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Trends of HIV-1 and HIV-2 prevalence among pregnant women in Guinea-Bissau, West Africa: possible effect of the civil war 1998-1999

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Keywords: HIV-1, HIV-2, surveillance, Guinea-Bissau, war

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Figure texts:
Figure 1: HIV-1 and HIV-2 total prevalence (with 95% confidence intervals) of all women for the years 1987 to 2004. HIV-1 and HIV-2 double infections are added into each group.

Figure 2: HIV-1, HIV-2 and HIV-1/2 prevalence stratified by age groups for the years 1992-2004, where age data were available.

Key messages:
1) HIV-2 prevalence is declining among pregnant women in Bissau
2) HIV-1 prevalence increased until 1999 and is now the dominant HIV type among pregnant women in Bissau
3) There was an increase in HIV-1 prevalence directly after the civil war 1998-1999 but HIV-1 prevalence stabilized in the subsequent years and there is no evidence of a long-term war effect

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ABSTRACT

Objectives: Sentinel surveys in Bissau, the capital of Guinea-Bissau, have shown low prevalence of HIV-1 but high HIV-2 prevalence before 1998. Guinea-Bissau experienced a civil war in 1998-1999. We specifically wanted to examine the trends of HIV-prevalence from antenatal surveys in Bissau, Guinea-Bissau 1987-2004, and examine if the civil war in 1998-99 could have an effect on HIV prevalence levels after the conflict.

Methods: Since 1987, anonymous HIV testing in delivering women has been performed at the maternity clinic, Simão Mendes National Hospital, Bissau, as part of the national sentinel surveillance program. Consecutive sampling was performed for approximately 3 months between September and December each year. Serological analyses were performed at the National Public Health Laboratory (LNSP) in Bissau.

Results: A total of 20422 women were tested for HIV between 1987 and 2004. The total HIV-1 prevalence increased from 0.0 % in 1987 to 4.8 % in 2004 and the total HIV-2 prevalence decreased from 8.3 % in 1987 to 2.5 % in 2004. The HIV-1 prevalence increased from 2.5 % in 1997 to 5.2 % in 1999, but stabilized in the subsequent years.

Conclusions: There was a significant increase in HIV-1 prevalence in the years 1987 – 2004 and a significant decline in HIV-2 prevalence over the same period. The civil war in 1998-99 may have sparked HIV-1 transmission, as HIV-1 prevalence more than doubled between 1997 and 1999 but there is no evidence of a long-term effect on the trends of HIV-1 or HIV-2 prevalence.

INTRODUCTION

At the beginning of the HIV epidemic when the infection spread rapidly in East and Central Africa, very few cases were found in West Africa.[1] In 1986, a similar virus with lower pathogenicity which was subsequently named HIV-2, was discovered in 2 patients from Guinea-Bissau and the neighbouring Cabo Verde.[2] Seroepidemiological surveys showed that HIV-2 was mainly confined to West Africa with the highest prevalence in Guinea-Bissau, 8.9% in the adult urban population of Bissau in the first survey of seroprevalence in 1987.[3] However, declining prevalence rates of HIV-2 along with increasing prevalence rates of HIV-1 have since then been reported from Guinea-Bissau.[4,5] In the neighbouring countries Senegal[6] and The Gambia[7], the prevalence of HIV-1 and HIV-2 has continued on a comparatively low level among pregnant women, around or below 1 %, while the HIV-2-prevalence declined from 7.0% in 1988-91 to 4.0% in 2001-03 in a recent study of clinical patients in The Gambia.[8]

The war in Guinea-Bissau in 1998-99 started on June 7, 1998, with a military uprising against the regime. Fierce fighting followed which made approximately 250.000 persons leave the capital city of Bissau for interior parts of the country. A cease-fire was agreed upon on July 24, 1998 after which parts of the people returned to Bissau but had to flee again during renewed outbreaks of fighting in October 1998 and February 1999.[9] The vast majority of the population resided inside the country and did not cross borders during the conflict. During the conflict there was a general decline in public services such as health institutions.[10,11] In this study we specifically wanted to examine the trends of HIV-prevalence from antenatal surveys in Bissau, Guinea-Bissau 1987-2004, and examine if the civil war in 1998-99 could have a

possible effect on HIV prevalence levels, as monitored in the antenatal surveys before and after the conflict.

METHODS

Study population: Guinea-Bissau is a country of ~1.3 million inhabitants. In the UNDP Human Development Report of 2005 it was ranked as the 6th poorest out of 177 countries.[12] The capital Bissau has 250.000 - 300.000 inhabitants. The majority of the women in the city normally give birth at the Simão Mendes National Hospital.[13] Within the national sentinel surveillance system, and according to the recommendations of WHO regarding sentinel surveillance of selected population groups, anonymous HIV testing of women giving birth at the Simão Mendes National Hospital has been performed since 1987. The testing has taken place every 1 or 2 years between August and December. During the first years sample size varied between 700 and 2500, but since 1992 the number of women tested per year has been approximately 1500. The data from 1989-1991 have been pooled in the presentation because of lower sampling numbers during these years. Questionnaires have differed slightly over the years, but age of the women has been recorded throughout. Age data are completely missing from the years 1987 –1991 and of the first 460 women from 1995 – probably they have been lost in a fire during the conflict in 1998-1999 when the national laboratory was almost totally burnt down after being hit by a missile. No sampling was planned for 1998, but even if it would have been planned it would probably not have been possible to perform during the civil war. For 1992 – 2004 where age records are available, age data are missing on average in 6.4 % of cases. Results for the years 1987-1997 have earlier been reported. [5]

Laboratory methods: HIV testing was performed at the National Public Health Laboratory (LNSP), Bissau. In 1987-1994 sera were screened for HIV-1 and HIV-2 antibodies by enzyme-linked immunosorbent assay (ELISA) with use of the Behring anti-HIV-1/HIV-2 (Behring, Marburg, Germany) and/or Wellcozyme recombinant anti-HIV-1 (Wellcome, Dartford, UK) and an in-house HIV-2 (SBL6669) ELISA assay.[14] From 1995 and onwards, screening has been performed with Behring Enzygnost HIV-1/HIV-2 Plus ELISA (Behring, Marburg, Germany). Confirmation of positive results was done with Western blot (WB) analysis (Diagnostic Biotechnology anti-HIV-1 blot 2.2, Science park, Singapore, or in-house anti-HIV-2)[15] and dually HIV-1/HIV-2 positive samples were confirmed by Pepti-lav (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) in the years 1987-1997. Since 1999, an alternative confirmation strategy has been used with Capillus HIV-1/HIV-2 (Cambridge Biotech Limited, Galway, Ireland) and Immunocomb II HIV-1 and 2 BiSpot RST (Orgenics, Yavne, Israel).[16] The three screening assays used have all been evaluated in parallel with each other in order to avoid changes to assays with too different performance characteristics. The change from Behring anti-HIV-1/HIV-2 to Enzygnost HIV-1/HIV-2 Plus ELISA was due to an upgrade of the assays by the manufacturer mainly to include HIV-1 subtype O (no cases of that subtype have been found in Guinea-Bissau). The alternative confirmatory strategy introduced in 1999 was found to have very similar sensitivity and specificity as the ELISA/WB – based strategy used until then. The new strategy allowed screening with rapid/simple tests and confirmation without WB [16]. Gold standard for the diagnostics has throughout the years been our in house WB for HIV-2 [14] and the Diagnostic Biotechnology anti-HIV-1 blot for HIV-1. The Pepti-lav and Immunocomb assays are virtually equal in their capacity to differentiate between HIV-1 and HIV-2. [16,17]

Statistical analysis: For the years 1987 to 2004 HIV-1 and HIV-2 prevalence and trends were calculated from the whole statistic material of tested women. 95 % confidence intervals (CI) were derived from approximation based on normal distribution. For the years where data on age were present (not 1987 to 1991) we analyzed the material stratified by age groups 15-19, 20-24, 25-29 and 30-45 years. The reason for grouping the women between 30 and 45 years in only one group was that there were very few individuals above age 35 years and grouping in the age groups 35-39 and 40-45 would have given very small groups. The data were also analyzed for the age groups 15-24 years and 25-45 years, as the age group 15-24 years is a core indicator recommended by UNAIDS guidelines.[18] The mean age was compared for the different years and an age-controlled analysis was made to exclude age-dependent reasons for change in HIV prevalence. When calculating the total HIV-1 and HIV-2 prevalence, dually HIV-1/HIV-2 reactive samples were added both to the HIV-1 and HIV-2 group. SPSS 12 software was used for statistical analysis.

Ethics: The study was ethically approved by the Ministry of Health of Guinea-Bissau.

RESULTS

A total number of 20422 women have been HIV tested over a period of 18 years. Between 1987 and 2004 the total HIV-1 prevalence (including dually HIV-1/HIV-2 infected) increased from 0% to 4.8 % ($p<0.0001$, x^2test for trend). From a total HIV-1 prevalence of 2.5 % (95 % CI, 1.7-3.3) in 1997, the prevalence more than doubled to 5.2 % (95 % CI, 4.1-6.3) in 1999 (OR 2.15 ($p=0.00012$), 95 % CI, 1.44 – 3.20). In comparison, a linear regression weighted by the number of observations for the years 1987 to 1997, gave a predicted HIV-prevalence value for 1999 of 3,2 % (95 % CI 2.6 – 3.7). In the subsequent years the total HIV-1 prevalence has been fairly stable around 5%. Between 1987 and 2004, the total HIV-2 prevalence (including dually HIV-1/HIV-2 infected) decreased from 8.3% to 2.5% (x^2test for trend, $p<0.0001$). The HIV-2 prevalence in 1999 was slightly higher compared to 1997 (5.4% versus 5.1%), but after 1999 the prevalence continued to decrease (figure 1).

Table 1 HIV prevalence among pregnant women giving birth at the maternity ward of Simão Mendes National Hospital 1987-2004.

Year	Number	HIV-1 % (n)	HIV-2 % (n)	HIV-1/2 % (n)	HIV-1 Total %[a]	HIV-2 Total %[a]
1987	707	0.0 (0)	8.3 (59)	0.0 (0)	0.0	8.3
1988	2539	0.1 (3)	6.0 (152)	0.0 (0)	0.1	6.0
1989-1991	1514	0.4 (6)	5.5 (84)	0.0 (0)	0.4	5.5
1992	1494	0.9 (14)	6.0 (89)	0.0 (0)	0.9	6.0
1993	1087	0.9 (10)	4.1 (44)	0.3 (3)	1.2	4.3
1994	1095	1.4 (15)	6.5 (71)	0.2 (2)	1.6	6.7
1995	1487	1.9 (28)	4.3 (64)	0.7 (11)	2.0	5.0
1996	No survey performed					
1997	1491	2.0 (30)	4.6 (69)	0.5 (7)	2.5	5.1
1998	No survey performed					
1999	1505	3.9 (59)	4.2 (63)	1.3 (19)	5.2	5.4
2000	1498	3.6 (54)	3.2 (49)	0.7 (10)	4.3	3.9
2001	1502	4.2 (63)	2.9 (44)	0.4 (6)	4.6	3.3
2002	1498	4.7 (70)	2.2 (32)	0.3 (5)	5.0	2.5
2003	1499	4.7 (71)	1.8 (27)	0.3 (5)	5.1	2.1
2004	1506	4.2 (63)	1.9 (28)	0.6 (9)	4.8	2.5

[a] HIV-1/HIV-2 double infected included.

For the surveys 1992 until 2004, the mean age calculated for all women (HIV-negative and HIV-positive) showed a slight increase over time that was slightly significant (Spearman's rank correlation coefficient 0.047). The maximum difference in mean age between the years was 0.87 years (23.08 in 1995 and 23.95 in 2003).
To exclude the possibility that the observed changes in HIV prevalence were confounded by age, an age-correlated logistic regression analysis was made, showing that trends of HIV-1 prevalence ($p<0.0001$) and HIV-2 prevalence ($p<0.0001$) were not depending on the overall mean age of the women.
We then stratified the women by the age groups 15-19, 20-24, 25-29 and 30-45 for the years 1992 to 2004, where 14225 women had recorded age. 64 subjects were < 15 years or > 45 years and were excluded from the analysis. The HIV-1 prevalence increased in all age groups in the 1999 sample, after that we observed a steady decline in the age group 15-19 years, from 5.1 % in 1999 to 1.8% in 2004. In contrast to this, in the age groups 20-24 and 25-29 years, the HIV-1 prevalence has continued around a level of 6%, and in the oldest age group (30-45 years), the HIV-1 prevalence has thereafter varied on a level around 4%. HIV-2 prevalence was higher in the older age groups throughout the observation period, and a gradual decline was seen in all age groups (figure 2).

Table 2: number of women for each age group for the years 1992-2004, where age data were available (see HIV prevalence in figure 1).

Age group	1992	1993	1994	1995	1997	1999	2000	2001	2002	2003	2004
15-19	432	293	323	272	386	373	368	368	371	383	379
20-24	366	276	311	273	435	505	544	496	517	489	511
25-29	276	200	211	188	303	352	266	312	346	363	357
30-45	214	156	137	137	251	220	212	251	242	251	246
Total	1288	925	982	870	1375	1450	1390	1427	1475	1486	1493

From 1992 to 2004, when stratified by only age groups 15-24 and 25-45 years, the HIV-1 prevalence among women aged 15-24 increased from 1.2 % to 5.7 %,while in the same time period the overall HIV-2 prevalence in the same age group decreased from 3.6 % to 1.7 %. In the age group 25-45 years the HIV-1 prevalence increased from 1.4 % to 6.9 % while the HIV-2 prevalence decreased from 10.4 % to 5.8 %.

DISCUSSION

We observed a significant increase in HIV-1 prevalence and a significant decrease in HIV-2 prevalence among 20422 women between 1987 and 2004. It is noteworthy that in the initial survey in 1987, no HIV-1-positive subject was found.
There are no exact data on the percentage of women giving birth at the Simão Mendes National Hospital. However, there is no indication of changing numbers of deliveries over the study period, so this would hardly bias the results. Age is self-reported in the surveillance system as in most studies of antenatal prevalence in African countries, thus the individual age might be disputable in some cases. As described in the methods part, HIV tests have changed over time with possible variation in sensitivity and specificity. A further weakness of the study is lack of systematically repeated behavioural data, creating difficulties in interpretation of the trends and limits the use of the data in responding to the epidemic.
Compared to the majority of other West African countries there was a sharper increase of HIV-1 prevalence until 1999. Since 2000 the HIV-1 prevalence has been fairly stable in the region, including Guinea-Bissau. In Bissau though, the prevalence

stays on a higher level compared to figures from sentinel studies from Senegal and The Gambia. In the whole West African sub-region only Ivory Coast, Burkina Faso and Togo have reported higher total HIV prevalence in antenatal surveys since 2000.[19] In a recent report from Ivory Coast, a decrease in overall HIV prevalence (no distinction was made between HIV-1 and HIV-2) in pregnant women was reported, from 14-15% in 1995-96 to 11% in 2002, with a drop from 15% to 8% in 18-22 year-old women. The changes were presumed to be the result of different phenomena such as ageing of the epidemic and behavioural changes.[20] Differences in resources for prevention programmes, general health services and education, levels of political stability and population migration as well as cultural, religious and behavioural differences makes comparisons between countries difficult. The HIV-1 epidemic in West Africa is generally dominated by an A/G recombinant strain,[21,22] which makes a biological explanation for differences less likely. In many countries no distinction is made between HIV-1 and HIV-2, in spite of substantial differences in transmission rates, disease progression and mortality between the 2 infections.[23] In this study, without distinction, there would only have been a recorded difference in overall HIV prevalence from 8.3% to 6.6% over these 18 years.

The differences in HIV-1 prevalence between the different age groups were mostly marked between the older groups and the age group 15-20 where HIV-1 prevalence increased in the first survey after the war (1999) but in the subsequent years declined to a level equivalent to the pre-war situation. An explanation for declining HIV-1 prevalence in this group could be behavioural change with better disease and prevention awareness, as reported from Ivory Coast,[20] but the recorded HIV-1 prevalence in 1999 might also be the recorded result of a group of younger women having been more sexually active during the war. The increase in HIV-1 prevalence between 1997 and 1999 in all age groups was striking, 5.2 % in 1999 compared to a predicted HIV-1 prevalence of 3.2 % based on the years 1987 – 1997. The stable HIV-1 prevalence level in the subsequent years shows us though the importance of long-term investigations to avoid precipitated conclusions. One possible scenario is that the HIV-1 epidemic now has entered a steady state and will continue at this level. Another possible scenario is that the HIV-1 epidemic curve reached a peak after the civil war in 1998-1999, stabilized in the subsequent years and is now returning to the increasing trend curve seen before the war with maybe further increase in the coming years.

Only speculation can be made on the amount of newly infected women during the war. The sampling of pregnant women giving birth in 1999 took place from August until December 1999, thus indicating conceptions from November 1998 to March 1999, i.e in a time that was characterized by repetitive major population displacements. The observed increase of HIV-1 prevalence in pregnant women might be a result of war-related effects such as changed sexual behaviour and increased female vulnerability as seen in other places [24] but there is no evidence that it has affected the long-term trend of HIV-1 prevalence. Conflicts have in some cases shown to increase the transmission of HIV in societies by mechanisms such as population movements, increase in commercial sexual activities, sexual abuse, transitory sexual relations as well as collapse of health structures leading to unsafe injections and blood transfusion routines.[25,26] Armed troops have been shown in some places to have significantly higher HIV prevalence than the general population,[27] and this has also been the case for peacekeeping forces.[28] However, there are also several examples of settings where armed conflict has retarded the

transmission of HIV by means such as reduced mobility and accessibility of populations and increased death rates among high-risk groups.[29,30]

We observed a decline in the HIV-2 prevalence among pregnant women in Bissau from 8.3 % to 2.5 % between 1987 and 2004. Similar results of declining HIV-2 prevalence have also been observed in surveys of the general population[4] and of a professional cohort[5] in Guinea-Bissau, as well as of patients attending clinics in The Gambia.[8] The reasons for the previously high HIV-2 prevalence in Guinea-Bissau are not clear, but could partly be a result of the prolonged war of independence in 1963-1974. It has been suggested that transmission then might have been driven by multiple mechanisms, such as inoculation campaigns, non-sterile surgical procedures or injections, blood transfusions and sexual transmission.[31] None of the neighbouring countries had a similar period of prolonged armed conflict during the sixties and the seventies and surveys in these countries have shown lower levels of HIV-2 prevalence in the eighties.[6,7] Thus, the declining prevalence of HIV-2 that we have observed in this study could be the return of the HIV-2 prevalence to a steady state level similar to levels in The Gambia and Senegal. Few data regarding changes in sexual behaviour are available to support any hypothesis of declining HIV-2 prevalence due to sexual behaviour changes, and increasing HIV-1 prevalence during the same period contradicts this hypothesis. An alternative explanation to the declining prevalence of HIV-2 is offered in a mathematical model by Anderson et al[32], which implies that in countries where both HIV-1 and HIV-2 are being transmitted, HIV-1 will outcompete and in a longer perspective displace HIV-2 because of higher reproductive rate. We could not find any evidence of the civil war in 1998-1999 halting the trend of long-term decline in HIV-2 prevalence. Only in the 1999 measurement a slight increase was recorded, but after came a clear post-war decline in all age-groups. Further research is needed to gain knowledge on behavioural patterns in Bissau and future surveillance will tell whether treatment and prevention efforts can help to stabilize or halt the HIV-1 epidemic, or if HIV-1 prevalence will again increase as seen before the civil war.

In conclusion, there has been a significant shift in prevalence rates of HIV-1 and HIV-2 in women giving birth at the maternity clinic in Bissau, away from the less virulent HIV-2 infection towards a dominance by the more aggressive HIV-1 infection. The observed increase of HIV-1 prevalence in pregnant women might have been sparked by war effects such as population movements but there is no evidence that the war affected the long-term trends nor HIV-1, neither HIV-2 prevalence.

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CONTRIBUTORS

AA was medically responsible for the clinical site, ZdS and FD were responsible for the HIV testing at the lab, SA developed the HIV testing algorithm, GB and EMF were responsible for project coordination. FM and HN coordinated the laboratory and clinical work and were lead authors of the manuscript, all other authors scrutinized the manuscript and suggested corrections.

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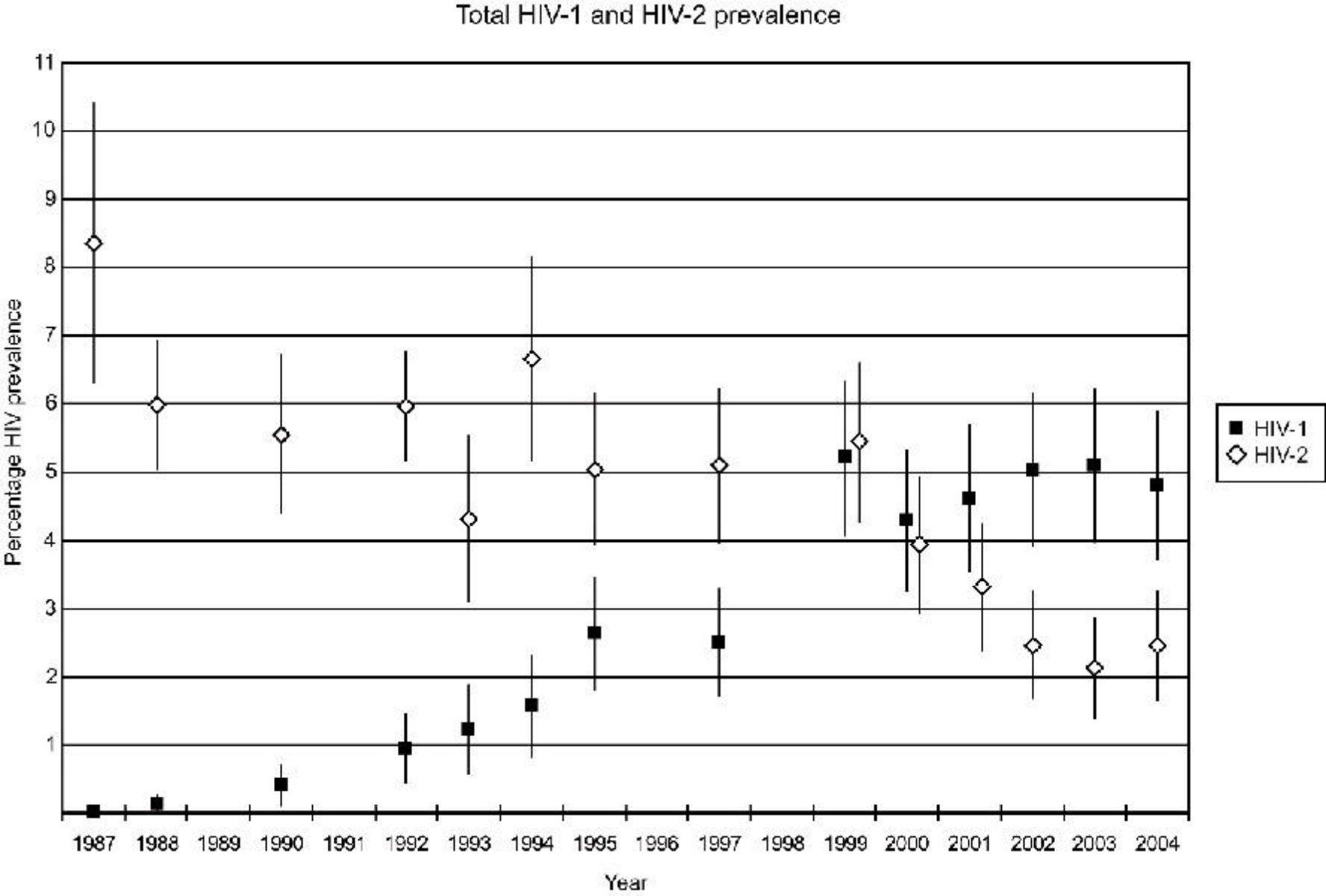
Total HIV-1 and HIV-2 prevalence
Percentage HIV prevalence
11
10
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1987
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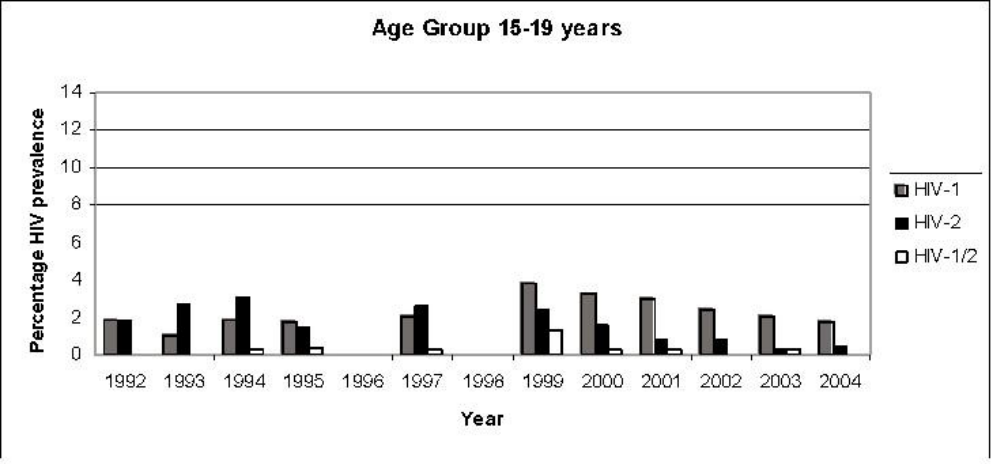
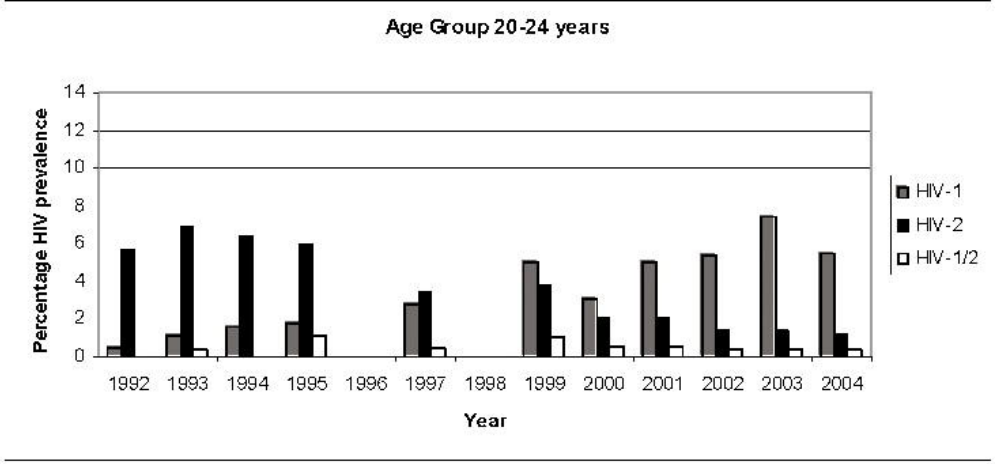
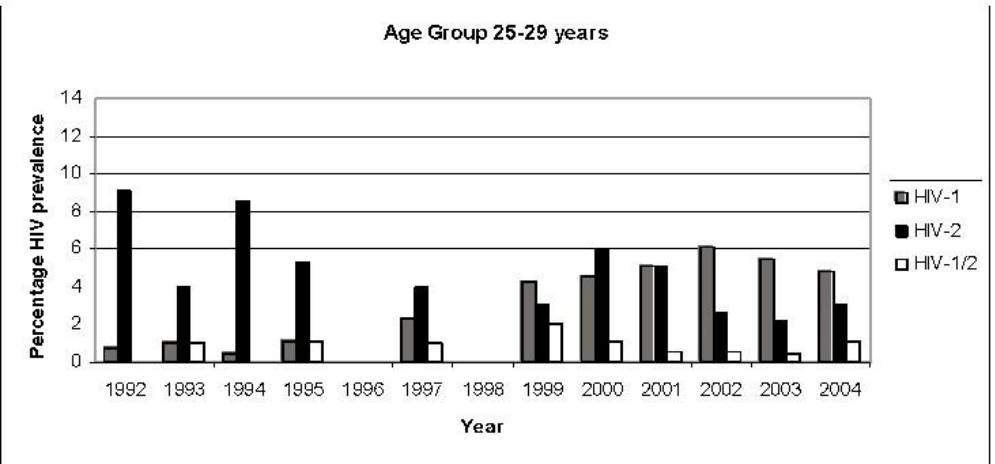
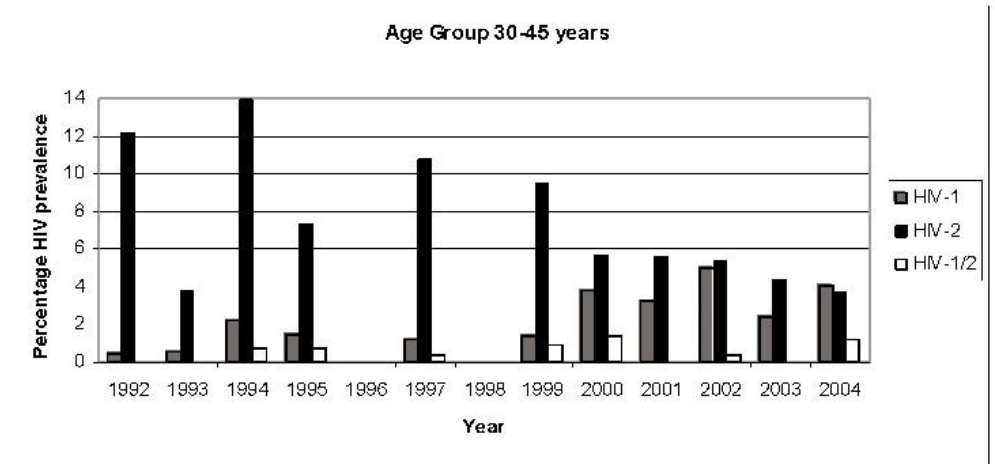
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
Year
HIV-1
HIV-2


Age Group 15-19 years
Percentage HIV prevalence
0
2
4
6
8
10
12
14
1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004
Year
HIV-1
HIV-2
HIV-1/2


Age Group 20-24 years
Percentage HIV prevalence
0
2
4
6
8
10
12
14
1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004
Year
HIV-1
HIV-2
HIV-1/2


Age Group 25-29 years
Percentage HIV prevalence
0
2
4
6
8
10
12
14
1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004
Year
HIV-1
HIV-2
HIV-1/2


Age Group 30-45 years
Percentage HIV prevalence
0
2
4
6
8
10
12
14
1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004
Year
HIV-1
HIV-2
HIV-1/2