High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region

MEMORIAS DO INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO, v. 107, supl. 1, pp. 60-67, DEC, 2012
http://www.producao.usp.br/handle/BDPI/38009

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High rates of undiagnosed leprosy and subclinical infection amongst school children in the Amazon Region

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Leprosy in children is correlated with community-level factors, including the recent presence of disease and active foci of transmission in the community. We performed clinical and serological examinations of 1,592 randomly selected school children (SC) in a cross-sectional study of eight hyperendemic municipalities in the Brazilian Amazon Region. Sixty-three (4%) SC, with a mean age of 13.3 years (standard deviation = 2.6), were diagnosed with leprosy and 777 (48.8%) were seropositive for anti-phenolic glycolipid-I (PGL-I). Additionally, we evaluated 256 household contacts (HHCs) of the students diagnosed with leprosy; 24 (9.4%) HHC were also diagnosed with leprosy and 107 (41.8%) were seropositive. The seroprevalence of anti-PGL-I was significantly higher amongst girls, students from urban areas and students from public schools (p < 0.0001). Forty-five (71.4%) new cases detected amongst SC were classified as paucibacillary and 59 (93.6%) patients did not demonstrate any degree of physical disability at diagnosis. The results of this study suggest that there is a high rate of undiagnosed leprosy and subclinical infection amongst children in the Amazon Region. The advantages of school surveys in hyperendemic areas include identifying leprosy patients at an early stage when they show no physical disabilities, preventing the spread of the infection in the community and breaking the chain of transmission.

Key words: leprosy - epidemiology - anti-PGL-I - subclinical infection - school children

Leprosy in children younger than 15 years old is correlated with recent disease and active foci of transmission in the community, reflecting the efficiency of local control programmes. In the state of Pará (PA), in the Brazilian Amazon Region, leprosy is hyperendemic in this age group. This state had an annual case detection rate of 20.4/100,000 people in 2008, which is much higher than the Brazilian average of 5.8/100,000 people (MS/SVS 2009), indicating that Mycobacterium leprae is circulating amongst the children in PA. Furthermore, in highly endemic areas, the prevalence of previously undiagnosed leprosy in the general population is six times higher than the registered prevalence (Moet et al. 2008).

In Brazil, the primary health service is responsible for diagnosing leprosy, finding active cases of leprosy, tracing the patients’ contacts, treating leprosy and preventing disability in the people affected by leprosy, but only 42% of the total population of PA is covered by these services (Department of Health Care/Department of Primary Care 2012 - dab.saude.gov.br/historico_cobertura_sf.php). This scenario suggests that there may be many patients with undiagnosed leprosy in PA who are perpetuating the transmission of the bacillus. Additionally, because of the long incubation period of M. leprae, more leprosy patients (LPs) are expected to emerge in the coming decades.

The diagnosis of leprosy is based primarily on a clinical examination and there is no laboratory test that detects all forms of leprosy. Because of the isolation and characterisation of phenolic glycolipid-I (PGL-I) (Hunter & Brennan 1981), a species-specific antigen from the M. leprae cell wall, various studies have demonstrated that serology could potentially be used to detect antibodies against PGL-I to classify patients for treatment purposes, monitor cases, identify the risk of relapse and identify the household contacts (HHCs) of LPs who are at a higher risk of contracting the disease than the general public (Moura et al. 2008). A positive test for anti-PGL-I is associated with an 8.6-fold higher risk of leprosy in HHCs and a 4.4-fold higher risk in non-contacts (Brasil et al. 2003).

Some studies have shown that subclinical infections with M. leprae are much more common than overt disease in endemic communities (Godal & Negassi 1973, Abe et al. 1990, Barreto et al. 2011) and that anti-PGL-I seropositivity is a marker of subclinical infection (Baumgart et al. 1993, Lobato et al. 2011). Van Beers et al. (1999) indicated that the seropositivity rates amongst school children (SC) may reflect leprosy incidence. Seroprevalence may be an appropriate indicator of the magnitude of the burden of leprosy in a selected area.

Therefore, screening to determine the seroepidemiology of anti-PGL-I in hyperendemic areas may be useful

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Financial support: CNPq (Neglected Diseases 576425/2008-7, scholarship for CGS), FAPESPA, SESPA, UFPA

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Received 16 March 2012

Accepted 18 July 2012

online | memorias.iof.fiocruz.br
in identifying subclinical infections amongst children in the general population. In addition, combined with clinical examinations of randomly selected subjects, screening tests could help to estimate the true burden of the disease in a specific region. Thus, the objectives of this study were to determine the prevalence of subclinical infection (defined in this study as seropositivity for anti-PGL-I IgM with no clinical signs or symptoms of leprosy) and the prevalence of undiagnosed leprosy amongst SC from selected municipalities in PA.

SUBJECTS, MATERIALS AND METHODS

Study design, setting and population - We conducted a cross-sectional study in eight inner counties of PA, from 2009-2011. Table I shows the demographics and epidemiological characteristics of the selected municipalities and Fig. 1 illustrates their geographic locations. The PA counties were selected based on their geographic position to sample all of the regions of the state and to accommodate PA’s leprosy clusters 1 and 7, as identified by Penna et al. (2009).

Participants - A team of health care professionals with experience in leprosy, including dermatologists, nurses, physical therapists, researchers and a lab technician, travelled to the municipalities and visited 37 randomly selected public elementary and high schools. At each school, two-four classes (approximately 60 subjects) of students aged six-20 years were randomly selected. The teachers, selected students and their parents received general information about leprosy and the main objectives of the study. The students who agreed to participate provided their written consent; for the participants younger than 18 years old, consent was obtained from the parents or another responsible adult within the family.

Next, the students were clinically evaluated by a dermatologist and a sample of peripheral blood was collected from each subject to determine the prevalence of IgM antibodies against PGL-I. When a new leprosy case was detected amongst the students, we travelled to their homes to evaluate the HHCs of the students diagnosed with leprosy. There were no restrictions on study participation based on gender, skin colour or comorbidities. To compare the anti-PGL-I titration levels of students with well-established leprosy cases, we included 51 patients (41 multibacillary (MB) and 10 paucibacillary (PB)) diagnosed at the Dr Marcello Candia Reference Unit in Sanitary Dermatology (UREMC) in PA. Additionally, 45 healthy students, aged seven-17 years, from private schools in Belém, PA’s capital, were sampled and evaluated for anti-PGL-I titration levels.

Diagnostic procedures - The diagnosis of a new leprosy case was based on the identification of a skin lesion with sensory loss. A case was classified as indeterminate leprosy if there was a hypopigmented macule, but no detection of nerve involvement, or the case was classified as one of the clinical forms defined by the Ridley and Jopling (1966) classification system (tuberculoid-tuberculoid (TT), borderline tuberculoid (BT), borderline-borderline, borderline lepromatous or lepromatous-lepromatous (LL)). Cases of indeterminate and TT leprosy were classified as PB cases, while the other forms were classified as MB cases. Primary neural leprosy was diagnosed if nerve enlargement was detected, but no skin signs were present. When only one nerve was affected, the case was classified as PB; two or more enlarged nerves defined the case as MB.

Laboratory procedures - Seropositivity was determined with an enzyme-linked immunosorbent assay

TABLE I
Characteristics of the selected municipalities

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Population (2010)(^a)</th>
<th>New cases detected (2006-2010)(^b)</th>
<th>Annual new case detection rate per 100,000 people (2009)(^c)</th>
<th>Children among new cases of leprosy (2006-2010)(^d)</th>
<th>Endemicity level(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altamira</td>
<td>99,075</td>
<td>611</td>
<td>108.3</td>
<td>56 (9.2)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Breves</td>
<td>92,860</td>
<td>233</td>
<td>42.0</td>
<td>34 (14.6)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Castanhal</td>
<td>173,149</td>
<td>380</td>
<td>48.2</td>
<td>35 (9.2)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Marituba</td>
<td>108,246</td>
<td>424</td>
<td>50.4</td>
<td>65 (15.3)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Oriximiná</td>
<td>62,794</td>
<td>68</td>
<td>18.7</td>
<td>5 (7.3)</td>
<td>Highly endemic</td>
</tr>
<tr>
<td>Paragominas</td>
<td>97,819</td>
<td>720</td>
<td>130.4</td>
<td>80 (11.1)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Parauapebas</td>
<td>153,908</td>
<td>1,397</td>
<td>142.0</td>
<td>143 (10.2)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Redenção</td>
<td>75,556</td>
<td>584</td>
<td>186.4</td>
<td>72 (12.3)</td>
<td>Hyperendemic</td>
</tr>
<tr>
<td>Total</td>
<td>863,407</td>
<td>4,491</td>
<td>55.7(^d)</td>
<td>490 (10.9)</td>
<td>Hyperendemic</td>
</tr>
</tbody>
</table>

\(^a\): source: Brazilian Institute for Geography and Statistics (ibge.gov.br/estadosal/); \(^b\): calculated from The National Notifiable Diseases 2012 (portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idxtx=31200); \(^c\): according to the parameters designated by the Brazilian Ministry of Health; \(^d\): average detection rate of the state of Pará (The National Notifiable Diseases 2012 - portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idxtx=31200).
(ELISA) using native PGL-I, which was generously provided by Dr. John Spencer of Colorado State University (USA). The cut-off for positive results was arbitrarily established at an optical density (OD) of 0.295, based on the average plus three times the standard deviation of the test results from 14 healthy subjects from the same hyperendemic area (endemic control). A detailed description of the laboratory procedures was previously reported (Barreto et al. 2011).

Data analysis - Descriptive statistics were calculated and various statistical inference procedures were performed with BioEstat 5.0 software (Institute for Sustainable Development Mamirauá, Tefé, Amazonas, Brazil). Statistical significance was assessed using a significance level of 0.05 (two-tailed). The Student’s t-test or the Mann-Whitney U test was used to assess the quantitative data from independent samples. A chi-square test or Fisher’s exact test was used to compare proportions between different groups when appropriate and Pearson’s coefficient was used to detect correlations between anti-PGL-I titres and the variables of interest.

Ethics - This study conforms to the Declaration of Helsinki and was approved by the Institute of Health Sciences Research Ethical Committee at the Federal University of Pará (protocol 197/07 CEP-ICS/UFPA).

RESULTS

A total of 1,592 students were examined (966 girls and 626 boys); the mean age was 12.3 [standard deviation (SD) = 3.2] years. Sixty-three (4%) of these students were clinically diagnosed with leprosy and 777 (48.8%) tested positive for anti-PGL-I IgM. We also examined 256 HHCs of the students diagnosed with leprosy at their homes (142 females and 114 males); the mean age was 25.7 (SD = 17.8) years. Twenty-four (9.4%) of the HHCs were also diagnosed with leprosy (Table II) and 107 (41.8%) tested positive for anti-PGL-I IgM. Table II shows the seroprevalence and the number of new cases detected amongst the students and HHCs in each municipality. Amongst the new cases detected, 51 (58.6%) were children younger than 15 years old. The distribution of cases by gender, age group, clinical classification, degree of physical disability, anti-PGL-I seropositivity and the presence of a BCG scar is shown in Table III.

The levels of anti-PGL-I were similar in the MB patients (median OD = 0.369; IQR = 0.409) and the PB patients [median OD = 0.394; interquartile range (IQR) = 0.445] diagnosed during the active survey of the students and their HHCs (p = 0.752) and the proportions of seropositivity were also similar (MB = 60.6%, PB = 66.7%);

Fig. 1A, B: sample pictures of team work on the school and on the field; C: one of the children diagnosed with borderline lepromatous leprosy with her father, detected after our visit to her house.

Fig. 2: geographic locations of the selected municipalities in the state of Pará, in the Brazilian Amazon Region.
p = 0.646). However, when we compared the MB and the PB patients diagnosed at the leprosy reference unit, significant differences were found in both the levels of anti-PGL-I (p = 0.007) and the proportions of seropositivity (MB = 95%; PB = 70%; p = 0.045). Figs 3, 4 illustrate the levels of anti-PGL-I in the different groups and Table IV shows the median OD values from the ELISA.

Regarding the degree of physical disability, there were no differences in the anti-PGL-I levels between those with grade 0 (median OD = 0.376; IQR = 0.410) and those with grade 1 or 2 (median OD = 0.301; IQR = 0.778) (p = 0.941). In addition, we did not find a significant association (p = 0.709) between the prevalence of seropositivity and BCG vaccination status (presence or absence of a BCG scar).

Amongst the study participants, 42.5% of the male students and 53.3% of the female students were seropositive (p < 0.0001). The median OD for the males was 0.256 (IQR = 0.262) and the median OD for the females was 0.323 (IQR = 0.354) (p < 0.0001), but the number of leprosy cases did not differ by gender (p = 0.792).

Thirty-four of the visited schools were in urban zones and three schools were in rural zones; we examined 1,428 urban students (54 new cases) and 164 rural students (9 new cases). There was no significant difference between the proportions of new cases detected in the two areas (p = 0.288). The level of anti-PGL-I was higher in the urban area (median OD = 0.301; IQR = 0.323) than in the rural area (median OD = 0.220; IQR = 0.276) (p < 0.0001) and the prevalence of seropositivity was also higher in urban areas (urban = 50.7%; rural = 32.3%; p < 0.0001).

Twenty newly diagnosed leprosy cases (23%) reported that they had experienced starvation (a full day without meals) at least once because they lacked the resources to buy food, 77 (89%) had family incomes of up to twice the Brazilian minimum wage, 73 (84%) were receiving some type of governmental financial assistance, such as a family allowance or retirement benefits, and 59 (68%) lived in a house with more than two people per bedroom. Additionally, 54.5% of the newly diagnosed students stated that they were aware of previous contact with at least one person affected by leprosy.

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## TABLE II

Seroprevalence and new cases detected in each municipality

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Public schools (enrolled students) n (%)</th>
<th>Selected schools (examined students) n (%)</th>
<th>Seroprevalence among students n (%)</th>
<th>New cases detected among students n (%)</th>
<th>HCSDL survey n (%)</th>
<th>Seroprevalence among HCSDL n (%)</th>
<th>New cases detected among HCSDL n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altamira</td>
<td>115 (24.137)</td>
<td>6 (282)</td>
<td>81 (28.7)</td>
<td>10 (3.5)</td>
<td>30</td>
<td>9 (30)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Breves</td>
<td>325 (30.290)</td>
<td>5 (229)</td>
<td>150 (65.5)</td>
<td>14 (6.1)</td>
<td>81</td>
<td>30 (37)</td>
<td>12 (14.8)</td>
</tr>
<tr>
<td>Castanhal</td>
<td>82 (39.331)</td>
<td>4 (188)</td>
<td>125 (66.5)</td>
<td>9 (4.8)</td>
<td>31</td>
<td>9 (29)</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Marituba</td>
<td>57 (24.978)</td>
<td>4 (199)</td>
<td>128 (64.3)</td>
<td>10 (5)</td>
<td>34</td>
<td>19 (55.9)</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>Oriximiná</td>
<td>89 (16.785)</td>
<td>6 (135)</td>
<td>57 (42.2)</td>
<td>6 (4.4)</td>
<td>25</td>
<td>14 (56)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Paragominas</td>
<td>93 (24.182)</td>
<td>4 (181)</td>
<td>84 (46.4)</td>
<td>10 (5.5)</td>
<td>44</td>
<td>22 (50)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Paraapebas</td>
<td>50 (33.300)</td>
<td>5 (146)</td>
<td>58 (39.7)</td>
<td>3 (2)</td>
<td>8</td>
<td>3 (37.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Redenção</td>
<td>37 (17.581)</td>
<td>3 (232)</td>
<td>94 (40.5)</td>
<td>1 (0.4)</td>
<td>3</td>
<td>1 (33.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>848 (210.584)</td>
<td>37 (1.592)</td>
<td>777 (48.8)</td>
<td>63 (4)</td>
<td>256</td>
<td>107 (41.8)</td>
<td>24 (9.4)</td>
</tr>
</tbody>
</table>

*a: elementary and high schools (Brazilian Institute for Geography and Statistics - ibge.gov.br/estadosat); HCSDL: household contact of student diagnosed with leprosy; PGL-I: phenolic glycolipid-I.
DISCUSSION

From 1991-2010, 88,805 new leprosy cases were diagnosed in PA alone (The National Notifiable Diseases Surveillance 2012 - portal.saude.gov.br/portal/saude/profissional/virtualizar_texto.cfm?txt=31200). Considering that there were approximately two million students enrolled in public schools in 2009 (Brazilian Institute for Geography and Statistics - ibge.gov.br/estadostat/), we examined only 0.08% of all the students. If the data we collected were extrapolated to the entire population of SC, there may currently be approximately 80,000 undiagnosed leprosy cases amongst PA students. According to the World Health Organization, the peak age range for the onset of leprosy is 20-30 years (WHO 2009). However, while the diagnosis may occur during this period of life, the onset may occur earlier and the patient may be sick for a long time. In PA, almost half of the patients have been diagnosed between the ages of 20-60 years and in the last 20 years, an average of 4,400 patients were diagnosed each year. If we assume that the 80,000 currently undiagnosed leprosy cases will be detected in the next 20 years, we may maintain this average for a long time.

In fact, these data may be corroborated by the high proportions of anti-PGL-I-positive SC, from 28.7-66.5%. These figures are similar to the prevalence of subclinical infection detected by Dayal and Bharadwaj (1995) amongst healthy children who were close contacts of PB and MB LPs (61% and 75%, respectively). Although these numbers are high compared with those of other studies involving the general population, there is little surveillance of leprosy amongst SC. Some studies report lower seroprevalence rates, even in highly endemic regions (Cartel et al. 1990, Bührer-Sékula et al. 2008), whereas other studies report high seroprevalence rates that are compatible with the high incidence rate of the surveyed population (Abe et al. 1990, Van Beers et al. 1999) and corroborate our results.

The other interesting findings, already presented in other studies (Fine et al. 1988, Krishnamurthy et al. 1991, Bakker et al. 2004), are the higher levels of anti-PGL-I seropositivity amongst children and young adults compared with older adults (i.e., an inverse correlation with age) and amongst girls compared with boys. There is no definite explanation for these results, but generally, IgM antibody levels vary with age and they are consistently higher in females than in males at every age (Oskam et al. 2003). Moreover, the difference in seropositivity between the urban and rural SC may be due to the poorer living conditions in urban areas, where the students live in more crowded houses and neighbourhoods and are more susceptible to food shortages. These observations, along with the high seropositivity rate amongst students in public schools, led us to question whether higher income students would have the same results. As expected, the prevalence of anti-PGL-I seropositivity amongst SC in private schools was significantly lower than the prevalence in public schools, confirming the strong correlation between leprosy and poverty.

Although the seroprevalence rate is high, we must remember that M. leprae is a highly infective, but low pathogenic bacterium that causes disease in only 10-20% of all infected people, considering a positive lepromin test in approximately 80% of the population protected by the “N-factor of Rotberg” (Rotberg 1989). According to our data, almost half of the SC in public schools have subclinical infections and respond by producing anti-PGL-I, which can be used as a marker of M. leprae dissemination into the community (Baumgart et al. 1993). Of the two million students enrolled in PA public schools, approximately one million may be positive for anti-PGL-I. A total of 15% of the healthy students in our sample had an anti-PGL-I titration that was two times higher than our threshold for positivity. If 5% of one million students become ill in the coming years, we will have an additional 50,000 new patients in the future, joining the 80,000 individuals who are currently ill. Some of these patients will experience mild signs and symptoms (e.g., single-lesion PB leprosy) and they may never become registered cases if their leprosy heals spontaneously, as described in the literature (Jesudasan & Christian 1985).

In fact, when examining the same population months after an initial screening for anti-PGL-I, as we did in the county of Oriximiná, in the western region of PA (Salgado et al. 2012), we found a high number of new leprosy cases amongst families with anti-PGL-I-positive individuals who were previously undiagnosed. Many factors may contribute to this high hidden prevalence. The clearest indicator is the low rate of contact examination, approximately 40% in PA, which is considered to be precarious by the Brazilian Ministry of Health (MS/ SVS 2009). Additionally, the low coverage of the population by the family health programme, with almost 60% of people lacking access to the system (Department of Health Care/Department of Primary Care 2012 - dab.)
saude.gov.br/historico_cobertura_sf.php), may explain the high number of undiagnosed leprosy cases. Furthermore, the majority of the cases we diagnosed were PB and many of them had only one lesion, sometimes a slightly hypochromic macule that was not recognised by the patient or family as a lesion. The family health programme team may be trained to detect well-established leprosy cases with clear symptoms, but the team may not be trained to diagnose cases in the early stages, such as those that we detected in this study.

![Graphs and diagrams showing the distribution and statistical significance of anti-phenolic glycolipid-I (PGL-I) IgM levels according to various factors such as gender, region, school category, and degree of physical disability.](image)

**Fig. 4A**: levels of anti-phenolic glycolipid-I (PGL-I) IgM according to the students gender; **B**: region of the schools; **C**: category (public or private); **D**: there was no significant difference according to the degree of physical disability; **E**: differences between cases detected during school survey [paucibacillary (PB) and multibacillary (MB)] and cases referenced to the reference unit (RU); **F**: correlation between the level of anti-PGL-I and the age of the household contacts of student diagnosed with leprosy; OD: optical density; UREMC: Dr Marcello Candia Reference Unit in Sanitary Dermatology.
Interestingly, the seropositivity rates amongst the PB and MB patients in our sample were almost the same. By contrast, the PB patients diagnosed at UREMC had a slight, but not significant increase in anti-PGL-I levels, while the UREMC MB patients had a significant increase in the median level of anti-PGL-I. These results confirm the early diagnosis because we detected more PB cases than MB cases and because the MB cases were mostly BT patients.

Surveys to detect leprosy among SC are not new. In 1947, researchers in British Guiana examined 42,811 students and found 94 (0.21%) new cases. The author proposed that surveying schools “should become a permanent part of the leprosy public health program” and concluded that without the study, the early cases might not have been identified until they were more advanced and difficult to cure (Wharton 1947). In a study similar to ours that was performed in India, Bhavasr and Mehta (1981) demonstrated that school surveys and contact examination of children could be considered to be a useful, inexpensive and rapid method for detecting leprosy cases in the community. In that study, visits to the homes of 24 new student cases revealed a family history of leprosy for 50% of the affected students. Similarly, Thirumalaikolundusubramanian and Prince (1983) examined 6,731 primary SC in India and 173 (2.7%) had leprosy. Silva et al. (2007) detected 20 new leprosy cases during an active search of 14,653 students, a case detection rate of 136/100,000 students, in Buriticupu, a hyperendemic municipality in the state of Maranhão (Brazil). In a cross-sectional survey of 1,114 students 11-20 years old in four districts in Timor-Lest, dos Santos et al. (2010) detected 17 (1.5%) new leprosy cases, which represents a case detection rate of 1,526/100,000 students. All five of these studies corroborate our findings. In addition to a high number of new student cases (63 cases out of 1,592 examined; 4%), we found 24 (9.4%) new cases amongst 256 HHCs of the students diagnosed with leprosy. This result clearly indicates that the contacts of infected students must be examined in addition to the students themselves.

Previously, we performed a study in the county of Oriximiná based on our serological data and found a high number of hidden cases (Salgado et al. 2012). Similarly, we performed a more thorough study in the county of Castanhal, where SC and family members of anti-PGL-I-positive individuals (identified 2 years ago) were examined. We identified more than 60 new cases of leprosy among approximately 400 examined individuals (unpublished observations), thus demonstrating the effectiveness of our strategy. In addition, we provided an in-service training session for the municipality health team workers.

Our results suggest that implementing school surveys for identifying leprosy cases is imperative in highly endemic areas. The advantage of our strategy is that it identifies early cases of leprosy (mainly PB cases) with no physical disabilities, thus preventing the spread of the infection in the community and breaking the chain of transmission that is responsible for the high incidence rate observed over time.
ACKNOWLEDGEMENTS

To André Luiz Correa de Sousa, Anna Elizabeth Martins Alves, Claudia Moacilene Penalber Tavares, Denis Vieira Gomes Ferreira, Márcia Leão, Patricia Ervedosa Ribeiro, Ricardo Braga Amorim and Sabrina Sampaio Bandeira, for collecting samples and data from patients, to Domingos Diniz, Miguel Canto and the PAI program at Oriximína, for all of the logistical support and fruitful discussions, to Moisés Batista da Silva, for assistance with ELISA protocols, to John Spencer, for supplying the native PGL-I and technical support with the ELISA, to the Secretary of Health and community health agents from the selected municipalities, and to the study participants.

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