

Occasional Survey

NATIONWIDE COMMUNITY-BASED SEROLOGICAL SURVEY OF HIV-1 AND OTHER HUMAN RETROVIRUS INFECTIONS IN A CENTRAL AFRICAN COUNTRY

RWANDAN HIV SEROPREVALENCE STUDY GROUP*

Summary In December, 1986, a nationwide serological survey of human immunodeficiency virus type 1 (HIV-1) infection in the general population of Rwanda was done in two parts—one in the rural and the other in the urban population. The sampling method was a modification of the cluster sampling technique developed for monitoring immunisation coverage. Antibodies to HIV-1 (and to HIV-2 and human T-cell leukaemia/lymphoma virus type I [HTLV-I]) were detected by immunoenzymatic assays and confirmed by western blot. The number of clusters surveyed was 30 in each setting, urban and rural. HIV-1 seroprevalence was 17.8% (95% confidence interval 14.3–21.2%) in the urban sample (n = 1870) and 1.3% (0.5–2.2%) in the rural sample (n = 742). In the urban sample, females were more frequently HIV-1 seropositive than males (21.0% *vs* 14.6%). Age-specific peaks of HIV-1 seroprevalence were identified at 0 to 5 years of age (10.1%) and at 26–40 years (30.0%). No differences in seroprevalence were observed in terms of age and sex in the rural sample. None of the sera were seropositive for HIV-2 and HTLV-I seroprevalence was 0.2% in the urban sample and 0.3% in the rural. Nationwide serological surveys could be effective in evaluating the spread of HIV infection and the efficacy of public health interventions against AIDS in developing countries.

INTRODUCTION

In urban East and Central Africa, where heterosexual contacts among sexually active adults and vertical transmission from mother to child account for most cases of AIDS, human immunodeficiency virus type 1 (HIV-1) infection is not confined to easily identified population groups.¹⁻³ Only careful estimation of seroprevalence in the general population can yield an estimate of the spread of HIV infection and help to select sentinel populations to be used in a serosurveillance programme. The purpose of our study was to evaluate the seroprevalence of HIV-1 in the general population of Rwanda and to test the efficiency of cluster sampling. HIV-2 and human T-cell leukaemia/lymphoma virus type I (HTLV-I) seroprevalence were evaluated on the same samples.

MATERIAL AND METHODS

Population

The 1978 national census and an estimated annual growth population rate of 3.5% yielded the total population of Rwanda of 6312 000 in December, 1986. 368 300 (5.8%) were living in urban

centres—about 220 000 in Kigali, the capital, and 148 000 in the other eleven cities in Rwanda. The country is divided in ten prefectures and 143 communes, and the communes are subdivided into sectors and then into cells, of 50–150 households.

As of December, 1986, 705 AIDS cases had been reported to the World Health Organisation (WHO) from Rwanda.⁴

Sampling Method

Two surveys were done, one in the rural population and the other in the cities. We modified a sampling method used for immunisation coverage surveys.⁵ The strategy is known as "probability proportionate to size" cluster sampling.⁶ A subset of geographical groups (clusters) is selected from the complete list of clusters by random numbers. The selection is done in such a way that it is possible for a large cluster to be selected more than once. A cell defined a cluster in our study. In each cluster, the selection of the households started at a random starting point and then door to door until all individuals corresponding to predetermined inclusion criteria of sex and age were enrolled. We selected only one individual per household, to avoid bias due to intrafamilial spread of HIV-1. If nobody from the appropriate age group was present in the dwelling, the adjacent house was studied. The sample size was calculated separately for each age group^{7,8} on certain assumptions about expected prevalence and about the increase in seroprevalence that a follow-up survey should be able to detect (details available on request). Previous serological studies had suggested a low variability in HIV-1 seroprevalence among various groups of urban-based adults in Rwanda.⁹

Data Collection

The fieldwork was done in the first 10 days of December, 1986, by a team of ninety investigators and supervisors. Demographic data (age, sex, marital status, profession, religion, education) and information on risk factors for HIV-1 infection¹⁰ were collected on a standardised precoded questionnaire. After verbal consent had been obtained, a 5–10 ml blood sample was collected.

Serological Methods

Blood was centrifuged within 24 hours. Serum was divided and sodium azide was added. HIV-1 antibodies were sought by enzyme immunoassay (EIA) ('Vironostika'; Organon Teknika) and EIA positives were confirmed by western blot (WB) (Biotech, Du Pont de Nemours). A positive WB was defined by the presence of at least one band reactive to a core protein (p17, p24, p55) plus at least one reactive to an envelope protein (gp41, gp120, gp160) of HIV-1; reactivity limited to HIV-1 core protein was labelled "uninterpretable" and counted as negative for statistical analysis. All sera were also tested for HIV-2 antibodies by EIA (reagents kindly provided by S. Sprecher, Pasteur Institute, Brussels) with confirmation by WB (Biotech, DuPont de Nemours). In HIV-1 WB reactive samples, a weaker reactivity to HIV-2 proteins by WB was considered as a cross-reactivity and not dual infection.¹¹ Sera were also tested for HTLV-I antibodies by EIA and WB (Biotech, Du Pont de Nemours). A specimen demonstrating WB reactivity to the HTLV-I *gag* gene product p24 and to an *env* gene product (gp46 and/or gp61/68) was considered as confirmed positive;¹² specimens with restricted reactivity (p19 alone or p19 and p24) were designated "indeterminate".

Statistical Methods

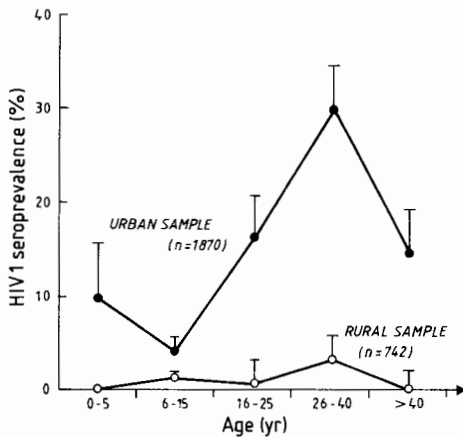
Fisher's exact test, Mantel-Haenszel chi-square test, and chi-square test for trend were used for comparison of proportions.

RESULTS

Sampling

60 clusters were surveyed (30 urban and 30 rural). In the urban survey 16 clusters were in Kigali and 14 were in smaller cities (including the main towns in all ten prefectures). The calculated sample size was 2040 for the

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HIV-1 seroprevalence by age in urban and rural samples.

urban survey and 780 for the rural survey. In the urban survey, 1870 out of the 2040 samples (91.7%) were available for analysis and 742 out of the 780 samples (91.1%) in the rural survey. Lost cases resulted from refusal of venepuncture (19% of lost cases in the urban sample, 29% in the rural), failed venepuncture (18% urban, 50% rural), or accidents during sample transport or handling (63% urban, 21% rural). The individuals whose blood was not available for analysis did not differ from the sample tested in respect of demographic features. Nor did our sample differ demographically from national census data.

Seroprevalence in Urban Survey

347 out of 1870 serum samples (18.6%) were HIV-1 EIA positive. 333 (17.8%; 95% CI 14.3-21.2%) were confirmed positive by WB; 7 were WB unreactive and 8 reacted only to core proteins. HIV-1 seroprevalence was greater in women than in men. HIV-1 seroprevalence was not homogeneously

TABLE I—HIV-1 SEROPREVALENCE BY AGE GROUP AND BY SEX IN URBAN SAMPLE

Age	No	% HIV-1 seroprevalence (and 95% CI)	Design effect
<i>Both sexes*</i>			
0-5	148	10.1 (4.5-15.8)	1.3
6-15	238	4.2 (1.7-6.7)	0.9
16-25	493	17.0 (12.8-21.3)	1.6
26-40	500	30.0 (24.1-35.9)	2.1
>40	491	14.9 (10.3-19.4)	2.0
<i>All ages†</i>			
Males	948	14.6 (11.5-17.6)	..
Females	922	21.0 (16.3-25.8)	..

* χ^2 for trend = 90.1; 4 degrees of freedom, $p < 0.001$.

†Mantel-Haenszel $\chi^2 = 13.45$, $p < 0.001$.

TABLE II—HIV-1 SEROPREVALENCE BY AGE GROUP AND BY SEX IN RURAL SAMPLE

Age	No	% HIV-1 seroprevalence (and 95% CI)	Design effect
<i>Both sexes*</i>			
0-5	109	0.0	..
6-15	115	1.7 (0.0-4.1)	1.0
16-25	168	1.2 (0.0-2.8)	1.0
26-40	178	2.8 (0.0-5.6)	1.3
>40	172	0.6 (0.0-1.7)	1.0
<i>All ages</i>			
Males	378	1.3 (0.2-2.4)	..
Females	364	1.4 (0.0-2.7)	..

* χ^2 for trend = 5.3, 4 degrees of freedom, $0.30 > p > 0.20$.

distributed in all age groups for there were two peaks, one in the under-5's and one in the sexually active age groups (figure, table 1).

A significant difference in HIV-1 seroprevalence between males and females was found only in 16-25-year-olds (Mantel-Haenszel chi square, $p < 0.001$). None of the 1870 sera was HIV-2 seroreactive. 30 (1.6%) were HTLV-I antibody positive by EIA; of these 3 samples (0.2%) were positive, 9 (0.5%) negative, and 18 (1.0%) indeterminate on WB.

Seroprevalence in Rural Survey

33 out of 742 samples (4.4%) were HIV-1 EIA positive. 10 (1.3%, 95% CI 0.5-2.2%) were positive by WB; 11 (1.5%) were WB unreactive; and 12 (1.6%) reacted only to HIV-1 core proteins. In contrast with the urban sample, there were no significant differences in seroprevalence by age (figure, table II) or sex. All 742 sera were HIV-2 seronegative. 13 (1.8%) were HTLV-I antibody positive by EIA; 2 (0.3%) were positive, 3 (0.4%) negative, and 8 (1.1%) indeterminate on WB.

DISCUSSION

Previous serological studies of HIV-1 infection in Africa have been limited to certain population strata or occupational groups, mainly in urban settings.^{9,13,14} A seroprevalence study in five central African countries¹⁵ was initially designed to assess the seroprevalence of haemorrhagic fever and these sera were subsequently tested for HIV-1 antibodies. Also, it did not cover various age groups in every cluster. A nationwide study has not been reported before. In such a study the sample size must be large, to give confidence intervals narrow enough to be usable for statistical comparisons in a later phase of the AIDS control programme. The calculation of size must also take into account the fact that prevalence has to be measured simultaneously in different age groups. Cluster sampling meets these requirements and can be easily organised where census data are limited, as they often are in developing countries. Cluster sampling does require large samples on the assumptions we used. Choosing only one individual per household was a modification of cluster sampling that might itself have introduced two biases—namely, over-representation of people living in small households and variability of HIV seroprevalence in individuals of similar age living in a household. The impact of the second potential bias could be assessed by testing all individuals in a given age group in a subset of households and comparing the results with those found by our method. However, we think that any bias due to under-representation of large households or variability of seroprevalence within an age group in a given household is much less important than the bias of intrafamilial spread of HIV-1 which our sampling method was designed to avoid.

As suggested in previous studies,^{9,16,17} HIV-1 seroprevalence is much lower in rural areas than in cities in Africa. In our urban sample, HIV-1 seropositivity was more frequent in women than in men, especially in young adults. The same trend had been observed in young women in Kinshasa¹³ but the reason remains obscure. Differences in the numbers of sexual encounters in a subset of these women as compared with men and/or the possibility of easier transmission of HIV from man to woman than from woman to man¹⁸ could expose urban-based women to HIV more frequently and/or more dangerously than men.

HIV-1 seroprevalence was not spread evenly in respect of age in our urban sample. The two age peaks observed (at 0-5 and 26-40 years) are consistent with sexual transmission in adults and vertical transmission to children, the two major modes of transmission of HIV in Africa.^{19,20} This pattern also suggests that other modes of contamination that are theoretically unrelated to age, such as transmission by arthropod vector or by therapeutic injections, do not play a major part in the spread of the epidemic.

In late 1986 more than 94% of the Rwandese population was rural so one can estimate that about 2% of the country's population was infected by HIV at that time. This gives a calculated ratio of AIDS cases to seropositives of 1:180, which is lower than the one observed in the United States.^{21,22} This suggests that national surveillance of AIDS is not as effective in developing countries as it is in the USA.

The seroprevalence of HTLV-I in the general population of Rwanda was low and similar to that observed in the South-eastern United States.²³ Also, no difference in HTLV-I seroprevalence existed between rural and urban areas, as previously shown in Ghana.²⁴ HTLV-I is widespread in many west African countries, however,^{24,25} and in Uganda, a country bordering on Rwanda.²⁵ This observation indicates a wide range of variability in HTLV-I seroprevalence in central Africa.

HIV-2 is endemic in west Africa^{26,27} but we found not one HIV-2 seropositive individual in Rwanda in late 1986. However, an HIV-2 infected AIDS case has recently been diagnosed in Kigali (unpublished).

A nationwide seroprevalence study such as ours should be done to provide a baseline for national AIDS control programmes in countries where HIV infection is a public health hazard. Cluster sampling allows the collection of such baseline data quite cheaply; our study cost less than \$50 000. However, with the method and sample size used it could be difficult to detect a small increase in seroprevalence in 0-15 year-olds in a follow-up study done only 1 or 2 years later. We suggest that the follow-up studies should focus on age-targeted populations such as the under-5's and the sexually active adults.

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Peptide Regulatory Factors

INTERLEUKINS AND THE IMMUNE SYSTEM I

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WHEN the immune system encounters antigens expressed by invading organisms such as parasites, bacteria, or viruses, it mounts a specific reaction that ultimately eradicates the infection. The antigen-specific cells of the immune system are lymphocytes, of which there are two main types: B cells synthesise antibody whereas T cells regulate antibody synthesis and also mediate effector functions such as direct cytotoxicity or the inflammatory response of delayed type hypersensitivity. Antigen is first taken up by phagocytic cells such as macrophages, processed in a complex fashion, and then re-expressed on the surface of the cell. In association with class II or class I major histocompatibility complex (MHC) molecules, antigen can then be recognised by helper T cells (T_H) or cytotoxic T lymphocytes (T_C), respectively. The specific encounter with antigen leads to production of growth and differentiation factors both by the cells that present the antigen and by the T cells themselves. These factors, known as cytokines, lymphokines, or interleukins (A. Green, this series, for discussion of terminology), are responsible for the rapid expansion and differentiation of the initially small numbers of antigen-specific lymphocytes present in a previously unchallenged host.

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