STATISTICAL METHODS FOR ASSESSING THE RISK AND TIMING OF VERTICAL TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS

A THESIS PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY UNIVERSITY OF LONDON

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ABSTRACT

Various methods have been used to estimate the HIV vertical transmission rate and the paediatric AIDS incubation period. These are reviewed, and the standard method of analysis, which ignores children of indeterminate infection status, is shown to be biased. A new method of estimation that is appropriate for prospective studies conducted in non-breastfeeding populations is described. The method, based on an EM algorithm, takes into account clinical, virological, and immunological data and is more efficient than previous approaches. The method was applied to data from the European Collaborative Study and revealed evidence of temporal changes in the transmission rate and the AIDS incubation period.

An individual patient meta-analysis of polymerase chain reaction (PCR) data on neonates subsequently shown to be HIV-infected was conducted. The main objectives were to estimate the age-specific sensitivity of the assay and the relative contributions of intrauterine and intrapartum transmission. Distributionfree and parametric approaches were used for the analysis of the data, which were interval-censored. The sensitivity of PCR was shown to be higher than previously thought. Approximately one-third of vertically-acquired HIV infection could be attributable to intrauterine transmission.

In a retrospective cohort study conducted in the State of Sao Paulo, Brazil, there was considerable variation in breastfeeding practice. Models, with structures reflecting the series of potential exposures to the virus (intrauterine, intrapartum, breastfeeding), were developed to estimate the risk of breastfeeding according to duration. Although breastfeeding per se was a strong risk factor for transmission, duration of breastfeeding was not. The extent to which this finding could be explained by variability in maternal infectivity was investigated.

TABLE OF CONTENTS

At	ostrac	t		2
Li	st of 1	Tables		6
Li	st of F	igures	5	7
Ac	know	ledger	nents	9
Pr	eface			10
1	A rev trans	view of smissio	f methods for estimating the HIV vertical on rate and the paediatric AIDS incubation period	11
	1.1	Histor	ical background	11
	1.2	Diagn	osing HIV infection in epidemiological studies	12
	1.3	Standa from p	ard method for estimating the vertical transmission rate prospective studies	13
	1.4	Alterna rate fr	ative methods for estimating the vertical transmission om prospective studies	14
	1.5	Estima neona	ating the vertical transmission rate from analysis of tal blood specimens	17
	1.6	Estima	ating the paediatric AIDS incubation period	18
	1.7	Studie mortal	es conducted in settings with high rates of infant ity	21
2	A ne	w metł	nod for estimating the HIV vertical transmission rate	28
	and f data	the pae	ediatric AIDS incubation period from prospective	
	2.1	Outlin	e of new method	28
	2.2	Theor	y	28
		2.2.1	The model.	28
		2.2.2	Identifiability	31
		2.2.3	Estimation	32
		2.2.4	Precision of estimates	36
		2.2.5	Covariates	37
	2.3	Examp	ble	38
		2.3.1	Description of study	38
		2.3.2	Application of new method	40
		2.3.3	Comparison with standard estimator	41

		2.3.4	Effect of calendar period	42
2.4 Conclusions			usions	43
		2.4.1	Discussion of findings and comparison with other studies	43
		2.4.2	Model assumptions	45
3	A st	udy to	determine when HIV DNA is first detectable in	58
	verti	ically-ir	nfected infants	
	3.1	Epide	miological background	58
	3.2	Data d	collection	59
	3.3	Statist	ical methods	60
		3.3.1	Non-parametric model	60
		3.3.2	Parametric models	63
		3.3.3	Locally-weighted regression	65
	3.4	Result	S	66
		3.4.1	Age-specific estimates of percentage of children ever DNA positive	66
		3.4.2	Effect of mode of delivery	68
		3.4.3	Comparison of methods for deriving confidence intervals	68
	3.5	Discus	ssion	69
		3.5.1	Timing of transmission	69
		3.5.2	Early diagnosis of HIV infection	71
		3.5.3	Related studies	72
4	The	risk of	HIV transmission by duration of breastfeeding	84
	4.1	Backg	round	84
	4.2	Descri	ption of Sao Paulo study and results of basic analyses	85
	4.3	Result	s using non-parametric analyses	87
	4.4	Serial	exposure model - constant hazard	88
		4.4.1	Theory	88
		4.4.2	Results	89
	4.5	Individ	ual variation in hazard rate	89
		4.5.1	Theory	90
		4.5.2	Results	92

	4.6	Incorporating variation into probability of prepartum 93 transmission		
		4.6.1 Theory		
		4.6.2	Results	95
	4.7 Interpretation of model as frailty parameter becomes arbitrarily large			96
	4.8	Estima	tes of frailty from other types of study	97
		4.8.1	Twin study	97
		4.8.2	Studies of viral load and vertical transmission	98
		4.8.3	Consistency of estimates	99
	4.9 Factors that could bias estimates of model parameters			100
		4.9.1	Reverse causality	100
		4.9.2	Indeterminate infection status	101
		4.9.3	Error in recording duration of breastfeeding	101
	4.10	Relatio	nship to partner studies of sexual transmission	102
	4.11	.11 Public health implications		
5	Gene	eral dis	cussion	120
Appendices				
	2.1	FORTR	AN 77 program to implement EM algorithm	124
	2.2	Partial c	louble derivatives in Section 2.2.4	129
	2.3 I	Listing c	of data used in Section 2.3	131
	3.1 I	Letter to	prospective investigators about PCR study	134
	3.2 I	_isting c	of data used in Section 3.4	135
	4.1 I	_isting c	of data used in Sections 4.2-4.6	136
Re	References 137			

Publications included as subsidiary matter

ı.

LIST OF TABLES

1.1	Estimated transmission rates from studies using non-standard method of analysis	25
1.2	Studies using AIDS surveillance data to estimate the paediatric AIDS incubation period	26
2.1	Classification of children and information required by the proposed method	48
2.2	Comparison of crude estimates and maximum likelihood estimates under proposed model	49
2.3	Comparison of time-homogenous and time-variant models	50
2.4	Sensitivity of virus culture and PCR according to age	51
2.5	Results of beta-binomial models applied to virus test data	52
3.1	Estimates of the cumulative percentage of children with detectable HIV DNA	78
3.2	Results of analyses using parametric models	79
3.3	90% confidence intervals obtained by Peto's method and by method of profile likelihood	80
3.4	Negative predictive value of PCR at selected ages	81
4.1	Transmission rate by whether breastfed	106
4.2	Transmission rate by duration of breastfeeding	107
4.3	Results of different serial exposure models	108
4.4	Effect of excluding potential influential observations	109
4.5	Mode of transmission as frailty parameter becomes arbitrarily large	110
4.6	Likely impact of proposed interventions to reduce mother-to- child transmission of HIV in breastfeeding populations	111

LIST OF FIGURES

1.1	Probability of developing AIDS before age t months conditional on AIDS developing before age 72 months	27
2.1	Estimates of the survivor functions for AIDS, antibody loss, and of the conditional probability of infection for a child aged t months who is antibody positive, has not developed AIDS, and has never received a virus test	53
2.2	Estimate of the survivor function for AIDS	54
2.3	Maximum likelihood estimator and standard estimator of the survivor function for AIDS	55
2.4	Estimates of the survivor function for AIDS among 415 children born between 1985-88 and 562 children born between 1989-94	56
2.5	Non-parametric estimator and parametric estimator of survivor function for antibody loss	57
3.1	Cumulative percentage DNA positive using non-parametric model	74
3.2	Cumulative percentage DNA positive conditional on being DNA negative at birth	75
3.3	Effect on log-logistic model of excluding outlying observations	76
3.4	Comparison of non-parametric model and parametric models	77
3.5	Locally-weighted regression models	78
3.6	Cumulative percentage DNA positive for caesarean section deliveries	79
4.1	Estimated probability of transmission by duration of breastfeeding - non-parametric analyses	112
4.2	Estimated probability of transmission by duration of breastfeeding - models without frailty in prepartum transmission	113
4.3	Assessment of goodness of fit	114
4.4	Estimated probability of transmission by duration of breastfeeding - comparison of models with and without frailty in prepartum transmission	115

4.5	Profile log-likelihood for frailty parameter	116
4.6	Probability density function for latent risk of prepartum transmission	117
4.7	Probability density function for latent hazard rate of transmission through breastfeeding	118
4.8	Simulation model to study the effects of child's HIV infection on duration of breastfeeding	119

ACKNOWLEDGEMENTS

I am very grateful to Tony Ades for his generous support and encouragement, not only in my Ph.D. but in my work generally. Beatriz Tess kindly allowed me to use data from the Sao Paulo Collaborative Study for Vertical Transmission of HIV-1. Finally, I am indebted to Noelene for showing far more tolerance and patience than I deserve.

PREFACE

Between 1991-1996 I was the statistician to The European Collaborative Study, a large prospective study of children born to HIV-infected mothers. I was also fortunate to have access to other important datasets connected with verticallyacquired HIV infection. Many of the statistical problems I encountered were non-standard and to solve these I had to either develop new approaches or to modify uncommonly applied methods. The thesis describes the analyses which I consider to be the most interesting from a statistical standpoint. A common theme in these analyses is the assessment of the risk of transmission, its determinants, or its timing. The focus of the thesis is statistical methodology, and I have not attempted to review comprehensively the epidemiological literature. I have not included two "standard" meta-analyses which I carried out of the risk of HIV transmission through breastfeeding (Dunn et al. 1992) and the effect of caesarean section delivery (Dunn et al. 1994).

Several of the analyses have already been published, and copies of the publications are attached at the end of the thesis. Formulae, but not the underlying theory, for estimating the rate of vertical transmission in studies conducted in settings with high rates of infant mortality (Section 1.5) are given in Dabis et al. (1993). The material in Chapter 2, which describes a new method for estimating the vertical transmission rate and the paediatric AIDS incubation period, is essentially contained in Dunn and Ades (1996), although some additional analyses are presented. This paper also contains a brief review of other approaches to this problem, which is developed more fully in Sections 1.1 and 1.2. The main results the distribution-free analysis of Chapter 3, a meta-analysis of polymerase chain reaction (PCR) data on neonates subsequently shown to be HIV-infected, were published in Dunn et al. (1995). The thesis provides more technical detail and describes alternative analytic approaches. The analyses of Chapter 4 have not been published but a manuscript is in preparation.

1. A REVIEW OF METHODS FOR ESTIMATING THE HIV VERTICAL TRANSMISSION RATE AND THE PAEDIATRIC AIDS INCUBATION PERIOD

This chapter is a review of different approaches that have been used to estimate the HIV vertical transmission rate and the paediatric AIDS incubation period¹. It covers prospective studies of children born to HIV-infected mothers, AIDS surveillance systems, and inference based on analysis of neonatal blood samples.

1.1 Historical background

The first cases of paediatric AIDS were described in 1982 (Centers for Disease Control 1982). Although HIV had not been isolated at this time, the fact that all their mothers had either AIDS or risk factors for AIDS suggested the involvement of an vertically-transmissible infectious agent. Opportunities for epidemiological research remained limited, however, until the development of an antibody assay in 1985.

Early studies of vertical transmission of HIV were often poorly designed, suffering in particular from selective inclusion of infected infants (European Collaborative Study 1991). The risk of transmission was therefore generally overestimated, with some estimates as high as 50% (Friedland and Klein 1987). It is now recognised that reliable estimates of the risk of transmission can be obtained only from prospective studies of children born to HIV-infected mothers who are enrolled at the time of delivery (birth cohort studies). Multicentre recruitment is usually essential to achieve adequate sample size. The first such studies were initiated in Europe (European Collaborative Study 1988, Blanche et al. 1989), although other studies have been conducted or are being conducted in most areas of the world where HIV is prevalent (Working Group of Mother-to-Child Transmission of HIV, 1995).

¹ Although the term paediatric AIDS incubation period tends to be used synonymously with age distribution at AIDS diagnosis, this is only strictly correct if all children are infected at the time of delivery

Birth cohort studies have also been used to examine risk factors for vertical transmission and to elucidate the natural history of vertically-acquired HIV infection, particularly the paediatric AIDS incubation period. An alternative approach is to utilise data reported to AIDS surveillance systems, although this requires the application of non-standard statistical techniques (see Section 1.5). With rare exceptions, AIDS surveillance systems exist only in industrialised countries (Commenges et al. 1992).

1.2 Diagnosing HIV infection in epidemiological studies

The standard diagnostic test for HIV infection is based on the detection of HIV IgG antibody. However, all children born to infected mothers initially test HIV antibody positive since maternal IgG antibody crosses the placenta. The absence of infection can be deduced from any subsequent negative antibody test, but a positive diagnosis is only possible from a test performed after the age when maternal antibody is no longer detectable. In an early report, Semprini et al. (1987) erroneously assumed a 9 months threshold and thus overestimated the transmission rate. In most studies a 15 months or 18 months threshold has been used (Boylan and Stein 1991), although maternal antibody has exceptionally been detected up to age 24 months (Kind et al. 1992).

In contrast, a positive virus test (p24 antigen, culture, polymerase chain reaction [PCR]) at any age is indicative of infection, although there have been rare reports of children with early positive virus tests who appear to have subsequently "cleared" the virus (Bryson 1995). As a safeguard against laboratory error the convention is to classify a child as infected only following a positive result on two or more separate samples. Virus tests have not been regarded as sufficiently sensitive to allow infection to be excluded on the basis of a negative test result. Sensitivity is particularly low in the first month of life (McIntosh et al. 1994, Dunn et al. 1995).

Since death may preempt a definitive laboratory diagnosis it is important to also consider clinical criteria when diagnosing HIV infection. A child who dies from

HIV infection or who is diagnosed with AIDS would always be classified as infected, although less rigorous criteria are sometimes used. For example, Goedert et al. (1989) defined as infected children who developed two or more signs in category P2A of the Centers for Disease Control (1987b) classification.

1.3 Standard method for estimating the vertical transmission rate from prospective studies

The standard method of estimating the vertical transmission rate first requires each child enrolled in the study to be classified as infected, uninfected, or of indeterminate infection status. The numerator for the transmission rate is the number of infected children, and the denominator is the number of infected plus uninfected children (Boylan and Stein 1991). This estimator is unaffected by the number of indeterminate children.

A child's infection status may be indeterminate because of loss to follow-up, death not clearly related to HIV infection, delay in the communication of data from the paediatrician to the study coordinator, or the fact that the child was born shortly before the date of analysis. Loss to follow-up is unlikely to be independent of infection status as parents may be less inclined to bring healthy children for regular clinical examinations. This could explain the 39% transmission rate reported from a study in Zambia which had a 47% rate of loss to follow-up (Hira et al. 1989). Even if infection status is missing at random, the standard estimator of the transmission rate will still generally be biased. This results from the <u>selective</u> use of information in classifying infection status. For example, if a child develops AIDS he is defined as infected. But if he does not develop AIDS, suggesting but not proving that he is uninfected, this information is not taken into account. Likewise, the conventions of ignoring negative virus test results and positive antibody results before the threshold age result in positive and negative bias respectively.

A technique to reduce bias in ongoing studies is to limit the analysis to children

whose infection status should in principle be known. Thus, if antibody persistence beyond 18 months was regarded as proof of infection then children born less than 18 months before the date of analysis would be excluded (European Collaborative Study 1991). A related technique is to base the transmission rate on children with an antibody test after the threshold age (Tsai et al. 1994). However, this may bias estimates upwards as there is no external motivation for continuing follow-up of seronegative children to beyond the threshold age. At the time these rules were developed, analysis centred on the results of antibody tests since virus tests were not widely available. This is no longer the case, and it is difficult to justify excluding from the analysis a child who has repeatedly tested virus positive merely because he is under 18 months of age at the date of analysis.

Dabis et al. (1992) proposed that potential bias should be examined by reestimating the transmission rate assuming all indeterminate children to be either uninfected or infected. This is an attractive idea, but as most studies have at least 20% loss to follow-up the upper limit is usually infeasibly high (Working Group on Mother-to-Child Transmission of HIV 1995).

1.4 Alternative methods for estimating the vertical transmission rate from prospective studies

In this section more sophisticated methods which have been used to estimate the transmission rate are considered. One analysis exploited the fact that infected children are persistently HIV antibody positive whereas uninfected children ultimately lose antibody (European Collaborative Study, 1991). The survivor function of children who remain antibody positive should therefore asymptote at a value equal to the transmission rate. The advantages of this approach over the standard method of analysis are (i) no need to assume a threshold age for antibody persistence (ii) all antibody test results are taken into consideration, including positive tests before the threshold age. Standard survival analysis techniques are not applicable, however, since the age at antibody disappearance is not observed but is known only to lie in the interval

between the last positive test and first negative test (interval censoring). The nonparametric estimator due to Turnbull (1976), which is the analogue of the Kaplan-Meier estimator for right censored data, was used. This method is discussed in detail in Section 3.3.1. Another difficulty in the analysis were children who died from HIV infection, since this censoring mechanism is not independent of the outcome event (antibody disappearance). This was addressed by setting the censoring age to the age the child would have been at the date of analysis had he not died. The same procedure should be applied to children who test virus positive if, in such cases, antibody testing may be discontinued before the threshold age for antibody persistence.

A related analysis was performed in the ACTG-076 trial, which was designed to assess the effectiveness of zidovudine in reducing vertical transmission (Connor et al. 1994). This analysis was based on the results of virus culture assays, which under the study protocol were conducted at birth and at ages 12, 24, and 78 weeks. Interval censoring was not taken into account, and separate Kaplan-Meier analyses were performed of age to first positive virus culture in the intervention and placebo groups. The transmission rates were estimated from the asymptote of the distribution function (one minus the survivor function). No child tested positive for the first time at the 78 week sample and the distribution functions asymptoted at age 24 weeks. In effect, a child who tested negative at the 24 week sample was deemed to be uninfected. This is valid if virus culture sensitivity at this age is close to 100%, but this is not supported by findings in other studies. For example, McIntosh et al. (1994) report a sensitivity of 87% between 1-6 months of age. The appropriateness of a survival analysis model, which requires that once virus can be cultured in an infected child it can always be cultured, is questionable. Two less important criticisms are the inadequate description of the analysis of the six children who died due to HIV infection and an apparent error in censoring children at their last negative antibody test.

Tsai et al. (1994) developed an estimator of the transmission rate under the somewhat artificial scenario where all children receive "continuous" clinical

follow-up but have only one HIV antibody test at the threshold age for antibody persistence (they assume 15 months). Their method is based on a competing risks model where the two events are development of clinical evidence of HIV infection and censorship. It was assumed that the censoring time is independent of both HIV infection status and the incubation period. This assumption, and the different mechanisms that may give rise to censoring, are discussed in detail in the paper. The maximum likelihood estimator of the transmission rate is shown to be

$$\hat{\pi} = 1 - \frac{n_{\rm U}}{N\,\hat{\rm S}_{\rm c}(15)}$$

where n_u is the number of children who test antibody negative, N is the size of the initial cohort, and $\hat{S}_c(15)$ is the survivor function for the censoring time C at age 15 months estimated by the Kaplan-Meier method. In effect, one minus the transmission rate is estimated as the proportion of children who test antibody negative at age 15 months, where $\hat{S}_c(15)$ is an adjustment to obtain the correct denominator. Tsai et al. argue that a negative antibody test result below the threshold age should not be used to infer the absence of infection as "... infants might subsequently revert to antibody positive status ... either because of technical failures in the testing procedure or because maternal antibodies may have disappeared before the infected children began to produce antibody". However, technical failures (i.e. false negatives) are no more likely at younger ages than at older ages, and although transient loss of antibody in infected children at around 3-6 months of age has been described, it is very uncommon (Simpson and Andiman 1994).

In all three papers reviewed in this section a "standard" estimate of the transmission rate was calculated as well an estimate using the new method. There was reasonable agreement between the estimators within each of the studies (Table 1.1).

1.5 Estimating the vertical transmission rate from analysis of neonatal blood specimens

Dried blood spot specimens are routinely collected from neonates in order to detect metabolic abnormalities. One way of monitoring the HIV epidemic in the heterosexual population is to test residual blood from the specimens for HIV antibody (Ades et al. 1995). This yields an estimate of the prevalence of HIV infection among child-bearing women.

Comeau et al. (1993) tested stored neonatal blood spots of children who developed AIDS by PCR. 35 (52%) of the 67 specimens were PCR positive. Based on this finding, it was proposed that a "real time" estimate of the transmission rate could be obtained by testing all antibody-positive blood spots by PCR, calculating the proportion of these specimens which were PCR positive, and multiplying this by a factor of 2.

The method was applied to 48,585 blood spot specimens from the New England Regional Newborn Screening Programme (Comeau 1994). 161 samples were Western Blot confirmed antibody positive, with 156 yielding sufficient material for PCR analysis, of which 10 were PCR positive. The transmission rate was therefore estimated as $2 \times 10/156 = 6.4\%$.

This method is sensitive to the assumption that 50% of vertically-infected children test PCR positive in the neonatal period. There is evidence that the proportion of infected children with detectable levels of HIV DNA at birth may be closer to 30-40% (see Section 3.5.3.). Moreover, PCR sensitivity changes rapidly in the first 2 weeks of life (Dunn et al. 1995), and the exact age at which the sample is obtained is therefore important. However, this information would normally be lost in the anonymisation of the specimens.

1.6 Estimating the paediatric AIDS incubation period

In some prospective studies an attempt is made to follow-up HIV-infected children after infection is diagnosed in order to estimate the pediatric AIDS incubation period. The product-limit method (Kaplan and Meier, 1958) is commonly used; children who do not develop AIDS are censored at their last clinical examination. As with the standard estimator of the vertical transmission rate, children of indeterminate infection are excluded from the analysis. Consequently, the rate of progression to AIDS is over-estimated as the indeterminate children who are actually infected should contribute to the AIDS risk sets. The degree of this bias can be surprisingly large; empirical results are given in Section 2.3.3.

A number of investigators have estimated the incubation period based on paediatric AIDS case reports to routine surveillance systems (Table 1.2). This problem is identical to that of estimating the AIDS incubation period for individuals infected through blood products with a known date of infection (Kalbfleisch and Lawless 1989). The principle limitation of such data is that we are aware only of those infected individuals who have already developed AIDS (i.e. right truncation) and thus patients with short incubation periods are more likely to be observed. Thus, the frequency distribution of incubation periods, as directly observed, will be misleading in that it underestimates the true distribution (Downs et al. 1995). Bias is particularly serious at the beginning of the epidemic.

Valid inference requires a conditional probability argument. Let (x_i,t_i) be the observed data, where for the ith individual, x_i is the date of birth, t_i is the date of AIDS diagnosis, and $s_i=t_i-x_i$ is the observed incubation period (i=1,...,n). Let $f(s;\theta)$ be the density function of the incubation period where θ is a vector of unknown parameters. Assume the analysis is based on AIDS cases diagnosed over the period (T_L,T_R) and that reporting delay can be ignored. Conditioning on

 $x_1,...,x_n$ the distribution of $s_1,...,s_n$ has the truncated density (Commenges et al. 1992),

$$\prod_{i=1}^{n} \left[\frac{f(s_i;\theta)}{\int\limits_{max(T_L,x_i)}^{T_n} f(t-x_i;\theta) dt} \right]$$
(1.1)

Unless a functional (parametric) form is specified, $f(s;\theta)$ is estimable only up to a constant of proportionality (Kalbfleisch and Lawless, 1989). That is, the data provide information only on incubation times <u>conditional on</u> AIDS developing by the age at the oldest observed AIDS diagnosis. This makes intuitive sense because, without external information, we have no knowledge about the number of HIV-infected children who have not yet developed AIDS. An algorithm for estimating $f(s;\theta)$ under a discrete time model is described by Kalbfleisch and Lawless (1989), and under a continuous time model by Lagakos et al. (1988).

Figure 1.1 shows non-parametric estimates of the conditional AIDS incubation period from the studies listed in Table 1.2, except that only the most recent of the three analyses of the New York data is shown. This study by MaWhinney et al. (1994) indicates age-specific AIDS incidence to be approximately constant in the first 6 years of life. In the other studies, cases in early childhood were over-represented, specifically between 6-24 months in Kigali and between birth-12 months in Europe.

As well as conducting non-parametric analyses, several authors also maximised the likelihood (1.1) assuming a functional form for f(s;0). Theoretically this allows estimation of the <u>unconditional</u> AIDS incubation period. The Weibull distribution has been used to model the adult AIDS incubation period (Alcabes et al. 1993), but the monotonicity of the hazard function makes it unsuitable for modelling the paediatric AIDS incubation period. Instead, a mixture of two Weibull distributions (double Weibull) has been widely used (Auger et al. 1988, Commenges et al. 1992, Downs et al. 1995). Its popularity may be partly due to the "bimodality" hypothesis (Blanche et al. 1994) which asserts that there two

discrete sub-populations of "rapid" and "slow" progressors among verticallyinfected children.

However, the detailed study of Kalbfleisch and Lawless (1989) of the reliability of unconditional estimation (using a single Weibull distribution) casts doubt on the value of these analyses. They found that the problem of identifiability is only partly circumvented by parametric modelling and that the estimated centiles of the distribution function of the unconditional incubation period are highly imprecise. It is therefore not surprising that very wide interval estimates are obtained for functions of the parameters of the double Weibull distribution. For example, Downs et al. (1995) report a 95% confidence interval of 5.5-115 years for the median incubation time among the "slow" progressing sub-population.

In two closely related papers, AIDS surveillance data was combined with information from anonymous HIV screening programmes of neonatal blood specimens (DeGruttola et al. 1992, MaWhinney and Pagano 1994). This information, which was available for a 2-year period only, was used to infer the total number of children born to HIV-infected mothers. Children born outside this period essentially provide information only on the <u>shape</u> of the incubation period, whereas children born during this period constitute a cohort who also provide information on its <u>scale</u>. The analyses yielded estimates of cumulative progression to AIDS among all children born to HIV-infected mothers, rather than just HIV-infected children. MaWhinney and Pagano also incorporated an external estimate of the vertical transmission rate to derive an estimate of the paediatric AIDS incubation period.

An alternative approach to estimating the unconditional AIDS incubation period is to utilise external estimates of progression to AIDS among HIV-infected children from birth cohort studies. Using an unconditional 1 year progression rate estimate of 0.26 from the European Collaborative Study (1991) and a conditional (given AIDS by 8 years) 1 year progression rate estimate of 0.27, Downs et al. (1995) estimated the unconditional 8 year progression as 0.96

(0.26/0.27).

A number of caveats regarding the use of routine surveillance data should be noted. First, it is assumed that the completeness of reporting of cases is independent of calendar time and age at diagnosis. However, in at least one data set there is a deficit of young cases in the early period of surveillance (Commenges et al. 1992). Second, it is important to account for delay in the reporting of cases. The simplest approach is to exclude all cases diagnosed less than X months before the date of analysis, where it is assumed that all cases are reported within X months of diagnosis. DeGruttola et al. (1992) found that even 2 years after diagnosis of AIDS, fewer than 80% of cases had been reported, calling into question the common use of a 6 months (Auger et al. 1988) or 12 months (Downs et al. 1995) exclusion rule. An alternative and more efficient approach is to model the reporting delay distribution. In this context, Wang (1992) generalised the work of Kalbfleisch and Lawless (1989). Finally, the paediatric AIDS case definition has undergone periodic revision (Centers for Disease Control 1987b), and unless analysis is restricted to AIDS cases meeting a standard definition, interpretation of the estimated incubation period is problematic (DeGruttola et al. 1992)

1.7 Studies conducted in settings with high rates of infant mortality

The standard method of estimating the vertical transmission rate can be applied only if HIV-related deaths can be distinguished from non-HIV-related deaths. However, in settings with a high underlying rate of infant mortality it may not be possible to classify deaths reliably, and some studies have therefore included a control group of children born to mothers who are not infected with HIV. By assuming that the mortality experience of this group is representative of uninfected children born to infected mothers, the excess mortality due to HIV infection can be estimated without ascribing cause of death in individual cases. Halsey et al. (1990) estimated the transmission rate by adding the estimated excess mortality to the seroprevalence rate among the survivors, but this is incorrect (Dabis et al. 1993).

In this section, a formula for the vertical transmission rate is developed for studies in which children receive their first antibody test at the threshold age when a definitive serological diagnosis can be made. In studies conducted in less-developed countries this is usually taken to be age 15 months. Notation is described in the following table. Mortality before the threshold age is estimated using standard survival analysis methods, assuming random loss to follow-up.

Symbol	Meaning
π	Probability of vertical transmission
Ν	Number of children born to HIV-infected mothers
I	Indicator variable denoting whether child is infected (I=1) or uninfected (I=0)
Х	Indicator variable denoting whether child died (X=1) or survived (X=0)
M ₁	Estimated risk of mortality before age 15 months among children born to HIV- infected mothers
M _o	Estimated risk of mortality before age 15 months among children born to HIV- uninfected mothers
S	HIV prevalence at age 15 months among surviving children who were tested serologically
N _s	Denominator for the estimator S
N ₁	Of children born to HIV-infected mothers, number followed-up to age 15 months
No	Of children born to HIV-uninfected mothers, number followed-up to age 15 months

The number of children who survive to age 15 months and are infected is estimated by N(1–M₁)S. The total number of children who die and are infected is estimated by NM₁P(I=1|X=1). Thus the transmission rate, π , is estimated by

$$\hat{\pi} = \frac{N(1 - M_1)S + NM_1P(I=1 | X=1)}{N} = (1 - M_1)S + M_1P(I=1 | X=1)$$
(1.2)

From Bayes' Theorem,

$$P(I=1 | X=1) = \frac{P(X=1) - P(X=1 | I=0) P(I=0)}{P(X=1)}$$

which is estimated by

$$\frac{M_{1} - M_{0}(1 - \hat{\pi})}{M_{1}}$$
(1.3)

From an initial estimate of π , updated estimates are obtained by iterating between equations (1.3) and (1.2). However, a closed form solution exists, for at convergence,

$$\hat{\pi} = (1 - M_1)S + M_1 \left[\frac{M_1 - M_0(1 - \hat{\pi})}{M_1} \right] = (1 - M_1)S + M_1 + M_0(1 - \hat{\pi})$$

Rearranging,

$$\hat{\pi} = \frac{(1 - M_1)S + M_1 - M_0}{1 - M_0} = 1 - (1 - S) \left[\frac{1 - M_1}{1 - M_0} \right]$$

An estimate of the variance of $\hat{\pi}$ is most easily derived via

$$\log(1-\hat{\pi}) = \log(1-S) + \log(1-M_1) - \log(1-M_0)$$

Using the delta method and the simple estimate for the variance of a survivor function suggested by Peto (Cox and Oakes 1984, Section 4.3),

$$\operatorname{var}[\log(1-\hat{\pi})] \approx \frac{S(1-S)/N_{s}}{(1-S)^{2}} + \frac{M_{1}^{2}(1-M_{1})/N_{1}}{(1-M_{1})^{2}} + \frac{M_{0}^{2}(1-M_{0})/N_{0}}{(1-M_{0})^{2}}$$

$$= \frac{S}{(1-S)N_{s}} + \frac{M_{1}^{2}}{(1-M_{1})N_{1}} + \frac{M_{0}^{2}}{(1-M_{0})N_{0}}$$

It is then straightforward to calculate a confidence interval for $\log(1-\hat{\pi})$ and thus for $\hat{\pi}$.

The key assumption in the method is that the unobserved mortality of uninfected children born to infected mothers can be validly estimated from the mortality of children born to uninfected mothers i.e. $E(M_0) = P(X=1|I=0)$. This assumption is questionable as maternal infection per se may have an adverse

effect on child mortality. Consequently, the excess mortality and thus the estimated transmission rate could be overestimated, although the extent of this bias is difficult to quantify.

Table 1.1Estimated transmission rates from studies using non-standardmethod of analysis

Study	Estimated transmission rate		
	New method	Standard method	
European Collaborative Study (1991)	0.134	0.129	
Connor et al. (1994)	0.255	0.224	
Tsai et al. (1994)	0.300	0.343	

* placebo arm

.

Table 1.2 Studies using AIDS surveillance data to estimate the paediatric AIDS incubation period

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Reference	Source of data	No. of AIDS cases
Auger et al. 1988	New York, 1979-1987	215
Commenges et al. 1992	Kigali (Rwanda), 1984-88	685
DeGruttola et al. 1992	New York, 1982-89	568
MaWhinney et al. 1994	New York, 1983-90	493
Downs et al. 1995	Europe, 1982-90	792

Figure 1.1 Probability of developing AIDS before age t months conditional on AIDS developing before age 72 months. Studies shown are Commenges et al. (solid line), MaWhinney et al. (dashed line), and Downs et al. (dotted line).



CHAPTER 2. A NEW METHOD FOR ESTIMATING THE HIV VERTICAL TRANSMISSION RATE AND PAEDIATRIC AIDS INCUBATION PERIOD FROM PROSPECTIVE DATA

2.1 Outline of new method

Standard methods of analysis for prospective studies of children born to HIVinfected women were described in Section 1.3. It was shown that these methods may lead to biased estimates of the rate of vertical HIV transmission and the paediatric AIDS incubation period because of complications introduced by the difficulty in diagnosing HIV infection in young children. Alternative methods based on modelling the disappearance of HIV antibody or appearance of virus are inefficient as they essentially rely on a single variable. In this chapter an alternative model, which takes into account clinical, immunological, and virological data, is developed.

The central feature of the new method is the estimation of the conditional probability of infection for every child in the study, given the observed data. This entails iterative and simultaneous estimation of the vertical transmission rate, the sensitivities of the virus tests, the AIDS incubation period, and the distribution function of antibody loss (taking account of interval censoring). All distributions are estimated non-parametrically and there is no need for an arbitrary threshold age at which antibody persistence is taken as proof of infection. By taking children of indeterminate infection status into account, the method avoids the biases that affect the standard estimators and should be more efficient.

2.2 Theory

2.2.1 The Model

Let π = probability of vertical transmission, T_A = age at AIDS diagnosis, and T_s = age when child first becomes seronegative. Although a continuous time model can be specified and estimated, a discrete formulation is more convenient for the purposes of exposition. Let $g_t = Pr(T_A = t | T_A > t-1, infected)$ be

the AIDS hazard at age t months given that the child is infected, and let $h_t = Pr(T_s=t \mid \underline{uninfected})$ be the probability density function of antibody loss at age t months given that the child is uninfected. Let $G_t = \prod_{u=0}^{t-1} (1-g_u)$ and $H_t = 1 - \sum_{u=0}^{t-1} h_u$ denote the survivor functions for AIDS and antibody loss respectively. Finally, up to V different types of virus tests may be applied with sensitivities $s = [s_1 \ s_2 \ \dots \ s_v]$. The tests are assumed to be perfectly specific, so that a child is inferred to be infected on the basis of one or more positive tests. A further assumption is that all virus test results are independent, conditional on infection status.

It is supposed that each child in a cohort of size N can be classified into one of four mutually exclusive groups (Table 2.1), although in practice this may not be straightforward (see Section 2.6). N_A children are observed to develop AIDS and the age at diagnosis is assumed to be accurate; N_s children become antibody negative (seronegative) at an unknown point between the last positive antibody test and the first negative antibody test; N_v children have not developed AIDS but are positive on one or more virus tests; the remaining N_c children have not developed AIDS, are never positive on a virus test, and remain antibody positive. This last group will be referred to as censored, which it should be noted, does not mean the same as indeterminate infection status as defined in Section 1.2.

The problem can be considered as a mixture model with two states (infected and uninfected). A special feature of the data, analogous to the problem considered by Hosmer (1973), is that state membership is known for some observations. Children who develop AIDS or test virus positive are assumed to be infected, and children who become seronegative are assumed to be uninfected. The contribution to the likelihood of a subject whose state is known is the product of the unconditional probability of belonging to that state and the conditional probability of the observed data. The contribution of a subject whose state is not known is the sum across states of all such terms.

Consider, for example, a censored child who has not developed AIDS by age a_k , remains antibody positive at age I_k , and who has had, by virus test v, a total of y_{kv} negative results (v=1,...,V). If the child is infected he provides no information about the antibody loss distribution H, and if he is uninfected he provides no information about the AIDS incubation period G or about the sensitivities of the virus tests **s**. His contribution to the likelihood function is thus the sum of two components. The first component is the product of the unconditional probability of being infected π , and of the conditional probabilities of not developing AIDS $\Pi(1-g_i)$ and of always testing virus negative

 $V_{t=0}^{V}$ (1-s_v)^{y_w}. The second component is the product of the unconditional probability of not being infected (1- π), and the conditional probability of remaining antibody positive (1- $\sum_{t=0}^{k} h_t$). The contribution to the likelihood function of children in the AIDS, VIR, and SERO groups are derived similarly:

Group	Contribution to likellhood
AIDS	$\pi g_{a_{k}} \prod_{t=0}^{a_{k}-1} (1-g_{t}) \prod_{v=1}^{V} s_{v}^{x_{kv}} (1-s_{v})^{y_{kv}}$
SERO	$(1-\pi) \sum_{t=l_{k}+1}^{r_{k}} h_{t}$
VIR	$\pi \prod_{t=0}^{a_{k}} (1-g_{t}) \prod_{v=1}^{V} s_{v}^{x_{kv}} (1-s_{v})^{y_{kv}}$
CENS	$\pi \prod_{t=0}^{a_{k}} (1-g_{t}) \prod_{v=1}^{V} (1-s_{v})^{y_{kv}} + (1-\pi) (1-\sum_{t=0}^{I_{k}} h_{t})$

The log-likelihood $L(\pi, \mathbf{s}, \mathbf{g}, \mathbf{h})$ is the sum of the logarithm of the individual contributions

$$\sum_{\substack{\text{AIDS} \\ k}} \log(\pi) + \log(g_{a_{k}}) + \sum_{\substack{t=0 \\ t=0}}^{a_{k}-1} \log(1-g_{t}) + \sum_{\nu=1}^{\nu} \left[x_{k\nu} \log(s_{\nu}) + y_{k\nu} \log(1-s_{\nu}) \right] \\ + \sum_{\substack{\text{SERO} \\ k}} \log(1-\pi) + \log \left[\sum_{\substack{t=l_{k}+1 \\ t=l_{k}+1}}^{r_{k}} h_{t} \right] \\ + \sum_{\substack{\text{VIR} \\ k}} \log(\pi) + \sum_{\substack{t=0 \\ t=0}}^{a_{k}} \log(1-g_{t}) + \sum_{\nu=1}^{\nu} \left[x_{k\nu} \log(s_{\nu}) + y_{k\nu} \log(1-s_{\nu}) \right] \\ + \sum_{\substack{\text{CENS} \\ k}} \log \left[\pi \prod_{\substack{t=0 \\ t=0}}^{a_{k}} (1-g_{t}) \prod_{\nu=1}^{\nu} (1-s_{\nu})^{y_{k\nu}} + (1-\pi) (1-\sum_{t=0}^{l_{k}} h_{t}) \right]$$
(2.1)

subject to $0 \le \pi \le 1$, $0 \le s_v \le 1$, $0 \le g_t \le 1$, $0 \le h_t \le 1$, $\Sigma h_t = 1$

2.2.2 Identifiability

There is a potential identifiability problem when virus tests are not conducted. Consider an alternative parameterisation (π, g, h) defined by

$$π^* = cπ$$
, G^{*}=1- $\frac{(1-G)}{c}$, H^{*}=1-(1-H) $\frac{1-π}{1-cπ}$ for t≤max(I_k; CENS), otherwise =1

where c is an arbitrary positive constant such that the range conditions in equation (2.1) are not violated. Noting that there is no VIR group in the absence of virus test data, the individual contributions to the likelihood under this parameterisation are:

AIDS:
$$\pi^*(G_{a_k}^* - G_{a_{k+1}}^*) = \pi^*[(1 - G_{a_{k+1}}^*) - (1 - G_{a_{k}}^*)] = \pi C \left[\frac{1 - G_{a_{k+1}}}{C} - \frac{1 - G_{a_{k}}}{C}\right]$$

= $\pi (G_{a_k}^* - G_{a_{k+1}}^*)$

SERO:
$$(1 - \pi^*)(H_{l_k}^* - H_{r_k}^*) = (1 - \pi^*)[(1 - H_{r_k}^*) - (1 - H_{l_k}^*)]$$

= $(1 - \pi c) \left[\frac{1 - \pi}{1 - \pi c} (1 - H_{r_k}) - \frac{1 - \pi}{1 - \pi c} (1 - H_{l_k}) \right] = (1 - \pi)(H_{l_k} - H_{r_k})$

CENS:
$$\pi^* G_{a_{k+1}}^* + (1 - \pi^*) H_{l_{k+1}}^* = \pi C \left[1 - \frac{1 - G_{a_{k+1}}}{C} \right] + (1 - \pi C) \left[1 - (1 - H_{l_{k+1}}) \frac{1 - \pi}{1 - \pi C} \right]$$

= $\pi G_{a_{k+1}}^* + (1 - \pi) H_{l_{k+1}}$

The equivalence of the likelihood under the alternative parameterisations (π , g, h) and (π , g, h) indicates a lack of identifiability in the model, and to obtain unique maximum likelihood estimates it is necessary to constrain either \hat{G} or \hat{H} . However, this is unnecessary if the oldest age at AIDS diagnosis exceeds the oldest censoring age for AIDS i.e. if max(a_k ; AIDS) > max(a_k ; CENS), since this removes the indeterminancy in the right tail of \hat{G} . Otherwise, it suffices to apply the mild constraint that all antibody loss in uninfected children occurs by the oldest age at which an antibody test was performed. This is a reasonable assumption provided at least one child has been followed to at least age two years. If virus test data are available the model is immediately identifiable provided at least one child is in the VIR group or at least one child in the CENS group has had one or more (negative) virus tests.

2.2.3 Estimation

Dempster et al. (1977, Section 4.3) showed that finite mixture models can be cast in the framework of an EM algorithm by introducing unobserved dummy variables to represent state membership. In the E-step, the conditional probabilities of belonging to each of the states are estimated for each observation, given current parameter estimates. The contribution of each observation to the complete-data log-likelihood is the weighted sum of the loglikelihood associated with each of the states, with weights given by the conditional probabilities. The M-step involves re-estimation of the parameters

by maximisation of the complete-data log-likelihood. A FORTRAN 77 program that implements the EM algorithm is given in Appendix 2.1.

E-step

The conditional probabilities of state membership are fixed (0 or 1) for children in the AIDS, SERO, and VIR groups. For children in the CENS group, the conditional probabilities of infection are, using Baye's Theorem,

$$\hat{\tau}_{k} = \frac{\hat{\pi} \, \hat{G}_{a_{k}+1} \prod_{\nu=1}^{V} (1 - \hat{s}_{\nu})^{y_{k\nu}}}{\hat{\pi} \, \hat{G}_{a_{k}+1} \prod_{\nu=1}^{V} (1 - \hat{s}_{\nu})^{y_{k\nu}} + (1 - \hat{\pi}) \, \hat{H}_{l_{k}+1}}$$
(2.2)

The complete-data log-likelihood L_c is identical to the observed-data log-likelihood (equation 2.1) except that the contribution from the CENS group is

$$\sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_{k} \left[\log(\pi) + \sum_{t=0}^{a_{k}} \log(1-g_{t}) + \sum_{v=1}^{v} y_{kv} \log(1-s_{v}) \right] + (1-\hat{\tau}_{k}) \left[\log(1-\pi) + \log(1-\sum_{t=0}^{l_{k}} h_{t}) \right]$$

instead of

$$\sum_{\substack{\text{CENS} \\ k}} \log \left[\pi \prod_{t=0}^{a_{k}} (1-g_{t}) \prod_{v=1}^{V} (1-s_{v})^{y_{kv}} + (1-\pi) (1-\sum_{t=0}^{l_{k}} h_{t} \right]$$

M-step

The M-step consists of four parts:

 Re-estimate π by the ratio of the estimated number of infected children to the total cohort size

$$\hat{\pi} = (N_A + N_V + \sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_k) / N$$

which is the solution of

$$\frac{\partial L_c}{\partial \pi} = \frac{N_A + N_V}{\pi} - \frac{N_s}{1 - \pi} + \sum_{\substack{\text{CENS} \\ k}} \left(\frac{\hat{\tau}_k}{\pi} - \frac{1 - \hat{\tau}_k}{1 - \pi}\right) = 0$$

(2) Re-estimate the components of s by the ratio of the number of positive test results to the estimated total number of tests performed on infected children

$$\hat{s}_{v} = \frac{\sum_{\substack{\text{AIDS} \\ k}} x_{kv} + \sum_{\substack{\text{VIR} \\ k}} x_{kv}}{\sum_{\substack{\text{AIDS} \\ k}} (x_{kv} + y_{kv}) + \sum_{\substack{\text{VIR} \\ k}} (x_{kv} + y_{kv}) + \sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_{k} y_{kv}}$$

which is the solution of

$$\frac{\partial L_{c}}{\partial s_{v}} = \sum_{\substack{AIDS \\ k}} \left(\frac{x_{kv}}{s_{v}} - \frac{y_{kv}}{1 - s_{v}} \right) + \sum_{\substack{VIR \\ k}} \left(\frac{x_{kv}}{s_{v}} - \frac{y_{kv}}{1 - s_{v}} \right) - \sum_{\substack{CENS \\ k}} \hat{\tau}_{k} \frac{y_{kv}}{1 - s_{v}} = 0$$

(3) Re-estimate G by the product-limit method where the discrete hazards are based upon the <u>estimated</u> number at risk of AIDS (estimated number infected) at each age

$$\hat{g}_{t} = \frac{\sum_{\substack{\text{AIDS} \\ k}} I(a_{k}=t)}{\sum_{\substack{\text{AIDS} \\ k}} I(a_{k}\geq t) + \sum_{\substack{\text{VIR} \\ k}} I(a_{k}\geq t) + \sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_{k} I(a_{k}\geq t)}$$

which is the solution of

$$\frac{\partial L_{c}}{\partial g_{t}} = \sum_{\substack{\text{AIDS} \\ k}} \frac{I(a_{k}=t)}{g_{t}} - \sum_{\substack{\text{AIDS} \\ k}} \frac{I(a_{k}\geq t+1)}{1-g_{t}} - \sum_{\substack{\text{VIR} \\ k}} \frac{I(a_{k}\geq t)}{1-g_{t}} - \sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_{k} \frac{I(a_{k}\geq t)}{1-g_{t}} = 0$$

where I is the indicator function.

(4) There is no closed form solution for \hat{H} but a standard algorithm for estimating a distribution function from interval censored data (Turnbull 1976) can be used. Although the original method assumed all observations have equal weight, DeGruttola et al. (1992, Section 2) pointed out that unequal weights are permissible. In this application, the weights are the current conditional probabilities of being uninfected. The method iteratively reallocates the weight associated with each child across all times when antibody loss could have occurred, in proportion to the current estimates of the probabilities of antibody loss for those intervals. Thus, the estimate $\hat{\mathbf{h}}^{(i+1)}$ at iteration i+1 is obtained from the estimate $\hat{\mathbf{h}}^{(i)}$ at iteration i by

$$\hat{h}_{t}^{(i+1)} = \sum_{\substack{\text{SERO}\\k:l_{k}+1 \leq t \leq r_{k}}} \frac{\hat{h}_{t}^{(i)}}{\sum_{u=l_{k}+1}^{r_{k}} \hat{h}_{u}^{(i)}} + \sum_{\substack{\text{CENS}\\k:l_{k}+1 \leq t}} (1 - \hat{\tau}_{k}) \frac{\hat{h}_{t}^{(i)}}{1 - \sum_{u=0}^{l_{k}} \hat{h}_{u}^{(i)}}$$
(2.3)

followed by re-scaling to ensure that $\Sigma \hat{h}_t^{(i+1)}=1$

The estimation of H is itself an EM algorithm and when algorithms are nested as here it is computationally inefficient to drive the inner algorithm to convergence (Healy, discussant to Dempster et al. 1977). For data arising from an exponential family where the M-step does not have a closed from solution, it has been proved that convergence to a local maximum is ensured with a single Newton-Raphson iteration in the M step (Rai and Matthews 1993). Although this problem does not fall within this framework, it was found empirically that convergence is achieved with one iteration of equation (2.3) per cycle of the main EM algorithm.
2.2.4 Precision of estimates

Methods have been described for obtaining the variance-covariance matrix when using the EM algorithm (Louis 1982, Meng and Rubin 1991). In this application, however, partial double derivatives of the observed-data loglikelihood can be evaluated directly (listed in Appendix 2.2.) It is necessary to consider only the non-zero elements of \hat{g} and \hat{h} (Cox and Oakes 1984 [Section 4.2], Turnbull 1976). Work on coverage probabilities of confidence intervals for the product-limit estimator suggests the use of a logarithmic scale (Miller 1981, Section 6.1.4). The AIDS survivor function has therefore been parameterised in terms of $\lambda_t = \log(1-g_t)$. Then,

$$\log G_t = \sum_{u=0}^{t-1} \lambda_u$$

$$var(\log \hat{G}_{t}) = \sum_{u=0}^{t-1} var(\hat{\lambda}_{u}) + 2 \sum_{u=0}^{t-1} \sum_{v=u+1}^{t-1} cov(\hat{\lambda}_{u}, \hat{\lambda}_{v})$$

Using the results and notation of Appendix 2.2, the observed information for π is

$$\frac{N_{A}+N_{V}}{\hat{\pi}^{2}} + \frac{N_{s}}{(1-\hat{\pi})^{2}} + N_{c} \left[\frac{T_{1}T_{2} - T_{3}}{T_{4}}\right]^{2}$$
(2.4)

From equation (2.2),

$$\hat{\tau}_{k} = \frac{\hat{\pi}T_{1}T_{2}}{\hat{\pi}T_{1}T_{2} + (1-\hat{\pi})T_{3}}$$

and equation (2.4) can be re-expressed as

$$\frac{N_{A}+N_{V}}{\hat{\pi}^{2}} + \frac{N_{S}}{(1-\hat{\pi})^{2}} + \sum_{\substack{\text{CENS}\\k}} \left[\frac{\hat{\tau}_{k}-\hat{\pi}}{\hat{\pi}(1-\hat{\pi})}\right]^{2}$$

The last term of this expression indicates the extra information that is gained by taking children of indeterminate infection status into account. This depends on

the extent to which the "posterior" estimate that the child is infected $(\hat{\tau}_k)$ differs from the "prior" probability of infection $(\hat{\pi})$. For censored children who receive no virus tests and who are last observed before the earliest age at AIDS diagnosis and earliest age at antibody loss, $\hat{\tau}_k = \hat{\pi}$. Thus, it is immaterial whether or not these children are retained in the analysis.

2.2.5 Covariates

There may be interest in assessing the effects of covariates on the model parameters, particularly the vertical transmission risk and the AIDS incubation period. For a discrete covariate it is straightforward to introduce categoryspecific parameters in the relevant steps of the algorithm. For the transmission risk or sensitivities of the virus tests, the significance of the covariate can be assessed by a likelihood ratio test.

A natural way to assess significance with respect to the AIDS incubation period is by the Σ (E-O)²/E variant of the log-rank test (Peto and Peto 1972), using the <u>estimated</u> numbers at risk of AIDS (under the null model). This procedure will be somewhat under-conservative as it does not take into account uncertainty in the estimated numbers at risk. For right-censored data, the logrank test can be derived as a score test under the proportional hazards model (Breslow 1975). However, in this application it is not possible to regard the underlying hazard as a nuisance distribution which can be conditioned out because of the contribution to the likelihood of the censored children.

Finkelstein (1986) developed a proportional hazards model for interval censored data and this could be considered for the antibody loss distribution. It would not be straightforward to modify the EM algorithm to accommodate this model and direct numerical maximisation of the likelihood function might need to be considered. Also, an essential feature of this model, unlike the analogous

model for right censored data, is the need to estimate the baseline distribution. This could affect the stability of the estimates of the effects of the covariates, particularly if age was recorded on a fine scale.

2.3 Example

2.3.1 Description of study

Since 1985, paediatricians in 10 centres participating in the European Collaborative Study have prospectively reported data on children born to mothers known to be HIV-infected at the time of delivery (European Collaborative Study, 1994). In principle, children are examined clinically and tested for HIV antibody every 3 months until 2 years of age and every 6 months thereafter. Virus tests are encouraged whenever an adequate sample is available, provided laboratory expertise and facilities exist. This analysis utilises the results of PCR and virus culture assays performed after the first month of life; the age restriction is applied to allow for the rapid rise in sensitivity after birth (McIntosh et al. 1994, Dunn et al. 1995). The study has consistently used the 1987 Centres for Disease Control pediatric AIDS definition (Centres for Disease Control 1987b) with two exceptions: it excludes children with asymptomatic lymphoid interstitial pneumonitis diagnosed by X-ray and includes children considered to have died as a consequence of HIV infection without fulfilling the precise AIDS case definition.

A total of 1055 children had been recruited by the date of analysis. To avoid problems of non-independence, 78 second-born twins or children with an older sibling in the study were excluded, leaving 977 subjects for analysis. Alternatively, these data could have been retained by including a random maternal effect in the model, but this would have complicated the analysis considerably. 41 children had developed AIDS (range 2-74 months). 639 children had become seronegative. The earliest negative antibody test was

observed at 2 months, and the latest positive test at 21 months. Ignoring tests performed on the seronegative children, there was a total of 591 virus cultures and 229 PCR assays. One or more positive virus test results were observed for 68 children who had not developed AIDS. The remaining 229 children were classified in the CENS group. A full listing of the data is given in Appendix 2.3.

The new method requires that each child is classified into one of the groups in Table 2.1, but in practice inconsistencies arise, even after eliminating obvious laboratory errors. In these data 5 apparently infected children were transiently HIV antibody negative, 2 children lost HIV antibody after developing AIDS, and 13 children had one or more positive virus tests despite becoming consistently antibody negative. Similar inconsistencies have been observed in other studies and explanations put forward (Simpson and Andiman 1994, Bryson 1995).

To resolve these inconsistencies the following hierarchical rule was applied:

- (1) if a child develops AIDS then classify in the AIDS group
- (2) otherwise, if the <u>last</u> antibody test is negative then classify in the SERO group
- (3) otherwise, if any virus test is positive then classify in the VIR group
- (4) otherwise, classify in the CENS group.

Giving precedence to AIDS diagnoses over laboratory findings is consistent with the standard classification of HIV infection (Centres for Disease Control 1987a) and serological tests are at present judged more reliable than virological tests. This hierarchy could be re-ordered without changing the general form of the likelihood. However, in studies in which the number of inconsistencies is small, such as the European Collaborative Study, results will not be sensitive to the precise choice of hierarchy.

2.3.2 Application of new method

The EM algorithm yielded maximum likelihood (ML) estimates of 0.153 (SE 0.012) for the vertical transmission rate, 0.768 (SE 0.022) for sensitivity of viral culture, and 0.920 (SE 0.029) for sensitivity of PCR. Figure 2.1 shows the estimated survivor functions for antibody loss and AIDS up to age 24 months. Figure 2.2 extends the AIDS survivor function up to age 48 months and indicates the precision of pointwise estimates. Strictly speaking, these distributions should be represented as step functions, but for clarity the age-specific estimates have been connected with straight lines. An estimated 0.150 (90% CI 0.097-0.199) of infected children are diagnosed with AIDS by age 6 months with a fairly constant hazard thereafter (approximately 0.08 per year). The median duration of antibody persistence among uninfected children is 11 months, with all children losing antibody by 22 months of age.

Figure 2.1 also shows the conditional probability of infection for a child who is antibody positive, has not developed AIDS, and for whom no virus test results are available. This function remains relatively flat until 10-11 months of age before rising rapidly. The data rule as infected all children who test antibody positive after 22 months. From equation (2.2) the conditional odds of infection is

$$\frac{\hat{\pi} \; \hat{G}_{a_{k}+1}}{(1-\hat{\pi}) \; \hat{H}_{I_{k}+1}} \prod_{\nu=1}^{\nu} (1-\hat{S}_{\nu})^{y_{k\nu}}$$

Thus the effect of each negative virus test result is to scale the odds of infection by (1-sensitivity); that is, by 0.232 for a negative virus culture result and by 0.080 for a negative PCR result.

2.3.3 Comparison with standard estimator

In drawing comparisons with the standard estimator described in Section 1.3, different critical ages at which antibody persistence is regarded as proof of infection were examined (Table 2.2). The usual convention of excluding children born less than 15, 18, 21, or 24 months before the date of analysis was applied, although their inclusion made little difference to the results (Dunn and Ades, 1996).

Considering first an analysis based solely on AIDS diagnoses and antibody test results (i.e. excluding virus test results), the crude estimates of the vertical transmission rate are lower than the ML estimate of 0.150, the difference being more pronounced the older the critical age. The reason for this is that the crude estimator ignores children of indeterminate infection status, who as they become older, are more and more likely to be infected (Figure 2.1). However, this can be compensated for by choosing a critical age that is too early, thereby including as infected a number of children who will eventually lose antibody. It would be difficult to justify, however, the use of a 15 months or 18 months threshold, as maternal antibody was detected after these ages.

Conversely, the crude estimates of the AIDS survivor function are too low (progression rate too high) because the number of children at risk of the event have been underestimated. The degree of bias can be substantial. For example, the standard estimate of the AIDS survivor function using an 18 months threshold is approximately 8% lower (relatively) than the maximum likelihood estimate (Figure 2.3).

As the standard method of analysis takes account of positive virus test results but ignores negative results, the inclusion of virus test data must increase estimates of the transmission rate and decrease estimates of the rate of

progression to AIDS. However, the estimates of the transmission rate are now too high and the rate of progression to AIDS is still overestimated, although the bias in the latter is now less severe.

The small differences between the crude and maximum likelihood estimators of the vertical transmission rate is due to the fact that the infection status of a large majority of the enrolled children has been established, the study having begun over 10 years ago. The gain in efficiency of the new method would be greater in studies of shorter duration, including randomised controlled trials of interventions to reduce vertical transmission.

2.3.4 Effect of calendar period

The analyses in Section 2.3.2 assumed that all parameters remained constant over the duration of the study. Using the methods described in Section 2.2.5, the 415 children born between 1985-88 were compared with the 562 children born between 1989-94 with respect to the rate of vertical transmission and the AIDS incubation period (Table 2.3).

Under the assumption of a stationary incubation period, the estimated transmission rate was 0.122 for 1985-88 and 0.177 for 1989-94. On the basis of a likelihood ratio test, this difference is marginally significant (χ^2 =4.92, P=0.03). Figure 2.4 compares the AIDS incubation periods between the two cohorts, under the assumption of period-specific transmission rates. This appears to indicate that rapid progression to AIDS was more common in the earlier period but that the hazard rates at older ages are fairly similar. An overall comparison provides moderate evidence of a difference between the AIDS incubation periods (log-rank χ^2 =2.85, P=0.09). A more significant result is obtained by restricting the comparison to the first 6 months of life (log-rank χ^2 =7.14, P=0.008), although <u>a posteriori</u> analyses should be interpreted

cautiously. Allowing period-specific AIDS incubation periods made little difference to the estimated transmission rates.

2.4 Conclusions

2.4.1 Discussion of findings and comparison with other studies

Among the large prospective studies, the European Collaborative Study has been notable for its low rate of vertical transmission. The analysis provides evidence of a rise in the rate in recent years, which is now close to estimates from other cohorts in Europe and the United States, although still lower than figures reported from Africa (Working Group on Mother-to-Child Transmission of HIV, 1995). As the risk of vertical transmission depends on the degree of maternal immune suppression (European Collaborative Study 1996) and as there is evidence for a secular decline in average maternal CD4 count (Thorne et al. 1995), the increase in the transmission rate is not unexpected. However, Ades (1995) showed that the maternal CD4 count distribution will eventually stabilise if the incidence of new infections remains constant. It is noted that our results derive from data obtained on women with a <u>recognised</u> HIV infection from selected centres, and do not necessarily generalise to all infected women in Europe.

Even in the largest cohort studies, an insufficient number of children have been observed to progress to AIDS to allow reliable characterisation of the incubation period. Although comparatively precise estimates have been derived for the <u>shape</u> of the distribution using data from AIDS surveillance systems, these data have a number of limitations (DeGruttola 1992). Among these are periodic revisions in the pediatric AIDS case definition, and comparisons between studies must take account of differences in definitions. Of the 41 AIDS cases according to the criteria used in European Collaborative Study, 3 died before fulfilling the formal AIDS definition (Centres for Disease Control, 1987) and

presumably would not have been notified. Conversely, our analysis excluded children with asymptomatic lymphoid interstitial pneumonitis (LIP) which is AIDS-defining. LIP was responsible for 26% of initial pediatric AIDS-defining diagnoses in the United Kingdom up to April 1995 (Fiona Holland, personal communication). In a recent paediatric HIV classification system (Centers for Disease Control and Prevention 1994) LIP is no longer considered a "severe" (category C) clinical manifestation.

The finding of an inflexion point around 6 months of age in the AIDS hazard rate is consistent with other reports (Figure 1.1), although this effect was less striking among children born later in the study. The secular fall in early AIDS incidence is mirrored in mortality rates (data not shown), apparently ruling out a diagnostic artifact. A possible explanation for this observation is an improvement in the clinical management of young infants with proven or suspected HIV infection. Life expectancy, for adults with a CD4 count below 350 cells/mm³, improved between 1985-88 and 1989-93 in the Multicenter AIDS Cohort Study, and was attributed to the use of antiretroviral and prophylactic therapy (Enger et al. 1996).

Analyses of AIDS surveillance data have generally assumed that the incubation period is stationary. Our analysis points to a need for critical examination of this assumption, although with retrospective data it is only possible to compare incubation periods conditional on developing AIDS by a given age (DeGruttola et al. 1992).

2.4.2 Model assumptions

Censoring and loss to follow-up

Estimates obtained under the new model are unbiased only if censoring is independent in the following senses:

- among children of indeterminate infection status, the probability of loss to follow-up does not depend on true infection status
- (2) among infected children who have not yet developed AIDS, the probability of loss to follow-up does not depend on the age at which AIDS will eventually be diagnosed
- (3) among uninfected children who have not yet lost antibody, the probability of loss to follow-up does not depend on the age at which antibody loss will eventually occur

It is not possible to examine these assumptions empirically (Lagakos 1979). However, assumptions (1) and (2) would be violated if ill children were more likely to remain in contact with the study paediatrician. This would result in over-estimation of the vertical transmission rate and the rate of progression to AIDS, although the extent of this potential bias is difficult to quantify. It is noted that the other estimators reviewed in Sections 1.3 and 1.4 also require these or stronger assumptions.

Although the report of a study should always describe the number of losses to follow-up (Dabis et al. 1992), the categorisation used by the new method (Table 2.1) does not reveal this information. As well as including children who are lost to follow-up, the CENS group includes children known to be infected on the basis of antibody persistence and children of indeterminate infection status born shortly before the date of analysis. Thus, if the new method is used, loss to follow-up should be described separately, although in ongoing studies this may be difficult to define. If it is arbitrarily assumed that a child is lost to follow-up if

no information has been received for over two years, then this would encompass 132 of the 229 children in the CENS group, or 14% of the total cohort of 977 children. Of these 132 children, 69 were not observed following discharge from hospital after delivery.

Reporting delay is an important factor in the analysis of AIDS surveillance data (Wang 1992). In the European Collaborative Study, paediatricians batch completed questionnaires to the coordinating centre on a regular basis. Although there is a delay between a patient being examined and the receipt of information, no bias is introduced unless there is <u>selective</u> rapid communication of information, for example, following an AIDS diagnosis. It was not suspected that this occurred.

Virus tests

In the analysis of the ACTG-076 trial (Connor et al. 1994) it was implicitly assumed that every infected child makes a discrete transition from virus culture negative to consistently virus culture positive (Section 1.4). The method developed in this chapter assumes that each virus test has a fixed sensitivity which, after the neonatal period, does not depend on age at testing. Table 2.4 shows the estimated age-specific sensitivity of virus culture and PCR, based on an analysis of known infected children in the European Collaborative Study. Apart from a lower sensitivity in neonates and a possible decline in viral culture sensitivity in the second year of life, there is no clear dependence of sensitivity on age at testing.

The assumption that sensitivity is homogenous across children was examined by fitting beta-binomial models (Crowder 1978). This specifies a child-specific latent sensitivity which is a realisation from a beta distribution. It is convenient to parameterise the beta distribution in term of its mean θ , and variance $\phi\theta(1-\theta)$.

Conditional on the latent sensitivity, the observed number of positive test results is binomially distributed. ϕ =0 corresponds to the homogenous model. The results of this analysis are shown in Table 2.5. The likelihood ratio statistic is only approximately chi-squared distributed on 1 degree of freedom since the null value lies on the edge of the parameter space (Self and Liang 1987). Nonetheless, the hypothesis of homogeneity of sensitivity is clearly incorrect i.e. some infected children are more likely to test virus positive than others. The failure to account for heterogeneity between children in sensitivity attaches too much diagnostic significance to <u>multiple</u> negative test results, thus underestimating the transmission rate. A realistic model would also need to allow for the possibility of serial dependence between virus test results within individuals.

Antibody loss distribution

Unsatisfactory estimates of the antibody loss distribution were obtained in some sub-analyses where the number of seronegative children was small relative to the number of censored children. This is due to the instability of estimators derived from interval censored data (Bachetti 1990). Although this problem is unlikely when modelling extensive data, modifications to the method may need to be considered for smaller data sets, such as imposing a smoothness penalty (Bachetti 1990) or estimating the antibody loss distribution parametrically (Swan 1969). The latter approach was explored by maximising the log-likelihood (2.1) in which H, after a power transformation of age, was assumed to be normally distributed. Figure 2.5 shows the result of the best fitting model (age^{0.7}) along with the non-parametric maximum likelihood estimate. There is close agreement in the tails of the distributions, although the parametric estimate is a little too low between 6 and 12 months of age. The estimates of the transmission rate under the two models were identical to the third decimal place.

Table 2.1 Classification of children and information required by the proposedmethod

Group	HIV infected	Number of children	Required information	
Clinical AIDS (AIDS)	Yes	N _A	Age at AIDS diagnosis Number of positive virus tests Number of negative virus tests	a _k X _{kv} Y _{kv}
Negative antibody test (SERO)	No	Ns	Age at last positive antibody test Age at first negative antibody test	l _k r _k
Positive virus test but not AIDS (VIR)	Yes	N _v	Age at last clinical assessment Number of positive virus tests Number of negative virus tests	a _k x _{kv} (≥1) y _{kv}
None of the above (CENS)	No assumption	N _c	Age at last clinical assessment Age at last (positive) antibody test Number of negative virus tests	a _k I _k Y _{kv}

Note: Subscript k denotes the k^{th} child within a group and subscript v denotes the v^{th} type of virus test.

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Table 2.2 Comparison of crude estimates and maximum likelihood estimates under proposed model.

	Including virus test data		luding virus test data Excluding virus test data	
	π	Ĝ ₂₄	π	Ĝ ₂₄
MLE's	.153 (.012)	.742 (1.056)	.150 (.014)	.738 (1.058)
STANDARD ESTIM	ATES [*] old			
15 months	.169 (.014)	.738 (1.056)	.146 (.013)	.705 (1.064)
18 months	.165 (.013)	.732 (1.057)	.141 (.013)	.695 (1.067)
21 months	.158 (.013)	.721 (1.060)	.135 (.013)	.677 (1.072)
24 months	.158 (.013)	.719 (1.061)	.134 (.013)	.670 (1.074)

* Analysis restricted to children born at least 15, 18, 21, or 24 months before date of analysis

Values in parenthesis are SE($\hat{\pi}$) and exp[SE(log \hat{G}_{24})]. An approximate (1- α) confidence interval for G_{24} is derived by dividing and multiplying the point estimate by exp[SE(log \hat{G}_{24})] raised to the power $\Phi^{-1}(\alpha/2)$.

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Model	Number of non-zero parameters	Deviance	Test statistic	Estimated transmission rate
Time homogenous	39	-1610.74		$\hat{\pi} = 0.153$
Time-variant transmission rate	40	-1608.29	Likelihood ratio statistic = 4.92 (1 df)	$\hat{\pi}_{_{85-90}} = 0.122$ $\hat{\pi}_{_{91-94}} = 0.177$
Time-variant transmission rate and incubation period	45	-1591.91	Log-rank test = 2.85 (1 df)	$\hat{\pi}_{85.90} = 0.122$ $\hat{\pi}_{91.94} = 0.178$

Table 2.3 Comparison of time-homogenous and time-variant models

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Age in completed months	Virus culture	PCR
. 0	47 (72)	45 (38)
1-2	84 (79)	90 (63)
3-5	84 (76)	88 (32)
6-11	85 (93)	92 (24)
12-23	70 (80)	88 (14)

Table 2.4 Sensitivity of virus culture and PCR according to age

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Table 2.5 Results of beta-binomial models applied to virus test data

Test	Parameter estimates	Likelihood ratio statistic
Virus culture	θ=0.793, φ=0.226	22.93
PCR	θ=0.922, φ=5.779	23.86

* Test that children are homogeneous with respect to test sensitivity. Under null hypothesis, distribution of test statistic is less extreme than chi-squared distributed on 1 degree of freedom (see text).

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Figure 2.1 Estimates of the survivor functions for AIDS (•), antibody loss (■), and of the conditional probability of infection for a child aged t months who is antibody positive, has not developed AIDS, and has never received a virus test (▲).



Figure 2.2 Estimate of the survivor function (logarithmic scale) for AIDS with 5% and 95% confidence limits (thin lines). Numbers at top of figure represent estimated number of children at risk of AIDS at 0, 6, 12, ... months.



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Figure 2.3 Maximum likelihood estimator (•) and standard estimator (■) of the survivor function (logarithmic scale) for AIDS.



Figure 2.4 Estimates of the survivor function (logarithmic scale) for AIDS among 415 children born between 1985-88 (•) and 562 children born between 1989-94 (■). Numbers at top of figure represent estimated number of children at risk of AIDS at 0, 6, 12, ... months; top line refers to 1985-88 and bottom line to 1989-94.



Figure 2.5 Non-parametric estimator (solid line) and parametric estimator (dotted line) of survivor function for antibody loss.



CHAPTER 3. THE DETECTION OF HIV DNA IN VERTICALLY-INFECTED INFANTS

3.1 Epidemiological background

As discussed in Section 1.2, the standard test for HIV infection, enzyme immunoassay for specific IgG antibody, is not useful for the early diagnosis of infants born to HIV-infected women. Early diagnostic tests which have been studied include viral culture, p24 antigen detection, in vitro antibody production, and DNA and RNA polymerase chain reaction (Consensus Workshop 1992). DNA PCR has emerged as the most useful of these tests and is increasingly used in clinical and epidemiological settings.

PCR is capable of detecting extremely low levels of viral genome and achieves very high sensitivity in adults with established infection (Owens et al. 1996a). However, negative PCR test results are often observed in neonates subsequently shown to be HIV-infected. This is presumably due to the absence of HIV genome in peripheral blood in early life rather than to a failure of the assay.

Birth cohort studies with serial PCR examinations are the only reliable way of assessing when HIV DNA first reaches detectable levels in vertically-infected infants. Follow-up should continue until HIV-infection status has been established using criteria independent of PCR. Although relevant data have been reported from a number of studies (Owens et al. 1996b), sample sizes have generally been small because of the limited number of children available for study within centres, the difficulty in obtaining blood samples at frequent intervals, and the fact that only 15-25% of children will eventually be found to be infected. In an early review of available data, the accuracy of PCR and other methods of early diagnosis were assessed (Consensus Workshop 1992). However, no formal statistical analysis was performed and many more studies have subsequently been published. A "meta-analysis" of prospective studies which had assessed the role of PCR as an early diagnostic test for HIV

infection was therefore undertaken.

The main objective of the analysis was to derive age-specific estimates of the proportion of HIV-infected children who had ever expressed detectable levels of HIV DNA as assessed by PCR. Also, it is generally considered that isolation of HIV or detection of HIV genome shortly after birth implies intrauterine transmission, and that failure to detect virus or genome implies intrapartum transmission (Bryson et al. 1992). If this hypothesis is correct then the rate of PCR positivity in samples taken shortly after delivery should reflect the relative contributions of intra-uterine and intra-partum transmission. Finally, if it could be shown that transition from PCR positive to PCR negative was rare then the analysis would yield approximate estimates of the "clinical sensitivity" of PCR. This information is essential when interpreting the diagnostic significance of an negative PCR test result.

3.2 Data collection

Studies were identified through a literature review, including an electronic search on EMBASE. The possibility of synthesising published data was first considered. However, serial data were presented in only a few studies and age was often recorded only to the nearest week or nearest month. More commonly, the data consisted only of the total number of tests and number of positive tests within arbitrarily-defined age groups, which often differed between studies. These limitations precluded an efficient statistical analysis, and it was therefore decided to request non-aggregated data from study investigators.

A letter was sent to the principle investigators of the 14 studies identified which had included 10 or more infected children (Appendix 3.1). Smaller studies were excluded as it was considered that the extra administrative effort would yield minimal additional information. The letter requested information on children tested before age 3 months as it was known that PCR could achieve a very high sensitivity after this age. As well as PCR test results, information was requested on breastfeeding, mode of delivery, and timing of diagnosis of

maternal infection. Breastfed children are at risk of postnatal infection (Dunn et al. 1992) which complicates the issue of early diagnosis and it was intended to exclude them from the main analysis. If caesarean section delivery reduces the risk of intrapartum transmission then a greater proportion of infected children in this group would have been infected in utero, and one would expect different patterns of DNA positivity by mode of delivery. The reason for asking about the timing of diagnosis of maternal infection is evidence of an association between early identification of virus and a rapid clinical course (De Rossi et al. 1993). Bias might, therefore, be introduced by including children whose infection was detected because they were symptomatic.

A response was received from 11 of the 14 investigators who were approached, of whom 8 sent additional unpublished data. In total, PCR test results were reported for 271 HIV-infected children. No child had a history of breastfeeding and all mothers were known to have been infected at the time of delivery i.e. all children were identified prospectively. The number of children per centre ranged between 9 and 53. Details of the laboratory procedures used are given in Dunn et al. (1995).

3.3 Statistical Methods

The age at which a child first has detectable levels of HIV DNA is not directly observed but falls between the last negative PCR test and first positive PCR test (interval censoring). For children whose first PCR test is positive, DNA could have first been detectable at any point between conception and the first test date (left censoring). A positive PCR result was observed on every child and these data do not therefore display right censoring. A listing of individual PCR test results is given in Appendix 3.2. No investigator reported a child who had a negative PCR test result after a positive PCR test result.

3.3.1 Non-parametric model

Non-parametric analyses were performed in the first instance because of uncertainty about the relationship between DNA positivity and age. Peto (1973)

developed a non-parametric model for interval censored data which is the analogue of the Kaplan-Meier estimator for right-censored data. To illustrate the method, the figure below shows hypothetical data observed on four subjects. The left and right endpoints of each individual are projected onto the time axis, creating seven time intervals. A probability density is associated with each of the seven intervals - thus the parameterisation is induced by the data. The contribution to the likelihood of each individual is the sum of the probability densities associated with the intervals spanned by that individual.



Developing algebraic notation, define H(t) as the proportion of children who have <u>not</u> expressed DNA by age t i.e. the survivor function. On subject i $(1 \le i \le N)$ we observe (I_i, r_i) , where I_i is the age at last negative test and r_i the age at first positive test. If subject i is left censored then I_i is set to an arbitrary negative number which represents the point of conception. Assume that the data induce M intervals $\zeta_1, ..., \zeta_M$. Let the indicator variable I_{ik} equal 1 if $\zeta_k \subseteq (I_i, r_i)$ and equal 0 otherwise. ζ_k is associated with probability density h_k although no assumption is made regarding the behaviour of H within the interval. Thus the probability of first becoming PCR positive in the interval (I_i, r_i) is $\Sigma h_k I_{ik}$.

The likelihood function is

$$L(h) = \prod_{i=1}^{N} \left(\sum_{k=1}^{M} h_{k} I_{ik} \right)$$
(3.1)

subject to

$$h_k \ge 0$$
, $\sum_{k=1}^{M} h_k = 1$ (3.2)

Peto (1973) outlined a constrained Newton-Raphson method to maximise the likelihood (3.1) subject to the non-negativity bounds and the linear constraint (3.2). Turnbull (1976) described an elegant EM algorithm which avoids the need to calculate first and second derivatives, but additional code is required to obtain estimates of standard error (Louis 1982, Meng and Rubin 1991). For this analysis, NAG subroutine E04UCF, which uses a sequential quadratic programming algorithm, was used.

Although the form of the likelihood is simple it is not a trivial maximisation problem. Firstly, the likelihood is heavily parameterised if event times are measured on a nearly continuous scale. Secondly, at the maximum likelihood estimate many components of **h** are identically zero i.e. the solution lies on the boundary of the parameter space. Indeed, it was only recently demonstrated that the maximum likelihood estimator of this model is consistent (Gentleman and Geyer 1994).

Peto (1973) and Turnbull (1976) both suggested the following approach to obtaining pointwise confidence limits for the survivor function. Suppose θ =H(t) is the parameter of interest where t is the right endpoint of ζ_{τ} ,

$$\theta = 1 - \sum_{k=1}^{T} h_k$$

Then

$$var(\hat{\theta}) = \sum_{k=1}^{T} var(\hat{h}_{k}) + \sum_{j=1}^{T} \sum_{k\neq j}^{T} cov(\hat{h}_{j}, \hat{h}_{k})$$
 (3.3)

and an approximate 100(1- α)% confidence interval for θ is given by $\hat{\theta} \pm z_{\alpha/2}SE(\hat{\theta})$.

The variances and covariance terms in equation (3.3) are obtained from the

inverse of the observed information matrix,

$$- \frac{\partial^2 \log L}{\partial h_j \partial h_k} = \sum_{i=1}^{N} I_{ij} I_{ik} \left(\sum_{u} \hat{h}_{u} I_{iu} \right)^{-2}$$

Peto states (without proof) that the variance-covariance contribution of only the non-zero elements of $\hat{\mathbf{h}}$ need be included in equation (3.3).

Confidence intervals based on the likelihood ratio test have desirable features not shared by confidence intervals based on the information matrix (Cox and Snell 1989, Section A1.5). The likelihood ratio test statistic for $H_o:\theta=\theta_o$ is

$$w(\theta_{o}) = 2 \times \{ \log L(\hat{h}) - \max[\log L(\tilde{h})] \}$$
(3.4)

where
$$1 - \sum_{k=1}^{T} \tilde{h}_{k} = \theta_{o}$$
 (3.5)

Subroutine E04UCF was used to maximise $\log L(\tilde{h})$ by imposing a second linear constraint (3.5) and thus to derive the likelihood ratio statistic (3.4). Approximate 100(1- α)% confidence limits for θ are given by the roots of the equation

$$w(\theta) - \chi^2_{1,\alpha} = 0$$
 (3.6)

where $\chi^2_{1,\alpha}$ is the upper α point of the chi-squared distribution on one degree of freedom. Rather than specifying a value for θ and solving by trial and error, an algorithm was written in which the E04UCF subroutine was nested inside a second NAG subroutine that locates a simple root of a continuous function on a given interval (C05AZF). Note that the derivation of confidence bands for the entire survivor function requires that equation (3.6) be solved for each of the M intervals. This approach is computationally highly intensive.

3.3.2 Parametric models

Even with extensive data, the non-parametric model described in the previous section concentrates probability density in a few intervals and results in a highly discontinuous estimated survivor function (Harris et al. 1950). One possibility is

to retain this basic formulation but to impose a smoothness penalty on the probability densities in neighbouring intervals. However, the form that this penalty should take is unclear. A parametric model, provided it was sufficiently flexible to describe the true survivor function, would have the advantages of producing smooth estimates of the rate of DNA positivity and yielding narrower confidence intervals. Mixture models were fitted assuming two sub-populations:

- (i) children who are infected in utero (who test PCR positive at delivery)
- (ii) children infected intra-partum (in whom the time to becoming PCR positive follows a specific parametric distribution).

Let π be the proportion of children infected in utero and $(1-\pi)$ the proportion infected intra-partum. Let $F(t;\mu)$ represent, for children infected intra-partum, the probability of becoming PCR positive before age t, where μ is a vector of unknown parameters.

The contribution to the likelihood for a child who is PCR positive when first tested at age I_i is

$$\pi$$
 + $(1 - \pi)F(I_i)$

The contribution to the likelihood for a child who is PCR negative when first tested and is observed to become PCR positive between ages I_i and r_i is

 $(1 - \pi)[F(r_i) - F(l_i)]$

The following parametric forms for F(t) were examined:

Distribution	F(t)
Exponential	1-exp(-λt)
Weibull	1-exp[-(λt) ^κ]
Pareto	$(\kappa/\lambda)^{\kappa} / (t+\kappa/\lambda)^{\kappa}$
Log-logistic	1-1/[1+(λt) ^κ]

The Pareto distribution arises by considering each individual's survival time to be exponentially distributed where the parameter is generated from a gamma density of mean λ and index κ (Cox and Oakes 1984, Section 2.3). An advantage of the log-logistic distribution not shared by the other distributions is that the hazard function can be non-monotonic. The exponential distribution is a special case of both the Weibull distribution (κ =1) and the Pareto distribution (κ →∞).

3.3.3 Locally-weighted regression

In Sections 3.3.1 and 3.3.2 survival models were used to estimate the probability of ever having had detectable levels of DNA. This can be interpreted as the "sensitivity" of PCR only if it is assumed that a child always tests PCR positive after initially testing PCR positive. This assumption is not realistic, although no investigator in this study reported a child who tested PCR negative after testing PCR positive. Estimates of PCR sensitivity that do not rest on this assumption can be obtained from "cross-sectional" analyses. To avoid the bias that would be introduced if the number of tests performed depended on the results of the tests, this analysis was based on the result of each child's <u>first</u> PCR test.

At each exact age there were too few children to reliably estimate sensitivity. Hastie and Tibsharani (1990, Section 2) describe, in the context of continuous variables, several ways to estimate the dependence of the mean of one variable on a second variable without assuming a functional relationship. One of these methods is locally-weighted regression:

- (1) select a value of the independent variable, say x_{o}
- (2) assign weights to each observation that decrease in a smooth fashion as one moves away from the target point, e.g. φ(|x_i-x_o|/λ) where φ() is the Gaussian density. λ is known as the bandwidth and controls the degree of smoothing.
- (3) perform a linear regression analysis using the weights derived in step 2 and obtain the predicted value at x_a

(4) repeat steps 1-3 across the range of the independent variable.

This approach was adapted to the present binary data problem by performing a logistic regression analysis in step (3) rather than normal regression. A range of values for the bandwidth was examined. Values that are too small give rise to curves with implausibly many turning points; values that are too large result in over-smoothing.

3.4 Results

3.4.1 Age-specific estimates of percentage of children ever DNA positive Table 3.1 and Figure 3.1 show estimates of the cumulative percentage of children with detectable levels of DNA under the non-parametric model. The figure was 38% for children tested on the day of birth. There was little change in the first week of life, but in the second week of life the percentage ever DNA positive rose rapidly, reaching 93% by age 14 days. The large discontinuities in Figure 3.1 are a characteristic of this method of analysis and are not necessarily biologically significant. In particular, the estimates at older ages are dictated by the data on the children with late negative test results. The cumulative percentage reaches 100% at 183 days since this was the oldest age when a negative PCR test was observed.

The flatness of the function in Figure 3.1 in the first week of life and the rapid rise in the second week is an important finding that lends support to the view that early PCR tests can discriminate between children infected intra-uterine and intra-partum. It also suggests that the interval between infection and the emergence of HIV DNA in peripheral blood is approximately 1-2 weeks.

Figure 3.1 gives no indication of the statistical uncertainty in <u>changes</u> in the level of DNA positivity. An additional analysis was undertaken to estimate the rate of DNA positivity among children putatively infected intra-partum. This was achieved by a re-parameterisation in terms of conditional probabilities,

Pr(ever DNA positive by X days | DNA negative at age 1 day).

The resulting confidence intervals (Figure 3.2) are wide, but indicate that the proportion of children infected intra-partum who express HIV DNA by age 9 days could be as low as 4% and by age 7 days is probably less than 34%

Late negative PCR results were uncommon, and only 7 children tested negative after age 29 days (Appendix 3.2). These outlying observations heavily influence the results of the parametric analyses. Figure 3.3 compares the log-logistic models in which these seven observations are included and excluded. Their inclusion caused the curve to rise too slowly at young ages and the deviance increased markedly (from 263.9 to 362.2). Although the mothers of all 7 children denied breastfeeding this could not be verified and it was suspected that the children could have been infected post-natally. These observations were therefore excluded from subsequent parametric analyses.

Table 3.2 and Figure 3.4 shows the results of fitting different parametric models. The exponential did not fit satisfactorily and the Pareto distribution converged to the exponential. The curves and deviances under the Weibull and log-logistic models were similar, although the latter best captured the lack of increase in DNA positivity in the first few days of life. The estimated mixing parameter from the log-logistic model (40.4%) is close to the estimate of DNA positivity at day 1 from the non-parametric model (39.0%) but the 90% confidence interval is narrower (33.3-44.6% as opposed to 29.0-46.1%).

The deviance under the non-parametric model was 14.08 units less than under the log-logistic model. Turnbull and Weiss (1978) examined the asymptotic properties of a likelihood ratio statistic for testing a parametric model with data subject to right or interval censoring. However, they considered the case when observations are grouped into discrete intervals. In the present problem it is not clear if the number of parameters associated with the non-parametric model should be the total number of intervals (81), the number of intervals with nonzero probability (12), or some intermediate value.

Figure 3.5 shows the results of cross-sectional analyses based on each child's first test result, using different degrees of smoothing. These are consistent with the results of the survival model analysis, except that the rise in DNA positivity between days 8 and 14 is less sharp. This difference is less marked when the bandwidth is small (less smoothing). The erratic behaviour of the smoothers after age 14 days is due to the relatively small number of children tested.

3.4.2 Effect of mode of delivery

Few investigators provided information on child's mode of delivery. 73 children were known to have been delivered vaginally, 19 by caesarean section, and for 179 children mode of delivery was unknown. Figure 3.6 shows the rate of DNA positivity estimated from the 19 children known to have been delivered by caesarean section. This is an estimated 0% at day 1, which is a consequence of all three children who were tested on this day being PCR negative. As would be anticipated with a small sample size, the estimates are subject to considerable statistical uncertainty. The 90% profile confidence interval on day 1 is 0.0-34.8% and on day 2 is 6.4-78.3%. Bearing this uncertainty in mind, the results of the analysis based on caesarean section deliveries is not strikingly different from the results of the overall analysis.

3.4.3 Comparison of methods for deriving confidence intervais

Table 3.3 compares the 90% confidence intervals obtained by the two methods described in Section 3.3.1. With Peto's method, but not with the likelihood based approach, the confidence limits change only in those age intervals with non-zero estimated probability. The confidence intervals are similar at day 1, but at other ages they are markedly different. For example, the lower 95% confidence limits at day 9 are 39.6% and 50.6% by profile likelihood and Peto's method, respectively. A disadvantage of Peto's method is that values do not necessarily lie between 0% and 100%, as occurs with the 95% upper confidence limit after day 12.

The discrepancy between these two approaches points to the need for further

research, particularly to assess the accuracy of their coverage probabilities. One approach would be to simulate data from an assumed survival distribution under a variety of censoring patterns. Such studies have been conducted in the context of exact and right censored data, and revealed the superiority of profile likelihood confidence intervals (Thomas and Grunkemeier 1975).

3.5 Discussion

3.5.1 Timing of transmission

It has been proposed that isolation of HIV-1 or detection of HIV-1 genome shortly after birth implies intra-uterine transmission and conversely, that failure to detect viral markers implies intra-partum transmission (Bryson et al. 1992). On this basis, the timing of HIV-1 transmission has been explored in a number of small studies but before this analysis reliable quantitative inference had not been possible. The estimated rate of DNA positivity on the first day of life (nonparametric analysis) indicates that 38% (90% CI 29-46%) of vertically-acquired infection is attributable to intra-uterine transmission. A similar figure (40%, 95% CI 33-45%) was obtained from the parametric analysis assuming a log-logistic distribution, the most satisfactory of the parametric models.

Using the detection of viral markers to infer timing of transmission is, however, potentially misleading. Firstly, a positive PCR test shortly after birth could theoretically result from intra-partum transmission of a large inoculum of virus. Secondly, a negative PCR test does not exclude intra-uterine transmission as virus could be sequestered by non-circulating target cells. Finally, without invasive fetal sampling, intra-partum transmission cannot be distinguished from intra-uterine transmission in late pregnancy. However, the most plausible explanation for the lack of increase in DNA positivity in the first week of life is the existence of two sub-populations.

The analysis suggests that approximately two-thirds of vertically-acquired infection is attributable to intra-partum transmission. However, this would appear to conflict with other evidence that caesarean section delivery confers

little or no protection against infection. In a meta-analysis of observational studies, the odds of transmission was estimated to be only 20% lower (95% Cl 0-37%) in children delivered by caesarean section (Dunn et al. 1994). One possible explanation is that the short but possibly intense exposure to maternal blood during the operation is no less hazardous than the relatively prolonged exposure to maternal cervical secretions during vaginal delivery. The pattern of DNA positivity observed in the sub-analysis of caesarean section deliveries is consistent with transmission around the time of delivery, although the numbers in this analysis were small.

The study showed that viral genome in peripheral blood is first detectable in the second week of life for most children putatively infected at delivery. Whether the same kinetics occur during primary infection in adults is unknown since it is rarely possible to pinpoint the date of infection. In the few cases where the date of infection has been ascertained the earliest virological tests have been performed 4-6 weeks after infection, by which time high levels of viral expression are frequently found (Tindall and Cooper 1991). The results from this study are similar to results from experiments on SIV infection in macaques. By analysing serial blood specimens it has been shown that viral genome and antigens can first be detected 1-2 weeks after inoculation with SIV (Fazely et al. 1991, Lundgren et al. 1991). There are, however, a number of differences between this animal model and vertically-transmitted infection in humans, including the dose and route of inoculum, and the presence of passively acquired HIV antibody.

Bryson et al. (1992) proposed a classification system for in utero versus intrapartum transmission to "help clarify the number of infants infected either in utero or during delivery". The classification, summarised in the following table, appears to be based on the assumption that children infected intrapartum do not express viral markers for at least 48 hours after birth.

Result by PCR or culture or p24 antigen		Transmission
<48 hours	48 hours - 6 days	
positive	no test	in utero
negative	no test	intrapartum
no test	negative	intrapartum
negative	positive	undefined
no test	positive	undefined

There would appear to be no advantage in using this or a related system over the assumption-free analysis used in the present study. In particular, it is not clear how unbiased estimates of the relative proportions infected in utero and intrapartum would be derived. To elucidate the timing of vertical transmission samples should be obtained in the first week of life and certainly no later than age 14 days.

3.5.2 Early diagnosis of HIV infection

PCR achieves very high specificity provided contamination is carefully avoided and quality control procedures are maintained (Brandt et al. 1992). A positive test result implies, therefore, that it is highly likely that the child is HIV-infected. The diagnostic interpretation of a negative PCR test result is more difficult because of uncertainty about the sensitivity of PCR. Although the percentage of children who have ever had detectable levels of DNA is not the same as "clinical sensitivity" the former appears to only slightly over-estimate the latter.

The validity of combining data from studies using different PCR methodologies and the generalisability of this analysis has been questioned (Kuhn et al. 1996). Before combining the data across studies, site-specific analyses were performed but did not reveal any clear differences according to any aspect of laboratory methodology. Some differences, which the study was not powerful enough to detect, probably do exist. However, to adopt the philosophical stance that aggregate analyses should be conducted only on perfectly homogeneous studies is to effectively never undertake an aggregate analysis.

The main new finding is that high levels of sensitivity can be achieved at earlier
ages than previously thought. A consensus meeting in 1992 judged that sensitivity was approximately 50% at 1 month of age and 90% at 3 months of age (Consensus Workshop 1992), but in this analysis these levels were surpassed by 9 and 13 days of age. This suggests that an early negative test result is strongly indicative that infection is absent. Negative predictive value was calculated assuming a specificity of 98% (Owen et al. 1996b) and a range of transmission rates (Table 3.4). This indicates that false-negative test results after the second week of life should be very rare.

3.5.3 Related studies

Kuhn et al. (1996) replicated the present analysis using data on 120 infected children enrolled in the New York City Perinatal HIV Transmission Collaborative Study. "Sensitivity" was estimated to be 22% at birth, and rose rapidly after age 4 days reaching 90% by 21 days. Confidence intervals were not reported in this analysis. A similar analysis was conducted by Kalish et al. (1997) on 140 children in the Women and Infants Transmission Study except that virus culture was used in place of PCR. The authors emphasised that they were estimating "the probability of observing at least one positive culture up to that age if cultures were performed frequently" rather than sensitivity. The estimated probabilities of a first positive culture by days 0, 7, and 16 were 27%, 45%, and 89%, respectively. The results of our study and these other two studies are broadly consistent, although the rapid rise in sensitivity began at an earlier age in the American datasets.

In a study in Rwanda, the same statistical model was used to analyse PCR data on 47 HIV-infected children diagnosed on the basis on antibody persistence (Simonon et al. 1994). All children in this cohort were breastfed. DNA was detected in an estimated 30% (95% CI 15-51) of children tested at birth, although this was based on a cord blood sample which carries a risk of maternal contamination. The rate of DNA positivity at age 3 months was an estimated 81% (95% CI 54-94). This is lower than the rate found in our analysis and is presumably due to postnatal transmission through breastfeeding.

This study provided no information on the pattern of change between birth and age 3 months because children were not tested in this period.

A Markov model was used by Rouzioux et al. (1996) in an analysis of 95 infected children in the French Prospective Study on Pediatric HIV Infection. This analysis was based on any viral marker (culture, PCR, or p24 antigenaemia) and not PCR results alone. The model assumed (1) a discrete proportion of children are infected on the day of delivery (2) for the remaining children infected before delivery, the interval between infection and delivery follows an exponential distribution (3) the interval between infection and detectable viral markers also follows an exponential distribution. Assumption (2) is difficult to justify; if there is a constant force of infection during pregnancy it would be natural to work forwards from the time of conception rather than backwards from the time of delivery. Also, the exponential model in Figure 3.4 indicates that assumption (3) is not tenable. Rouzioux et al. concluded that 35% of children are infected in utero, of whom most are infected shortly before delivery (median interval of 14 days). However, this is an artefact of the model and there is no empirical evidence to support that most in utero transmission is in late gestation.

Recently, Owens et al. (1996b) published a meta-analysis of published studies which had assessed the accuracy of PCR for the diagnosis of HIV infection in children. From 1735 abstracts that were reviewed, 32 studies met their inclusion criteria. Each study was plotted on a graph of sensitivity versus (1-specificity) from which a summary receiver operating characteristic (ROC) curve was derived. This method of analysis is appropriate for assays in which each sample is classified as negative or positive on the basis of some continuous measurement with a cutoff under the control of the investigator. This is not obviously applicable to PCR, and there was no evidence that studies with low sensitivity had high specificity, and vice versa. The analysis of the effect of age was also inadequate, being limited to a comparison of ages <30 days with ages 30 days-13 years.

AGE	MLE	CL_5	CL_95	AGE	MLE	CL_5	CL_95
1	37.98	28.99	46.12	54	96.86	94.60	98.42
2	43.34	35.22	50.70	55	96.86	94.60	98.42
3	43.34	35.22	50.70	56	96.86	94.60	98.42
4	43.34	35.22	50.90	57	96.86	94.60	98.42
5	44.43	36.94	56.41	59	96.86	94.60	98.42
6	44.43	36.94	56.44	60	96.86	94.60	98.42
7	44.43	36.94	58.37	62	96.86	94.60	98.42
8	44.43	36.94	75.56	63	96.86	94.60	98.42
9	69.53	39.62	84.42	64	96.86	94.60	98.42
10	69.53	51.64	85.84	65	96.86	94.60	98.42
11	69.53	51.64	89.30	66	96.86	94.60	98.42
12	78.59	57.57	94.56	68	96.86	94.60	98.42
13	92.52	69.31	96.73	70	96.86	94.60	98.42
14	92.52	75.65	96.73	71	96.86	94.60	98.42
15	92.52	82.16	96.73	72^{-72}	96.86	94,60	98.42
16	93.69	85.67	97.88	75	96.86	94.60	98.42
17	93.69	85.81	97.88	77	96.86	94.61	98.97
18	93.69	86.09	97.88	80	96.86	94.63	98.97
19	93.69	86.09	97.88	82	96.86	94.60	98.97
20	93 69	86.09	97 88	83	96.86	94 62	98 97
21	93.69	86 09	97 88	84	96.86	94 62	98.97
23	96 09	87 02	98 28	85	96.86	94 62	98 97
24	96.09	87.78	98.28	86	96.86	94.62	98.97
25	96.09	88 18	98 28	87	96.86	94 62	98 97
27	96.09	88 92	98 28	88	96.86	94 62	98.97
29	96.09	89 16	98 28	90	96.86	94 62	98 97
30	96.86	92.89	98 42	91	96.86	94.62	98.97
31	96.86	93.12	98.42	94	96.86	94.62	98.97
32	96.86	93.62	98.42	95	96.86	94.62	98.97
33	96.86	93.83	98.42	96	97.98	96.01	99.18
34	96.86	94.06	98.42	98	97.98	96.01	99.18
35	96.86	94.22	98.42	106	98.54	96.03	99.51
36	96.86	94.61	98.42	113	98.54	96.05	99.51
37	96.86	94.60	98.42	114	98.54	96.25	99.51
38	96.86	94.60	98.42	131	98.54	96.80	99.51
39	96.86	94.61	98.42	138	98.54	96.80	99.51
40	96.86	94.60	98.42	145	98.54	96.80	99.51
42	96.86	94 60	98 42	183	98 54	96.80	99 51
43	96.86	94.60	98 42	190	100.00	97.60	
44	96.86	94 60	98 42	203	100.00	97 63	•
45	96 86	94 60	98 42	204	100 00	97 69	•
46	96 86	94 60	98 12	245	100 00	97 71	•
47	96.86	94 60	98 12	252	100.00	98 93	•
49	96.00	94 60	98 13	260	100 00	98 95	•
51	96.86	94 60	98.42	200	100.00	99 27	•
52	96.00	94.00	90.442 98 19	271 272	100.00	99.27	•
52	96.86	94 60	90.442	213	100.00	22.40	•
55	20.00	241.00	20.442	557	100.00	•	•

Table 3.1 Estimates of the cumulative percentage (and 90% profile likelihood confidence interval) of children with detectable HIV DNA

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Table 3.2	Results of	analyses	usina	parametric	models ¹
				p	

Model	Mixing parameter (90% confidence Interval)	Parameters describing F(t)	Deviance	Parame- ters
Exponential	0.210 (0.106-0.313)	λ =0.117	272.04	2
Weibull	0.368 (0.263-0.455)	λ=0.0815, κ=1.739	264.67	3
Pareto	Converged to e	exponential	272.04	3
Log-logistic	0.404 (0.333-0.446)	λ=0.1015, κ=3.6292	263.95	3
Non-parametric	0.390 ² (0.298-0.473)		249.87	?

1. 7 children who tested PCR negative after 29 days were excluded from all analyses.

2. Estimated sensitivity on day 1

i

Age (days)	Ever DNA positive (%)	90% Cl: profile likelihood	90% CI: Peto's method
1	38.0	29.0 - 46.1	28.4 - 47.6
2	43.3	35.2 - 50.7	33.9 - 52.8
3	43.3	35.2 - 50.7	33.9 - 52.8
4	43.3	35.2 - 50.9	33.9 - 52.8
5	44.4	36.9 - 56.4	31.7 - 57.2
6	44.4	36.9 - 56.4	31.7 - 57.2
7	44.4	36.9 - 58.4	31.7 - 57.2
8	44.4	36.9 - 75.6	31.7 - 57.2
9	69.5	39.6 - 84.4	50.6 - 88.4
10	69.5	51.6 - 85.8	50.6 - 88.4
11	69.5	51.6 - 89.3	50.6 - 88.4
12	78.6	57.6 - 94.6	51.8 - 105
13	92.5	69.3 - 96.7	80.9 - 104
14	92.5	75.6 - 96.7	80.9 - 104

Table 3.3 90% confidence intervals obtained by Peto's method and by method of profile likelihood

Table 3.4 Negative predictive value of PCR at selected ages.

Age (days)	Vertical transmission rate ("prevalence")			
	10%	15%	20%	
1	93.4 (92.5)	90.0 (88.7)	86.3 (84.7)	
5	94.1 (93.3)	90.9 (89.8)	87.6 (86.1)	
10	96.7 (94.8)	94.8 (92.0)	92.8 (89.0)	
14	99.2 (97.3)	98.7 (95.8)	98.1 (94.2)	
21	99.3 (98.4)	98.9 (97.6)	98.4 (96.6)	
28	99.6 (98.9)	99.3 (98.0)	99.0 (97.3)	

Specificity = 98%.

i.

1

Sensitivity is maximum likelihood estimate or 95% lower confidence limit (value in parenthesis) from Table 2.1.

Figure 3.1 Cumulative percentage DNA positive (90% CI) using non-parametric model



Figure 3.2 Cumulative percentage DNA positive (90% CI) conditional on being DNA negative at birth

.



Figure 3.3 Effect on log-logistic model of excluding outlying observations. Nonparametric model (solid line), log-logistic model applied to full dataset (dashed line), log-logistic model excluding 7 children who tested PCR negative after age 29 days (dotted line)



Figure 3.4 Comparison of non-parametric model and different parametric models. Non-parametric model (solid line), exponential model (dashed line), Weibull model (dotted line), log-logistic model (irregular dashed line)



Figure 3.5 Locally-weighted regression models. Non-parametric model (solid line), smoother with bandwidth=3 days (dashed line), smoother with bandwidth=4 days (dotted line)



Figure 3.6 Cumulative percentage DNA positive for caesarean section deliveries. All deliveries (solid line), caesarean section deliveries (dotted line)



CHAPTER 4. THE RISK OF HIV TRANSMISSION BY DURATION OF BREASTFEEDING

4.1 Background

Although it is well established that HIV can be transmitted through breastfeeding whether the mother is infected prenatally or postnatally (Van de Perre et al. 1992), precise quantitative estimates of the risk of transmission are not yet available. It is difficult to envisage how estimates could be obtained other than from birth cohort studies, but these have tended to recruit either very few breastfed children (studies conducted in industrialised countries) or very few bottlefed children (studies conducted in less-developed countries). In a meta-analysis of all informative studies, the 95% confidence interval for the additional risk of transmission through breastfeeding from prenatally infected mothers ranged widely from 7% to 22% (Dunn et al. 1992). Furthermore, selection bias and confounding bias, which are of especial concern in view of the imbalance within studies in the numbers of breastfeed and bottlefed children, could not be ruled out.

It could be argued that it is meaningless to attach a single figure to the risk of transmission through breastfeeding since this must depend on the age at weaning, which varies greatly within and between populations. de Martino et al. (1992) presented breastfeeding duration-specific estimates of the risk of transmission based on data reported to the Italian Register for HIV Infection in Children. The Register includes HIV-infected and HIV-antibody-positive children irrespective of when maternal infection was diagnosed. The findings of this analysis are of questionable validity since they largely reflect the breastfeeding experience of children born to mothers whose infection was diagnosed postnatally (Dunn and Newell 1992).

Preliminary findings from cohort studies conducted in Johannesburg (South Africa) and Sao Paulo (Brazil) have recently been presented (Gray et al. 1996, Tess et al. 1997). Being the first studies with substantial numbers of both

breastfed children and bottlefed children, they represent an important advance in our knowledge about breastfeeding and HIV transmission. There is less concern about selection and confounding biases, and inference about the effect of the duration of breastfeeding is possible because of variation in age at weaning.

This chapter describes an analysis of the data from the Sao Paulo study, with particular reference to duration of breastfeeding. Non-parametric analyses are described in sections 4.2 and 4.3, but the results of these were unsatisfactory due to inadequate sample size. Subsequent sections describe parametric models which encapsulate two biological features: the fetus/child potentially experiences a <u>series</u> of exposures to the virus (in utero, intrapartum, breastfeeding), and heterogeneity in maternal infectivity.

4.2 Description of Sao Paulo study and results of basic analyses

Between October 1993 and April 1995 Dr. Beatriz Tess conducted a retrospective cohort study of children born to HIV-infected woman at one of seven obstetric hospitals in Sao Paulo State, Brazil. The study was restricted to children born between January 1988 and April 1993, the later date being chosen so that all children were old enough to have had their infection status determined serologically.

To avoid selection bias, enrolment to the study was based solely on identification of HIV-infected pregnant women from a review of hospital obstetric registers. Information on potential risk factors for transmission was extracted from obstetric notes. Clinical findings and serological results on each child were extracted from paediatric notes. Finally, home visits were carried out whenever a contact address was available. A standard questionnaire was administered to the mother which included detailed questions on breastfeeding history. If consent was given, blood and saliva samples were obtained from the child (Tess et al. 1996).

A total of 553 mother-child pairs were recruited to study. The infection status of 434 (78%) children was determined, 415 on serological criteria and 19 (all infected) on clinical criteria alone. Of the 119 children of indeterminate infection status, 29 died before a serological diagnosis had been made and where the death could not be definitely ascribed to HIV infection, 78 children were not located, and 12 parents refused to participate in the study. Of the 434 children of known infection status, it was known whether or not the child had ever been breastfed in all but two cases, and the duration of breastfeeding was unknown in a further 17 cases.

Findings on breastfeeding in relation to transmission risk are summarised in Tables 4.1 and 4.2, and a listing of the individual data is given in Appendix 4.1. Approximately one-third of children were breastfed although the duration of breastfeeding tended to be short, with 73% of breastfed children being weaned before age 3 months. The rate of transmission was significantly higher among breastfed children than among exclusively bottlefed children ($\chi_1^2=7.37$, P=0.007). However, there was no clear evidence of a dose-response relationship with duration of breastfeeding among children who were breastfed (χ^2 test for trend=1.85, P=0.17). The only confounder of the association between breastfeeding and HIV infection status was maternal symptoms of HIV infection; a proportionately smaller number of mothers who breastfed had symptoms than mothers who exclusively bottlefed. Thus, a univariate analysis slightly underestimates the true risk of breastfeeding.

Contrary to what is generally found (Dunn et al. 1994), a lower rate of transmission was found among children delivered vaginally (41/279, 15%) than among children delivered by caesarean section (28/150, 19%), although this difference is not statistically significant (χ_1^2 =0.86, P=0.35).

4.3 Non-parametric analyses

The association between the duration of breastfeeding and the risk of transmission is not clear from Table 4.2, and alternative non-parametric approaches which do not require the specification of arbitrary age groups were explored.

Firstly, the locally-weighted regression method described in Section 3.3.3 was applied. Figure 4.1 (solid line) shows the predicted transmission rate by duration of breastfeeding using a bandwidth of 60 days. It is evident that this analysis is unsatisfactory, and although the line of prediction could be made less irregular by increasing the bandwidth, this would tend towards a standard linear logistic regression model.

Secondly, the problem can be set in a survival analysis framework. Infected children are left-censored observations where infection must have occurred between conception and date of cessation of breastfeeding, or date of delivery if not breastfed. Assuming that indefinite breastfeeding would inevitably result in infection, uninfected children are right-censored at date of cessation of breastfeeding. In the special case when all observations are either left- or right-censored the "pool adjacent violators" algorithm due to Ayer et al. (1955) can be used instead of the computionally intensive EM algorithm suggested by Turnbull (1976). The results of this analysis are also shown in Figure 4.1 (dashed line). The estimated probability of transmission is a step function with only two steps, at ages 10 and 120 days.

Parametric models are justified in view of the unsatisfactory results obtained from these non-parametric analyses. To maximise the information for the estimation of these models, duration of breastfeeding was imputed for the 17 children lacking this information by sampling with replacement from the empirical frequency distribution, conditional on infection status. If data are missing at random, imputation does not introduce bias but does result in artificially precise parameter estimates. At the expense of additional complexity,

correct standard errors can be obtained by the method of multiple imputation (Rubin and Schenker 1986). However, this was not considered to be necessary in view of the small number of imputed observations.

4.4 Serial exposure model - constant hazard

All of the models subsequently considered take account of the fact that the fetus/child of an HIV-infected mother is potentially exposed to the virus in utero, then intrapartum, then through breastfeeding i.e. serially. In the Sao Paulo study, however, there is no motivation to distinguish in utero and intrapartum transmission since no diagnostic tests were performed at birth and no protective effect of caesarean section delivery was observed. For convenience, in utero and intrapartum transmission will be referred to collectively as prepartum transmission.

4.4.1 Theory

The simplest model assumes that each child experiences a constant hazard rate of infection throughout the period of breastfeeding. For child i, let Y_i denote infection status at the end of follow-up (0=uninfected, 1=infected), let π_i denote the probability of infection, and let t_i denote the duration of breastfeeding in days (t_i =0 if exclusively bottlefed). Let the parameter u represent the probability of prepartum transmission, and λ the hazard rate of transmission through breastfeeding, initially assumed to be constant. The probability of not being infected after t_i days of breastfeeding is the survivor function for the exponential distribution, $exp(-\lambda t_i)$.

A child is ultimately uninfected if he avoids both prepartum <u>and</u> breastfeeding transmission (assuming no risk of casual transmission):

$$Pr(Y_i=0) = 1 - \pi_i = (1 - u) exp(-\lambda t_i)$$

$$\log(1-\pi_i) = \log(1-u) - \lambda t_i$$
 (4.1)

This is a generalised linear model with infection status as the dependent

binomial variable (uninfected coded as the "event"), a log link, and a single covariate t_i . The intercept yields an estimate of log(1-u) and the slope, multiplied by -1, an estimate of the breastfeeding hazard, λ . In SAS this model is easily estimated using PROC GENMOD. In GLIM the log link is non-standard for a binomial error distribution, although relevant macros have been published by Wacholder (1986).

4.4.2 Results

ML estimates of the probabilities of prepartum transmission and the hazard rate through breastfeeding were $\hat{u}=0.142$ (95% Cl 0.103-0.179) and $\hat{\lambda}=0.488\times10^{-3}$ (95% Cl 0.000-1.054x10⁻³) (Table 4.3). Such a low hazard rate results in an almost linear relationship between the predicted probability of transmission and duration of breastfeeding (Figure 4.2).

The goodness of fit of the model was informally assessed by ranking children by duration of breastfeeding and comparing the cumulative observed and cumulative predicted numbers of infected children (Figure 4.3). This indicates that the constant hazard model underestimates (overestimates) the risk of transmission for children with a short (long) duration of breastfeeding.

4.5 Individual variation in hazard rate

The assumption of a constant hazard rate of transmission through breastfeeding would appear to incorrect and a more complex model is required. One obvious solution would be to allow the hazard rate to vary intrinsically with age e.g. using the Weibull distribution. However, it is well known that variation between individuals in their underlying hazard ("frailty") induces an apparent decrease in the <u>average</u> hazard rate over time, even if the individual hazard rates remain constant (Aalen 1994). Variation in the underlying hazard is most readily explained by heterogeneity in maternal infectivity, for which there is compelling evidence.

Firstly, viral load is strongly associated with the risk of transmission and varies

by up to 1000-fold in pregnant woman (Sperling et al. 1996), while analysis of serial samples obtained from infected adults has shown viral load to be relatively stable over intervals of 4-16 months (Weiser et al. 1994). Secondly, there is a high degree of concordance (odds ratio of 11.8) in the infection status of twins born to HIV-infected women (Duliège et al. 1995). The observation that the degree of concordance was similar for monozygotic and dizygotic twins implicates maternal factors rather than shared genetic susceptibility to infection.

4.5.1 Theory

In initial models the hazard rate of transmission through breastfeeding, but not the probability of prepartum transmission, was allowed to vary between motherchild pairs. The latent hazard rate λ_i for the ith mother-child pair was assumed to be generated from (a) the gamma distribution, or (b) the log-normal distribution. It was assumed that the ith child was exposed to the hazard rate λ_i throughout the period of breastfeeding i.e. the hazard rate is conditionally <u>independent</u> of age. If $f(\lambda)$ is the probability density function for λ , then

$$Pr(Y_{i}=0) = 1 - \pi_{i} = \int_{0}^{\infty} Pr(Y_{i}=0 | \lambda) f(\lambda) d\lambda$$

$$= \int_{0}^{\infty} (1-u) \exp(-\lambda t_{i}) f(\lambda) d\lambda$$

To avoid boundary constraints on the parameter u, let

$$\alpha = \log[-\log(1-u)] \implies 1-u = \exp[-\exp(\alpha)]$$
(4.2)

......

so that

$$1 - \pi_{i} = \exp[-\exp(\alpha)] \int_{0}^{\infty} \exp(-\lambda t_{i}) f(\lambda) d\lambda \qquad (4.3)$$

Under the gamma model,

$$f(\lambda) = \frac{\rho(\rho\lambda)^{\kappa-1} \exp(-\rho\lambda)}{\Gamma(\kappa)} \qquad (\kappa > 0, \rho > 0)$$

and (4.3) can be integrated analytically (Cox and Oakes 1984, Section 2.3) to give

$$\pi_{i} = 1 - \exp[-\exp(\alpha)] \left[\frac{\rho}{t_{i} + \rho}\right]^{\kappa}$$
(4.4)

The frailty parameter κ reflects the degree of heterogeneity in maternal infectivity. Low values of κ correspond to high frailty, while the model converges to the constant hazard model (4.1) as $\kappa \rightarrow \infty$.

It is convenient to formulate the log-normal model as

$$\log(\lambda_i) = \beta + \sigma z_i$$

where $z_i \sim N(0,1)$ is a random effect and σ reflects the degree of frailty. The "underlying" hazard exp(β) is divided (z_i negative) or multiplied (z_i positive) by exp($\sigma |z_i|$). Under this model

$$Pr(Y_i=0|z_i) = (1-u)exp(-\lambda_i t_i) = exp[-t_i exp(\beta+\sigma z_i) - exp(\alpha)]$$

Thus

$$Pr(Y_i=0) = 1 - \pi_i = \int_{-\infty}^{\infty} Pr(Y_i=0|z) f(z) dz$$

$$\pi_{i} = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \exp[-t_{i} \exp(\beta + \sigma z) - \exp(\alpha)] \exp(-z^{2}/2) dz$$

$$= 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \exp[-t_i \exp(\beta + \sigma z) - \exp(\alpha) - z^2/2] dz$$
 (4.5)

The log-likelihood function is

$$\sum_{i} [y_{i} \log(\pi_{i}) + (1 - y_{i}) \log(1 - \pi_{i})]$$

where π_i is replaced by expression (4.4) or (4.5).

Maximisation of the log-likelihood functions was carried out by the simplex method (Nelder and Mead, 1965), using NAG subroutine E04CCF. Numerical integration within the maximisation subroutine, which was required for the lognormal model, was performed by subroutine D01AKF. This is an adaptive procedure in which the interval of integration is repeatedly divided into a number of smaller sub-intervals and integration rules are applied separately to each subinterval.

Considerable time was spent identifying the appropriate maximisation and integration subroutines from the NAG library. Other subroutines either failed to converge, produced warnings about numerical accuracy, or produced results that were sensitive to specification of input parameters. These problems stem from the substantial frailty in these data, which gives rise to a flat likelihood function that is difficult to maximise (Babiker and Cuzick 1994). Moreover, the integrand in equation (4.5) is ill-conditioned for large values of σ and accurate numerical integration difficult.

4.5.2 Results

On the basis of the maximised log-likelihood, the log-normal model fitted marginally better than the gamma model (Table 4.3). The frailty parameters (κ and σ) indicate substantial heterogeneity in maternal infectivity. This is also evident in Figure 4.2, which shows the predicted probability of infection by duration of breastfeeding. Most infection through breastfeeding is attributable to transmission in the first one or two months of life, suggesting the existence of a group of highly infectious breastfeeding mothers whose children get infected early in infancy. As the constant hazard model is a special case of the gamma frailty model, its adequacy can be tested by a likelihood ratio test. This gives

 $\chi_1^2 = 5.14$ which is certainly significant at the 5% level as the test of a null value on the edge of the parameter space is conservative.

4.6 Incorporating individual variation into probability of prepartum transmission

The models of the previous section are implausible because the probability of prepartum transmission, u, is assumed to be independent of the level of maternal infectivity. A more realistic model would require that women who are more likely to transmit the infection through breastfeeding are also more likely to transmit the infection during pregnancy or at delivery. It is not clear how the gamma model could be generalised to accommodate this but the log-normal model is more flexible.

4.6.1 Theory

Assume that the hazard rate of transmission from the ith mother to her fetus at time t after conception is

$$\omega_{i}(t) = \omega(t) \cdot \exp(\sigma z_{i})$$

where $\omega(t)$ represents the baseline hazard and z_i and σ are defined as before. Note that this model allows for the possibility of an intense hazard at the time of delivery. Using a standard result (Cox and Oakes 1984, Section 5.3), if the baseline survivor function

$$1 - u = \exp\left(-\int_{\text{conception}}^{\text{delivery}} \omega(u) \, du\right)$$

then

$$1 - u_i = (1 - u)^{\exp(\sigma z_i)}$$

Using the transformation (4.2),

$$1 - u_i = (\exp[-\exp(\alpha)])^{\exp(\sigma z_i)}$$

which simplifies to

$$\exp[-\exp(\alpha + \sigma z_i)] \tag{4.6}$$

It is then straightforward to show that

$$\pi_{i} = 1 - \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} \exp[-t_{i} \exp(\beta + \sigma z) - \exp(\alpha + \sigma z) - z^{2}/2] dz \qquad (4.7)$$

analogous to equation (4.5). This model is effectively a proportional hazards model where the frailty random effect $exp(\sigma z_i)$ scales the underlying hazard of transmission throughout pregnancy, delivery, and breastfeeding. The model was estimated as described in Section 4.5.

The (latent) probability density functions for u_i and λ_i are derived as follows. From equation (4.3),

$$u_i = 1 - \exp[-\exp(\alpha + \sigma z_i)] = g(z_i) \text{ say,}$$

where the probability density function for \boldsymbol{z}_i is

$$f(z_i) = \frac{1}{\sqrt{2\pi}} exp(-z_i^2/2)$$

Using a standard result for transforming random variables,

$$f(u_i) = \left| \frac{dz_i}{du_i} \right| f(g^{-1}(u_i))$$

$$= \left| \frac{1}{\sigma (1-u_i) \log(1-u_i)} \right| \frac{1}{\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{\log(-\log(1-u_i)-\alpha)}{\sigma} \right)^2 \right]$$

The probability density function for $\boldsymbol{\lambda}_i$ is the log-normal density

$$f(\lambda_i) = \left| \frac{1}{\sigma \lambda_i} \right| \frac{1}{\sqrt{2\pi}} \exp \left[-\frac{1}{2} \left(\frac{\log(\lambda_i) - \beta}{\sigma} \right)^2 \right]$$

4.6.2 Results

Incorporating frailty in prepartum transmission caused the ML estimate of σ to increase from 6.9 to 11.8 (Table 4.3). However, there was virtually no difference in the maximised likelihood function or in the estimated relationship between transmission risk and the duration of breastfeeding (Figure 4.4). It is clearly not possible to identify empirically the "best" model. A comparison of the expected and observed cumulative number of infected children, in the model which allows for prepartum frailty, shows adequate goodness of fit (Figure 4.3).

The profile-likelihood function for the frailty parameter σ (Figure 4.5) indicates that it is imprecisely estimated, with values between 1.3 and at least 30 being consistent with the data. It is difficult to estimate the likelihood function for values of σ which are larger than this for the reasons outlined in Section 4.6.1. Confidence intervals for the parameters α and β , both of which are highly negatively correlated with σ , are also very wide.

The median duration of breastfeeding was only 45 days. However, there were six atypical children who breastfed for over 2 years, all of whom were uninfected (Appendix 4.1). Sensitivity analyses were performed for selected models to investigate the "leverage" of these observations. Table 4.4 gives a comparison of parameter estimates when the observations are included and excluded. Their exclusion results in a 2.4-fold increase in the estimate of the parameter λ under the constant hazard model. Essentially, the data on these children force the estimated hazard rate to be low as the model would otherwise predict that they would almost certainly be infected.

There were also large changes in the estimates of the parameters of the lognormal frailty model. However, although the profile log-likelihood for the frailty parameter σ is now more concave (Figure 4.5), it is still imprecisely estimated. Even the most extreme values, corresponding to the constant hazard model (σ =0) and the dichotomous model (σ →∞) cannot be rejected.

4.7 Interpretation of model as frailty parameter becomes arbitrarily large

Figures 4.6 and 4.7 show the probability density functions for $\{u_i\}$ and $\{\lambda_i\}$. The U-shaped relationship in Figure 4.6 shows that most women have a probability of prepartum transmission which is close to either 0 or to 1, again reflecting the high frailty in the data. It is interesting to consider the behaviour of the model as $\sigma \rightarrow \infty$. Let ε represent some arbitrarily small constant. Then

$$Pr(u_i < \varepsilon) = Pr(1-u_i > 1-\varepsilon)$$

= $\Pr(\log[-\log(1-u_i)] < \log[-\log(1-\varepsilon)])$

=
$$\Pr(\alpha + \sigma z_i < \log[-\log(1 - \varepsilon)]]$$

= $\Pr(z_i < \frac{\log[-\log(1-\epsilon)] - \alpha}{\sigma})$

$$\rightarrow \Phi(-\frac{\alpha}{\sigma})$$
 as $\sigma \rightarrow \infty$ (4.8)

By a similar argument,

$$\Pr(u_i > 1 - \varepsilon) \rightarrow 1 - \Phi(-\frac{\alpha}{\sigma}) \text{ as } \sigma \rightarrow \infty$$
 (4.9)

From equations (4.8) and (4.9) it follows that

$$Pr(\varepsilon < u_i < 1 - \varepsilon) \rightarrow 0$$
 as $\sigma \rightarrow \infty$

This asserts that each woman either inevitably transmits or inevitably does not transmit the infection prepartum. If σ increases indefinitely it can also be shown that the probability of transmission through breastfeeding tends to

$$1 - \Phi(-\frac{\beta}{\sigma})$$

<u>independent</u> of the duration of breastfeeding t_i . In the limiting case, therefore, the model defines three subgroups of women, with group membership being determined by the realisation of the random effect (Table 4.5).

Because the duration of breastfeeding is irrelevant in the limiting case $(\sigma \rightarrow \infty)$, an equivalent and simpler formulation is the dichotomous model with one parameter for the effect of prepartum transmission and a second parameter for the additional effect of breastfeeding transmission. The fit of this model is only marginally inferior to the more complex models that were tried (Table 4.3). This is not unexpected as the conventional test for a dose-response relationship did not attain statistical significance (Section 4.2).

4.8 Estimates of frailty from other types of study

As already discussed, the concordance of HIV infection status among twins and the strong relationship between maternal viral load and the risk of vertical transmission lend some justification for the frailty models that have been fitted. Furthermore, information from these studies can, with careful analysis, be used to make quantitative inference about the frailty parameter.

4.8.1 Twin study

The latest report from The International Registry of HIV-exposed Twins (Duliège et al. 1995). describes 115 prospectively followed twins sets with known infection status. Information on breastfeeding status was frequently missing, but it can reasonably be assumed that almost all of the 98 sets notified from Europe or North America were exclusively bottlefed. Of these 98 sets, both twins were infected in 9, neither was infected in 73, and in the remaining 16 sets the children were of discordant infection status.

Let Y_{ij} denote the infection status (0=uninfected, 1=infected)of the jth twin (j=1,2) of the ith mother (i=1,...,98). Let R_i denoted the number of infected twins (R_i=0,1,2) and let z_i be the frailty random effect as previously defined. If the simplifying assumption is made that no child was breastfed then from equation (4.6),

$$Pr(Y_{i1}=1 | z_i) = Pr(Y_{i2}=1 | z_i) = \pi_i = 1 - exp[exp(\alpha + \sigma z_i)]$$

Letting $\xi_i = \exp(\alpha + \sigma z_i)$,

$$Pr(R_i=0|z_i) = Pr(Y_{i1}=0|z_i)$$
 and $Pr(Y_{i2}=0|z_i) = [exp(-\xi_i)]^2 = exp(-2\xi_i)$

By a similar argument,

$$Pr(R_i=1|z_i) = 2[exp(-\xi_i) - exp(-2\xi_i)]$$
 and

$$Pr(R_i=2|z_i) = 1 - 2 \exp(-\xi_i) + \exp(-2\xi_i)$$

Integrating out the z_i terms and dropping subscripts, the log-likelihood function for the twin data under the model of Section 4.6.1 is, up to a constant,

$$73 \times \log \left[\int_{-\infty}^{\infty} \exp(-2\xi) \exp(-z^{2}/2) dz \right] + 16 \times \log \left[\int_{-\infty}^{\infty} 2\left[\exp(-\xi) - \exp(-2\xi) \right] \exp(-z^{2}/2) dz \right]$$
$$+ 9 \times \log \left[\int_{-\infty}^{\infty} \left[1 - 2\exp(-\xi) + \exp(2\xi) \right] \exp(-z^{2}/2) dz \right]$$

The ML estimate of σ is 2.0, with a 95% confidence interval (profile loglikelihood) of (1.1,3.5).

4.8.2 Studies of viral load and vertical transmission

Differences between women in viral load is probably the most important source of heterogeneity in maternal infectivity. In this section an estimate of the frailty parameter σ is derived assuming that infectivity is entirely mediated through viral load. This requires information on between-individual variability in viral load and the strength of association between viral load and the risk of vertical transmission. The most extensive data are available from the ACTG-076 trial (Sperling et al. 1996). No child in the trial was breastfed, so the focus is again on prepartum transmission.

Recall from equation (4.6) that

$$\log_{e}[-\log_{e}(1-u_{i})] = \alpha + \sigma z_{i} \quad \text{where } z_{i} \sim N(0,1) \quad (4.10)$$

Sperling et al. fitted linear logistic models of the form

$$\log_{0} \frac{1 - u_{i}}{u_{i}} = a + b \log_{10} (RNA \text{ copies})$$
(4.11)

The complementary log-log transformation (4.10) and the logistic transformation (4.11) are indistinguishable for values of u_i below 0.3 (Collet 1991, Section 3.5). If it is assumed that $z_i \equiv \log(\text{RNA copies})$, then the distributions of values on the right hand side of equations (4.10) and (4.11) must be approximately equal. In particular,

$$var[\alpha + \sigma z_i] \approx var[a + blog_{10}(RNA copies)]$$

and it follows that

$$\sigma \approx b \times SD[log_{10}(RNA \text{ copies})]$$
 (4.12)

Sperling et al. present the distribution of RNA copies per ml as assessed by reverse-transcription PCR in subjects at study entry (14-34 weeks gestation). Reading-off the values from this graph, SD(\log_{10} RNA copies) is estimated as 0.7736. The strongest association between viral load and the risk of vertical transmission was seen in the placebo group using values at the time of delivery, with an odds ratio of 2.21 (95% CI 1.45-3.36) for every 5-fold increase in RNA level. This slope b is therefore estimated as 1.13 [log_e(2.21)/log₁₀(5)], giving an estimate for σ of 0.88 (1.13×0.7736). Ignoring uncertainty in the estimate of SD(log₁₀ RNA copies), the 95% confidence interval for σ is 0.41-1.42.

4.8.3 Consistency of estimates

These external estimates of the frailty parameter are considerably lower than, although not inconsistent with, the direct estimates. In principle, a Bayesian analysis could be performed, using the results from Section 4.8.1 or 4.8.2 to derive a prior distribution for σ . However, in view of the flatness of the likelihood function (Figure 4.5), the posterior distribution for σ would be dominated by the prior distribution.

4.9 Factors that could bias estimates of model parameters

4.9.1 Reverse causality

The objective of this chapter has been to determine the effect of duration of breastfeeding on the risk of HIV transmission. However, causality may also operate in the other direction. For instance, illness or death in the mother or child could curtail breastfeeding. This would be most likely to affect mothers with advanced HIV infection and who are at higher risk of transmitting the virus, and children who are HIV-infected rather than uninfected.

It is not obvious how this mechanism might affect the estimates of interest. Consider a child who dies from HIV infection at age 6 months who would otherwise have been breastfed to age 12 months. Is bias introduced or does the death merely allow the conclusion that infection occurred prepartum or before age 6 months rather than prepartum or before age 12 months (inference is simply more accurate)? To shed light on this, data were simulated for 10,000 notational children as shown in Figure 4.8. Transmission was assumed to follow the constant hazard model (4.1) with u=0.15 and λ =1.0×10⁻³. For each child, an <u>intended</u> duration of breastfeeding was simulated, which was curtailed if the child died prematurely from HIV infection. Extreme hypothetical distributions were used to illustrate the qualitative effects of bias.

Estimating the constant hazard model from the <u>actual</u> duration of breastfeeding gave $\hat{u}=0.172$, $\hat{\lambda}=0.826\times10^{-3}$. Thus, apparently, the risk of prepartum transmission is overestimated and the hazard rate of transmission through breastfeeding is underestimated. This results from a weakening of the association between duration of breastfeeding and risk of transmission due to the premature deaths.

This source of bias is unlikely to be of quantitative importance in the Sao Paulo study because duration of breastfeeding was typically short. Of the children who died, there were only five (all infected) who, as far as can be judged, were still being breastfed. However, the interpretation of studies conducted in Africa,

where children are often not weaned from the breast until the second year of life, is more problematic. The curtailment of breastfeeding is one explanation for the absence of a correlation between duration of breastfeeding between transmitting mothers (mean=15.8 months) and non-transmitting mothers (14.4 months) reported by Guay et al. (1996) in a Ugandan study.

Adjusting for such an effect is not straightforward. The simulation indicates the need to (a) treat duration of breastfeeding as a random variable rather than a fixed covariate (b) model the timing of mother-to-child transmission (c) model the interval between HIV infection and death. This poses severe problems of identifiability, and external data would be essential to develop this further.

4.9.2 Indeterminate infection status

The analysis excluded 119 children of indeterminate infection status, of whom 29 died without a definitive diagnosis of HIV infection, although frequently with respiratory symptoms. In view of the low infant mortality rate in Sao Paulo (approximately 20 per 1000 live births), most of the 29 children are likely to have been infected. Although the study did not have a control group of children born to uninfected mothers, applying the "indirect" method of Section 1.5 assuming M_0 =0.002 gives an estimated transmission rate of 19.7%. Recall that the crude estimate of the transmission rate was 16.0%. There was a high prevalence of breastfeeding (n=17) among the 29 indeterminate deaths, suggesting that the true association between breastfeeding and risk of transmission may have been slightly underestimated.

4.9.3 Error in recording duration of breastfeeding

There is likely to be inaccuracy in the recorded duration of breastfeeding as mothers were asked to recall this information up to seven years after the birth of the study child. <u>Random</u> measurement error is subsumed by and inflates the frailty parameter σ in model (4.7) (Jewell and Shiboski 1990), and attenuates estimates of the hazard rate under the constant hazard model (4.1). The effect of systematic error or differential error between infected and uninfected children

is more difficult to predict.

4.10 Relationship to partner studies of sexual transmission

Much of our knowledge about the sexual transmission of HIV derives from partner studies, which are based on individuals who are known to be HIVinfected (index case) and their sexual partners. The most informative studies have involved partners of patients with transfusion-associated HIV infection, since the date of infection of the index case can then usually be determined (Peterman et al. 1988). Following diagnosis of HIV infection of the index case, a blood sample is obtained from the partner for serological testing and information is collected on factors that may affect the likelihood of transmission. A key variable is the number of sexual contacts subsequent to infection of the index case. Note that if the partner is found to be infected then, because of the retrospective design of these studies, the cumulative number of sexual contacts by the time of infection is not known.

The problem of estimating the risk of transmission by duration of breastfeeding is closely related to the problem of estimating the risk of transmission by number of sexual contacts, and many ideas in this chapter have been borrowed from the literature on partner studies. Minor modifications were required to account for the measurement of the covariate on a continuous rather than a discrete scale, and the need for an intercept term to encompass prepartum transmission.

If a constant probability of infection per sexual contact is assumed then estimation is carried out by a complementary log-log model (Jewell and Shiboski 1990, Kaplan 1990),

$$\log(-\log(1-\pi_i)) = \log(-\log(1-\nu)) + \log x_i$$

where π_i is the probability that the partner is infected, x_i is the number of sexual contacts, and v is the probability of infection per contact. This is analogous to the constant hazard model of Section 4.4. In no partner studies has the

assumption of a constant probability of infection per contact been found to be tenable, and this basic model overestimates the risk of infection for a large number of contacts. Shiboski and Jewell (1992) allowed infectivity of the index case to depend on time since the index case acquired the infection, but the relationships estimated from various data sets do not appear to be biologically plausible.

Closer to the ideas developed in this chapter, Wiley et al. (1989) and Shiboski and Jewell (1990) examined the effect of heterogeneity in infectivity across partnerships. Wiley et al. assumed the per contact transmission probability for each partnership to be a random variable from a beta distribution, and applied this model to data on 55 partnerships reported by Peterman et al. (1988). It was estimated that all partnerships had either a probability of infection per sexual contact of zero (proportion 81%) or one (19%), signifying frailty at its most extreme. Identical results were obtained from an alternative two group mixture model in which partnerships were assigned a constant infectivity of either β or zero. This is a special case of a model proposed by Babiker and Cuzick (1994). A non-parametric analysis of Peterman's data using the pool adjacent violators algorithm (Section 4.3) gave an estimated 19% transmission probability which was independent of the number of sexual contacts (Kaplan 1990), consistent with the findings of Wiley et al.

Shiboski and Jewell (1990) assessed the validity of the constant risk model by fitting the more general model

$$\log(-\log(1-\pi_i)) = \alpha + \beta \log x_i \qquad (4.13)$$

and testing the null hypothesis $\beta=1$. This family corresponds to the discrete Weibull distribution. The authors investigated the consequences of fitting (4.10) to data generated from the beta model of Wiley et al., and tabulated the values of β according to the variance:mean ratio of the underlying beta distribution.

4.11 Public health implications

It has been shown that the decline in the <u>average</u> hazard rate with age can be accounted for by heterogeneity in maternal infectivity without introducing ageeffects per se. However, this is just one interpretation of the data, and a model with age effects ignoring maternal infectivity could probably be identified which described the data equally well. In reality, it is likely that the average hazard rate is influenced by both maternal infectivity and age.

There are several reasons for anticipating an intrinsic effect of age, although this could be complex. Firstly, exposure to HIV depends on the volume of breast milk consumed, which increases after birth then decreases as other foods are introduced. Secondly, colostrum is rich in white cells and, if HIV is transmitted intracellularly, the risk of infection could be particularly high in the first few days of life. Thirdly, the neonate's gut is less acidic than that of an older child, and thus more permeable to infection. Finally, the introduction of weaning foods, typically between 3-6 months of age, may introduce pathogens into the gut and damage the epithelium, thus increasing susceptibility to infection.

Some measure of maternal infectivity, ideally viral load, is essential to examine the independent effect of age. The constant hazard model (4.1), <u>stratified</u> by maternal viral load, could be used to test individual level age effects rather than population level age effects. Extensive data would be required for reliable estimation, although inference would be sharper if children were regularly tested for infection (e.g. by PCR) to enable assessment of the approximate timing of transmission.

Tables 4.6 lists several interventions which have been proposed to reduce mother-to-child transmission of HIV in populations where exclusive bottlefeeding is not feasible (Cohen 1995). If implemented, their impact will depend on the relative importance of the effects of heterogeneity in maternal infectivity and age. Consider, for example, the proposal to withhold colostrum from

neonates (Van de Perre et al. 1992). If the apparent intense transmission in early infancy is due to high susceptibility of the neonate and/or a high level of virus in colostrum, then this intervention should have a sizeable impact. On the other hand, if the reason is a sub-group of highly infectious mothers, then delaying the onset of breastfeeding will merely delay the transmission of infection.

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Table 4.1 Transmission rate by whether breastfed

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Ever breastfed	Total number (%)	Number (%) infected
No	278 (64)	34 (12)
Yes	154 (36)	35 (23)
Total	432 (100)	69 (16)

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Duration breast -feeding (days)	Total number (%)	Number (%) Infected
1-7	37 (27)	5 (14)
8-30	28 (20)	6 (21)
31-90	34 (25)	6 (18)
91-365	25 (18)	8 (32)
>=366	13 (9)	3 (23)
Total	137 (100)	

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Table 4.3 Results of different serial exposure models

Model	Number of parameters	Maximum likelihood estimates	-2 × Log- likellhood
Constant hazard	2	^ u=0.142, λ=0.488×10 ⁻³	375.39
Dichotomous model (ever/never breastfed)	2	a =0.122, b =0.105 ¹	371.62
Gamma frailty ²	3	$\hat{\alpha}$ =-2.01, $\hat{\rho}$ =4.952, $\hat{\kappa}$ =5.04×10 ⁻³	370.25
Log-normal frailty ²	З	$\hat{\alpha}$ =-2.04, $\hat{\beta}$ =-12.6, $\hat{\sigma}$ =6.9	370.03
Log-normal frailty ³	3	$\hat{\alpha}_{=-14.3}, \hat{\beta}_{=-13.2}, \hat{\sigma}_{=11.8}$	370.07

1. Additional risk of transmission through breastfeeding

2. Frailty around breastfeeding hazard only

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3. Frailty around breastfeeding hazard and probability of prepartum transmission

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Table 4.4. Effect of excluding potential influential observations

Model	Parameter	Effect on estimate of potential influential observations						
		Included	Excluded					
Constant hazard	u	0.142	0.133					
	λ	0.488×10 ⁻³	1.167×10 ⁻³					
Log-normal frailty ¹	α	-14.3	-4.3					
	β	-13.2	-7.4					
	σ	11.8	3.0					

1. Frailty around breastfeeding hazard and probability of prepartum transmission

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Table 4.5. Mode of transmission as frailty parameter becomes arbitrarily large $(\sigma \rightarrow \infty)$

Subgroup	Random effect	Proportion of women, among women who breastfeed
Women who transmit HIV prepartum	$z_i > -\frac{\alpha}{\sigma}$	$1 - \Phi(-\frac{\alpha}{\sigma})$
Women who transmit HIV through breastfeeding but not prepartum	$-\frac{\beta}{\sigma} < z_i < -\frac{\alpha}{\sigma}$	$\Phi(-\frac{\alpha}{\sigma}) - \Phi(-\frac{\beta}{\sigma})$
Women who do not transmit HIV	$z_i < -\frac{\beta}{\sigma}$	$\Phi(-\frac{\beta}{\sigma})$

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Table 4.6 Likely impact of proposed interventions to reduce mother-to-child transmission of HIV in breastfeeding populations

Intervention	Reason for decline in average hazard rate with age							
	age effect per se	heterogeneous maternal infectivity						
Withhold colostrum	sizeable	weak						
Limit duration of breastfeeding (e.g. to 6 months)	weak	weak						
Discourage early introduction of weaning foods	unclear	weak						
Antiretroviral therapy just before and/or at delivery	sizeable	weak						

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Figure 4.1 Estimated probability of transmission by duration of breastfeeding non-parametric analyses. Locally-weighted logistic regression (solid line), Ayer's method (dashed line).



duration of breastfeeding (days)

Figure 4.2 Estimated probability of transmission by duration of breastfeeding models without frailty in prepartum transmission. Constant hazard (solid line), gamma frailty (dotted line), log-normal frailty (dashed line).



Figure 4.3 Assessment of goodness of fit. Observed (solid line), predicted number under constant hazard model (solid circles), predicted number under log-normal frailty model (open circles).



Figure 4.4 Estimated probability of transmission by duration of breastfeeding - comparison of models with (solid line) and without (dashed line) frailty in prepartum transmission.



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Figure 4.5 Profile log-likelihood for frailty parameter. All observations (solid line), excluding six outlying observations (dashed line).







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Figure 4.7 Probability density function for latent hazard rate of transmission through breastfeeding



Figure 4.8 Simulation model to study the effects of child's HIV infection on breastfeeding duration



Distributions of random variables: X ~ Mixture model $\pi_1=0.5$: X=0 (i.e. not breastfed) $\pi_2=0.5$: X ~ Normal, E(X)=360 days, SD(X)=150 days Y ~ lognormal, E(log Y)=75 days, SD(log Y)=30 days Z ~ exponential, E(Z)=1000 days

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5. GENERAL DISCUSSION

The most unsatisfactory features of the new method described in Chapter 2 to estimate the HIV vertical transmission rate and the AIDS incubation period are (i) the assumptions about the sensitivity and specificity of virus tests, and (ii) a limitation in accommodating only a single categorical covariate. The first problem could be addressed by introducing a latent distribution that allows for individual-specific sensitivities, similar to the models of Chapter 4. The presence and effect on estimates of serial correlation within individuals would also need to be examined. Concerning the second problem, π in equation (2.1) could, in principle, be replaced by an arbitrarily complex function of covariates. With these extensions to the basic model an EM algorithm, or even direct maximisation of the likelihood using standard subroutines, is likely to be so complex that Markov Chain Monte Carlo methods may be a more attractive approach to estimation. However, for reasons outlined below it is not clear if it would be worthwhile investing considerable effort in these lines of research.

The analysis of Chapter 3 indicates that the sensitivity of PCR after the neonatal period is apparently close to 100%, which has also been reported in related studies (Section 3.5.3). This would appear to conflict with the results of Chapter 2 where PCR sensitivity was estimated as only 92%. A likely explanation for this discrepancy is that the tests in the European Collaborative Study were performed for routine clinical diagnosis whereas the data in Chapter 3 were obtained primarily from research laboratories. The collection of an early blood sample for PCR analysis should now be a standard feature of all vertical transmission study protocols. Where this is done, and provided laboratory quality control procedures are maintained, a reliable early diagnosis should be available for all children. This effectively eliminates any difficulties in the statistical analysis and thus nullifies advantages of the method described in Chapter 2. This is unlikely, therefore, to find wide application in the context of HIV infection, although ad hoc applications could be envisaged.

Analyses of epidemiological studies of other vertically-transmitted infections,

such as <u>Toxoplasma gondii</u>, HTLV-1, and hepatitis C virus have generally been unsophisticated. In principle, the new method could, with suitable modification, be applied to any of these infections. A common feature is that reliable diagnosis can eventually be made using antibody assays but that direct tests for the virus or parasite (e.g. PCR) are highly inaccurate. Detailed consideration would need to be given to the biology of, and tests for, each particular infection. For example, a model for hepatitis C would need to take account of the possibility of complete clearance of the virus (Ni et al. 1994).

Returning attention to HIV infection, it is generally held, partly on the basis of the analysis of Chapter 3 and related studies, that transmission around the time of delivery is more frequent than transmission in utero. This is the rationale for a number of studies that have evaluated or are evaluating interventions against intrapartum transmission, including antiseptic cleansing of the birth canal, zidovudine started shortly before the expected date of delivery, and caesarean section delivery (Biggar et al. 1996, Cohen 1995). Results from these studies should shed further light on the critical question of the timing of transmission.

Early PCR tests still have an important role to play. For example, caesarean section delivery is of no value if the fetus has already been infected. A powerful way of assessing the effectiveness of this intervention would be compare transmission rates by mode of delivery in infants who apparently avoided prepartum transmission, as reflected by an early negative PCR test result. Also, data which have been used to assess the sensitivity of PCR largely predate publication of the ACTG-076 trial (Connor et al. 1994) and thus mainly include children of women who did not receive zidovudine during pregnancy. However, zidovudine could be more effective at blocking some mechanisms of transmission than others and thus affect the age at which viral markers are first detectable. Kuhn et al. (1997) explored these and other issues in a recent paper, defining presumed intrauterine and presumed intrapartum infection as a positive or negative PCR within 3 days of birth, respectively. Their findings were inconclusive, possibly due to the small sample size (48 infected infants).

This is an interesting approach and there is a need for collaborative analyses. Using an arbitrary cut-off, as in the paper of Kuhn et al., is wasteful of information, and the paucity of data warrants use of one of the more efficient methods described in Chapter 3.

The most difficult challenges in terms of study design and statistical analysis concern studies conducted in breastfeeding populations. For example, the primary endpoint in an African trial to assess the effectiveness of antiseptic cleansing of the birth canal was infection status as assessed by PCR at six weeks of age (Biggar et al. 1996). Rates of PCR positivity were almost identical in the intervention and control arms and it was inferred that antiseptic cleansing is not effective in reducing vertical transmission. However, if a difference had been found the usefulness of this intervention in public health terms would have been uncertain because the effect of subsequent transmission through breastfeeding had not been measured. The frailty models of Chapter 4 suggest that breastfeeding will tend to nullify the effect of an intervention which reduces the risk of transmission during or shortly after delivery. It has been strongly argued, therefore, that follow-up should continue in all intervention studies throughout the entire breastfeeding period (Ekpini et al. 1997).

In this situation the statistician would have data available on antibody tests and clinical status, but the new method for estimating the vertical transmission rate that was developed in Chapter 2 would not be directly applicable. Firstly, a negative antibody test result implies that a child has ultimately escaped infection <u>only</u> if he has already been weaned and is not in the window period between infection and antibodies appearing in peripheral blood. Secondly, the method was developed in a context where the clinical diagnosis of AIDS can reasonably be assumed to be 100% specific for HIV infection. In settings with high underlying infant morbidity and mortality rates, for example parts of Africa, this assumption would not be valid. Further work is required to develop appropriate methods of estimation for these circumstances. This need is pressing in view of

the large number of studies of "affordable" interventions that are being carried out in less-developed countries (Cohen 1995). To my knowledge, none of these studies are using a control group of children born to HIV-uninfected mothers, precluding use of the "indirect" approach described in Section 1.5 to estimate the absolute risk of transmission within each intervention arm.

Finally, there remains the important issue of the extent and timing of transmission through breastfeeding in itself. The analysis in Chapter 4 of data from the Sao Paulo study appears to indicate that this is an important mode of transmission in the first few months after delivery but that the risk of infection is small at older ages. This conclusion must be tentative since the accuracy of the information on duration of breastfeeding, which was recalled retrospectively, is open to question. However, a recent <u>prospective</u> study in Soweto, South Africa also found a strong effect of ever breastfeeding on transmission risk without a clear dose-response relationship with duration (Glenda Gray, personal communication).

An alternative approach to studying transmission through breastfeeding is to test serial blood samples by PCR or antibody tests. Serological tests can provide information only on late post-natal transmission as few uninfected children have cleared maternal antibodies before 6 months of age. However, as HIV DNA can be detected in almost all infected, non-breastfed children by one month of age, PCR can be used to demonstrate earlier post-natal transmission. Buterys et al. (1995) estimated a transmission rate of 5.8 per 100 child-years after age 20 months. Ekpini et al. (1997) estimated that among children who escape infection during the first 6 months of life, 20% of those who are breastfed until age 24 months will acquire infection by that age. These findings suggest, unlike the studies in Sao Paulo and Soweto, that substantial transmission through breastfeeding is not limited to early infancy.

Appendix 2.1 FORTRAN 77 program to implement EM algorithm

С	Scalars:	
С	PI	- vertical transmission rate
С	NTESTS	- number of different virus tests
С	MAXINTS	- maximum follow-up (months)
С	MAXLOOP	- maximum allowed number of main iterations
С	L	 individual log-likelihood contribution
С	LL	- log-likelihood
С	CONVERGE	- program stops when log-likelihood changes by less than
С		CONVERGE between successive iterations
С	Vectors:	
С	G	- survivor function for AIDS
C	LOGG	- log (survivor function for AIDS)
C	AFREQ	- age-specific number of children diagnosed with AIDS
С	HAZARD	- age-specific hazards for AIDS (g)
С	ATRISK	 estimated number of children at risk of AIDS
С	H	 survivor function for antibody loss
С	PDF	 probability density function for antibody loss (h)
С	TAU	 conditional probability of infection
С	S	- sensitivity of virus tests
	INTEGER PARAMETE INTEGER * A DOUBLE P * P * T * A PARAMETE G(0)=ONE	NCHILD, MAXINTS, LOOP, MAXR, NTESTS, MAXLOOP R (NCHILD=977, MAXINTS=120, NTESTS=3, MAXLOOP=10000) GROUP(NCHILD), A(NCHILD), L(NCHILD), R(NCHILD), FREQ(MAXINTS), X(NTESTS, NCHILD), Y(NTESTS, NCHILD) RECISION ONE, ZERO, EPS, CONVERGE, L, LL, LL2, V1, V2, V3, NINFECT, I, RESCALE, H(MAXINTS), PDF(MAXINTS), PDF2(MAXINTS), AU(NCHILD), G(0:MAXINTS), LOGG(0:MAXINTS), HAZARD(MAXINTS), TRISK(MAXINTS), S(NTESTS), SUMP(NTESTS), SUMN(NTESTS) R (ZERO=0.0D0, ONE=1.0D0, EPS=1.0D-6, CONVERGE=1.0D-8)
	LOGG(0) = ONE	ZERO
с	*****	*********
~		

```
С
С
   Read in data
С
   See JASA article for notation and required information
С
   GROUP must be coded as 1 (AIDS), 3 (VIR), 4 (SERO), 5(CENS)
С
DO 20 J=1,NCHILD
      READ(4,*) GROUP(J), A(J), L(J), R(J), X(1,J), Y(1,J), X(2,J),
               Y(2,J), X(3,J), Y(3,J)
       IF (GROUP(J).EQ.1) THEN
        AFREQ(EXAM(J)) = AFREQ(EXAM(J)) + 1
       ENDIF
       IF (GROUP(J).EQ.5) THEN
         R(J)=MAXINTS
       ENDIF
20
     CONTINUE
C ******
С
С'
  ASSIGN STARTING VALUES
C
C ******
     DO 160 J=1, MAXINTS
      PDF(J)=ONE/DBLE(MAXINTS)
```

```
160 CONTINUE
    DO 180 J=1,NCHILD
      IF (GROUP(J).EQ.1.OR.GROUP(J).EQ.3) TAU(J)=ONE
      IF (GROUP(J).EQ.4) TAU(J)=ZERO
      IF (GROUP(J).EQ.5) TAU(J)=ZERO
 180 CONTINUE
C ********
C
С
  START OF MAIN LOOP
C
C *************
    DO 10000 LOOP=1, MAXLOOP
C
С
   RE-ESTIMATE OVERALL PROB OF INFECTION
C
NINFECT=ZERO
    DO 1000 J=1,NCHILD
     NINFECT=NINFECT+TAU(J)
1000 CONTINUE
    PI=NINFECT/DBLE(NCHILD)
С
С
   RE-ESTIMATE SURVIVOR FUNCTION FOR AIDS INCUBATION PERIOD
C
DO 1010 J=1, MAXINTS
1010
     ATRISK(J) = ZERO
    DO 1020 J=1, MAXINTS
    DO 1020 J1=1,NCHILD
      IF (EXAM(J1).GE.J) ATRISK(J)=ATRISK(J)+TAU(J1)
1020 CONTINUE
    DO 1040 J=1, MAXINTS
      IF (AFREQ(J).EQ.0) THEN
       HAZARD(J) = ZERO
      ELSE
       HAZARD(J)=DBLE(AFREQ(J))/ATRISK(J)
      ENDIF
      IF (HAZARD(J).LT.ONE-EPS)
       LOGG(J) = LOGG(J-1) + DLOG(ONE-HAZARD(J))
      G(J) = DEXP(LOGG(J))
1040 CONTINUE
С
C
   RE-ESTIMATE PDF FOR DISTRIBUTION OF ANTIBODY LOSS
C
 С
     DO 2000 J=1, MAXINTS
       PDF2(J) = ZERO
2000
     CONTINUE
     DO 2060 J=1,NCHILD
```

```
125
```

```
IF (GROUP(J).EQ.1.OR.GROUP(J).EQ.3) GOTO 2060
        V1=ZERO
        DO 2030 J2=L(J)+1, R(J)
2030
          V1=V1+PDF(J2)
        DO 2040 J_2=L(J)+1, R(J)
          IF (V1.GT.EPS) PDF2(J2)=PDF2(J2) + (ONE-TAU(J))*PDF(J2)/V1
2040
2060 CONTINUE
      RESCALE=ZERO
      DO 2080 J=1, MAXINTS
       PDF(J) = PDF2(J)
       RESCALE = RESCALE + PDF(J)
2080
      CONTINUE
      DO 2090 J=1, MAXINTS
       PDF(J)=PDF(J)/RESCALE
2090
      CONTINUE
      V1=ZERO
      DO 3000 J=MAXINTS, 1, -1
       V1=V1+PDF(J)
       H(J) = V1
3000
      CONTINUE
С
С
   RE-ESTIMATE SENSITIVITIES OF VIRUS TESTS
C
DO 3500 K=1,NTESTS
      SUMP(K) = ZERO
      SUMN(K)=ZERO
3500 CONTINUE
     DO 3700 J=1,NCHILD
     DO 3700 K=1,NTESTS
       SUMP(K) = SUMP(K) + TAU(J) * X(K, J)
       SUMN(K) = SUMN(K) + TAU(J) * Y(K, J)
3700 CONTINUE
     DO 3800 K=1,NTESTS
       S(K) = SUMP(K) / (SUMP(K) + SUMN(K))
3800 CONTINUE
C
С
     RE-ESTIMATE THE CONDITIONAL PROBABILITIES OF BEING INFECTED
С
 C
     DO 4000 J=1,NCHILD
       IF (GROUP(J).EQ.5) THEN
        V1=ONE
        DO 4001 J1=1, EXAM(J)
          V1=V1*(ONE-HAZARD(J1))
4001
        V2=ZERO
        DO 4002 J1=1, L(J)
4002
          V2=V2+PDF(J1)
        V3 = ZERO
        DO 4003 J1=1,NTESTS
4003
          V3=V3+DBLE(Y(J1,J))*DLOG(ONE-S(J1))
          V3 = DEXP(V3)
        TAU(J) = (PI*V1*V3) / (PI*V1*V3 + (ONE-PI)*(ONE-V2))
       ENDIF
```

```
4000 CONTINUE
```

LL=ZERO

```
DO 6000 J=1,NCHILD
        IF (GROUP(J).EQ.1) THEN
          V1=ZERO
          DO 5010 J2=1, EXAM(J)-1
            V1=V1+DLOG(ONE-HAZARD(J2))
5010
        CONTINUE
          L=DLOG(PI)+DLOG(HAZARD(EXAM(J)))+V1
          DO 5012 K=1,NTESTS
            L=L+DBLE(X(K,J))*DLOG(S(K))
            L=L+DBLE(Y(K, J))*DLOG(ONE-S(K))
5012
          CONTINUE
        ENDIF
        IF (GROUP(J).EQ.3) THEN
          V1=ZERO
          DO 5020 J2=1, EXAM(J)
            V1=V1+DLOG(ONE-HAZARD(J2))
5020
          CONTINUE
          L=DLOG(PI)+V1
          DO 5022 K=1,NTESTS
            L=L+DBLE(X(K,J))*DLOG(S(K))
            L=L+DBLE(Y(K, J))*DLOG(ONE-S(K))
5022
          CONTINUE
        ENDIF
        IF (GROUP(J).EQ.4) THEN
          V1=ZERO
          DO 5030 J2=L(J)+1, R(J)
            V1 = V1 + PDF(J2)
5030
          CONTINUE
          L=DLOG(ONE-PI)+DLOG(V1)
        ENDIF
        IF (GROUP(J).EQ.5) THEN
          V1=ONE
          DO 5040 J1=1,EXAM(J)
            V1=V1*(ONE-HAZARD(J1))
5040
          V2=ZERO
          DO 5042 J1=1,L(J)
5042
            V2 = V2 + PDF(J1)
          V3=ZERO
          DO 5044 J1=1,NTESTS
5044
            V3=V2+DBLE(Y(J1,J))*DLOG(ONE-S(J1))
            V3 = DEXP(V2)
          L=DLOG( PI*V1*V3 + (ONE-PI)*(ONE-V2))
        ENDIF
        LL=LL+L
6000
       CONTINUE
С
 ******
С
С
    TEST FOR CONVERGENCE
С
С
  *****
      IF (DABS(LL-LL2).LT.CONVERGE) GOTO 20000
```

LL2=LL

10000 CONTINUE

```
С
С
                  WRITE OUT RESULTS AT CONVERGENCE
С
20000 CONTINUE
      WRITE(6,*) 'Number of loops =',LOOP
      WRITE(6,*) 'Last change in L =',LL-LL2
WRITE(6,*) 'Log likelihood = ',LL
WRITE(6,*) 'Estimated VTR = ',PI
WRITE(6,*) 'Sensitivities: ',S(1),S(2),S(3)
      WRITE(6,9901)
      DO 20010 J=1, MAXINTS
20010 IF (AFREQ(J).GT.0) WRITE(6,*) J,AFREQ(J),ATRISK(J),
* HAZARD(J) G(J)
            HAZARD(J),G(J)
      WRITE(6,9902)
      DO 20020 J=1, MAXINTS
         IF (PDF(J).GT.EPS) WRITE(6,*) J,PDF(J),H(J)
20020
      STOP
 9901 FORMAT(//,' Survivor function for AIDS onset ',/)
9902 FORMAT(//,' PDF and survivor function for antibody loss ',/)
      END
```



Appendix 2.2 Partial double derivatives in Section 2.2.4

SERO group



VIR group

$$-\frac{\partial^2 L}{\partial \pi^2} = \frac{1}{\pi^2}$$
$$-\frac{\partial^2 L}{\partial s_v^2} = \frac{X_{kv}}{s_v^2} + \frac{Y_{kv}}{(1-s_v)^2}$$

CENS group

Define	$T_1 = \exp\left[\sum_{t=0}^{a_k} \lambda_t\right]$	$\int_{2}^{V} = \prod_{v=1}^{V} (1 - s_{v})^{y_{kv}}$
	$T_3 = 1 - \sum_{t=0}^{l_k} h_t$	$T_4 = \pi T_1 T_2 + (1 - \pi) T_3$
$-\frac{\partial^2 L}{\partial \pi^2} =$	$\left[\frac{T_{1}T_{2}-T_{3}}{T_{4}}\right]^{2}$	
$-\frac{\partial^2 L}{\partial \lambda_t \partial \lambda_t},$	$= \frac{-\pi(1-\pi)T_{1}T_{2}}{T_{4}^{2}}$	T ₃ 0≤t,t′≤a _k
_∂²L _	$-\pi(1-\pi)T_1T_2y_{kv}$	$[(1-\pi)T_3 - T_4]$
∂s _v ²	(1-s _v	r_{4}^{2} T ₄ ²
∂²L ∂h,∂h,,	$= \frac{(1-\pi)^2}{T_4^2}$	$0 \le t, t' \le I_k$
$-\frac{\partial^2 L}{\partial \pi \partial \lambda_t}$	$= \frac{-T_1 T_2 T_3}{T_4^2}$	0≤ t≤ a _k
$-\frac{\partial^2 L}{\partial \pi \partial s_v}$	$= \frac{T_1 T_2 T_3 y_{kv}}{(1 - s_v) T_4^2}$	
$-\frac{\partial^2 L}{\partial \pi \partial h_1}$	$=\frac{-T_1T_2}{T_4^2}$)≤t≤l _k
$-\frac{\partial^{2}L}{\partial\lambda_{t}\partial h_{t},}$	$= \frac{-\pi(1-\pi)T_{1}T_{2}}{T_{4}^{2}}$	0≤t≤a _k , 0≤t′≤I _k
$-\frac{\partial^2 L}{\partial \lambda_t \partial s_v}$	$= \frac{\pi(1-\pi)T_1T_2T_3}{(1-s_v)T_4^2}$	$\frac{y_{kv}}{dt}$ $0 \le t \le a_k$
_ <mark>∂²L</mark> ∂h _t ∂s _v	$= \frac{\pi(1-\pi)T_1T_2y_k}{(1-s_v)T_4^2}$	⊻ 0≤t≤l _k
− <u>∂²L</u> <u>∂s_v∂s_v,</u>	$= \frac{-\pi(1-\pi)T_{1}T_{2}}{(1-s_{v})(1-s_{v})}$	$\frac{T_{3}y_{kv}y_{kv'}}{s_{v'})T_{4}^{2}}$

Children in AIDS group (n=41): columns are age at AIDS diagnosis, positive virus tests, negative virus tests, positive PCR tests, negative PCR tests

5	0	3	0	0	3	0	0	0	0	4	0	0	0	0
2	0	0	0	0	42	12	8	0	0	2	0	0	0	0
10	2	0	0	0	4	0	0	0	0	5	0	0	0	0
3	1	0	0	0	2	0	0	0	0	11	0	0	0	0
27	1	0	0	0	5	0	0	0	0	12	0	0	0	0
2	3	0	3	0	27	4	1	1	0	5	0	0	0	0
11	1	0	0	0	5	0	0	0	0	2	0	0	0	0
4	2	0	1	0	74	0	0	0	0	19	3	0	3	0
5	3	0	0	0	10	0	0	0	0	3	8	0	0	0
30	3	4	0	0	17	0	0	0	0	9	8	1	1	0
6	2	2	0	0	3	5	3	0	0	18	0	0	0	0
4	0	0	0	0	2	2	1	0	0	30	0	0	0	0
44	3	0	1	0	13	7	0	0	0	42	0	0	0	0
33	0	1	0	0	12	4	0	0	0					

Children in SERO group (n=639): age at last positive antibody test - age at first negative antibody test (value in paretheses represents number of children, if more than one)

0-2 ³	0-3²	0-44	0-5²	0-6	0-7 ⁵	0-84	0-9⁵
0-10 ¹	0-11 ²	0-12 ³	0-13	0-14 ³	0-15²	0-16²	0-18
0-19	0-20	0-23	0-28	0-36	$0 - 41^{2}$	0-60	1-2
1-3	1-5	1-6²	1-11²	1-14	1-16	2-3	2-4
2-5 ⁸	2-6°	2-7	2-8	2-9	2-113	2-13	2-15
2-16	2-21	2-24 ²	3-5 ³	3-6°	3-7 ³	3-9 ³	3-10
3-11²	3-12	3-13 ²	3-14 ²	3-17	3-18 ²	3-27	4-5
4-64	$4 - 7^{2}$	4 - 8 ³	4 – 9 ³	4-10²	4-11	4-12 ²	4-14
4-15 ²	4-17	4-19	5-6²	5-7 ³	5-8 ¹⁸	5-911	5-10 ⁵
5-116	5 -12 ⁵	5-13	5-15	5-16²	5-17	5-18	5-21
5-28	6-75	6-811	6-9 ³⁸	6-106	6-116	6-124	6-134
6-144	6-15	6-18 ²	6-21	6-23²	6-24	6-35	7-84
7-9 ⁵	7-1010	7-11°	7-12⁴	7-13	7-14 ³	7-15²	8-10 ³
8-1124	8-1231	8-134	8-14 ³	8-15 ³	8-18 ²	8-20 ²	8-21
8-41	9-10	9-11 ¹⁹	9-1248	9-1311	9-14 ³	9-15°	9-16²
9-18²	9-19	9-20	9-22	9-23	10-1210	10-136	10-14 ³
10-15 ⁵	10-164	10-17	10-26²	10-30	11-1417	11-15'	11-16 ⁵
11-17²	11-18	11-19	11-24²	11-26	12-147	12-1522	12 - 16⁵
12-17 ³	12-18 ³	12-19	12-20 ²	12-52	13-16²	13-18 ²	13-19
14-17 ³	14-18 ²	14-24	15-174	15-18²	15-19²	15-21	15-28
15-30	18-21	$18 - 24^{2}$	19-30	20-22	21-32		

ł

Child	ren	in	VII	R grou	ıp (:	n=	68)	: 0	colu	mna	s are ag	ge a	at	last	: cl :	inical	exam,	
posit:	ive	vir	rus	tests	s, n	eg	ati	Lve	vir	us	tests,	pog	sit.	ive	PCR	tests,	negati	lve
PCR te	esta	3																
							_								•			
16	1	0	0	0	4	9	2	0	2	0	12	2	0	1	0			
101	4	3	2	0	4	2	2	0	2	0	9	5	1	3	0			
102	5	0	2	0	4	8	3	1	0	0	5	4	0	2	0			
99	4	0	0	0	3	6	3	0	0	0	75	1	1	0	0			
81	1	0	2	0	3	0	2	2	0	0	54	2	0	0	0			
75	4	2	2	0	2	9	3	0	0	0	52	2	0	0	0			
70	4	0	1	0	1	8	1	0	0	0	58	1	0	0	0			
58	1	0	3	0	2	0	2	1	0	0	7	1	0	0	0			
56	2	0	2	0	3	4	2	1	3	0	32	3	1	2	0			
53	1	0	2	0	2	0	1	0	1	0	7	1	1	0	2			
4	1	0	1	0	1	1	0	0	1	0	21	1	0	1	0			
44	2	0	2	0		5	0	0	2	0	18	2	0	1	2			
45	2	0	1	0	8	3	9	4	1	0	9	1	0	0	0			
33	1	1	2	0	6	2	6	3	0	0	3	1	0	1	0			
40	1	0	1	0	5	3	6	3	1	0	9	1	2	0	0			
38	0	0	1	0	4	4	3	11	1	0	24	3	0	0	0			
6	2	0	2	0	5	0	7	1	0	0	12	3	0	0	0			
79	12	0	0	0	3	6	11	2	3	0	13	1	4	0	3			
62	2	2	0	0	3	2	5	1	2	0	9	2	0	0	0			
3	1	0	Ō	Ō	2	3	2	0	0	Ō	6	4	Ó	0	Ó			
105	12	4	ŏ	Õ	1	5	7	1	4	Õ	3	2	Ō	2	Õ			
49	3	5	Ō	Ō	1	9	8	ō	4	Ō	4	2	Ō	1	Ō			
11	4	Ō	Ō	0	1	8	8	Ō	4	Ō	_	_	-					

Children in CENS group (n=229): columns are age at last clinical exam, age at last antibody test, negative virus tests, negative PCR tests. For 69 children, values of all variables were 0 i.e. lost to follow-up shortly after birth.

2	2	0	0	52	47	0	0	7	7	5	2	14	3	3	0
33	33	0	0	44	44	0	0	8	6	4	1	11	5	5	0
7	7	1	0	38	33	0	0	5	5	4	2	11	11	5	0
14	14	1	0	35	35	0	0	5	5	3	2	12	9	4	0
9	9	0	0	43	43	0	0	5	5	4	2	12	10	4	0
3	1	1	1	5	5	0	0	4	3	2	1	2	1	1	0
6	6	2	2	38	38	0	0	6	1	3	3	17	8	3	3
18	18	3	2	40	36	0	0	6	6	4	3	12	9	2	2
7	7	0	0	3	3	0	0	6	0	2	2	11	1	1	1
2	2	2	1	8	1	0	0	5	5	4	2	2	2	1	0
2	2	1	1	2	2	0	0	5	5	3	2	14	6	3	3
7	2	1	2	14	14	2	2	1	1	1	1	14	14	1	1
3	0	0	0	11	11	1	1	8	8	4	3	6	6	3	3
2	0	0	0	11	11	0	2	5	2	2	2	10	10	5	2
3	3	1	1	11	11	0	2	5	5	4	3	4	3	0	0
3	3	0	0	6	6	0	0	5	5	4	4	2	2	0	0
2	2	1	1	9	5	0	1	6	0	3	2	4	3	0	0
3	3	0	0	6	1	0	1	2	2	2	2	6	6	2	2
3	1	0	0	7	7	0	1	5	2	2	1	1	1	1	1
5	5	0	0	3	3	0	2	3	3	1	1	6	6	4	4
5	5	0	0	2	2	0	0	5	4	4	4	1	1	1	1
2	0	0	0	12	10	4	0	2	2	1	1	6	6	1	1
9	7	0	0	6	0	0	0	1	0	1	1	5	5	3	3

2	2	0	0	12	12	5	0	1	0	0	0	3	3	2	2
9	9	1	0	11	11	4	0	1	1	0	0	1	1	1	1
3	3	0	0	6	6	4	2	37	37	0	0	1	1	2	2
26	26	0	0	15	15	10	4	4	3	0	0	3	3	2	2
3	3	0	0	11	8	8	2	73	72	0	0	1	1	1	1
1	0	0	0	3	3	6	3	7	7	0	0	1	1	1	1
8	6	0	0	6	6	4	2	2	0	0	0	3	1	1	1
13	13	0	0	1	0	0	0	6	5	0	0	2	1	0	0
5	5	0	0	12	10	4	2	5	3	0	0	14	14	6	6
3	3	0	0	5	5	4	2	3	2	0	0	5	5	1	2
18	18	1	0	9	9	4	2	47	47	0	0	4	3	1	2
109	109	0	0	25	25	1	0	4	4	0	0	4	4	2	3
84	84	0	0	2	0	0	0	45	42	0	0	6	6	2	0
100	100	0	0	15	0	0	0	3	3	0	0	8	6	2	0
57	53	0	0	21	21	0	0	5	5	0	0	7	6	2	0
59	56	0	0	48	48	0	0	2	2	0	0	12	4	0	0
6	6	0	0	8	8	1	2	5	5	0	0	16	14	3	0

Appendix 3.1 Letter to prospective collaborators about PCR study

We have recently reviewed studies which have reported the results of PCR tests performed on young infants born to HIV-infected mothers, including your paper which appeared in (). As such studies have typically involved between 10 and 20 infected children, the sensitivity of PCR as estimated in the individual studies is subject to considerable statistical error. Moreover, in the different studies the tests were often scheduled at different ages and reported in broad age bands. We think that it is worthwhile to conduct a quantitative analysis of all the published data. In order to do this in the most efficient statistical manner we would require slightly more detail than you presented in your paper.

We would be most grateful if you could send us the following information on each child who was known to be infected - by criteria other than PCR - and who had at least one PCR result (positive or negative) before age 3 months. (We would also gratefully receive more recent data if you wished to make this available.)

- (a) The exact age in <u>days</u> at the last negative PCR and first positive PCR (having the data in this form would enable us to use the optimal statistical technique).
- (b) Whether the child's mother was known to be infected at the time of delivery, and if not, the reason why the child was tested.
- (c) Whether the child was breastfed.
- (d) Whether the child was delivered vaginally or by caesarean section.

You might question the validity of combining data from methodologically different PCR systems. We agree that this is a concern and we plan to first examine the studies for systematic differences. Our interest is more in describing the proportion of children, by age, with detectable levels of virus than in making precise statements about the sensitivity of specific systems. This could help clarify the question of the timing of transmission.

The reason for asking why the child was tested is the evidence of association between early identification of virus and a rapid clinical course. There might be bias, therefore, by including children whose infection was recognised because of symptoms due to HIV. It may also be worthwhile exploring whether laboratory data can give indirect evidence on the role of caesarean section in reducing transmission. If caesarean section really does reduce the risk of intrapartum transmission then a greater proportion of infected children in this group will have been infected in utero. Therefore, if the age at which virus is first detectable is related to the timing of transmission one would expect to see different patterns by mode of delivery.

We hope that you will be able to contribute to this exercise. A list of other researchers to whom we have written is attached. Naturally, we would include you as an author on any papers that derive from this work, would send you drafts for comments prior to submission for publication, and would acknowledge your key collaborators.

<u>175 chi</u>	<u>ldren wh</u>	o tested	I PCR p	ositive at	initial as	sessmen	<u>t</u> : age a	at initial	
assessi	nem								
1 ²⁰	2 ¹⁴	3 ^₅	4 ⁴	5 ¹⁶	6²	7	8	9 ³	10⁴
11	12 ²	13²	14	15 ⁹	16	17	18	19	20 ²
23	24 ³	27⁵	29 ⁵	30 ³	31 ³	32 ²	33	34 ²	36 ²
37 ³	38 ⁴	39²	40	43	44	45	46 ²	47 ⁴	49
51	52	54	56	57 ²	59	60 ⁴	62²	63	64
65	66²	68	70	71	72 ³	77	80	82	83
85	86	87 ²	88 ²	90 ²	91				
96 child	lren who	tested	PCR ne	gative at i	initial ass	essment	: age a	at last ne	egative
PCR - a	age at fir	rst positi	ve PCR	l					
1-2	1-14	1-	·29 ²	1-30 ³	1-36	1-44	1	-60 ⁶	1-84
1-91	1-94	l 1-	106	1-138	1-204	2-16	2	-30²	2-32 ²
2-35	2-53	2-	·60⁵	2-113	3-10	3-15 ⁸	² 3	-304	3-33
3-34	3-39	3-	45	4-16	4-21	4-23	4	-25	4-30 ²
4-32	4-35	5 4-	-36	4-46	4-55	4-60 ²	² 5	-12	5-30 ³
5-33	5-35	5 5-	-36	5-40	5-42 ²	5-60	6	-13	6-34
6-39	6-60	6-	·83	7-15	7-59	7-60 ²	² 7	-203	8-39
8-245	9-16	5 10	D-18	10-260	11-29	12-4	51	5-36	21-114

Appendix 3.2 Listing of data used in Section 3.4

Age recorded in days.

29-337 65-98

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Superscript denotes number of children with this pattern, if more than one.

75-96 95-190 98-131 145-271 183-253 183-273

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Appendix 4.1 Listing of data used in Sections 4.2-4.6

Observed					
0 (34,244)	1 (2,10)	2 (1,5)	3 (1,4)	4 (0,3)	5 (0,2)
7 (1,8)	8 (0,3)	10 (1,0)	12 (0,1)	14 (1,1)	15 (2,5)
20 (0,4)	26 (0,1)	30 (2,7)	40 (0,3)	41 (0,1)	45 (2,1)
55 (0,1)	56 (0,1)	60 (2,8)	75 (0,1)	86 (0,1)	90 (2,11)
105 (0,1)	116 (0,2)	120 (4,1)	150 (1,1)	180 (0,2)	210 (2,2)
240 (0,3)	270 (0,1)	330 (0,1)	335 (0,1)	365 (1,2)	455 (1,1)
485 (0,1)	515 (2,1)	545 (1,0)	730 (0,2)	790 (0,1)	1095 (0,3)
Imputed					
1 (1,0)	7 (0,1)	30 (1,1)	40 (0,1)	60 (0,3)	75 (0,1)
90 (0,2) 515 (0,1)	120 (1,0)	180 (1, 0)	210 (1,0)	365 (1,0)	455 (1,0)

Values are duration of breastfeeding in days (no. infected, no. uninfected)

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MEETING REPORT

Estimating the rate of mother-to-child transmission of HIV. Report of a workshop on methodological issues Ghent (Belgium), 17–20 February 1992

François Dabis, Philippe Msellati, David Dunn*, Philippe Lepage[†], Marie-Louise Newell*, Catherine Peckham*, Philippe Van de Perre[‡] and the Working Group on Mother-to-Child Transmission of HIV[§]

Purpose: In the last 8 years, numerous cohort studies have been conducted to estimate the rate of mother-to-child transmission (MTCT) of HIV. Many of these have faced problems in data collection and analysis, making it difficult to compare transmission rates between studies. This workshop on methodological aspects of the study of MTCT of HIV-1 was held in Ghent (Belgium) in February 1992.

Study selection and data extraction: Fourteen teams of investigators participated, representing studies from Central (five) and Eastern Africa (three), Europe (two), Haiti (one) and the United States (three). A critical evaluation of the projects was carried out, under four headings: (1) enrollment and follow-up procedures, (2) diagnostic criteria and case definițions, (3) measurement and comparison of MTCT rates and (4) determinants of transmission.

Results of data analysis: Reported transmission rates ranged from 13 to 32% in industrialized countries and from 25 to 48% in developing countries. However, no direct comparisons could be made because methods of calculation differed from study to study. Based on this review, a common methodology was developed. Agreement was reached on definitions of HIV-related signs/symptoms, paediatric AIDS and HIV-related deaths. A classification system of children born to HIV-1-infected mothers according to their probable HIV infection status during the first 15 months of life, allowed the elaboration of a direct method of computation of the transmission rate and of an indirect method for studies with a comparison group of children born to HIV-seronegative mothers. This standardized approach was subsequently applied to selected data sets.

Conclusions: The methodology can now be applied to all studies with sufficient follow-up and comparisons made between transmission rates. This step is essential for assessing determinants of transmission and for the development of a common approach for the evaluation of interventions aimed at reducing or interrupting MTCT of HIV.

AIDS 1993, 7:1139-1148

Keywords: HIV, children, mother-to-child transmission, methodology, Africa.

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Note: This paper was presented in part at the VIII International Conference on AIDS, Amsterdam, July 1992 [abstract PoC4218]. Sponsorship: This initiative was sponsored by the European Economic Community AIDS Task Force, the European Association for Cooperation, the Global Programme on AIDS of the World Health Organization and the United Nations Children's Fund. Requests for reprints to: Dr F. Dabis, INSERM U 330, Université de Bordeaux II, 146 rue Léo-Saignat, 33076 Bordeaux Cédex, France. Date of receipt: 23 March 1993; accepted: 5 May 1993.

© Current Science Ltd ISSN 0269-9370

1139

Introduction

The worldwide spread of HIV through heterosexual contacts and intravenous drug use has lead to a high prevalence of infection among women of child-bearing age, particularly in Africa [1]. Mother-to-child transmission (MTCT) of HIV, often referred to as perinatal transmission, is a direct consequence of this epidemiological pattern. The World Health Organization (WHO) estimates that by 1992, almost 1 million infected children had been born to HIV-infected mothers since the beginning of the HIV epidemic. Almost half a million have already developed AIDS and subsequently died [2]. A larger number of uninfected children are likely to become orphans because of the loss of one or both parents from HIV disease [1,3].

Many researchers have been engaged in the study of MTCT of HIV since the mid-1980s [4–7]. These studies are necessary for at least five reasons: (1) to estimate the MTCT rate of HIV for demographic projections [1,2]; (2) for planning health resources allocation; (3) to obtain a reliable range of values for MTCT of HIV and to compare rates of transmission in various settings; (4) to understand the determinants of MTCT of HIV so that factors amenable to interventions which could decrease transmission can be identified; (5) to improve individual counselling and the case management of mothers and children.

Investigators of MTCT studies confront many practical problems, including difficulties with survey instruments, definitions used for calculating the rate of transmission and the protocol of clinical and biological follow-up of children. The need for a methodological workshop on MTCT of HIV with special reference to Africa was recognized by several investigators, the European Economic Community (EEC) AIDS Task Force and WHO in 1988 [8]. Most of the ongoing studies had insufficient follow-up at that time to provide definite results for the formulation of recommendations for public health and clinical practice.

A meeting was held in 1992 in Ghent (Belgium) under the auspices of the EEC AIDS Task Force, in collaboration with the WHO Global Programme on AIDS and UNICEF. This workshop included 40 scientists from three continents who were experts in paediatrics, obstetrics, virology, epidemiology and biostatistics. The objectives were: (1) to address methodological issues in the estimation of the rate of MTCT of HIV-1 with special reference to developing countries; (2) to present a critical evaluation of selected perinatal studies using a standardized methodological approach. This report provides a summary of the discussions and recommendations made during the workshop for further analysis and follow-up of ongoing studies and for the direction and design of future studies.

Overview of the completed and ongoing studies

The principal investigators of 14 studies participated in this workshop. Five reviewed studies were from Central Africa [9–14], three from Southern and Eastern Africa [15–17], one from the Caribbean [18], three from the USA [19–22] and two from Europe [23–25]. With the exception of one study in Central Africa [13], these were based in large urban centres where HIV seroprevalence is high. In developing countries, there was a wide range in seroprevalence among pregnant women screened, from 3.9% in Brazzaville (Congo) in 1987–1989 [10] to 30% in Kigali (Rwanda) in 1988–1989 [11]. Few seroprevalence figures were available for industrialized countries.

All studies reviewed included as an objective the measurement of the rate of HIV MTCT and the description of the natural history of paediatric HIV infection. However, only about half were able to collect data on potential determinants of HIV MTCT. All studies started between 1985 and 1989. The availability of early diagnostic tools was limited at the start and varied according to site, influencing the design of these studies. Eight studies enrolled livebirths and six enrolled pregnant women, either during pregnancy or at delivery. Enrollment took place in prenatal clinics or maternity wards.

The number of children born to HIV-seropositive mothers enrolled in each study varied from 112 to 1060 in industrialized countries and from 118 to 679 in developing countries. Only two studies from industrialized countries included a comparison group [21,22], compared with the nine studies in developing countries. Various criteria were used to select the comparison group (next delivery, age, parity, geographical origin, etc.). The number of children born to HIV-seronegative mothers enrolled in the comparison group varied from 40 to 3589 per study. Formal sample size calculation was rarely performed before the study was started.

In all studies, there was a regular clinical follow-up throughout the first year of life but there was more variation in the timing and frequency of blood sampling and in the overall length of follow-up. In most studies, mortality in the comparison group (children born to HIV-seronegative mothers) was lower than anticipated, due to surveillance bias. The different estimations of the rate of HIV MTCT reported ranged from 13 to 32% in industrialized countries and from 26 to 48% in developing countries (Fig. 1). However, in each study, the method used to estimate the MTCT rate of HIV was study-specific.





Rate of transmission (%)

Fig. 1. Reported rates of mother-to-child transmission of HIV-1 (%) in five studies in industrialized countries (\blacksquare) and in nine studies in developing countries (\Box). Ghent, February 1992.

Summary of recommendations

Clinical and laboratory procedures Enrollment

Women should be identified during pregnancy in preference to delivery to study the timing of transmission and all children enrolled at birth. A strict enrollment definition with clear inclusion criteria should be established and all enrolled children described in any publication. Strictly defined exclusion criteria should be used to reduce the loss to follow-up. Women refusing to participate in the study should be excluded, either at the initial enrollment or if they are unwilling to attend the first follow-up. In addition, it may be appropriate to exclude women and children not permanently resident in the catchment area. Children should be excluded if their mother was not tested at br before the time of delivery. Stillbirths should always be excluded from the calculation of the rate of HIV MTCT.

No specific recommendation with regard to sample size could be made because the sample size depends on study-specific objectives. A comparison group is necessary for the calculation of excess mortality of children born to HIV-seropositive mothers (see Measurement and comparison of rates of transmission). Although it was recognized that the morbidity and mortality of the comparison group may not reflect that of the population from which it was derived, both cohorts have the same access to health care and special services provided by the study. It was recommended that the two groups should be made as comparable as possible in terms of maternal age and parity although individual matching was not considered essential. Another justification for a comparison group is to identify maternal seroconversions and to study postnatal transmission [26].

Follow-up

Clinical information should aim to identify paediatric AIDS, HIV-related signs/symptoms and HIV-related deaths (see proposed definitions in Diagnostic criteria and case definitions). Regular specimen collection (blood or other samples) up to at least 15 months of life is important in the analysis of loss of maternal antibody.

The estimation of mortality should always use survival analysis methods (for example, Kaplan–Meier product limit method [27]). An effort should be made to establish causes of death of children who died at home, using verbal autopsies [28]. A standard verbal autopsy report should be used for this purpose.

Loss to follow-up is a major problem in prospective studies and may bias the estimates of MTCT rates. It should be clearly defined and described. Where possible, comparisons should be made between the clinical and immunological characteristics of children lost to follow-up and those not lost to follow-up, in order to look for potential biases.

Diagnostic criteria and case definitions

Clinical assessment

Two paediatric clinical AIDS definitions applicable to settings with scarce diagnostic facilities have been used in the past (Table 1): the WHO clinical definition [29] (the so-called Bangui definition) and a modified version [8]. In the modified version, persistent cough is not included as a minor sign, but recurrent pneumonia is included as a major sign. These definitions have been evaluated cross-sectionally in various African countries using HIV-antibody test results as the gold standard [30-32]. They have shown low sensitivity and positive predictive value. In cohort studies where repeated cross-sectional assessments are performed, the combination of signs and symptoms necessary to classify a child as fulfilling the AIDS criteria should be identified during the same observation period and not by cumulating morbidity experience over time. A comparative evaluation of the original WHO paediatric AIDS clinical definition and of the modified version should be performed within existing cohort studies. Meanwhile, the use of the original WHO case definition should be recommended.

HIV-related signs and symptoms in children born to HIV-seropositive mothers

It is often not possible to establish persistence of signs and symptoms in young infants. It is for this reason that a scoring system was developed in one study, to take into account the relative weight and the dynamics of the symptoms [9]. This scoring system needs to be evaluated against clinical AIDS definitions and new methods for early diagnosis of HIV infection before it is recommended for use in other research projects. Some signs and symptoms have been shown to be highly predictive of paediatric HIV infection. A list of HIV-related signs and symptoms is summarized in Table 2.

Table 1. World	Health Organization	case definitions for	paediatric AIDS*
used for the Gh	ent classification of	paediatric HIV infec	ction, 1992.

WHO clinical case definition for paediatric AIDS [29]
Major signs
Weight loss or failure to thrive
Chronic diarrhoea (>1 month)
Prolonged fever (>1 month)
Minor signs
Generalized lymphadenopathy
Oro-pharyngeal candidiasis
Repeated common infections
Persistent cough
Generalized dermatitis
Confirmed maternal HIV infection
Modified WHO clinical case definition for paediatric AIDS [8]
Major signs
Weight loss or failure to thrive
Chronic diarrhoea (>1 month)
Prolonged fever (>1 month)

Chronic diarrhoea (> 1 month) Prolonged fever (> 1 month) Severe or repeated pneumonia Minor signs Generalized lymphadenopathy Oro-pharyngeal candidiasis

Repeated common infections Generalized pruritic dematitis

Confirmed maternal HIV infection

With both definitions, paediatric AIDS is suspected in a child presenting with a least two major signs and two minor signs in the absence of known causes of immunosuppression.

 Table 2. HIV-related signs and symptoms in children born to HIV-seropositive mothers used for the Ghent classification of paediatric HIV infection, 1992.

Persistent diarrhoea (\geq 15 days)

Oral candidiasis (beyond the neonatal period)

Generalized lymphadenopathy (enlarged lymph nodes in at least two independent anatomic sites)

Failure to thrive (no weight gain for a period of 3 months

or crossing two percentiles lines on the growth chart) Chronic parotitis (> 1 month)

Herpes zoster infection ('shingles')

Recurrent pneumonia (two or more episodes)

HIV-related deaths in children born to HIV-seropositive mothers

For the purpose of calculating the rate of HIV MTCT, it is important to establish whether a child who dies before 15 months is HIV-infected. Three definitions were proposed for children who died before their infection status could be determined by serology (Table 3).

Laboratory assessment

A combination of HIV serum antibody enzyme-linked immunosorbent assay (ELISA) and Western blot (WB) tests was used in most cohort studies. A consensus was reached to assess WB according to the WHO criteria (positive WB if at least the gp41 and gp120/160 bands are reactive). More importantly, a negative WB should be defined by the complete absence of any band. Indeterminate WB consists of all other situations. Some Table 3. Definitions for children born to HIV-seropositive mothers who died before infection status could be determined by serology. Chent classification, 1992.

Probable HIV-related death Either AIDS or At least one HIV-related sign/symptom** when last seen and Dying from severe infection[†] or persistent diarrhoea beyond the first 4 weeks of life‡ Probable non-HIV-related death No HIV-related sign/symptoms** when last seen and Dying from cause other than severe infection⁺ or persistent diarrhoea after the first 4 weeks of life Death with indeterminate relation to HIV infection All deaths occurring within the first 4 weeks of life or Beyond this period, all the deaths not classified above

See definition in Table 1. **See definition in Table 2. [†]It may be necessary to establish a list of severe paediatric infections for this purpose. [‡]Children who died from persistent diarrhoea but do not present any other HIV-related sign/symptom should be considered as a death with indeterminate relation to HIV infection.

questions were raised about the necessity of using a confirmation test (WB) for longitudinal follow-up of children born to HIV-seropositive mothers [33]. Simpler and cheaper methods, such as two different types of ELISA, have been used in some MTCT studies. Different diagnostic strategies should therefore be compared to WB on serum samples collected between 12 and 18 months of age.

There was a consensus to use 15 months of age as the cut-off point for antibody loss for estimating MTCT rates. However, in infants lost to follow-up before 15 months of age, a negative WB result obtained at or after 9 months could be considered as seroreversion in the absence of clinical AIDS. In the proposed definition, such infants are categorized as uninfected with. HIV. The group recognized that a small percentage of HIV-infected infants transiently serorevert before again becoming HIV-seropositive [16,34]. In most studies, the windows of seronegativity have occurred between 3 and 8 months of age with a few exceptions. Omitting the small number of infants with longer windows should not have a significant impact on the overall estimation of the MTCT rate.

Various methods for the early diagnosis of HIV infection in infants born to HIV-seropositive mothers were discussed [viral culture, polymerase chain reaction, p24 antigen, *in vitro* antibody production, HIV-specific serum immunoglobulin A (IgA)] [35]. It was agreed not to integrate any of these criteria in the proposed methods of estimation of the MTCT rate of HIV at the present time. However, the recommendations made at the Sienna meeting held in January 1992 on the early diagnosis of HIV infection in infants, should be used by the Working Group in the future [36]. These new diagnostic tools, particularly HIV-specific IgA antibody tests [37], should first be evaluated in available stored serum samples from existing studies. The confirmation of the diagnostic value of specific IgA antibody tests on large numbers of serum samples collected at 6 and 9 months of age in children born to HIV-seropositive mothers and performed in a single laboratory facility was considered a research priority. It was felt that this result could be used to validate and revise the Ghent classification.

Measurement and comparison of transmission rates General approach to the problem

Ideally, the transmission rate (TR) for MTCT of HIV should be computed as: TR = number of HIV-infected children/total number of children enrolled. Based on the experience of the workshop participants, it was decided that calculations should be made using all the available information for the first 15 months of followip. In studies that do not have serum samples available at 15 months of age, the 12-month cut-off should be used. There are problems in estimating both the numerator (how to define an HIV-infected child?) and the denominator of this rate (how should deaths before 15 months of age and losses to follow-up be considered?).

The definitions proposed in Tables 1 and 3 to classify a child as HIV-infected can be recommended. A classification of children according to their probable HIV infection status was developed and is presented in Table 4 and Fig. 2. This classification should be used to compare cohort studies and to minimize the number of children whose HIV infection status remains indeterminate. The risk of misclassification of HIV-infected children was recognized. In some studies, several children who had seroreverted were shown later to have detectable HIV antibodies again [16,34]. Before taking this into account in the present classification, detailed description of these sequential laboratory results, as well as further laboratory testing using new diagnostic tools, were felt to be necessary in those studies reporting this phenomenon frequently.

Methods for estimating the MTCT rate of HIV

Several methods have been used to estimate the MTCT rate, including: (1) the use of antibody tests results only; (2) the use of antibody test results combined with an estimate of excess mortality observed in children born to HIV-seropositive mothers; (3) the use of antibody test results combined with a clinical assessment of HIV infection status in children without definitive serology (either because they died or were lost to follow-up); (4) the use of other virological and immunological methods. The latter will not be considered further in order to allow a standardized methodology to be applied to many of the studies in developing countries without access to advanced virological testing. Also, quantitative antibody test

Table 4. Chent classification* of children born to HIV-seropositive mothers according to their probable HIV infection status, 1992.

HIV-infected

or

or

01

or

or

HIV WB-antibody positive at 15 months

HIV-related death (see definition in Table 3)

AIDS (see definition in Table 1)

HIV-non-infected

HIV WB-serum-antibody-negative at 15 months or

HIV WB-negative \geq 9 months in a child lost to follow-up without AIDS

HIV WB negative \geq 9 months in a child who died from probable non-HIV-related cause (see definition in Table 3)

Indeterminate HIV infection status

Death before 15 months with indeterminate relation to HIV infection (see definition in Table 3)

Child died of probable not HIV-related cause while WB-positive or indeterminate before 15 months or WB-negative \leq 9 months (when last seen)

Child lost to follow-up while WB-positive or indeterminate before 15 months or WB-negative < 9 months (when last seen)

Child with indeterminate WB and alive at 15 months

This classification is designed to be used in the estimation of the rate of mother-to-child transmission of HIV and the comparisons between studies and not in the management of children born to HIV-seropositive mothers. The use of this classification must be restricted to a cohort of children born to HIV- seropositive mothers, all of whom were born more than 15 months before analysis. All children of the cohort should be accounted for using the above classification. In those studies where other methods of diagnosis such as viral culture are used, the results of these methods should be described if modifying the classification, but should be ignored for the purpose of comparisons. WB, Western blot.

results should be interpreted as negative, positive or indeterminate.

In method 1, clinical information is not considered. Each child is defined as infected (WB-positive after 15 months), uninfected (WB-negative after 9 months) or indeterminate (otherwise). The number of children in these three categories is denoted as n +, n- and n?, respectively. The total size of the cohort of children born to HIV-seropositive mothers is denoted as N. There are three possible ways to estimate the transmission rate (TR). The lower estimate assumes that all indeterminates are uninfected: TR = n + /N. The upper estimate assumes that all indeterminates are infected: TR = (n +) + (n?)/N. The intermediate estimate assumes that indeterminates provide no information on HIV infection status: TR = (n +)/(n +) + (n -). This first method will yield an unacceptably wide range between the lower and upper estimates if a study has a high background or HIV-specific infant mortality rate



Fig. 2. Algorithm used to classify children born to HIV-seropositive mothers according to their probable HIV infection status. Ghent classification, 1992. "See definition in Table 1; "see definition in Table 3. WB, Western blot.

or a high rate of loss to follow-up. In these circumstances, the number of indeterminate children will be very high. Consequently, the use of method 1 is not recommended since most of the studies considered suffer from either or both of these problems.

Method 2 requires a comparison group of children born to HIV-seronegative mothers. It will be referred to as the indirect method. There is no need to make a clinical assessment of HIV infection with this approach. It addresses a weakness of method 1, namely that infected children of HIV-seropositive mothers, the exposed group, may be more likely to die before 15 months than uninfected children of HIV-seropositive mothers. To avoid having to determine whether the death was HIV-related, the comparison - unexposed group is used to model the mortality experience of uninfected children of HIV-seropositive mothers. The indirect method is applicable to settings with a comparison group of at least the same size and similar socio-economic conditions as the exposed group. Let mO (m1) denote the probability of dying before 15 months for children born to HIV-seronegative (seropositive) mothers; these probabilities should

be estimated using survival analysis methods, regarding loss to follow-up as a censoring event [27]. If 12 rather than 15 months is used to define definitive serology, then the probabilities of dying before 12 month should be calculated instead. The excess mortality is computed as: (m1 - mO). Let p denote the proportion of children who are HIV-antibody-positive at 15 months (or 12 months when appropriate). The standard way of estimating the TR by this formula has been [18]: $TR = (m1 \cdot mO) + p$, with p estimating the proportion of HIV-antibody-positive children in the entire cohort, which includes children who died because of HIV infection. However, this effectively counts children who died as a consequence of HIV infection twice. An attempt was made to improve on this method by applying the prevalence of antibody carriage to the total number of children in the cohort minus the estimated number of children who died of causes related to HIV infection. Thus, the preceding formula should be modified as follows:

$$TR = \frac{m1 - m0 + (p \times (l - m1))}{1 - m0}$$

This indirect method does not resolve the problem that loss to follow-up may be related to HIV infection status but lost to follow-up is taken into account in the computation of probabilities of dying in both groups. A key assumption for this method is that uninfected children of HIV-infected mothers have the same mortality experience as children of uninfected mothers. It is very likely that maternal HIV infection per se may have an adverse effect on the mortality of a child. Consequently, the excess mortality and thus the MTCT rate of HIV could be overestimated with the indirect method, although the extent of this bias is difficult to quantify. The 95% confidence interval (CI) of TR obtained with the indirect method can be calculated as: TR \pm 1.96 \times \sqrt{Var} (TR). The variance of the transmission rate, Var (TR), can be calculated as (details are available upon request):

$$\int \text{Var}(\text{TR}) = \left((1-p) \times \text{SR}^2\right) \times \left\{\frac{p}{n} + (1-p) \times \left(\frac{\text{var} S1}{S1^2} + \frac{\text{var} S0}{S0^2}\right)\right\}$$

SO and S1 are the probabilities of surviving in both groups computed by survival techniques, with their variance usually computed by Rothman's formula [38]. SR is the survival ratio: SR = S1/SO = (1 - ml)/(1 - mO). Other information required is: n, the denominator upon which the rate of seropositivity (p) is based, i.e., the number of children born to HIV-seropositive mothers and surviving at 15 months of age.

Method 3 uses the classification of children born to HIV-seropositive mothers according to their probable HIV infection status (Table 4 and Fig. 2). Method 3 will be referred to as the direct method. The three formulas under method 1 can be applied, with n + denoting HIV-infected children, n-denoting HIV-uninfected children and n? denoting children with indeterminate HIV infection status. The success of this method depends on the accuracy of clinical assessment. As most early deaths or early losses to follow-up will be classified as indeterminate HIV infection status, this method will yield disparate lower and upper estimates if there are substantial numbers of such children. The 95% CI of TR obtained with the direct method is easily calculated with the standard methods for proportions:

95% CI (TR) = TR ± 1.96 ×
$$\sqrt{\frac{\text{TR} \times (1 - \text{TR})}{D}}$$

TR is the transmission rate, ranging between 0 and 1; D is the denominator used for its computation, N or (n+) + (n-) depending of the formula used to calculate TR.

Application of the methods of calculation of the transmission rate

The data collected in Kigali (Rwanda) between 1988 and 1990 during an ongoing MTCT study [12] were used to illustrate the use of the three methods previously described. In this cohort, 218 (N) children born to HIV-seropositive mothers and 218 children born to HIV-seronegative mothers were followed from birth. In the group of children born to HIV seropositive mothers, 27 were alive and HIV-antibody-positive at 15 months (n +). At that time, 136 were alive and HIVantibody-negative (n-). Seven had been lost to followup between 9 and 15 months, at a time when they were already HIV-antibody-negative. The remaining 48 children could not be classified as HIV-antibody-positive or negative at 15 months because of early death in 31 or loss to follow-up in 17.

Using method 1, the minimum value was: TR = 27/218 = 12.4% (CI = 8.0–16.8%). The maximum value was TR = (27 + 48)/218 = 34.4% (CI = 28.1–40.7%) and the intermediate value was TR = 27/(27 + 136) = 16.6% (CI = 10.9–22.3%). This shows clearly why the use of method 1 cannot be recommended in a context where the number of indeterminate children is high (48 children or 22% of the cohort).

To use the indirect method (method 2), the following information was obtained using Kaplan–Meier techniques: m1 = 0.1462, mO = 0.0416 and the excess of mortality, m1–mO = 0.1046. The number of children alive at 15 months of age was 174 (n1) in the cohort of children born to HIV-seropositive mothers and 201 (nO) in the comparison group. An estimate of TR can be obtained with the indirect method, using as the prevalence of antibody at 15 months (p), the intermediate value of TR obtained with method 1: p = 27/(27 + 136) = 0.1656. The estimate of TR is therefore TR = 25.7% (CI = 18.8–32.5%). The 95% CI of TR is obtained by computing Var (TR) with the following figures: n = 163, S1 = 0.8539, Var S1 = 0.00059, So = 0.9584 and Var So = 0.00018.

The use of Ghent classification (method 3) for the 218 children born to HIV-seropositive mothers in Kigali gave the following three groups: 46 HIV-infected children, 140 non-HIV-infected and 32 with indeterminate HIV infection status. For the definition of HIV-infected children, the 1989 revision of the WHO clinical definition of paediatric AIDS [8] rather than the 1986 Bangui definition was used. Of the 46 children classified as HIV-infected, 16 were AIDS cases regardless of their HIV serological status (seven of those subsequently died before 15 months of age); 23 were alive, followed up to 15 months and WB-positive at that time; finally, eight died of HIV-related causes before 15 months of age. For the uninfected group, 135 were negative at 15 months and five were lost to follow-up before but were WB-negative at or after 9 months. Thus, the use of the direct method gave the following three estimates of TR: a minimum estimate of 46/218 = 21.1% (CI = 15.7–26.5%), a maximum estimate of (46 + 32)/218 = 35.8% (CI = 29.4-42.1%) and an intermediate estimate of 46/(218-32) 24.7% (CI = 18.5-30.9%). It is worth noting that the intermediate estimate obtained with the direct method is similar to that obtained with the indirect method, with comparable confidence intervals. However, the range of possible values remains wide with both methods. The investigators chose to report the following range of values around their point estimate of 25.7% (upper and lower limits of the 95% CI obtained by the direct method, 18.8–32.5%) [12].

The direct method was also applied to the European Collaborative Study (ECS) dataset (N = 720 children born to HIV-seropositive mothers) using the 1987 Centers for Disease Control case definition of paediatric AIDS [39]. A cut-off point of 18 months was chosen for the interpretation of the positive WB results. The direct method yielded an intermediate estimate of TR of 12.2% (CI = 9.8-14.5%). This range of values includes the transmission rate of 14.4% reported by these investigators using more sophisticated clinical and laboratory criteria [25]. The indirect method could not be applied in this study, which did not have a comparison group.

Application of the direct and indirect methods to other African data sets gave preliminary results in the range of 24% for the Uganda study [14] to 32% for the Malawi study (data not shown). i.e., comparable to the Kigali figures and twice the ECS intermediate estimate. Table 5 summarizes the different estimates of the transmission rate obtained with three datasets. Looking at the intermediate estimates, one can see a substantial difference between the African studies and the European study which cannot be attributed to differences in methodology and sample size.

 Table 5. Range of possible values for the mother-to-child transmission rate (TR) of HIV-1 using two methods of calculation proposed by the Working Group on Mother-to-Child Transmission of HIV. Ghent, 1992.

 Kigali, Kampala and European Collaborative Study (ECS) datasets.

Study TR(%)		Minimum (s.d.*)	Intermediate (s.d.*)	Maximum (s.d.*)	
Kigali	IM	_	25.7% (3.51%)	_	
0	DM	21.1% (2.75%)	24.7% (3.16%)	35.8% (3.27%)	
Kampala	IM	_	26.5% (2.51%)		
	DM	18.7% (1.94%)	24.2% (2.43%)	41.5% (2.46%)	
ECS	DM	10.0% (1.1%)	12.2% (1.2%)	28.3% (1.7%)	

Proposed scheme for data analysis

For the purpose of comparing MTCT rates between studies, the direct method should be applied and results presented with three estimates, each with their 95% CI. For the purpose of internal validation of the measurement of the MTCT rate with several methods in the same dataset, the indirect method should also be applied if an adequate comparison group is available and results presented with a 95% CI. Both methods should be applied to those groups of children born at least 15 months before the date of analysis. This scheme for data analysis can also be applied to MTCT of HIV-2, which has been much less studied until now [40].

Determinants of mother-to-child transmission of HIV-1

Before maternal risk factors for HIV MTCT can be assessed, it is necessary to ensure that the overall estimate of MTCT rate for a particular cohort is reliable, as well as the case-by-case classification of children born to HIV-seropositive mothers according to their probable HIV infection status. For example, a high loss to follow-up could invalidate these results. Few studies in developing countries have included the determination of risk factors for MTCT as a specific objective [41]. Specifically designed studies with systematic and comprehensive data collection in pregnancy, at delivery and in the post-partum period are therefore a priority, with strict enrollment criteria and follow-up procedures designed to minimize loss to follow-up. Finally, the workshop participants recognized the difficulty in determining the exact role of each possible factor during data analysis - confounder, effect modifier, marker or true risk factor. Furthermore, the timing, route(s) and cellular mechanisms by which transmission occurs remained fundamental questions to be answered [42,43].

Factors that have been identified as possible risk factors for HIV MTCT include impaired maternal clinical and immunological status [9,11,25], HIV-seroconversion during pregnancy [26], shortened duration of pregnancy [9,18,44], choriamnionitis [9], vaginal delivery [21], prolonged and/or complicated labour [25] and breast-feeding [45]. Maternal age and parity do not appear to be associated with MTCT in most studies. In Rwanda [12], Kenya [16] and Malawi [17], sexually transmitted diseases were not associated with an increased risk of HIV MTCT. The role of other factors, such as viral characteristics, protective maternal antibodies and genetic factors, is not yet clear [41].

In future, special consideration should be given to the relative importance of each mechanism of transmission and to the study of MTCT of HIV infection through breast-milk [45,46], in particular. Only four studies have included both breast- and formula-fed infants [9,21,23,25]. Three showed an increased transmission rate in breast-fed infants [9,23,25]. However, the magnitude of the risk has not been described accurately and further studies are needed [45-48]. Their design should consider the nutritional and immunological benefits of breast-milk. Post-natal transmission of HIV through breast-mik in women who seroconverted after delivery has been demonstrated clearly [26] and highlights the importance of following-up HIV-seronegative mothers to observe maternal seroconversion and possible subsequent post-natal transmission of HIV.

Acknowledgements

The scientific secretariat acknowledges the help of Dr Davis Hom (Case Western Reserve University, Cleveland) and Paolo Miotti (Johns Hopkins School of Hygiene and Public Health, Baltimore) for providing updated data on their studies. Special thanks to Mr Daniel Commenges (INSERM U 330, Bordeaux) for his invaluable assistance in the development of the statistical aspects of the report. The authors acknowledge the advice and criticisms provided by Professor Neal Halsey (Johns Hopkins School of Hygiene and Public Health, Baltimore), Professor Roger Salamon (INSERM U 330, Bordeaux), Dr Lieve Fransen (EEC AIDS Task Force, Brussels) and Dr Benjamin Nkowane (WHO, Geneva) in their review of the manuscript. This workshop would not have taken place without the encouragement and the support provided by Mr L Guerrato (Commission of European Communities, Brussels), Dr David Heymann (WHO Global Programme on AIDS, Geneva) and Dr James Sherry (UNICEF, New York).

The authors wish to acknowledge the secretariat assistance of Marijke Bontinck (EEC ADS Task Force, Brussels), Mane-Pierre Martin (IN-SERM U 330, Bordeaux) and Dominique Nicolini (Institut du Cerveau, Bordeaux) in the organization of the workshop. Special thanks to Evelyne Bloch, Fara Rafidimanana and Dominique Touchard for their help in the preparation of the manuscript.

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Appendix

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The following persons also participated in the Ghent meeting

Georgette Adjorlolo (Côte d'Ivoire), Jean-Paul Butzler (Belgium), Joan Casanova (EEC AIDS Task Force), Eric Delaporte (France), Joseph Fumbi (UNICEF), William Heyward (WHO Global Programme on AIDS), Normand Lapointe (Canada), Peter Piot (Belgium), Anna-Maria Stevens (Belgium), Marc Tardieu (France) and Marleen Temmerman (Kenya).

FAST TRACK

The sensitivity of HIV-1 DNA polymerase chain reaction in the neonatal period and the relative contributions of intra-uterine and intra-partum transmission

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Objective: To derive reliable estimates of the sensitivity of HIV-1 DNA polymerase chain reaction (PCR) in the neonatal period and to quantify the relative contributions of intra-uterine and intra-partum transmission.

Methods: After reviewing studies on the early diagnosis of HIV-1 infection, investigators were asked to provide published and unpublished PCR test results on prospectively followed, non-breastfed, vertically infected children. Age-specific estimates of the sensitivity of PCR were derived using distribution-free methods for interval-censored data.

Results: Data on 271 infected children were combined for analysis. PCR detected HIV-1 DNA in an estimated 38% [90% confidence interval (CI), 29–46] of HIV-infected children tested on the day of, or day after, birth. Sensitivity was observed to rise rapidly in the second week of life, reaching 93% (90% CI, 76–97) by 14 days of age.

Conclusion: The sensitivity of PCR in the neonatal period is higher than previously reported. This affects the clinical interpretation of an early negative test result and encourages the use of PCR as an endpoint for trials to evaluate interventions to reduce vertical transmission in non-breastfed populations. Approximately one-third of vertically acquired HIV-1 infection could be attributable to intra-uterine transmission.

AIDS 1995, 9:F7-F11

Keywords: Polymerase chain reaction, sensitivity, timing of transmission, vertical transmission

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Date of receipt: 31 May 1995; revised: 23 June 1995; accepted: 27 June 1995.

© Rapid Science Publishers ISSN 0269-9370

Introduction

DNA genome detection by polymerase chain reaction (PCR) is one of the most promising approaches for the early diagnosis of HIV-1 infection in infants [1]. With the development of non-radioactive indicator systems [2], the availability of standardized commercial kits [3], and the potential for testing dried blood filter-paper specimens [3–7], it is increasingly used in clinical and epidemiological settings.

PCR achieves high specificity provided meticulous care is taken to avoid contamination and appropriate quality control procedures are maintained [2,8]. Consequently, a positive test result implies that it is highly likely that the child is HIV-infected. However, negative PCR test results are often observed in neonates subsequently shown to be HIV-infected, probably because levels of circulating viral nucleotides are too low to be detected [9–11]. The interpretation of a negative test result is therefore difficult, and depends primarily on the sensitivity of the assay at the age at which the specimen was obtained [12].

The sensitivity of PCR below the age of 3 months is not clearly defined. One retrospective study, which assayed the archived newborn filter-paper specimens of 67 children (median age, 2 days) subsequently found to be HIV-infected, reported a sensitivity of 52% [5]. However, this study was not designed to provide information on sensitivity at older ages and selectively included children with rapid clinical progression, who appear to show higher rates of PCR positivity in neonatal specimens [5,13,14]. Prospective studies avoid this bias and cover a wider range of ages, but information is often based on small numbers of HIV-infected children. This is due to the limited number of children available for study at any one laboratory, the difficulty in obtaining repeated blood samples from young infants, and the fact that most tests are conducted on children who are subsequently found to be uninfected. In a review of available data in 1992, the sensitivity of PCR and other methods of diagnosis at different ages were assessed [1]. However, no formal statistical analysis was performed and many more studies have subsequently been published. In this study we present age-specific estimates of the sensitivity of PCR. resulting from a collaborative exercise with researchers from 12 centres and, on the basis of this analysis, discuss the relative contributions of intrauterine and intrapartum transmission.

Methods

A literature review was carried out to identify studies in which the role of PCR in the early diagnosis of HIV-1 infection had been examined. We included only prospective studies published since 1990 which described nine or more HIV-infected children. The principal investigators were contacted and information sought on the results of PCR tests, including the exact ages (days) when the blood samples were obtained for vertically infected children meeting all the following criteria: (1) the mother was known to be HIV-infected at the time of delivery; (2) at least one PCR test was performed within the first 3 months of life; and (3) there was no history of breastfeeding. Results on cord-blood specimens are not included in the analysis. Several investigators provided additional unpublished data.

Details of the PCR methods used in the different studies are shown in Table 1. The specimens were usually fresh peripheral blood mononuclear cells (PBMC), although filter-paper specimens, glycigel stored PBMC, and whole-blood lysates were also used. In all studies PCR was directed at HIV-1 DNA sequences using inhouse systems, apart from one study which investigated the Roche Amplicor kit (Roche Diagnostic Systems, Basel, Switzerland). Specimens were always assessed with at least two primer/probe sets. All assays included standard positive reference samples and were capable of detecting at least 1–10 HIV-1 copies per 10⁵ cells.

Sensitivity is conventionally estimated as the rate of PCR positivity, within defined age bands, on samples from HIV-infected children. Here, a more efficient survival analysis approach of the transition from PCR-negative to PCR-positive was employed. The age when this transition occurs is not directly observed but falls between the last negative and the first positive test (interval censoring). For children whose first test is positive, the transition could have occurred at any time between conception and the first test date. Maximum likelihood estimates of sensitivity were derived without imposing a functional relationship with age [23], with likelihood ratio-based confidence intervals (CI) [24].

The assumption that once a child tests positive he or she will always test positive is idealized, and the estimates may therefore be biased upwards. However, for no child was a negative test result observed to follow a positive test result, and any bias is therefore assumed to be minor.

Results

Data were available on 271 HIV-infected children, ranging between nine and 53 per study (Table 1). Before combining the data across studies, sensitivity curves were derived for each site. This did not reveal any clear pattern according to the primers used, the type of sample, or any aspect of laboratory methodology. However, the data are too sparse to conclude that differences do not exist.

At the initial assessment, 175 children tested PCR-positive and 96 tested PCR-negative. All children who initially tested negative subsequently tested PCR-positive. Fig. 1 shows the estimated sensitivity of PCR according to age, with 90% CI. An estimated 38% of infected children tested on the day of birth or day after birth are PCR-positive (90% CI, 29–46). No major change in sensitivity over the first week of life was apparent, but in

Reference	No. children	Sample	No. cells amplified	Duplicate sample	Primers	Probes	No. cycles	Indicator system
Brandt <i>et al.</i> [2,15]	53	Fresh PBMC (< 36 h)	2×10 ⁵ -2×10 ⁶	Retested negatives after 1:2–1:25 dilution with water	SK 29/30 SK 38/39 SK 68/69 SK 145/150	SK31 SK19 SK70 SK102	40	Biotin-based
Krivine et al. [10]	35	Fresh PBMC	2.5×105	Yes	SK38/39 SK68/69 pol3/4		40	Hybridization with digoxigenin (labelled) probe
Cassol et al. [3,4]*	33	Filter-paper specimens (< 3 years)	1.2×10 ⁵	Yes	BioSK431/462 SK145i/150 SK68i/69i2	SK102 SK102 SK70	35	Biotin/ELISA-based and/or autoradiography [32P]
Roques et al. [16]	29	Fresh PBMC (< 15 h)	2×10 ⁵ -1×10 ⁶	Retested if discrepancy with virus culture or between different primers	SK01/39 pol3/4 Elenvnp/cp		35	Autoradiography [³² P]
Borkowsky et al. [17]	24	PBMC	1.5×10 ⁵	Positives retested	SK38/39 SK29/30 SK68/69	SK19 SK31 SK70	30	Autoradiography (32P)
De Rossi <i>et al.</i> [9,11]	20	Fresh (< 24 h) or frozen PBMC	1.0×10 ⁵	Yes	SK29/30 SK38/39 SK68/69	SK31 SK19 SK70	30	Autoradiography [32P]
Newell <i>et al.</i> [18]	19	Glycigel-stored PBMC (< 6 months)	1.0×10 ⁵	Yes	GAG1/2 MH5/6 MH1A/2A POL1/2		35 + 25	Visualization of ethidium bromide stained bands
Denamur <i>et al.</i> [19]	16	Fresh PBMC	1.5×10 ⁵	Positives retested (one primer/probe)	LAV1/2 SK68/69 SK38/39	TARG LAV SK70 SK19	35	Autoradiography [32P]
Ehrnst <i>et al.</i> [20] Sönnerborg <i>et al.</i> [21]	13	Fresh PBMC (< 24 h)	2×10 ⁵	Yes	BLgag1-3 BLpol1-3		30 + 30	Visualization of ethidium bromide stained bands
C. Loveday†	11	Fresh or frozen PBMC	1.0×10 ⁵	Yes	GAG1/2 MH5/6 MH1A/2A POL1/2		35 + 25	Visualization of ethidium bromide stained bands
Comeau et al. [8]	9	Fresh PBMC (< 4 h)	1.5×10 ⁵	Yes	SK38/39 SK68/69		35	Autoradiography [32P]
Guettari et al. [22]	9	Fresh or frozen whole-blood lysates	2.5 × 10 ⁵		3 in-house primers		30 + 30	Direct detection of ¹²⁵ I gamma radioactivity

Table 1. Details of the polymerase chain reaction methods used in the different studies.

*Specimens tested by Roche Amplicor (Roche Diagnostic Systems) and/or in-house system. [†]Unpublished observations from University College London Medical School, London, UK, 1995. PBMC, Peripheral blood mononuclear cells.



Fig. 1. Estimated sensitivity of HIV-1 polymerase chain reaction (PCR; ----) with 90% confidence interval (- - - -).

the second week sensitivity was observed to rise rapidly, reaching 93% (90% CI, 76–97) by 14 days of age. At 28 days the estimated sensitivity was 96% (90% CI, 89–98). Only seven children had negative test results after the

neonatal period, the age at the last negative test ranging between 65 and 183 days.

As information was not sought on children who were subsequently found to be HIV-uninfected, conclusions about the specificity of PCR cannot be drawn.

Discussion

The ability to detect HIV-1 DNA in an HIV-infected child may be influenced by the sample collected and the methodology used. For example, diluting lysates to reduce the effect of inhibitors has been reported to increase sensitivity [15], and the numbers of cells and amplifications may be important for children with low viral load [5]. Although this raises the question of the validity of combining information across the different studies, it was reassuring that no study was clearly atypical.

In an earlier review, which was based on less extensive data, the sensitivity of PCR was assessed to be approx-

imately 50% at 1 month of age and 90% at 3 months of age [1]. Our estimates of sensitivity are much higher and these levels were surpassed by 9 and 13 days of age, respectively, suggesting that an early negative PCR result is of diagnostic significance. It is important to emphasize, however, that the data for this analysis were obtained in the research context by experienced virologists and that caution is required in extrapolating the findings [1]. Also, the extent to which standard primers recognize the geographical variants of HIV-1 is not yet resolved [3]. Late negative test results were observed in several HIV-infected children, some of whom may have been infected postnatally, although the analysis was restricted to children with no history of breastfeeding. Horizontal transmission of HIV-1 has been described but is thought to be rare [25]. Alternative explanations include technical errors and very low levels of circulating virus [10,11].

It has been proposed that isolation of HIV-1 or detection of HIV-1 genome shortly after birth implies intrauterine transmission and conversely, that failure to detect viral markers implies intrapartum transmission [26]. On this basis, the timing of HIV-1 transmission has been explored in a number of small studies [10,11,15,17,20]. This large dataset permits reliable quantitative inference and indicates that 38% of vertically acquired HIV infection could be attributable to intrauterine transmission, with the 46% upper estimate appearing to confirm that most transmission occurs around the time of delivery [20]. However, it is important to recognize several caveats with this approach. First, a positive PCR test shortly after birth could conceivably result from intrapartum transmission of a large inoculum of virus. Second, a negative PCR test does not exclude intrauterine transmission as virus could be sequestered by non-circulating target cells [1,10]. Finally, intrapartum transmission cannot be distinguished from intrauterine transmission in late pregnancy. The rapid rise in the proportion of children with detectable levels of HIV-1 DNA highlights the importance of obtaining specimens soon after delivery in order to examine the issue of the timing of transmission.

To date, epidemiological studies of vertical transmission of HIV-1 have largely relied upon HIV-antibody assays to determine infection status of the children. In common with PCR, high sensitivity can be achieved by virus culture as early as 1 month of age [27], and results from these assays are beginning to be used in primary analyses [6,28]. The appeal of these methods is the ability to reduce the overall duration of the study, and to minimize the proportion of children lost to follow-up before the determination of their infection status. However, the testing of breastfed infants should be continued beyond weaning because of possible acquisition of the virus from breastmilk [29]. As well as being cheaper, a further important advantage of PCR over virus culture in intervention trials and other large-scale studies is the opportunity to use filter-paper specimens. This greatly simplifies the logistics of sample collection, storage, and transport, and facilitates the centralized testing of specimens [3].

Acknowledgements

We would like to thank Dr Tony Ades and Dr Di Gibb (Institute of Child Health, London, UK), Hélène Cavé, Martine Lévine and Etienne Vilmer (Hôpital Robert Debré, Paris), D. Marcé (Commissariat a L'Energie Atomique, Fontenay-aux-Roses, France), Nancy Karthas (Harvard Medical School, Boston, USA), Dr Stanley Read (St. Margaret's Hospital, Nassau), Carol Major (Ontario Ministry of Health, Canada), Dr Normand Lapointe (St. Justine's Hospital, Montreal, Canada), Micheline Fauvel (Quebec Ministry of Health, Canada), Carlo Giaquinto (University of Padova, Italy), Dr Ann-Britt Bohlin and Dr Susanne Lindgren (Huddinge Hospital, Sweden), Dr Keith Krasinski and Dr Henry Pollack (New York University Medical Center, New York, USA), and Professor Richard Tedder and Dr Steve Kaye (University College London Medical School, London, UK).

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Estimating the HIV Vertical Transmission Rate and the Pediatric AIDS Incubation Period From Prospective Data

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This article considers the estimation of the rate of vertical human immunodeficiency virus (HIV) transmission and the pediatric acquired immune deficiency syndrome (AIDS) incubation period using data from birth cohort studies. Standard methods of analysis, which ignore children whose infection status is not established, may lead to biased estimates. Methods based on modeling the disappearance of HIV antibody or appearance of virus are inefficient, as they essentially rely on a single variable. We describe an alternative model that takes into account clinical, immunological, and virological data. Maximum likelihood estimates of rate of vertical transmission, sensitivity of virus tests, pediatric AIDS incubation period, and age distribution at antibody loss are readily obtained by an EM algorithm. The method was applied to data from the European Collaborative Study and revealed evidence of temporal changes in the transmission rate and AIDS incubation period. This new approach, with possible modification, should allow efficient analysis of data from randomized controlled trials of interventions to reduce vertical transmission. It may also be applicable to other vertically transmissible infections.

KEY WORDS: Acquired immune deficiency syndrome; Human immunodeficiency virus; Incubation period; Prospective study; Vertical transmission.

1. INTRODUCTION

This article considers the problems of estimating the rate of vertical (i.e., mother-to-child) transmission of human immunodeficiency virus (HIV) and, for children who acquire the virus, the age distribution at diagnosis of acquired immune deficiency syndrome (AIDS). This distribution, also known as the pediatric AIDS incubation period, has been estimated using data from AIDS surveillance systems (Auger et al. 1988; Downs, Salamina, and Ancelle-Park 1995), sometimes supplemented with data from anonymous neonatal HIV screening programs (DeGruttola, Tu, and Pagano 1992; MaWhinney and Pagano 1994). However, our focus is on prospective studies of children born to known HIVinfected mothers. The analysis of these studies may initially appear straightforward, but complications are introduced by the difficulty in diagnosing HIV infection in young children (Consensus Workshop 1992).

The standard diagnostic test for HIV infection is based on the detection of HIV-specific immunoglobulin class G (IgG) antibody, but because maternal IgG antibody crosses the placenta, all children born to infected mothers initially test antibody positive. Maternal antibody is usually assumed to have cleared by age 15 months or 18 months, although a 12month and a 24-month threshold have also been used (Dabis et al. 1993). In some studies, results of tests that directly measure the presence of the virus (i.e., p24 antigen, virus culture, polymerase chain reaction [PCR]) are also available. A positive virus test result at any age is indicative of infection, but these tests have not generally been regarded as sufficiently sensitive to allow the absence of infection to be inferred from a negative test result. Certain clinical signs and symptoms also provide evidence of infection status, but a definitive diagnosis would usually be made only following the diagnosis of AIDS. Other data that could potentially discriminate between infected and uninfected children, such as CD4 T-cell count or total immunoglobulin levels, generally have not been taken into account.

The first stage in a standard analysis is to classify children as infected, uninfected, or of indeterminate infection status, on the basis of clinical, immunological, and virological information (Dabis et al. 1993). Although standard classification systems have been proposed (e.g., Centers for Disease Control 1987a), different studies have used slightly different criteria (Matheson et al. 1995). The vertical transmission rate is then estimated as the ratio of the number of infected children to the number of infected plus uninfected children (Boylan and Stein 1991). Children whose infection status has not been determined because they were lost to follow-up, died from causes unrelated to HIV infection, or simply were born shortly before the date of analysis do not enter the calculation. This results in a biased estimate, because the unconditional probability of vertical transmission generally is not equal to the probability of transmission given that no infection-defining event has been observed.

The pediatric AIDS incubation period is usually estimated by the product-limit method (Kaplan and Meier 1958), in which the analysis is based on known infected children and children who do not develop AIDS are rightcensored at their last clinical examination (Blanche et al. 1994; European Collaborative Study 1994). Exclusion of indeterminate children again gives rise to biased estimates,

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^{© 1996} American Statistical Association Journal of the American Statistical Association September 1996, Vol. 91, No. 435, Applications & Case Studies

as these children do not enter the risk set at any age despite the fact that some are truly infected and thus at risk of AIDS.

A technique to reduce these biases in ongoing studies is to limit the analysis to children whose infection status should in principle be known. For example, if antibody persistence beyond age 18 months were regarded as proof of infection, then children born within 18 months of the date of analysis would be excluded. A disadvantage of this technique is a loss of information, which may be particularly serious when a comparatively high number of children are born after the inclusion date.

Two alternative methods of analysis that are connected with cure models (Laska and Meisner 1992) have been described. The first method exploited the fact that infected children are persistently HIV antibody positive, whereas uninfected children ultimately lose antibody (European Collaborative Study 1991). Thus the survivor function of the proportion of children who remain antibody positive should, when applied to the entire cohort, asymptote at a level that corresponds to the transmission rate. This method has the advantage of including all antibody test results, irrespective of whether the child had been followed to age 18 months. For children who died due to HIV infection (i.e., nonindependent censoring), the censoring age was set to the age they would have been at the date of analysis had they not died.

A different analytic approach was used in the ACTG-076 trial, which aimed to assess the efficacy of zidovudine in preventing vertical HIV transmission (Connor et al. 1994). Here transmission rates were estimated from a Kaplan-Meier analysis of time to first positive virus culture. A concern with this analysis is that few infected children were followed beyond the age at which the curves asymptoted (24–36 weeks), and the possibility that later events will be observed cannot be excluded. Moreover, because interval censoring was not taken into account, the estimated distribution functions depend on the frequency of testing, although whether this has an important effect on the asymptote is unclear. Also, no justification was given for censoring children at their latest negative *antibody* test.

The objective of this article is to describe a new method of analysis, central to which is the estimation of the probability of infection for every child in the study, conditional on his or her observed data. This entails iterative and simultaneous estimation of the vertical transmission rate, the sensitivities of the virus tests, the AIDS incubation period, and the distribution function of antibody loss, taking into account interval censoring. The distributions are estimated nonparametrically, and there is no need for an arbitrary threshold age at which antibody persistence is taken as proof of infection. By taking children of indeterminate infection status into account, the method avoids the biases intrinsic to the crude estimators described previously. Furthermore, because it utilizes clinical, immunological, and virological data, it should be more efficient than the methods based on cure models, which essentially rely on a single variable. However, in common with the other estimators, the method does require the assumption of independent censoring (Lagakos 1979).

2. PROPOSED METHOD

2.1 The Model

Let π denote the probability of vertical transmission, T_A denote age at AIDS diagnosis, and T_S denote age when the child first becomes seronegative. Although a continuoustime model can be specified and estimated, a discrete formulation is more convenient for the purposes of exposition. Let $g_t = \Pr(T_A = t | T_A > t - 1, infected)$ be the AIDS hazard at age t months given that the child is infected, and let $h_t = \Pr(T_S = t | uninfected)$ be the probability density function of antibody loss at age t months given that the child is uninfected. Let $G_t = \prod_{u=0}^{t-1} (1-g_u)$ and $H_t = 1 - \sum_{u=0}^{t-1} h_u$ denote that survivor functions for AIDS and antibody loss. Finally, up to V different types of virus tests may be applied with sensitivities $s = [s_1 s_2 \dots s_V]$. The tests are assumed to be perfectly specific, so that a child is inferred to be infected on the basis of one or more positive tests. A further assumption is that all virus tests results are independent, conditional on infection status.

It is supposed that each child in a cohort of size N can be classified into one of four mutually exclusive groups (see Table 1). N_A children are observed to develop AIDS, and the age at diagnosis is assumed to be accurate; N_S children become antibody negative (seronegative) at an unknown point between the last positive antibody test and the first negative antibody test; N_V children have not developed AIDS but are positive on one or more virus tests; and the remaining N_C children have not developed AIDS, are never positive on a virus test, and remain antibody positive. This last group will be referred to as censored—which, it should be noted, does not mean the same as indeterminate infection status (see Sec. 1).

The problem can be considered as a mixture model with two states: infected and uninfected. A special feature of the data, analogous to the problem considered by Hosmer (1973), is that state membership is known for some observations. Children who develop AIDS or test virus positive are assumed to be infected, and children who become seronegative are assumed to be uninfected. The contribution to the likelihood of a subject whose state is known is the product of the unconditional probability of belonging to that state and the conditional probability of the observed data. The contribution of a subject whose state is not known is the sum across states of all such terms. Table 1 shows each child's contribution to the likelihood function, ignoring terms that do not involve the unknown parameters.

Consider, for example, a censored child who has not developed AIDS by age a_k , remains antibody positive at age l_k , and who has had, by virus test v, a total of y_{kv} negative results ($v = 1, \ldots, V$). If the child is infected, he provides no information about the antibody loss distribution H, and if he is uninfected, he provides no information about the AIDS incubation period G or about the sensitivities of the virus tests s. His contribution to the likelihood function is

Dunn and Ades: Estimating HIV Transmission and Pediatric AIDS Incubation

Group	HIV infected	Number of children	Required information	Contribution to likelihood
Clinical AIDS (AIDS)	Yes	NA	Age at AIDS diagnosis a_k Number of positive virus tests $x_{k\nu}$ Number of negative virus tests $y_{k\nu}$	$\pi g_{a_k} \prod_{l=0}^{a_k-1} (1-g_l) \prod_{\nu=1}^{V} s_{\nu}^{x_{k\nu}} (1-s_{\nu})^{y_{k\nu}}$
Negative antibody test (SERO)	No	N _S	Age at last positive antibody test I_k Age at first negative antibody test r_k	$(1-\pi)\sum_{t=l_k+1}^{l_k}h_t$
Positive virus test but not AIDS (VIR)	Yes	N _V	Age at last clinical assessment a_k Number of positive virus tests x_{kv} (\geq Number of negative virus tests y_{kv}	1) $\pi \prod_{t=0}^{a_k} (1-g_t) \prod_{\nu=1}^V s_{\nu}^{x_{k\nu}} (1-s_{\nu})^{y_{k\nu}}$
None of the above (CENS)	No assumption	N _C	Age at last clinical assessment a_k Age at last antibody test l_k Number of negative virus tests y_{kv}	$\pi \prod_{t=0}^{a_k} (1 - g_t) \prod_{v=1}^{V} (1 - s_v)^{y_{kv}} + (1 - \pi) \sum_{v=1}^{\infty} h_i$

Table 1. Classification of Children and Information Required by the Proposed Method

NOTE: Subscript k denotes the kth child within a subgroup and subscript v denotes the vth type of virus test.

thus the sum of two components. The first component is the product of the unconditional probability of being infected, π , and of the conditional probabilities of not developing AIDS, $\prod_{t=0}^{a_k} (1-g_t)$, and of always testing virus negative, $\prod_{v=1}^{V} (1-s_v)^{y_{kv}}$. The second component is the product of the unconditional probability of not being infected, $(1-\pi)$, and the conditional probability of remaining antibody positive, $(\sum_{t=l_k+1}^{\infty} h_t = 1 - \sum_{t=0}^{l_k} h_t)$. The contribution to the likelihood function of children in the AIDS, VIR, and SERO groups are derived similarly.

The log-likelihood $L(\pi, s, g, h)$ is the sum of the logarithm of the individual contributions

$$\sum_{\substack{\text{NIDS}\\k}} \left\{ \log(\pi) + \log(g_{a_k}) + \sum_{t=0}^{a_k-1} \log(1-g_t) + \sum_{v=1}^{V} \log(x_v) + y_{kv} \log(1-s_v) \right\} + \sum_{v=1}^{V} \left\{ x_{kv} \log(s_v) + y_{kv} \log(1-s_v) \right\} + \sum_{\substack{\text{NIR}\\k}} \left\{ \log(\pi) + \sum_{t=0}^{a_k} \log(1-g_t) + \sum_{v=1}^{V} \left[x_{kv} \log(s_v) + y_{kv} \log(1-g_t) \right] \right\} + \sum_{\substack{\text{NIR}\\k}} \left\{ \log\left(\pi\right) + \sum_{t=0}^{a_k} \left[1 - g_t \right) \prod_{v=1}^{V} (1-s_v)^{y_{kv}} + (1-\pi) \sum_{t=l_k+1}^{\infty} h_t \right],$$

subject to $0 \le \pi \le 1, 0 \le s_v \le 1, 0 \le g_t \le 1, 0 \le h_t \le 1, \sum_{t=0}^{\infty} h_t = 1.$

2.2 Identifiability

In the absence of virus test data, it can be shown that scaling π , 1 - G, and 1 - H by the factors c, 1/c, and $(1 - \pi)/(1 - c\pi)$ has no effect on the value of the likelihood function. This signifies a lack of identifiability, and the existence of unique maximum likelihood estimates requires that either \hat{G} or \hat{H} be fixed at a point t. However, it suffices to apply the mild constraint that all antibody loss occurs by the oldest age at which an antibody test was performed. In fact, even this constraint is unnecessary if the oldest age at AIDS diagnosis exceeds the oldest censoring age for AIDS, as this removes the indeterminancy in the right tail of \hat{G} (Kaplan and Meier 1958). The issue of identifiability does also not arise if there is at least one child in the VIR category or at least one child in the CENS category who has had a negative virus test result.

2.3 Estimation

(1)

Dempster, Laird, and Rubin (1977, sec. 4.3) showed that finite-mixture models can be cast in the framework of an EM algorithm by introducing unobserved dummy variables to represent state membership. In the E step, the conditional probabilities of belonging to each of the states are estimated for each observation, given current parameter estimates. The contribution of each observation to the complete-data log-likelihood is the weighted sum of the log-likelihood associated with each of the states, with weights given by the conditional probabilities. The M step involves reestimating the parameters by maximizing the complete-data loglikelihood.

2.3.1 *E Step.* The conditional probabilities of state membership are fixed (0 or 1) for children in the AIDS, SERO, and VIR groups. For children in the CENS group, the conditional probabilities of infection are, using Bayes's

938

theorem,

$$\hat{r}_k = \frac{\hat{\pi}\hat{G}_{a_k+1}\prod_{\nu=1}^V (1-\hat{s}_{\nu})^{y_{k\nu}}}{\hat{\pi}\hat{G}_{a_k+1}\prod_{\nu=1}^V (1-\hat{s}_{\nu})^{y_{k\nu}} + (1-\hat{\pi})\hat{H}_{l_k+1}}$$

The complete-data log-likelihood L_C is identical to the observed-data log-likelihood L (Eq. 1), *except* that the contribution from the censored children becomes

$$\sum_{\substack{\text{CENS} \\ k}} \hat{\tau}_k \left[\log(\pi) + \sum_{t=0}^{a_k} \log(1 - g_t) + \sum_{v=1}^{V} y_{kv} \log(1 - s_v) \right] \\ + (1 - \hat{\tau}_k) \left[\log(1 - \pi) + \log\left(\sum_{t=l_k+1}^{\infty} h_t\right) \right],$$

2.3.2 M Step. The M step consists of four parts:

1. Reestimate π by the ratio of the estimated number of infected children to the total cohort size:

$$\hat{\pi} = \left(N_{\rm A} + N_{\rm V} + \sum_{\substack{\rm CENS\\k}} \hat{\tau}_k \right) \middle/ N,$$

which is the solution of

$$\frac{\partial L_C}{\partial \pi} = \frac{N_{\rm A} + N_{\rm V}}{\pi} - \frac{N_{\rm S}}{1 - \pi} + \sum_{\substack{\text{CENS}\\k}} \left(\frac{\hat{\tau}_k}{\pi} - \frac{1 - \hat{\tau}_k}{1 - \pi}\right) = 0.$$

2. Reestimate the components of s by the ratio of the number of positive test results to the estimated total number of tests performed on infected children:

$$\hat{s}_{v} = \frac{\sum_{\text{AIDS}_{k}} x_{kv} + \sum_{\text{VIR}_{k}} x_{kv}}{\sum_{\text{AIDS}_{k}} (x_{kv} + y_{kv}) + \sum_{\text{VIR}_{k}} (x_{kv} + y_{kv})} ,$$
$$+ \sum_{\text{CENS}_{k}} \hat{\tau}_{k} y_{kv}$$

which is the solution of

$$\frac{\partial L_C}{\partial s_v} = \sum_{\substack{\text{AIDS}\\k}} \left(\frac{x_{kv}}{s_v} - \frac{y_{kv}}{1 - s_v} \right) + \sum_{\substack{\text{VIR}\\k}} \left(\frac{x_{kv}}{s_v} - \frac{y_{kv}}{1 - s_v} \right) - \sum_{\substack{\text{CENS}\\k}} \hat{\tau}_k \frac{y_{kv}}{1 - s_v} = 0.$$

3. Reestimate G by the product-limit method where the discrete hazards are based on the *estimated* number at risk of AIDS (estimated number infected) at each age:

$$\hat{g}_t = \frac{\sum_{AIDS_k} I(a_k = t)}{\sum_{AIDS_k} I(a_k \ge t)} ,$$

+
$$\sum_{VIR_k} I(a_k \ge t) + \sum_{CENS_k} \hat{\tau}_k I(a_k \ge t) ,$$

which is the solution of

$$\frac{\partial L_C}{\partial g_t} = \sum_{\substack{\text{AIDS}\\k}} \frac{I(a_k = t)}{g_t} - \sum_{\substack{\text{AIDS}\\k}} \frac{I(a_k \ge t + 1)}{1 - g_t} - \sum_{\substack{\text{VIR}\\k}} \frac{I(a_k \ge t)}{1 - g_t} - \sum_{\substack{\text{CENS}\\k}} \hat{\tau}_k \frac{I(a_k \ge t)}{1 - g_t} = 0,$$

Journal of the American Statistical Association, September 1996

where I is the identity function.

4. There is no closed-form solution for \hat{H} , but a standard algorithm for estimating a distribution function from interval-censored data (Turnbull 1976) can be used. Although the original method assumed that all observations have equal weight, DeGruttola et al. (1992, sec. 2) pointed out that unequal weights are permissible. In this application, the weights are the current conditional probabilities of being uninfected. The method iteratively reallocates the weight associated with each child across all times when antibody loss could have occurred, in proportion to the current estimates of the probabilities of antibody loss for those intervals. Thus the estimate $\hat{\mathbf{h}}^{(i+1)}$ at iteration i+1 is obtained from the estimate $\hat{\mathbf{h}}^{(i)}$ at iteration i by

$$\hat{h}_{t}^{(i+1)} = \sum_{\substack{\text{SERO}\\k:l_{k}+1 \le t \le \tau_{k}}} \frac{\hat{h}_{t}^{(i)}}{\sum_{u=l_{k}+1}^{\tau_{k}} \hat{h}_{u}^{(i)}} + \sum_{\substack{\text{CENS}\\k:l_{k}+1 \le t}} (1 - \hat{\tau}_{k}) \frac{\hat{h}_{t}^{(i)}}{\sum_{u=l_{k}+1}^{\infty} \hat{h}_{u}^{(i)}}, \quad (2)$$

followed by rescaling to ensure that $\sum_{t=0}^{\infty} \hat{h}_t^{(i+1)} = 1$.

The estimation of H is itself an EM algorithm, and when algorithms are nested as here, it is computationally inefficient to drive the inner algorithm to convergence (see Healy's comments in Dempster et al. 1977). For data arising from an exponential family where the M step does not have a closed-form solution, it has been proved that convergence to a local maximum is ensured with a single Newton-Raphson iteration in the M step (Rai and Matthews 1993). Although this problem does not fall within this framework, our empirical observation is that convergence is achieved with one iteration of Equation (2) per cycle of the main EM algorithm.

2.4 Precision of Estimates

Methods have been described for obtaining the variancecovariance matrix for parameters when the complete-data information matrix is more tractable than the observed-data information matrix (Meng and Rubin 1991). In this application, however, partial double derivatives of the observeddata log-likelihood can be evaluated directly. When calculating pointwise standard errors for the product-limit estimator, only observed event times need be considered. Also, the pointwise standard errors of a survivor function estimated from interval censored data are a function of the nonzero elements of the empirical probability density function (Turnbull 1976). Therefore, we need to compute only the derivatives of the nonzero elements of g and h. Work on coverage probabilities of confidence intervals for the product-limit estimator suggests the use of a logarithmic scale for analysis (Miller 1981, sec. 6.1.4.). Thus the AIDS survivor function has been parameterized in terms of $\lambda_t = \log(1 - g_t)$. Then

$$\log G_t = \sum_{u=0}^{t-1} \lambda_u$$

Dunn and Ades: Estimating HIV Transmission and Pediatric AIDS Incubation

and

$$\operatorname{var}(\log G_t) = \sum_{u=0}^{t-1} \operatorname{var}(\lambda_u) + 2 \sum_{u=0}^{t-1} \sum_{\nu=u+1}^{t-1} \operatorname{cov}(\lambda_u, \lambda_\nu).$$

The observed information for π can be expressed as

$$\frac{N_{\rm A}+N_{\rm V}}{\hat{\pi}^2}+\frac{N_{\rm S}}{(1-\hat{\pi})^2}+\sum_{\rm CENS}\left[\frac{\hat{\tau}_k-\hat{\pi}}{\hat{\pi}(1-\hat{\pi})}\right]^2$$

The last term of this expression indicates the extra information gained by taking children of indeterminate infection status into account. For censored children who receive no virus tests and who are last observed before the earliest age at AIDS diagnosis and earliest age at antibody loss, $\hat{\tau}_k = \hat{\pi}$. Thus it is immaterial whether or not these children are retained in the analysis.

2.5 Covariates

There may be interest in assessing the effects of covariates on the model parameters, particularly the vertical transmission risk and the AIDS incubation period. For a discrete covariate, it is straightforward to introduce categoryspecific parameters in the relevant steps of the algorithm. For the transmission risk or sensitivities of the virus tests, the significance of the covariate can be assessed by a likelihood ratio test.

A natural way to assess significance with respect to AIDS incubation period is by the $\sum (E - O)^2/E$ variant of the log-rank test (Peto and Peto 1972), using the *estimated* numbers at risk of AIDS (under the null model). This procedure will be somewhat underconservative, as it does not take into account uncertainty in the estimated numbers at risk. In the general case, the log-rank test can be derived as a score test under the proportional hazards model (Breslow 1975). However, in this application it is not possible to regard the underlying hazard as a nuisance distribution that can be conditioned out, because of the contribution to the likelihood of the censored children.

Finkelstein (1986) developed a proportional hazards model for interval censored data that could be considered for the antibody loss distribution. It would not be straightforward to modify the EM algorithm to accommodate this model, and direct numerical maximization of the likelihood function might need to be considered. Also, an intrinsic feature of this model (unlike the analogous model for exact or right-censored data) is the need to estimate the baseline distribution. This could affect the stability of the estimates of the covariate coefficients, particularly if age was recorded on a fine scale.

3. EXAMPLE

3.1 Description of the Study

Since 1985, pediatricians in 10 centers participating in the European Collaborative Study have prospectively reported data on children born to mothers known to be HIV infected at the time of delivery (European Collaborative

Study 1994). In principle, children are examined clinically and tested for HIV antibody every 3 months until age 2 years and every 6 months thereafter. Virus tests are encouraged whenever an adequate sample is available, provided that laboratory expertise and facilities exist. This analysis utilizes the results of PCR and virus culture assays performed after the first month of life; the age restriction is applied to allow for the rapid rise in sensitivity after birth (Dunn et al. 1995; McIntosh et al. 1994). The study has consistently used the 1987 Centers for Disease Control (CDC) pediatric AIDS definition (Centers for Disease Control 1987b) with two exceptions: It excludes children with asymptomatic lymphoid interstitial pneumonitis diagnosed by X-ray only and includes children considered to have died as a consequence of HIV infection who did not fulfill the precise AIDS case definition.

A total of 977 children had been recruited by the date of analysis (February 1995), excluding twins or children with an older sibling in the study. Of these, 41 children had developed AIDS (range age 2–74 months), and 639 children had become seronegative. The earliest negative antibody test was observed at 2 months (3 children), and the latest positive test at 21 months (1 child). Ignoring tests performed on the seronegative children, there were 591 virus cultures and 229 PCR assays. One or more positive virus test results were observed for 68 children who had not developed AIDS. The remaining 229 children were censored.

The proposed method requires that each child be classified into one of the groups in Table 1, but in practice inconsistencies arise, even after eliminating obvious laboratory errors. In these data, 5 apparently infected children were transiently HIV antibody negative, 2 children lost HIV antibody after developing AIDS, and 13 children had one or more positive virus tests despite becoming consistently antibody negative. Similar inconsistencies have been observed in other studies, and explanations for them have been conjectured (Bryson 1995; Simpson and Andiman 1994).

To resolve these inconsistencies, we applied the following hierarchical rule:

1. If a child develops AIDS, then classify him or her in the AIDS group.

2. Otherwise, if the *last* antibody test is negative, then classify him or her in the SERO group.

3. Otherwise, if any virus test is positive, then classify him or her in the VIR group.

4. Otherwise, classify him or her in the CENS group.

Giving precedence to AIDS diagnoses over laboratory findings is consistent with the CDC classification of HIV infection (Centers for Disease Control 1987a), and serological tests are at present judged more reliable than virological tests. This hierarchy could be reordered without changing the general form of the likelihood. However, in studies in which the number of inconsistencies is small, such as the European Collaborative Study, results will not be sensitive to the precise choice of hierarchy. The method of Section 2.1 was developed in a context in which the clinical diagnosis of AIDS can reasonably be assumed to be 100% specific for HIV infection. In settings with high underlying infant



Figure 1. Estimates of the Survivor Functions for AIDS (\bullet), Antibody Loss (\blacksquare), and of the Conditional Probability of Infection for a Child Age t Months Who is Antibody Positive, has not Developed AIDS, and has Never Received a Virus Test (\blacktriangle).

morbidity and mortality rates (e.g., parts of Africa), this assumption would not be valid, and general modifications to the method would be required.

3.2 Results

The EM algorithm yielded maximum likelihood (ML) estimates of .153 (standard error [SE] .012) for the vertical transmission rate, .768 (SE .022) for sensitivity of viral culture, and .920 (SE .029) for sensitivity of PCR. Figure 1 shows the estimated survivor functions for antibody loss and AIDS up to age 24 months. Figure 2 extends the AIDS survivor function up to age 48 months and indicates the precision of pointwise estimates. Strictly speaking, these distributions should be represented as step functions, but for clarity, the age-specific estimates have been connected with straight lines. An estimated .150 (90% confidence interval of .097-.199) of infected children are diagnosed with AIDS by age 6 months, with a fairly constant hazard thereafter (approximately .08 per year). The median duration of antibody persistence among uninfected children is 11 months, with all children losing antibody by 22 months of age.

Figure 1 also shows the conditional probability of infection for a child who is antibody positive, who has not developed AIDS, and for whom no virus test results are available. This function remains relatively flat until age 10-11 months before rising rapidly. The data rule as infected all children who test antibody positive after 22 months. The effect of each negative virus test result is to scale the odds of infection by (1 - sensitivity); that is, by .232 for a negative virus culture result and by .080 for a negative PCR result.

These analyses have assumed that all parameters remained constant over the duration of the study. Using the methods described in Section 2.5, the 415 children born in 1985–1988 were compared to the 562 children born in 1989–1994 with respect to the rate of vertical transmission and the AIDS incubation period. Under the assumption of a stationary incubation period, the estimated transmission rate was .122 for 1985–1988 and .177 for 1989–1994 (likelihood ratio $\chi^2 = 4.92, P = .03$). Figure 3 compares the



Figure 2. Estimate of the Survivor Function (Logarithmic Scale) for AIDS (•) with 5% and 95% Confidence Limits (•). Numbers at the top of the figure represent the estimated number of children at risk of AIDS at 0, 6, 12, ... months.

AIDS incubation periods between the two cohorts, allowing time-specific transmission rates. This appears to indicate that rapid progression to AIDS was more common in the earlier period but that the hazard rates at older ages are fairly similar. An overall comparison provides moderate evidence of a difference between the AIDS incubation periods (log-rank $\chi^2 = 2.85$, P = .09). A more significant result is obtained by restricting the comparison to the first 6 months of life (log-rank $\chi^2 = 7.14$, P = .008), although a posteriori analyses should be interpreted cautiously. With time-specific AIDS incubation periods, the estimated transmission rates were .122 and .178.

3.3 Comparison with Crude Estimators

In drawing comparisons with the crude estimators described in Section 1, different critical ages at which anti-



Figure 3. Estimates of the Survivor Function (Logarithmic Scale) for AIDS Among 415 Children Born 1985–1988 (•) and 562 Children Born 1989–1994. Numbers at the top of the figure represent the estimated number of children at risk of AIDS at 0, 6, 12, . . . months. The top line refers to 1985–1988; the bottom line, to 1989–1994.

Dunn and Ades: Estimating HIV Transmission and Pediatric AIDS Incubation

		Including virus test data		Excluding virus test data		
		$\hat{\pi}$	Ĝ ₂₄	$\hat{\pi}$	Ĝ ₂₄	
Maximum likelihood estimates		.153 (.012)	.742 (1.056)	.150 (.014)	.738 (1.058)	
Crude estimates						
Critical age	Restricted*					
15 months	No	.172 (.014)	.740 (1.055)	.146 (.013)	.705 (1.064)	
	Yes	.169 (.014)	.738 (1.056)	.146 (.013)	.705 (1.064)	
18 months	No	.171 (.014)	.738 (1.056)	.141 (.013)	.695 (1.067)	
	Yes	.165 (.013)	.732 (1.057)	.141 (.013)	.695 (1.067)	
21 months	No	.169 (.014)	.734 (1.057)	.134 (.013)	.677 (1.072)	
	Yes	.158 (.013)	.721 (1.060)	.135 (.013)	.677 (1.072)	
24 months	No	.168 (.013)	.732 (1.058)	.132 (.012)	.670 (1.074)	
	Yes	.158 (.013)	.719 (1.061)	.134 (.013)	.670 (1.074)	

Table 2. Vertical Transmission Rate and Proportion of Infected Children Who Have Not Developed AIDS By Age 24 Months

* Analysis restricted to children born at least 15, 18, 21, or 24 months before date of analysis.

NOTE: Values in parentheses are SE($\hat{\pi}$) and exp(SE(log \hat{G}_{24})). An approximate (1 - α) confidence interval for G_{24} is derived by dividing and multiplying the point estimate by exp(SE(log \hat{G}_{24})) raised to the power $\Phi^{-1}(\alpha/2)$.

body persistence is regarded as proof of infection were examined (Table 2). Considering first an analysis based solely on AIDS diagnoses and antibody test results (i.e., excluding virus test results), the crude estimates of the vertical transmission rate are lower than the ML estimate (under the proposed model) of .150, the difference being more pronounced the older the critical age. The reason for this is that the crude estimator ignores children of indeterminate infection status, who as they become older are more and more likely to be infected (Fig. 1). However, this can be compensated for by choosing a critical age that is too early, thereby including as infected a number of children who will eventually lose antibody. But it would be difficult to justify using a 15-month or 18-month threshold, as maternal antibody was detected after these ages. Conversely, the crude estimates of the AIDS survivor function are too low (progression rate too high) because the number of children at risk of the event has been underestimated. Excluding children who had not reached the critical age for antibody persistence by the date of analysis did not alter the estimated AIDS incubation period (no child born within 24 months of the data of analysis had developed AIDS) and had only a marginal effect on the estimated transmission rate.

As the standard method of analysis takes account of positive virus test results but ignores negative results, the inclusion of virus test data must increase estimates of the transmission rate and decrease estimates of the rate of progression to AIDS. However, the estimates of the transmission rate are now too high and the rate of progression to AIDS is still overestimated, although the bias in the latter is now less severe. The exclusion of recently born children had a greater effect than in the analysis in which the virus test data were not considered, bringing estimates of the transmission rates closer to the ML estimates but increasing the bias of the estimated AIDS incubation period.

3.4 Computing

For the main analysis in Section 3.2, the log-likelihood changed by less than 1.0×10^{-10} after 650 iterations. This

required 6 seconds of CPU time on a Fujitsu VPX240 vector processor. No attempt was made to optimize the starting values, which were obtained simply by setting the conditional probability of infection for all censored children, $\hat{\tau}_k$, equal to .5 and proceeding with the *M* step of the algorithm. It is noted that using a finer age scale would have resulted in commensurately longer CPU time.

4. DISCUSSION

Among the large prospective studies, the European Collaborative Study has been notable for its low rate of vertical transmission. This analysis provides evidence of a rise in the rate in recent years, which is now close to estimates from other cohorts in Europe and the United States, although still lower than figures reported from Africa (Working Group on Mother-to-Child Transmission of HIV 1995). Because the risk of vertical transmission depends on the degree of maternal immune suppression (European Collaborative Study 1992), it would be surprising if the transmission rate did not change as the epidemic evolves. These results from the European Collaborative Study may not be generalizable, as the collaborating centers were not randomly chosen and there are many pregnant women with an unrecognized HIV infection (Holland et al. 1994), who by definition cannot be enrolled in the study.

Even in the largest cohort studies, an insufficient number of children have been observed to progress to AIDS to allow reliable characterization of the incubation period. Although comparatively precise estimates have been derived (at least for the shape of the distribution) using data from AIDS surveillance systems, these data have a number of limitations (DeGruttola et al. 1992). Among these are the periodic revisions in the pediatric AIDS case definition, and comparisons between studies must take account of differences in definitions. Of the 41 AIDS cases according to the criteria used in European Collaborative Study, 3 died before fulfilling the 1987 CDC definition and presumably would not have been notified. Conversely, our analysis excluded children with asymptomatic lymphoid interstitial pneumonitis on the grounds that AIDS is supposed to reflect a clinically significant manifestation of HIV infection. This condition was responsible-for 26% of initial pediatric AIDS-defining diagnoses in the United Kingdom up to April 1995 (Fiona Holland, personal communication).

The finding of an inflexion point in the AIDS hazard rate at around age 6 months is consistent with other reports (Auger et al. 1988; DeGruttola et al. 1992), although this effect was much less striking among children born in the later part of the study. The reasons for this are unclear, although one possible explanation is an improvement in the clinical management of young infants. Analyses of routine AIDS data have generally assumed that the incubation period is stationary. Our analysis points to a need for critical examination of this assumption, although with retrospective data it is possible only to compare incubation periods conditional on developing AIDS by a given age (DeGruttola et al. 1992).

The algorithm is not guaranteed to converge to estimates that are biologically reasonable. For example, unsatisfactory estimates of the antibody loss distribution may be obtained if either the number of seronegative children is small relative to the number of censored children or there are children who are antibody positive beyond, say, age 2 years who have repeatedly tested virus negative. This gives rise to a "conflict" between the antibody test results and virus test results, and if the latter predominate, then the antibody loss distribution may have a too-long tail. Although these problems are unlikely when modeling extensive data, modifications to the method may need to be considered for smaller data sets, such as estimating the antibody loss distribution parametrically or imposing a smoothness penalty. With the European Collaborative Study data, fitting a normal distribution with a power transformation of age made virtually no difference to estimates of the transmission rate or of the incubation period (results not shown).

In common with other estimators, our method assumes that the censoring mechanism is independent of infection status, time to AIDS (if infected), and time to antibody loss (if uninfected). But it is not possible to directly examine these assumptions (Lagakos 1979). Children can be censored either because they were born shortly before the date of analysis or because they were genuinely lost to followup. For the former group the assumptions would appear reasonable, but for the latter group there is concern that ill children, including a disproportionate number of HIVinfected children, may be more likely to remain in contact with the study pediatrician. If this is true, then the vertical transmission rate and the rate of progression to AIDS would both be overestimated.

Survival analysis was used for virus culture data in the ACTG-076 trial (Connor et al. 1994). This implicitly assumes a transition age for each infected child, before which he or she always tests negative and after which he or she always tests positive. In contrast, our model assumes that each virus test has a fixed sensitivity that is independent of age (after the neonatal period). This is obviously a simplification, but it would be extremely difficult to model all

the technical facets of the assays that influence sensitivity. A more productive development would be to include test results on neonates and-to model the effects of age. It is also plausible that some infected children, averaging over a series of virus test assessments, are more likely to test positive than others. Failure to model "frailty" would result in attaching too much diagnostic significance to *multiple* negative test results, thereby underestimating the transmission rate. Furthermore, estimates of marginal sensitivity would be affected if virus tests were selectively performed on infected children who were more, or less, likely to test positive.

We found differences between the crude estimates and the ML estimates under the proposed model that were of moderate size (Sec. 3.3). This partly reflects the long duration of the European Collaborative Study (10 years), so that the infection status of a large majority of the enrolled children has been established. The method that we have described should be particularly useful in studies of shorter duration, particularly in the efficient analysis (possibly interim) of randomized controlled trials to reduce vertical transmission. Finally, although the method has been developed in the context of HIV, it may also be applicable to other vertically transmissible infections, such as hepatitis C virus.

[Received August 1993. Revised February 1996.]

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Dunn and Ades: Estimating HIV Transmission and Pediatric AIDS Incubation

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