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Epidemiology and the emergence of human immunodeficiency virus and acquired immune deficiency syndrome

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Although acquired immune deficiency syndrome (AIDS) was first described in the USA in 1981, there is evidence that individual cases occurred considerably earlier in Central Africa, and serological and virological data show human immunodeficiency virus (HIV) was present in the Democratic Republic of Congo (DRC) as far back as 1959. It is likely that HIV-1 infection in humans was established from cross-species transmission of simian immunodeficiency virus of chimpanzees, but the circumstances surrounding this zoonotic transfer are uncertain. This presentation will review how causality is established in epidemiology, and review the evidence (a putative ecological association) surrounding the hypothesis that early HIV-1 infections were associated with trials of oral polio vaccine (OPV) in the DRC. From an epidemiological standpoint, the OPV hypothesis is not supported by data and the ecological association proposed between OPV use and early HIV/AIDS cases is unconvincing. It is likely that Africa will continue to dominate global HIV and AIDS epidemiology in the near to medium-term future, and that the epidemic will evolve over many decades unless a preventive vaccine becomes widely available.

Keywords: human immunodeficiency virus; Africa; acquired immune deficiency syndrome; polio; origins; causality

1. INTRODUCTION

Persuasive evidence exists that human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) represent zoonotic infections whose African, non-human primate hosts are the chimpanzee and the sooty mangabey, respectively (Hahn *et al.* 2000). Although the origins of HIV-1 and HIV-2 seem academic questions compared with the urgent needs for prevention and care, public health cannot ignore how the acquired immune deficiency syndrome (AIDS) pandemic emerged. UNAIDS estimates that at the end of 2000 there were 36.1 million persons living with HIV/AIDS; and 21.8 million cumulative deaths, indicating an estimated 57.9 million cumulative infections (UNAIDS 2000). Circumstances promoting potential human exposure to simian retroviruses have increased rather than decreased recently (Hahn *et al.* 2000). In addition, a theory that oral polio vaccine (OPV) trials in the late 1950s resulted in cross-species transmission has gained widespread publicity (Curtis 1992; Hooper 1999), with potential detrimental effects on vaccination programmes in general.

This paper reviews concepts of association and causality from an epidemiological perspective, applying these to hypotheses concerning HIV and AIDS origins, especially the one that OPV trials caused HIV-1 and HIV-2 to become established and spread in African populations.

2. HYPOTHESES CONCERNING THE ORIGINS OF HIV-1 AND HIV-2

Hahn *et al.* (2000) recently reviewed the evidence, primarily virological and phylogenetic, that HIV-1 and HIV-2 represent infections of simian origin that crossed into humans. HIV-2 and the simian immunodeficiency virus of sooty mangabeys (SIVsm) are essentially the same virus; animals in the wild are known to be infected; there is geographical overlap between human and non-human infections in western Africa; considerable contact is known to occur between humans and the non-human hosts of SIVsm; and unintentional infection of a laboratory worker with an SIVsm isolate has been documented.

Although the number of chimpanzee isolates studied has been fewer, virological and phylogenetic data support chimpanzees as the source of HIV-1 groups M, N and O, with their precursor group of viruses referred to as SIVcpz. The available phylogenetic data indicate that the divergence of group M into its subtypes, now numbering ten, occurred after cross-species transmission, while infections with groups N and O must have resulted from independent transmission events.

The majority of researchers who accept HIV-1 and HIV-2 as zoonotic infections envisage that cross-species transfer occurred under natural circumstances, as a result of human exposure to infected simian blood or secretions. There are no epidemiological data upon which to base claims for when, where, or how frequently such transmissions

occurred, other than that routes of transmission of retroviruses are known and include exposure to infected blood and secretions. Theories explaining HIV and AIDS emergence must accommodate the following: (i) based on phylogenetic analyses, at least three transmissions of HIV-1 and five of HIV-2 must have occurred; (ii) transmissions are likely to have occurred in the geographical ranges of the original hosts; and (iii) cross-species transmissions must have preceded the documented HIV-1 and HIV-2 epidemics and the postulated duration from phylogenetic studies of the respective retroviral infections in humans (Hahn *et al.* 2000).

An alternative and more specific hypothesis is that live OPV preparations used in Africa in the late 1950s were grown in primate tissue cultures contaminated with SIVcpz and SIVsm; that the vaccine products derived were themselves infected; that these products were fed to hundreds of thousands of persons in different parts of Africa, especially in the Democratic Republic of Congo (DRC); and that human infections with HIV-1 and HIV-2 established in this way led to the HIV and AIDS pandemic. The most widely quoted versions of this hypothesis appeared in the magazine *Rolling Stone* (Curtis 1992) and, more recently, in a widely publicized book authored by Hooper (1999). Evidence cited in favour of the hypothesis is that the earliest documented AIDS cases occurred some time after the OPV campaigns, in the same region, and that opportunity existed to use chimpanzee kidneys for necessary tissue culture work. Most of the focus of attention has been on HIV-1 group M and the DRC. Since the discovery of HIV-1 groups N and O the hypothesis has been widened to suggest that these infections also, as well as HIV-2, resulted from human exposure to contaminated OPV.

3. EPIDEMIOLOGY AND INFERENCE OF CAUSALITY

Epidemiology is concerned with the study of associations between exposures and disease and the interpretation of such associations. If an association exists, it may be causal or due to chance, bias, or confounding factors. Proof of causality is difficult and involves judgement, but there is widespread acceptance of the value of the criteria proposed by the late Sir Bradford Hill for assessing whether associations are causal (Hill 1965). The remainder of this paper examines how the OPV hypothesis meets these criteria for causality. The concept of specificity (an exposure not being associated with more than one outcome) has been largely abandoned as a causal criterion and is not further considered.

(a) *Strength of association*

Strength of association is assessed by comparing the incidence of disease (HIV or AIDS) in those exposed to a risk factor (OPV recipients) and those not exposed, or the frequency of exposure in persons who are ill and not ill. Causal factors result in an increased risk of disease in exposed versus unexposed populations. Evidence that vaccine recipients suffered an increased incidence of HIV or AIDS is lacking because there are no data concerning the Congolese vaccine cohort, nor is it possible to compare the vaccination status of early HIV or AIDS cases and controls. There are no direct data, therefore, to show that any human infections occurred in any vaccine recipients, nor that any vaccine recipients were exposed to chimpanzee or sooty mangabey material, SIVcpz or SIVsm infected or not.

The association proposed by Hooper (1999) is an ecological one; that is, an association based on proposed geographical overlap between where likely AIDS cases and epidemic HIV or AIDS were first described and where the OPV trials were conducted. Ecological associations can be useful for generating hypotheses or providing support for causal inference, but of themselves are insufficient to establish causality. Ecological associations are especially vulnerable to the reasons for association other than cause, namely bias, chance and confounding factors.

(i) *Bias in reporting of early AIDS cases*

In assessing the association between OPV exposure and HIV or AIDS incidence, consideration is required of HIV or AIDS incidence in persons not exposed, and in exposure without disease. OPV trials by the same investigators who worked in the former Belgian Congo were also conducted elsewhere, including in the USA and Europe. There is no evidence that materials used in Africa were only used there, yet early HIV or AIDS cases were restricted to Africa, questioning the association.

Acceptance of the proposed ecological association assumes that early, predominantly Congolese AIDS cases (mostly documented in the medical literature as case reports) were reasonably representative and complete. This is improbable and under-recognition, and thus ascertainment bias is likely to have been considerable. The DRC, a country measuring 2×10^6 km^2 and approximately the size of Western Europe, today has a population of 52 million people. In 1960, the year of independence, Kinshasa had a population of half a million, which by the mid-1980s had grown to 2.5 million. There were only about 400 hospitals and 1200 Belgian doctors in the country at that time, and very few Congolese professionals. The ratio of doctor to population must have been one per many tens and possibly hundreds of thousands. Case reports are generally only written up by academically orientated physicians, and where access to services is difficult, unusual diseases and even extensive outbreaks easily go unreported. The 29 early, possible AIDS cases in the Congo upon which Hooper bases his arguments are most unlikely to have been complete (i.e. probably represented only a fraction of the real number of cases) or representative (i.e. did not necessarily match the distribution of all cases at that time). An indication of probable bias in ascertainment is that five (17%) of these possible 29 early AIDS cases in the Congo were in Europeans, whose better access to medical care would have made them especially likely to be described in the literature. Early in an epidemic the ratio of asymptomatic HIV infections to AIDS cases is of the order of 50:1 or more, so that drawing conclusions about HIV epidemiology from a small number of early AIDS cases may be injudicious.

HIV and AIDS in the late 1950s and in the 1960s had not been recognized and no diagnostic tests existed, so that many cases, irrespective of their location or true incidence, would have gone undetected. Even today, with an understanding of the clinical features of AIDS and established surveillance, the sensitivity of the clinical case

definition for AIDS used in Africa is only 30–40%, and less than 15% of diagnosed AIDS cases get reported (meaning less than 5% of all AIDS cases are reported today in Africa). The 29 early, possible cases of AIDS quoted by Hooper, therefore, are unlikely to have been representative of all HIV disease in the Congo at that time. The first indication of epidemic AIDS in the Congo was a report of increased cases of cryptococcal meningitis in Kinshasa in 1979, illustrating how HIV disease essentially went unrecognized for decades, and questioning the representativeness of individual case reports. Specificity of diagnosis may also have been a problem, since not all the AIDS cases quoted by Hooper were serologically tested for HIV.

(ii) *Bias in reporting of early HIV infections*

Similar lack of representativeness plagues early cases of serologically diagnosed HIV infection, reports of which are based on retrospective testing of collections of stored serum specimens whose availability reflects chance and bias. The 23 seropositive Congolese specimens prior to 1981 quoted by Hooper as closely associated with OPV sites came from four serum collections dating from 1959, 1970, 1976 and 1980, which amounted to approximately 2500 specimens. Three of these four collections were from Kinshasa, reflecting the bias of expatriate researchers working in urban centres; one was from rural Equateur Province, available only by chance because the CDC had investigated an epidemic of Ebola haemorrhagic fever there in 1976. If the prevalence detected in those collections is at all representative, several hundred or several thousand HIV infections may already have existed in Kinshasa in 1959 and 1970, several tens of thousands by 1980; and tens or hundreds of HIV infections in Equateur by 1976. It is impossible to comment on when these infections would have been acquired, where, what the seroepidemiology of HIV would have been earlier, or what levels of infection were at this time in other parts of this enormous country, because material has not been available for systematic testing.

(iii) *Chance and confounding factors*

Misinterpretation due to confounding factors is also likely. Areas chosen for establishment of infrastructure for vaccination trials would have been selected for logistic convenience and access, development and communications, and other professional considerations. The River Congo, 2700 km long, acted as a main transportation route and it is not surprising many towns and cities where AIDS cases may have occurred were in proximity to the river. The same areas are likely to have concentrated different populations and infrastructures, as well as human activities including scientific and commercial work, allowing for numerous weak, non-causal associations at a population level.

Clear examples exist outside of HIV and AIDS of ecological associations that are striking but non-causal. The distribution of HIV and AIDS in women in the USA is reminiscent of that of syphilis and gonorrhoea, and sexually transmitted diseases are known to potentiate HIV transmission. Nevertheless, the epidemiology of HIV in women in the USA has been determined more than anything else by the distribution of HIV in male injecting drug users.

A striking resemblance exists between the distribution of cumulative AIDS cases and that of aeroplanes flying over the USA at one point in time (K. M. De Cock, unpublished data), another ecological association that is real but not causal.

A final caution concerns deductions about prior trends in HIV and AIDS from case series and surveillance data documented later. Wherever the first human infections occurred, it seems likely that the DRC was the first country to suffer epidemic HIV and AIDS, the epidemic then extending into other former Belgian and British colonies eastwards. The epidemic in Africa today is very heterogeneous, with several countries in southern Africa having rates of HIV infection many times higher than those in the DRC where HIV-1 has been present much longer. Deductions based on the current epidemiology could give quite erroneous insight into earlier patterns of spread or descriptive characteristics. Similarly, it is injudicious to draw conclusions about the origins or early spread of HIV-1 based simply on the first available Congolese case reports or surveillance data, which may have been unrepresentative of all HIV-1 infections in the region.

(b) *Consistency*

Consistency implies that studies in different settings support the association between an exposure and an outcome. In this case, the absence of early HIV infections in areas outside of the DRC where the group from the Wistar Laboratory (Philadelphia, USA) conducted research can only be reconciled with the OPV hypothesis if there was something specifically different about the vaccine used in the Congo from other preparations. Conversely, the occurrence of HIV-1 groups O and N cases in western Central Africa, especially in Cameroon and Gabon, as well as HIV-2 in western Africa, requires that associations exist between vaccination programmes and early HIV and AIDS cases, as well as analogous contamination of vaccine preparations specifically used in different parts of Africa. Data do not exist to support these requirements.

(c) *Temporality*

Temporality is the most stringent of all the criteria for causality, since for an exposure to be causal it must occur before the postulated outcome. The earliest known human infected blood sample dates from 1959, although the duration of infection in that case is unknown. It is unlikely that this represents the world's first ever HIV infection bearing in mind the very limited testing of early specimens that has occurred. It is also unlikely that after the amount of time that has elapsed we will find extensive collections of relevant stored African specimens for retrospective testing. Further insights into the timing of early HIV-1 or HIV-2 infections come from phylogenetic analyses and modelling; the most recent molecular clocks date the ancestry of HIV-1 group M to around 1931 with confidence intervals spanning the first half of the 20th century (Korber *et al.* 2000). If these estimates are correct this would invalidate the hypothesis that OPV trials in the Congo initiated the HIV-1 group M epidemic, since the OPV exposures occurred in the late 1950s.

(d) *Biological gradient*

This essentially refers to a dose response, such as exists, for example, between cigarette smoking and the incidence of lung cancer. While we know that viral load is associated with the risk of HIV-1 transmission, putative SIV load in OPV would presumably have been equal across doses from individual vaccine lots. Since children were the main, although not the exclusive, target group for OPV, it is in children that the greatest number of infections would have been expected, although the early AIDS cases reported in the areas where OPV trials were conducted were all in adults.

(e) *Biological plausibility and coherence*

These necessarily subjective criteria (distinct but here discussed together) require that the hypothesis be plausible based on generally accepted knowledge as well as consistent with other available information. Biological arguments against the OPV hypothesis include the fact that no direct evidence exists that either chimpanzee or sooty mangabey tissues were used in vaccine preparation; and that if SIVcpz or SIVsm had been included in tissue culture material it is unlikely to have survived the OPV preparation, storage and distribution. Since children were the main target group for the OPV campaigns, most cases of HIV or AIDS would have been expected in the paediatric age group, for which there is no evidence: HIV and AIDS epidemiology shows disease to present in early childhood as a result of mother-to-child transmission, and from adolescence onwards as a consequence of sexually transmitted infection. Transmission from children to adults is most unusual, and the hypothesis that children could have become subclinically infected and survive for many years to go on and spread HIV-1 when adult is improbable.

If the common ancestor of group M viruses existed in humans, this would preclude the OPV hypothesis based on temporal considerations (see § 3(c)). If the common group M ancestor and its evolution into different subtypes occurred in chimpanzees, then at least ten cross-species transmissions would have had to occur through OPV to explain the distribution of subtypes in humans. Yet further OPV-related transmissions would have to have occurred to accommodate group O and N infections. Postulating that HIV and AIDS in Africa represent several discreet point-source epidemics is not consistent with most interpretations of the evolution of the HIV and AIDS epidemic across the African continent.

(f) *Experimental evidence*

Often the most rigorous test of an epidemiological hypothesis is an intervention study in which an exposure is eliminated. This is not possible in this case, putative exposure having occurred long ago. Hooper (1999) argued that OPV remnants used in Africa should be tested for SIV and for chimpanzee DNA. This has now been done and materials were negative for both. The materials were positive, however, for macacque DNA, confirming that the tissue source for vaccine production of the lot that was tested was a monkey species that does not naturally harbour SIV.

(g) *Analogy*

Analogies can be useful for further generation of hypotheses for testing, but provide little in support of causality. Human-initiated epidemics do occur, including with HIV-1, witnessed by the international epidemiology of HIV-1 in haemophiliacs. Nosocomially infected children have apparently occasionally transmitted HIV-1 to their mothers but there are no obvious analogies for oral infection with a pathogen predominantly in childhood with later epidemic spread among adults. This criterion adds little in favour or against the OPV hypothesis.

4. CONCLUSIONS

Scientific proof of causality is difficult. Acceptance that an exposure led to an outcome requires evidence as well as judgement. Rothman & Greenland (1999) remind us of Peter Medawar's warning that the intensity of conviction that a hypothesis is true has no bearing on whether it is true or not. In the absence of data supporting an association, epidemiology favours the null hypothesis, that no association exists. Data supporting an association between OPV and subsequent HIV and AIDS in Africa are epidemiologically inadequate, and there is no evidence in favour of a causal link.

There is widespread consensus, based on virological evidence, that HIV-1 and HIV-2 are zoonotic infections that crossed species from their African non-human primate sources to humans. Modes of human exposure and infection exist, and unintentional human infections with SIV have occurred in the laboratory. Human infections with SIVcpz and SIVsm sometime in the past, through routes of transmission such as exposure to blood that have been documented to occur, are plausible. However, epidemiology cannot provide data about events that perhaps happened long ago, and is a discipline that avoids speculation.

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