Estimated Timing of Mother-to-Child Human Immunodeficiency Virus Type 1 (HIV-1) Transmission by Use of a Markov Model

C. Rouzioux,1 D. Costagliola,2 M. Burgard,1 S. Blanche,3 M. J. Mayaux,4 C. Griscelli,3 A.-J. Valleron,2 and the HIV Infection in Newborns French Collaborative Study Group5

It has been shown that mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission can occur both during pregnancy and at delivery, but the respective frequencies in these periods are unknown. Moreover, it is difficult to determine the timing of mother-to-child HIV-1 transmission by direct sampling. The use of an elaborate statistical method is therefore necessary. The authors studied 495 consecutive infants born between May 1988 and August 1991 who were included, at birth, in the French Prospective Study on Pediatric HIV Infection. At least one blood sample was obtained from every infant during the first 14 days of life. All samples obtained within 3 months of birth were tested by at least two of the following methods: viral culture, polymerase chain reaction (PCR), and antigenemia, as well as by Western blot test. Data for the 95 infected infants (those seropositive at 18 months and those who died of HIV disease before this age), and who were exclusively bottle-fed, were analyzed in a Markov model to estimate the timing of viral transmission, the time from birth to the emergence of detectable virus, and the time from birth to seroconversion. The model indicated that one-third of the infants were infected in utero, less than 2 months before delivery (95th percentile). In the remaining 65% of cases (95% confidence interval (CI) 22-92), the date of infection was estimated as the day of birth. The estimated median period between birth and the emergence of viral markers was 10 days (95% CI 6-14) and the 95th percentile was estimated at 56 days. These results support the view that HIV infection can be diagnosed during the first 3 months of life. The authors conclude that mother-to-child HIV-1 transmission appears to occur late in pregnancy or at delivery. Am J Epidemiol 1995;142:1330-7.

The mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission rate has been estimated at between 10 and 39 percent in epidemiologic studies (1). The timing of transmission is unknown, yet it is crucial to know for effective prevention. Lentiviruses, including HIV-1, replicate and can be detected in the blood throughout the course of the infection; HIV-1 is continuously present in the form of complete infectious virions in the plasma and as proviruses integrated in host lymphocyte DNA. This means that the fetus of an infected woman is, in theory, continuously exposed to the virus. Early transmission has been suggested by reports of HIV-1 DNA detection in aborted fetuses and fetal tissues taken during the first 6 months of pregnancy (2, 3), while other studies have pointed to later transmission in utero or during delivery (4, 5). HIV-1 is detectable at birth in only about 40 percent of infected infants (6, 7), suggesting that infection occurs close to or during delivery in the remaining cases. Firstborn twins are more frequently infected than their siblings, which...
strongly suggests that contamination can occur during passage through the birth canal (8).

The problem of timing is difficult to address for several reasons. First, fetal sampling in utero is ruled out by the risk of inoculating the fetus with infected maternal cells. Similarly, it is ethically unacceptable to take numerous samples from neonates. The results of existing studies of this type are difficult to interpret because samples were usually obtained at different times and in different numbers from each infant.

In this study, we based our assessment of the timing of transmission on the kinetics of viral replication and the infant’s immune response. We did so by using a Markov model to analyze prospective virologic and immunologic data for infants enrolled at birth in the French pediatric cohort (9). We also used this approach to estimate the time lapse between birth and the emergence of viral markers, and the time lapse between birth and antibody production in infants.

MATERIALS AND METHODS

Patients

This study was part of the French Prospective Study on Pediatric HIV Infection (9) conducted under the auspices of the Agence Nationale de Recherches sur le Sida (ANRS). From May 1988 to August 1991, more than 1,500 children—all those born to mothers known to be infected with HIV who attended 62 obstetric and pediatric centers in the Paris, Toulouse, Bordeaux, and Nice areas—were included at birth in the prospective cohort. This study involved the subgroup of all infants enrolled in the 41 centers in the Paris area that send blood specimens to the virology laboratory of the Hôpital Necker-Enfants Malades, Paris. During this period, the laboratory received specimens from 495 consecutive neonates. The cut-off date for the analysis was chosen as March 1, 1993, so that at least 18 months had elapsed since birth in every case. During the study period, 99 infants were diagnosed as being infected by HIV-1 (on the basis of specific antibodies present at age 18 months (n = 90) and/or death from acquired immunodeficiency syndrome (AIDS) (n = 9) before this age), and 396 were classified as uninfected. One infected infant who was breast-fed and three infected infants from whom no blood specimens were available within the first 14 days of life were excluded from this analysis. The remaining 95 infected infants (no siblings) formed the group on which the statistical analyses were performed. As demanded by the protocol of the cohort study, all infants underwent clinical examinations and laboratory tests at specified intervals; at least one blood specimen was taken in the first 14 days of life. One specimen was available in 28 of these 95 cases, two specimens in 47 cases, three in 18 cases, and four in two cases; a total of 184 specimens were thus available for the group of infected children, all taken before 3 months of life. Specimens from 73 mothers of the infected babies, taken on the day of delivery, were also available.

Viral markers of HIV-1 replication

Viral replication was studied by testing for p24 antigenemia and by viral culture or polymerase chain reaction (PCR). Viral culture was performed on fresh patients’ peripheral blood mononuclear cells (PBMC) as previously described (7). Briefly, 3–5 × 10⁶ PBMC were co-cultured with 5 × 10⁶ fresh donor cells; viral replication was monitored by measuring p24 antigen production (enzyme immunosorbent assay from Abbott Laboratories, N. Chicago, Illinois) in the culture supernatants after ultracentrifugation. PCR analysis was done as previously reported (10): briefly, 2 × 10⁶ PBMC were lysed, then each sample was tested in duplicate with at least two HIV-1 primer pairs (pol 3/pol 4 and gag SK 38/39); the amplified products were detected by Southern blot test with a radiolabeled oligobprobe. PCR analysis was done on frozen samples when viral culture was not possible. Viral culture or PCR was done on heparinized samples. Antigenemia was tested on all samples (plasma and serum), and all positive results were confirmed by means of a neutralization test. Viral culture (or PCR) and antigenemia tests were negative on all specimens from the 396 uninfected children (i.e., those seronegative at 18 months of age).

Anti-HIV-1 antibody production in infants

Due to transfer of maternal immunoglobulins, the HIV-1 Western blot pattern in a neonate is generally identical to the maternal pattern. Seroconversion can nonetheless be identified in infants by the detection of antibodies against proteins produced by the HIV-1 gag and pol genes, provided that the maternal antibody pattern at the time of delivery is incomplete (absence of anti-p18 and/or anti-p24 and/or anti-p40 and/or anti-p55 and/or anti-p34 and/or anti-p68 or presence of very faint bands). We thus compared the neonatal pattern of antibody to HIV-1 (anti-HIV-1) to that of the mother, and then the successive anti-HIV-1 patterns for the specimens obtained from each infant. All tests were done on the same day, with the same batch (Diagnostics Pasteur, Marnes-La-Coquette, France) and in the same conditions. Western blot tests were carried out on all samples (plasma or serum). Seroconversion patterns were never detected in the 396 uninfected children.
Statistical analyses

The statistical analysis had to deal with the fact that the different time points or periods of interest were either interval-censored or right-censored. Indeed, when infants are exclusively bottle-fed, mother-to-infant HIV-1 transmission occurs within a known time interval, namely between conception and birth. Similarly, viral markers emerge between the date of infection and the date of the first specimen, between two consecutive specimens, or later (the last specimen obtained during the first 3 months may be taken before the emergence of viral markers). The same notion applies to antibody production. As a result, statistical techniques that are generally used to estimate the occurrence of endpoints, such as survival analysis (11), were not appropriate. The Markov modeling technique, however, is well suited to analysis of ordered clinical processes subject to interval or right censoring. The technique has already been used in regard to HIV to model the dynamics of CD4+ cell counts (12) and the time between infection and seroconversion in adults with a known date of infection (13). We generalized this approach (14) to the case in which the "date" of infection is interval-censored; because of the censoring, we used a time-homogenous model.

The time from infection to detectable antibody production was divided into three stages: *stage 1*, in which the infant was infected, but was negative in viral culture or PCR, and produced no HIV-1 specific antibodies; *stage 2*, in which the infant was positive for viral culture or PCR but produced no specific antibodies; and *stage 3*, in which the infant was positive for viral culture or PCR and produced detectable specific antibodies (figure 1). Infants with positive p24 antigenemia were placed in stage 2 when results of viral culture or PCR were unavailable.

Each specimen from each infant was classified into one of the three stages on the basis of virologic findings and Western blot test results. By definition, all the infants enter stage 1 when infected, and the time origin is therefore the date of HIV infection.

To estimate the time interval $x_k$ between infection and delivery, we postulated that it would have a value of 0 (transmission on the day of delivery) for a proportion $P$ of infants and that it would be distributed according to a continuous probability law in a proportion $1 - P$ of infants (transmission in utero).

The probability of an infant $k$ being in stage $i$ ($i = 1, 2, 3$) at the time of the first specimen ($t_{ik}$ after birth) is expressed as:

$$
P(1)_{i(t_{ik})} = \left[ (1 - P) \times \frac{\int_0^\infty [P(t_{ik} + x_k) = u] \times \varphi(u) \times du}{\int_0^\infty \varphi(u) \times du} \right] + (P) \times \left\{ \frac{P(t_{ik} + x_k)}{x_k = 0} \right\},
$$

where $P_i$ is the usual transition probability in a Markov model for a given $x_k$ (15):

$$
P_1(t_{ik} + x_k) = \exp[-\lambda_1(t_{ik} + x_k)]$$

$$
P_2(t_{ik} + x_k) = -\lambda_1 \left[ \frac{\exp[-\lambda_1(t_{ik} + x_k)]}{(\lambda_1 - \lambda_2)} + \frac{\exp[-\lambda_2(t_{ik} + x_k)]}{(\lambda_2 - \lambda_1)} \right]$$

$$
P_3(t_{ik} + x_k) = -\lambda_1 \lambda_2 \left[ \frac{1 - \exp[-\lambda_1(t_{ik} + x_k)]}{\lambda_1(\lambda_1 - \lambda_2)} + \frac{1 - \exp[-\lambda_2(t_{ik} + x_k)]}{\lambda_2(\lambda_2 - \lambda_1)} \right]$$

\[Am J Epidemiol\] Vol. 142, No. 12, 1995
and $\varphi(u)/\int \varphi(u) \, du$ is the density of probability of the time interval between infection and delivery for an infant infected in utero, $\delta_k$, the period of gestation (for the mother-infant pair $k$), and $\lambda_1$ and $\lambda_2$ are the transition intensities from stage 1 to stage 2 and from stage 2 to stage 3, respectively (this permits the different length of gestation for each pair to be taken into account).

For subsequent specimens, the probability of being in state $j$ at time $t'_k$ (time at which the second or a later specimen was taken) when the infant was in state $i$ at time $t_k$ (time of the previous specimen), is $P_{ij}(t'_k - t_k)$, the usual probability of a Markov model.

The likelihood is the product of the contributions of all informative specimens from all infants (14). When seroconversion was not "observable" (i.e., when the maternal Western blot pattern was complete), it was not possible to place infants in either stage 2 or 3 (this case was defined as stage 2/3); these infants thus contribute to the likelihood in the form of $1 - P_{u}(t'_k - t_k)$. It was not possible to place four infants in either stage 1 or 2 (this case was defined as stage 1/2) on the basis of their first specimen, because neither viral culture nor PCR were available (clotted samples, inadequate blood, bacterial contamination during viral culture); these infants thus contribute to the likelihood in the form of $1 - P_{l3}(t_k - t_k)$.

We used the maximum likelihood method to estimate the probability of transmission during delivery, the density of probability of the time of contamination in utero, and the transition intensities from stage 1 to stage 2 and from stage 2 to stage 3. The estimations of all the parameters were obtained simultaneously by maximizing the likelihood by the pseudo-Gauss-Newton algorithm in the BMDP (3R) statistical package. Confidence intervals were obtained by using the likelihood ratio statistics (11).

Cumulative distributions of the time from birth to the emergence of viral markers and the time from birth to antibody production were thus derived from the Markov model and parameter estimates (see Appendix).

### RESULTS

**Virologic and immunologic results in infected infants**

The viral marker results and antibody patterns in some infants were typical of the primary phase of HIV-1 infection in adults (16). The classification of the informative specimens for each infant into the three stages is presented in table 1.

Among the 95 infected infants, 85 were tested by means of viral culture or PCR on the first sample, and 29 were positive (26/73 by viral culture and 3/12 by PCR). Among the 91 infected infants tested for the presence of antigenemia, eight were positive on the first sample (seven of the eight were tested by viral culture and all were positive).

Seroconversion was observed in 21 of the 57 infected infants whose mother's antibody pattern was incomplete. The antibodies involved were anti-gag proteins in 21 cases (p55, p40, p24, and p18) and anti-pol proteins (p34 and p68) in three cases. These seroconversions were always confirmed when a later specimen was available.

**Proportions of infants infected in utero and intrapartum, and distribution of the dates of transmission**

For the probability law of $\varphi(u)$, we first used a gamma law,

$$(\lambda^\delta u^{\delta-1} e^{-\lambda u})/\Gamma(u).$$

Because the estimation of $\delta$ was close to 1 (with a not significant likelihood ratio test), we simplified the model by using an exponential law ($\lambda e^{-\lambda u}$).

The estimated distribution of the dates of HIV transmission expressed in weeks before delivery is presented in figure 2. About two-thirds, 65 percent (95

---

**TABLE 1. Number of observed paths for 95 infants with human immunodeficiency virus type 1 (HIV-1) infection, obtained on 184 blood samples (159 samples were contributing to the likelihood): French Prospective Study on Pediatric HIV infection, May 1988-August 1991**

<table>
<thead>
<tr>
<th>Stage of first specimen taken before day 14</th>
<th>Stages of subsequent specimen when available</th>
<th>No. of cases ($n = 95$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>2 → 2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>2 → 2/3</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>1/2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>2 → 2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2 → 2 → 2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2 → 2 → 3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2 → 3</td>
<td>10</td>
</tr>
<tr>
<td>2/3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* The contribution to the Markov model is different for each infant depending on the number of samples available, the observed path, and the date of the samples.
percent confidence interval (CI) 22–92) of the infants in this population sample were considered to have been infected during delivery (or on the day of birth). In the case of the infants estimated to have been contaminated in utero, the median period between infection and delivery was estimated as 14 days (95 percent CI 5–75), and the 95th percentile was estimated as 59 days before delivery.

**Time lapse between birth and the emergence of viral markers and antibody production**

The cumulative distribution of the estimated period between birth and the emergence of viral markers is shown in figure 3, along with the cumulative distribution of the time between birth and detectable antibody production. The estimated median period between

---

**Figure 2.** Estimated distribution of the dates of transmission.

**Figure 3.** Estimated proportions of human immunodeficiency virus type 1 (HIV-1)-infected infants presenting at a given age: 1) at least one viral marker (culture or polymerase chain reaction (PCR), and/or antigenemia positivity); 2) production of antibody to HIV-1 (anti-HIV-1).
birth and the emergence of viral markers was 10 days (95 percent CI 6–14) and the 95th percentile was estimated at 56 days. The estimated median period between birth and seroconversion was 62 days (95 percent CI 40–72) and the 95th percentile was estimated as 215 days. The data presented in figure 3 can be used to estimate the value of viral culture or PCR to date the time of transmission. It was calculated that 17 percent of the infected infants were positive for one of the viral markers on the day of birth, with a 100 percent risk of having been infected in utero. A positive sample taken on day 2 of life (25 percent of the infected infants) had a high probability of indicating transmission in utero (75 percent). In contrast, a positive sample taken on day 7 of life (42 percent of the infected infants) had about the same probability of indicating infection in utero as infection on the day of delivery (54 percent vs. 46 percent).

A key feature of the model is that it allows the diagnostic sensitivity of viral markers at a given age to be estimated (even though observations were made at various uncontrolled times), precluding the use of a Kaplan-Meier estimator. Figure 3 shows that 96 percent of the infections could have been diagnosed before age 2 months by means of viral culture or PCR.

DISCUSSION

The results of this study suggest that mother-to-child HIV-1 transmission occurs late in utero in one-third of cases, and on the day of delivery in the remainder. With overall transmission rate estimated to be 20 percent, the risk of transmission in utero was estimated to be about 7 percent and the risk of transmission on the day of delivery 13 percent. However, our data suggest that the period of transmission is a continuum, with no frontier between infection in utero and infection during delivery.

The Markov model we devised allowed us to estimate data prior to birth, in particular the date of infection, well before the first sample in some cases. As a corollary of the period between the estimated date of infection and the time of sampling, the confidence intervals for the percentage of infants infected in utero and for the median period between infection and delivery are wide, and would probably be difficult to reduce even with a larger population. To check the validity of the Markov model, we used methods summarized by Gentleman et al. (17). Our model assumes time-homogeneous transition intensities because time-dependent transition intensities are difficult to fit from incomplete data. To check whether or not transition intensities between stage 1 and stage 2 and between stage 2 and stage 3 were similar before and after birth, we adjusted a model with different transition intensities. This piecewise constant model did not fit the data significantly better than the time-homogeneous model ($\chi^2_{(2, df)} = 4.6, p = 0.100$). Another assumption included in the model was the distribution of the period between infection and birth. The gamma distribution was not found to be significantly better than the exponential one, but the true distribution could be different. To assess goodness-of-fit, we compared expected prevalence counts of the presence of viral markers after birth to observed prevalence counts. Only approximate observed prevalence counts can be evaluated. As recommended by Gentleman et al. (17), we assumed that an infant not actually observed on day $t$ remained in the same state as at his/her preceding inspection time in order to compute these approximate observed prevalence counts. In the case in hand, this assumption would tend to underestimate the true figure. We chose to count the number of infants with viral markers on day 7. The expected percentage of infants positive for viral markers on day 7 was estimated at 41 percent, and we found that 30 infants (37 percent) were positive among the 82 infants that could be evaluated (i.e., those with an informative specimen before 8 days of life) (mean squared error (MSE) = 0.804). We also evaluated the prevalence counts on day 60 from viral data for infants observed between day 15 and day 60. Among the 52 evaluable infants, it was predicted from the model that 50 infants would be positive by day 60; in fact, 48 infants were positive (MSE = 2.080). In both cases, as expected, the observed percent of infants with viral markers was below the expected value, although not markedly so. In conclusion, the various diagnostic checks used to assess the adequacy of the model did not reveal any large departure from the assumptions included in the model.

The results of the statistical analysis do not dismiss the possibility of early transmission, although the estimated frequency was low: it was estimated that less than 2 percent of infected infants were infected more than 2 months before birth. Our estimation is at variance with studies (2, 18) in which the HIV-1 detection rate in aborted fetuses was between 15 and 70 percent, but these results were obtained in nonrepresentative series as clearly shown by transmission rates higher than prospective pediatric cohorts. In contrast, our estimations appear to be compatible with results of a recent large study of 99 fetuses from HIV-1 seropositive women (19), in which the HIV-1 genome was detected in two thymuses (one from a fetus that died in utero, and one from a child stillborn at 26 weeks of pregnancy). This large study of fetal tissue demonstrated the potential lethality of HIV-1 in utero, but did not reveal a high rate of early infection in utero. Moreover, it must be kept in mind that mother-to-child
HIV-1 transmission rates are only estimated for live births in cohort studies. The distribution of probable dates of infection in our study is also in keeping with the fact that most infants born to HIV-1 seropositive mothers have a normal birth weight and length, and that very few have early clinical signs or immune deficiency (9, 20). The recent results of the ACTG-076 trial support this analysis, because zidovudine administration late in pregnancy cuts the transmission rate by about two-thirds (21). Similarly, the preventive effect of cesarean section is often discussed and points to a high frequency of late transmission (22). We also observed the typical pattern of primary infection in the first 3 months of life. Although only 21 seroconversions were identified in the 57 evaluable cases, immunologic immaturity may have played a role in some cases, and only one sample was available from certain infants during the study period, i.e., the first 3 months of life. Specimens taken between 6 and 10 months were available for 19 infants in whom seroconversion was not detected in the specimens taken before age 3 months and all had seroconverted. In contrast to a widely held general belief, these results show that infants can mount a specific immune response to HIV-1 during the first 3 months of life (a phenomenon documented for other viruses acquired in utero or during delivery, e.g., rubella and herpes simplex). In addition, these findings are in keeping with previous studies of HIV-1 antibody production in vitro (23) and with the production of anti-HIV immunoglobulin A by infected infants (24, 25).

The way in which the virus infects the fetus is unclear. One possibility is maternofetal blood exchange, especially during the days preceding the onset of labor. Passage through the birth canal also carries a risk of infection. When the infection occurs on the day of delivery, both mechanisms may play a role. The Markov model also revealed that neonatal HIV-1 infection can be diagnosed through the use of viral markers, directly during the first 3 months of life in more than 95 percent of cases. Given the excellent sensitivity and specificity of viral culture and PCR, the positive predictive value of two consecutive positive results is high. It is thus no longer necessary to wait until age 18 months to make a firm diagnosis of HIV-1 infection. Early diagnosis enables preventive treatment of opportunistic infections to be started in all infected babies and possibly anti-retroviral therapy in those likely to develop an early severe form of HIV-1 infection.

The model we devised can be used to estimate the timing of mother-to-child HIV-1 transmission. Further studies are now required to determine whether maternal factors influence the course of HIV-1 infection through the timing of transmission or its mechanism (26). In conclusion, this study provides strong evidence of a high frequency of late mother-to-child transmission and should provide the basis for preventive trials.

ACKNOWLEDGMENTS

This work was supported by grants from ANRS (Agence Nationale de Recherche sur le Sida).

This work is dedicated to Denis Bucquet, who died of AIDS on July 17, 1993. The authors gratefully acknowledge his constant support, enthusiasm, and stimulating discussions. They also thank Guy Thomas and Patrick Berche for their helpful comments; the technicians of the Virology Laboratory of Hôpital Necker for technical assistance; and Anne Doussin, Corinne Laurent, and Yasmine Moudoub for their collaboration in the Pediatric Prospective study.

REFERENCES

APPENDIX

It follows from the model that the cumulative distribution of the time $t$ from birth to emergence of viral markers can be calculated from

$$P \times \left\{1 - \exp[-\lambda_1 t]\right\} + (1 - P) \times \frac{\{\int_U \varphi(u) \times du\} - \exp[-\lambda_1 (t + u)]}{\int_U \varphi(u) \times du}$$

and the cumulative distribution of the time $t$ from birth to detectable antibody production from

$$P \times \left\{-\lambda_1 \lambda_2 \left[\frac{1 - \exp[-\lambda_1 t]}{\lambda_1 (\lambda_1 - \lambda_2)} + \frac{1 - \exp[-\lambda_2 t]}{\lambda_2 (\lambda_2 - \lambda_1)}\right]\right\} + (1 - P) \times \frac{\left\{\int_U \varphi(u) \times du\right\} - \exp[-\lambda_1 (t + u)] + \exp[-\lambda_2 (t + u)]}{\int_U \varphi(u) \times du} \times \varphi(u) \times du,$$

where $\varphi(u)/\int \varphi(u) \, du$ is the density of probability of the delay between infection and delivery for an infant infected in utero, $P$ is the probability of transmission during delivery, and $\lambda_1$ and $\lambda_2$ are the transition intensities from stage 1 to stage 2 and from stage 2 to stage 3, respectively.