Title: Surveys, serology and sequences reveal history of iatrogenic transmission of HIV-1

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Emerging infections are receiving an increasing amount of both scientific and popular attention, with epidemics of Ebola virus in West Africa, and MERS Coronavirus in the Middle East. For these acute infections, the time between 'patient zero' and recognition of an epidemic was short. Conversely, epidemics caused by chronic infections, such as caused by human immunodeficiency virus type 1 (HIV-1), have taken much longer to recognise. HIV-1 was recognized in June 1981 in the United States (1), but emerged from primate reservoirs in Central Africa in the first half of the twentieth century (2).

Given the scarcity of epidemiological data, sequence data have played a major role in consolidating our understanding of the emergence of HIV-1, as the evolutionary relatedness between viruses sampled from different individuals allows insights into the transmission dynamics of infection before the time of the first sample. Based on identification of genetically similar viruses in chimpanzees and gorillas in Cameroon, HIV-1 jumped from apes to humans at least four times (2), resulting in divergent groups of HIV-1 known as M, N, O and P. Given samples over a sufficient time span, the evolutionary rate of the virus can be estimated, which can then be used to infer the time to the most recent common ancestor, or TMRCA, of a set of viruses. This approach was used to estimate the TMRCA of group M HIV as being in the early 20th century (3). Sequence data can also be used to monitor changes in epidemiological dynamics over time, as larger epidemics tend to be associated with higher genetic diversity. Based on a diverse set of sequences from the Democratic Republic of Congo (DRC, formerly Zaire), this approach was used to show an increase in the epidemic growth rate in the 1950s (4). Information on the geographical location of samples can be used to reconstruct spatial spread, which has argued that Kinshasa (formerly Leopoldville) in the DRC was the source of group M sequences that went on to seed the HIV-1 pandemic (5). Furthermore, sequence data can be used to identify recombinant viruses, which emerge when an individual is infected with two or more viral strains; the high frequency of recombinant viruses from Kinshasa in the 1980s suggests that transmission was occurring at a high rate (6). Yet despite these insights, sequence data alone cannot tell us the causes for this spread.

Why did HIV-1 emerge in Central Africa? Given the presence of multiple simian immunodeficiency viruses in different primate species in Africa (7), as well as the increasing amount of contact with wildlife during the Scramble for Africa, with the cultivation of cash crops and the exploitation of natural resources (8). However, human-to-human transmission is required in order for a cross-species transmission event to result in an epidemic, and during the earliest stages of an epidemic, the infection may well 'fade out' due to chance. The routes of transmission of HIV are well characterised, yet their epidemiological importance in Africa has remained controversial. This controversy stems in part from the low probability of transmission of HIV per sexual contact (10). It is only with repeated exposures to an infected person that the cumulative risk of transmission becomes significant. The exception to this is during primary HIV infection, when the infectivity is higher; with high rates of partner change, chains of more efficient transmission involving primary infection may occur. This may have contributed to the rapid emergence of HIV among female sex workers in Nairobi, where

the prevalence rose from 4% in 1981 to 61% in 1985 (11). In contrast, infection via the parenteral route is more efficient, even in chronic infection (12).

What caused HIV-1 to emerge? Early modeling work (13) investigated two possible explanations for how HIV transmission may increase sharply. Firstly, a spatially heterogeneous model where the population is divided into villages can give rise to an increase in the epidemic growth rate, without any change in the epidemiological characteristics of the model. However, the increase in growth rate probably occurred in an urban environment, rather than an interconnected network of villages. Secondly, even a modest increase in contact rate can lead to greatly increased transmission rates, as more transmissions occur during acute HIV infection, when infectivity is high. One proposed pathway of increased contact rates is that iatrogenic transmission played a role in 'jumpstarting' the epidemic. The high rate of transmission of HIV via blood or contaminated needles (12), coupled with the potentially high number of exposures to a single infected needle could potentially amplify a small number of cases into a large number, capable of sustaining further transmission. Western style biomedicine was used as a tool of empire by the colonial powers, and the 1950s was a time of widespread biomedical interventions, including injections for treatment of tuberculosis, sleeping sickness, syphilis, and yaws. Some have argued that iatrogenic transmission must have been important in the very earliest stages of HIV-1 emergence from primate reservoirs (14). A competing, although non-exclusive, pathway is sexual transmission; high rates of sex work that accompanied urbanisation following World War II may have boosted HIV-1 prevalence.

In this issue of the *Journal of Infectious Diseases*, Hogan et al. (15) present evidence that iatrogenic exposure may have played a role in the apparently rapid increase of HIV-1 in the 1950s in Kinshasa, using a combination of viral sequence, serological and epidemiological survey data relating to two other chronic infections - hepatitis C virus (HCV) and human T cell lymphotropic virus 1 (HTLV-1). Elderly residents of Kinshasa, who may have been exposed to blood-borne viruses in the 1950s, were administered a questionnaire, and blood samples taken for serological testing and sequencing of HCV and HTLV-1. For two subtypes of HCV, 4k and 4r, sequence analysis demonstrated a burst of infection starting in the 1950s. Analysis of the serological and survey data demonstrated statistical associations of infection with HCV subtypes 4k and 4r, as well as HTLV, with potential iatrogenic exposure, particularly those reported to have occurred before 1960. A high proportion (12/26) of HTLV infected individuals were also infected with HCV, although the specific iatrogenic associations differed for the three different viruses, suggesting distinct but overlapping epidemics perhaps associated with transmission at different hospitals or clinics. The study team had used similar approaches elsewhere (16), but the focus on Kinshasa, the crucible where group M HIV-1 emerged makes it more relevant for studying pandemic HIV-1.

The retrospective approach of Hogan et al. suffers from some unavoidable limitations. The sampling frame for the study was biased away from those individuals who had already died, such that HIV+ individuals and HCV+ individuals who had already developed cirrhosis or hepatocellular carcinoma are underrepresented. The sequence

analysis was also prone to bias due to the age of participants, which would inherently result in large 'effective population sizes' close to the present. Tips of the tree represent a combination of within-host. Although individuals co-infected with HCV and HTLV-1 were overrepresented, suggesting that individuals may have been infected with both viruses at the same time, there was insufficient information in the sequence data to compare phylogenetic trees with confidence. The recall of events in the distant past is often prone to forward telescoping - thinking that an event occurred more recently than it actually happened, although Hogan et al. used events to anchor an individual in time, and hence minimise this effect. Despite these issues, the level of concordance in the survey and sequence data is reassuring. Another strength is that the sequence and survey data were collected from the same individuals, although it is difficult to integrate sequence data and behavioral data, and the sample size for phylogenetics was restricted to those infected, this was not a problem for HCV, as the prevalence was high (25.9%), but HTLV prevalence was much lower (3.1%) and may have limited statistical power.

Why did group M viruses, which went on to become pandemic, emerge in Kinshasa, rather than Cameroon, where they presumably originated? Recent results on group O HIV-1 add to the puzzle. Like group M, group O originated in southern Cameroon, although from gorillas rather than chimpanzees (17), and like group M HIV-1 in Zaire, group O HIV-1 also underwent an increase in genetic diversity in the 1950s, consistent with an increasing epidemic (18). Unlike groups N and P, which are extremely rare and appear to have limited transmissibility, individuals infected with group O HIV-1 have a high viral load. Biomedical interventions were widespread in Cameroon, with rapid HCV diversification (19), consistent with epidemiological data demonstrating a cohort effect (20), with individuals born in 1940 having the highest HCV prevalence, and an association between trypanosomiasis treatment and HCV and HTLV infection amongst individuals 55 years and older (21). There may have been competition between group M and group O HIV-1, although individuals dually infected with groups M and O have been identified (22), or the emergence of HIV-1 in Zaire rather than Cameroon may simply reflect a founder effect, like those associated with HIV-1 epidemics outside of Africa.

What about today? The widely held view is that sexual transmission is the predominant route, supported by mathematical models that exhibit relatively low rates of iatrogenic transmission (23) and a high risk associated with patterns of concurrency, although this remains contentious (24). Nevertheless, we should strive for the reduction of iatrogenic transmission, although there are barriers to be overcome in terms of provision of sufficient medical supplies and infrastructure, and improvements in biosecurity may be offset by the large reservoir of individuals harboring blood-borne viruses. In Egypt, which has the largest HCV epidemic in the world (27), probably as a result of parenteral-antischistosomal-therapy (PAT) mass-treatment campaigns (28), most new infections are iatrogenic in origin (29).

What lessons should we glean from studies like Hogan *et al.*? All too often, such studies are erroneously interpreted or viewed with scepticism, such as following the publication of molecular epidemiological evidence implicating Haiti as a stepping stone for the

dissemination of HIV from Africa to the United States (31). Such studies should be treated more than a history lesson. Iatrogenic risks are not unique to Africa; Mongolia, for example, has a rate of hepatocellular carcinoma six times the global average, driven by high HCV and hepatitis B prevalence; while the exact reasons for the high prevalence of viral hepatitis are not known, epidemiological studies point to dental treatment as a risk factor (33). In an era of increasing population mobility, which drove the dissemination of HIV from Africa to the rest of the world, we have to take shared responsibility for development of health services and appropriate prevention strategies. With current discussions of vaccination to protect against Ebola virus (34), iatrogenesis should remain a concern in the global health agenda.

Notes

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