The proportion of HIV incidence due to unsafe injections, unsafe blood transfusions and mother to child transmission in rural Masaka, Uganda

Final report for UNAIDS

London School of Hygiene and Tropical Medicine
London
UK
29th Oct 2004

Richard G White[1], Anusha Kedhar[1], Kate K Orroth[1],
Sam Biraro[2], Rebecca Baggaley[5], Jimmy Whitworth[1,2],
Eline L Korenromp[3,4], Marie-Claude Boily[5,6], Richard J Hayes[1]

[1] London School Of Hygiene and Tropical Medicine, London, UK
[2] Medical Research Council Programme on AIDS, Uganda Virus Research Institute, Entebbe, Uganda
[3] World Health Organization, Roll Back Malaria Department, Switzerland
[4] Erasmus Medical Center, University Medical Center, Dept. of Public Health, Rotterdam, The Netherlands
[5] Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College, London
[6] Unité de recherche en santé des populations, Hôpital du Saint-Sacrement, Centre Hospitalier Affilié Universitaire de Québec, 1050, Chemin de Sainte-Foy, Québec G1S 4L8, Canada

Abstract

Objectives

To estimate the proportion of all-age HIV incidence attributable to unsafe injections, unsafe blood transfusions and mother-to-child transmission (MTCT) in rural Masaka, Uganda, during the early 1990s.

Methods

Data: Observed HIV incidence and prevalence, and injection and transfusion rates were calculated using data from a general population cohort study in Masaka (1989-2000). Injection and blood transfusion safety was estimated from observational surveys within Uganda and East Africa. HIV transmission probabilities were estimated from scientific literature review. Model: A model was used to estimate the incidence via unsafe injections (assuming random or age-dependent mixing of injection equipment) and unsafe transfusions. An age-specific model of fertility was used to estimate the incidence via MTCT.

Results

Unsafe injections accounted for 5.1% [95% uncertainty bounds (UB) 0.0-10.3] or 12.4% [95%UB 0.0-27.0] of all-age HIV incidence in the random and age-dependent mixing scenarios respectively. Unsafe blood transfusions accounted for 0.4% [95%UB 0.2-0.6], and MTCT accounted for 23.4% [95%UB 15.3-31.5]. 64-71% of all-age HIV incidence was left unexplained by these three routes of transmission.

Among 13+ year olds, unsafe injections accounted for 1.4% [95%UB 0.0-2.8] or 12.1% [95%UB 0.0-26.5] of HIV incidence in the random and age-dependent mixing scenarios respectively. Unsafe blood transfusions accounted for 0.3% [95%UB 0.1-0.4], leaving 87.6-98.3% of HIV incidence left unexplained by these three routes of transmission.

Conclusion

This study does not support the hypothesis that unsafe injections or blood transfusions played a major role in HIV transmission in this population during the study period. The safety of both injections and transfusions should be improved to reduce HIV transmission via these routes still further, but particular efforts should be made to reduce the larger proportion of HIV transmission due to MTCT, and among 13+ year olds, the unexplained incidence, presumably primarily due to sexual transmission.

Table of contents

List of tables

List of figures

Abbreviations

CI	Confidence interval
CSW	Commercial sex worker
GUD	Genital ulcer disease
HIV	Human immunodeficiency virus, type 1
MTCT	Mother to child transmission
OR	Odds ratio
PAF	Population attributable fraction
pppy	Per person, per year
pyar	Person-years at risk
RR	Rate ratio
SD	Standard deviation
STI	Sexually transmitted infection
UB	Uncertainty bound
WHO	World Health Organization
y	year

1. Introduction

1.1. Rationale

Most scientists have assumed that heterosexual transmission is the predominant route of HIV transmission in sub-Saharan Africa, and that unsafe injections and the use of other inadequately sterilised skin-piercing instruments have caused less than 5% of HIV-1 infections in the region (Chin et al. 1990; Hauri et al. 2004). Recently, however, a group of scientists have suggested that unsafe medical injections may be a major route of HIV transmission in sub-Saharan Africa (Gisselquist et al. 2002b; Gisselquist et al. 2002a; Gisselquist 2002; Brewer et al. 2003; Gisselquist et al. 2003a; Gisselquist et al. 2003b). The plausibility of these claims have been discussed in detail (Boily et al. 2003; Schmid et al. 2004). This study aims to contribute to the debate by assessing the contribution of unsafe injections, unsafe blood transfusions and mother-to-child transmission to the HIV incidence in a specific African population in rural Masaka, Uganda, from which high quality population based cohort data are available.

1.2. Objectives

To estimate the proportion of HIV incidence due to unsafe injections, unsafe blood transfusions and mother-to-child transmission in rural Masaka, Uganda

2. Methods

2.1. Definitions

An *unsafe injection* was defined as the reuse of unsterilised injection equipment (syringe and/ or needle) that had previously been used on another individual, and a *contaminated unsafe injection* was defined as the reuse of unsterilised injection equipment previously used on an HIV infected individual. Similarly, an *unsafe blood transfusion* was defined as a transfusion of blood or blood products from a donor to the recipient in the absence of screening, and a *contaminated unsafe blood transfusion* was defined as the transfusion of unscreened blood or blood products from a HIV infected donor into a patient.

2.2. Data

Masaka District is in rural southwest Uganda and has a population of 874,200, residing in 119 administrative units, each with approximately 10 to 12 villages. As of 2000, there were 3 hospitals, 46 health units, and 937 hospital beds.

Data on injection and transfusion rates and HIV prevalence and incidence were obtained from a rural general population cohort in Masaka. The study population and study methods have been described in detail previously (Kamali et al. 2000; Mbulaiteye et al. 2002; Whitworth et al. 2002). In

brief, it is an ongoing open cohort of all children (0-12 years old) and adults (13+ years old) who are resident in a cluster of 15 neighbouring villages. The present study used data from annual survey rounds between 1989 and 2000. All ages were eligible to be surveyed and serotested at all rounds between 1989 and 1993, and again in 2000. Injection and blood transfusion safety were estimated from independent clinic-based surveys within Uganda and East Africa. Transmission probabilities for contaminated injections, contaminated blood transfusions and mother to child transmission among HIV infected mothers were obtained from scientific literature review.

2.3. Modelled HIV incidence due to unsafe injections, unsafe transfusions and mother-to-child transmission

2.3.1. Unsafe injections and unsafe blood transfusions

The modelled HIV incidence due to exposure to unsafe injections or unsafe blood transfusions was estimated using two separate models of the form:

$$I = 1 - (1 - p_c * p_t)^{n_u}$$

Where, I is the modelled annual incidence risk among HIV negatives due to unsafe injections or unsafe blood transfusions, p_c is the probability that an unsafe injection or unsafe blood transfusion is contaminated, p_t is the probability of transmission from a contaminated unsafe injection or a contaminated unsafe blood transfusion, and n_u is the number of unsafe injections or unsafe blood transfusions per person per year. Where,

$$n_u = n * p_u$$

Where n is the number of injections or blood transfusions per person per year and p_u is the probability that an injection or blood transfusion is unsafe.

Incidence risks per year were calculated for 0-4, 5-12, and 13+ years olds, and for all-ages.

2.3.2. Mother-to-child transmission

The annual HIV incidence risk I among HIV negative 0-4 year olds due to mother to child transmission (MTCT) was calculated by estimating the annual number of MTCT infections among 0 to 4 year olds and dividing this by the number of person years among 0-4 year olds:

$$I = \frac{r * p_t * \sum_{MothersAge} s * F}{N}$$

Where *r* is the ratio of fertility among HIV infected to HIV uninfected women, p_t is the probability of MTCT of HIV from HIV infected mothers, *s* is the number of HIV infected women by age, *F* is the age specific fertility rate among HIV uninfected women, and *N* is the annual person years at risk among HIV negative 0-4 year olds. We assumed all MTCT transmission occurred among 0-4 year olds, including transmission that occurred prior to birth.

The modelled incidence risks from all three routes of HIV transmission were converted to rates for comparison with empirical data using the standard formula:

Rate = - ln (1 – Risk)

2.3.3. Model implementation

The model was implemented in Microsoft Excel 2002 (Microsoft Corporation 2002). Model parameter values were randomly selected using Latin Hypercube sampling from uniform distributions between the quantified minimum and maximum bounds (*'Parameter constraints'* in Table 3, section 2.5), using Crystal Ball v5.5 (Decisioneering 2004). Each randomly selected combination of parameters (*parameter set*) was used to calculate the modelled HIV incidence attributed to each route of transmission in each age group.

The mean HIV incidence in each age group from each of the three routes of transmission was calculated. The total modelled HIV incidence in each age group was the sum of these three means. The all-age HIV incidence from each route of transmission was calculated as the sum of the mean incidence in each age group weighted by the population size of each age group. The total all-age incidence was calculated as the sum of the all-age HIV incidence from each route of transmission.

Sufficient model iterations were performed to allow us to be 95% certain that the modelled mean HIV incidence for each age group was within 1% of the 'true' value the model would predict if the whole parameter space was exhaustively sampled.

A parameter set was rejected if the modelled total HIV incidence in any age group was greater than the upper bound of the 95% confidence interval (CI) associated with the observed HIV incidence in that age group (see *'Validation constraints'* in Table 3, section 2.5). To allow for the possibility that the sum of the HIV incidence from these three routes of transmission was not sufficient to explain the observed HIV incidence in any age group, a parameter set was not rejected if the modelled HIV incidence was smaller than the lower bound of the 95% CI.

For each parameter, the 'best fitting' value was defined as the mean of all the parameter values that were consistent with the parameter and validation constraints, when all parameters were allowed to

vary simultaneously. To summarise the variability of model predictions of HIV incidence that result from uncertainty in the parameters (ie from the plausible ranges we assumed for the parameters), we also report the *95% uncertainty bound* (95%UB) defined as the modelled mean HIV incidence ± 1.96 * the standard deviation of the modelled HIV incidence predicted by all parameter sets used to calculate the mean.

In scenarios in which the sum of the modelled mean HIV incidence via all three routes was smaller than the observed mean HIV incidence in any age group, the difference was attributed to 'Other causes'. This allowed us to calculate the proportion of all-age observed HIV incidence the model could explain by transmission via unsafe injections, unsafe transfusions and MTCT, and thus the proportion of observed HIV incidence left unexplained by these three routes of transmission.

2.4. Model quantification

We quantified the model by analysing and collating data to allow us to propose model parameter and validation constraints.

2.4.1. Observed HIV incidence and prevalence

We calculated HIV prevalence for the age groups surveyed at each survey round between 1989 and 2000. For each annual survey round, age- and sex-specific prevalence were derived from the number of resident children and adults testing HIV positive and negative at that round. Individuals with missing serology were excluded from the analysis.

We calculated all-age seroincidence rates for the three inter-survey periods between 1989 and 1993. All children and adults identified as HIV negative at a given survey round who had a further definitive HIV test result at one or more subsequent rounds were eligible for inclusion in the incidence analyses. Serostatus and person-years were imputed if missing between two negative HIV test results. Date of seroconversion was imputed for cases with missing data between a negative HIV test result and a positive HIV test result. Dates of seroconversion for all incident events were estimated as the midpoint between the date of the last negative and first positive HIV test result. Person-years at risk (pyar) commenced at the date when an HIV-negative individual first gave a blood sample and ceased at the date of their last blood sample, or, for seroconverters, their estimated date of seroconversion.

These incidence rates were then adjusted to include the additional cases of HIV transmission among infants (children less than 1 year old) who were already HIV-positive at their first sero-survey. When these infants are not added to the seroconversions observed in between repeated surveys, the proportion of HIV transmission due to MTCT is grossly underestimated. In this analysis, all infants found seropositive for HIV were defined as incident HIV cases. They were

assumed to have been surveyed at 0.5 years old, and to have seroconverted at 0.25 years old, and therefore have contributed 0.25 of a year at risk before seroconversion.

As the numbers of seroconversions in each age group were small, exact 95% confidence intervals were calculated using the Poisson distribution.

An average of 3958 adults aged 13 years and over were censused as resident and bled annually between 1989 and 2000. HIV prevalence among adults has been declining steadily from the mid 1990s, from around 8% in the early 1990s to 6% by the end of the decade (Figure 1). An average of 1513 0-4 year olds and 2406 5-12 years olds were censused as resident and bled between 1989 and 1993 and again in 1999/2000. HIV prevalence declined from 1.5% to 0.8% among 0-4 year olds during the early 1990s, but had not declined further by 1999/2000. HIV prevalence among 5-12 year olds increased from 0.4% to 0.9% in the early 1990s, but had fallen again to 0.4% by the late 1990s.

All-age HIV prevalence was relatively stable at around 4.5% in the early 1990s, but had fallen to 3.5% by the end of the decade.

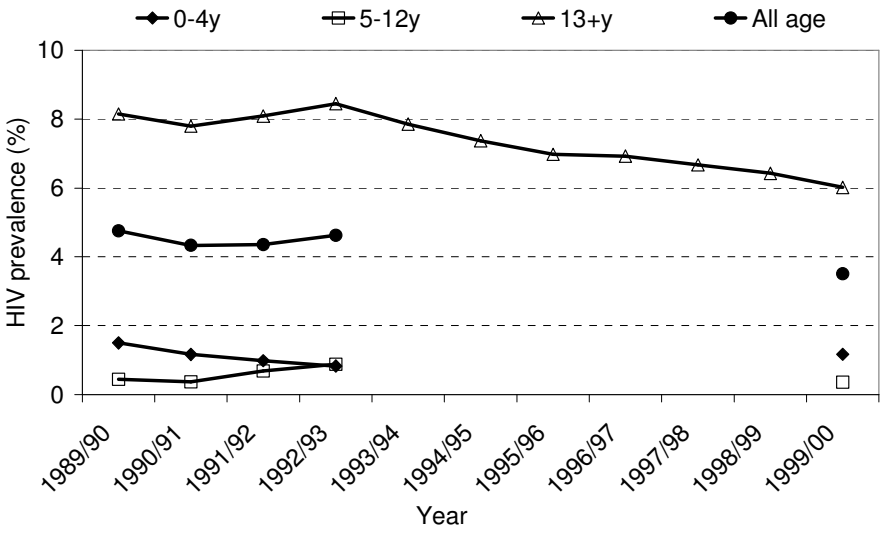

Figure 1 HIV prevalence in the Masaka cohort over time, by age group (%)
y=years

Between 1990 and 2000, 245 HIV-1 seroconversions were identified in the 53,726 person years at risk. Between 1993 and 2000, 144 seroconversions in 25,960 pyar were observed among adults (aged 13+ years). Between 1990 and 1993 incidence data were available from all ages, and 101 seroconversions in 27,765 pyar were observed. Of these, 99 occurred among adults in 15,507 pyar. In the same period, among 0-4 years olds, 1 incident case was observed in 3,406 pyar and 16

infants (<1 year old) found to be HIV positive at first test were also considered as HIV seroconversions in their first year of life. Among 5-12 year olds 1 incident case was observed in 8,852 pyar.

The incidence rates for adults fluctuated around 0.65/100pyar until 1996, after which incidence declined markedly to 0.36/100pyar in 1999 (Figure 2). Between 1990 and 1993, data were available from all ages. Mean adult incidence in this period was 0.64/ 100pyar (95% CI 0.52 to 0.78). Among the 0-4 years olds, the mean observed all-age HIV incidence (excluding the unobserved incidence from infants that tested HIV-positive at their first survey) was 0.03/100pyar (95% CI 0.00 to 0.16). If infants that were HIV-positive at their first survey were included as seroconversions in their first year, the adjusted HIV incidence in this age group was 0.47/100pyar (95% CI 0.27 to 0.76). In the same period, among children aged 5 to 12 years old, the mean HIV incidence was 0.01/100pyar (95% CI 0.00 to 0.06), and all-age HIV incidence fluctuated around 0.42/100pyar (95% CI 0.35 to 0.50).

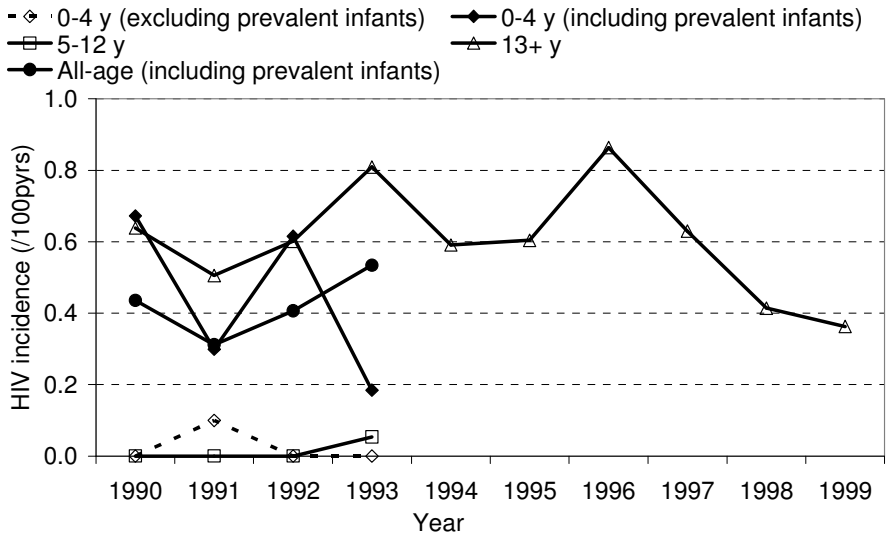

Figure 2 HIV incidence in the Masaka cohort over time, by age group (/100pyar)
y=years

2.4.2. Modelled HIV incidence via unsafe injections and unsafe blood transfusions

2.4.2.1. The number of unsafe injections or unsafe blood transfusions per person per year, n_u

The number of unsafe injections or unsafe blood transfusions per person per year was estimated from the product of *n,* the number of injections or blood transfusions per person per year, and p_u, the probability that an injection or blood transfusion was unsafe.

2.4.2.1.1. The number of injections or blood transfusions per person per year, *n*

Data from the Masaka cohort on the number of injections and blood transfusions were available for 1999/2000. Injection rate data were obtained from a structured questionnaire administered to all adults (13+ years) present in 1998/99, and all children (0-12 years) in 1999/2000 and in 2000/01. Respondents or their proxies were asked how many injections were received in the previous 12 months. Injection rates reduced with age (Table 1). Males and females reported similar numbers of injections at all ages. Children below 5 years were reported to have received 5.5 injections per person per year (pppy). Children 5-12 years old reported receiving 3.7 injections pppy, and adults (13+ years) reported 1.1 injections pppy. We assumed that injection rates were constant over time.

The surveys in Masaka measured the proportion of individuals aged less than 13 years old who had ever had a blood transfusion. 1.5% of 0 to 12 year olds had ever had a blood transfusion (Whitworth et al. Draft Aug 2004). The maximum number of blood transfusions in one individual under 13 years old was three (1 child, pc Linda Morison, June 4th 2004). Annual rates were not available. However 0.5% of a rural community cohort in the neighbouring rural Rakai District reported one or more blood transfusions in the previous year (Kiwanuka et al. 2004), and the Global Burden of Disease study estimated an annual all-age rate of 0.5% per person per year in the region (Rapiti E et al. Draft May, 2004). These data appear inconsistent, as an annual-rate of 0.5% per year would correspond with an ever-rate of around 3% among the under 13s. However, to be conservative (to tend to overestimate incidence from this source) we assumed an annual all-age blood transfusion rate of 0.5% per person per year for all age groups.

	Male						Female						Male and Female						Male and Female	
	0-4y	n	5-12y	n	13+y	n	0-4y	n	5-12y	n	13+y	n	0-4y	n	5-12y	n	13+y	n	All ages	n
Mean number of injections (pppy)	5.5	1173	3.7	489	1.0	2034	5.6	1207	3.4	501	1.1	2215	5.5	2380	3.7	935	1.1	4248	2.8	7563

Table 1 Self-reported mean number of injections in the Masaka cohort by age (per person per year).

13+y data from 1999/1999. Data for under 13 years olds is the weighted mean of data from two surveys in 1999/2000 and 2000/01; y=years

2.4.2.1.2. The probability an injection or blood transfusion is unsafe, p_u

In the absence of directly observed data on injection safety practices in these two populations, the safety of injections in the early 1990s was estimated from two clinic-based surveys within Uganda.

An observational survey in Ankole (Mbarara and Bushenyi Districts within Uganda) reported that 72% of the government and private clinics observed did not '*observe the minimum hygienic conditions before injection administration*', where '*minimum hygienic conditions'* were those who used saucepans instead of sterilisers, and encouraged patients to keep and sterilise their own

equipment at home (Birungi et al. 1994). A survey in Busoga District, reported that *'Use of the same equipment on multiple patients was observed in over 50% of the health facilities…'* (page 27, Birungi et al. 1994). We therefore assumed that the probability that an injection was unsafe lay between 50% and 72%.

The probability that a blood transfusion was unsafe was assumed to be one minus the proportion screened for HIV. Data from the Global Database on Blood Safety on WHO region Africa 'E' suggests 96% of all blood was screened. Therefore, we assumed that the probability that a blood transfusion was unsafe lay between 4% and 8%, the latter to allow for the overestimation of safety from this data source.

2.4.2.2. The probability that an unsafe injection or unsafe blood donation is contaminated, p_c

We assume that the probability that an unsafe injection is contaminated is equal to the probability that the unsafe injection was previously used on an HIV infected individual. This can be estimated from the age-specific HIV prevalence, age-specific injection rates, and age-specific pattern of mixing between consecutive recipients of unsafe injections.

Consecutive recipients of unsafe injections may not be selected randomly from the population. Children, for example, may be more likely to visit the same clinic for immunisations as other children, and therefore may be more likely to receive unsafe injections previously used on other children than previously used on other adults. Similarly, adults may visit sources frequented by other adults, such as STI clinics or injectionists. However, since these data (not shown) do not allow an accurate quantification of the true mixing pattern, we explored two extreme mixing scenarios (i) a *random mixing* scenario, in which consecutive recipients of unsafe injections are selected randomly from the population, and (ii) an *age-dependent mixing* scenario, in which consecutive recipients of unsafe injections are selected only from others in their age group (categorised into 0-4, 5-12 and 13+ years).

It is also plausible that symptomatic HIV/AIDS infected individuals may seek medical services more frequently than HIV negative or asymptomatic individuals may and therefore the probability that an unsafe injection is contaminated may be higher than the HIV prevalence in the general population (Thoma et al. 2004). Therefore, in the age-dependent mixing scenario we assumed the probability that an unsafe injection was contaminated lay between 100% and 150% of the HIV prevalence in that age group (Table 2).

Similarly, in the random mixing scenario, we assumed that the probability that an unsafe injection was contaminated lay between 100% and 150% of the all-age HIV prevalence. However, as the

probability an unsafe injection is contaminated will tend towards the HIV prevalence in the groups receiving most injections, we weighted the age specific prevalence by the frequency of injections in each age group to calculate the probability that an unsafe injection was contaminated. In rural Masaka, this lowered the lower bound of the plausible range of the probability an unsafe injection was contaminated from 4.5% to 1.7%, as injection rates were higher among younger, lower HIV prevalence age groups. The upper bound of the plausible range was 1.7% * 150% = 2.5% (Table 2).

	Age			
	0-4y	**5-12y**	**13+y**	**All-age**
Observed HIV prevalence (%)	1.1	0.6	8.1	4.5
Age-dependent mixing scenario				
Probability unsafe injection contaminated (%)				
Min	1.1	0.6	8.1	4.5
Max	1.7	0.9	12.2	6.8
Random mixing scenario				
Weight (Observed injection rate (pppy))	5.5	3.7	1.1	2.8
Probability unsafe injection contaminated (%)				
Min	1.7	1.7	1.7	1.7
Max	2.5	2.5	2.5	2.5

Table 2 Probability an unsafe injection is contaminated in random and age-dependent scenarios of mixing of injection equipment

y= years; pppy=per person per year

Unlike unsafe injections, it is less plausible that the probability an unsafe transfusion was contaminated is higher than the HIV prevalence in the general population, as blood donors are not the recipients of care. In Uganda, most blood donors are adults and during routine national blood collection, potential blood donors are screened using a sexual behaviour history, which should reduce the probability that they are HIV infected. Locally however, it is quite likely that relatives donate blood when required, and in these circumstances, behavioural screening may not be as rigorous. Therefore, we assume that the probability that an unsafe (ie prior to testing for HIV) blood transfusion is contaminated lies between the adult prevalence (8.1%) and half this prevalence (4.1%). We also assume random mixing between donors and recipients of blood transfusions, because the very limited number of sources of blood transfusions prevents non-random mixing by preventing higher and lower risk individuals choosing different locations to donate or to receive blood transfusions.

2.4.2.3. The probability of transmission from a contaminated unsafe injection or contaminated unsafe blood transfusion, p_t

Our transmission probability review (*Systematic Review of HIV-1 Transmission Probabilities in absence of antiretroviral therapy, Baggaley et al, 25th Aug 2004, accompanying this report*) suggested that the median per contact transmission probability (over studies) from a needle-stick

and other accidental sharp instrument injury is 0.00% (range 0.00% – 5.80%). This included many small studies with no transmissions and wide confidence intervals. Adjusting for the size of the smaller studies, the weighted mean per-contact transmission probability (over studies) from a needle-stick and other accidental sharp instrument injury is 0.29% (95%CI 0.21% – 0.50%; 18/6225; calculated using data from Table 15 of the report). Since most unsafe injections are not accidents with blood-containing equipment but usually occur with (at least) water-rinsed equipment, and there will be a delay between injections during which the virus may become non-infectious, this transmission probability is likely to overestimate the transmission probability from unsafe injections. Conversely, transmission probabilities from unsafe injections as high as 1.2% (Hauri et al. 2004) and 2.3% (Gisselquist 2002) have been proposed. Therefore, to encompass this range of uncertainty we assume that the plausible range in which the transmission probability from a contaminated unsafe injection lies between 0.1% and 2.3%.

Our review also suggested that the median per contact transmission probability from a contaminated blood transfusion is 95.2% (range 88.3% – 100.0%). Therefore, we assume that the plausible range in which the transmission probability from a contaminated unsafe blood transfusion lies between 88.3% and 100%. For comparison, the Global Burden of Disease study assumed that the transmission probability was 89% (Rapiti E et al. Draft May, 2004).

2.4.3. Modelled HIV incidence via mother to child transmission

2.4.3.1. Ratio of fertility among HIV infected to HIV uninfected women, *r*

Studies in Ugandan and Tanzanian populations have shown the fertility rate among HIV positive women to be between 45% and 75% of HIV negative women (Carpenter et al. 1997; Gray et al. 1998; Ross et al. 1999; Hunter et al. 2003). Thus, we assume this ratio lay between 45% and 75%.

2.4.3.2. Probability of mother to child transmission, p_t

In the absence of antiretroviral treatment, in developing countries, the probability of vertical transmission of HIV-1 from HIV infected mothers to their children has been found to be between 20% and 40%. The wide range may be explained by variation between studied populations in feeding practices and nutritional status of mothers and babies, the viral load of pregnant women, or the prevalence of sexually transmitted diseases. We assumed this probability lay between 20% and 40%.

2.4.3.3. Number of HIV infected women by age, *s*, number of person years at risk among HIV negative 0-4 year olds, *N*, and age specific fertility rates, *F*

The number of HIV infected women, and the number of person years at risk among HIV negative 0 to 4 year olds was calculated using the mean of data from the general population cohort between

1989 and 1993; age specific fertility rates for Masaka District were obtained from the 1990/91 Uganda census (Uganda Statistics Dept. 1995).

2.5. Summary of parameter and validation constraints

Table 3 summarises the parameter and validation constraints.

	Symbol in formula	Minimum	Maximum
Parameter constraints			
Injections			
Injection rate (per person per year)	n		
0-4 years		5.5	5.5
5-12 years		3.7	3.7
13+ years		1.1	1.1
Probability injection equipment unsafe	p_u	50%	72%
Probability injection equipment contaminated	p_c		
Random mixing scenario			
All-ages		1.7%	2.5%
Age-dependant mixing scenario			
0-4 years		1.1%	1.7%
5-12 years		0.6%	0.9%
13+ years		8.1%	12.2%
Transmission probability from contaminated injection	p_t	0.10%	2.30%
Transfusions			
Transfusion rate (per person per year)	n	0.005	0.005
Probability transfusion unsafe	p_u	4.0%	8.0%
Probability transfusion contaminated	p_c	4.1%	8.1%
Transmission probability from contaminated transfusion	p_t	88.3%	100.0%
Mother-to-child transmission			
Ratio of HIV+ to HIV- fertility	r	45.0%	75.0%
Transmission probability from mother-to-child	p_t	20.0%	40.0%
Validation constraints			
Projected HIV incidence (/100pyar)	I_{total}		
0-4 years		0.0000	0.7610
5-12 years		0.0000	0.0629
13+ years		0.0000	0.7773
All-age		0.0000	0.5010

Table 3 Summary of parameter and validation constraints

2.6. Sensitivity analysis

We explored the robustness of our results by investigating how sensitive our results were to each individual parameter, and what unsafe injection parameters values would attribute 30%, 50% or 70% of all-age HIV incidence to unsafe injections.

2.6.1. How sensitive was the modelled HIV incidence to each parameter?

The modelled HIV incidence in each scenario, using the best fitting value for all parameters, was compared to the modelled HIV incidence using the minimum and maximum value of each parameter in turn (see Table 3 for range), while using the best fitting values of all other parameters. Validation constraints were ignored.

2.6.2. What unsafe injection parameters values would attribute 30%, 50% or 70% of all-age HIV incidence to unsafe injections?

The values of the unsafe injection parameters were increased in turn, while using the best fitting values of all other parameters, until the model attributed 30%, 50% or 70% of all-age HIV incidence to unsafe injections. Validation constraints were ignored.

3. Results

3.1. Modelled and observed HIV incidence

The model was run twice, once for the random mixing scenario and once for the age-dependent mixing scenario. In the random mixing scenario, 9,507 of 40,100 parameter sets resulted in a modelled HIV incidence consistent with the observed data (according to the criteria defined in *'Validation constraints',* Table 3). In the age-dependent mixing scenario 13,606 of 34,500 parameter sets resulted in a modelled HIV incidence consistent with the observed data. The mean observed and modelled HIV incidence is shown in Figure 3.

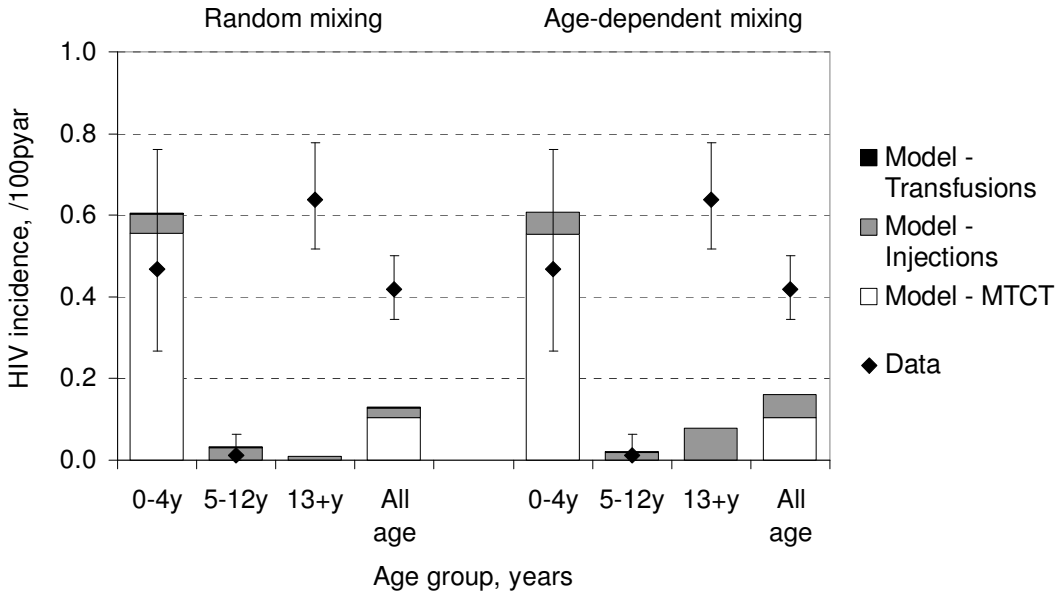

Figure 3 Observed HIV incidence by age (♦ with 95% CI) and modelled HIV incidence by age group and route of transmission (stacked columns), /100pyar, in random and age-dependent scenarios of mixing of injection equipment

Among children under 13 years old the modelled HIV incidence attributed to mother-to-child transmission, unsafe injections and unsafe blood transfusions adequately explained all of the observed HIV incidence.

Among 0-4 year olds, the sum of the modelled HIV incidence from these three routes of transmission was 0.604/100pyar [*95% uncertainty bounds* or *95%UB* (ie 1.96 * the standard deviation of the modelled HIV incidence) lay between 0.412 and 0.796/100pyar] in the random mixing scenario and 0.608/100pyar [95%UB 0.417-0.800] in the age-dependant mixing scenario. This is compared to an observed overall HIV incidence in this age group of 0.469/100pyar [95%CI 0.268-0.761].

Among 5-12 year olds, the sum of the modelled HIV incidence from these three routes of transmission was 0.033/100pyar [95%UB 0.001-0.065] in the random mixing scenario and 0.020/100pyar [95%UB 0.000-0.042] in the age-dependant mixing scenario. This is compared to an observed HIV incidence in this age group of 0.011/100pyar [95%CI 0.000-0.063].

However, among 13+ year olds, the modelled incidence from the three routes of transmission was able to explain only a small proportion of the observed HIV incidence. The sum of the modelled HIV incidence from these three routes was 0.011/100pyar [95%UB 0.002-0.020] in the random mixing scenario and 0.079/100pyar [95%UB 0.000-0.171] in the age-dependant mixing scenario. This is compared to an observed HIV incidence in this age group of 0.638/100pyar [95%CI 0.519-0.777].

Similarly, over all-ages, the modelled incidence from these three routes of transmission was able to explain only a small proportion of the observed HIV incidence. The sum of the modelled HIV incidence from these three routes was 0.130/100pyar [95%UB 0.089-0.170] in the random mixing scenario and 0.161/100pyar [95%UB 0.092-0.230] in the age-dependant mixing scenario (vs. 0.418/100pyar [95%CI 0.345-0.501] observed).

3.2. Proportion of modelled HIV incidence attributed to each route of transmission

By attributing the difference between the mean observed HIV incidence among 13+ year olds, and the modelled HIV incidence due to the three modelled routes to 'other causes', we calculated the proportion of the total observed HIV incidence explained by unsafe injections, unsafe transfusions, MTCT, and other causes, as shown in Figure 4

Among 0-4 year olds the majority of HIV incidence was explained by mother-to-child transmission (91.9% [95%UB 60.1-123.7] and 90.8% [95%UB 59.2-122.3] in the random and age-dependent mixing scenario respectively). A much smaller proportion was explained by unsafe injections (7.8% [95%UB 0.0-15.8] and 8.9% [95%UB 0.0-19.5] in the random and age-dependent mixing scenario respectively), and a very small proportion was explained by unsafe transfusions in both mixing scenarios (0.3% [95%UB 0.1-0.5].

Figure 4 Modelled proportion of HIV incidence attributable to each route of transmission, by age group, %, in random and age-dependent scenarios of mixing of injection equipment

Among 5-12 year olds, the majority of the very low HIV incidence in this age group was explained by unsafe injections (94.6% [95%UB 0.0-191.9] and 91.4% [95%UB 0.0-197.1] in the random and age-dependent mixing scenario respectively). The remainder was explained by unsafe transfusions (5.4% [95%UB 2.4-8.3] and 8.6% [95%UB 3.9-13.4] in the random and age-dependent mixing scenario respectively).

Among 13+ year olds, both mixing scenarios suggested that the majority of HIV incidence was left unexplained by these three routes of transmission (98.3% [95%UB 96.9-99.8] and 87.6% [95%UB 73.3-102.0] in the random and age-dependent mixing scenario respectively). Unsafe injections accounted for a small proportion of HIV incidence in the randomly mixing scenario (1.4% [95%UB 0.0-2.8]), but accounted for a larger proportion in the age-dependent mixing scenario (12.1% [95%UB 0.0-26.5]). In both scenarios, the proportion of HIV incidence explained by unsafe transfusions was very low (0.3% [95%UB 0.1-0.4]).

Over all-ages, the majority of HIV incidence was left unexplained by these three routes of transmission (71.2% [95%UB 70.1-72.2] and 63.8% [95%UB 53.4-74.3] in the random and age-dependent mixing scenario respectively). The largest proportion of the explained HIV incidence was attributed to mother-to-child transmission in both mixing scenarios (23.4% [95%UB 15.3-31.5]). Unsafe injections explained 5.1% [95%UB 0.0-10.3] in the randomly mixing scenario, and 12.4% [95%UB 0.0-27.0] in the age-dependent mixing scenario. Unsafe blood transfusions explained very little all-age HIV incidence in both mixing scenarios (0.4% [95%UB 0.2-0.6]).

3.3. Fitted parameter values

Table 4 shows the minimum, maximum, mean and standard deviation of the parameter values that fitted the validation constraints (ie predicted an HIV incidence smaller than the 95% CI upper bound of the observed HIV incidence in all age groups).

For most parameters, the best fitting parameter value was approximately the midpoint of the constraint range (Table 4), suggesting that the parameter constraints we quantified a-priori were consistent with the observed age-specific HIV incidence data.

	Fitted parameter values							
	Random mixing scenario				Age dependent mixing scenario			
Parameter (constraints lower bound; upper bound; midpoint)	Min	Max	Mean	SD	Min	Max	Mean	SD
Injections								
Probability injection equipment unsafe (50;70;60%)	50%	72%	60%	6%	50%	72%	61%	11%
Probability injection equipment contaminated								
Random mixing scenario								
All-ages (1.7;2.5;2.1%)	1.70%	2.50%	2.07%	0.23%	na	na	na	na
Age-dependant mixing scenario								
0-4 years (1.1;1.7;1.4%)	na	na	na	na	1.10%	1.70%	1.40%	0.30%
5-12 years (0.59;0.88;0.75%)	na	na	na	na	0.59%	0.88%	0.73%	0.14%
13+ years (8.1;12.2;10.2%)	na	na	na	na	8.1%	12.2%	10.1%	2.0%
Transmission probability from contaminated injection (0.10;2.30;1.2%)	0.10%	1.88%	0.68%	0.37%	0.10%	2.30%	1.12%	1.10%
Transfusions								
Probability transfusion unsafe (4.0;8.0;6.0%)	4.0%	8.0%	6.0%	1.1%	4.0%	8.0%	6.0%	2.0%
Probability transfusion contaminated (4.1;8.1;6.1%)	4.1%	8.1%	6.1%	1.2%	4.1%	8.1%	6.1%	2.0%
Transmission probability from contaminated transfusion (88.3;100;94.2%)	88.3%	100.0%	94.2%	3.4%	88.3%	100.0%	94.1%	5.8%
Mother-to-child transmission								
Ratio of HIV+ to HIV- fertility (45;75;60%)	45.0%	75.0%	55.9%	7.9%	45.0%	75.0%	55.8%	15.2%
Transmission probability from mother-to-child (20;40;30%)	20.0%	38.9%	25.5%	4.0%	20.0%	38.5%	25.4%	9.5%

Table 4 Summary of fitted parameter values

The minimum, maximum, mean and standard deviation (SD) of the parameter values that fitted the validation constraints. na= not applicable in this scenario

The exceptions were the *transmission probability from contaminated injections*, the *transmission probability from MTCT*, and the *ratio of HIV+ to HIV- fertility*. For each of these parameters the best fitting parameter value was smaller than the midpoint of the constraint range. The best fitting *transmission probability from a contaminated injection* was 0.68% (SD 0.37) in the random mixing scenario and 1.12% (SD 1.10) in the age-dependent mixing scenario, both lower than the midpoint of parameter constraints (1.2%). Similarly, the best fitting *transmission probability from MTCT* was 25%, and the best fitting *ratio of HIV+ to HIV- fertility* was 56%, both below the midpoint of parameter constraints (30% and 60%, respectively).

The best fitting value of the *transmission probability from an unsafe injection* was lower in the random mixing scenario (0.68%) than the age dependent mixing scenario (1.12%). Similarly, the all-age *probability injection equipment was contaminated* was lower in the random mixing scenario (2.07%) than in the age-dependent mixing scenario (2.87%; calculation not shown). For all other

parameters, the best fitting values were roughly the same in the two scenarios of mixing of unsafe injections (Table 4).

Figure 5 shows the distribution of the parameter values that were consistent with the validation constraints for these three parameters in the two mixing scenarios (top 6 graphs), and for comparison the distribution for *the probability a transfusion was unsafe*, a parameter that retained its uniform probability distribution during model fitting (bottom two graphs).

The top two graphs in Figure 5 show the fitted *contaminated injection transmission probability* values in the two mixing scenarios. In the random mixing scenario (top left graph), parameter values above 1% were markedly less likely to result in a modelled HIV incidence that fitted the observed data. Indeed, in this scenario no transmission probability greater than 1.88% resulted in a modelled HIV incidence consistent with these data. This was lower than the upper bound of the parameter constraint for this parameter (2.30%). Similarly, in the age-dependent mixing scenario (top right graph), higher transmission probabilities were less likely to be consistent with the observed HIV incidence data. However, in this scenario, because the *probability an injection was contaminated* was assumed to be lower among 5-12 year olds, all values within the parameter constraints were consistent with the observed HIV incidence data (albeit at a lower frequency).

Larger values of the *transmission probability from mother-to-child*, and the *ratio of HIV+ to HIV- fertility* were also markedly less likely to result in a modelled HIV incidence that fitted the observed HIV incidence (middle four graphs). In contrast, for comparison, all values of the *probability a transfusion is unsafe* (bottom two graphs) were equally likely to result in a modelled HIV incidence that fitted the observed HIV incidence (primarily because the HIV incidence via this route was so small).

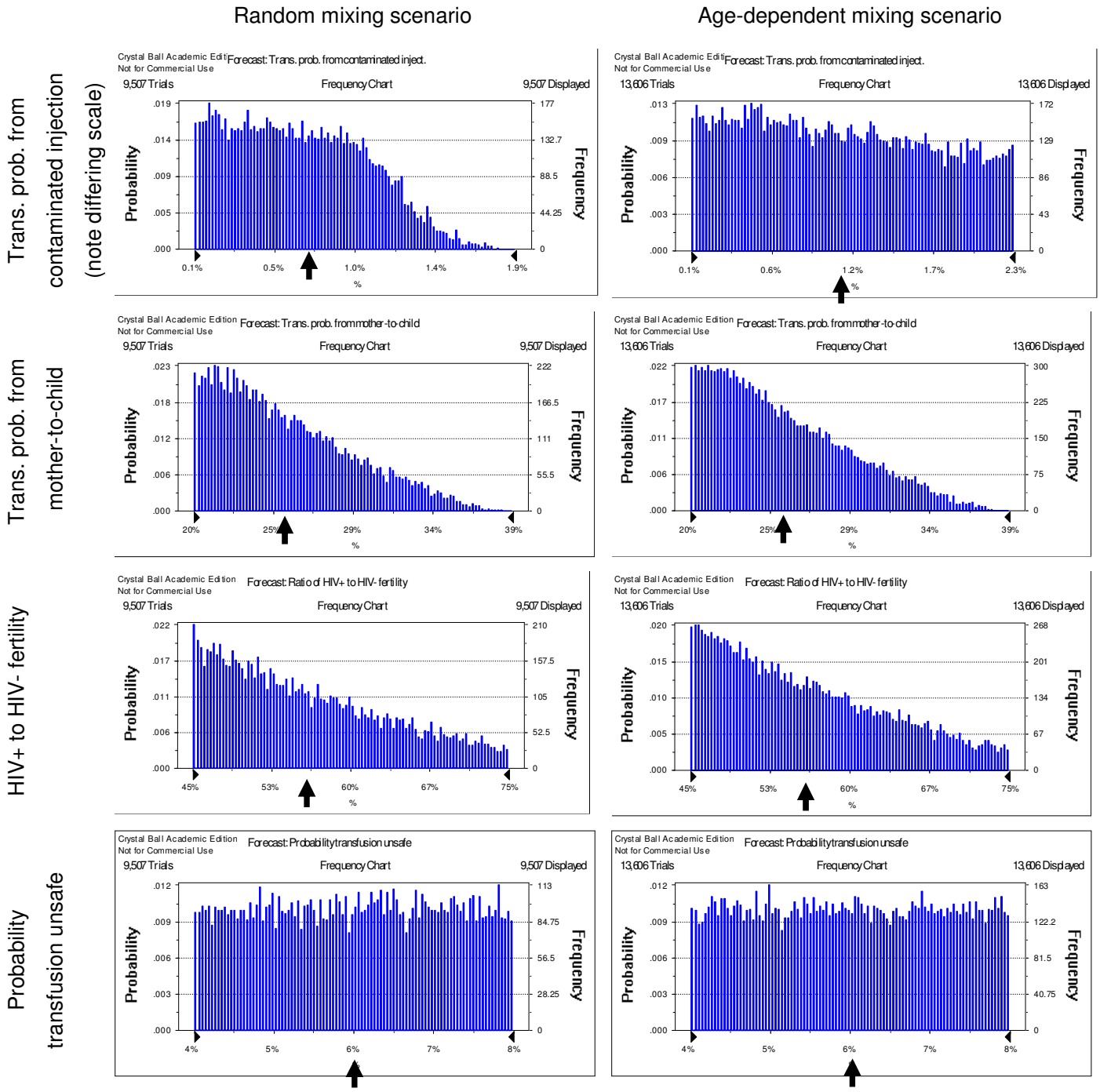

Figure 5 Probability (and frequency) distributions of parameter values consistent with the observed HIV incidence for four selected parameters, in both scenarios of mixing of injection equipment. Parameters were varied simultaneously.

Large arrows highlight fitted mean parameter value.

3.4. Sensitivity analysis

3.4.1. How sensitive was the modelled HIV incidence to each parameter?

The y-axis (vertical line) in each graph in Figure 6 shows the modelled all-age incidence using the fitted parameter values (summarised in Table 4) in the two mixing scenarios, ie it identifies an HIV

incidence of 0.130/100pyar in the random mixing scenario and 0.161/100pyar in the age-dependent mixing scenario. Either side of the y-axis, the horizontal grey bars show the minimum and maximum all-age HIV incidence if the proposed minimum and maximum parameter value were used (shown in the row headings).

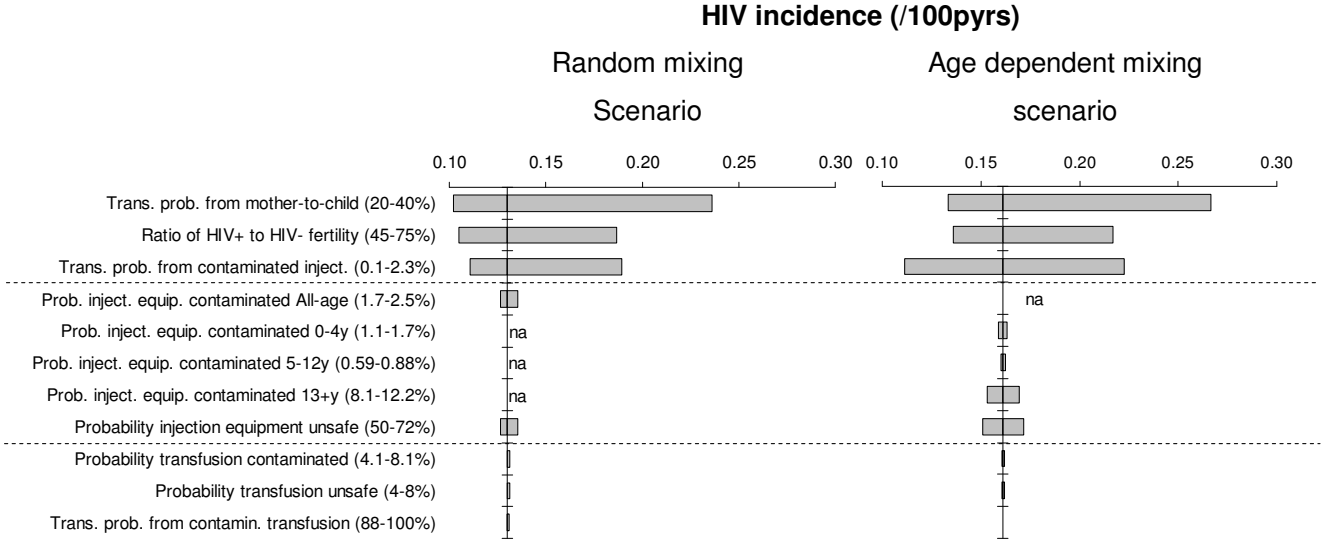

Figure 6 Sensitivity of modelled HIV incidence to each model parameter

y = years.

In both mixing scenarios, all-age HIV incidence was most sensitive to the two MTCT parameters (the *transmission probability from mother to child,* and the *ratio of HIV+ to HIV- fertility*) and the *transmission probability from contaminated injections* (Figure 6). All-age incidence was relatively insensitive to other parameters related to unsafe injections and all blood transfusion parameters when varied within their plausible range.

Comparing the sensitivity of the results to individual parameters in the two scenarios, the modelled all-age HIV incidence is equally sensitive to the two MTCT parameters in both mixing scenarios (ie the widths of the bars are equal in both scenarios). However, all-age HIV incidence is more sensitive to the *transmission probability from a contaminated injection* in the age-dependent mixing scenario than in the random mixing scenario. This is because, while in both scenarios the lowest transmission probability (0.1%) virtually eliminates HIV incidence from this route, the upper bound (2.3%) models higher HIV incidence in the age-dependent mixing scenario than in the random mixing scenario because the best fitting *probability an injection is contaminated* is also higher in the age-dependent mixing scenario (2.87%; calculation not shown) than in the random mixing scenario (2.07%, Table 4).

3.4.2. What unsafe injection parameters values would attribute 30%, 50% or 70% of all-age HIV incidence to unsafe injections?

Table 5 shows the parameters values that would attribute 30%, 50% or 70% of all-age HIV incidence to unsafe injections.

	Proportion of HIV incidence due to unsafe injections		
Parameter (constraint range)	30%	50%	70%
Random mixing scenario			
Trans. prob. from contaminated inject. (0.1-2.3%)	4.5%	8.5%	13.0%
Prob. inject. equip. contaminated all-age (1.7-2.5%)	13.7%	25.7%	39.5%
Probability injection equipment unsafe (50-72%)	na	na	na
Mean projected/ observed HIV incidence among 5-12 year olds	2070%	4366%	8047%
Age dependant mixing scenario			
Trans. prob. from contaminated inject. (0.1-2.3%)	2.9%	4.6%	6.3%
Prob. inject. equip. contaminated 0-4y (1.1-1.7%)	3.4%	5.7%	8.0%
Prob. inject. equip. contaminated 5-12y (0.59-0.88%)	1.8%	3.0%	4.2%
Prob. inject. equip. contaminated 13+y (8.1-12.2%)	24.3%	41.5%	57.7%
Probability injection equipment unsafe (50-72%)	na	na	na
Mean projected/ observed HIV incidence among 5-12 year olds	414%	708%	994%

Table 5 Scenarios in which 30%, 50% or 70% of all-age HIV incidence would be attributed to unsafe injections.

Parameter values were varied in turn, while holding other parameter values at the mean fitted value (as shown in Table 4). The validation constraints were ignored. The age-specific probabilities injection equipment were contaminated were varied together in the age-dependent mixing scenario. na = no value of the probability injection equipment was unsafe was found able to explain this proportion of incidence due to unsafe injections.

30% of all-age HIV incidence could be attributed to unsafe injections in the random mixing scenario if the *transmission probability from contaminated injections* was 4.5% or the *probability that injection equipment was contaminated* was 13.7%. In either scenario however, the HIV incidence among 5-12 year olds would be 2070% larger than observed.

Similarity, 30% of all-age HIV incidence could be attributed to unsafe injections in the age-dependent mixing scenario if the *transmission probability from contaminated injection* was 2.9% or in the age-dependent mixing scenario the *probability that injection equipment was contaminated* was 3.4% among 0-4 year olds, 1.8% among 5-12 year olds and 24.3% among 13+ year olds. In either scenario, the HIV incidence among 5-12 year olds would be 414% larger than observed.

No larger value of the *probability that injection equipment was unsafe* was found that could attribute 30% or more of HIV incidence to unsafe injections.

Higher proportions of HIV incidence (50% and 70%) could be attributed to unsafe injections by increasing the *transmission probability from contaminated injections* or the *probability that injection*

equipment was contaminated, as shown in Table 5. However, this further worsened the fit of the modelled and observed HIV incidence.

4. Discussion

Our study does not support the hypothesis that unsafe injections played a major role in HIV transmission in rural Masaka in the early 1990s. We found that unsafe injections accounted for 5% or 12% of all-age HIV incidence depending whether we assumed random or age-dependent mixing between consecutive recipients of unsafe injections (Figure 4). This proportion is higher than other studies have suggested for the region as a whole (Chin et al. 1990; Hauri et al. 2004), but remains a small proportion of all-age HIV incidence.

The primary reason why the proportion of all-age HIV incidence attributed to unsafe injections was constrained at this low level was the low HIV incidence observed among 5 to 12 year olds (Figure 2). Any claim that HIV incidence from unsafe injections is a large proportion of all-age HIV incidence, must provide a plausible explanation how this age group escapes infection, as injection rates are higher in this group than among adults in this population (Table 1). Low incidence among these ages is likely to be common to many other sub-Saharan African populations (Killewo et al. 1990; Fontanet et al. 1998; Glynn et al. 2001), and therefore this finding is likely to be generalisable beyond rural Masaka to other populations in sub-Saharan Africa.

Our estimates of HIV incidence were obtained from cohort data, and as such tend to underestimate incidence since the most mobile individuals, who are at higher risk of HIV infection (Kengeya Kayondo et al. 1996), are the least likely to be bled repeatedly and therefore be included in the sample. However, mobility rates among 5-12 year olds are typically lower than at younger and older ages, and therefore the impact of this bias is likely to be smallest in this age group. As incidence in this age group was the limiting constraint for the proportion of HIV incidence due unsafe injections, this bias may have led us to overestimate the proportion of all-age HIV incidence attributed to unsafe injections.

Among adults aged 13 years and above we estimated that unsafe injections accounted for a small proportion of observed HIV incidence in the early 1990s (1% or 12% in the random and age-dependent mixing scenario respectively, Figure 4). A statistical association between injection history and HIV acquisition among adults has been reported for this population in a recent case-control study of adult HIV incidence between 1990 and 2002. The study reported that among adults there was a significant association between seroconversion in the previous 3 months and one or more injections in the same period, after adjusting for gender and sexual behaviour (OR=6.4%, 95%CI 1.2-33.1) (Whitworth et al. Draft Aug 2004). However, the authors noted that other explanations could not be excluded, as most of these injections were given at formal clinics, using

the same stock of injection equipment used for children (in which seroconversions are very rare), and that the reasons given for these injections were either for acute febrile illness which could have been due to HIV seroconversion illness or alternatively, for genital ulcers and vaccinations among pregnant women, suggesting recent unprotected sexual activity, during which HIV transmission could have occurred (Whitworth et al. Draft Aug 2004). Conversely, in two nearby rural populations, no significant association between HIV incidence and recent injection history was found among adults. In the late 1990s in Rakai District adult HIV acquisition was not associated with having received one or more unsafe injections in the past year (RR=1.05, 95% CI=0.75–1.46) (Kiwanuka et al. 2004). Similarly, an analysis of HIV incidence data from the early 1990s in rural Mwanza, Tanzania showed no significant association between HIV incidence and one or more injections in the past two years among males or females (Todd et al. Draft June 2004).

We estimated that 23% of all-age HIV incidence was attributable to mother to child transmission (Figure 4). It was an appreciable proportion, because in this population, the risk of transmission from mother to child is relatively high and the exposure, birth to a HIV infected mother, is relatively common. This size of this proportion was primarily constrained by our estimate of the observed HIV incidence among 0 to 4 year olds. This estimate must be treated with caution however, as we assumed all HIV prevalent infants were HIV incident cases, whereas some of these infants would have sero-reverted after the survey. On the other hand, some HIV positive infants would have already died before they were tested for HIV (due to the high mortality of HIV infected children in their first year). These factors may have led to an over or under-estimate (respectively) the proportion of all-age HIV incidence due to MTCT.

Mother to child transmission was the dominant cause of HIV infection among children aged 0-4 years old in this population. We estimated over 90% of HIV incidence in this age group was due to MTCT (Figure 4). This is consistent with the findings of two studies in the same population (Kengeya Kayondo et al. 1995; Whitworth et al. Draft Aug 2004). In the more recent study, Whitworth and colleagues showed that among HIV infected children aged under 6 years old, for whom the mother's HIV serostatus was known, the population attributable fraction (PAF) of MTCT for HIV prevalence was 100% (Whitworth et al. Draft Aug 2004).

The proportion of all-age HIV incidence attributed to unsafe blood transfusions was very small (0.4%, Figure 4). This was because although the risk of transmission from a contaminated transfusion is very high, unsafe blood transfusions are uncommon. Our estimate of a small proportion of HIV incidence due to unsafe blood transfusions is consistent with the findings of two recent studies. Among children in rural Masaka, ever having a blood transfusion was associated with HIV prevalence (adjusted OR=7.7, 95% CI 0.98-61.8), but the exposure was rare (1.5% of children) (Whitworth et al. Draft Aug 2004), and thus the PAF would be small. Similarly, among

adults in rural Rakai, blood transfusions in the last year was found to be an independent risk factor for HIV acquisition (adjusted RR=3.9, 95% CI=1.22–12.58) but was also rare (0.5% of those interviewed) (Kiwanuka et al. 2004). In this study, the PAF of HIV acquisition due to transfusions among adults was estimated to be 1.6%, similar to the proportion of HIV incidence estimated to be due to blood transfusions among adults in Masaka (0.4%) in the present study.

Importantly, a large proportion of all-age HIV incidence (between 64% and 71%, Figure 4) was left unexplained by these three routes of transmission. As all unexplained incidence was among those aged 13 years and above, sexual transmission is likely to be the most credible route of transmission.

The great strength of this study in making informed predictions for the proportion of all-age HIV incidence attributable to unsafe injections was the availability of age-specific HIV incidence data. This was illustrated in the sensitivity analysis in which values of the *probability of transmission from unsafe injections* or the *probability injection equipment was contaminated* only slightly higher than was a-priori considered plausible were shown to be consistent with 30% or more of HIV incidence due to unsafe injections (section 3.4.2). However, these parameter values would result in a much higher than observed HIV incidence among 5-12 years olds.

Further, the present study revealed that the upper bound we proposed for the *probability of transmission from a contaminated injection* (2.3%) was too high. 2.3% has been considered plausible by other authors (Gisselquist 2002), but was found to be inconsistent with the observed HIV incidence data in this population during the study period. The best fitting transmission probability was 0.68% in the random mixing scenario or 1.12% in the age-dependent mixing scenario (Table 4).

In addition to those already mentioned, a number of further limitations of this study must be recognised. First, we explored only two extreme patterns of mixing between consecutive recipients of unsafe injections, random mixing, and age-dependent mixing in which consecutive recipients of unsafe injections are selected exclusively from within their own age group (0-4, 5-12 and 13+ years). If the 'true' mixing pattern of unsafe injection equipment further reduced the exposure of 5 to 12 year olds to unsafe injections, then the proportion of all-age HIV incidence attributed to unsafe injections may be higher than we estimated. However, we believe this is not a serious limitation since the 5-12 year olds were themselves the group with the lowest HIV prevalence and it is difficult to postulate which lower prevalence group 5-12 year olds could share unsafe injections with. Second, based on our exhaustive systematic review (*Baggaley et al, 25th Aug 2004*) the risks of HIV transmission from contaminated unsafe injections, blood transfusions and mother-to-child transmission are still not accurately known. However, we do believe the wide risk ranges we used in this study would have included the true risks in rural Masaka. Third, our model only estimated HIV incidence from the

reuse of injection equipment on one patient, it did not take into account the fact that injection equipment can be reused on multiple patients (Simonsen et al. 1999), or that multi-dose medication vials may be used unsafely (Katzenstein et al. 1999). However, again, for this to be a serious limitation, these increased risks would have to increase exposure among 0-4 and 13+ years olds relative to 5 to 12 year olds, else the limiting incidence among 5-12 year olds would still constrain the all-age proportion of HIV incidence attributed to unsafe injections to the levels estimated in this study. Finally, we used mean injection rates reported from all individuals. Data suggest that injection rates are higher among HIV positive individuals (not shown), who are not at risk of HIV infection, presumably because they are sicker. This simplification will have resulted in an overestimate of HIV incidence from unsafe injections in this study.

Previous studies have estimated the contribution of the various routes of transmission to all-age HIV incidence globally, but used data from different populations at different times. Few studies have attempted to address this question in a single site with as much high quality data as we have for rural Masaka.

Our study does not support the hypothesis that unsafe injections played a major role in HIV transmission in rural Masaka in the early 1990s. This finding appears to be generalisable to other sub-Saharan African populations, although it does not preclude the possibility that contaminated injections and transfusions may play a more significant role in HIV incidence in other populations, or that small local outbreaks may result from unsafe injections or transfusions in this or other populations.

HIV infections via unsafe injections and transfusions are preventable and the safety of both injections and transfusions should be improved to reduce HIV transmission via these routes still further, but particular efforts should be made to reduce the larger proportion of HIV transmission attributed to MTCT, and the incidence left unexplained by these three routes of transmission among 13+ year olds, presumably primarily due to sexual transmission.

5. Acknowledgements

We would like to thank Azra Ghani and Christophe Fraser for comments on the study methodology, Peter Ghys for comments on an earlier draft, Linda Morrison, Jim Todd and Yvan Hutin for access to unpublished data, and Carolyn Hartley for administrative support and copious patience. This project was in part funded by UNAIDS (APW number HQ/03/463823).

6. References

Birungi, H., D. Asiimye, et al. (1994). Injection use and practices in Uganda (WHO/DAP/94.18). Geneva, WHO.

Boily, M.-C., R. White, et al. (2003). "Transmission of HIV via unsafe injection or unsafe sex? Anomalies or Misunderstanding?" International Journal of STD & AIDS **15**: 61-62.

Brewer, D. D., S. Brody, et al. (2003). "Mounting anomalies in the epidemiology of HIV in Africa: cry the beloved paradigm." Int J STD AIDS **14**(3): 144-7.

Carpenter, L., J. Nakiyingi, et al. (1997). "Estimates of the impact of HIV infection on fertility in a rural Ugandan population cohort." Health Transitioin Review **7, suppl 2**: 113-126.

Chin, J., P. Sato, et al. (1990). "Projections of HIV infections and AIDS cases to the year 2000." WHO Bull: 68.

Decisioneering (2004). Crystal Ball v5.5. Denver.

Fontanet, A. L., T. Messele, et al. (1998). "Age- and sex-specific HIV-1 prevalence in the urban community setting of Addis Ababa, Ethiopia." AIDS **12**(3): 315-22.

Gisselquist, D. and J. J. Potterat (2003a). "Confound it: latent lessons from the Mwanza trial of STD treatment to reduce HIV transmission." Int J STD AIDS **14**(3): 179-84.

Gisselquist, D. and J. J. Potterat (2003b). "Heterosexual transmission of HIV in Africa: an empiric estimate." Int J STD AIDS **14**(3): 162-73.

Gisselquist, D., R. Rothenberg, et al. (2002a). "HIV infections in sub-Saharan Africa not explained by sexual or vertical transmission." Int J STD AIDS **13**(10): 657-66.

Gisselquist, D., R. Rothenberg, et al. (2002b). "Non-sexual transmission of HIV has been overlooked in developing countries." Bmj **324**(7331): 235.

Gisselquist, D. P. (2002). "Estimating HIV-1 transmission efficiency through unsafe medical injections." Int J STD AIDS **13**(3): 152-9.

Glynn, J. R., J. Ponnighaus, et al. (2001). "The development of the HIV epidemic in Karonga District, Malawi." Aids **15**(15): 2025-9.

Gray, R. H., M. J. Wawer, et al. (1998). "Population-based study of fertility in women with HIV-1 infection in Uganda." Lancet **351**(9096): 98-103.

Hauri, A., G. Armstrong, et al. (2004). "The global burden of disease attributable to contaminated injections given in health care setttings." International Journal of STD & AIDS **15**: 7-16.

Hunter, S. C., R. Isingo, et al. (2003). "The association between HIV and fertility in a cohort study in rural Tanzania." J Biosoc Sci **35**(2): 189-99.

Kamali, A., L. M. Carpenter, et al. (2000). "Seven-year trends in HIV-1 infection rates, and changes in sexual behaviour, among adults in rural Uganda." AIDS **14**(4): 427-34.

Katzenstein, T. L., L. B. Jorgensen, et al. (1999). "Nosocomial HIV-transmission in an outpatient clinic detected by epidemiological and phylogenetic analyses." AIDS **13**(13): 1737-44.

Kengeya Kayondo, J. F., A. Kamali, et al. (1996). "Incidence of HIV-1 infection in adults and socio-demographic characteristics of seroconverters in a rural population in Uganda: 1990-1994." International Journal of Epidemiology **25**(5): 1077-82.

Kengeya Kayondo, J. F., S. S. Malamba, et al. (1995). "Human immunodeficiency virus (HIV-1) seropositivity among children in a rural population of south-west Uganda: probable routes of exposure." Ann Trop Paediatr **15**(2): 115-20.

Killewo, J., K. Nyamuryekunge, et al. (1990). "Prevalence of HIV-1 infection in the Kagera region of Tanzania: a population based study." AIDS **4**: 1081-1085.

Kiwanuka, N., R. Gray, et al. (2004). "The incidence of HIV-1 associated with injections and transfusions in a prospective cohort, Rakai,Uganda." AIDS **AIDS**.

Mbulaiteye, S. M., C. Mahe, et al. (2002). "Declining HIV-1 incidence and associated prevalence over 10 years in a rural population in south-west Uganda: a cohort study." Lancet **360**(9326): 41-6.

Microsoft Corporation (2002). Microsoft Excel 2002.

Rapiti E, Hutin Y, et al. (Draft May, 2004). The global burden of HBV, HCV and HIV infections attributable to unsafe blood transfusions. Geneva, WHO.

Ross, A., D. Morgan, et al. (1999). "Reduced fertility associated with HIV: the contribution of pre-existing subfertility." AIDS **13**: 2133-2141.

Schmid, G. P., A. Buve, et al. (2004). “Transmission of HIV-1 infection in sub-Saharan Africa and effect of elimination of unsafe injections.” Lancet **363**(9407): 482-8.
Simonsen, L., A. Kane, et al. (1999). “Unsafe injections in the developing world and transmission of bloodborne pathogens: a review.” Bull World Health Organ **77**(10): 789-800.
Thoma, M., R. H. Gray, et al. (2004). “Unsafe injections and transmission of HIV-1 in sub-Saharan Africa.” Lancet **363**(9421): 1650-1.
Todd, J., H. Grosskurth, et al. (Draft June 2004). “Risk factors for HIV incidence in a rural African population.”.
Uganda Statistics Dept. (1995). The 1991 Population and Housing Census Analytical Reports, Vol 1, Demographic Characteristics. Entebbe, Uganda, Statistics Department.
Whitworth, J. A., S. Biraro, et al. (Draft Aug 2004). “The contribution of unsafe injections to HIV-1 transmission in Masaka district, Uganda.”.
Whitworth, J. A., C. Mahe, et al. (2002). “HIV-1 epidemic trends in rural south-west Uganda over a 10-year period.” Trop Med Int Health **7**(12): 1047-52.