

Seroprevalence of Human T Cell Leukemia Virus in HIV Antibody–Negative Populations in Rural Cameroon

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Seven hundred forty-seven serum samples collected from humans in 4 separate rural village areas in Cameroon were examined for antibody to human T cell leukemia viruses (HTLVs) by use of an enzyme immunoassay followed by a Western blot assay. Of the 88 serum samples that the enzyme immunoassay found to be repeatedly reactive, the HTLV status of 49 samples was confirmed by Western blot assay to be HTLV type I, and the status of 6 samples was confirmed to be HTLV type II.

Human T cell leukemia virus (HTLV) type I (HTLV-I) and type II (HTLV-II), the retroviruses identified as the cause of human adult T cell leukemia, have been found in various parts of the world. One strain of the virus (HTLV-I) has also been shown to cause a polio-like syndrome known as “HTLV-I–associated myelopathy” or “tropical spastic paraparesis.” Because HTLVs are highly cell associated, their presence in a population is usually identified through the testing of plasma or serum samples for HTLV antibody. A recent Dutch review article [1] lists only 9 studies of HTLV-I/HTLV-II prevalence performed in Africa, and 2 of those 9 studies were performed in North Africa. The Dutch study cites studies from sub-Saharan Africa that suggest that the region is an area where HTLVs are endemic. HTLV seropositivity

rates are 5%–30% in areas of endemicity, whereas such rates are generally <1% in areas of nonendemicity. Studies of retroviruses performed in Cameroon have determined that HIV strains are more diverse in this area than in any other African venue [2]. This finding has led to extensive collection of blood samples from rural areas to determine whether simian viruses might cross into human hosts. Indeed, studies of the prevalence of antibody to simian foamy viruses in these and similar rural areas have revealed extensive blood contact between human hunters of monkeys and their prey [3, 4].

In a cooperative study with the Johns Hopkins School of Public Health, the US Military HIV Research Program, and the Cameroon Army Medical Research, the US Food and Drug Administration Laboratory of Molecular Virology (Rockville, MD) tested >700 serum samples collected from humans in rural areas of Cameroon and tested them for the presence of antibody to HTLV by use of a US Food and Drug Administration–licensed EIA manufactured by Abbott Laboratories. The HTLV virus exists as 2 distinct serotypes (HTLV-I and HTLV-II), and, for some time, a Western blot assay existed only for identification of HTLV-I. Although “research-use-only” assays that discriminate between HTLV-I and HTLV-II have found some application in clinical laboratories, they do not resolve the HTLV status of all specimens [5]. Some Western blots remain classified as having an indeterminate status, and that status can be resolved only if a sufficient number of T cells or the DNA of T cells is available for genomic testing of the HTLV-integrated provirus by PCR [6].

Materials and methods. Serum samples were collected from people living in rural areas of Cameroon, according to a protocol approved by human use committees of both Johns Hopkins University and the Cameroonian Ministry of Health. Only HIV antibody–negative serum samples obtained from humans in rural areas of Cameroon were used. The HIV antibody–positive serum samples that were obtained (1%–4% of all samples) were used in other studies. Sites of serum sample collection in 4 separate village areas in different parts of Cameroon are shown in figure 1.

Serum samples were initially tested using the EIA for detection of HTLV-I and HTLV-II that was manufactured by Abbott Laboratories. If samples were found to be repeatedly reactive, they were then tested using the HTLV-I Western blot assay previously marketed by Calypte. Serum samples that were found to have an indeterminate status by the Calypte Western blot assay were then tested using HTLV Western Blot 2.4 (Genelabs), another research-use-only test, in an effort to fur-

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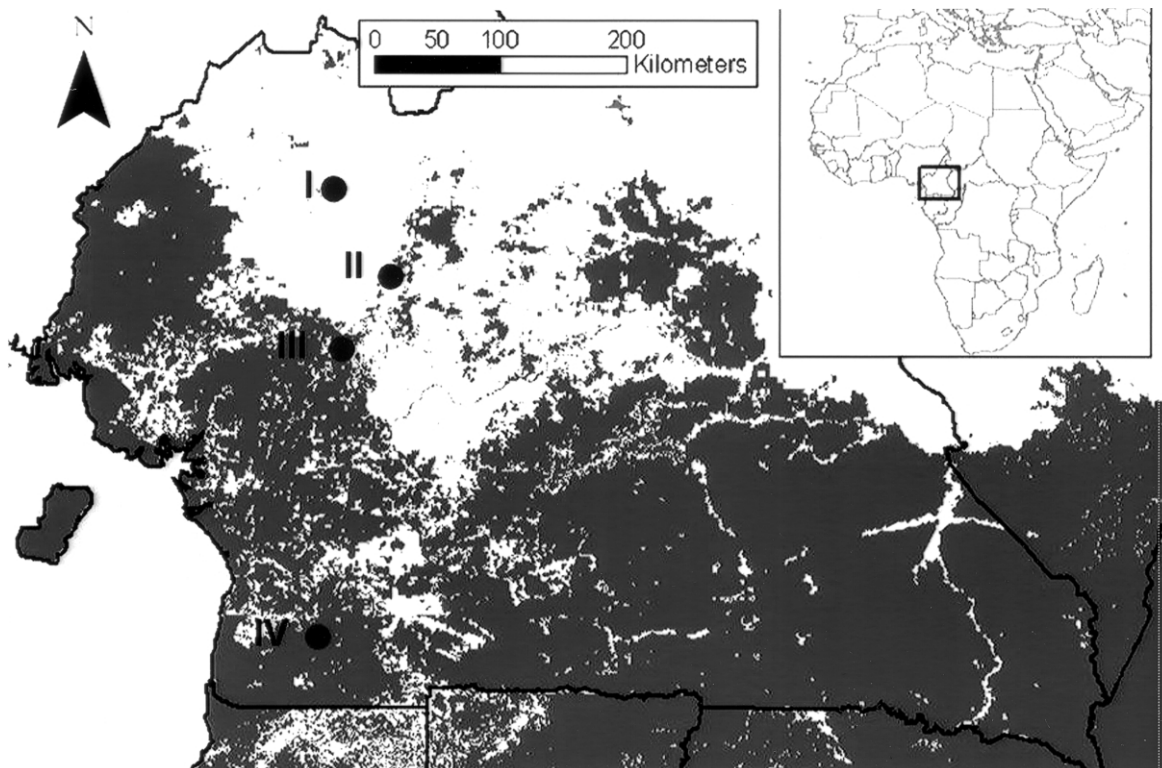


Figure 1. Map of study sites. Study sites (●) are shown in relation to distribution of lowland tropical forest in central Africa (gray-shaded areas).

ther resolve whether some indeterminate results could be resolved and an HTLV-I or HTLV-II could be identified. The criteria for interpretation of results were those specified by the manufacturer of the Western blot assay.

Results. Of 747 specimens that underwent testing, 113 were initially found to be reactive on the basis of results of the EIA manufactured by Abbott Laboratories; this finding resulted in an initial seropositivity rate of 15%. When these serum samples were tested in duplicate a second time, only 88 were found to be repeatedly reactive. Thus, the prevalence of repeatedly reactive results was 11.8%. Results of testing are summarized in figure 2. When the 88 serum samples were tested using the Calypte blot assay, only 24 showed band patterns consistent with HTLV-I. Two serum samples did not show any reactivity, and the remaining 61 samples produced band patterns but were considered to have indeterminate results. The serum samples of indeterminate status were then tested using the Western blot assay manufactured by Genelabs, which had the capability to resolve some of the indeterminate results and identify HTLV-I or HTLV-II. Of these 61 serum samples, 25 were identified as HTLV-I, 6 were identified as HTLV-II, 9 were classified as possible dual infections with HTLV-I and HTLV-II, and 16 had indeterminate results. Four serum samples did not display a reaction, and, because 1 sample did not have sufficient volume

to undergo the Genelabs assay, its status therefore remained unresolved.

When the 24 serum samples that were found by the second EIA to be nonreactive were tested using the Calypte assay, 18 did not have sufficient volume, and their status remained unresolved. Another 5 serum samples had an indeterminate band pattern, and 1 showed no bands. When the 5 serum samples with indeterminate band patterns were tested using the Genelabs blot assay, 2 samples appeared to have a dual-infection status, 2 had results that remained indeterminate, and 1 showed no banding. A flowchart of the tests performed and their results is shown in figure 2.

Discussion. That 11.8% of the serum samples were found to be repeatedly reactive by an EIA for the detection of HTLV strongly suggests that rural Cameroon should be considered an area of endemicity for HTLVs. Twenty-four serum samples were confirmed to be HTLV-I positive by use of the Calypte Western blot assay, and an additional 26 serum samples were identified as HTLV-I positive by the Genelabs assay; these findings indicate that HTLV-I is the predominant HTLV type (HTLV-I seropositivity rate, 5.57%) and further support the idea that HTLV-I is very likely endemic in rural Cameroon. Only 6 of the serum samples showed definite antibody to HTLV-II, whereas 11 serum samples seemed to show dual infection with both HTLV-

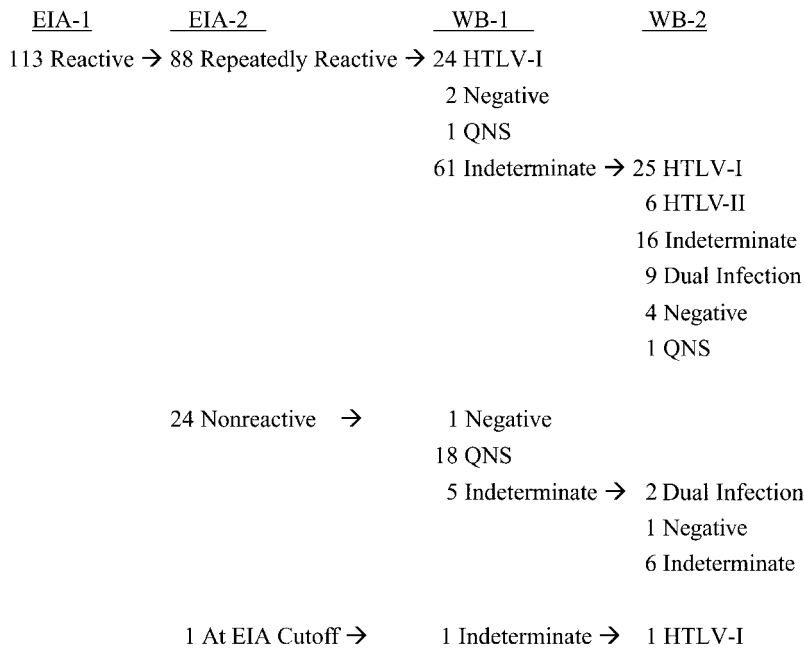


Figure 2. A flowchart of the tests performed for the detection of human T cell leukemia virus (HTLV) and their results. Dual infection, infection with HTLV type I (HTLV-I) and HTLV type II (HTLV-II); QNS, quantity not sufficient; WB-1, Western blot assay manufactured by Calypte; WB-2, HTLV Western Blot 2.4 (a Western blot assay manufactured by Genelabs).

I and HTLV-II, according to the Genelabs assay. The difference between identification of HTLV-I by the Calypte assay and identification by the Genelabs assay is the result of a better separation of the gp46 protein by the Genelabs assay.

In the present study, the prevalence of HTLV-I appeared to be double that reported in 2 other African studies from Mozambique and Uganda [1]. Although the Cameroon coast was visited by early European explorers, the presence of HTLV-I in remote rural areas argues against its introduction by foreign visitors or settlers.

The significance of indeterminate band patterns is not yet well understood, but such patterns are of interest in understanding retroviral infections in these populations. Because HIV is postulated to have been introduced into the human population from a wild primate source [7], the question might be asked whether the indeterminate results of Western blot assays performed for the detection of HTLV in serum samples collected in rural Cameroon might denote the presence of new and as-yet-unknown retroviruses that have entered humans from a forest reservoir [3]. Even if mononuclear cells were available, resolution of indeterminate results of Western blot analysis might still be difficult, because, although HTLVs are highly cell associated, they infect relatively few T cells. It is possible to derive and sequence a viral genome by use of PCR and other molecular methods; however, the usually low level of infected cells poses significant challenges for identification by PCR. Studies are underway to further investigate samples

with indeterminate patterns and to understand whether such patterns denote the presence of new retroviral agents potentially transmitted across species.

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