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Are maternal re-infections with *Trypanosoma cruzi* associated with higher morbidity and mortality of congenital Chagas disease?

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Summary

BACKGROUND Comparing two surveys performed in Bolivia in 1992–1994 and 1999–2001, we reported a significant decrease in the proportions of severe and mortal forms of congenital Chagas disease. This might be due to a reduction of vectorial density (VD) in maternal residence area, raising the question of a possible causal relationship between such VD, maternal parasitaemia and prognosis of congenital infection with *Trypanosoma cruzi*.

METHOD Comparisons of haematological and parasitological data obtained from Bolivian mothers infected with *T. cruzi,* and of clinical and biological data obtained from their infected and uninfected newborns, stratified according to VD in the area of maternal residence.

RESULTS i) Blood hematocrit rates or hemoglobin amounts were within the normal ranges and similar in all the maternal groups, whatever the VD in their areas of residence; ii) mothers living in high VD areas displayed a higher frequency of hemocultures positive for *T. cruzi;* iii) newborns congenitally infected with *T. cruzi,* but not uninfected babies born from infected mothers, displayed higher frequencies of very low Apgar scores, low birth weights, prematurity, respiratory distress syndrome or anasarca, as well as higher mortality rates when their mothers lived in areas of high VD.

CONCLUSION Frequent bites of blood sucking *Reduvidae* during pregnancy do not induce maternal anaemia, but, likely through multiple maternal re-infections with *T. cruzi,* increase maternal parasitemia and worsen congenital Chagas disease. Maternal dwelling in areas of high VD is associated with a serious increased risk of severe and mortal congenital Chagas disease.

keywords *Trypanosoma cruzi*, congenital Chagas disease, vectorial density, maternal residence, parasitaemia

Introduction

The protozoan parasite *Trypanosoma cruzi*, the agent of Chagas'disease, infects 16–18 million people in Latin America. Parasites are mainly transmitted by blood-sucking vector bugs releasing excreta containing infectious agents, or transfusion of infected blood. The materno-fetal transmission of parasites is another mode of contamination occurring in 1–12% of infected mothers (Carlier *et al.* 2002; Carlier & Torrico 2003).

In a previous clinico-epidemiological study on congenital *T. cruzi* infection, comparing two surveys performed in Bolivia in 1992–1994 and 1999–2001, we reported a significant decrease in the proportions of severe and mortal forms of congenital Chagas disease in the second survey compared with the first one, whereas the materno-fetal transmission rates remained similar (Torrico *et al.* 2004). Though, a sensitive decrease of poverty occurred in Bolivia during the time separating both surveys, qualitative ameliorations of housing limiting their colonization by bugs, and increasing efforts of the Bolivian public health authorities for controlling peri- and intra-domiciliary vectors were noted. This raises the question of a possible causal relationship between high vectorial density (VD) in maternal dwelling and the development of more severe forms of congenital Chagas disease.

Indeed, frequent bites of blood-sucking infected and uninfected Reduvidae in pregnant women, might result in maternal anaemia (Schofield 1981) known to be highly

pejorative for the fetal development (Allen 2000). Moreover, it can be expected that close contacts with infected bugs in dwellings displaying high VD, might result in frequent maternal re-infections with *T. cruzi*. As mothers transmitting parasites to their fetuses display a specific immunodeficiency limiting their capacity to control infection (Hermann *et al.* 2004), such re-infections might favour the occurrence of higher maternal parasitaemia and subsequent repeated transmissions of parasites towards fetuses, which in turn would develop more severe pathology. Indeed, the role of such re-infections with parasites on the severity of Chagas disease has been already suggested in human adult infection (Pinto Dias 2000), but remains controversial in experimental infections (Revelli *et al.* 1990; Lauria-Pires & Teixeira 1997; Cabrine-Santos *et al.* 2001; Machado *et al.* 2001; Bustamante *et al.* 2002).

In order to test the hypothesis of a role of VD in maternal dwelling on maternal parasitaemia and prognosis of congenital infection, in the present study, we have stratified the parasitological data obtained from Bolivian mothers infected with *T. cruzi*, and the clinical and biological data obtained from their infected and uninfected newborns, according to VD in the area of maternal residence. Our results show that mothers living in high-VD areas displayed a higher frequency of haemocultures positive for *T. cruzi*, and that newborns congenitally infected with *T. cruzi* presented more frequently low Apgar scores, low birth weights, prematurity, respiratory distress syndrome (RDS) or anasarca and higher mortality rates when their mothers live in areas of high VD. However, uninfected babies born from infected mothers displayed similar maturity or clinical patterns whatever the VD in the area where their mothers lived. Finally, maternal haematocrit rates or haemoglobin amounts were similar in all maternal groups.

Material and methods

Patient cohort

The present work takes into account newborns from women living in the Bolivian departments of Cochabamba, Chuquisaca and Tarija, considered as areas endemic for *T. cruzi* infection (Figure 1). Most data came from one study run in 1992–1994 and another one performed in 1999–2001, and are reported elsewhere (Torrico *et al.* 2004). *Trypanosoma cruzi* infection (determined by serology as indicated below) was the criterion used to include mothers in the study, without exclusion criteria. Congenital cases were defined by the detection of live parasites in blood using parasitological methods (see below). Seventy-one congenitally *T. cruzi*-infected babies (so called M+B+) and 578 uninfected newborns from infected mothers (so called M+B−) came from women residing in areas displaying known intra-domiciliary vector infestation (see below), and admitted to the maternity hospital German Urquidi (Universitary Hospital Vietma, Universidad Mayor de San Simon) in Cochabamba, Bolivia. Seven M+B+ and 146 M+B− babies came from infected mothers residing in a peculiar wet and warm Bolivian area (Bermejo, Department of Tarija) known to be free of vectors (see below), and admitted in the district hospital. They were enrolled in the study according to the same criteria as for the patients admitted at the Cochabamba maternity.

Assessment of *Trypanosoma cruzi* infection in mothers

Maternal infection was assessed by standard *T. cruzi*-specific serological tests (indirect haemagglutination and/or immunofluorescence), as previously described (Breniere *et al.* 1985; Torrico *et al.* 2004). The serologically positive mothers were all asymptomatic and, particularly, they did not display clinical evidences of cardiac or digestive involvements of chronic Chagas'disease. As parasitaemias in chronically-infected patients are generally low and undetectable by direct microscopical examination of blood (Carlier *et al.* 2002), we evaluated the presence of parasites in infected mothers using haemocultures of 2 ml of blood for 2–8 weeks, as previously described (Basso & Moretti 1984). This was possible in 23 and 42 mothers living in high- and low/medium-VD areas respectively.

Biological diagnosis of congenital *Trypanosoma cruzi* infection and management of newborns

Congenital infection with *T. cruzi* was diagnosed either by microscopical examination of buffycoat from cord or peripheral blood (obtained before the 30th day of life) collected in microhaematocrit tubes, and/or haemoculture, as previously described (Basso & Moretti 1984; Torrico *et al.* 2004). The absence of *T. cruzi* infection in parasitologically negative newborns was confirmed by polymerase chain reaction (Virreira *et al.* 2003). As previously reported, complementary serological investigations allowed us to discard possible co-infections of newborns with other TORCH pathogens frequently infecting neonates, as *Toxoplasma gondii*, cytomegalovirus, rubella virus, herpes simplex virus, *Treponema pallidum* and HIV-1 and -2 viruses (Torrico *et al.* 2004).

Whenever possible, the congenitally infected newborns were treated during 30 or 60 days with benznidazole (7–10 mg/kg/day) as soon as the diagnosis was estab-

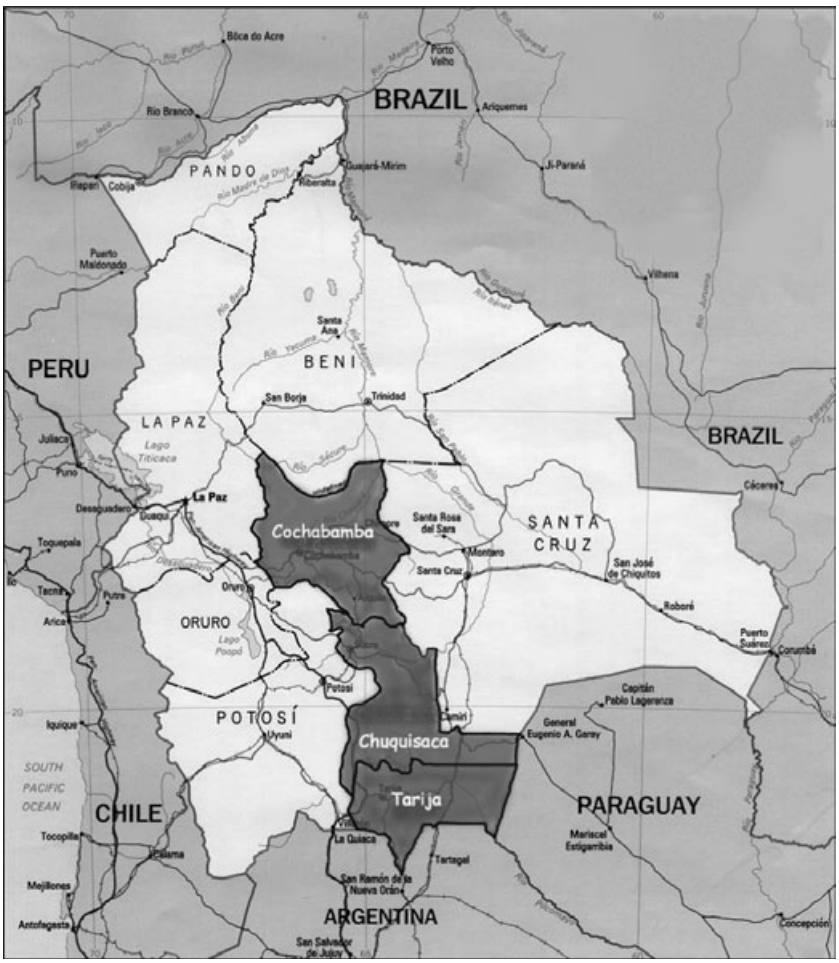

Figure 1 Map of Bolivia indicating the departments from which the mothers are coming from (in dark).

lished, and circulating parasites were no more detected 1 month later. This study has been approved by the scientific/ethic committees of UMSS and ULB and informed writing consent of the mothers was obtained before collection of blood.

Clinical examination of newborns

Newborns were weighed at birth. A physical examination was performed in the 24 h following delivery according to standard procedures described elsewhere (Torrico *et al.* 2004). Particularly, the Apgar score at 1 min, hepatomegaly (when ≥2-cm below the right costal margin), splenomegaly (spleen size under the left costal margin), the occurrence of anasarca (palpebral, genital or leg edema alone were not considered) or petechiae (whatever their localization) were investigated. The diagnosis of RDS was considered when at least one of the following signs was present: tachypnea, throbbing of the ala nasi, expiratory grunting, intercostal retraction and/or facial or systemic cyanosis (peripheral cyanosis was not considered). Neurological examination included assessment of tone, level of alertness, Moro and other primary neonatal reflexes, deep tendon reflexes, spontaneous motor activity, bulging of fontanelles and convulsions (Huttenlocker 1987). The determination of gestational age was based on physical signs and neurological characteristics of newborns (Ballard *et al.* 1991) related to data obtained from the maternal last menstruation date.

Other biological investigations in newborns and their mothers

In newborns, blood haematocrit rates, haemoglobin amounts and white blood cells (WBC), neutrophil, lymphocyte and monocyte counts, as well as plasmatic levels of direct and indirect bilirubin, aspartate amino-

transferase, alanine aminotransferase, alkaline phosphatase, urea and creatinine were determined by standard tests of clinical biology. Blood haematocrit rates or haemoglobin amounts were also determined in *T. cruzi*-infected mothers.

Epidemiological investigations

Data on peri- and intra-domiciliary VD in the villages or town districts where mothers resided at the time of pregnancy were collected from reports of the Bolivian ministry of health, regional health authorities and/or surveys performed by the UMSS team to design vector control campaigns. They were obtained using the standard inspection method by one person catching each observed bug, and the results were expressed as the number of triatomines caught per man-hour of search (Minter *et al.* 1973). These data allowed us to classify the residence places of infected mothers into high-, medium-, low- or nil-VD areas. The low-VD area corresponds to invasion in <20% of houses, i.e. occasional and sporadic infestations with rates <1 bug caught per hour/man/house. The medium-VD area includes 20–40% of infested houses with intra- or peri-domiciliary infestations, i.e. well set-up infestations, with rates between one and five bugs caught per hour/man/house. In areas of high VD, more than 40% of houses or surrounding buildings (peri-domicile) were infested, with rates >5 bugs caught per hour/man/house. All caught bugs belonged to the *Triatoma infestans* species and their infestation rates, determined by optical microscopical examination for trypanosomes of the rectal content (Wisnivesky-Colli *et al.* 1982), ranged between 40% and 60%.

The M+B+ group included 7, 12, 28 (one delivered twins), and 30 mothers living in areas of nil, low, medium and high VD respectively. The M+B− group counted 146, 109, 270 and 199 mothers living in areas classified as of nil, low, medium and high VD respectively. As previously reported (Torrico *et al.* 2004), significant differences could be observed between the mean ages and numbers of previous pregnancies of the M+B+ (mean ± SEM: age, 23.7 ± 0.7; previous pregnancies: 2.7 ± 0.2) and M+B− maternal groups (mean ± SEM: age, 26.4 ± 0.2; previous pregnancies: 3.5 ± 0.1; $P < 0.05$). However, any significant differences in mean ages or numbers of previous pregnancies were noted between the VD subgroups within M+B+ or M+B− maternal groups.

Statistical analysis

Differences in proportions were tested with the chi-squared, chi-squared for trend or Fisher exact tests, and a value of $P < 0.05$ was considered statistically significant.

Results

Parasitic load and haematological parameters of mothers living in areas of different vectorial density

Mothers living in high- and low/medium-VD areas displayed 56.5% (13/23) and 23.8% (10/42) of haemocultures positive for *T. cruzi* respectively. Such difference was highly significant (Fischer test: $P < 0.001$). A similar difference in the frequency of positive haemocultures was also observed within the M+B+ maternal subgroup (high VD: 6/9, i.e. 66.7%; low/medium VD: 8/23, i.e. 34.8%), though it was not statistically significant, likely by the too low numbers of studied mothers.

In order to test the hypothesis of an eventual role of multiple bites from haematophagous triatomines on haematological status of mothers, blood haematocrit rates or haemoglobin amounts of M+B+ and M+B− mothers living in areas of high-, medium- or low-VD were compared. The mean haematocrit rates or haemoglobin amounts were similar in all maternal subgroups and within the normal range, in M+B+ as well as in M+B− mothers (data not shown).

Apgar scores and maturity parameters of newborns from *Trypanosoma cruzi*-infected mothers living in areas of different vectorial density

Figure 2 shows the proportions of babies displaying Apgar scores at 1 min <7, low birth weight (LBW < 2500 g), prematurity (gestational age <37 weeks) or prematurity + LBW (gestational age <37 weeks; birth weight <2500 g) distributed according to VD in the maternal residence area. Figure 2a indicates that 27–47% of M+B+ babies from mothers residing in areas of high VD displayed such poor Apgar scores, LBW, prematurity or prematurity + LBW. By contrast, such alterations were found only in 7–17% and 0–8% of M+B+ newborns from medium- and low-VD areas, respectively, and not at all in the nil VD area. Chi-squared test for trend indicated such differences as highly significant ($0.05 < P < 0.0001$). As shown in Figure 2b, such differences were not observed in the uninfected M+B− babies, which displayed altered parameters only in 3–11% of them, whatever the VD in the maternal residence area ($P > 0.05$). Comparison of data from M+B+ and M+B− babies of high-VD areas highlighted significant differences ($0.01 < P < 0.0001$), whereas comparisons of M+B+ and M+B− data for nil, low- and medium-VD did not show significant differences ($P > 0.05$).

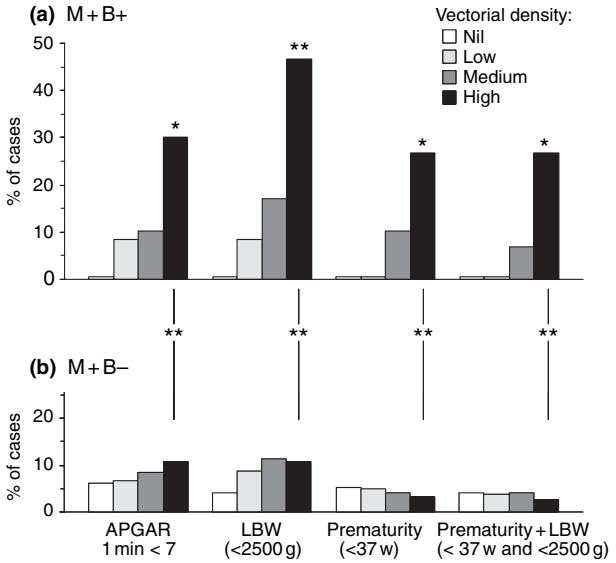

Figure 2 Apgar scores and maturity parameters in infected newborns of *Trypanosoma cruzi*-infected mothers (congenital cases M+B+) (a), and uninfected neonates from infected mothers (M+B−) (b), distributed according to the vectorial density in maternal residence area. LBW, low birth weight. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.001$) between M+B+ VD subgroups (chi-squared test for trend), or M+B+ and M+B− groups (chi-squared test). *n* for M+B+: VD nil, 7; low, 12; medium, 29; high, 30. *n* for M+B−: VD nil, 146; low, 109; medium, 270; high, 199.

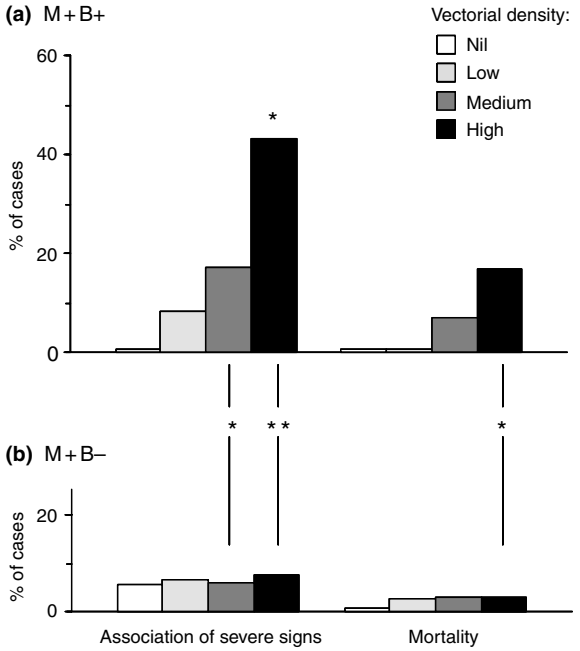

Figure 3 Morbidity and mortality rates in infected newborns of *Trypanosoma cruzi*-infected mothers (congenital cases M+B+) (a), and uninfected neonates from infected mothers (M+B−) (b), distributed according to the vectorial density in maternal residence area. The association of severe signs included at least two of the signs frequently associated with prematurity (LBW, Apgar score at 1 min <7, RDS or anasarca). See Figure 1 for significance of asterisks and *n* values.

Mortality and morbidity of newborns from *Trypanosoma cruzi*-infected mothers living in areas of different vectorial density

Forty-one to 50% of congenitally infected newborns from mothers living in nil-, low-, medium- or high-VD areas displayed one or more of the following signs/syndrome: RDS, hepatomegaly, splenomegaly, neurological signs (other than convulsions), anasarca, petechiae. No differences could be noted in the proportions of symptomatic babies according to the VD areas (data not shown). As indicated in Figure 3a, when the presence of at least two of the following severe signs frequently associated with prematurity: LBW, Apgar score at 1 min <7, RDS or anasarca, was considered, a clear increase in the proportions of positive M+B+ babies appeared from nil- to high-VD groups. Indeed, three to six times more babies presented such association of signs in the group of mothers residing in high-VD areas (high VD: 43%, medium VD: 17%; low VD: 8%, $P < 0.001$), whereas no symptomatic cases were detected in the nil VD area. Though not statistically significant (likely by the few numbers of cases), mortality rates in M+B+ babies also increased progressively from <1% in nil- (0/7) and low-VD (0/12), to 2/29 (6.9%) in medium- and 5/30 (16.7%) in high-VD areas (Figure 2a). The previously performed analysis of data available from these seven fatal cases (Torrico *et al.* 2004) indicated that the death of four of them (occurring within 24–48 h after birth) could be reasonably associated with congenital Chagas disease. Three of these four fatal cases concerned babies of mothers living in high-VD areas.

Six to 20% of M+B− babies from mothers living in nil-, low-, medium- or high-VD areas displayed at least one of the signs/syndrome, mentioned above, whereas 6–7% of them presented at least two of the severe signs associated with prematurity (Figure 3b), without any differences in the proportions of positive babies according to the VD of maternal residence areas. Mortality rates were between <1% and 3% in M+B− babies, whatever the VD area where mothers came from. As expected from our previous study (Torrico *et al.* 2004), comparisons of symptomatology in M+B+ and M+B− babies showed significant differences, particularly high in the group of high VD ($0.05 < P < 0.0001$) (Figure 3).

The haematological parameters of neonates (haematocrit rates, haemoglobin, WBC, neutrophil, monocyte and lymphocyte levels), as well as those exploring liver (plasmatic direct and indirect bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) and renal functions (plasmatic urea and creatinin) of M+B+ and M+B− babies, were within normal ranges and without significant differences when distributed according to the VD of maternal areas (data not shown).

Discussion

Our results show that high VD in maternal residence area is associated with greater frequency of positive haemocultures in mothers, and more severe clinical forms of congenital Chagas disease, as M+B+ babies displayed more frequently Apgar scores at 1 min <7, LBW, prematurity and/or prematurity + LBW, association of severe signs and higher mortality rates. However, this high-VD maternal environment was not associated with lower maternal haematocrit rates or haemoglobin amounts, or morbi-mortality of uninfected newborns from infected mothers. The used data of VD per village or town district can be considered as reliable, as they have been collected by highly skilled personnel belonging to specialized health departments in order to design vector control campaigns.

Poverty might play a role in the association between poor housing conditions, favouring the installation of large populations of *T. cruzi* vectors (Minter *et al.* 1973; Mott *et al.* 1978), and poor maternal health, with harmful consequences on perinatal morbidity and mortality of newborns. However, our observation that the morbi-mortality of *T. cruzi*-unrelated pathologies observed in the M+B− newborns was similar in nil-, low-, medium- and high-VD groups, does not favour such hypothesis. Indeed, the analysed clinical parameters (mainly Apgar <7, LBW and prematurity) are not specific of congenital Chagas disease (Torrico *et al.* 2004). If poverty would have played a strong harmful effect on neonatal health, it would have affected also these general clinical markers in the M+B− control group living in high-VD areas.

Accordingly, a causal relationship between high VD in maternal environment and severe congenital Chagas disease can be considered, raising the question of the possible involved mechanisms. Though a complete haematological profile of mothers was not performed in the present study, the fact that blood haematocrit rates or haemoglobin amounts were within the normal range and similar in all the maternal groups, whatever the VD in houses, rules out a possible role of vector-induced blood loss and a subsequent maternal anaemia as a factor worsening congenital Chagas disease.

Another possible explanation for a relationship between the density of infected bugs in maternal residence area and morbi-mortality of congenital Chagas disease, relates to more frequent *T. cruzi* re-infections in pregnant women. Indeed, the relationship between VD, the numbers of human-triatomines contacts and *T. cruzi* inoculation has been well established (Minter *et al.* 1973; Mott *et al.* 1978; Piesman *et al.* 1985; Rabinovich *et al.* 1990). The higher frequency of *T. cruzi*-positive haemocultures observed in mothers living in high-VD areas (>5 bugs/h/man), as well as the high infection rate of captured bugs (roughly an half of them were infected) argue for such a possibility. Indeed, it can be admitted that a higher frequency of positive haemocultures reflects higher amounts of circulating parasites in peripheral blood (Hermann *et al.* 2004). Moreover, as M+B+ mothers, as we recently showed (Hermann *et al.* 2004), display a parasite-specific immunodeficiency, these recurrent re-infections with *T. cruzi* might favour repeated transmissions of parasites towards immunologically immature fetuses, jeopardizing them by increasing their own parasitaemia. This is in line with others data of our laboratory indicating that the severity of congenital Chagas disease is associated with higher neonatal parasitaemias (Torrico *et al.* unpublished observation). Moreover, this also relates to observations gathered in adult cases suggesting the worsening role of re-infections, as less severe chagasic cardiopathies are observed in patients living in areas submitted to vectorial control, i.e. in which possibilities of re-infections have been seriously reduced (Pinto Dias 2000). Some experimental data have shown that re-infections induce higher parasitaemia associated with worsened acute Chagas disease (Cabrine-Santos *et al.* 2001; Bustamante *et al.* 2002), whereas other works, likely by using different experimental conditions, have not observed such an association (Revelli *et al.* 1990; Lauria-Pires & Teixeira 1997; Machado *et al.* 2001).

In conclusion, our study suggests that maternal dwelling in areas of high VD is associated with higher maternal parasitaemia and a serious increased risk of severe and mortal congenital Chagas disease. This should strongly encourage pursuing programmes of vector controls in endemic areas, of which one unexpected collateral effect might contribute to decrease the morbidity and mortality of *T. cruzi* congenital infection.

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Les réinfections maternelles par *Trypanosoma cruzi* sont-elles associées à une morbidité et une mortalité plus élevée de la maladie de Chagas congénitale?

DONNEES DE BASE En comparant deux enquêtes réalisées en Bolivie en 1992–1994 et 1999–2001, nous reportions une diminution significative des proportions de formes sévères et mortelles de la maladie de Chagas congénitale. Ceci pourrait être du à une réduction de la densité vectorielle (VD) dans la zone de résidence maternelle, soulevant la question d'une relation causale possible entre cette DV, la parasitémie maternelle et le pronostic de 1'infection congénitale à *Trypanosoma cruzi*.

METHODE Comparaisons des données hématologiques et parasitologiques obtenues de mères boliviennes infectées par *T. cruzi*, et des données cliniques et biologiques obtenues de leurs nouveaux-nés infectés et non infectés, stratifiées selon la DV de la zone de résidence, maternelle.

RESULTATS i) Les taux d'hématocrite ou d'hémoglobine étaient normaux et similaires dans tous les groupes maternels, quelque soit la DV dans leurs zones de résidence; ii) Les mères vivant dans les zones de haute DV montraient une plus grande fréquence d'hémocultures positives pour *T. cruzi*; iii) Les nouveaux-nés congénitalement infectés avec *T. cruzi*, mais pas les bébés non infectés nés de mères infectées, montraient des fréquences plus élevées de scores d'Apgar bas, de faibles poids de naissance, de prématurité, de syndrome de détresse respiratoire, ou d'anasarque, ainsi que des taux plus élevés de mortalité quand leurs mères vivaient dans des zones de haute DV.

CONCLUSION Les piqûres fréquentes de réduvidés hématophages pendant la grossesse n'induisent pas d'anémie maternelle, mais, vraisemblablement par de multiples réinfections par *T. cruzi*, augmentent les parasitémies maternelles et aggravent la maladie de Chagas congénitale. La résidence maternelle dans des zones à haute DV est associée à une augmentation sérieuse du risque de maladie de Chagas congénitale sévère et mortelle.

mot clés *Trypanosoma cruzi*, maladie de Chagas congénitale, densité vectorielle, résidence maternelle, parasitémie

Estan las reinfecciones maternas con *Trypanosoma cruzi* asociadas con una mayor morbilidad y mortalidad en la enfermedad de Chagas congénita?

ANTECEDENTES Comparando encuestas realizadas en Bolivia en 1992–1994 y 1999–2001, monstramos una disminución significativa de las proporciones de formas severas y mortales de la enfermedad de Chagas congénita. Esto puede ser debido a una reducción de la densidad vectorial (DV) en el área de residencia materna, originando la pregunta sobre una posible relación causal entre esta DV, la parasitemia materna y el pronóstico de la infección congénita por *Trypanosoma cruzi*.

METODOS Comparaciónes de los datos hematológicos o parasitológicos obtenidos de madres bolivianas infectadas por *T. cruzi,* y de los datos clínicos y biológicos obtenidos de sus recién nacidos infectados y non infectados, estratificados según la DV en la zona de residencia materna.

RESULTADOS i) Las tasas de hematocrito y de hemoglobina eran normales y similares en todos los grupos maternos, independientemente de la DV en sus zonas de residencia; ii) Las madres que vivían en áreas de alta DV monstraron una mayor frecuencia de hemocultivos positives por *T. cruzi*; iii) Los recién nacidos infectados congenitalmente por *T. cruzi,* pero no los bebés no infectados nacidos de madres infectadas, presentaron con mayor frecuencia una puntuación Apgar muy baja, bajo peso al nacer, prematuridad, síndrome de distrés respiratorio o anasarca, asi como una mayor tasa de mortalidad cuando sus madres vivían en áreas con alta DV.

CONCLUSION Las picaduras frecuentes de *Reduvidae* hematófagos durante el embarazo no inducen anemia materna, pero probablemente por múltiples reinfecciones con *T. cruzi,* aumentan la parasitemia materna y empeoran la enfermedad de Chagas congénita. La morada materna en áreas con alta DV está asociada con un serio aumento del riesgo de enfermedad de Chagas congénita severa y mortal.

palabras claves *Trypanosoma cruzi*, enfermedad de Chagas congénita, densidad vectorial, residencia materna, parasitemia

