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SHORT PAPER

## Prevalence of HIV-1, HIV-2 and HTLV antibody, in Fortaleza, Ceara, Brazil, 1993-1994

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**Summary:** To evaluate the respective part of HIV-1, HIV-2 and human T lymphotropic virus (HTLV) infection in Fortaleza, the principal city of the Ceara state (Northeast of Brazil), a cross-sectional seroepidemiological survey was conducted from July 1993 to February 1994 in 6 selected groups: pregnant women, tuberculosis (Tb) patients, sexually transmitted disease (STD) patients, female and male commercial sex workers (CSWs) and prisoners. Sera were screened by Mixt HIV-1/HIV-2 commercial enzyme immunoassay and ELISA HTLV I/II. Each serum found positive by ELISA was confirmed by Western blot. A total of 2917 persons were interviewed, of whom 2754 (94.4%) agreed to participate and gave a blood sample. Twenty-eight were found to be HIV-1 antibody positive. The prevalence ranged from 0.25% in pregnant women to 2.9% in male CSWs. The prevalence was 1% in STD patients and 0.44% in Tb patients. None of the sera was found positive for HIV-2. The prevalence of antibodies to HTLV-I varied from 0.12% in pregnant women to 1.21% in female CSWs. Five sera were positive for HTLV-II. These results confirm the hypothesis that the HIV epidemic in Northeastern Brazil is still limited to high risk groups. Repeated cross-sectional surveys of this type should be performed as a surveillance tool to study the dynamics of this epidemic in low prevalence areas. Defining risk factors should allow targeting of intervention strategies.

**Keywords:** Retrovirus, HIV-1, HIV-2, HTLV I, HTLV II, epidemiology, South America, Brazil

### INTRODUCTION

In Latin America and the Caribbean, there were an estimated 1-1.5 million adults infected with human immunodeficiency virus (HIV) as of late 1993<sup>1</sup>. The largest number of people infected was in Brazil, with an estimated number of 750 000 in January 1992<sup>2</sup> and a prevalence of 0.5% in the general population. The first cases of AIDS were diagnosed in Brazil in 1982, in the states of São Paulo and Rio de Janeiro. As of June 1994<sup>3</sup>, 49 312 cases of AIDS had been notified in Brazil, with 77% of these notified by the Southeastern region (including São Paulo and Rio de Janeiro), and 8% by the Northeast. Ceara is a Northeastern state, that notified 15% of the cases of this region or 1.3% of the overall

number of cases reported nationwide. Fortaleza, the principal city of the Ceara accounted for 514 cases, i.e. more than 80% of the cases reported in that state. Because no data are available to analyse the dynamics of the epidemic, the present study was conducted to evaluate, through seroprevalence surveys of selected population groups, the respective part played by HIV-1, HIV-2 and human T lymphotropic virus (HTLV) infection in the population of Fortaleza.

### POPULATION AND METHODS

A cross-sectional seroepidemiological survey was conducted in Fortaleza, from July 1993 to February 1994 in 6 selected population groups.

The sample size was calculated from an estimated prevalence of AIDS of 18.3/1000 000 among the general population of the Ceara state, in March 1990, according to the National AIDS Programme. We supposed that half of the AIDS cases were notified,

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that the number of AIDS cases in the city of Fortaleza was 3 times higher than in the rural area of the Ceara and that for each AIDS case there were 100 HIV infected individuals<sup>4</sup>. From these data and from studies carried out in Southeastern Brazil<sup>5</sup>, we calculated that the overall prevalence rate of HIV infection was 1% in Fortaleza with wide variations in the sub-groups studied (Table 1). The sample size was calculated according to the 95% confidence interval formula with an acceptable alpha-type one error of 5%.

The first 3 groups, pregnant women, tuberculosis (Tb) patients and sexually transmitted disease (STD) patients were recruited from Public Health Centres and Public Hospitals. A total of 17 centres were chosen, according to the number of new cases notified each month and the geographical location in the city: 3 centres for pregnant women, 7 reference treatment centres for Tb patients and 7 clinics for STD patients. Beginning in August 1993, all new cases diagnosed were systematically asked to participate in the study until the appropriate sample size was obtained. The study of commercial sex workers (CSWs) was conducted with the help of prostitute associations. We delineated all the areas of the town known for male or female street prostitution, then asked each CSW identified if they wanted to participate in the study. We then visited 80% of the bars, houses of prostitution and social clubs, to recruit the rest of the sample of CSWs. The prisoners were volunteers from 3 state prisons of Ceara: 2 prisons for men and one prison for women.

People were individually informed of the study objectives and context. They then gave oral consent, and completed a 15–20 minutes oral questionnaire collecting socio-demographic variables, and information on risk factors, STD history and sexual behaviour. A blood sample was collected immediately after the interview and the pre-test counselling. The questionnaire was anonymous. People were informed that the results were available upon request (through a confidential numbering system) from nurses or social workers trained for this purpose. Post-test counselling, psychological and medical support and follow-up was offered at the HIV consultation of the São José Infectious Diseases Teaching Hospital.

Sera were screened by Mixt HIV-1/HIV-2 commercial enzyme immunoassay (ELISA, Genelavia Mixt Sanofi/Diagnostic Pasteur, Narnes la Coquette, France) and ELISA HTLV I and II (Ortho Diagnostic System/Cambridge Biotech Corp. Worcester, USA). Each serum positive by Genelavia Mixt was analysed by HIV-1 (Wellcozyme HIV-1 Recombinant, Murex, Dartford, UK) and HIV-2 (Clonatec Ab HIV-2, Paris, France) monospecific ELISA. The positive sera were confirmed by Western blot (New LAV-Blot 1 and New LAV-Blot 2, Sanofi, Diagnostics Pasteur, Narnes La Coquette, France). Each serum positive by HTLV I and II ELISA was confirmed by Western blot HTLV I/II (Cambridge Biotech, Worcester, Mass, USA). World Health Organization criteria were used to define indetermined sera<sup>6</sup>. It was not possible to retest indeterminate results. A serum was suspected to be positive for HTLV-II if it produced a positive p24 and rgp 21 band, and was negative for the p19 band by Western blot. The serum was then confirmed by a reactivity on a specific HTLV-II gp 46 recombinant protein (HTLV Blot 2.3, Diagnostic Biotechnology, Singapore).

Only samples from individuals who had completed a questionnaire and given a blood specimen were used in the data analysis. Data were compared using the Mantel-Haenszel Chi-square for trend, and the Student *t*-test, when appropriate. The 95% confidence interval of the prevalence in each group was computed by the Fleiss quadratic method, because of the small number of positives and the large sample size.

## Results

A total of 2917 persons were interviewed of which, 2754 (94.4%) accepted to have a blood sample drawn. The details of the participation rate by study groups are presented in Table 1. For all groups, more than 80% were born in Ceara and all had been living in the city of Fortaleza for more than 10 years. The study population was poorly educated: 15% were illiterate and 54% did not complete primary school; only 2% had been to university. In our sample, the socio-economical level was also very low: the median income (US \$180) represented 3 times the national minimum

Table 1. Calculated sample size, according to estimated prevalence of HIV-1, precision needed and effective participation rate of the studied populations, Fortaleza, Ceara, Brazil, 1993–1994

| Group          | Estimated prevalence | Precision | Sample size (100%) | Number of people interviewed (%) | Blood sample collected (%) |
|----------------|----------------------|-----------|--------------------|----------------------------------|----------------------------|
| Pregnant women | 0.005                | 0.005     | 800                | 833 (104)                        | 814 (101)                  |
| Tb patients    | 0.05                 | 0.02      | 500                | 494 (99)                         | 451 (90)                   |
| STD patients   | 0.05                 | 0.02      | 500                | 428 (85)                         | 395 (79)                   |
| Female CSWs    | 0.06                 | 0.02      | 600                | 529 (88)                         | 496 (83)                   |
| Male CSWs      | 0.13                 | 0.03      | 500                | 189 (38)                         | 171 (34)                   |
| Prisoners      | 0.05                 | 0.02      | 500                | 444 (89)                         | 427 (85.4)                 |

Tb=tuberculosis, STD=sexually transmitted diseases, CSWs=commercial sex workers

wage in 1993, and 40% were without any official income. The age of the respondents ranged between 12 and 79 years (mean: 28 years, median: 26 years); 29% of them were married.

Among the 2754 serum samples, 28 were found to be HIV-1 antibody positive. The prevalence of antibodies to HIV-1 by population group ranged from 0.25% in pregnant women to 2.92% in male CSWs (Table 2). The overall prevalence of indeterminate results was 0.4%; it was significantly higher among Tb patients (1.1%) than among other groups (0.3%) ( $P=0.023$ ). None of the study participants had detectable antibodies to HIV-2. Among the seropositives for HIV-1, 40% earned less than 3 times the minimum wage and 24% said they had no income; 78% did not complete primary school.

The prevalence of antibodies to HTLV-I is presented in Table 3; it ranged from 0.12% in pregnant women to 1.21% in female CSWs. We also found 5 sera positive for HTLV-II: one in a pregnant woman, one in a Tb patient, one in a female CSW and 2 in prisoners (Table 3). A 45-year-old, homosexual, CSW, intravenous drug user (IVDU), who had Tb presented coinfection with HIV-1 and HTLV-I; he had had a negative test for HIV-1 in 1988.

The associations between risk factors and HIV-1 infection are presented in Table 4. Several risk factors could be present in any one individual. However, the commonest reported risk factor for HIV-1 seropositivity was 'never used condoms'.

## Discussion

Our results show that Fortaleza and consequently Ceara state is a region of low endemicity for HIV-1 as possibly is the case for all the North and Northeastern states of Brazil as of 1994.

The HIV epidemic in this part of Brazil is recent, as it has been shown by Ishak, *et al.*<sup>7</sup>. These authors did not identify a single positive for HIV among 640 sera from different population groups of the North of Brazil, that had been collected between 1974 and 1980. In 1983, another serological survey carried out in Para state (Amazon region) among gold miners coming from all parts of Brazil (including the Southeastern region) did not show evidence of HIV-1 in this part of Brazil<sup>8</sup>. Indeed, in Ceara state as in most North-northeastern states, the majority of the initial AIDS cases have acquired their infection in either São Paulo or Rio de Janeiro<sup>9</sup>: in our study, 98% of the population tested was born in Ceara and among them, 90% had never left Ceara, a feature which has considerably minimized their risk so far. People at risk who travel to the South of Brazil are likely to be educated and wealthy, and were therefore unlikely to be represented in our sample.

A bias exists in relation to the recruitment in public health clinics, which are attended primarily by low income populations, particularly pregnant women, and therefore do not represent the general population. The population studied was informed that HIV testing would be performed, introducing another possible selection bias, particularly among

Table 2. Prevalence of antibodies to HIV-1 by selected population groups, Fortaleza, Ceara, Brazil, 1993–1994

|                | Sample size | Number of positives | Prevalence (%) | 95% CI*   | Number of indeterminate results |
|----------------|-------------|---------------------|----------------|-----------|---------------------------------|
| Pregnant women | 814         | 2                   | 0.25           | 0.04–0.99 | 0                               |
| Tb patients    | 451         | 2                   | 0.44           | 0.08–1.77 | 5                               |
| STD patients   | 395         | 4                   | 1.01           | 0.32–2.75 | 1                               |
| Female CSWs    | 496         | 8                   | 1.61           | 0.75–3.28 | 2                               |
| Male CSWs      | 171         | 5                   | 2.92           | 1.08–7.05 | 1                               |
| Prisoners      | 427         | 7                   | 1.64           | 0.72–3.50 | 2                               |

\*CI=confidence interval calculated by the Fleiss quadratic method, Tb=tuberculosis, STD=sexually transmitted diseases, CSWs=commercial sex workers

Table 3. Prevalence of antibodies to HTLV-I and HTLV-II, by selected population groups, Fortaleza, Ceara, Brazil, 1993–1994

|                | Sample size | Number of positives (HTLV I) | Prevalence (%) | 95% CI*   | Confirmed HTLV-II | Number of indeterminate results |
|----------------|-------------|------------------------------|----------------|-----------|-------------------|---------------------------------|
| Pregnant women | 814         | 1                            | 0.12           | 0.01–0.79 | 1                 | 1                               |
| Tb patients    | 451         | 2                            | 0.44           | 0.08–1.77 | 1                 | 1                               |
| STD patients   | 395         | 2                            | 0.51           | 0.09–2.02 | 0                 | 2                               |
| Female CSWs    | 496         | 6                            | 1.21           | 0.49–2.75 | 1                 | 3                               |
| Male CSWs      | 171         | 1                            | 0.58           | 0.03–3.71 | 0                 | 0                               |
| Prisoners      | 427         | 2                            | 0.47           | 0.08–1.87 | 2                 | 2                               |

\*CI=confidence interval calculated by the Fleiss quadratic method, Tb=tuberculosis, STD=sexually transmitted diseases, CSWs=commercial sex workers

Table 4. Risk factors of HIV-1 infection among the studied group of population, Fortaleza, Ceara, Brazil 1993-1994

|                      | Pregnant women<br>(814/2 HIV+ve) |        | Tb patients<br>(451/2 HIV+ve) |        | STD patients<br>(395/4 HIV+ve) |        | Female CSWs<br>(496/8 HIV+ve) |        | Male CSWs<br>(171/5 HIV+ve) |        | Prisoners<br>(427/7 HIV+ve) |        |
|----------------------|----------------------------------|--------|-------------------------------|--------|--------------------------------|--------|-------------------------------|--------|-----------------------------|--------|-----------------------------|--------|
|                      | n (%)                            | HIV+ve | n (%)                         | HIV+ve | n (%)                          | HIV+ve | n (%)                         | HIV+ve | n (%)                       | HIV+ve | n (%)                       | HIV+ve |
| Multiple partners    | 1 (0.1)                          | 0      | 4 (0.8)                       | 0      | 22 (5.1)                       | 1      | 317 (60)                      | 6      | 111 (59)                    | 2      | 17 (3.8)                    | 0      |
| Blood recipients     | 45 (5.4)                         | 0      | 48 (9.9)                      | 0      | 27 (6.3)                       | 0      | 56 (11)                       | 0      | 8 (4.3)                     | 1      | 58 (16)                     | 1      |
| Had at least one STD | 40 (4.9)                         | 0      | 105 (22)                      | 1      | 451 (100)                      | 4      | 121 (23)                      | 4      | 60 (32)                     | 3      | 136 (38)                    | 1      |
| Had or have Tb       | 14 (1.7)                         | 0      | 451 (100)                     | 2      | 9 (2.4)                        | 0      | 37 (7)                        | 2      | 17 (9)                      | 1      | 34 (10)                     | 1      |
| IVDUs                | 4 (0.5)                          | 0      | 14 (2.9)                      | 0      | 6 (1.4)                        | 0      | 23 (4)                        | 0      | 9 (5)                       | 1      | 103 (29)                    | 3      |
| Had sex with IVDU    | 33 (4)                           | 0      | 26 (5.4)                      | 0      | 20 (4.7)                       | 2      | 96 (18)                       | 0      | 24 (13)                     | 0      | 106 (30)                    | 2      |
| Prostitution         | 19 (2.3)                         | 0      | 21 (4.4)                      | 0      | 22 (5.2)                       | 2      | 529 (100)                     | 8      | 189 (100)                   | 5      | 48 (14)                     | 0      |
| Never used condoms   | 582 (71)                         | 2      | 385 (85)                      | 2      | 353 (89)                       | 3      | 256 (48)                      | 5      | 112 (66)                    | 5      | 318 (74)                    | 6      |

Multiple partners was defined as more than 1 sexual partner in the last month. STD=sexually transmitted diseases, Tb=tuberculosis, IVDUs=intravenous drug users

male CSWs (whose low participation level, especially that of transvestites, made the interpretation of results difficult) and prisoners. Consequently, the prevalence of antibodies to HIV-1 in these 2 groups is certainly lower than it should be. Even though, the prevalence figures are slightly different from those found by other studies in Southeastern Brazil and Bahia state. Our prevalence rate in pregnant women of 0.12% is in marked contrast to a rate of 3.6% found in a study in Santos (São Paulo state)<sup>10</sup>. A further study in Salvador (Bahia state)<sup>2</sup> reported a prevalence of 1%. The results among Tb patients also show a low prevalence when compared with studies conducted in Africa<sup>11</sup>. However, a study performed in 1987 and 1988 in Rio de Janeiro, in 389 patients with active pulmonary tuberculosis showed a prevalence of 0.51%<sup>12</sup>. We cannot explain the significantly high number of indeterminate sera for HIV-1 among tuberculosis patients. This phenomenon has already been reported in infectious diseases<sup>13</sup>; in this study, seroconversion would be an important hypothesis to consider, but the study design did not allow retesting. For female CSWs our prevalence is similar to the Cortes study in upper class prostitutes<sup>5</sup>. In relation to male CSWs, our results show a much lower prevalence than in Rio de Janeiro and São Paulo state<sup>5</sup>, but are difficult to interpret because of the selection bias explained earlier and because the number required for significance has not been achieved.

The prevalence of HTLV-I was higher for the groups with high risk sexual behaviour: STD patients, female and male CSWs and prisoners. It was very close, for pregnant women and Tb patients, to the prevalence found in Minas Gerais among blood donors<sup>14</sup>. This could mean, as stated by Cortes<sup>5</sup>, that HTLV-I is relatively common in Brazil and particularly in the North and Northeastern regions where this infection seems to be associated with sexual habits. Until now, HTLV-I has been more studied in Brazil among patients with tropical spastic paraparesis<sup>15-17</sup>. Further studies are needed to define the epidemiology of this retrovirus and, in particular, to define the transmission routes. Conclusions can not be drawn from the single case of co-infection by HIV 1 and HTLV-I.

Identification of the presence of HTLV-II was an important observation coming out of this study. This retrovirus has only been reported in Brazil among Amerindians (South and Central American Indian tribes) with a prevalence varying among tribes from 12 to 33%. This finding has led to the hypothesis that HTLV-II is an ancient human virus in the Americas, these tribes having very few contacts with non-Indians<sup>18</sup>. This is the first time that HTLV-II has been identified in Northeastern Brazil and this deserves further investigation.

There were few IVDUs in our sample, because this method of drug intake is not common in Northeastern Brazil, in contrast to the South and

Southeastern parts of the country. Indeed, in this part of Brazil, sexual transmission of HIV is predominant and the main risk factor identified for HIV-1 transmission was the absence of regular condom use.

Our results confirm the hypothesis that the HIV epidemic in Northeastern Brazil is still limited to high risk groups such as CSWs and has not spread yet in the general population. The prevalence of HIV-1 may possibly be higher in the high income and intellectual class of bisexuals and homosexuals. Certainly, the spread of the HIV epidemic in the lower middle class has already started and a sharp increase of the prevalence should be expected before the year 2000. Repeated cross-sectional surveys of this type should be performed as a surveillance tool to study the dynamics of this epidemic in low prevalence areas. Defining risk factors should also allow targeting of resources for maximum benefit. At this point, it is of primary importance to focus on STD and Tb control, and to develop diagnosis, treatment, counselling and HIV prevention units in primary health centres, and in particular to educate the population in the preventive benefits of condoms.

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