differences are not statistically significant (\( p > 0.1 \)).

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[[Image: Figure 2 - Serological prevalence to T. cruzi infection according to age-group: a) (-----) San Martin 2, Formosa: without chemical treatment; b) (--------) Lobatón y Acheral, Jujuy: with chemical treatment and continuous surveillance.]]

Serological reactivity of those populations which were chemically treated with insecticides compared to non-treated areas are analysed in Figure 3. A significant difference was observed between both regions, where children from 0 to 4 years of age belonging to chemically treated areas show an average prevalence rate of 2.3%, whereas children of the same age living in non-treated areas show a prevalence rate of 17.5% (\( p < 0.005 \)).

[[Image: Figure 3 - Distribution of average serological prevalence to T. cruzi infection obtained in Argentine in 6 localities without chemical treatment (blank columns) and 7 localities with chemical treatment (striped columns).]]
DISCUSSION

The serological prevalence rates for Chagas' disease in rural areas without insecticidal treatment shown in this study are coincident with the relative values obtained by Rosenbaum and Cerisola at the end of the fifties\(^1\)\(^,\)\(^2\)\(^,\)\(^1\) and confirm those obtained by Bonet et al\(^3\). In all cases serological prevalence rates increase with the age of the individuals, reaching their maximum in the age groups older than 40.

Data obtained confirm previous findings in Argentina which show that the highest risk of infection, in areas of active transmission, is below 10 to 14 years of age, reflected on the fact that at least 50% of the total infected population is already infected at this age\(^2\)\(^,\)\(^1\). These data are coincident among others with those found in Bambui, Brasil\(^1\)\(^,\)\(^1\) and Venezuela\(^1\)\(^,\)\(^8\).

The results shown here present, according to Rosenbaum, "Information about what happened in the past with individuals studied in the present", where each individual under study represents a cross section of the different stages of the evolutive cycle of the disease\(^1\)\(^,\)\(^2\)\(^,\)\(^1\).

This assertion becomes demonstrated by the increasing prevalence with steeper slopes for areas without treatment than for chemically treated regions.

In those areas where chemical treatment was performed, a prevalence rate of 2.3% was found in children 0 to 4 years of age, whereas in non-treated areas a prevalence rate of 17.5% was found in this age group, evidencing the effects of control measures against domestic triatominne with promotion of community participation. Once more the use of chemical control actions ensures the interruption of \(T.\) cruzi human transmission and the surveillance appears necessary to maintain this condition in the long-term. These assertions are reflected in locality of Chancani, Córdoba, where the area was sprayed every 4-5 years, and in Lobaton where surveillance was performed continuously from 1966 up to the present time by the current programme of health of the province of Jujuy. In both localities interruption of the transmission was successfully achieved, in the early eighties in the former and at the end of the sixties in the latter.

Research work on the serological prevalence of \(T.\) cruzi infection in children gives information about the status of transmission at the moment of sample collection, about its history and its future evolution, the latter, depending on the actions to be implemented.

In chemically treated areas where continuous entomological surveillance activities were carried out, serological prevalence rates of \(T.\) cruzi infection in children up to 4 years of age tended to decrease, whereas this trend in those areas where no surveillance measures were taken was not observed. Thus serology is a suitable indicator of the state of the transmission of infection by \(T.\) cruzi in rural communities before or after any control campaign intervention\(^4\).

Control Programmes current surveillance and evaluation activities are based on the use of the results of their own entomological surveys as indicators of the state of transmission. The extent of the endemic area and control programme constraints concerning trained personnel and its mobilization to the areas makes it difficult to achieve regular evaluations by this methodology. Serology, on the other hand, would allow the undertaking of surveys in the hands of local trained human resources, becoming a useful tool to help in a Control Programme current activities.

Control Programmes have, at present, a variety of improved and new technologies compared with those available at their origins that open new possibilities. This fact would permit the beginning of a gradual change in the philosophy of control Programmes and the use of new laboratory's methodologies, like serology in the hands of their own well-trained field agents, this would effectively accomplish the scope and objectives of the programmes.

In summary, the results of prevalence of \(T.\) cruzi infection obtained from serological sampling performed in rural areas from thirteen departments of seven endemic provinces for Chagas' disease in Argentina, qualify serology as an appropriate technology, easily-available, economic, and a sensitive indicator of the status of the transmission, providing a tool for the evaluation of control actions performed and the planning of future activities.

RESUMO

Treze comunidades de 7 províncias argentinas foram escolhidas para avaliação de sorologia como indicador da transmissão da Doença de Chagas. Das comunidades mencionadas, seis não tinham história prévia de tratamento com inseticidas e sete tinham recebido tratamento esporádico ou continuado.

Vinte por cento dos moradores das casas de cada localidade foram estudados por sorologia. As amostras foram obtidas por punção da ponta do dedo e 50 microlitros de sangue foram misturadas com 150 microlitros de uma solução conservadora de glicerin a 50% em meio de cultivo, para serem estudados por hemaglutinação indireta, e imunofluorescência indireta. Nas áreas não tratadas a prevalência da infecção em crianças de 0-4 anos foi de 17,5% chegando a mais de 22% para as de 5-9 anos e a 33,3% no grupo 10-14 anos. A prevalência nas áreas tratadas e sob vigilância foi de 2,6% em crianças de 0-4 anos, 5,4% anos de 5-9 anos e de 6,2% em jovens de 10-14 sendo as diferenças entre os dois tipos de áreas estatisticamente significativas (< 0,005).
Este estudo propõe a sorologia como um indicador válido para a avaliação da transmissão da Doença de Chagas em áreas rurais.


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REFERENCES


