

## Seroprevalence of *Trypanosoma cruzi* (TC) and risk factors in Colima, Mexico

Oscar Alberto Newton-Sánchez<sup>1,3\*</sup>, Francisco Espinoza-Gómez<sup>1,3</sup>, Valery Melnikov<sup>1</sup>, Iván Delgado-Enciso<sup>1,4</sup>, Fabián Rojas-Larios<sup>1,3</sup>, Eric Dumonteil<sup>2</sup>, Benjamín Trujillo-Hernández<sup>1</sup> and Miriam de la Cruz-Ruiz<sup>1</sup>

<sup>1</sup>Grupo de Estudio de las Enfermedades Transmisibles, Facultad de Medicina de la Universidad de Colima, Colima, Col.; <sup>2</sup>Universidad Autónoma de Yucatán, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Mérida, Yuc.; <sup>3</sup>Hospital Regional Universitario, Servicios de Salud del Estado de Colima, Colima, Col.; <sup>4</sup>Instituto Estatal de Cancerología, Servicios de Salud del Estado de Colima, Colima, Col. Mexico

### Abstract

**Introduction:** The present study was conducted to estimate the incidence of seropositivity to anti-*Trypanosoma cruzi* antibodies and analyze potential risk factors in Colima, on the western coast of Mexico. **Methodology:** Longitudinal studies of 209 subjects with negative serology in 1999 for anti-*Trypanosoma cruzi* antibodies by hemagglutination inhibition test were tested again in 2005. At the same time, 716 children under six years of age were surveyed serologically (total  $n = 925$ ); the history of *Trypanosoma cruzi* infection was determined by the same hemagglutination inhibition test. The variables analyzed were age, sex, living in triatomine-infested places, type of community, quality of housing, presence of pets, and number of inhabitants per house. **Results:** *Trypanosoma cruzi* seropositivity in the period of six years was 22/925 cases, with a point prevalence of 2.73% and an adjusted rate of 7.3/1,000 person-years. The variable living in triatomine-infested areas showed association with seropositivity anti-*Trypanosoma cruzi* antibodies (RR: 5.5; 95% CI: 1.28-23.5). The remaining variables showed no significant association. **Conclusions:** This study confirms the active transmission of Chagas disease in Mexico's west-central region, which merits greater epidemiological surveillance and vector control, particularly in localities infested with triatomines. (Gac Med Mex. 2017;153:179-84)

**Corresponding author:** Oscar Alberto Newton-Sanchez, onewton@uocol.mx

**KEY WORDS:** American trypanosomiasis. Chagas disease. *Trypanosoma cruzi*. Colima.

### Resumen

**Introducción:** El estudio se realizó para estimar la incidencia de la positividad a anticuerpos contra *Trypanosoma cruzi* y analizar los potenciales factores de riesgo en Colima, en el Occidente de México. **Método:** Estudio longitudinal de 209 sujetos con serología negativa en 1999 para anticuerpos contra *T. cruzi* por hemaglutinación indirecta y examinados de nuevo en 2005. Al mismo tiempo fueron examinados serológicamente por el mismo método de hemaglutinación indirecta 716 niños menores de 6 años ( $n$  total = 925). Las variables analizadas fueron edad, sexo, vivir en lugares infestados por triatomíneos, tipo de comunidad, calidad de vivienda, presencia de mascotas y número de habitantes por casa. **Resultados:** La seropositividad para anticuerpos contra *T. cruzi* en el periodo de 6 años fue de 22/925 casos, con una prevalencia de

#### Correspondencia:

\*Oscar Alberto Newton-Sanchez  
Facultad de Medicina  
Universidad de Colima  
Avenida Universidad, 333  
Col. Las Víboras  
C.P. 28040, Colima, Col., México  
E-mail: onewton@uocol.mx

Fecha de recepción en versión modificada: 09-01-2016

Fecha de aceptación: 27-01-2016

punto de 2.73% y una tasa ajustada de 73/1000 personas-año. Vivir en áreas infestadas por triatominos mostró una asociación con la seropositividad de anticuerpos contra *T. cruzi* (riesgo relativo [RR]: 5.5; intervalo de confianza del 95% [IC 95%]: 1.28-23.5). El resto de las variables no mostraron asociación significativa. **Conclusiones:** El presente estudio confirma la transmisión activa de la enfermedad de Chagas en la región centro-occidente de México, donde se requiere mayor vigilancia epidemiológica y control del vector, en especial en las localidades infestadas por triatominos.

**PALABRAS CLAVE:** *Tripanosomiasis americana. Enfermedad de Chagas. Trypanosoma cruzi. Colima.*

## Introduction

Infection by TC and its clinical outcome, Chagas disease (ChD), remains a serious public health problem in America even though some regions of South America have made important progress in its control<sup>1,2</sup>. Traditionally, ChD is considered a health problem characteristic of rural regions in South America<sup>1</sup>. However, its transmission has been documented in both in Central and North America, including the southern United States<sup>3</sup>. While Andean and Southern Cone countries have made significant progress in the control of the disease<sup>4</sup>, other countries like Mexico have limited the application of control programs<sup>1</sup>.

The persistence of TC infection in several regions could be explained in part because it is not easily identified since the acute infection is often limited to a non-specific febrile event, which seldom arouses sufficient clinical suspicion to call for specific studies aimed at identifying the parasite. On the other hand, in its chronic phase, ChD is usually characterized by cardiac or gastrointestinal manifestations that are often confused with other chronic degenerative diseases. Therefore, TC infection is often underreported and its epidemiologic surveillance is challenging. A review of the literature revealed that the average prevalence of TC infection in Mexico could reach 5.88%<sup>5</sup>, while the National Center for Epidemiological Surveillance of the Mexican Secretary of Health recorded only 3,551 cases during the period from 2000 to 2010, or about a few hundred cases per year<sup>6</sup>.

Although TC can be transmitted through blood transfusions, organ transplantation, laboratory accidents, ingestion of food contaminated with the parasite<sup>7,8</sup> or transplacentally in neonates<sup>9</sup>, the main form of infection occurs through contact between humans and feces of the triatomine vector (Hemiptera, Reduviidae). Studies estimating the risk posed to persons living in communities infested by these insects and ChD's subsequent development in humans, have demonstrated that to live in rural zone, with a low socioeconomic level, and a greater number of people per dwelling are the most

frequent risk factors detected<sup>10,11</sup>. However, the association of these variables directly with the incidence of human infection has not been sufficiently studied.

The state of Colima, located on the west central Pacific coast of Mexico, is an area where the presence of *Triatoma pallidipennis* (Stål) and *T. longipennis* (Ussinger) infected with TC in indoor spaces has been documented<sup>12</sup>. Also, as much as 2% of the human population in Colima has resulted positive for anti-TC antibodies, which indicates transmission of the parasite in the region<sup>13</sup>. Unfortunately, in Mexico to date, there are no accurate estimates of the temporal distribution of ChD and if its incidence is increasing or decreasing. This information is significant because it would help health authorities to implement policies for vector control and systematic strategies to diminish the transmission of TC, but also would allow evaluating the risk of the disease around to other localities, including those where it has previously been eradicated.

In order to estimate the incidence of seropositive anti-TC antibodies in the inhabitants of the state of Colima, Mexico, and analyze the possible factors associated with its incidence, we followed up a cohort of the region's population from 1999 to 2005. We also evaluated seropositive anti-TC antibodies in children aged less than six years with the premise that they were TC seronegative at birth.

## Material and methods

### Study populations and design

The study involved the follow-up of two cohorts of inhabitants of the state of Colima, located on Mexico's Pacific coast (North 19° 31' South 18° 41' latitude, East 103° 29' West 104° 41' longitude) with a population of 650,555 inhabitants<sup>14</sup>. One of the cohorts was formed by a group of 209 previously studied subjects who participated in a survey conducted in Colima in 1999<sup>13</sup>, who had tested negative for the presence of TC antibodies and continued to live in areas that had previously been registered (Table 1). The second cohort

**Table 1. Demographic characteristics of the people sampled in Colima, Mexico during 1999 throughout 2005 for detection of seropositivity to anti-*T. cruzi* antibodies (n = 925)**

Cohort	Male	Female	Mean age (years)	Urban/Rural
Seronegative to <i>T. cruzi</i> in 1999	69	140	37.7	119/90
Children < 6 years old	358	358	2.4	385/331
Totals	427	498	10.4	504/421

**Table 2. Association between seropositivity to *T. cruzi* and potential risk factors**

Variable	Sampled subjects	Positive Anti- <i>T. cruzi</i> antibodies	Exposure (months/persons)	Rate ratio (95% CI)	Wald's p
Female	498	12	41.6	1.13	0.76
Male	427	10	35.6	(0.5-2.6)	
Age ≤ 6 years old	716	9	29.16	2.0	0.1
> 6 years	209	13	72.0	(0.85-4.7)	
Rural settlement	504	14	37.94	0.65	0.33
Urban settlement	421	8	39.9	(0.27-1.55)	
Residence in an infested locality with triatomine bugs	498	20	46.5	5.5	0.021*
Without triatomine	427	2	29.85	(1.28-23.5)	
Incomplete housing	265	5	38.8	0.73	0.73
Complete housing	660	17	38.8	(0.27-1.98)	
Blood transfusion Yes	35	1	60.62	0.63	0.65
Blood transfusion No	714 <sup>†</sup>	21	39.68	(0.09-4.7)	
Domestic animals	432	17	47.8	0.64	0.39
No domestic animals	224 <sup>‡</sup>	5	41.9	(0.23-1.75)	
Habitants per house ≥ 4	334	12	47.9	1.05	0.9
Habitants per house < 4	308 <sup>§</sup>	10	45.5	(0.45-2.43)	

Associations were analyzed by means of univariate Poisson regression.

\*Significance test:  $\chi^2 = 8.27$ ,  $p = 0.004$ . <sup>†</sup>Whether or not 176 subjects had received blood transfusions could not be determined. <sup>‡</sup>Recorded in 656 houses. <sup>§</sup>Number of permanent inhabitants reported was 642 persons.

consisted of a group of 716 children under six years of age who were randomly selected from rural and urban populations of the state of Colima (Table 1). This selection was stratified according to the presence or absence of triatomines in the subjects' homes. In addition, cord blood samples of a total of 165 newborns were taken from the same hospital unit headquarters of the investigators to verify the absence of neonatal TC infection and to support the premise of seronegativity in the children's cohort (all were seronegative by hemagglutination inhibition).

### Variables studied

The exposure factor of the cohorts was defined as the time they had resided in an area infested with

intra-domestic triatomine. These areas were defined as being villages in which at least two houses showed the presence of indoor triatomines. The longest time of exposure per person was 72 months for the 1999-2005 cohorts and the age in months of children six years or younger. Also, the following variables were recorded: rural or urban locality (less or more than 2,500 inhabitants, respectively); complete or incomplete housing, based on the criteria suggested by De Andrade<sup>15</sup>; presence of pets (dogs or cats); and previous contact with triatomines. These data were obtained from previous surveys carried out at the time of the study in the selected households and at least in five neighboring houses, by means of the man-hour method proposed by Schofield<sup>16</sup>. Other potentially intervening variables recorded were: number of occupants per dwelling, age, sex,

previous history of fever for more 10 days, and having received a blood transfusion. The number of occupants per dwelling and age were expressed as continuous variable, while the other variables were coded into dichotomous nominal scales. The dependent variable was the presence of antibodies to TC by the hemagglutination inhibition test (HAI) with Chagatest kit, Wiener laboratory, considering a dilution  $\geq 1:16$  with mercaptoethanol, according to the manufacturer's specifications<sup>17</sup>, with the aim to determine the change of seronegative to seropositive and not to diagnose the clinical disease.

### Statistical analysis

The crude incidence rate was calculated with its corresponding 95% confidence interval (95% CI), based on the subjects who tested seropositive for anti-TC antibodies over the total number of subjects sampled and adjusted for the duration of exposure. The association between anti-TC antibodies (positive seroprevalence) and the rest of the variables was explored by adjusting the total exposure time for months/person or months/age and was analyzed by means of Poisson univariate regression, estimating the rate of transmission and its relative risk and respective 95% CI. The Wald's p-value and  $\chi^2$  were used to assess statistical significance using the PEPI 2 software<sup>18</sup>.

### Ethics

The study was approved by the Institutional Commission for Research and Bioethics of the Secretary of Health of the State of Colima, and each participant signed an informed consent for participation in the study. The TC seropositive subjects were sent to the Secretary of Health for confirmation of ChD and its treatment.

### Results

The total crude incidence for the two cohorts was of 2.37% (95% CI: 1.4-3.3), based on 22/925 cases, which when adjusted for exposure corresponded to 7.3 cases per 1000 persons per year. The cohort of adult subjects who were previously seronegative in 1999 that was studied included 209 individuals, of which 140 were woman and 69 were men, with an average age of 37.7 years (95% CI: 34.9-40.5) and an exposure time of 72 months/person (period 1999-2005). The seropositive anti-TC antibodies rate in the group was 13/209 (6.22%; 95% CI: 2.9-9.5), corresponding to 1.03% cases per year of follow-up.

For the cohort of 716 children under six years of age, 358 were female and 358 were male, with an average age of 2.4 years (95% CI: 2.27-2.51) and an average exposure period of 29.16 months/person (95% CI: 27.7-30.6). In this group, the seropositive anti-TC antibodies rate was 9/716 cases (1.25%; 95% CI: 0.43-2.0), which resulted in an incidence of 0.51% per year of exposure. It should be noted that there were three positive cases of anti-TC antibodies among 402 children younger than two years of age.

The incidence adjusted for months of exposure seemed somewhat higher in adults compared to children, but this difference was not significant (RR: 2.0; 95% CI: 0.85-4.7;  $p = 0.1$ ). Finally, as specified in the section of material and methods, no positive case of anti-TC antibodies was found in the newborns that were tested in the study.

Importantly, we found that of the 925 total individuals studied, 498 resided the entire time in communities with intra-domiciliary triatomine infestation, and thus were exposed, while 427 lived in areas without triatomines. The triatomines found were of the same species identified in the 1999 survey (*T. pallidipennis* and *T. longipennis*). The localities that were positives for triatomines and cases of TC infection were located within a region of approximately 25 km in diameter, in the center of the state. Table 2 shows the results of seropositive anti-TC antibodies rates adjusted for exposure (living in a locality with triatomines), and its association with potential risk factors assessed by univariate Poisson regression. The different variables included sex, type of settlement, housing quality, presence of domestic pets, transfusions, and self-reported contact with triatomines.

Secondarily a subgroup of 661 people was surveyed specifically for the occurrence of febrile symptoms for more than 10 days in the previous five years. Of this subgroup, 12 reported having had this febrile event, of which four tested positive for anti-TC antibodies. The association between the febrile symptoms and anti-TC antibodies was very significant, even after adjusting for exposure time (RR: 7.62;  $p = 0.0002$ ).

### Discussion

This study confirms that TC is actively transmitted in Colima, Mexico. Indeed, the presence of at least three children under the age of two years with anti-TC antibodies, as well as an incidence of 7.3 cases per 1000 persons per year, reveal that transmission continues to be active in the region, which differs considerably from the

idea that Chagas disease is undergoing a process of remission and, thus, a low priority in Mexico's health programs. With this information and considering that Colima has a population of 650,555 inhabitants<sup>14</sup>, our results allow us to estimate that at least 4,500 people could be infected each year and of these, 900 may develop some form of chronic disease, which may well be diagnosed as cardiopathy caused by some other etiology.

The hemagglutination inhibition test used to detect anti-TC antibodies in our study has a sensitivity of 74.9% and specificity of 99.2%<sup>19</sup>. Thus, the expected 25% false negatives suggested that our incidence data are very likely underestimated, whereas virtually all subjects testing positive were true positive. Therefore, a single HAI test is highly reliable as a screening test for TC infection, information that coincides with other studies<sup>20-23</sup>, although additional tests would be required for confirmatory diagnosis of Chagas disease and treatment, as WHO recommended<sup>24</sup>. The absence of seropositive anti-TC antibodies in newborns suggest that vertical transmission is very rare in Mexico, and in turn indicates that virtually 100% of children surveyed in this study were seronegative at birth and the infection observed was recent.

The exposure factor investigated, i.e. living in localities with the presence of triatomines within households, has been documented to be highly associated with the incidence of TC infection, regardless of whether the households were located in rural or urban areas<sup>2</sup>. Furthermore, the quality of housing did not prove to be a significant causal factor of TC infection. Previous studies show that the presence of intra-domestic triatomine and people testing seropositive is geographically limited to a small area in the central part of the state of Colima<sup>12,13</sup>. On this occasion, we were able to confirm the new cases of seropositive anti-TC antibodies only in the same localities where it had previously been identified. The very specific geographic area infested by the vector and, therefore, the localized transmission of TC could be explained by the tendency of *T. pallidipennis* and *T. longipennis* to occupy small patches within specific geographic areas rather than in more diffuse geographically distributed regions. However, this assumption of clustering in triatomine populations deserves a more detailed exploration.

Importantly, although TC infection has been associated with poverty in marginalized rural areas in Mexico, living in a town infested with triatomines appears to place the population at a greater risk of acquiring TC than other risk factors, including poverty, incomplete housing, and having more than three persons per household, as suggested by other authors<sup>8,11,22,25</sup>, as well as resistance of

the vector to insecticides<sup>26</sup>. This study suggests that entomological surveillance, epidemiologic and control measures should focus on communities where triatomines have been detected in intra-household conditions, regardless of the physical conditions of the home or the socioeconomic status of its inhabitants.

On the other hand, our study showed no association at the individual level of TC infection with any of the variables such as age, sex, having received transfusions, presence of pets, or contact with triatomines, except in cases of resident sites infested with triatomines.

Association between seropositive anti-TC antibodies and fever of long duration should alert clinical suspicion of Chagas disease as a possibility in the differential diagnosis of fever in communities infested with triatomines, which needs to be studied further.

Confirming the active transmission of TC in Colima, in the western coast of Mexico, by identifying newly acquired seropositive anti-TC antibodies in patients, requires the implementation of intensive control measures, as is contemplated by the Official Mexican Normativity for monitoring and controlling vector-borne diseases<sup>27</sup>, especially in the communities where the presence of intra-household triatomines has been identified, since currently there are no formal active programs for triatomine control.

To date, Chagas disease is considered a problem of low priority in Mexico. However, the persistence of foci of active transmission, such as the one we described in Colima, should alert the health authorities about the possibility of persistence and re-emergence of this serious health problem. This situation has been stressed by some authors<sup>5,22,23,25</sup> who urge to make a wide national revision of the strategies to be used for the control of Chagas disease in Mexico<sup>28</sup>. Furthermore, it is imperative to follow-up and provide treatment to patients infected with TC, in spite of the absence of symptoms, because the medical treatment in this phase could halt the progression of chronic disease<sup>2</sup>.

Finally, containment of TC infection in Mexico would not only reduce the socioeconomic burden of this disease on the system, but would also make an important contribution to the global eradication initiative of this disease.

## Declaration of interest

Financial support: Fondo Ramon Alvarez Buylla de Aldana # 255/04 (University of Colima) and the Integral Program for Institutional Strengthening (PIFI) of the Secretary of Education, Mexico.



## References

- Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. *Mem Inst Oswaldo Cruz*. 2009;104:17-30.
- Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. *Lancet*. 2010;375:1388-402.
- Sarkar S, Strutz SE, Frank DM, Rivaldi CL, Sissel B, Sánchez-Cordero V. Chagas disease risk in Texas. *PLoS Negl Trop Dis*. 2010;4:e836.
- Dias JCP, Silveira AC, Schofield CJ. The impact of Chagas disease control in Latin America: a review. *Mem Inst Oswaldo Cruz*. 2002;97:603-12.
- Cruz-Reyes A, Pickering-López JM. Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years - A review. *Mem Inst Oswaldo Cruz*. 2006;101:345-54.
- Bottazzi ME, Dumonteil E, Valenzuela JG, Betancourt-Cravioto M, Tapia-Conyer R, Hotez PJ. Bridging the innovation gap for neglected tropical diseases in Mexico: capacity building for the development of a new generation of antipoverty vaccines. *Bol Med Hosp Infant Mex*. 2010;68:138-46.
- Wendel S. Transfusion transmitted Chagas disease: is it really under control? *Acta Trop*. 2010;115:28-34.
- Belaunzarán ML. Chagas disease: Globalization and new hope for its cure. *Rev Argent Microbiol*. 2015;47: 85-7.
- Oliveira I, Torrico F, Muñoz J, Gascon J. Congenital transmission of Chagas disease: a clinical approach. *Expert Rev Anti Infect Ther*. 2010; 8:945-56.
- Buekens P, Almendares O, Carlier Y, et al. Mother-to-child transmission of Chagas' disease in North America: Why don't we do more? *Matern Child Health J*. 2008;12:283-6.
- Medina-Torres I, Vázquez-Chagoyán JC, Rodríguez-Vivas RI, de Oca-Jiménez RM. Risk factors associated with triatomines and its infection with *Trypanosoma cruzi* in rural communities from the southern region of the State of Mexico, Mexico. *Am J Trop Med Hyg*. 2010;82:49-54.
- Espinoza-Gómez F, Maldonado-Rodríguez A, Coll-Cárdenas R, Hernández-Suárez CM, Fernández-Salas I. Presence of triatominae (Hemiptera, Reduviidae) and risk of transmission of Chagas disease in Colima, México. *Mem Inst Oswaldo Cruz*. 2002;97:25-30.
- Coll-Cárdenas R, Espinoza-Gómez F, Maldonado-Rodríguez A, Reyes-López PA, Huerta-Viera M, Rojas-Larios F. Active transmission of human Chagas disease in Colima Mexico. *Mem Inst Oswaldo Cruz*. 2004;99:363-8.
- Instituto Nacional de Estadística Geografía e Informática (INEGI). (2010). Censo Nacional de población 2010. INEGI, México, 2010. <http://www3.inegi.org.mx/sistemas/sisept/Default.aspx?t=mdemo148&s=est&c=29192>
- De Andrade AL, Zicker F, De Oliveira RM, et al. Evaluation of risk factors for house infestation by *Triatoma infestans* in Brazil. *Am J Trop Med Hyg*. 1995;53:443-7.
- Schofield CJ. A comparison of sampling techniques for domestic populations of Triatominae. *Trans R Soc Trop Med Hyg*. 1978;72:449-55.
- Wiener labs. Chagatest. HAI. Prueba de hemaglutinación indirecta para la detección de anticuerpos contra el *Trypanosoma cruzi*. Rosario, Argentina. 2000. Available at: <http://www.wiener-lab.com.ar>
- Abramson JH, Gahlinger PM: Computer Programs for Epidemiologists: PEPI Version 3.00. Llanidloes, Wales: Brixton Books; 1999.
- Blejer JL, Saguier MC, Dinapoli RA, Salamone HJ. [Prevalence of *Trypanosoma cruzi* antibodies in blood donors]. *Medicina (B Aires)*. 1999;59:129-32.
- Bergmann-Araújo A, Aires-Berne MA. Conventional serological performance in diagnosis of Chagas' disease in southern Brazil. *Braz J Infect Dis*. 2013;17:174-8.
- Duarte LF, Flores O, Rincón G, González CI. Comparison of seven diagnosis tests to detect *Trypanosoma cruzi* infection in patients in chronic phase of Chagas disease. *Colombia Medica*. 2014;45:61-6.
- Sosa-Jurado F, Zumaquero-Ríos JL, Reyes PA, Cruz-García A, Guzmán-Bracho C, Monteón VM. Factores bióticos y abióticos que determinan la seroprevalencia de anticuerpos contra *Trypanosoma cruzi* en el municipio de Palmar de Bravo, Puebla, México. *Salud Publica Mex*. 2004;46:39-48.
- Salazar PM, Rojas G, Bucio M, et al. [Seroprevalence of *Trypanosoma cruzi* antibodies and associated risk factors among the population under 18 years of age in Veracruz, Mexico]. *Rev Panam Salud Publica*. 2007;22:75-82.
- Organización Mundial de la Salud. Control de la Enfermedad de Chagas. Segundo Informe del Comité de Expertos de la OMS. Ginebra 2002:26-31.
- Ramsey JM, Alvear AL, Ordoñez R, et al. Risk factors associated with house infestation by the Chagas disease vector *Triatoma pallidipennis* in Cuernavaca metropolitan area, Mexico. *Med Vet Entomol*. 2005;19:219-28.
- Mougabure-Cueto G, Picollo MA. Insecticide resistance in vector Chagas disease: evolution, mechanisms and management. *Acta Tropica*. 2015;149:70-85.
- Comité Consultivo Nacional de Normalización de Prevención y Control de Enfermedades. Norma Oficial Mexicana NOM-032-SSA2-2010, Para la vigilancia epidemiológica, prevención y control de las enfermedades transmitidas por vector. *Diario Oficial de la Federación, México*, 01 junio 2011:22-24.
- Salazar-Schetinno PM, Cravioto A, Tapia-Conyer R. Iniciativa México: propuesta para el control y vigilancia epidemiológica de la enfermedad de Chagas en México. *Bol Chil Parasitol*. 2011;56:76-9.