Nested case-control study in a serological survey to evaluate the effectiveness of a Chagas disease control programme in Brazil

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Objective To identify risk factors associated with *Trypanosoma cruzi* infections in areas under surveillance in the State of Minas Gerais, Brazil.
Methods A model using a nested case-control design incorporated within a serological survey of schoolchildren which was employed to evaluate the effectiveness of the Chagas disease control programme.
Findings In a sample of 40 374 schoolchildren (aged 7–14 years) surveyed, 16 children tested positive for *T. cruzi* antibody (by indirect immunofluorescence and indirect haemagglutination). In the case-control study, each case was randomly matched to three seronegative controls (classroom and age $\pm$ 1 year). Compared to controls, *T. cruzi*-seropositive children were more likely to have a seropositive mother (odds ratio (OR) = 6.8; 95% confidence interval (CI) = 0.71–63.9) or a seropositive family member (OR = 8.6; 95% CI = 1.0–75.5).
Conclusion Use of the nested case-control model in a sero-epidemiological survey to evaluate risk factors for *T. cruzi* transmission was adequate for assessing the effectiveness of a Chagas disease control programme.

Keywords Chagas disease/prevention and control; Child; Program evaluation/methods; Risk factors; Logistic models; Seroepidemiologic studies; Case-control studies; Brazil (*source: MeSH*).

Mots clés Trypanosomiase sud-américaine/prévention et contrôle; Enfant; Evaluation programme/méthodes; Facteur risque; Modèle logistique; Etude séro-épidémiologique; Etude cas-témoins; Brésil (*source: INSERM*).

Palabras clave Enfermedad de Chagas/prevención y control; Niño; Evaluación de programas/métodos; Factores de riesgo; Modelos logísticos; Estudios seroepidemiológicos; Estudios de casos y controles; Brasil (*fuente: BIREME*).

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Introduction

Epidemiological studies are often used to evaluate the impact of health programmes, by estimating their efficacy and effectiveness after controlling for confounding factors. External comparison (i.e. comparison between areas with and without an intervention) can identify changes in health indicators of populations covered by the programme and can associate programme actions with the observed changes (*1–5*). The quasi-experimental model has been proposed as the method of choice to evaluate public health programmes when comparison areas are available, especially for programmes affecting large populations (*3, 6*). When a control area is not available, internal comparison methods may be used. One such approach is the use of several cross-sectional studies in the same area at different times. Unlike conventional cross-sectional studies, this method — known as a panel study — can attribute changes in morbidity or mortality to the intervention programme because time trends can be analysed (*7*). Another design that uses internal comparison to assess impact is the case-control study, which compares infected and uninfected individuals and their exposure to the programme. An advantage of the case-control method is that it can be initiated relatively early in relation to programme actions, thereby providing results more rapidly. Furthermore, the method is quick, inexpensive, easy to carry out, and effective in identifying risk factors associated with infection (*8–11*).

The Chagas disease control programme in Brazil employs insecticide spraying of residences to control the triatomine population — the intermediate host of *Trypanosoma cruzi*, the etiological agent of Chagas disease. Programme assessment has been based mainly on entomological indicators, such as measuring the reduction in the household triatomine population. The programme has been successful in

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controlling *Triatoma infestans,* the most important vector of *T. cruzi* in Brazil (*12–17*). In addition to the entomological results, epidemiological studies using a quasi-experimental model and panel studies conducted in the intervention area have shown reductions in *T. cruzi* infection in the cohort of children born after programme implementation (*18–20*).

Sero-epidemiological surveys in surveillance areas have been part of the Chagas disease control programme in Brazil since 1992. Serological evaluations of selected cohorts (e.g. children born post-intervention) can estimate the risk of parasite transmission in the community (*13, 16*). In 1998, the prevalence of *T. cruzi* infection in the 7–14-year age group was 0.04%. There was a 99.1% reduction compared to the 1980 age-seroprevalence rate (*17*). Finding children identified as positive through serological surveys raise the question of why transmission of *T. cruzi* occurred in the areas under control. The present study describes a model that uses a nested case-control design incorporated in the serological survey of schoolchildren, which was used to evaluate the effectiveness of the Chagas disease control programme, in order to identify the risk factors associated with *T. cruzi* infections in surveillance areas.

Materials and methods

Serological survey of schoolchildren

Study population and data collection. The rationale, organization and data collection methods employed in the serological survey of schoolchildren of the Chagas disease control programme in Brazil have been described in detail elsewhere (*21, 22*). Briefly, however, the survey was carried out on 7–14-year-old children in rural surveillance areas. The sample size estimate considered the frequency of *T. cruzi* infection in this age group as estimated by the Brazilian Chagas disease serological survey conducted from 1975 to 1980 (*23*), the error levels employed (α = 0.05 and 1– β= 0.90), and the 7–14-year-old rural population covered. To ensure validity, we carried out the survey in all rural schools using a two-stage sampling scheme. A stratified random sample included a number of schoolchildren selected in proportion to the number of students enrolled in each school; a simple random sample of classes was used. All students registered in the selected classes took part in the study.

Participant data were collected using a questionnaire developed specifically for the survey. A blood sample was collected from all participants by fingerprick onto filter-paper rectangles (10 cm x 5 cm) containing two 2-cm circles. The filter-paper was dried at room temperature, placed in a plastic bag with silica gel, and stored at 4 °C until examination. The serological tests were processed at the Chagas Disease Reference Laboratory, Fundação Ezequiel Dias, Minas Gerais. The filter-paper eluates were tested for anti-*T. cruzi* antibodies using indirect immunofluorescence (IIF) with a cut-off at 1:20. The results were considered negative when the reading was non-reactive at this cut-off. When it was reactive, the sample was re-tested simultaneously with IIF and indirect haemagglutination (IHA). A sample was considered positive when the following conditions were met: IIF >1:40 and IHA minimum discriminative titre. Reactions that had IIF titres of 1:20 or 1:40 and a minimum discriminative titre on IHA were considered borderline. In those cases, a blood sample was collected by venous puncture and the serum was processed for two reactions. Results were considered positive using the criteria described above.

Nested case-control. The nested case-control study was conducted in the state of Minas Gerais, south-eastern Brazil, from 1994 to 1998, the same period that the survey of schoolchildren was performed. Cases and controls were identified from the serological survey.

Selection of cases and controls. Cases were defined as those who tested positive for *T. cruzi* antibody according to the criteria adopted for the serological survey (i.e. concordance in two immunological tests on filter-paper and, when necessary, confirmation by venous blood testing). Controls were selected from subjects who tested negative for *T. cruzi* based on the adopted criteria. For each case, three seronegative controls were randomly selected and matched for classroom and age $\pm$1 year.

Data collection. The study procedures were approved by the ethical committee of the Institute of Biological Sciences of the Federal University of Minas Gerais. Informed consent was obtained before data collection. Interviews were conducted with the parents of cases and controls using a pre-coded questionnaire developed for the study. The questions covered personal data of the cases and controls, characteristics of the household (type of walls, roof, floor and outbuildings), the presence of animals, period of residence in the house, and other risk factors associated with *T. cruzi* infection (presence of triatomines in the house and a history of blood transfusion). At the time of the interview, blood samples from family members of cases and controls were collected by fingerprick onto filter-papers and tested for anti-*T. cruzi* antibodies by IHA and IFA using the same diagnostic criteria adopted for cases and controls.

Statistical analysis. Cases and matched controls were compared in terms of the following variables: personal characteristics, parent and sibling serology, housing characteristics, peridomestic environment and presence of animals in the household. Matched odds ratios (OR) and 95% confidence intervals (CI) were estimated and variables whose levels were statistically significant (P <0.005) on univariate matched analysis or were a biologically plausible risk for *T. cruzi* infection were entered jointly into a multivariate conditional logistical regression. Statistical significance was determined by likelihood tests.

Results

The serological survey was conducted in 168 counties in the state of Minas Gerais, i.e. 32.2% of the control programme area. Blood samples were collected from 40 374 schoolchildren. Using IIF as a screening test with a cut-off at 1:20, we found that 41 samples were reactive while 16 were confirmed positive for the serological criteria. The children who were positive for *T. cruzi* came from seven counties where the control programme had been in progress for 15 years and where epidemiological surveillance began in 1988.

Of the 16 seropositive *T. cruzi* infections identified in the survey of schoolchildren, 11 were included in the nested case-control study. Five cases were not included because, at the time that the investigation was carried out, they had moved from the area and could not be contacted. Each case was matched (age and classroom) to three randomly selected seronegative children. The personal characteristics of cases (mean age, 10.7 years) and matched controls (mean age, 11.2 years) are shown in Table 1.

Table 2 shows the conditional OR (matched analysis), comparing selected variables. The OR for period of residence — in two categories, 1–6 years and 7–14 years (reference) — was 9.38 (95% CI, 1.10–80.16). Cases did not differ from controls on housing characteristics including type of walls, roof, floor and outbuildings, and presence of animals.

Table 3 lists the conditional OR (univariate matched analysis) for the serology of relatives among *T. cruzi* cases and matched controls. We first compared the serological results of having a seropositive mother among cases and controls and found a OR of 6.8 (95% CI, 0.71–63.9; $P = 0.095$). Because only two siblings were positive, we defined one variable as a positive mother or sibling which resulted in a OR of 8.6 (95% CI, 1.00–74.5; $P = 0.05$). Only these two variables were associated with the infection in the tested models.

Table 1. **Characteristics of seropositives (cases) and matched seronegatives (controls), *Trypanosoma cruzi* infections, Minas Gerais State, Brazil**

Characteristic	Cases ($n = 11$)	Controls ($n = 33$)
Mean age ± SD (years)	10.7 ± 2.24	11.2 ± 1.93
Sex		
Males	8 (72.7)[a]	21 (63.6)
Females	3 (27.3)	12 (36.4)
Birthplace in an area with >10 years of control programme[b]		
Yes	9 (90.0)	31 (96.9)
No	1 (10.0)	1 (3.1)
Mean period of residence in years ± SD	9.2 ± 2.9	10.4 ± 2.7
Blood transfusion recipient		
No	10 (100)	32 (96.9)
Yes	0	1 (3.03)

[a] Figures in parentheses are percentages.

[b] Current surveillance area.

Discussion

Our results show that use of the nested case-control model in a sero-epidemiological survey to evaluate risk factors for *T. cruzi* transmission was adequate for assessing the effectiveness of a Chagas disease control programme. The model rapidly provided answers to study questions, and it can be extended to areas under surveillance or intervention. Furthermore, the model is operationally feasible and can be routinely included in the planning phase of programme evaluation by public health agencies.

The results of the serological survey of schoolchildren demonstrated the effectiveness of the Chagas disease control programme in interrupting vector transmission in surveillance areas. The seropositive rate in Minas Gerais State was 0.06% (*16*). Owing to the success of the control programme in reducing transmission, only 16 out of the 40 374 children tested were positive for *T. cruzi*. The case-control study was therefore conducted with a reduced number of positive subjects, which led to very large confidence intervals for risk variables and an unstable OR precision.

Selection bias could have been introduced in the nested case-control study because five *T. cruzi*-seropositive cases were not included in the investigation. However, there is no evidence that these losses (due to migration) were associated with *T. cruzi* infection. Migration from rural to urban areas has been increasing in Brazil in recent years. A possible explanation for this is the fact that rural schools offer only elementary level education; in order to continue their studies, children therefore have to migrate to urban areas. The demographic characteristics of lost cases were similar to those included in the study.

Compared to the controls, *T. cruzi*-seropositive children were more likely to have a seropositive mother or sibling (OR = 8.6; 95% CI, 1.0–75.5), while the OR for a seropositive mother was 6.8 (95% CI, 0.71–63.9). Similar results have been reported in a case-control study conducted in an intervention area: children of seropositive mothers were 3.9 times more likely to have anti-*T. cruzi* antibodies after adjusting for confounding variables like the presence of triatomines, mother's age, and family size (*24*).

One limitation in interpreting the results of epidemiological studies on Chagas disease is that the serological antibody marker indicates past infection, while the evaluation of behaviour and the environment does not necessarily correspond to conditions at the time of infection. The interview included questions relating to past exposure, such as type of house and presence of triatomines in the residence, but we could not identify exactly when the infection

Table 2. **Univariate analysis of housing characteristics among seropositives (cases) and matched seronegatives (controls), *Trypanosoma cruzi* infections, Minas Gerais State, Brazil**

Housing characteristic	Odds ratio	95% CI[a]	*P*-value
Length of residence			
7–14 years	1.0		
1–6 years	9.4	1.10–80.2	0.041
Triatomines reported indoors or outdoors[b]			
No	1.0		
Yes	1.92	0.42–8.882	0.401
House building			
Brick (walls) and tiles (floor)	1.0		
Mud (walls) and dirt (floor)	0.90	0.25–3.29	0.869
Presence of chicken coop			
No	1.0		
Yes	0.20	0.04–1.01	0.051
Presence of pigsty			
No	1.0		
Yes	0.27	0.05–1.39	0.118
Presence of silo			
No	1.0		
Yes	0.60	0.14–2.45	0.477
Presence of barn			
No	1.0		
Yes	1.30	0.28–6.11	0.737
Presence of animals			
Dog			
No	1.0		
Yes	2.6	0.29–26.62	0.387
Cattle			
No	1.0		
Yes	1.68	0.327–8.63	0.534
Cat			
No	1.0		
Yes	0.25	0.03–2.60	0.249

[a] 95% CI = 95% confidence interval.

[b] Previous reports of triatomines, indoors or outdoors, by the householders.

Table 3. **Univariate analysis of serology among seropositives (cases) and matched seronegatives (controls) among relatives, *Trypanosoma cruzi* infections, Minas Gerais State, Brazil**

	Odds ratio	95% CI[a]	*P*-value
Mother			
Negative	1.0		
Positive	6.8	0.71–63.9	0.095
Mother or sibling			
Negative	1.0		
Positive	8.6	1.00–74.5	0.050

[a] 95% CI = 95% confidence interval.

occurred and age was used as a proxy. It is also important to consider the role of recall bias in case-control studies.

The mean age of cases was 10.7 years, so that transmission could have occurred during the initial phase of the implementation of the control programme. The results showed a strong association between a seropositive mother and seropositive offspring, which was not seen with respect to the father's serology. We could not exclude vertical transmission because *T. cruzi*-seropositive children were more likely to have a seropositive mother than seronegative subjects. Congenital transmission of *T. cruzi,* although numerically low, is the third most important mechanism of transmission. Up to 4% of births from infected mothers can result in infected babies due to parasites crossing the placenta (*25–27*). The mean age of cases in our study is more indicative of vectorial transmission, i.e. exposure to triatomines, which had occurred in the past; all family members of cases and controls were tested and no positives were identified who were younger than the cases. The risk of mother's versus father's seropositivity could be explained by increased exposure to triatomines because of maternal behavioural patterns. However, vertical transmission cannot be ruled out, as previously reported (*28*).

The sero-epidemiological survey is a sensitive tool for monitoring *T. cruzi* transmission when it is performed on a representative population sample in intervention areas. The development of the survey methodology used for the 7–14-year-old cohort of schoolchildren has made *T. cruzi* diagnosis easier and more affordable for large-scale field studies in rural areas. A sero-epidemiological survey carried out in ten Brazilian states since 1994 indicated that the incidence of positive reactions in this age group was less than 0.5% in nine of these states, representing a reduction of over 96% since 1980 (*16*). The cohort of schoolchildren studied here represents a sample of the population born before the attack phase of the vector control programme (1979–82). A serological survey of children younger than 6 years of age would have provided more accurate information on the impact of the programme since only the incidence of cases would have been detected. However, house-to-house rather than school-based surveys are operationally difficult to conduct.

The main limitation of a survey or cross-sectional study in evaluating health programme effectiveness is that they do not permit causal inferences because time factors were not evaluated. Surveys can identify negative and positive *T. cruzi* infections and can estimate prevalence rates that can be compared to different time periods. When positive children are identified in a serological survey, it raises the question of why transmission occurred in areas under control. Impact assessment attempts to discern changes in health indicators and whether these changes could be attributed to the intervention. One problem is separating programme actions from social, economic and environmental changes that may have occurred in the intervention areas. The case-control design has recently been suggested as an alternative for evaluating health interventions. Its major advantages

include reduced cost, ease of execution, and capacity to identify different infection risk factors (*8–11*). However, distortions are often associated with case-control studies, such as those resulting from the classification of subjects with respect to disease and exposure, and those resulting from the effect of the study factor mixed with the effects of confounding factors. But when control areas are not available, the case-control method is the most effective one for evaluating control programmes.

This case-control study within a serological survey can be seen as a sentinel event capable of detecting temporal changes that occurred in *T. cruzi* transmission in surveillance areas, particularly regarding the replacement of domesticated triatomine species by sylvatic species in household infestations, which could possibly lead to recrudescence in *T. cruzi* infection rates. ■

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Conflicts of interest: none declared.

Résumé

Utilisation d'une étude cas-témoins emboîtée dans une enquête sérologique pour évaluer l'efficacité d'un programme de lutte contre la maladie de Chagas au Brésil

Objectif Identifier les facteurs de risque associés aux infections par *Trypanosoma cruzi* dans des zones couvertes par la surveillance dans l'Etat de Minas Gerais (Brésil).

Méthodes Un modèle faisant appel à une étude cas-témoins emboîtée dans une enquête sérologique réalisée sur des écoliers a été utilisé pour évaluer l'efficacité du programme de lutte contre la maladie de Chagas.

Résultats Dans un échantillon de 40 374 écoliers (âgés de 7 à 14 ans) enquêtés, 16 possédaient des anticorps dirigés contre *T. cruzi* (détectés par immunofluorescence indirecte et hémagglutination indirecte). Dans l'étude cas-témoins, chaque cas était apparié à 3 témoins séronégatifs choisis par tirage au sort parmi les élèves de leur classe et ayant le même âge à un an près. Par rapport aux témoins, les enfants ayant une sérologie positive pour *T. cruzi* avaient une probabilité plus grande d'avoir une mère également positive (odds ratio (OR) = 6,8 ; intervalle de confiance (IC) à 95 % : 0,71-63,9) ou un membre de la famille positif (OR = 8,6 ; IC 95 % : 1,0-75,5).

Conclusion L'inclusion d'une étude cas-témoins emboîtée dans une enquête sérologique afin de rechercher les facteurs de risque de transmission de *T. cruzi* était utile pour évaluer l'efficacité d'un programme de lutte contre la maladie de Chagas.

Resumen

Estudio de casos y testigos anidado en una encuesta serológica para evaluar la eficacia de un programa de lucha contra la enfermedad de Chagas en el Brasil

Objetivo Identificar los factores de riesgo asociados a las infecciones por *Trypanosoma cruzi* en zonas sometidas a vigilancia en el Estado de Minas Gerais (Brasil).

Métodos Para evaluar la eficacia de un programa de lucha contra la enfermedad de Chagas, se empleó un modelo anidado de casos y testigos incorporado en una encuesta serológica de escolares.

Resultados En la muestra de 40 374 escolares (de 7–14 años) encuestados, 16 niños dieron positivo para los anticuerpos contra *T. cruzi* (por inmunofluorescencia indirecta y hemaglutinación indirecta). En el estudio de casos y testigos, cada caso se emparejó al azar con tres testigos seronegativos (de la misma clase y con una diferencia de edad máxima de un año). En comparación con los testigos, los niños seropositivos para *T. cruzi* presentaban una mayor probabilidad de tener una madre seropositiva (razón de posibilidades (OR) = 6,8; IC95%: 0,71–63,9) o un familiar seropositivo (OR = 8,6; IC95%: 1,0–75,5).

Conclusión El uso de un modelo de casos y testigos anidado en una encuesta seroepidemiológica para evaluar los factores de riesgo de transmisión de *T. cruzi* fue un medio adecuado para determinar la eficacia de un programa de lucha contra la enfermedad de Chagas.

References

1. **Ibrahim MA.** Epidemiology: application to health services. *Journal of Health Administration Education,* 1983, **1**: 37–69.
2. **Holland WW, ed.** *Evaluation of health care.* Oxford, Oxford University Press, 1983.
3. **Omran AR.** Health systems research: approaches and pitfalls. *World Health Forum,* 1990, **11**: 286–292.
4. **Shapiro S.** Randomized controlled trials in health services research. In: Armenia HK et al., eds. *Epidemiology and health services.* New York, Oxford University Press, 1998, 157–181.
5. **Habicht JP, Victora CG, Vaughan JP.** Evaluation designs for adequacy, plausibility and probability of public health programme performance and impact. *International Journal of Epidemiology,* 1999, **28**: 10–18.

6. **Kleinbaum DG, Kupper LL, Morgenstern H.** *Epidemiological research: principles and quantitative methods.* New York, VNB, 1982.
7. **Kelsey JL et al.** *Methods in observational epidemiology*, 2nd ed. New York, Oxford University Press, 1996.
8. **Smith PG, Rodrigues LC, Fine PEM.** Assessment of the protective efficacy of vaccines against common diseases using case–control and cohort studies. *International Journal of Epidemiology*, 1984, **13**: 87–93.
9. **Selby JV.** Case–control evaluation of treatment and program efficacy. *Epidemiological Reviews*, **16**: 90–101.
10. **Armenian HK. Gordis L.** Future perspectives on the case–control method. *Epidemiological Reviews*, 1994, **16**: 163–164.
11. **Armenia HK.** Case control methods. In: Armenia HK et al., eds. *Epidemiology and health services.* New York, Oxford University Press, 1998: 135–155.
12. **Dias JCP.** Control of Chagas disease in Brazil. *Parasitology Today*, 1987, **3** (11): 336–341.
13. **Silveira AC, de Rezende DF.** [Epidemiology and control of transmission of vectors of Chagas disease in Brazil]. *Revista da Sociedade Brasileira de Medicina Tropical*, 1994, **27** (suppl. 3): 11–22 (in Portuguese).
14. **Silveira AC, Vinhaes M.** [Chagas disease: epidemiologic aspects of control.] *Revista da Sociedade Brasileira de Medicina Tropical*, 1998, **31** (suppl. 2): 15–60 (in Portuguese).
15. **Moncayo A.** Progress towards the elimination of transmission of Chagas disease in Latin America. *World Health Statistics Quarterly*, 1997, **50** (3–4): 195–198.
16. **World Health Organization.** Chagas disease — interruption of transmission. *Weekly Epidemiological Record*, 1997, **72** (1/2):1–5.
17. **UNDP/World Bank/WHO/TDR.** Chile and Brazil to be certified free of transmission of Chagas disease. *TDR News*, 1999, No. 59 (June): 10.
18. **Carneiro M, Antunes CMF.** A quasi-experimental model for evaluating public health programmes: efficacy of Chagas disease control programme in Brazil. *Bulletin of the World Health Organization*, 1994, **72**: 721–728.
19. **Carneiro M, Antunes CMF.** [Evaluation of the efficacy of a Chagas disease control programme: methodological aspects]. *Cadernos de Saúde Pública*, 1994, **10** (suppl. 2): 261–272 (in Portuguese).
20. **Costa FC et al.** Chagas disease control programme in Brazil: a study of the effectiveness of 13 years of intervention. *Bulletin of the World Health Organization*, 1998, **76**: 385–391.
21. **National Health Foundation.** [*Epidemiological enquiry on the evaluation of the Chagas disease control programme*]. Brasilia, Ministry of Health, 1994 (in Portuguese).
22. **Moreno EC, Carneiro M, Antunes CMF.** [Methodological aspects of a serological study of schoolchildren and a nest case-control study for evaluating the efficacy of the Chagas Disease Control Programme]. *Informe Epidemiológico do SUS*, 1996, **5**: 49–59 (in Portuguese).
23. **Camargo ME et al.** [Serological enquiry of the prevalence of Chagas infections in Brazil]. *Revista da Sociedade Brasileira de Medicina Tropical*, 1984, **26**: 192–204 (in Portuguese).
24. **Andrade ALSS et al.** Risk factors for *Trypanosoma cruzi* infection among children in central Brazil: a case-control study in vector control settings. *American Journal Tropical Medicine and Hygiene*, 1995, **52**: 183–187.
25. **Bittencourt AL.** Possible risks factors for vertical transmission of Chagas disease. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 1992, **34**: 403–408.
26. **Andrade ALSS, Zicker F, Martelli CM.** An epidemiological approach to study congenital Chagas disease. *Cadernos de Saúde Pública*, 1994, **10** (suppl.2): 345–351.
27. **Schmunis GA.** *Trypanosoma cruzi*, the etiological agent of Chagas disease: status in the blood supply in endemic and non-endemic countries. *Transfusion*, 1991, **31**: 547–557.
28. **Dias JCP.** Epidemiology of Chagas disease. In: Wendel S et al., eds. *Chagas disease (American trypanosomiasis): its impact on transfusion and clinical medicine.* São Paulo, ISBT, 1992: 49–80.