Prevalence and risk determinants of HIV-1 and HIV-2 infections in pregnant women in Bissau

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Summary  Objectives: To analyse the risk determinants of HIV-1 and HIV-2 infections in pregnant women in Bissau.
Methods: Pregnant women attending the antenatal clinics of Bissau between January 2002 and June 2006 were consecutively tested unless they opted out.
Results: Among 23,869 tested women the overall prevalence of HIV-1 was 5.7%, that of HIV-2 was 2.4%, and these included the 0.7% prevalence of HIV-1 and HIV-2 duals. The main factors associated with the risk of HIV-1 infection were older age, occupation and number of sexual partners. Beafada and Mandinga ethnic groups were at greater risk of presenting HIV-1, and Bijago and Papel at lower risk. The factors associated with the risk of HIV-2 were age, literacy and occupation; the Beafada were at greater risk than the other ethnic groups.
Conclusions: The prevalence of HIV-2 infection decreased overtime, whereas that of HIV-1 infection remained substantially stable, but was higher than that observed in previous studies. The rapid decline in the rates of HIV-2 infection suggests that many of the factors that allowed its exponential growth in the past have now been partially removed, and that sexual and vertical transmission have not been sufficient to maintain and extend the epidemic.

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Introduction

Guinea-Bissau is one of the few sub-Saharan African countries in which the rates of both HIV-1 and HIV-2 infections have reached significant levels.1–4

Molecular analysis suggests that HIV-2 emerged about 1940s and increased exponentially after 19555 to reach the highest prevalence in the world.2,6–8 It is thought that the long war that ravaged the country from 1963 to 1974 increased the risk of sexual abuse and promiscuity, parenteral transmission through female ritual excision and multiple injections for the treatment of tuberculosis and the prevention of trypanosomiasis significantly contributed to its spread,1,2,9 but its prevalence is now declining10–12. The reasons for this decline are not clear as various data converge to show that survival is longer in patients infected with HIV-2 than in those infected with HIV-1, and that a larger percentage of HIV-2 infected individuals become long-term non-progressors13,14 and therefore potentially contribute to the spread of the infection for a long time. On the other hand, the modest genital shedding of HIV-215–17 probably reduces the efficiency of sexual transmission, as suggested by the considerably slower spread of HIV-2 among sex workers16,18 and the marked reduction in HIV-2 in Angola and Mozambique at the end of 1990s.1

HIV-1 in Guinea-Bissau is mainly represented by the A/G (CRF 02_AG) recombinant strain19,20 and the finding of clusters, which contained sequences separated by short branches, suggests that the virus had time to evolve in the country.20 However, it is likely that HIV-1 arrived some years after HIV-2 as it was completely absent in a study population already showing a significant prevalence of HIV-2 in 1980s,21 and was first documented in three out of 2539 pregnant women (0.1%) by Månsson et al. in 1988.22 Interestingly, this study (which tested 20,422 pregnant women in Bissau between 1982 and 2004) found that the prevalence of HIV-2 was 8.3% in 1987 and decreased to 1.9% in 2004, whereas the prevalence of HIV-1 increased and passed 4% in 1999. It has been suggested that the civil war in 1998–1999 played a role in spreading HIV-1 infection.22

This study data collected during a programme for the prevention of mother-to-child transmission (PMTCT) of HIV-1 infection in Bissau City between 2002 and 2006 provides further information concerning the trend of the two HIV infections, and has allowed us to speculate on the factors associated with the risk of HIV infection.

Materials and methods

The study started in ten public antenatal clinics (ANCs) in Bissau City in 2002 with the ethical approval of the Scientific Committee of the Ministry of Public Health of Guinea-Bissau. The study was part of a Nevirapine (NVP) based PMTCT program offered to all HIV-1 infected women. Both HIV-1 and HIV-2 infected women were also offered free artificial feeding and nutritional support. All pregnant women attending the ANC were invited to participate in a voluntary counselling and testing programme by local counsellors. During the counselling, a nurse completed a questionnaire covering age, ethnic group, school attendance, profession, marital status, the number of sexual partners in the year preceding the study, and the number of previous pregnancies (excluding abortions and stillbirths).

When no personal documents or official registrations were available, the date of birth or presumptive age was assessed on the basis of the indications given by the women. When their parents belonged to different ethnic groups, the women were allowed to define themselves as being of 'mixed' ethnicity; the women were also allowed to define themselves as not belonging to any specific ethnic group. The women whose nationality was not Guinea-Bissauan formed a separate group. All of the women were asked to state their previous school attendance; those who had never attended school and were unable to read the items of the questionnaire were defined 'illiterate'. Teachers, healthcare workers, traders, and public and private employees were grouped as 'professionals' and compared with students and housewives. Marital status was categorised as casada (regularly married), amígada (cohabitant or having a stable partner) and solteira (single, no stable partner reported). The nurse who filled the questionnaire was asked to evidence every item refused by the counselled woman.

The enrolled women were asked to give their informed consent to HIV testing and the use of their personal data to analyse the factors associated to risk of HIV-1 and HIV-2 infections. Before analyses, all data were made anonymous by using an alpha-numeric code.

A coding system based on name and date of birth was adopted by all the ANCs, and the data were entered in a centralised database.

HIV testing

If the woman accepted to be tested, a rapid test was performed as soon as possible during the first physical examination and the results were given immediately after. HIV infection was detected using the WHO protocols for developing countries23 and two rapid screening tests: Capillus HIV-1/HIV-2 (Cambridge 5 Biotechnology, Galway, Ireland) (Sensitivity: 98.6–99.9%, specificity: 98.2–99.6%) or Genie II HIV-1/2 (BioRad Laboratories, Redmond, Washington, USA) (Sensitivity: 97.8–100%, specificity: 99.7–100%); an enzyme-like immunosorbent assay (ELISA, BioRad Laboratories, USA) was used for confirmation.

Statistical analysis

Linear by linear association X² test was used to determine the trend of HIV-1 and HIV-2 infections during the study period 2002–2006. Subjects with dual HIV-1/HIV-2 infections were included in both single HIV-1 and HIV-2 infected groups.

A logistic model was applied in order to compare the overall risk of HIV infection between the ethnic groups frequently practising excision and those which do not.

Univariate and multiple polytomous logistic regression analyses were used to analyse the associations between HIV positivity (dependent variable, classified as HIV-1, HIV-2, HIV-1/2 or HIV negative) and the independent variables, which included the sociodemographic data (age, literacy,
HIV-1 and -2 infections in pregnant women in Bissau

occupation, marital status, the number of sexual partners, ethnic group, and the number of previous pregnancies) and the year of the test. For each sociodemographic factor and the year of testing, polytomous logistic regression models were used to estimate the odds ratios (ORs) of being HIV-1, HIV-2 or HIV-1/2 positive vs HIV negative (reference category), and their 95% confidence intervals (CIs).

All of the independent variables in the multiple logistic regression analyses were forced to enter the model simultaneously; no variable (stepwise, forward or backward) selection procedure was applied.

All of the statistical analyses were made using SAS statistical software (version 9.1).

Results

Between January 1, 2002 and April 30, 2006, a total of 27,166 pregnant women attended the ANCs. Three hundred and sixty-nine of them (1.4%) refused to participate; the percentage of refusals per calendar year did not change significantly during the study period (range 1.1–1.5%). Agreement to participate was not actually followed by HIV testing in 2928 cases (10.8%).

Table 1 shows the demographic characteristics of the 23,869 women (median age 23 years) who were actually counselled and tested for HIV-1 and HIV-2. The characteristics did not significantly change overtime. The women belonging to ethnic groups that accounted for fewer than 1% of the total (Cristão de Gebo [60 women], Djacanca [44], Nhomínca [28], Nalu [77], Saraculê [177] and Sussó [54]) were grouped together and defined as ‘other’, which also included the women whose ethnicity was unclear (1.7%). The foreigners mainly came from Cape Verde (109, 0.5%) and Senegal (32, 0.1%).

HIV-1 and HIV-2 prevalence, and variations overtime

Throughout the study period, testing showed that the HIV-1 infected women (including HIV-2 co-infected) were 1360 (5.7%). The HIV-2 infected women (including HIV-1 co-infected) were 575 (2.4%). HIV-duals were 170 (0.7%).

Figure 1 shows the prevalence of HIV-1 and HIV-2 infections by calendar year. There was a significant decrease in the prevalence of HIV-2 (p = 0.006) during the study period, but no significant change in the prevalence of HIV-1 infection.

The polytomous logistic regression in the univariate model revealed that the risk of a positive test was influenced by the calendar year of the test itself (p = 0.0010), but only the risk of co-infection significantly decreased per calendar year (OR 0.797; 95% CI 0.704–0.901). The multivariate model confirmed the influence of the calendar year of testing (p = 0.003) and the reduced risk of co-infection per calendar year (AOR 0.758, 95% CI 0.664–0.865) (Table 2).

Age of testing and the risk of HIV infection

The women were divided into four age groups (15–19, 20–24, 25–29 and ≥30 years); the youngest group, which accounted for 24.6% of the total, was taken as the reference category. Age strongly influenced the risk of testing HIV positive in both the univariate (p = 0.0001) and multivariate models (p = 0.0001), with a clearly increased risk of HIV-1, HIV-2 and co-infection. Interestingly, the women aged ≥30 years were more than six times more likely to be HIV-2 positive than those aged 15–19 years, and more than eight times more likely to be co-infected, whereas their risk of being HIV-1 positive was only twice as high.

Occupation and the risk of HIV infection

Just over 80% of the women were housewives, 2137 (9.7%) defined themselves as students, and only 411 (1.7%) were working (policewomen, clerks, teachers, art dealers and merchants) (Table 1). Nevertheless, working proved to be a significant correlate of the risk of HIV infection in the univariate model (p = 0.0001). In particular, the OR of the risk of HIV-1 infection was 2.011 (95% CI 1.424–2.839) in professionals vs housewives, and students were at significantly lower risk of being HIV-2 positive than housewives (OR 0.352, 95% CI 0.210–0.591); both of these findings were confirmed by the multivariate analyses (Table 2). Univariate analysis showed that, in comparison with the housewives, the women who refrained to report their occupation were at higher risk of HIV-1 infection (OR 1.497, 95% CI 1.244–1.802) and HIV-1/HIV-2 co-infection (OR 1.673, 95% CI 1.072–2.610), and this was confirmed by the multivariate model (Table 2).

Number of sexual partners and the risk of HIV infection

As can be seen in Table 1, 74.1% of the women declared they had only one sexual partner in the 12 months before enrolment. The number of partners significantly influenced the risk of HIV infection in the univariate model (p = 0.0001). In particular, the OR of the risk of HIV-1 infection was 2.240 (95% CI 1.059–4.535) in the group refraining from reporting the number of sexual partners and 1.416 (95% CI 1.193–1.691) in the group reporting ≥2 partners. In the univariate analyses the women reporting two or more partners were also at increased risk of HIV-2 infection (OR 1.792, 95% CI 1.374–2.332) and HIV-1/HIV-2 co-infection (OR 1.655, 95% CI 1.221–2.239). The multivariate analyses confirmed the increased risk associated with the number of partners (p = 0.0005) and the increased risk of HIV-1 infection and HIV-1/HIV-2 co-infection in women reporting ≥2 partners.

Number of previous pregnancies and the risk of HIV infection

40.9% of the enrolled women had two or more previous pregnancies, while 38.6% were at first pregnancy (Table 1). The number of pregnancies significantly influenced the risk of infection in the univariate model (p = 0.0001). In particular, the risk of HIV-1 infection was higher in the women undergoing their second pregnancy (OR 1.428, 95% CI 1.222–1.670) and those reporting ≥2 previous pregnancies (OR 1.281, 95% CI 1.119–1.467). The women with ≥2
previous pregnancies were also at higher risk of HIV-2 infection (OR 2.796, 95% CI 2.196–3.561) and HIV-1/HIV-2 co-infection (OR 1.930, 95% CI 1.357–2.745). The multivariate model showed that the number of pregnancies influenced the risk of HIV infection, but only confirmed a significantly increased risk for HIV-1 in women undergoing their second pregnancy in comparison with those at their first pregnancy (Table 2).

**Ethnic groups and the risk of HIV infection**

The Balantas were the most represented ethnic group (24.1%), and were used as the reference category. The univariate and multivariate models both showed that the different ethnic groups were at significantly different risk of HIV infection ($p = 0.0001$). The univariate analyses indicated that the risk of being infected with HIV-1 was higher among the Mandinga (OR 1.378, 95% CI 1.114–1.705) and Beafada (OR 1.651, 95% CI 1.197–2.277) and lower among the Papel (OR 0.784, 95% CI 0.639–0.964) and Bijago (OR 0.506, 95% CI 0.267–0.959). The multivariate analyses confirmed all of these findings (Table 2).

Univariate analysis showed that the risk of HIV-2 infection was higher among the Beafada than among the Balanta (OR 2.066, 95% CI 1.293–3.300), and this difference was confirmed by the multivariate model. An increased risk of HIV-1/HIV-2 co-infection was found in women belonging to the ethnic groups accounting for fewer than 1% of the total sample and in women of unclear ethnicity, but this was not significant in the multivariate model.

### Table 1

Demographic characteristics and prevalence of HIV infection in 23,869 women enrolled in the study.

<table>
<thead>
<tr>
<th>HIV-1</th>
<th>HIV-2</th>
<th>HIV-1/2</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 1190</td>
<td>N = 405</td>
<td>N = 170</td>
<td>N = 22104</td>
<td>N = 23869</td>
</tr>
<tr>
<td>N (row%)</td>
<td>N (row%)</td>
<td>N (row%)</td>
<td>N (row%)</td>
<td>N (column%)</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–19</td>
<td>169 (2.9)</td>
<td>31 (0.53)</td>
<td>11 (0.19)</td>
<td>5618 (96.38)</td>
</tr>
<tr>
<td>20–24</td>
<td>437 (3.33)</td>
<td>102 (1.24)</td>
<td>46 (0.56)</td>
<td>7621 (92.87)</td>
</tr>
<tr>
<td>25–29</td>
<td>349 (6.03)</td>
<td>118 (2.04)</td>
<td>55 (0.59)</td>
<td>5269 (90.99)</td>
</tr>
<tr>
<td>≥30</td>
<td>220 (5.71)</td>
<td>151 (3.92)</td>
<td>53 (1.38)</td>
<td>3430 (89.00)</td>
</tr>
<tr>
<td>Missing</td>
<td>17 (9.73)</td>
<td>3 (1.60)</td>
<td>5 (2.64)</td>
<td>166 (87.83)</td>
</tr>
<tr>
<td><strong>Literacy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterates</td>
<td>674 (4.9)</td>
<td>236 (1.71)</td>
<td>112 (0.81)</td>
<td>12747 (92.58)</td>
</tr>
<tr>
<td>Literates</td>
<td>516 (5.11)</td>
<td>169 (1.67)</td>
<td>58 (0.57)</td>
<td>9357 (92.64)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>905 (4.73)</td>
<td>347 (1.81)</td>
<td>135 (0.71)</td>
<td>17752 (92.75)</td>
</tr>
<tr>
<td>Professional</td>
<td>37 (9.00)</td>
<td>10 (2.43)</td>
<td>3 (0.73)</td>
<td>361 (87.83)</td>
</tr>
<tr>
<td>Students</td>
<td>110 (4.75)</td>
<td>15 (0.65)</td>
<td>9 (0.39)</td>
<td>2183 (94.22)</td>
</tr>
<tr>
<td>NR</td>
<td>138 (6.89)</td>
<td>33 (1.65)</td>
<td>23 (1.15)</td>
<td>1808 (80.31)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>433 (4.92)</td>
<td>160 (1.82)</td>
<td>64 (0.73)</td>
<td>8150 (92.54)</td>
</tr>
<tr>
<td>Amigada’</td>
<td>530 (4.74)</td>
<td>174 (1.56)</td>
<td>77 (0.69)</td>
<td>10399 (91.58)</td>
</tr>
<tr>
<td>Single/NR</td>
<td>227 (5.85)</td>
<td>71 (1.83)</td>
<td>29 (0.75)</td>
<td>3555 (91.58)</td>
</tr>
<tr>
<td><strong>Number of sexual partners</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>820 (4.64)</td>
<td>291 (1.65)</td>
<td>109 (0.62)</td>
<td>16455 (93.10)</td>
</tr>
<tr>
<td>NR</td>
<td>202 (5.69)</td>
<td>52 (1.47)</td>
<td>27 (0.76)</td>
<td>3268 (92.08)</td>
</tr>
<tr>
<td>Ethnics groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanta</td>
<td>275 (4.78)</td>
<td>103 (1.79)</td>
<td>33 (0.57)</td>
<td>5343 (92.86)</td>
</tr>
<tr>
<td>Beafada</td>
<td>47 (7.52)</td>
<td>22 (3.52)</td>
<td>3 (0.48)</td>
<td>553 (88.48)</td>
</tr>
<tr>
<td>Bijagos</td>
<td>10 (2.49)</td>
<td>6 (1.50)</td>
<td>1 (0.25)</td>
<td>384 (95.76)</td>
</tr>
<tr>
<td>Fuepe</td>
<td>22 (4.25)</td>
<td>7 (1.35)</td>
<td>2 (0.39)</td>
<td>487 (94.02)</td>
</tr>
<tr>
<td>Fula</td>
<td>214 (4.86)</td>
<td>71 (1.61)</td>
<td>36 (0.82)</td>
<td>4082 (92.71)</td>
</tr>
<tr>
<td>Mancanha</td>
<td>90 (5.75)</td>
<td>31 (1.98)</td>
<td>14 (0.89)</td>
<td>1430 (91.37)</td>
</tr>
<tr>
<td>Mandjaco</td>
<td>89 (4.84)</td>
<td>28 (1.52)</td>
<td>15 (0.82)</td>
<td>1705 (92.81)</td>
</tr>
<tr>
<td>Mandinga</td>
<td>135 (6.46)</td>
<td>36 (1.72)</td>
<td>17 (0.81)</td>
<td>1903 (91.01)</td>
</tr>
<tr>
<td>Papel</td>
<td>143 (3.81)</td>
<td>52 (1.38)</td>
<td>19 (0.51)</td>
<td>3542 (94.30)</td>
</tr>
<tr>
<td>Foreigners</td>
<td>14 (7.04)</td>
<td>7 (3.52)</td>
<td>1 (0.50)</td>
<td>177 (88.94)</td>
</tr>
<tr>
<td>Others</td>
<td>66 (5.66)</td>
<td>18 (1.54)</td>
<td>13 (1.11)</td>
<td>1069 (91.68)</td>
</tr>
<tr>
<td>NR</td>
<td>85 (5.47)</td>
<td>24 (1.54)</td>
<td>16 (1.03)</td>
<td>1429 (91.96)</td>
</tr>
<tr>
<td><strong>Previous pregnancies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>389 (4.22)</td>
<td>90 (0.97)</td>
<td>47 (0.51)</td>
<td>8682 (94.28)</td>
</tr>
<tr>
<td>1</td>
<td>289 (5.90)</td>
<td>57 (1.16)</td>
<td>30 (0.61)</td>
<td>4519 (92.31)</td>
</tr>
<tr>
<td>≥2</td>
<td>512 (5.24)</td>
<td>258 (2.64)</td>
<td>93 (0.95)</td>
<td>8913 (91.27)</td>
</tr>
</tbody>
</table>

NR: not responding.

- Including teachers, healthcare workers, traders, employees.
- Cohabitant with a stable sexual partners.
- in the last year.
- including "mixed" ethnicity and ethnic group accounting for <1% of the total population.
- Reference category.
A logistic model, comparing the overall risk of HIV infection between the ethnic groups frequently practising excision (Fula, Mandinga, Beafada, and Nalu) and those which do not (Balanta and Papel), showed that the former had a risk of 1.33 (95% CI 1.09–1.64, \( p = 0.008 \)) for HIV-2 and a risk of 1.38 (95% CI 1.2–1.59, \( p < 0.0001 \)) for HIV-1.

**Discussion**

Our findings confirm the previously reported decline in the prevalence of HIV-2 in Guinea-Bissau.11,12,22

In a recent study, Schmidt et al. used a mathematical model to evaluate the putative role of temporary changes in sexual behaviour and the competitive role of HIV-1 as a factor determining HIV-2 decline.24 Assuming that the competitive reduction induced by HIV-1 was 30%, the prevalence of HIV-2 in women aging less than 45 years should have been about 3% in 2005 and 2.5% in 2010.24 Conversely, we found a prevalence of 2.3% in 2005, thus suggesting that the decline in HIV-2 may have been more rapid and possibly involved factors other than those considered in the model. The overall prevalence of HIV-2 infection in our 15–19-year-old women was 0.7%, and its prevalence in 2005 was 0.6%, about six times less than that found by Månsson et al. in the same age group in 1995.22 It can therefore be supposed that the HIV-2 epidemic has profoundly changed over the last 20–30 years.

The modest genital shedding of HIV-2 and the consequent inefficiency of sexual transmission have been claimed as the main cause of the decline in HIV-2 infection in Guinea-Bissau and its disappearance in other African countries.1,15–18 This suggests that parenteral spreading was crucial at the beginning of the epidemic and a reduction in parenteral risk factors may have played a substantial role.9

Serological data show that HIV-1 entered the country several years after HIV-2: it was absent in 1980s21 and reached a prevalence of 6.3% in less than twenty years, which is higher than that recently reported in neighbouring countries such as Senegal (1.0%) and Guinea Conakry (2.0%).25 During our study period (2002–2006), the prevalence of HIV-1 infection did not significantly change, but this finding does not seem to be enough to suggest that the epidemic has now reached a steady-state.

The overall prevalence of HIV-1/HIV-2 co-infection in our study population was relatively low (0.7%), but still sufficient to identify a significant degree of overlapping because it is about five times higher than expected if the two epidemics were fully independent: (prevalence of HIV-1) * (prevalence of HIV-2) = 0.057 * 0.024 = 0.0014 (0.14%). These data confirm previous findings that HIV-2 infection did not protect against subsequent HIV-1 infection.26,27

Our multivariable analyses revealed an approximately 24% decrease in the risk of being co-infected (95% CI 14–34%) per each more recent calendar year. The phenomenon probably reflects the reduction of HIV-2 circulation in Bissau in the recent years.

Analysis of the potential factors associated with the risk of HIV infection in the population of Bissau City showed that, although they share the same routes of transmission, the two viruses have been influenced by quite different social and behavioural factors. As expected, age was an independent correlate of the risk of HIV-1 infection.

However, although the risk of HIV-1 infection was similar in the three groups of women aged >20 years and about twice as low in those aged 15–19 years, the risk of HIV-2 infection and HIV-1/HIV-2 co-infection was six and 8.5 times higher in the women aged ≥30 years than in those aged 15–19 years, respectively.

In our study, the rate of illiteracy of 57.7% suggests that access to school has improved over the last 15 years, at least among the women living in Bissau City.28 Against our expectations, the multivariate model identified a slightly increased risk of HIV-2 infection among literate women. There is no obvious explanation for this finding, particularly as it partially conflicts with other studies, which designated a higher prevalence of HIV-2 infection in rural areas, where the rate of illiteracy is presumably higher than in the capital.

It has been reported that professional occupations, population mobility and higher socioeconomic status are associated with a higher risk of HIV-1 infection in sub-Saharan Africa.28–31 Although they were a minority in the enrolled population (1.7%), women with a professional occupation were at significantly higher risk of HIV-1 infection than housewives, whereas there was no significant difference in the prevalence of HIV-2 or co-infection between the two groups. The lower risk of HIV-2 infection among students might be due to mainly urban location. This is in agreement with other studies on the same topic.29,31

In our analyses, marital status did not affect the risk of HIV infection: however, most of the women defined themselves as "amigadas", and many of those who were formally married belonged to Muslim ethnic groups in which the polygamy is very common.

The number of sexual partners during the 12 months before enrolment can be considered a factor associated with sexual transmission but, although it closely correlated...
with the risk of HIV-1 infection and HIV-1/HIV-2 co-infection, the correlation with HIV-2 infection was only of borderline statistical significance, possibly because the sexual transmission of HIV-2 is less efficient.\textsuperscript{1,15–18}

In the multivariate model, the women at their second pregnancy were at significantly higher risk of being infected with HIV-1 than those at their first pregnancy, whereas the women who had experienced >2 previous pregnancies were not. Further investigations, including timing and modality of previous deliveries, could help to confirm the role of pregnancies and deliveries as factors increasing the risk of HIV-1 transmission.

The population of Guinea-Bissau includes about 34 different ethnic groups, each with its own language,\textsuperscript{28} and cultural identity affecting personal behaviour, social life and geographical distribution.\textsuperscript{28} Both HIV-1 and HIV-2 were differently distributed among the ethnic groups. When we considered the Balanta as the reference category (the largest ethnic group accounting for 21% of the country’s total population and 24.1% of our study population), it was found that the Beafadas were at greater risk of both HIV-1 and HIV-2 and Mandinga were at higher risk of HIV-1 infection. This was quite surprising because these two ethnic groups are prevalently Islamic and some studies have found that to be Muslim is a correlate of protection against HIV-1 infection.\textsuperscript{31,32} However, our findings pointed out that the risk of both HIV-1 and HIV-2 was higher in the prevalent Muslim ethnic groups which frequently practise female excision than in the groups that do not.\textsuperscript{33} Female circumcision takes place during an initiation ritual, the \textit{fanado}.\textsuperscript{34} The Beafada usually undergo the ritual twice (usually at aged 9 and 12), whereas the women belonging to

\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
 & HIV-1 & & & HIV-2 & & & HIV-1/2 & \\
 & OR & 95% CI & & OR & 95% CI & & OR & 95% CI & \\
\hline
Year of test \textsuperscript{a} & 1.02 & 0.97–1.8 & & 0.95 & 0.87–1.03 & & 0.76 & 0.66–0.86 & 0.0003 & \\
Age & & & & & & & & & & \\
15–19\textsuperscript{f} & 1 & & & 1 & & & 1 & & & \textless 0.0001 & \\
24–29 & 2.21 & 1.77–2.76 & & 3.34 & 2.10–5.32 & & 5.69 & 2.74–11.80 & & & \\
Literacy & & & & & & & & & & \\
Illiterates \textsuperscript{f} & 1 & & & 1 & & & 1 & & & 0.301 & \\
Literate & 1.13 & 0.99–1.29 & & 1.26 & 1.01–1.57 & & 0.85 & 0.59–1.21 & & & \\
Occupation & & & & & & & & & & \\
Housewife \textsuperscript{f} & 1 & & & 1 & & & 1 & & & <0.0001 & \\
Professional\textsuperscript{b} & 1.72 & 1.21–2.45 & & 0.96 & 0.50–1.84 & & 0.64 & 0.29–2.94 & & & \\
Student & 1.10 & 0.88–1.37 & & 0.56 & 0.32–0.96 & & 0.83 & 0.39–1.78 & & & \\
NR & 1.93 & 1.41–2.64 & & 1.44 & 0.79–2.63 & & 2.83 & 1.31–6.12 & & & \\
Marital status & & & & & & & & & & \\
Married \textsuperscript{f} & 1 & & & 1 & & & 1 & & & 0.687 & \\
Amigada \textsuperscript{c} & 1.04 & 0.91–1.19 & & 1.02 & 0.81–1.28 & & 1.03 & 0.73–1.46 & & & \\
Single/NR & 1.12 & 0.92–1.38 & & 1.15 & 0.82–1.60 & & 0.68 & 0.37–1.24 & & & \\
No sexual partners\textsuperscript{d} & 1\textsuperscript{f} & 1 & & 1 & & & 1 & & & & \\
& ≥2 & 1.34 & 1.12–1.60 & & 1.27 & 0.96–1.69 & & 1.87 & 1.25–2.76 & & & \\
& NR & 1.05 & 0.86–1.29 & & 0.81 & 0.55–1.18 & & 0.85 & 0.47–1.56 & & & \\
Ethnic group & & & & & & & & & & \\
Balanta \textsuperscript{f} & 1 & & & 1 & & & 1 & & & <0.0001 & \\
Bijagos & 0.46 & 0.24–0.87 & & 0.75 & 0.32–1.72 & & 0.36 & 0.05–2.66 & & & \\
Fulpe & 0.88 & 0.56–1.37 & & 0.85 & 0.39–1.84 & & 0.69 & 0.16–2.90 & & & \\
Mandinga & 1.38 & 1.11–1.71 & & 1.04 & 0.71–1.54 & & 1.35 & 0.75–2.44 & & & \\
Mandjaco & 0.94 & 0.73–1.21 & & 0.77 & 0.50–1.18 & & 1.24 & 0.66–2.31 & & & \\
Mancanha & 1.09 & 0.85–1.40 & & 1.01 & 0.66–1.53 & & 1.44 & 0.75–2.77 & & & \\
Papel & 0.74 & 0.60–0.91 & & 0.73 & 0.52–1.03 & & 0.83 & 0.46–1.48 & & & \\
Fula & 1.12 & 0.93–1.35 & & 1.09 & 0.79–1.48 & & 1.50 & 0.93–2.43 & & & \\
Foreigners & 1.35 & 0.77–2.37 & & 1.89 & 0.86–4.19 & & 0.85 & 0.11–6.28 & & & \\
N\textsuperscript{D} & 0.62 & 0.43–0.91 & & 0.92 & 0.46–1.81 & & 1.18 & 0.45–3.08 & & & \\
Others \textsuperscript{e} & 1.10 & 0.83–1.46 & & 0.78 & 0.47–1.30 & & 1.68 & 0.88–3.24 & & & \\
Beafada & 1.63 & 1.18–2.25 & & 2.15 & 1.33–3.49 & & 0.86 & 0.26–2.81 & & & \\
Previous pregnancies & 0\textsuperscript{f} & 1 & & 1 & & & 1 & & & & \\
& 1 & 1.29 & 1.08–1.55 & & 0.94 & 0.64–1.38 & & 1.04 & 0.60–1.79 & & & \\
& ≥2 & 0.98 & 0.81–1.19 & & 1.26 & 0.88–1.80 & & 0.90 & 0.53–1.53 & & & \\
\hline
\end{tabular}
\end{table}

NR: not responding, OR: odds ratio, 95% CI: 95% confidential Interval.

\textsuperscript{a} Risk assessment of testing HIV positive according to the calendar year, considered as continuous variable.

\textsuperscript{b} Including teachers, health care workers, traders, employees.

\textsuperscript{c} Cohabitant with a stable sexual partner.

\textsuperscript{d} In the last year.

\textsuperscript{e} Including “mixed” ethnicity and ethnic groups accounting for <1% of the total population.

\textsuperscript{f} Reference categories.
the other ethnic groups are circumcised only once, and we found that the Beafada were at highest risk for both HIV-1 and HIV-2 infections. The women participating in our study were not asked to specify whether they had been excised or not, and so we do not have any direct evidence concerning the question.

The fanado has recently been partially replaced by a ‘small initiation’, which is performed at an earlier age and involves smaller groups of girls. Such changes in rituals may have reduced transmission of HIV-2, but not of viruses having a more efficient parenteral transmission, such as HIV-1 and perhaps HTLV-1.

The Bijago, a prevalently Animist group living in an archipelago off Bissau City, were at lower risk of HIV-1 infection than the groups living in the mainland, and a reduced risk of HIV-1 infection has been reported in other African islands. The Papel, a group that traditionally lives in the urban area of Bissau, also proved to be at lower risk of HIV-1 infection, but the explanation for this will require further study.

The main purpose of our project was to provide a mother-to-child transmission prevention programme and, as the collection of epidemiological data was only a secondary aim, this study may have some biases. In particular, no information was collected concerning the use of condoms, although its use seems very rare also on the basis of the data in a recent UNAIDS report. Furthermore, despite the large number of enrolled women, possible sampling biases include the under-representation of pregnant women aged >35 years; however, the reported ages were frequently unsupported by official documents and, in the opinion of the health counsellors, the women may have tended to report a younger age. Finally, the women’s reports of their sexual behaviour and the number of sexual partners may have been affected by recall or reticence biases.

In conclusion, our data show that the prevalence of HIV-2 infection is currently declining in Bissau City and, although currently stable, that the prevalence of HIV-1 is significantly higher than in the recent past, which could imply the possibility of a further increase in the future. Although HIV-1 and HIV-2 share the same transmission routes, our data suggest that the overlapping of the two epidemics is only partial.

The rapid decline in the rates of HIV-2 infection suggests that many of the factors that allowed its exponential growth in the past have now been partially removed, and that sexual and vertical transmission have not been sufficient to maintain and extend the epidemic.

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Ethical approval

The scientific committee of the Ministry of Public Health in Guinea-Bissau granted permission to conduct the study.

Conflict of interest

The authors declare that they have no conflict of interest.

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