**Objectives.** To evaluate the geographic distribution of human T-lymphotropic virus types 1 and 2 (HTLV-1/2) in the State of Minas Gerais, Brazil, in puerperal women whose newborns were tested for HTLV-1/2 during neonatal screening, and to overlap seropositivity with social and economic status determinants.

**Methods.** During September–November 2007, the dry-blood samples taken from newborns on filter paper for routine screening were also tested for maternal IgG anti-HTLV-1/2 antibodies. For reactive samples, the mothers of the newborns had blood drawn to test for these viruses.

**Results.** The study analyzed 55,293 specimens taken from newborns. Of these, 52 (9.4 per 10,000) were reactive and 42 mothers (7.6 per 10,000) were confirmed with HTLV-1/2 infection. HTLV-1/2 geographic distribution was heterogeneous, with a tendency to be higher in the North and North-East parts of Minas Gerais. The highest rates of seropositivity were observed in Vale do Mucuri (55.9 per 10,000) and in Jequitinhonha (16.0 per 10,000), overlapping with the State’s worst social and economic indicators.

**Conclusions.** To our knowledge this was the first time that neonatal screening for HTLV-1/2 was performed in Brazil. This model could be used in other areas with high HTLV-1/2 prevalence rates. The detection of carrier mothers can enable intervention measures, such as providing infant formula to newborns, to be implemented expeditiously to reduce vertical transmission.

**Key words** Human T-lymphotropic virus 1; human T-lymphotropic virus 2; neonatal screening; infectious disease transmission, vertical; Brazil.
Infective dermatitis—a disease that occurs mainly in childhood—is also associated with HTLV-1 (1). HTLV-2 seems to have less pathogenicity, although there are reports of association with neurological diseases similar to HAM/TSP (4). There is no effective anti-viral treatment for HTLV-1/2, and treatments in use for ATL and HAM/TSP have not presented satisfactory results (1).

HTLV-1 is globally distributed; 15–20 million individuals are estimated to be infected (1). Its exact prevalence is unknown, as there have been few population-based primary studies. Estimates of HTLV-1 prevalence, therefore, are mainly derived from research data obtained from specific populations, such as blood-donor candidates and pregnant women (1). HTLV-1 is considered endemic in southeast Japan (where seroprevalence reaches 10%); Caribbean countries (Cuba, Jamaica, and Trindad and Tobago: up to 6%); Sub-Saharan African countries (Benin, Cameroon, and Guinea Bissau: up to 5%); parts of Iran and Melanesia (less than 5%); and in South American countries (Brazil, Colombia, and Peru: 1–5%) (1, 2). An epidemiological pattern has been consistently reported that includes clustering of seropositive individuals in different geographic areas of the world; prevalence variation according to geographic area; an increase in prevalence with age; and higher seroprevalence in females (1, 5). HTLV-2 has a distinct pattern of distribution, with higher prevalence among Native American groups, various African tribes, and intravenous-drug users in the United States of America and Europe (4).

Several studies have reported that indicators of an individual’s low social and economical position, such as few years of education, are associated with HTLV-1/2 infection in endemic and non-endemic areas (5–8). These findings suggest that determinants of greater vulnerability—both as they pertain to the individual and to the physical and social environment—may be associated with a higher risk of infection by these viruses (2).

In Japan, the seroprevalence for HTLV-1 in pregnant women varies from 400–500 per 10 000 in hyperendemic areas to 10–100 per 10 000 in non-endemic areas (9). In a study of seven Western European countries, during prenatal and neonatal period, seropositivity for HTLV-1/2 was 4.4 per 10 000, ranging from 0.7 per 10 000 in Germany to 11.5 per 10 000 in France (10). Research in several countries indicated the following seroprevalence for HTLV-1/2 for every 10 000 pregnant women: 2 in Spain (11), 19 in Argentina (12), 193 in Martinique (13), 170 in Peru (14), 200 in Jamaica (15), 210 in Gabon (16), 250 in Ghana (17), 344 in French Guiana (18), 370 in Zaire (19), and 1 670 in Nigeria (20). This epidemiologic data demonstrates the heterogeneity seen in the prevalence of HTLV-1/2 across these geographically-dispersed populations.

For both types of viruses, routes of transmission are well established: sexual (by unprotected sexual relations), parental (by transfusion of hemocomponents or by sharing contaminated needles), and vertical (from mother-to-child, more frequently through nursing) (1). Several studies conducted in populations endemic for HTLV-1 demonstrated that vertical transmission rates range from 15%–25% (21). Elevated maternal proviral load and high maternal antibody titers have been described as risk factors associated with vertical transmission of HTLV-1 (22).

HTLV-1 infection in the first decade of life is a risk factor for infective dermatitis and ATL (1). Approximately 1%–5% of children infected through vertical transmission will develop ATL (1). Replacing breastfeeding with infant formula can reduce the rate of mother-to-child transmission to nearly 3% (23–25). In Japan, HTLV-1 vertical transmission was reduced 80% by instituting recommendations that infected mothers not breastfeed their newborns (26). The duration of breastfeeding is also an important factor in vertical transmission: infants nursed by infected mothers for more than 6 months have infection rates exceeding 20%, while those nursed for 6 months or fewer have HTLV-1 seropositivity rates around 5% (24, 27). The low seroconversion of the children nursed for less time or not at all can be attributed to the protective effect of the mother’s IgG class anti-HTLV-1 antibodies, passively transferred during gestation. High levels of these antibodies are present in newborns during the first post-partum months, but tend to dissipate over the course of months 6–12 (9, 23, 27). The presence of specific anti-HTLV-1/2 antibodies in newborns is considered a reliable indicator of maternal infection. A study in Japan demonstrated the presence of maternal IgG anti-HTLV-1 antibodies in 98% of umbilical cord blood samples of infants born to mothers that were HTLV-1 carriers (23).

The importance of implementing strategies to prevent and control the spread of HTLV-1/2 in the Americas has been previously discussed by members of the Interdisciplinary Research Group on HTLV (abbreviated “GIPH” in Spanish and Portuguese) (2). Public health measures to control the spread of HTLV-1/2 are justified given the geographic distribution of HTLV-1/2 in the Americas, its modes of transmission, the morbidity and mortality attributed to HTLV-1/2 infection, and the fact that there is currently no effective treatment (2). Interventions to prevent mother-to-child transmission—e.g., prenatal/neonatal screening in geographic areas with high prevalence and encouraging seropositive mothers to provide formula rather than breast milk for their infants—would likely reduce the incidence of vertical transmission and HTLV-related diseases (2). Public policies designed to prevent perinatal transmission of HTLV-1/2 have not been widely implemented in Brazil, or in other countries in the Americas, in part because information on the real risk of perinatal transmission is lacking. In response, the Interdisciplinary Research Group on HTLV conceived the present study as part of its continuing efforts to put into practice the vertical transmission control measures proposed by the leading study (2).

The main objectives of this study were: (a) to identify HTLV-1/2 positive mothers by detecting maternal antibodies in blood samples taken from newborns in the Minas Gerais State; and (b) to determine if there is geographic overlapping between HTLV-1/2 seropositivity rates and several aggregate-level indicators of social and economical position, using sub-areas of the state as the unit of analysis.

**MATERIALS AND METHODS**

A descriptive, observational study was conducted to determine the distribution of HTLV-1/2 among mothers of newborns screened by the Minas Gerais State Neonatal Screening Program in September–November 2007. For each of Minas Gerais’ 12 sub-areas, seropositivity was calculated and compared to the
aggregate-level social and economic indicators of each.

**Study setting**

The State of Minas Gerais, located in Southeastern Brazil, has an area of 586,528 km² and a population of 19,273,506 (2007 estimate) (28). The State’s 853 municipalities are grouped into 12 sub-areas, as defined by the Brazilian Institute of Geography and Statistics (IBGE) (29). These were the geographical units of analysis for this study.

**Minas Gerais Neonatal Screening Program**

The Minas Gerais Neonatal Screening Program is a publically-funded program that performs routine screening for phenylketonuria, hypothyroidism, cystic fibrosis, and hemoglobinopathies using dried blood spots. Samples are collected from newborns on the 5th day of life at state-funded primary health care centers. For newborns who remain hospitalized on the 5th day of life, the blood spot specimens are collected in the hospital. The State of Minas Gerais Neonatal Screening Program has high coverage, testing about 95% of the state’s newborns (30, 31).

**Ethical aspects**

This study project was approved by the Federal University of Minas Gerais Committee for Ethics in Research (COEP-UFGM — Evaluation code ETIC 482/06) and by the Municipal Health Secretariat’s Committee for Ethics in Research (Protocol Number 082/2007).

**Research description and data collection**

The Health Secretariats in all of Minas Gerais’ 853 municipalities agreed to cooperate with the study. The permission of parents/legal guardians to test for maternal IgG anti-HTLV-1/2 antibodies using the same filter paper specimen used for routine screening was obtained for all children tested by the state’s Neonatal Screening Program during the 3-month study period. Collection of the children’s data (date of birth, name, and address) and sample coding was done according to the Program’s routine protocol.

For each positive or indeterminate HTLV-1/2 reaction on the filter paper, a sample of the mother’s peripheral blood was requested from the primary health care center that had submitted the blood spots. Prior to blood collection, mothers received written guidelines informing them of HTLV-1/2 infection and transmission, including recommendations and instructions related to preventing transmission. Mothers whose blood tested positive for HTLV-1/2—by enzymatic immunoassay (ELISA)—were instructed to discontinue breastfeeding to avoid transmission to their child. A 6-month supply of infant formula was provided for all newborns whose mothers had been identified as HTLV-1/2 carriers; plus, seropositive mothers were referred to a specialized clinic for HTLV-related medical care.

**Laboratory testing**

All dried blood-spot filter papers were delivered to the Federal University of Minas Gerais’ Diagnostic and Research Center, the reference laboratory for the Neonatal Screening Program. Anti-HTLV IgG antibodies were detected by an ELISA developed specifically to detect anti-HTLV-1 and/or anti-HTLV-2 antibodies in dry blood samples collected on S&S 903 filter paper (Q-PREVEN HTLV 1+2–IgG-DBS, Advanced Laboratory Diagnostic Systems, Rome, Italy). Test results were interpreted according to manufacturer’s specifications as seropositive, seronegative, or indeterminate. This study is based on the premise that anti HTLV-1/2 specific IgG antibodies in neonates is indicative of maternal infection.

Prior to its use in this study, the Q-PREVEN HTLV 1+2–IgG-DBS kit was compared to the HTLV ELISA screening test used by the Hemominas Foundation, the State’s blood bank. Forty specimens were collected simultaneously in vacutainer tubes and on S&S 903 filter paper. Nineteen specimens were known negative for HTLV-1/2 and 21 positive, according to prior testing done by the Hemominas Foundation. Kappa statistics that considered both test results was 0.9, revealing an excellent concordance (32).

Blood specimens obtained from mothers of newborns with positive or indeterminate HTLV-1/2 results were tested with the ELISA test (HTLV-1/2 Ab-Capture ELISA Test System, Ortho Clinical Diagnostics Inc., New Jersey, United States). Seropositive samples were subjected to confirmatory Western Blot (HTLV Blot 2.4, MP Diagnostics, Singapore) testing. Both test results were interpreted according to the manufacturers’ specifications.

**Data analysis**

Using the statistics software, Stata 11.0 (StataCorp, College Station, Texas, United States) the proportion of HTLV-1/2 seropositive mothers was calculated for the State and each of its 12 sub-areas. Due to the small number of seropositive mothers, a Poisson distribution for the analysis was assumed. The filter paper samples taken from newborn twins were excluded from the analysis so that each infant specimen would match only one maternal specimen.

The HTLV-1/2 spatial distribution across the state for the 3-month collection was then overlapped with selected socioeconomic macroindicators for the participants’ residential sub-area. The socioeconomic indicators were derived from the 2000 census (33) and from the 2005 maternal death and newborn statistics from DATASUS, the demographic and utilization database of Brazil’s national health system (31). The indicators selected were considered important measures of socioeconomic status and showed substantial variability among the 12 sub-areas analyzed: (a) the percentage of heads of household whose incomes did not exceed the minimum wage (about US$ 83 at the time of the census) (33); (b) the percentage of heads of household whose formal education was equal to or less than 1 year (33); and, (c) the 2005 maternal mortality rate calculated by dividing the number of female deaths related to pregnancy by 100,000 live births (31). Tabwin 3.5 software (State Health Department, Brasilia, Brazil) was used to dereference the data. Map scales were defined according to quintiles of the data plotted on maps.

**RESULTS**

All eligible mothers gave their permission to have the blood spots tested for HTLV-1/2. Therefore, all 56,076 dried blood filter paper specimens received by the State’s Neonatal Screening Program in September–November 2007 under-
went HTLV-1/2 testing. Although tested (and found to be seronegative), 783 specimens were excluded from the study because they were obtained from twins. Of the remaining 55 293 specimens, a total of 52 were reactive and 1 was indeterminate. Peripheral blood samples were obtained from all 53 mothers and tested for HTLV-1/2.

Seropositivity was confirmed in 42 mothers (79.2%), including the mother of the child with the indeterminate result. The statewide HTLV-1/2 prevalence among mothers of singleton newborns in 2007 was 7.6 per 10 000 (95% CI: 5.5–10.3). Eleven (20.8%) were seronegative for HTLV-1/2. Forty mothers were identified as seropositive for HTLV-1, and two as seropositive for HTLV-2. Table 1 shows the frequency distribution of results by sub-area. In two sub-areas, Campo das Vertentes and Central Minera, all samples were non-reactive for HTLV-1/2. In the other 10 sub-areas, HTLV-1/2 seropositivity among the post-partum mothers ranged from 1.4–55.9 per 10 000 live singleton births.

The highest rates of maternal seropositivity were observed in Vale do Mucuri (55.9 per 10 000; 95% CI: 22.5–115.1) and Jequitinhonha (16.0 per 10 000; 95% CI: 4.4–41.0) sub-areas; and the lowest rates in the Sul/Sudoeste de Minas (1.4 per 10 000; 95% CI: –0.04–8.1) and Zona da Mata (1.8 per 10 000; 95% CI: –0.05–9.8) sub-areas. The two HTLV-2 positive specimens came from two mothers of indigenous Brazilian ethnicity, the Maxacalis tribe which lives in the Vale do Mucuri sub-area.

Looking at socioeconomic parameters—as measured by the percentage of heads of household with income below the minimum wage—it was noted that the poorest sub-areas, i.e., Jequitinhonha and Mucuri, match the sub-areas with the highest HTLV-1/2 seropositivity (Figure 2). Figure 3 shows a similar geographic correlation, in which sub-areas with heads of household with formal education equal to or lesser than 1 year overlap with the ones presenting higher proportions of HTLV-1/2 seropositive mothers, again Jequitinhonha and Mucuri.

**DISCUSSION**

To our knowledge, this is the first time that a neonatal screening program, covering all newborns in a population of almost 20 million inhabitants, was used to both identify HTLV-1/2 seropositive mothers and to intervene to reduce the risk of vertical transmission of the virus from mother to child.

In 2007, the seropositivity in the HTLV-1/2 screening test (ELISA) was 8 per 10,000, for blood donors at the Hemominas Foundation, and 2 per 10,000 with a confirmatory Western Blot test according to Sônia Mara Nunes da Silva, Biochemist, Hemominas Foundation Serology Lab (personal communication, 10 November 2008). The HTLV-1/2 seroprevalence rate of puerperal women was four times higher than that of blood donors.

The percentage of reactive samples among newborn blood spots that were not confirmed in the maternal specimen was 20.8%, which was not unexpected, given that in low-prevalence populations, the positive predictive value of a test—even a test with high sensitivity and specificity—is low. This phenomenon is seen at blood banks, where only about 10%–50% of samples reactive in the screening test are subsequently reactive in the confirmatory test (34).

In Brazil, studies have reported the following seroprevalences for HTLV-1/2 per 10 000 pregnant women: 10 in Botucatu (São Paulo State) (35), 13 in Mato Grosso do Sul State (36), 10 in Goiânia (Goiás State) (37), 24 in Fortaleza (Ceará State) (38), 87 in Salvador (Bahia State) (39), 98 in Cruz das Almas (Bahia State) (40) and 134 in Vitória (Espírito Santo State) (41). In the Vitória study, which reported the highest rates, confirmatory tests were not performed; both puerperal and pregnant women were tested (41). In two other studies conducted in Brazilian women during the puerperal period, one in Cuiabá (42) and the other in Belo Horizonte (where confirmatory tests were not performed on all positive specimens) (43), reported prevalences were 2 per 10 000 and 100 per 10 000, respectively. A study among nursing mothers of Paraíba State reported prevalence of 68 per 10 000 (44). Although the study samples and methodology are not directly comparable, the HTLV-1/2 seropositivity rates in mothers of newborns in Minas Gerais are among the lowest reported by Brazilian studies.

Minas Gerais is referred to by some as a mirror of Brazil. Like the country itself, the state’s poorest sub-areas are in the North and Northeast. The geographic distribution of seropositivity was heterogeneous across the state’s 12 sub-areas, with a trend toward higher seropositivity rates in Noroeste de Minas and Norte de Minas, a finding observed in a prior study, which investigated the geographic distribution of HTLV-1/2 infection among blood donors in Brazil (8).
The sub-areas with the highest HTLV-1/2 seropositivity rates overlap with those that have the worst macroindicators of socioeconomic position. However, the modest numbers of seropositive mothers constrains a more detailed analysis of a possible correlation with contextual and compositional indicators of socioeconomic status.

Previous studies reported the association between HTLV-1/2 seropositivity and individual (5–7) and macro level indicators (8) of socioeconomic vulnerability. In this sense, it is plausible that differences in HTLV-1/2 seroprevalence may be partly attributable to the socioeconomic variation across the sub-areas. Certainly, other compositional and individual-level characteristics, such as gender, age, and migration, may contribute to the HTLV-1/2 seroprevalence heterogeneity observed in the present study.

Both of the two HTLV-2 positive samples were from mothers of indigenous Brazilian ethnicity, specifically the Maxacalis tribe that lives in the Vale do Mucuri sub-area. Although only two cases, this finding is consistent with reports in the literature about HTLV-2 endemicity among indigenous populations of the Americas (4). Coincidently, these two cases occurred in the sub-area with the highest HTLV-1/2 seropositivity rate (55.9 per 10,000; 95%CI: 22.5–115.1).

Considering the diseases being screened for by neonatal programs, the prevalence of HTLV-1/2 infection (7.6 per 10,000) found in mothers of newborns in the Minas Gerais State is similar to the incidence of sickle cell disease (7.1 per 10,000), and is greater than the incidence of the other diseases: congenital hypothyroidism (2.9 per 10,000), phenylketonuria (0.5 per 10,000), and cystic fibrosis (1.1 per 10,000) (30). Therefore, HTLV-1/2 prevalence in Minas Gerais and the documented efficacy of vertical transmission interventions in other countries call for the development of long-term public health actions to identify HTLV-1/2 carriers and to implement proven interventions.

The HTLV-1/2 seropositivity found in the present study probably reflects the infection prevalence among pregnant women in the state of Minas Gerais. However, some study limitations should be acknowledged. Even though the screening assay used is specific for anti-HTLV-1/2 antibody detection on dried blood filter paper samples, the possibility of false negative results cannot be excluded. Therefore, newborns of mothers who are HTLV-1/2 carriers with low antibody levels could have false-negative results. Thus, this study’s findings might underestimate the prevalence in Minas.
Gerais; however, preliminary evaluation of the test showed high correlation with the conventional serology screening, known to have high sensitivity and specificity, used by Brazilian blood banks. It is estimated that about 5% of the Minas Gerais newborns are not screened in the public program. Most of these newborns are thought to be tested in the private program. Since these tests are not forwarded to the state health neonatal screening system. Since these are thought to be tested in the private program. Most of these newborns are not screened in the public program. Most of these newborns are thought to be tested in the private program. Since these tests are not forwarded to the state health department, information on these newborns is unknown. Also, women who had abortions, miscarriages, or whose children were stillborn were not included in this study. Although there is no reported association between HTLV-1/2 infection and abortion or stillbirth, either could cause an underestimation of HTLV-1/2 seroprevalences. A study evaluating 6,754 pregnant women in the city of Salvador, a Brazilian state capital, found that in the general population of Salvador, Brazil: a city with African ethnic and sociodemographic characteristics. J Acquir Immu

In summary, this study’s results indicate that in the state of Minas Gerais, the geographic distribution of mothers of HTLV-1/2 seropositive newborns is heterogeneous, with the highest rates overlapping in the areas with the worst indicators of socioeconomic position.

Brazil’s neonatal screening programs are well organized, attain high population coverage, and are widely known and trusted by the public. Adding an HTLV-1/2 immunoassay to neonatal screening programs may be an excellent strategy to leverage public resources to control the spread of HTLV-1 and HTLV-2. Systematic collection of information on HTLV-1/2 seroprevalence among pregnant women would permit better public health planning. The detection of virus carriers through prenatal or neonatal screening would enable intervention measures, such as providing formula for newborns of seropositive mothers, to be implemented expeditiously and could help reduce vertical transmission (26), especially in sub-areas with higher seropositivity rates.

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Objetivos. Evaluar la distribución geográfica del virus linfotrópico de células T humanas tipos 1 y 2 (HTLV-1/2) en el estado de Minas Gerais (Brasil), en mujeres puérperas en cuyos recién nacidos se analizó la presencia del HTLV-1/2 durante las pruebas neonatales de detección sistemática, y superponer la seropositividad con determinantes del estado socioeconómico.

Métodos. Entre septiembre y noviembre de 2007, en las muestras de sangre seca extraída a los recién nacidos en papel de filtro para un tamizaje sistemático, se analizaron también los anticuerpos maternos de tipo IgG anti-HTLV-1/2. En el caso de las muestras reactivas, se extrajo la sangre de las madres de los recién nacidos para realizar pruebas de detección de estos virus.

Resultados. En el estudio se analizaron 55 293 muestras extraídas de los recién nacidos. De estas, 52 (9,4 por 10 000) fueron reactivas y en 42 madres (7,6 por 10 000) se confirmó la infección por el HTLV-1/2. La distribución geográfica del HTLV-1/2 fue heterogénea, con una tendencia a ser mayor en el norte y el noreste de Minas Gerais. Las tasas más elevadas de seropositividad se observaron en Vale do Mucuri (55,9 por 10 000) y en Jequitinhonha (16,0 por 10 000), superponiéndose con los peores indicadores socioeconómicos del estado.

Conclusiones. Esta fue la primera vez que se realizó un tamizaje neonatal para el HTLV-1/2 en Brasil. Este modelo podría usarse en otras regiones con tasas de prevalencia altas del HTLV-1/2. La detección de las madres portadoras puede permitir la aplicación rápida de medidas de intervención, como por ejemplo, el suministro de leche maternizada a los recién nacidos, a fin de reducir la transmisión vertical.

Palabras clave. Virus linfotrópico de células T humanas tipo 1; virus 2 linfotrópico T humano; tamizaje neonatal; transmisión vertical de enfermedad infecciosa; Brasil.