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Identification of Rare HIV-1 Group N and HTLV-3 Strains in Rural South Cameroon

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Abstract

Background South Cameroon is a hot spot for newly emerging strains of HIV and HTLV, making this region a critical location for monitoring circulating variants that have the potential to spread globally. In this study we conduct surveillance of HIV/HTLV in South Cameroon to monitor the prevalence and diversity of HIV and HTLV. Data obtained from surveillance studies can inform diagnostics and research efforts.

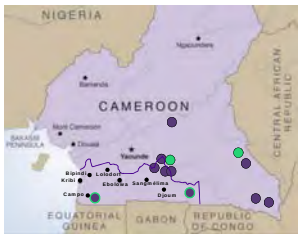
Methodology Study participants, recruited in 7 towns in South Cameroon, were screened for HIV infection using the national algorithm from 2010-2015. All collected specimens from 2010 were subjected to further serological testing; however, in 2011-2015 the selection criteria was changed such that 30-40% of collected specimens were HIV positive. All specimens were tested with ARCHITECT HIV Ag/Ab Combo assay (Abbott Diagnostics), and a subset of samples with remaining volume was tested with ARCHITECT rHTLV I/II assay. HIV serotype was determined by a peptide multiplex immunoassay, which classified infections as either HIV-1 group M, N, O or HIV-2. Molecular characterization was performed on a subset of reactive HIV and HTLV specimens.

Results In 2010, the prevalence of HIV in South Cameroon was 8.5% amongst study participants. The serotype immunoassay identified the majority of HIV infections in 2010-2015 as group M (n=3937, 99.4%); group O (n=24, 0.55%) and group N infections (n=2, 0.05%) were also identified and confirmed by sequence classification of *env* gp41 or *pol*-integrase regions. Of the 488 HIV-1 group M specimens sequenced 6 subtypes (A, C, D, F2, G, and H), 27 URFs and 12 CRFs were identified, with CRF02_AG accounting for 60.7% (n=296). From 2010-2015, the prevalence of HTLV was 1% (95% confidence interval: 0.70-1.33), including HTLV-I (n=38, 86.4%), HTLV-II (n=5, 11.4%), and HTLV-III (n=1, 2.3%) specimens. HIV-1 co-infection had no significant impact on prevalence of HTLV in this study population.

Conclusions Our findings confirm that South Cameroon has a high burden of HIV infection with a high level of HIV-1 and HTLV strain diversity. Only 4 previous HTLV-III infections have been reported, making our identification and sequencing of a new HTLV-III infection significant. Likewise, the 2 HIV-1 group N infections we report are 9% of the 22 total group N infections reported globally and add to the limited sequence information available. Continued HIV/HTLV surveillance in South Cameroon will be essential to ensure diagnostic tests and HIV research keep pace with these emerging viruses.

Study Population

Figure 1: Map of South Cameroon



Study participants were recruited during organized voluntary HIV counseling and testing campaigns carried out in several towns in the South Region from 2010 to 2015; district hospitals located in Kribi, Ebolowa and Sangmélima were the primary recruitment and blood collection sites. The South Region is demarcated by the purple line and collection towns are shown. Circles show primate colonies in southern Cameroon where SIV has been found: ● SIVcpz; ● SIVgor; ● both SIVcpz and SIVgor

Serological Testing

Serological testing for HIV infection was performed on all collected specimens; a subset of the specimens were screened for HTLV.

HIV and HTLV Screening

Field Sites:
Determine™ HIV1/2[a] (Alere; Bedfordview, South Africa)
Murex HIV Ag/Ab Combo[a] (DiaSorin; Saluggia, Italy)

Abbott Diagnostics:
ARCHITECT HIV Ag/Ab Combo (Abbott Park, IL,)
ARCHITECT *r*HTLV I/II (Abbott Diagnostics; Wiesbaden, Germany)
Research use only assay using *env* V3 and IDR peptides (Peptide EIA) was used to serotype HIV reactive specimens into HIV-1 groups (M, N, O, and P) and HIV-2.

Table 1: HIV and HTLV ARCHITECT Testing Results

	HIV		
Year	**N Tested**	**Total Positive**	**% Positive**
2011	2826	241	8.5
2012	1558	604	38.8
2013	4908	1723	35.1
2014	1896	685	36.1
2015	2524	1077	42.7
Total	13712	4330	

	HTLV		
Year	**N Tested**	**Total Positive**	**% Positive**
2011	2543	15	0.6
2012	1321	20	1.5
2013	2543	27	1.1
2014	1884	22	1.2
2015	2509	19	0.8
Total	10800	103	

Table 2: Serotype of HIV Infected and Grayzone Specimens

	Years Specimens Collected					
HIV Serotype	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
M	364	456	1536	607	974	3937
O	4	6	9	4	4	27
N			4		2	6
P			2			2
Unclassified HIV-1	203	69	63	29	68	432
HIV-2			6	3	2	11
Non binders	23		69	42	26	160
Total	594	531	1689	685	1076	4575

Molecular Characterization

Molecular characterization of HIV specimens:

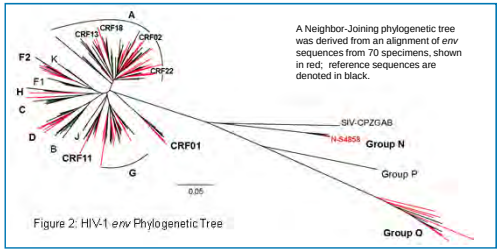
RT-PCR was performed using HIV-1 specific primers that amplify a 676 nt region of *env* gp41 including the IDR. If the *env* RT-PCR failed, alternative primers in *env* were used. For rare subtypes, RT-PCR was also performed for *gag* p24 (468 nt) and *pol* integrase (864 nt).

Viral sequences were aligned with reference strains and phylogenetic analysis was performed using PHYLIP 3.5c (J. Felsenstein, U of Wash, Seattle).

Table 3: HIV-1 Strain Diversity in South Cameroon

Classification by Viral Sequence		
Classification	% of Group M	N=
A	5.4	25
C	0.4	2
D	4.1	19
F2	2.8	13
G	3.7	17
H	0.2	1
CRF01	0.4	2
CRF02	64.2	296
CRF06	0.7	3
CRF11	2.4	11
CRF13	1.1	5
CRF18	0.7	3
CRF19	0.2	1
CRF22	5.9	27
CRF25	0.2	1
CRF36	0.7	3
CRF37	0.7	3
CRF43	0.7	3
URF	5.9	27
Group O	4.9	24
Group N	0.4	2
Total		488

Six different subtypes,12 CRFs, and 27 URFs were present in the population. CRF02 was the most prevalent strain. One HIV-1 group N specimen was classified by *pol* gene sequences only



A Neighbor-Joining phylogenetic tree was derived from an alignment of *env* sequences from 70 specimens, shown in red; reference sequences are denoted in black.

Figure 2: HIV-1 *env* Phylogenetic Tree

HTLV Surveillance and Molecular Characterization

103 of 10,800 specimens collected in Cameroon between 2011 and 2015 were classified *reactive* in the ARCHITECT rHTLVI/II assay. DNA was extracted from whole blood of 80 selected specimens and PCR amplification was done using primers designed to amplify *gag* (293 nt) or *tax* (467 nt) from HTLV I-IV. Fragments amplified successfully were sequenced directly (ABI 3130xl). Viral sequences were aligned with reference strains and phylogenetic analysis was performed using PHYLIP 3.5c (J. Felsenstein, University of Washington, Seattle).

Classification by *gag* or *tax* sequences	
HTLV-I	38
HTLV-II	5
HTLV-III	1
Negative	36
TOTAL	**80**

These results include identification of the fifth HTLV-III strain. Similar to the previous four HTLV-III strains, NKO39 was collected from southern Cameroon.

Figure 3A:
HTLV-*gag*
Phylogenetic Tree

This Neighbor-Joining phylogenetic tree was derived from an alignment of 45 *gag* sequences; reference sequences are denoted in black.

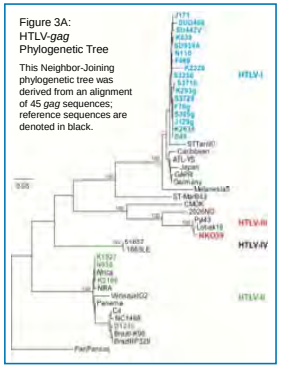
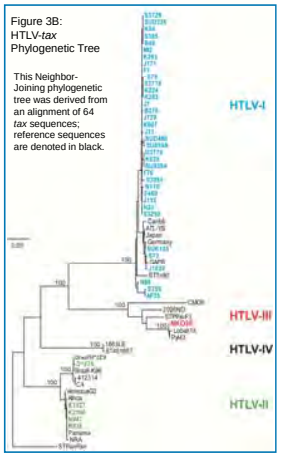


Figure 3B:
HTLV-*tax*
Phylogenetic Tree

This Neighbor-Joining phylogenetic tree was derived from an alignment of 64 *tax* sequences; reference sequences are denoted in black.



Conclusions

- Overall prevalence of HIV infection in the South Region among study participants is 8.5%
- Two HIV-1 Group N (0.046%) and N=24 Group O (0.55%) infections were identified
- HIV-1 group M strain diversity is high; 6 subtypes (A, C, D, F2, G, and H), 12 CRFs and URFs were found
- Overall prevalence of HTLV infection in the South Region among study participants is 1%.
- HTLV-I N=38 (86.4%), HTLV-II N=5 (11.4%), and HTLV-III N=1 (2.3%) infections were found
- The new HTLV-III specimen identified in this study is the fifth HTLV-III strain identified globally to date