

# Seroincidence of HIV-1 infection in African women of reproductive age: a prospective cohort study in Kigali, Rwanda, 1988–1992

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**Objective:** To estimate the seroincidence of HIV-1 infection among women of reproductive age in Kigali, Rwanda.

**Design:** Fixed prospective cohort followed for 36 months between November 1988 and June 1992, as part of an ongoing study of mother-to-child transmission of HIV-1.

**Setting:** Centre Hospitalier, Kigali, Rwanda.

**Subjects:** A total of 216 HIV-seronegative women were enrolled at delivery between November 1988 and June 1989.

**Methods:** A blood sample was obtained at delivery to test for HIV antibodies (by enzyme-linked immunosorbent assay and Western blot). Serum was tested every 3 months during follow-up. Incidence density rates of HIV seroconversion were estimated.

**Results:** The follow-up rate after 3 years was 89%, assessed by the maximum person-years method. The seroincidence density rate was 3.5 per 100 women-years (95% confidence interval, 1.9–5.0). It decreased linearly from 7.6 during the first 6-months postpartum to 2.5 per 100 women-years during the last 6 months of the third year of follow-up. Maternal age did not affect HIV incidence rates. We examined the role of the cohort, counselling, and the first 6-month postpartum effects on this estimate.

**Conclusion:** This fixed cohort provided an overall estimation of the HIV infection incidence rate and its dynamics. These figures could be used for programming future HIV preventive vaccine efficacy trials in Rwanda.

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**Keywords:** HIV-1 infection, seroincidence, women of reproductive age, Africa, vaccine trials

## Introduction

HIV-1 infection is a major public-health problem in sub-Saharan Africa [1]. A national serosurvey conducted in Rwanda in December 1986 found high HIV-antibody seroprevalences among urban popu-

lations, with an overall rate of 18% reaching 30% among 26–40-year-olds [2]. Among women specifically, the seroprevalence was 21% [2]. In early 1993, the HIV seroprevalence was 33% among pregnant women in the antenatal clinic of the Centre Hospitalier de Kigali in Kigali, the capital city of Rwanda

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[3]. Since December 1988, the progression of the HIV epidemic in Rwanda has been monitored through a network of sentinel posts, surveying a sample of pregnant women representative of the population of reproductive age [4]. This serosurveillance system has shown that HIV seroprevalence among urban-based Rwandan adults has increased in recent years in Kigali [4]. However, the real dynamics of this epidemic remain unclear. Data on the spread of HIV infection, particularly among young adults, are needed to assess the impact of prevention programs. Furthermore, trials designed to evaluate the efficacy of anti-HIV vaccines require precise estimates of the HIV incidence in potential target populations [5]. Because Rwanda has been designated to implement such HIV vaccine trials [6] by the World Health Organization (WHO), we analysed the incidence of HIV infection over a 3-year period, in an already existing prospective cohort of women of reproductive age in Kigali.

## Subjects and methods

A study of mother-to-child transmission of HIV-1 has been ongoing at the Centre Hospitalier de Kigali since November 1988. For this study, all HIV-seropositive women were enrolled at delivery at the maternity ward until June 1989, based on the following inclusion criteria: delivering a livebirth, living permanently within the city limits and granting verbal informed consent to participate. A group of HIV-seronegative women matched by maternal age and parity was consecutively selected as a comparison group. Details of the enrolment and follow-up procedures have been published elsewhere [7].

Briefly, the mean maternal age was 25.1 years (SD, 4.5 years) and the total number of pregnancies was 2.7 (SD, 1.8). The proportion of women living in a stable relationship, legal marriage or common law union tended to be lower in the HIV-seropositive group (83%) than in the HIV-seronegative group (89%;  $P=0.06$ ). HIV-seropositive mothers did not differ from seronegative mothers in terms of occupation, monthly income and place of origin [7]. Seronegative mothers, even if not enrolled, were similar in terms of socioeconomic status. Between delivery and day 15 postpartum, the drop-out rate in the seronegative group enrolled was not statistically different from that among the HIV-seropositive group. Predominant reasons for drop out were refusal by the husbands and false addresses given at the time of delivery in the maternity ward. Pre-test information about HIV prevention was given to each woman at delivery by a trained social worker. The participants were also informed about the objectives, constraints and benefits of the study. Post-test counselling was given only to those who wanted

to know their HIV serostatus or the HIV serostatus of their children during the study period.

At delivery, a blood sample was collected for HIV-antibody detection by a commercial enzyme-linked immunosorbent assay (ELISA; Vironostika, Organon Teknika, Boxtel, The Netherlands). Positive samples by ELISA were confirmed by a commercial Western blot technique (DuPont de Nemours, Wilmington, Delaware, USA) using the Centers for Disease Control and Prevention criteria for interpretation [8]. All women were retested for HIV antibodies every 3 months during the first 36 months of follow-up.

## Statistical analysis

All seronegative women were included in the framework of the study described above. In this group, we determined the incidence density rates of seroconversion over time and by age group. Incidence density rates of seroconversion were computed every 6 months by calculating the number of seroconversions/women-years at risk of seroconversion over 3 years (excluding women who died or had already seroconverted before the time of analysis). We used the mid-point of the 6-month follow-up to estimate the time of seroconversion and the time lost to follow-up. These results were computed with their 95% confidence intervals (CI) according to Miettinen's method [9]. A trend analysis of the evolution of incidence over time was performed using the  $\chi^2$  test for linear trends [10]. In order to calculate the follow-up rate, we used a single referenced method of maximum person-years described elsewhere [11]. Using this method, the follow-up rate was calculated by actual follow-up person-years/maximum expected follow-up person years  $\times 100$ .

## Results

After 36 months of follow-up, 178 of the 216 women, seronegative at inclusion, were still under clinical surveillance and 160 women were under serological follow-up. Two women had died after 1 day and 27 months of follow-up, respectively. The mean length of follow-up was 32 months (SD, 9.6 months; range, 1 day–36 months). This cohort accounted for 577 women-years of follow-up, excluding women who died. The maximum expected follow-up person-years was taken as the 216 women at study entry multiplied by 3 years, assuming that none were lost to follow-up during the study period, minus the follow-up of women who died (1.25 women-years). Thus, our follow-up rate was 89.2% (577/646.75). There was no significant difference between those lost to follow-up compared with those who remained in the study, with respect to age ( $P=0.10$ ), marital status ( $P=0.73$ ), and socioeconomic status ( $P=0.9$ ).

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A preliminary report on the first cases of maternal seroconversion in relation to postnatal transmission of HIV-1 has been published elsewhere [12]. Twenty seroconversions were documented during the first 36 months of follow-up among the 216 women seronegative at inclusion (Table 1), yielding a cumulative incidence of 11.2%. The largest number of seroconversions (eight out of 20; 40%) was observed in the first 6 months of the postpartum period. The overall incidence rate of seroconversion was 3.5 per 100 women-years (95% CI, 1.9–5.0). This seroincidence peaked at 7.6 per 100 women-years during the first 6 months of the postpartum period (95% CI, 2.3–12.9), with a substantial reduction during the following 6 months (4.0 per 100 women-years; 95% CI, 0.1–8.0). The incidence decreased linearly with time to 2.5 per 100 women-years (95% CI, 0.0–5.9) during the last 6 months of the third year of follow-up ( $\chi^2$  for linear trends,  $P=0.01$ ).

**Table 1.** Three-year incidence of HIV-1 seroconversion among 216 women HIV-seronegative after 577 women-years of follow-up postpartum, Kigali, Rwanda, 1988–1992.

Period of observation (months)*	No. women tested†	No. seroconversions	Follow-up‡	Six-month ID per 100 WY‡	95% CI
0–6	208	8	105	7.6	2.3–12.9
7–12	195	4	99	4.0	0.1–8.0
13–18	189	4	95	4.2	0.1–8.3
19–24	180	1	91	1.0	0.0–3.3
25–30	169	1	86	1.1	0.0–3.4
31–36	160	2	81	2.5	0.0–5.9

\*After delivery of a live child; †at the end of the 6-month period, excluding women who died or had already seroconverted before the time of analysis; ‡women-years (WY), excluding women who died or had already seroconverted before the time of analysis. ID, incidence density rate; CI, confidence interval.

Seroconversion rates over the 3 years in relation to age groups are shown in Table 2. Although higher rates were observed below 20 and above 30 years of age, no significant difference was documented between age groups.

**Table 2.** Incidence of HIV-1 seroconversion in relation to maternal age at delivery ( $n=216$ ), Kigali, Rwanda, 1988–1992.

Age at delivery (years)	No. women	No. seroconversions	Follow-up*	ID per 100 WY*	95% CI
15–19	26	4	93	4.3	0.1–8.5
20–24	74	9	262	3.4	1.2–5.7
25–29	79	3	159	1.9	0.0–4.0
≥30	37	4	63	6.4	0.1–12.6
Total	216	20	577	3.5	1.9–5.0

\*Women-years (WY), excluding women who died. ID, incidence density rate; CI, confidence interval.

None of the women who seroconverted received blood products during follow-up.

## Discussion

Several methods have been proposed to estimate the seroincidence of HIV infection in a given population. Most studies, including this one, choose to enroll 'fixed' cohorts in which no further entries are permitted once follow-up has begun. This type of cohort is well-suited to studying the natural history of a disease [13]. They are also easier to manage and less expensive than 'dynamic' cohorts in which participants are continuously entering or leaving the cohort. Other studies have used repeated HIV seroprevalence surveys to estimate incidence rates of HIV infection [4,14]. This is a less sensitive technique than the use of 'dynamic' or 'fixed' cohorts and provides a good approximation of the incidence rate, only if the prevalent populations are similar at all times surveyed.

The main objective of our study was to evaluate the mother-to-child transmission rate of HIV using this fixed prospective cohort design. We should take into account several biases when looking at seroincidence as a secondary objective among the comparison group of HIV-seronegative women. The most important bias was the cohort effect (Table 1). When the follow-up period is long, subjects at risk seroconvert at a higher rate early in the follow-up period while the remaining subjects are likely to have a lower risk for HIV infection. Consequently, the HIV incidence rate decreases artificially with time in our study. In addition, interventions such as counselling and education may have played a role in this decreasing incidence. The existence of another potential bias is suggested by the fact that more than one-third of the seroconversions were observed in the immediate postpartum period, namely the first 6 months after delivery. This early postpartum period might be a high-risk period for the acquisition of HIV infection, because of the hormonal changes and mucosal frailty of the cervix at this time. An unstable sexual partnership because of the absence of the father from home in the postpartum period could also play a role in the transmission of HIV to women, as suggested previously [12]. An alternative hypothesis is that in the months preceding delivery, women abstain from sexual activity and their partner may start other relationships incurring a higher risk of HIV infection. Thus, when postpartum sexual activity is resumed, the woman is at risk of infection from her partner.

Our estimate of HIV incidence, obtained over a longer period of time, is consistent with those obtained in other fixed cohorts in the same population [15] and with repeated prevalence studies [4]. A study with the primary objective of documenting the seroincidence and conducted with a 'dynamic' cohort could give a more accurate estimation of the HIV incidence rate. With such a design, the cohort effect would be controlled and the effects

of interventions and counselling taken into account. Also, the postpartum effect could be studied in more detail, without any attempt to reduce it. 'Dynamic' cohorts are currently being considered by the National Plan for HIV/AIDS Vaccine Development in Rwanda.

Our population study of prenatal women was approximately representative of women of reproductive age in Rwanda for several reasons. First, the fertility rate is one of the highest in the world: 8.6 children per woman at the end of a woman's reproductive life [16]; second, only 2% of Rwandan women are sterile [17]; third, only 14% of Rwandan women use a modern contraceptive method [16] and fourth, 92% of urban pregnant women seek prenatal medical services during the last trimester of pregnancy [16]. Consequently, prenatal women are a representative group among urban Rwandan women of reproductive age, which may explain why this group was chosen by the Rwandan National AIDS Control Program for sentinel serosurveillance of HIV-1 infection. However, the main selection bias in our analysis was the choice of an HIV-seronegative group matched to HIV-seropositive women after delivering a livebirth. Thus, our seroconversions rates cannot be generalized to all seronegative women who deliver in Kigali.

Studies on HIV incidence are critical in order to design HIV preventive vaccine efficacy trials. Our study confirms that pregnant women may represent a population in which the HIV seroincidence is high, and appears to be concentrated in the immediate postpartum period. Because pregnant women are also easy to recruit and relatively easy to follow, they should become a potential target group for future large scale vaccination trials and programs with adequate follow-up.

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