Epidemiology of HIV and selected blood-borne infections in East-Africa

Hladik, W.

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General Discussion
Chapter 12

Research undertaken for this thesis focused on HIV and selected blood-borne infections in East-Africa, the region with the second highest burden of HIV disease worldwide after Southern Africa. In this low-resource setting, health information systems tend to be very incomplete, i.e. lacking vital registration systems and reporting HIV related events such as new HIV diagnoses, HIV morbidity and mortality inadequately. Surveys as well as estimates based on modelling and projections are therefore essential to fill the information gaps. It is also a region that, despite substantial improvements in recent decades, remains mired with public health challenges, including access to health care and prevention services. The result is significant under-five and premature adult mortality, in no small part also due to HIV and related co-infections such as TB or human herpesvirus 8 (HHV-8). Thematically, the work in this thesis may be summarised as focusing in the following areas: Modelling and projections of the HIV epidemic to inform public health, the epidemiology of HIV in key populations within generalised HIV epidemics, the use of secondary HIV programme data for HIV surveillance, HIV incidence and surveillance, and the interactions of HIV and various co-infections.

The studies in this thesis have shown that HIV extols an enormous burden of disease on the general population in East-African countries. Still, key populations at increased risk for HIV, such as men who have sex with men (MSM) in Uganda, are the most heavily affected population segments and have poor access to tailored HIV services. Family planning, often neglected as an explicit tool for averting vertical HIV transmissions, is demonstrated to have enormous potential in mitigating the paediatric HIV epidemic, especially in countries with high total and unwanted fertility rates. While HIV surveys and surveillance underwent radical changes since the 1980s, antenatal clinic (ANC) based HIV surveillance continues to be the backbone in many countries’ surveillance system. We examined the potential of Prevention of Mother-To-Child Transmission (PMTCT) programmes in Kenya to eventually replace ANC HIV surveillance which relies on unlinked anonymous testing that provides no direct benefits to the tested pregnant
women. HIV recency assays have the potential to revolutionise HIV surveillance which traditionally was confined to measure prevalent HIV infections. We evaluated the effect of CD4 cell counts and anti-retroviral treatment (ART) on the specificity of the BED HIV recency assay.

The second part of this thesis focuses on select blood-borne infections. In a prospective cohort study we showed that HHV-8, the causative agent for Kaposi’s sarcoma, likely is transmissible by blood transfusion. Using data from the same study, we also demonstrated an increased risk of death following transfusion with HHV-8-antibody-positive blood, an observation that warrants further research. Both studies were conducted in Uganda where HHV-8 infection appears to be highly prevalent. We estimated the seroprevalence of HHV-8 among candidate blood donors in Kampala, Uganda and confirmed that approximately one in three blood donations test positive for antibodies to HHV-8. In another study among candidate Ugandan blood donors we found that the prevalence of HCV infection is likely below 1% and estimated the associated screening costs. The last study in this thesis evaluated paediatric transfusion recipients with severe malarial anaemia and demonstrated an increased mortality risk among HIV-infected compared to HIV-uninfected transfusion recipients.

Part 1 Epidemiology of HIV infection

12.1.1 Modelling and projections of HIV disease to inform policy, programming, and the public

Two chapters focus on the use of survey and programme data to model, extrapolate, and project HIV disease estimates on a national or subnational level. Tailored modelling software such as the Epidemic and Projection Package [1], as well as software originally developed for demography [2], are widely used by Ministries of Health, researchers and UN agencies to estimate a wide range of HIV-related issues, including new infections, care and
treatment needs, mortality but also the need for human resources in public health and accompanying funding requirements. As acute HIV infection often lacks specific symptoms or is asymptomatic, and terminal HIV disease occurs without accessing the health care system or without being diagnosed and reported, such modelling and projection work is essential in all of East Africa to quantify the estimated burden of HIV disease. UNAIDS routinely relies on the same software packages to produce regional and global HIV estimates which drive the universal discussion of this pandemic.

In our work on estimating and forecasting the HIV epidemic in Uganda, we determined that by around 2010, the absolute burden of HIV disease probably reached or eclipsed the corresponding burden during Uganda’s peak HIV prevalence in the early 1990s, despite a substantial reduction in HIV prevalence since then. To the largest part, the answer for this observation lies in Uganda’s high population growth that rapidly increases the absolute burden of HIV disease even while relative HIV measures (HIV prevalence) are held constant, or indeed may decline. This work and population dynamics also made clear that both AIDS mortality and the rate of new HIV infections remain high despite expansion of anti-retroviral treatment programs.

The other work using projection software focused on examining the effect of family planning (FP) on vertical (mother-to-child) HIV infections, and compared it to the conventional ART based PMTCT programme in Uganda. By its very nature, HIV infections averted through FP or anti-retroviral medications (ARVs) are non-events and as such not directly measurable. The effect of FP and PMTCT can only be inferred by creating and comparing projections with and without such programmes. This work demonstrated that FP has a substantial HIV prevention effect, derived from HIV-infected women opting to use family planning to delay or prevent any new pregnancies which may result in vertical HIV transmission. Despite the large unmet need in Uganda, FP in the last 5-10 years in this country averted substantially more HIV infections than ARV-based PMTCT programmes. Notably, our FP based estimates likely are
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Conservative as we assumed fertility rates would not differ by HIV status. Literature however suggests that HIV-infected women of reproductive age who know of their HIV-positive status express much lower wanted fertility rates than HIV uninfected women. Ever increasing HIV testing programmes likely will therefore lead to an increase in FP uptake among HIV-infected women, if adequate access to FP services is provided. The difference (number of infections averted by FP versus ARV-based PMTCT) is very dynamic as maternal and child health programmes in East Africa are hard at work to promote FP whereas donors and policy makers are increasingly committed to virtually eliminate paediatric HIV by aiming at 100% coverage of ARV-based PMTCT programmes. An unexpected, if less significant finding in this work was that ARV-based PMTCT may actually have increased the number of orphans because the short-term administration of ARVs among women and neonates for PMTCT results in a greater survival benefit for the offspring compared to their mothers. The recent shift in PMTCT ARV regimens away from single nevirapine or dual ARV regimens towards life-long ART for HIV-positive mothers may make this a transient phenomenon.

Future research lies in part in the further refinement of the projection software packages available. New biomedical interventions – treatment for prevention and pre-exposure prophylaxis – need to be considered in future software upgrades to allow for the routine estimation of the effect of these interventions on the population level.

Also, behavioural parameters in the prevailing projection models usually do not play a significant role, in part because of the wide variation and uncertainty of efficacies of behavioural interventions across time, place, and person, and, in part because the levels of effectiveness (programme quality) may vary even more and are difficult to monitor compared to ARV-based or surgical interventions. There is no question however that behaviour change remains key in HIV prevention in generalized epidemics. With the prominent role that projection-based estimates play in policy and
funding decisions and target setting, it is paramount to aim for fully comprehensive software packages as indicators or interventions that cannot be estimated with these software packages risk being neglected in HIV control programmes.

Key populations at higher risk for HIV (formerly known as “Most at Risk Populations”, i.e., men who have sex with men (MSM), sex workers, and persons who inject drugs) are another example of relative neglect in the projection work of many countries and institutions in East Africa. This fact is less due to the difficulties of incorporating key population estimates in national HIV disease projections but more often reflect the relative lack of survey and surveillance data in these populations. Because most surveillance work in East Africa focuses on the general population or declared proxies thereof (e.g., pregnant women) the survey data available on key populations are usually inadequate to be incorporated in projection packages. Surveillance officers and donors therefore need to devote more effort and resources towards these population groups which in turn will facilitate separate projection estimates for these often very different HIV sub-epidemics.

Further, the relative neglect of the contributions of FP towards PMTCT needs to be addressed, for example by generating and mandating FP-related indicators within or related to PMTCT programmes. FP is still primarily viewed in maternal and child health terms and has yet to be fully recognized as a key HIV prevention tool that merits its own indicators for monitoring and evaluation within the PMTCT framework.

12.1.2 Surveillance of HIV epidemics among key populations in generalized epidemics

We examined the burden of HIV disease in MSM in Kampala, Uganda. During the conduct of the survey, sampling was particularly challenging as lesbian, gay, bisexual, transgender, and intersex (LGBTI) activists and alleged homosexual persons were arrested twice in Kampala. Each time, sampling of MSM slumped while
at the same time concurrent sampling of sex workers and other groups continued at roughly the same levels. A previous MSM survey conducted in Kampala in 2004 by different investigators [3], was aborted after media reports questioned whether survey respondents may have been paid to have sex with other men (Phoebe Kajubi, 2008, personal communication). Such challenges put surveys at risk or may discourage investigators to initiate sampling, which in turn contributes to the paucity of survey data to inform the public health response.

The achieved sample size in our survey was just under 300 respondents, far lower than the target sample size of 600. To a large part this may have been due to the noted arrests during the survey period. However, respondent driven sampling (RDS) surveys may fail for various reasons, including insufficient networking among members of the target population, insufficient incentives, or lack of trust in the survey staff and investigators. Further, the extent to which RDS surveys are representative for the target population are subject to ongoing debate and research [4, 5]. Where several networks of the target population are isolated and separated from each other, RDS may only penetrate those networks from which seeds (initial respondents) were recruited from. Consequently, and usually unknown to the investigators, the resulting estimates are only representative for these networks. Subsequent surveys with seeds identified from different networks may subsequently yield estimates (e.g. HIV prevalence) that may be substantially different from previous surveys and falsely suggest increasing or decreasing trends. Networks that are linked to each other but only poorly so (few members of one network are connected to members of another network) may greatly increase such surveys' design effects.

Debate also continues as to the validity of RDS based estimates adjusted for the sampling frame. Tailored software packages (downloadable at http://www.respondentdrivensampling.org) or estimators developed in conventional software packages (such as R) facilitate estimates adjusted for the complex sampling frame.
RDS is a relatively new technique for network based sampling and research is ongoing as to the correct computation of variance, design effects and resulting confidence limits. Although peer referral is the common characteristic of both RDS and snowball sampling, RDS is deemed superior in that it yields a probability sample. Work is also ongoing to use RDS data directly to estimate population sizes, using methods that have yet to be more validated or piloted [6].

RDS surveys may also have potential to be carried out in cyberspace as web-based surveys [7]. Web-based RDS surveys importantly demonstrate the feasibility of peer-referral without the need for the recruiter to physically meet the recruitee. Although web-based RDS seem to make the measurement of biomarkers difficult or impossible, conventional RDS surveys using a physical survey office would benefit from identifying ways to facilitate peer-referral without necessitating physical contact between recruiter and recruitee. RDS guidelines mandate that survey participants receive a limited number of coupons (typically three per respondent) which they are asked to hand over to individuals they personally know within a set coupon expiration date. The peer-referred candidate then has to present the paper coupon to the survey office in order to be considered for enrolment. Hence, recruiter and recruitee often have to make an effort to physically meet and exchange the coupon which may be time consuming and may incur costs to both parties. Future survey research should investigate ways to convert paper-based coupons into an electronic format that may be emailed or sent by short message service (SMS). Maintaining anonymity and preventing the electronic coupon to be forwarded across several persons appear to be among the main challenges for such "e-Coupons". Internet penetration (the proportion of individuals having internet access) and cell phone coverage will determine the resulting degree of systematic sampling bias.
Currently, the main alternative to RDS for sampling "hidden" key populations (i.e. populations with low social visibility) is time location sampling (TLS) [8], an active sampling design where survey staff identify and sample members of the target populations at social venues, such as bars, clubs, or parks. In Kampala, formative assessment quickly suggested that TLS may be unsuitable due to the high level of stigmatization and homophobia, which may prompt venue owners to refuse sampling take place on their premises, and candidate survey participants hesitate to enrol in such surveys. Additionally, it was unclear whether the majority of target population members indeed would frequent such venues. Sampling hidden populations continues to be a challenge in Africa and elsewhere and efforts to either improve existing methods or identify new techniques are urgently needed.

Surveillance and surveys of key populations generally need to meet three principal information needs: the burden of disease and risk factors thereof, the population size, and the geographic distribution of key population members. In this MSM survey we attempted to estimate the population size using the multiplier method [9], by including survey questions about membership in community based organizations, whose absolute membership sizes were known to the investigators. The resulting estimates however were unrealistically small (data not shown). Other methods for population size estimation include capture-recapture analysis [10], measuring the prevalence of high risk behaviours in general population based surveys, and, recently, network scale-up [11-14]. None of these methods is without limitations and challenges; nevertheless, efforts to produce such estimates are necessary to advocate for and inform funding and programming.

The estimated HIV prevalence among Kampala MSM was about 3-4 times that of men in the general population. Throughout the world MSM appear to have substantially larger HIV prevalence ratios compared to the general population [15]. The resulting prevalence ratios (HIV prevalence in MSM : general population) vary substantially across world regions and appear highest in South
East and East Asian countries, or in Latin America, and are perhaps the lowest in sub-Saharan Africa, where generalized epidemics produce the highest HIV prevalence ratios world-wide. Put another way, HIV prevalence among MSM across different countries and world regions appear more similar to each other than to the same-country general population HIV prevalence. This observation may lend credit to the possibility that HIV epidemics among MSM are largely de-linked from epidemics in the general population and therefore largely independent from the “background” (general population) HIV prevalence. In contrast, HIV prevalence ratios in female sex workers in African countries appear to be more closely related to those seen in the general population. Confirmation of such “separate” HIV epidemics among MSM through further research including molecular epidemiological work examining HIV subtype distribution would make a stronger case for tailored and intensified HIV prevention interventions in this highly vulnerable population.

In contrast, the current global funding landscape for HIV services (such as PEPFAR, Global Fund, or the World Bank) suggests that HIV interventions for MSM (as other key populations) are underfunded compared to services for the general population [16]. In Uganda, the government does not allocate any specific funding towards HIV-related services for MSM, often citing the illegality of homosexuality as the reason for non-funding, despite the fact that (low-level) services are available for sex workers when sex work is criminalised as well. International donors need to provide specific funding for key populations and tie the adequate use of these funds to the receipt of all or part of the remaining funds. Survey investigators need to do their part by putting key populations “on the map” by regularly surveying them and disseminating these data. Policy makers and United Nations agencies should consider separate indicators for key populations, including serostatus knowledge, care and treatment coverage, to increase attention of national policy makers and programmers to these sub-populations that often warrant their own and tailored services.
One intriguing finding in our survey was that MSM with a lifetime history of homophobic abuse were more likely to be HIV-infected than MSM who did not report such abuse, after controlling for age. The cross-sectional survey design does not allow us to speculate about a causal relationship between homophobic abuse and subsequent HIV infection. It should prompt however further research in possible pathways, such as mental health measures that may mediate sexual behaviour.

12.1.3 Use of routine HIV programme data for HIV surveillance

We assessed accessibility and quality of PMTCT programme data in Kenya, compared HIV prevalence estimates from PMTCT programmes with those from ANC sentinel surveillance and identified determinants of observed differences in HIV prevalence estimates. In this study, both PMTCT data quality and HIV testing acceptance for PMTCT varied substantially by clinic, leading us to the conclusion that in such situations PMTCT data cannot replace unlinked anonymous testing (UAT) for the purpose of HIV surveillance.

In sub-Saharan Africa, HIV surveillance since the late 1980s relied on UAT of pregnant women who attend ANCs where they would routinely be bled for syphilis screening or haemoglobin measurement [17]. The remaining blood is de-linked from personal identifiers, basic data are transcribed from the ANC clinic registries (e.g., age, marital status, parity, syphilis result), and both data and specimens are transported to a more central laboratory for UAT. The lack of informed consent and the technical inability to return the HIV test result to the pregnant woman always exposed this surveillance system to some level of criticism. In the USA, UAT was discontinued in the 1990s largely because politicians criticized the programme as unethical.

With the expansion of HIV testing for PMTCT, a renewed discussion began in the 2000s, questioning not only the ethics of UAT without returning the test result that could be used for PMTCT-
related services but also about the potential utility of PMTCT data to either complement or replace UAT data. The former criticism is usually addressed by ensuring that UAT sites also offer PMTCT to the same ANC attendees. In addition, evaluations are ongoing about the utility of PMTCT data for the purpose of surveillance. Clear advantages for the use of PMTCT data – return of test results, far larger sample sizes and a larger number of PMTCT sites – have to be weighed against challenges and potential biases, including quality of routine PMTCT data, accessibility of these data, and self-selection bias for HIV testing in PMTCT. Self-selection bias has become a smaller issue since PMTCT programmes switched from opt-in to opt-out for HIV testing (i.e. informed consent is assumed unless the health care provider is told by the patient otherwise), which in many countries meanwhile yields HIV testing acceptance rates of more than 90% at most sites.

With PMTCT programmes aiming at universal coverage, individual data transcription from all PMTCT sites is unfeasible in most countries. Instead, operational research should consider the utility of using aggregate PMTCT data for surveillance. Aggregate PMTCT data are often reported monthly by clinics up to a more central level, usually reaching the ministry of health level eventually. Because HIV surveillance often only uses overall HIV prevalence or HIV prevalence among 15-24 year olds, the utility of individual PMTCT data may be overestimated. As aggregate PMTCT HIV testing data likely is reported from a great number of clinics, this offers the prospect of a much expanded surveillance system compared to UAT. Future PMTCT-based HIV surveillance systems can be envisioned incorporating both individual data transcription (for a small number of clinics) and aggregate data analysis (for all clinics).

Because of the large number of pregnant women that PMTCT programmes serve, this system also should be considered for laboratory-based HIV incidence surveillance. Relatively new assays offer the prospect of estimating HIV incidence from cross-sectionally collected blood samples. A PMTCT based HIV incidence
surveillance system would call for the routine collection of left-over HIV-positive blood specimens in PMTCT settings and the transcription of a few essential individual data elements (from both HIV-positive and negative patients), such as patient age, antiretroviral treatment (ART) status, and, possibly CD4 count; with the latter two variables aiding the determination of HIV recency. A concerted effort towards the direction of HIV incidence surveillance has the potential of complementing or replacing conventional HIV prevalence based surveillance, offering the advantage of detecting changes in the dynamics of HIV epidemics in a much timelier manner.

In a larger context, the massive expansion of HIV testing through various services deserves examination of its utility for surveillance. Beyond PMTCT, voluntary counselling and testing (VCT) programmes may have such potential. Although VCT clients to a large extent self-select themselves for HIV testing, trend observations in HIV prevalence may offer valid observations, and risk factors for HIV infection may yield useful findings for HIV programming. Similar to PMTCT systems, VCT sites with large sample sizes offer the prospect of subjecting remaining blood specimens (of HIV-infected clients) to HIV recency testing. This can facilitate the categorization of HIV-infected clients as either recently infected (depending on the time period during which assays or algorithms for HIV recency would label a specimen as “recent”) or long-term infected. Correlating VCT clients’ behavioural characteristics with HIV recency status would allow examination of HIV acquisition behaviour (for recently infected clients) or transmission behaviour (for long-term infected clients). In summary the massive sample size of HIV testing programmes, such as VCT and PMTCT, may offer new or additional avenues for HIV surveillance.
12.1.4 HIV incidence surveillance

We examined the specificity of the BED IgG capture immunosorbent assay using stored blood specimens and data from known long-term HIV-infected Ugandan adults before and after initiation of ART. In this study, the proportion of specimens testing false-recent was substantial and increased with time on ART.

Traditionally, HIV incidence is measured through cohorts where individuals are followed prospectively over time. Other methods include back calculating [2] or modelling [1] HIV prevalence data to infer the rate of new infections, as well as comparing HIV prevalence over adjacent age bands across two subsequent surveys [18]. Cohorts, often assumed to be the gold standard for incidence estimates, rarely are representative beyond their catchment area and lose representativeness even within their catchment area over time as the act of observation and measurement tend to alter people’s behaviours. Back calculation, modelling, and age band comparisons across comparable serial surveys have the distinct disadvantage of not lending themselves to individual level data analysis.

The newest alternative to the aforementioned methods for incidence measurements are laboratory assays that can distinguish recent from long-term HIV infections by testing a single blood specimen. These assays exploit the fact that the immune response to HIV infection in the first few months differs from that in mature infections. Early HIV infections are characterized immunologically by lower levels of immunoglobulin G (IgG), by anti-HIV antibodies with lower avidity (binding strength), by high CD4 cell counts, and by different time points during which certain HIV peptides start being expressed.

One of the most widely used HIV recency assays is the BED HIV-1 capture EIA. Specimens testing “positive” by the BED assay have relatively low levels of IgG compared to the total IgG. One challenge however is that total IgG may vary across individuals.
and world regions, which may affect the rate of false recent test results. Differences in the incidence of various other co-infections (e.g. malaria, trypanosomiasis, leishmaniasis) affect the level of total IgG and hence the frequency of false recent test results. This is the primary reason why “local” determination of the false-recent rate (FRR, also known as “epsilon”) is recommended. The local FRR can then be applied to adjust locally determined population level HIV incidence estimates. Further, individuals with advanced HIV disease or successful ART tend to have lower levels of HIV specific IgG, again leading to false recent test results.

Meanwhile, further development of HIV recency assays continues. One such recently developed assay, the limiting antigen avidity (LAg) EIA[19] appears to be more promising than the BED assay, as it seems to have a much lower FRR, possibly low enough to ignore it in incidence calculations, and stable enough across world regions so that a local FRR determination may not be warranted. Other recently developed assays and techniques include the V3-IDE EIA [20, 21], antibody avidity-based assays [22], certain molecular methods [23], or exploiting HIV’s genetic diversity over time as a marker for individual HIV infection [24]. In addition, efforts are also under way to evaluate algorithms to increase specificity; either by including a combination of different HIV recency assays or the consideration of additional laboratory markers, such as CD4 counts and viral load, both of which vary over the course of natural HIV infections as well.

HIV recency assays with acceptable accuracy will have potential beyond surveillance, such as for the evaluation of HIV interventions or for inclusion in individual HIV counselling and testing programmes. In research and surveillance, the main impediment for these assays’ implementation is and will be the challenge of satisfying their much larger sample size needs, compared to activities using prevalent HIV infection as the outcome of interest. For example, the LAg avidity EIA’s period of recency of 141 days or approximately 0.4 years leads to much smaller numerators (number of recent HIV infections) than HIV prevalence
surveys or surveillance systems, as the average life expectancy with
for individuals with untreated HIV-infection is currently estimated
at 11 years [25], some 25 to 30 times that of the LAg avidity EIA’s
period of recency. The resulting sample size needs are particularly
difficult to achieve in evaluations of small target populations, such
as MSM, or people who inject drugs, even when HIV incidence
may well be substantially higher in these groups compared to the
general population.

Part 2 Epidemiology of selected blood-borne infections

12.2.1 HIV, co-infections, and blood transfusion

Already prior to sub-Saharan Africa’s onset of generalized HIV
epidemics, poverty, inadequate access to health care, poor
nutrition and sanitation, and poor health literacy were important
determinants for its excess morbidity and mortality, much of it
cau sed by infectious diseases. Several of these infections, including
malaria, TB, hepatitis B and C, or human herpesvirus 8 (HHV-8),
are recognized as HIV co-infections, defined as infectious diseases
that accelerate HIV disease progression and/or causing diseases
that traditionally were part of the classifying criteria for a clinical
AIDS diagnosis. Several studies conducted for this thesis focused on
blood transfusion related aspects of some of these co-infections.

12.2.2 HHV-8 and blood transfusion

Much of this thesis’ work focused on HHV-8, an infection not
only endemic in East Africa, but also likely having its origin in this
world region, as evidenced by the highest prevalence estimates of
HHV-8 infection worldwide stemming from this region. As a cell-
associated virus belonging to the herpes virus family, HHV-8 is
believed to cause life-long infection. The vast majority of healthy
HHV-8-infected individuals remain asymptomatic throughout their
life and HHV-8 is usually controlled well enough to make viraemia
undetectable most of the time.
This thesis’ HHV-8 research examined three key aspects: the presence of HHV-8 infection in blood donors, the risk of HHV-8 transmission by transfusion, and the potential acute sequelae following transfusion of HHV-8 antibody positive blood. Our studies’ findings suggest that Ugandan blood donors have high HHV-8 seroprevalence; that HHV-8 likely is transmissible by blood transfusion; and that post-transfusion mortality is associated with transfusion of HHV-8 antibody positive blood. Our concurrent work on morbidity-related signs and symptoms associated with HHV-8 seroconversion in Ugandan transfusion recipients yielded no substantial findings (data not shown) possibly due to confounding by frequently present signs and symptoms in all transfusion recipients (HHV-8 seroconverters and non-seroconverters) due to their underlying disease leading to transfusion.

HHV-8 causes several distinct pathologies, foremost among them Kaposi’s sarcoma, or KS. One type of KS includes African KS, defined as KS in individuals residing in sub-Saharan Africa without any other known risk factor [26, 27], African KS was recognised in East-Africa decades prior to the rise of HIV [28, 29], and KS rates are much higher than in other parts of the world. The exact reasons for these high background rates are not known although one underlying factor certainly are the high HHV-8 seroprevalences seen in East Africa, often exceeding 30% in the general population [30-32]. The high HHV-8 seroprevalence is also reflected in healthy blood donors as evidenced in our and other studies on the continent [33, 34].

Studies involving HHV-8 serologic testing are limited nevertheless, in no small measure due to the unavailability of accurate, commercially available assays. HHV-8 serology generally suffers from both sub-optimal sensitivity and specificity. The sensitivities of assays appear limited mainly to the low level of anti-HHV-8 antibodies. Testing in the studies presented in this thesis included three different assays, all non-commercial, with one of
them microscopy-based and carried out at two separate dilutions. This form of testing is not feasible in routine settings, such as for the screening of candidate blood donors and impedes HHV-8 related research.

We found that the estimated HHV-8 seroprevalence was approximately ten times that among US blood donors, suggesting that excluding HHV-8 antibody-positive donors in Uganda would dramatically sharpen the existing short supply of screened blood.

Importantly, the exact transmission modes for HHV-8 remain elusive. The available literature points to vertical, sexual, and non-sexual, horizontal transmission [35]. Prevalence studies suggest different transmission patterns in different parts of the world. In East Africa, HHV-8 seroprevalence rises sharply beginning in early childhood; in Southern Africa, this rise commences later in age [36], whereas in Europe or North-America HHV-8 prevalence estimates are both lower and without an apparent rise during childhood [37]. The reasons for these differences in HHV-8 prevalence by age and magnitude are not understood. Our research examined HHV-8 transmissibility by blood transfusion. Previous studies were usually cross-sectional in nature, focused on either donors or recipients, and were often hampered by low background HHV-8 prevalence in blood donors. Our study's strength lied in its setting, a region with very high HHV-8 seroprevalence, its prospective design with serial recipient blood samples collected both before and after blood transfusion, and the linkage of blood donors and recipients which allowed for the determination of exposure status for each transfusion recipient. The study design may serve as a reminder about the strong potential offered by prospective, linked donor-recipient studies.

Although our data provided good evidence about the transmissibility of HHV-8 via transfusion, no blood bank worldwide has initiated routine screening for this virus. That no blood bank screens for HHV-8 may be due to mainly three reasons: the lack of evidence that HHV-8 is transmissible by blood transfusion, lack of
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evidence that HHV-8 in donated blood can cause significant harm to transfusion recipients, and the unavailability of commercially available assays suitable for high throughput testing of donated blood. Other considerations include the varying background prevalence among blood donors world-wide, which strongly affects the risk of transmission per unscreened blood bag, as well as the varying practices of processing blood. Blood banks in Uganda and many other countries in sub-Saharan Africa do not or not fully remove the buffy coat a thin layer of blood where white blood cells are concentrated and thus may harbour cell-associated HHV-8. In contrast, removal of the buffy coat is routine practice in industrialized setting, which, along with the lower HHV-8 seroprevalence see there, may further reduce the risk of HHV-8 transmission. Buffy coat removal or leukocyte depletion may represent a cost effective intervention in the absence of specific screening assays and provide additional (if partial) protection against other cell-associated viruses, including agents perhaps not yet identified. It should be noted again that transmission risk estimated at 2.0%-2.5% per HHV-8 seropositive blood bag transfused is likely conservative due to the testing algorithm and classification tree that emphasized specificity over sensitivity. The need for donated blood is high in Uganda and throughout East Africa, where malaria remains endemic and the most frequent cause of anaemia requiring transfusion. In Uganda, approximately 200,000 blood donations are collected every year, many of which are split in multiple blood bags for paediatric use. The high HHV-8 seroprevalence in blood donors and the low age of many transfusion recipients (who are more likely to be HHV-8 negative) implies a large number of transfusion-related transmissions each year. Although the number of transfusion-associated infections is most likely dwarfed by horizontal, community-based HHV-8 transmissions, it is worth remembering that these are iatrogenic infections in patients with poor health status.

Our findings on the transmissibility of HHV-8 by transfusion have different implications for industrialised settings. The lower HHV-8 prevalence in blood donors and routine leucodepletion likely
result in substantially lower per bag transmission risk compared to our estimate in Uganda. The findings however add to the ongoing discussion about the merit of donor referral. For example, in the U.S., MSM are deferred from blood donation for life [38]. The Food and Drug Administration (FDA) cites several reasons for this decision, among them the relatively high HHV-8 seroprevalence among U.S. MSM. Our study’s findings were discussed shortly after its publication during a hearing at the FDA on blood donor deferral [39].

The third aspect of our transfusion related research on HHV-8 in Uganda examined the risk of mortality among recipients of HHV-8 antibody positive blood [40]. Our finding that such recipients were almost twice as likely to die within six months post-transfusion is not described elsewhere in the literature to our knowledge. Several limitations need to be noted when considering this finding, including the lack of cause of death data, the lack of a demonstrated seroconversion risk among exposed deceased study participants, and the lack of demonstrable HHV-8 viraemia in either donor or recipient blood.

Conversely, several observations indicated that the observed overall mortality risk may not be a chance finding: the risk of death was observed only for transfusion of HHV-8 antibody positive blood bags stored four days or less between donation and transfusion, supporting the notion that a storage temperature labile agent is responsible for the elevated mortality risk. A similar observation was made previously with other infectious agents, including cytomegalovirus [41], another herpes virus family member. Further, we demonstrated a dose-response relationship as each additional transfusion of HHV-8 antibody positive blood increased the mortality risk. Because the number of transfusions likely was a strong marker for ongoing or increasing illness among recipients we also analysed recipients of a single transfusion and found that the HHV-8 related mortality risk also remained. When the reference group (recipients of HHV-8 antibody negative blood regardless of storage time) was limited to those whose transfused blood was
stored four days or less as well, the HHV-8 associated mortality risk remained. In the main analysis we had removed all deaths occurring in the first seven days post transfusion on the assumption that any deaths due to transfusion with HHV-8 antibody positive blood would take some time to materialize. When, in a separate sub-analysis, we restricted the data set to the first seven days post transfusion, we found our assumption confirmed as transfusion with HHV-8 antibody positive blood was not associated with any excess risk of death.

Our study’s findings raise the possibility that HHV-8 may cause serious acute sequelae. To date, HHV-8 is best known for causing serious chronic disease usually in immunosuppressed individuals, including KS, multicentric Castleman’s disease, and pleural effusion lymphoma [42]. Severe acute outcomes have been reported only anecdotally in the literature [43-45]. It seems worth remembering that all our study participants who received blood were severely ill at time of exposure. At this time however, the exact potential mechanism of HHV-8 causing death within a short time of infection, if at all, remains speculative and calls for more research in this area. A confounder is the most likely alternative explanation for our observed mortality risk. Our study’s donated blood already was routinely screened for HIV, syphilis, and hepatitis B surface antigen (HBsAg). Plasmodial parasites in donated blood were evenly distributed and not associated with transfusion of short-stored HHV-8 antibody positive blood. Epstein Barr virus is near ubiquitous in Uganda, including blood donors (data not shown). Cytomegalovirus remains another possible agent worth considering although its seroprevalence in Uganda likely is very high as well.

What do our findings mean for transfusion medicine? Because our study’s findings are unique and not confirmed elsewhere, and since our study lacked cause of death data and could not detect HHV-8 viraemia in deceased study participants, we cannot recommend any change in transfusion practice, such as HHV-8 related donor deferral or removal of blood. In resource limited setting where
donated blood is often scarce and HHV-8 seroprevalence can be high, blood transfusions save many lives and shortages of donated blood is a frequent challenge. More research is needed on the cause of death in such exposed patients, on HHV-8 DNA in linked donor-recipient pairs, and on the possible preventive effect of leucodepletion and/or irradiation. A similar study design evaluating such potential interventions in a high prevalence setting should be considered, perhaps with a three or four arm design: HHV-8 seronegative transfusion recipients exposed to 1) HHV-8 antibody negative blood, 2) HHV-8 antibody positive, not leucodepleted blood, and 3) HHV-8 antibody positive leucodepleted (or irradiated) blood. Better follow-up that ascertains cause of death (most of which are likely to occur outside the hospital setting) is needed. However, ethical implications of such a study design may not be overcome, as institutional review boards may insist on leucodepletion of, or irradiating all donated blood. If such a study design were found ethical, investigators would need to be prepared to halt the study as soon as a significant protective effect would emerge from leucodepletion and an ensuing ethical dilemma may arise if local blood banks would not have the (financial) means to routinely implement such a protective processing step. Alternatively, a study utilising autopsy on deceased (former) transfusion recipients and their linked blood donors’ blood samples may shed light on the cause and mechanism of death among transfusion recipients exposed to HHV-8 antibody positive blood.

12.2.3 Hepatitis C virus infection and blood transfusion

At the time when work for this thesis commenced, laboratory screening of candidate blood donors in Uganda included HIV, HBsAg, and Treponema pallidum. For many years, Ugandan blood banks’ primary challenge was to solicit sufficient numbers of candidate volunteer blood donors to satisfy the ever increasing demand for blood, fuelled by malarial anaemia, population growth, and an expanding access to health care. Historically, many blood donations stemmed from family members of hospitalised patients requiring transfusion. More recently, international
donors facilitated the expansion of blood bank infrastructure, the procurement of laboratory consumables for blood screening, and donor drives to identify more volunteer donors.

Screening for hepatitis C virus (HCV) in Ugandan candidate blood donors has meanwhile been added. Before that, the prevalence of HCV in candidate blood donors was unknown. In our evaluation we found that the HCV prevalence was comparatively low and that some of the HCV-positive blood bags were screened out as they were more likely to also test positive for HIV or HBsAg, both of which blood banks in Uganda already screened for. A simple cost analysis for a hypothetical screening suggested substantial costs for such routine testing, prompting us to speculate about its merit vis-à-vis other as yet to be implemented preventive measures, such as routine leucodepletion of all donated blood. The implementation of routine HCV screening makes the case for leucodepletion more compelling, especially in light of the HHV-8 related findings reported here and given the fact that Uganda, with its long history of virus discoveries, may harbour other transfusion transmissible viruses, described or not, and as yet unscreened for.

12.2.4 *Plasmodium falciparum*, HIV, and blood transfusion

Sub-Saharan Africa is the world region with the highest burden of both malarial and HIV disease. Any interaction of these two frequently occurring infections may therefore have significant public health impact. In our study examining the effect of *P. falciparum* parasitaemia in donated blood on HIV-infected paediatric transfusion recipients, we found that these young children, compared to HIV-uninfected children, face higher all-cause and higher malaria-related mortality than their HIV-uninfected peers.

These findings call for routine HIV testing of all candidate paediatric transfusion recipients in HIV-endemic settings and for aggressive treatment in such patients with an HIV diagnosis. The study serves as a reminder of the immunosuppressive effects of HIV and certain antimalarial drugs such as chloroquine, a frequently
used over-the-counter medication at the time of this study. Also worth remembering is that none of the study participants had received co-trimoxazole prophylaxis or ART at time of enrolment or during follow-up. In the years since this study was undertaken, Uganda’s policy on HIV testing shifted to a universal opt-out testing scheme where all persons accessing health care are considered for HIV testing by default. Similarly, there is a steady increase of the proportion of HIV-infected children under co-trimoxazole prophylaxis and more so under ART, which may alter the observed association between HIV infection, malaria, and blood transfusion. Furthermore, the recent success in malaria control in Uganda should result in a large number of blood transfusions averted – which, arguably, is the safest type of “transfusion”.

References


