JCM Accepts, published online ahead of print on 24 September 2008
J. Clin. Microbiol. doi:10.1128/JCM.01249-08

New insights into the prevalence, genetic diversity and proviral load of human T-cell leukemia viruses types 1 and 2 in pregnant women in Gabon, equatorial central Africa

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Running head: HTLV-1/2 in pregnant women in Gabon

Keywords: HTLV-1, HTLV-2, Gabon, pregnant women, seroprevalence, proviral load, genetic diversity, phylogenetic analysis.

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ABSTRACT

Human T-cell leukemia virus type 1 (HTLV-1) is highly endemic in areas of central Africa; mother-to-child and sexual transmission are considered to be the predominant routes. To determine the prevalence and subtypes of HTLV-1/2 in pregnant women in Gabon, we conducted an epidemiological survey in the five main cities of the country. In 907 samples, the HTLV-1 seroprevalence was 2.1%, which is lower than that previously reported. Only one case of HTLV-2 infection was found. The HTLV-1 seroprevalence increased with age and differed between regions ($p \leq 0.05$), with the highest prevalence (5%) in the south-eastern region. A wide range of HTLV-1 proviral loads was observed among the infected women. The level of the proviral load was correlated with a high HTLV-1 antibody titer ($p \leq 0.02$). Sequencing of HTLV-1 *env* and LTR fragments showed that all but one strain belonged to the central African subtype B; the outlier was of cosmopolitan subtype A. The new strains of subtype B exhibited wide genetic diversity, but there was no evidence of clustering of specific genomes within geographical regions of the country. Some strains were closely related to STLV-1 strains of great apes, suggesting that in these areas some HTLV-1 strains could arise from relatively recent interspecies transmission. The sole HTLV-2 strain belonged to subtype B. In this study we showed that the prevalence of HTLV-1 in the south-east is one of the highest in the world for pregnant women.

INTRODUCTION

The human T-cell lymphotropic viruses type 1 (HTLV-1) and 2 (HTLV-2) are members of a group of primates retroviruses that share some common epidemiological and biological properties, including tropism for T lymphocytes. HTLV-1 is the causative agent of adult T-cell leukemia/lymphoma (ATL) (46) and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) (15). It has also been associated with a number of inflammatory diseases, including pediatric infectious dermatitis (29, 32), uveitis (37) and some cases of myositis (38, 44). HTLV-2 may be responsible for rare neurological syndromes that are clinically related to TSP/HAM (22, 40), but no tumors have been linked definitively to such infection (12, 23).

HTLV-1 is endemic in certain areas, such as southern Japan, some regions of sub-Saharan Africa and of the Caribbean Basin as well as some parts of South America and the Middle-East (16), with an estimated 15–20 million infected persons worldwide. In the foci, the overall HTLV-1 prevalence is usually more than 2% of the adult population, and 2–8% of these infected persons will develop a severe HTLV-1-associated disease, such as ATL or TSP/HAM, during their lifetime (14).

HTLV-2 has been shown to be endemic in various American Indian populations (4, 53) and has also been endemic for the past 15–25 years among intravenous drug users in Europe and North America (41, 50). Furthermore, since 1991, sporadic cases of HTLV-2 infection have been detected in west and central Africa, where the presence of this infection in isolated rural populations, including some Pygmies, suggests an ancient presence of HTLV-2 (17). Interfamilial transmission was also reported in sporadic cases in Gabon, central Africa (55).

HTLV-1 and -2 are transmitted in three ways: (i) between sexual partners, mainly from man to woman; (ii) through blood transfusion with HTLV-infected cells and (iii) from mother to child

during prolonged breastfeeding. In highly endemic areas, mother-to-child transmission is sometimes the predominant route. In Japan, an area highly endemic for HTLV-1 infection, antenatal screening and a recommendation for formula-feeding of infants of HTLV-1-seropositive mother have been instituted successfully since 1987 (20). Similar recommendations were proposed in Europe and the Caribbean (19).

Since the original reports by the International Center of Medical Research (CIRMF) teams, Gabon has been considered an endemic area for HTLV-1. The seroprevalence varies considerably by sex, age and region (5% in urban adults areas, 8.5–10.5% in rural adults) (1-3, 7, 30), and there have been reports of some patients with ATL and TSP/HAM (8, 10, 45). The prevalence of HTLV-1 infection among pregnant women was estimated to be 5.5–6.8% (2, 51). Most previous studies, however, have been carried out in only one region of the country, the south-east, and the results may therefore not reflect the national prevalence, due to possible regional foci, a hallmark of HTLV-1 infection. Furthermore, the reported rate might be under- or rather overestimated, as in most cases confirmatory testing with strict western blot criteria and/or PCR were not used for HTLV-1 detection.

The aims of this study were to evaluate, with validated serological and molecular confirmatory assays, the prevalence of HTLV-1 and -2 in pregnant women living in the main cities of Gabon and to determine the HTLV-1 proviral load in the infected mothers, as this is a major factor for viral transmission to infants. We also investigated the molecular characteristics of the HTLV-1 strains in each area. As there is still no defined treatment for HTLV-1 infection, accurate seroprevalence rates in pregnant women will be helpful for establishing prophylactic measures to reduce the rate of mother-to-child viral transmission and thus later on the occurrence of ATL in adults.

MATERIALS AND METHODS

Area, study population and blood sampling

Gabon, a central African country, occupies 270 000 km^2 and is located on the Gulf of Guinea near the Equator. Tropical forest covers three-quarters of the territory. The population has been estimated to be 1 273 000, consisting of more than 40 ethnic groups. HTLV-1/2 seroprevalence in pregnant women and the circulating molecular subtypes were measured in five sentinel sites: Libreville, the capital, in the north-west; Port-Gentil, the main harbor and economic capital, in the centre-north; Lambaréné, in the centre of the country; Franceville in the south-east; and Oyem in the north-east (Figure 1). Between January and March 2005, venous blood samples were collected from all pregnant women who received their first antenatal examination, and sera and buffy coat were separated and kept frozen. Before testing, fully informed consent was obtained from each woman; when the women were younger than 18 years, informed consent was obtained from their parents. The samples were anonymous, but age and geographic origin were retained. The study obtained ethical clearance from the public health authorities.

Serological tests

An enzyme immunoassay (Vironostika HTLV-1/2, Bio-Mérieux) was performed, and positive or borderline-positive samples were analyzed by western blotting (HTLV blot 4, Diagnostic Biotechnology, Singapore) to confirm HTLV seropositivity and to differentiate between HTLV-1, HTLV-2 infections and indeterminate western blot serology (6).

Polymerase chain reaction, HTLV-1 proviral load and molecular studies

DNA was extracted from the buffy coat, and the HTLV-1 proviral load was measured as described previously (13) by an accurate, reproducible, quantitative polymerase chain reaction (PCR) method involving a dual-labelled fluorogenic probe (ABI Prism 7700 Sequence detection system).

For sequencing and phylogenetic analysis, PCR was performed with several specific HTLV-1 primers located within the long terminal repeat (LTR) and *env* regions, as described previously (47). HTLV-2 LTR and *env* amplification was performed as described previously (26). Purified PCR products were cloned with the pCR2.1 TOPO plasmid (Invitrogen, Carlsbad, California, USA), and positive clones were selected, extracted, purified and sequenced with an automatic sequencing system as described previously (26, 47).

For the phylogentic analysis, the *env* and LTR sequences were aligned with the ClustalX program and then analyzed manually with the editor program of the MEGA package (28). Phylogenetic relationships were reconstructed by the distance neighbor-joining method (49), and confidence levels were estimated for 1000 replicates with the MEGA package.

Statistical analysis

HTLV-1 serological status in relation to the age group and geographic origin of the pregnant women was analyzed statistically by the chi-squared test with Yates correction, and prevalences and odds ratios were calculated. The corresponding 95% confidence intervals (CIs) were reported as measures of statistical significance. Analyses were performed with Epi-Info (version 6.04dfr, ENSP-Epiconcept-InUS, 2001).

RESULTS

HTLV-1 and HTLV-2 serological studies and proviral loads

Between January and April 2005, 907 sera from pregnant women were screened for antibodies directed against HTLV-1 and HTLV-2 antigens. Only one case of HTLV-2 infection (0.1%) was found, while 19 pregnant women (2.1%) were HTLV-1 positive, as confirmed by strict western blot criteria (Table 1). The seroprevalence differed significantly among the five regions ($p \leq 0.05$), being 1.0% in Libreville, 1.2% in Port-Gentil, 2.1% in Lambaréné and 0% in Oyem; the highest prevalence (5%) was found in Franceville (Table 1). The sole HTLV-2 infection originated from the same area.

HTLV-1 seroprevalence increased with age, being 1.7% at 14–20 and 21–25 years, 1.8% at 26–30 years and 3.4 > 31 years (Table 1). Ten women (1.0%) had an indeterminate western blot profile, with various observed patterns, including some Gag indeterminate profiles (35). The seroprevalence of indeterminate western blot did not differ significantly by region or by age (data not shown).

The HTLV-1 and -2 antibodies titers and proviral loads are shown in Table 2. The HTLV-1 western blot-positive samples had higher antibody titers on MT-2 (HTLV-1) cells than on C19 (HTLV-2) cells, whereas the sole HTLV-2 sample had a higher immunofluorescence antibody titer in C19 than in MT-2 cells. The HTLV-1 proviral load was measured in all HTLV-1 samples with a complete western blot pattern and in one western blot-indeterminate sample. The latter sample exhibited reactivity against all HTLV-1 western blot proteins but not against the recombinant HTLV-1 peptide (MTA-1). The HTLV-1 proviral load varied widely; the mean

proviral copy number of the 19 samples with a positive signal was 10 310 copies (standard error of the mean, ± 20 119) per µg of DNA (150 000 cell equivalents; range, 10–71 100 copies; median, 500 copies). Of the 19 samples, 13 had a relatively low proviral load (mean, 412 ± 480 copies per µg of DNA), 2 samples had a moderate proviral load (mean 5 485 ± 2 877 copies per µg of DNA) and 4 had very high proviral load, with a mean of 44 890 ± 22 922 copies per µg of DNA, representing 30% of HTLV-1 infected cells in the blood. Furthermore, as seen in Figure 2, pregnant women with high antibody titers had a significantly ($p \leq 0.02$) higher proviral load.

PCR with specific or degenerated primers (5, 59) did not reveal HTLV-1, -2 or -3 provirus in DNA obtained from samples with indeterminate western blot profiles.

Molecular and sequence homology analyses

A 522-bp fragment of the gp21 HTLV-1 *env* sequence was obtained from 16 of the 19 infected women, and a 479-bp fragment of the LTR was amplified and sequenced for 18 of these women (Table 2). Sequence comparison analysis of the *env* and LTR with different HTLV-1 prototypic subtype strains demonstrated that all but one of these strains belonged to the central African subtype B (98.2–96.8% homology with the EL subtype B prototype *env* sequence and 97.4–95.8% homology with the EL LTR sequence). Only one sample (Gab112LM) belonged to transcontinental subtype A, with 98.4% *env* sequence homology and 97.3% LTR homology with the ATK strain. This new strain was also closely related to other sequences in the cosmopolitan group, with 99.2–97.7% homology with the *env* sequences and 100–96.3% homology with the LTR. Among the the newly described sequences, a wide diversity of subtype B strains was observed for both *env* and LTR genomic fragments. Some sequences were identical, while others exhibited 1 to more than 4% divergence for both *env* and LTR.

As described above, one pregnant woman was infected with HTLV-2. Sequence comparison indicated that the strain belonged to HTLV-2 subtype B, with 99.3–97.3% and 99.5–98.0% homology with other sequences of group B for *env* and LTR, respectively. Furthermore, this HTLV-2 sequence was nearly identical to the few other HTLV-2 sequences originating from Gabon, such as GabII (98.1% and 99.3% homology for *env* and LTR, respectively).

Phylogenetic analyses

The phylogenetic analyses of the *env* and LTR sequences confirmed the findings described above. As shown in Figures 3 (A and B), all but one HTLV-1 strain clustered in the large-well, phylogenetically supported HTLV-1 subtype B clade. Furthermore, the strains were distributed among at least four groups of this subtype.

Interestingly, the new strains from Gabon were more closely related to the known HTLV-1 strains originating from Gabon (such as GAB7 or Lib3) and to sequences from neighboring central African countries, such as Cameroon (such as Ph236, T49, H24) and the Central African Republic (such as 12504), than to strains originating from the Democratic Republic of the Congo (such as EL) (Figure 3). Some strains were closely related to STLV-1 strains isolated from chimpanzees and gorillas (PTR-CAR.875, 02CM-3157 and GGO-Cam12) (Figure 3).

For the sole HTLV-2 strain, both *env* and LTR sequence analyses showed that it belonged to HTLV-2 subtype B, which includes very few African strains and several Amerindian ones. As seen in the *env* phylogenetic tree (Figure 4A), this new strain was nearly identical to another strain (JPS-II) from the same region (Haut Ogooué) and previously described by our group (18). In the LTR phylogenetic analysis (Figure 4B), the new HTLV-2 strain was in the same cluster as

a strain isolated from Gabon (HTLV-II-Gab) and another found in southern Cameroon (PYGCAM-1).

DISCUSSION

In this study, based on a national survey of a series of comparable samples from pregnant women in the five provinces of Gabon, we found a focus of HTLV-1 infection in the south-east of the country. Using strict western blot serological criteria, as well as specific molecular detection, we found an HTLV-1 prevalence of 5% in the Haut-Ougoué region, as compared with 0–2.1% in the four other provinces. A study published in 1988 indicated that Gabon is endemic for HTLV-1 infection, with an overall higher seroprevalence in adults (5–10% according to area) (7). Furthermore, the prevalence of HTLV-1 infection in pregnant woman was reported previously to be 6.8–10% (51), on the basis of one epidemiological study performed in one region of the country (south-east). Our studies suggest that the overall national prevalence of HTLV-1 in pregnant women is lower than previously reported, probably because we used validated serological and molecular confirmatory assays and because the prevalence differs geographically, being particularly high in the south-east. However, in future studies in Gabon, the national HTLV-1 prevalence should be considered to be 2.1%.

The prevalence of HTLV-1 in the Haut Ogooué region (5%) is one of the highest in the world for pregnant women and is higher to those observed in highly endemic areas, such as southern Japan (3.7%) (25), and among the Noir Marrons in French Guiana (4.2%) (27, 54) and in Jamaica (2.0%) (11). The reason for the high prevalence in Haut Ogooué is unknown. Foci of high prevalence located near areas of low or very low endemicity is a hallmark of HTLV-1/-2 epidemiology but has never been explained. Several hypotheses have been proposed, including

genetic, environmental and socioeconomic factors (48).

The observed increase in HTLV-1 prevalence with age, especially after 30 years, is classical. It may have several explanations, but accumulation of new HTLV-1 infections through sexual activity over life is considered the most probable (39, 48).

In our study, molecular analysis demonstrated that all but one of the HTLV-1 strains belonged to molecular subtype B. This large group of sequences is well supported phylogenetically in both LTR and *env* analyses. These strains, found exclusively in central Africa, have been reported to be endemic in Cameroon, the Central African Republic and the Democratic Republic of the Congo (34). High genetic diversity is found within this group, and in some cases clear geographical clustering of strains with specific molecular features (e.g. mutation, insertion, deletion) and high bootstrap values has been observed. In our study, despite relatively high genetic diversity (up to 4% nucleotide difference) among the new strains, we observed no geographical clustering, and HTLV-1 strains from the Franceville or Lambaréné area were distributed among the different subgroups of HTLV-1, except the clade from the Democratic Republic of the Congo (EL prototype).

What is the origin of the genetic diversity? At least two hypotheses can be proposed. In the first, genetic diversity is based on transmission of slightly different STLV-1 strains from infected apes to humans. If such events are relatively recent and if the genetic variability of STLV-1 and HTLV-1 is very low over time, this will lead to some genetic diversity in human HTLV-1, mimicking the diversity originally present in ape STLV-1. This might be the case in some instances, as some HTLV-1 strains in central Africa have been found to be nearly identical to the STLV-1 found in chimpanzees or gorillas in Gabon or Cameroon (42, 58), and some of our samples were closely related to the STLV-1 sequences obtained from chimpanzees and gorillas

with less than 1% divergence.

In the second hypothesis, interspecies transmission occurred a long time ago, followed by a relatively long evolution with slow interhuman dissemination by intrafamilial transmission. This mechanism would lead to a certain diversity with, in some cases, a star or a founder effect. The latter situation is exemplified, with much greater HTLV-1 diversity, by the situation in Melanesia, where interhuman evolution can be dated in tens of thousands of years after initial interspecies transmission from monkey STLV-1, which probably occurred during the original migrations of the proto-Melanesians in South-East Asia (24).

We did not find HTLV-1 subytpe D, the second typical subtype from central Africa. This is not unexpected, as strains of this subtype have been found mainly among Pygmy populations in Cameroon and the Central African Republic but not in Bantus (34). Interestingly, these subtype D strains are also endemic among some mandrills, especially in Gabon (33). A large serological and molecular survey on HTLV-1 is under way in Gabon, in both humans and non-human primates, to obtain new insight into the origin, evolution and modes of dissemination of primate T lymphotropic viruses (34, 52, 57).

In our study, one pregnant woman was infected with HTLV-2 subtype B. This strain is highly endemic in several Amerindian populations but has also been found in central African populations, either sporadically (9, 43) or endemically, especially among Bakola Pygmies (17). The origin and age of these viral strains in central Africa have been a matter of debate (17, 52). Interestingly, some of us reported in the 1990s the intrafamilial transmission of this virus in a large family living in Franceville, with evidence of mother-to-child and sexual transmission (18, 55). Other ongoing studies should confirm the widespread circulation of HTLV-2 subtype B in various region of Gabon, with a relatively low prevalence, suggesting that this virus may have

been endemic for a long time in central Africa.

We have also shown a wide range of HTLV-1 proviral loads in the blood of infected pregnant women. Five of the 19 pregnant women had a very high proviral load and a high HTLV-1 titer. The high load found in our study has rarely been found in studies of women living in highly endemic areas such as Jamaica (21, 31), French Guiana (56) and Japan (36). It was reported previously that the rate of HTLV-1 transmission to children increased significantly when women had a high HTLV-1 proviral load (31, 56).

A large study is therefore being performed in the Haut-Ogooué area to assess precisely the level of mother-to-child transmission and to characterize the associated risk factors, such as length of breastfeeding, proviral load, antibody titer, molecular subtype, and genetic signature. The results of this study will be useful for designing preventive measures, such as specific educational programmes adapted to the local situation, aiming to decrease the spread and transmission of HTLV-1 in this highly endemic area, where associated diseases, such as ATL and TSP/HAM, have already been reported (8).

ACKNOWLEDGMENTS

Sonia Lekana-Douki Etenna is the recipient of a fellowship from the European Community and the CIRMF. We thank Marie-Thérèse Bedjabaga and Virginie Thuillier for technical help. The Centre International de Recherches Médicales Franceville (CIRMF), Franceville, Gabon, is funded by the Gabonese Government, Total-Gabon and the French Foreign Ministry. This work was supported by funds from the Service de Coopération et d'Action Culturelle, French Embassy, Libreville, Gabon. Part of this study was also supported by funds from the Agence Nationale de

la Recherche (2006 Microbiologie – 06-MIME-017-01).

REFERENCES

1. **Berteau, F., J. Mention, J. Tissedre, B. Narraido, C. Grall, E. Glowaczover, A. Keita, Y. Laval, I. Bedjabaga, and S. Ossari.** 1993. Evaluation of the seroprevalence of human immunodeficiency virus (HIV) and human t-cell lymphotropic virus (HTLV) in Haut Ogooué Province in Gabon in pregnant women and blood donor control groups. Bull. Soc. Pathol. Exot. **86:**12-5.
2. **Berteau, P. F., Y. Martin-Prevel, and I. Bedjabaga.** 1994. Vertical transmission of the human T-cell leukemia virus in an endemic area. An epidemiological study in children from 0 to 5 years in Gabon. Bull. Soc. Pathol. Exot. **87:**217-221.
3. **Bertherat, E., M. Makuwa, A. Renaut, R. Nabias, and M. Georges-Courbot.** 1998. HIV-1, HTLV-I, and HTLV-II in a semiurban population in East Gabon. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. **19:**430-432.
4. **Biglione, M., O. Vidan, R. Mahieux, M. de Colombo, M. de Los Angeles, A. de Basualdo, M. Bonnet, G. Pankow, M. Avila de Efron, A. Zorrilla, F. Tekaia, E. Murphy, G. de Thé, and A. Gessain.** 1999. Seroepidemiological and Molecular Studies of HTLV-II, subtype b, in isolated groups of Mataco and Toba indians of Northern Argentina. AIDS Res. Hum. Retroviruses **15:**407-417.
5. **Calattini, S., S. A. Chevalier, R. Duprez, S. Bassot, A. Froment, R. Mahieux, and A. Gessain.** 2005. Discovery of a new human T-cell lymphotropic virus (HTLV-3) in Central Africa. Retrovirology **9:**30.
6. **Cassar, O., C. Capuano, S. Bassot, F. Charavay, R. Duprez, P. V. Afonso, M. Abel, H. Walter, W. Mera, P. M. Martin, E. Chungue, and A. Gessain.** 2007. Human T lymphotropic virus type 1 subtype C melanesian genetic variants of the Vanuatu Archipelago and Solomon Islands share a common ancestor. J. Infect. Dis. **15:**510-521.
7. **Delaporte, E., A. Dupont, M. Peeters, R. Josse, M. Merlin, D. Schrijvers, B. Hamono, L. Bedjabaga, H. Cheringou, and F. Boyer.** 1988. Epidemiology of HTLV-I in Gabon (Western Equatorial Africa). Int. J. Cancer. **15:**687-689. .
8. **Delaporte, E., F. Klotz, M. Peeters, Y. Martin-Prevel, L. Bedjabaga, B. Larouzé, C. Nguembi-Mbina, P. Walter, and P. Piot.** 1993. Non-Hodgkin lymphoma in Gabon and its relation to HTLV-I. Int. J. Cancer. **53:**48-50.
9. **Delaporte, E., N. Monplaisir, J. Louwagie, M. Peeters, Y. Martin-Prével, J. P. Louis, A. Trebucq, L. Bedjabaga, S. Ossari, C. Honoré, B. Larouze, L. Auriol, G. Van der Groen, and P. Piot.** 1991 Prevalence of HTLV-I and HTLV-II infection in Gabon, Africa: comparison of the serological and PCR results. Int. J. Cancer **30:**373-376.
10. **Delaporte, E., M. Peeters, M. Simoni, and P. P.** 1989. HTLV-I infection in western equatorial Africa. Lancet **18:**1226.
11. **Dowe, G., S. D. King, M. F. Smikle, H. H. Wynter, R. Chout, and K. W.** 1998 Prevalence of viral and bacterial sexually transmitted pathogens in Jamaican pregnant women. West. Indian. Med. J. **47:**23-25.

12. **Fouchard, N., B. Flageul, M. Bagot, M. F. Avril, O. Hermine, F. Sigaux, H. Merle-Beral, X. Troussard, J. F. Delfraissy, G. de Thé, and A. Gessain.** 1995. Lack of evidence of HTLV-I/II infection in T CD8 malignant or reactive lymphoproliferative disorders in France: a serological and/or molecular study of 169 cases. Leukemia **9:**2087-2092.
13. **Gabet, A. S., F. Mortreux, A. Talarmin, Y. Plumelle, I. Leclercq, A. Leroy, A. Gessain, E. Clity, M. Joubert, and E. Wattel.** 2000. High circulating proviral load with oligoclonal expansion of HTLV-1 bearing T cells in HTLV-1 carriers with strongyloidiasis. Oncogene **19:**4954-60.
14. **Gessain, A.** 1996. Epidemiology of HTLV-I and associated diseases, p. 33-64. *In* P. Höllsberg and D. A. Hafler (ed.), Human T-cell Lymphotropic Virus Type 1. John Wiley & Sons Ltd, Chichester.
15. **Gessain, A., F. Barin, J. C. Vernant, O. Gout, L. Maurs, A. Calender, and G. de Thé.** 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet **2:**407-410.
16. **Gessain, A., R. Mahieux, and G. de Thé.** 1996. Genetic variability and molecular epidemiology of human and simian T cell leukemia/lymphoma virus type I. Journal of Acquired Immune Deficieny Syndromes and Human Retrovirology **13 Suppl 1:**S132-145.
17. **Gessain, A., P. Mauclère, A. Froment, M. Biglione, J. Y. Le Hesran, F. Tekaia, J. Millan, and G. de Thé.** 1995. Isolation and molecular characterization of a human T-cell lymphotropic virus type II (HTLV-II), subtype B, from a healthy Pygmy living in a remote area of Cameroon: an ancient origin for HTLV-II in Africa. Proc. Natl. Acad. Sci. U S A. **25:**4041-5.
18. **Gessain, A., P. Tuppin, M. Kazanji, J. Y. Cosnefroy, M. C. Georges-Courbot, A. J. Georges, and G. De Thé.** 1994. A distinct molecular variant of HTLV-IIB in Gabon, Central Africa. AIDS Res. Hum. Retroviruses. **10:**753-755.
19. **Hanchard, B.** 1996. Adult T-cell leukemia/lymphoma in Jamaica: 1986-1995. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. **13:**S20-25.
20. **Hirata, M., J. Hayashi, A. Noguchi, K. Nakashima, W. Kajiyama, and S. Kashiwagi.** 1992. The effects of breastfeeding and presence of antibody to p40tax protein of human T cell lymphotropic virus type-I on mother to child transmission. Int. J. Epidemio. **21:**989-994.
21. **Hisada, M., E. M. Maloney, T. Sawada, W. J. Miley, P. Palmer, B. Hanchard, J. J. Goedert, and A. Manns.** 2002. Virus markers associated with vertical transmission of human T lymphotropic virus type 1 in Jamaica. Clin. Infect. Dis. **34(15):**1551-1557.
22. **Hjelle, B., O. Appenzeller, R. Mills, S. Alexander, N. Torrezmartinez, R. Jahnke, and G. Ross.** 1992. Chronic Neurodegenerative Disease Associated with HTLV-II Infection. Lancet **339:**645-646.
23. **Hjelle, B., R. Mills, S. Swenson, G. Mertz, C. Key, and S. Allen.** 1991. Incidence of Hairy Cell Leukemia, Mycosis Fungoides, and Chronic Lymphocytic Leukemia in First Known HTLV-II- Endemic Population. J. Infec. Dis. **163:**435-440.
24. **Ibrahim, F., G. de Thé, and A. Gessain.** 1995. Isolation and characterization of a new simian T-cell leukemia virus type 1 from naturally infected celebes macaques (Macaca tonkeana): complete nucleotide sequence and phylogenetic relationship with the Australo-Melanesian human T-cell leukemia virus type 1. J. Virol. **69:**6980-6993.
25. **Kashiwagi, K., N. Furusyo, H. Nakashima, N. Kubo, N. Kinukawa, S. Kashiwagi, and J. Hayashi.** 2004. A decrease in mother-to-child transmission of human T lymphotropic virus type I (HTLV-I) in Okinawa, Japan. Am. J. Trop. Med. Hyg. **70:**158-163.

26. **Kazanji, M., B. Benoit, M. Meddeb, L. Meertens, C. Marty, A. Gessain, and A. Talarmin.** 2001. Molecular characterization and phylogenetic analysis of a human T cell leukemia virus type 2 strain from French Guiana. AIDS Res. Hum. Retroviruses **17:**563-8.
27. **Kazanji, M., and A. Gessain.** 2003. Human T-cell Lymphotropic Virus types I and II (HTLV-I/II) in French Guiana: clinical and molecular epidemiology. Cad. Saude Publica. **19:**1227-1240.
28. **Kumar, S., M. Nei, J. Dudley, and K. Tamura.** 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinform. **9:**299-306.
29. **Lagrenade, L., B. Hanchard, V. Fletcher, B. Cranston, and W. Blattner.** 1990. Infective Dermatitis of Jamaican Children - A Marker for HTLV-I Infection. Lancet **336:**1345-1347.
30. **Le Hesran, J. Y., E. Delaporte, C. Gaudebout, A. Trebuck, D. Schrijvers, R. Josse, M. Peeters, H. Cheringou, A. Dupont, and B. Larouze.** 1994. Demographic factors associated with HTLV-1 infection in a Gabonese community. Int. J. Epidemiol. **23:**812-7.
31. **Li, H. C., R. J. Biggar, W. Miley, E. M. Maloney, B. Cranston, B. Hanchard, and M. Hisada.** 2004. Provirus load in breast milk and risk of mother-to-child transmission of human T lymphotropic virus type I. J. Infect. Dis. **190:**1275-1278.
32. **Mahé, A., L. Meertens, F. Ly, P. S. Sow, C. T. Diop, N. D. Samb, O. M. Diop, F. Valensi, and A. Gessain.** 2004. Human T-cell leukaemia/lymphoma virus type 1-associated infective dermatitis in Africa: a report of five cases from Senegal. Br. J. Dermatol. **150:**958-965.
33. **Mahieux, R., C. Chappey, M. C. Georges-Courbot, G. Dubreuil, P. Mauclere, A. Georges, and A. Gessain.** 1998. Simian T-cell lymphotropic virus type 1 from Mandrillus sphinx as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. J. Virol. **72:**10316-10322.
34. **Mahieux, R., F. Ibrahim, P. Mauclere, V. Herve, P. Michel, F. Tekaia, C. Chappey, B. Garin, E. van der Ryst, B. Guillemain, E. Ledru, E. Delaporte, G. de Thé, and A. Gessain.** 1997. Molecular epidemiology of 58 new African human T-cell leukemia virus type 1 (HTLV-1) strains: identification of a new and distinct HTLV-1 molecular subtype in Central Africa and in Pygmies. J. Virol. **71:**1317-1333.
35. **Mauclère, P., J. Y. Le Hesran, R. Mahieux, R. Salla, J. Mfoupouendoun, E. T. Abada, J. Millan, G. de Thé, and A. Gessain.** 1997. Demographic, ethnic, and geographic differences between human T cell lymphotropic virus (HTLV) type I-seropositive carriers and persons with HTLV-I Gag-indeterminate Western blots in Central Africa. J. Infect. Dis. **176:**505-9.
36. **Miyata, H., T. Kamahora, S. Iha, S. Katamine, T. Miyamoto, and S. Hino.** 1995 Dependency of antibody titer on provirus load in human T lymphotropic virus type I carriers: an interpretation for the minor population of seronegative carriers. J. Infect. Dis. **171:**1455-1460.
37. **Mochizuki, M., K. Yamaguchi, K. Takatsuki, T. Watanabe, S. Mori, and K. Tajima.** 1992. HTLV-I and Uveitis. Lancet **339:**1110.
38. **Morgan, O., P. Rodgers-Johnson, C. Mora, and G. Char.** 1989. HTLV-I and polymyositis in Jamaica. Lancet **ii:**1184-1186.
39. **Murphy, E., J. Figueroa, W. Gibbs, A. Brathwaite, M. Holding-Cobham, D. Waters, B. Cranston, B. Hanchard, and W. Blattner.** 1989. Sexual transmission of human T-lymphotropic virus type I (HTLV-I). Ann. Intern. Med. **1:**555-560.
40. **Murphy, E. L., J. Fridey, J. W. Smith, J. Engstrom, R. A. Sacher, K. Miller, J. Gibble, J. Stevens, R. Thomson, D. Hansma, J. Kaplan, R. Khabbaz, and G. Nemo.** 1997.

HTLV-associated myelopathy in a cohort of HTLV-I and HTLV-II-infected blood donors. The REDS investigators. Neurology **48:**315-320.

41. **Murphy, E. L., R. Mahieux, G. de Thé, F. Tekaia, D. Ameti, J. Horton, and A. Gessain.** 1998. Molecular epidemiology of HTLV-II among United States blood donors and intravenous drug users: an age-cohort effect for HTLV-II RFLP type aO. Virology **242:**425-434.
42. **Nerrienet, E., L. Meertens, A. Kfutwah, Y. Foupouapouognigni, A. Ayouba, and A. Gessain.** 2004 Simian T cell leukaemia virus type I subtype B in a wild-caught gorilla (Gorilla gorilla gorilla) and chimpanzee (Pan troglodytes vellerosus) from Cameroon. J. Gen. Virol. **85:**25-29.
43. **Nyambi, P., Y. Ville, J. Louwagie, I. Bedjabaga, E. Glowaczower, M. Peeters, D. Kerouedan, M. Dazza, B. Larouze, G. van der Groen, and E. Delaporte.** 1996 Mother-to-child transmission of human T-cell lymphotropic virus types I and II (HTLV-I/II) in Gabon: a prospective follow-up of 4 years. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **1:**187-192.
44. **Ozden, S., V. Mouly, M. C. Prevost, A. Gessain, G. Butler-Browne, and P. E. Ceccaldi.** 2005 Muscle wasting induced by HTLV-1 tax-1 protein: an in vitro and in vivo study. Am. J. Pathol. **167:**1609-1619.
45. **Perret, J. L., J. B. Moussavou-Kombila, E. Delaporte, S. Coniquet, C. Nguemby-Mbina, and P. Normand.** 1996. Mycosis fungoides in a Gabonese patient infected with HTLV-I. Med. Trop. **56:**66-8.
46. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type-C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. U S A. **77:**7415-7419.
47. **Pouliquen, J. F., L. Hardy, A. Lavergne, E. Kafiludine, and M. Kazanji.** 2004. High seroprevalence of human T-cell lymphotropic virus type 1 in blood donors in Guyana and molecular and phylogenetic analysis of new strains in the Guyana shelf (Guyana, Suriname, and French Guiana). J. Clin. Microbiol. **42:**2020-2026.
48. **Proietti, F. A., A. B. Carneiro-Proietti, B. C. Catalan-Soares, and E. Murphy.** 2005 Global epidemiology of HTLV-I infection and associated diseases. Oncogene **5:**6058-6068.
49. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406-425.
50. **Salemi, M., A. Vandamme, C. Gradozzi, K. Van Laethem, E. Cattaneo, G. Taylor, C. Casoli, P. Goubau, J. Desmyter, and U. Bertazzoni.** 1998. Evolutionary rate and genetic heterogeneity of human T-cell lymphotropic virus type II (HTLV-II) using isolates from European injecting drug users. J. Mol. Evol. **46:**602-611.
51. **Schrijvers, D., E. Delaporte, M. Peeters, A. Dupont, and A. Meheus.** 1991. Seroprevalence of retroviral infection in women with different fertility statuses in Gabon, western equatorial Africa. J. Acquir. Immune Defic. Syndr. **4:**468-470.
52. **Slattery, J. P., G. Franchini, and A. Gessain.** 1999. Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. Gen. Res. **9:**525-40.
53. **Switzer, W. M., F. L. Black, D. Pieniazek, R. J. Biggar, R. B. Lal, and W. Heneine.** 1996. Endemicity and phylogeny of the human T cell lymphotropic virus type II subtype A from the Kayapo Indians of Brazil: evidence for limited regional dissemination. AIDS Res. Hum. Retroviruses **12:**635-640.

54. **Tortevoye, P., P. Tuppin, G. Carles, C. Peneau, and A. Gessain.** 2005 Comparative trends of seroprevalence and seroincidence rates of human T cell lymphotropic virus type I and human immunodeficiency virus 1 in pregnant women of various ethnic groups sharing the same environment in French Guiana. Am. J. Trop. Med. Hyg. **73:**560-565.
55. **Tuppin, P., A. Gessain, M. Kazanji, R. Mahieux, J. Cosnefroy, Y., F. Tekaia, M. C. Georges-Courbot, A. Georges, and G. de Thé.** 1996. Evidence in Gabon for an intrafamilial clustering with mother-to-child and sexual transmission of a new molecular variant of human T-lymphotropic virus type-II subtype B. J. Med. Virol. **48:**22-32.
56. **Ureta-Vidal, A., C. Angelin-Duclos, P. Tortevoye, E. Murphy, J. F. Lepere, R. P. Buigues, N. Jolly, M. Joubert, G. Carles, J. F. Pouliquen, G. de The, J. P. Moreau, and A. Gessain.** 1999. Mother-to-child transmission of human T-cell-leukemia/lymphoma virus type I: implication of high antiviral antibody titer and high proviral load in carrier mothers. Int. J. Cancer **82:**832-6.
57. **Van Dooren, S., M. Salemi, and A. M. Vandamme.** 2001 Dating the origin of the African human T-cell lymphotropic virus type-i (HTLV-I) subtypes. Mol. Biol. Evol. **18:**661-671.
58. **Van Dooren, S., E. J. Verschoor, Z. Fagrouch, and A. M. Vandamme.** 2007. Phylogeny of primate T lymphotropic virus type 1 (PTLV-1) including various new Asian and African non-human primate strains. Infect. Genet. Evol. **7:**374-81.
59. **Wolfe, N. D., W. Heneine, J. K. Carr, A. D. Garcia, V. Shanmugam, U. Tamoufe, J. N. Torimiro, A. T. Prosser, M. Lebreton, E. Mpoudi-Ngole, F. E. McCutchan, D. L. Birx, T. M. Folks, D. S. Burke, and W. M. Switzer.** 2005. Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. Proc. Natl. Acad. Sci. U S A. **31:**7994-7999.

FIGURE LEGENDS

Figure 1. Map of Gabon, with the main cities and "departments" in which the study was conducted.

Figure 2. Comparison of HTLV-1 copy numbers in blood of infected pregnant women and HTLV-1 titer as measured by immunofluorescence assay with the HTLV-1 infected MT-2 cell line.

Figure 3. Phylogenetic trees for HTLV-1 env and LTR.

(A) *env* phylogenetic tree constructed by the neighbor-joining method on a fragment of 522 bp encompassing the end of the carboxyl terminus of *gp46* and most of *gp21* in 47 HTLV-1 strains, including 16 new strains from pregnant women in Gabon (Gab, in bold).

(B) LTR phylogenetic tree constructed by the neighbor-joining method with 40 HTLV-1 strains, including 18 new strains from pregnant women from Gabon (Gab, in bold), for a portion of 450 bp from the LTR.

PTM3 (STLV-I strain) was used to root the tree. Numbers along ancestral segments indicate the robustness of each node as estimated by 1000 bootstrap samplings of the data. The accession numbers of all sequences included in the phylogenetic tree are given in brackets.

In subtype B, sequences from non-human primates are indicated by *.

Figure 4. Phylogenetic trees for HTLV-2 *env* and LTR sequences, including the newly isolated strain (Gab1080FC) from central Africa.

(A) The phylogenetic tree was constructed with a 537-bp region of HTLV-2 *env*, including the 19 major strains for HTLV-2 subtypes A and B. The *env* sequence of the STLV-2 (PP1664) isolate was used as an outgroup to root the tree.

(B) Phylogenetic tree of LTR sequences (645 bp), including the new HTLV-2 sequence with 21 published sequences for subtypes A and B. The LTR of the STLV-2 (PP1664) isolate was used as an outgroup to root the tree.

Table 1. Prevalence of HTLV-1 in pregnant women in Gabon by geographic area and age group

Variable	HTLV-1				
	No. positive / No. tested	%	95% CI	OR	95% CI
Sentinel site					
Libreville	2/196	1.0	0.6–1.4	0.42	0.10–1.83
Port Gentil	2/162	1.2	0.7–1.7	0.54	0.12–2.36
Lambaréné	7/326	2.1	1.6–2.6	1.04	0.41–2.67
Oyem	0/62	0	–	0	–
Franceville	8/161	5.0	3.9–6.1	3.49	1.38–8.82
Age range (years)					
14–20	4/227	1.7	1.2–2.3	0.80	0.26–2.44
21–25	5/281	1.7	1.3–2.3	0.79	0.28–2.22
26–30	4/221	1.8	1.2–2.3	0.82	0.27–2.50
31–40	6/178	3.4	2.5–4.2	1.92	0.72–5.12
Total	**19/907**	**2.1**	**1.8–2.4**		

CI, confidence interval; OR, odds ratio

Table 2. Epidemiological status, antibody titer and molecular screening results of HTLV-1/-2 positive or indeterminate western blot profiles in pregnant women in Gabon, central Africa

ID	Locality	Age (years)	Western blot pattern	Titer		Proviral load Copy number /µg of DNA	GenBank accession No.	
				MT-2	C19		Env	LTR
Gab1392PG	Port-Gentil	18	Complete HTLV-1	1/80	1/40	58	EU444088	EU444107
Gab683LB	Libreville	21	Complete HTLV-1	1/160	1/40	1200	EU444087	EU444102
Gab722LB	Libreville	31	Complete HTLV-1	1/320	1/160	7520	EU444098	EU444112
Gab958LM	Lambarené	25	Complete HTLV-1	1/320	1/80	36	EU444096	EU444117
Gab35LM	Lambarené	22	Complete HTLV-1	1/160	1/80	400	EU444083	EU444106
Gab70LM	Lambarené	27	Complete HTLV-1	1/640	1/160	71100	EU444089	EU444108
Gab109LM	Lambarené	28	Complete HTLV-1	1/160	1/80	76	EU444095	EU444116
Gab826LM	Lambarené	17	Complete HTLV-1	1/40	< 1/20	370	EU444090	EU444103
Gab197LM	Lambarené	32	Complete HTLV-1	1/80	< 1/20	10	ND	EU444110
Gab112LM	Lambarené	21	Complete HTLV-1	1/320	1/20	22	EU444091	EU444101
Gab1014FC	Franceville	24	Complete HTLV-1	1/640	1/160	30800	EU444097	EU444113
Gab1058FC	Franceville	32	Complete HTLV-1	1/320	1/40	3450	EU444084	EU444105
Gab1144FC	Franceville	19	Complete HTLV-1	1/2540	1/80	21260	EU444085	EU444104
Gab1123FC	Franceville	19	Complete HTLV-1	1/640	1/80	900	EU444092	EU444111
Gab1008FC	Franceville	30	Complete HTLV-1	1/320	1/80	1450	EU444086	EU444109
Gab1089FC	Franceville	23	Complete HTLV-1	1/640	1/80	56400	ND	EU444118
Gab1037FC	Franceville	35	Complete HTLV-1	1/320	1/80	254	EU444093	EU444114
Gab1077FC	Franceville	39	Complete HTLV-1	1/160	< 1/20	500	ND	ND
Gab1080FC	Franceville	17	Complete HTLV-2	1/20	1/160	ND	EU444099	EU444100
Gab1314PG	Port-Gentil	40	Indeterminate	1/640	1/80	85	EU444094	EU444115
Gab1390PG	Port-Gentil	29	Indeterminate	1/640	< 1/20	0	–	–
Gab1527OY	Oyem	20	Indeterminate	1/640	1/20	0	–	–
Gab652LB	Libreville	16	Indeterminate	1/640	< 1/20	0	–	–
Gab715LB	Libreville	33	Indeterminate	1/80	< 1/20	0	–	–
Gab79LM	Lambarené	19	Indeterminate	1/80	< 1/20	0	–	–
Gab155LM	Lambarené	39	Indeterminate	1/80	< 1/20	0	–	–
Gab1129FC	Franceville	23	Indeterminate	1/320	1/20	0	–	–
Gab1004FC	Franceville	26	Indeterminate	1/160	1/20	0	–	–
Gab1199FC	Franceville	24	Indeterminate	1/160	< 1/20	0	–	–

ND, not done because not enough DNA available

Figure 1

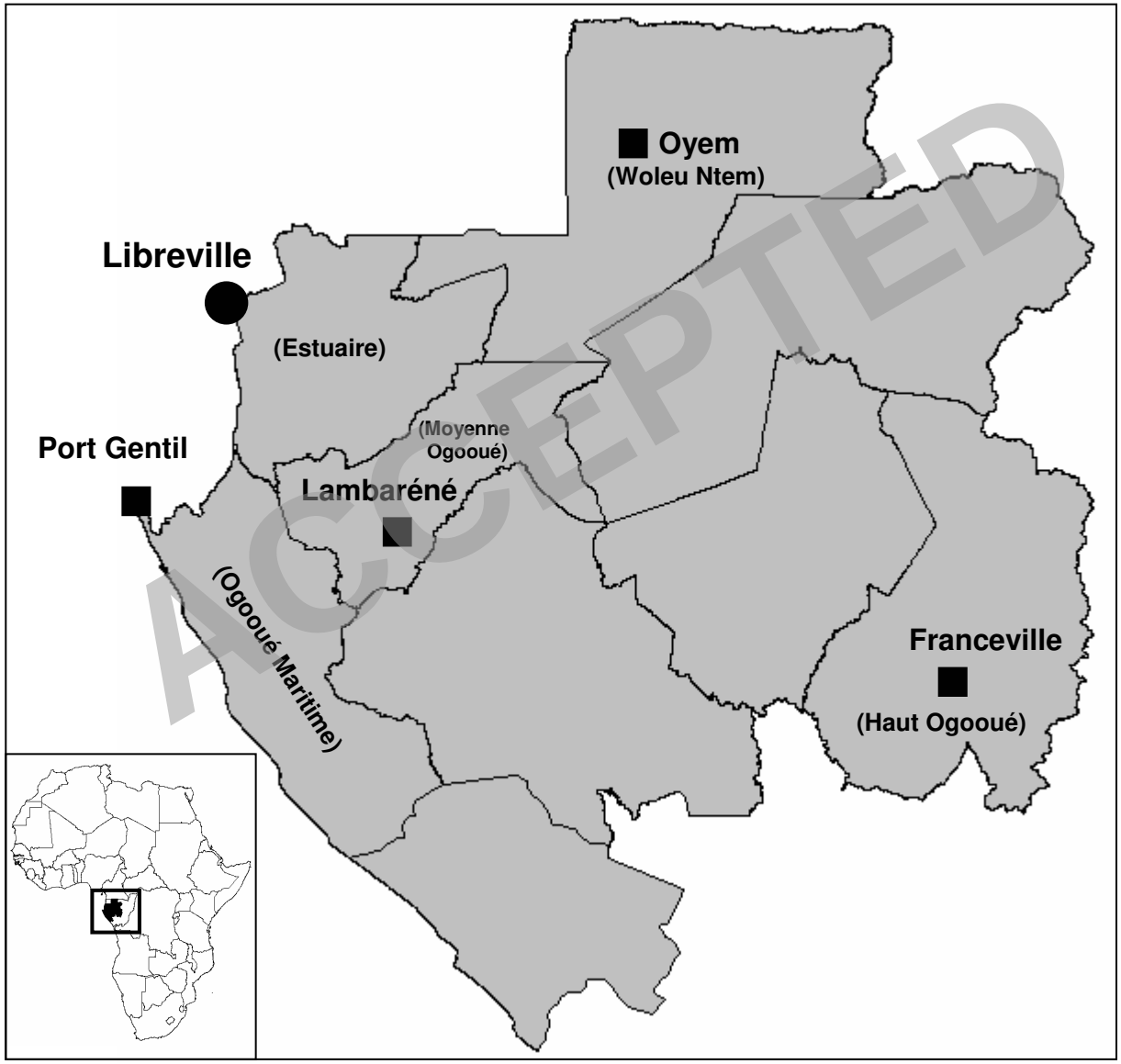

Figure 2

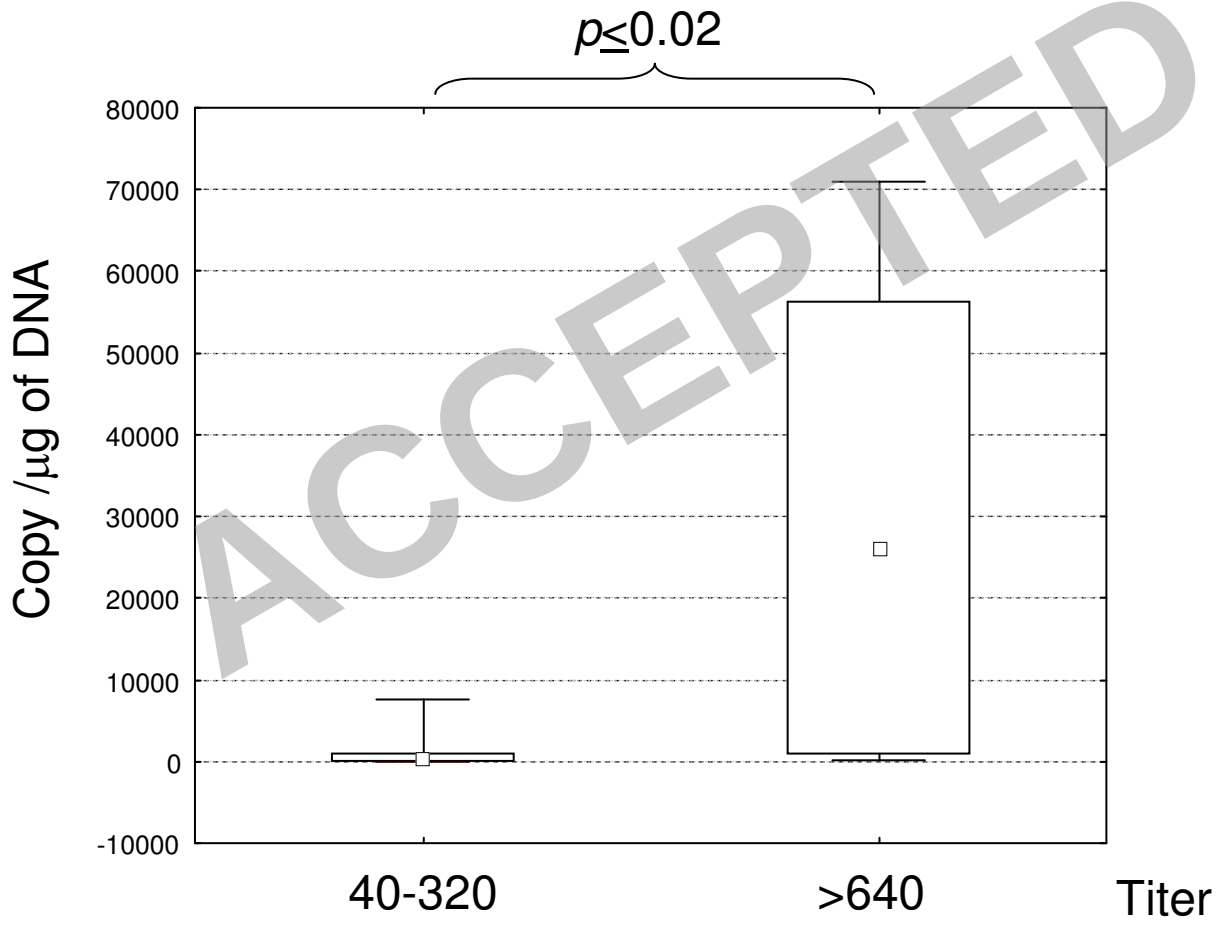

Figure 3

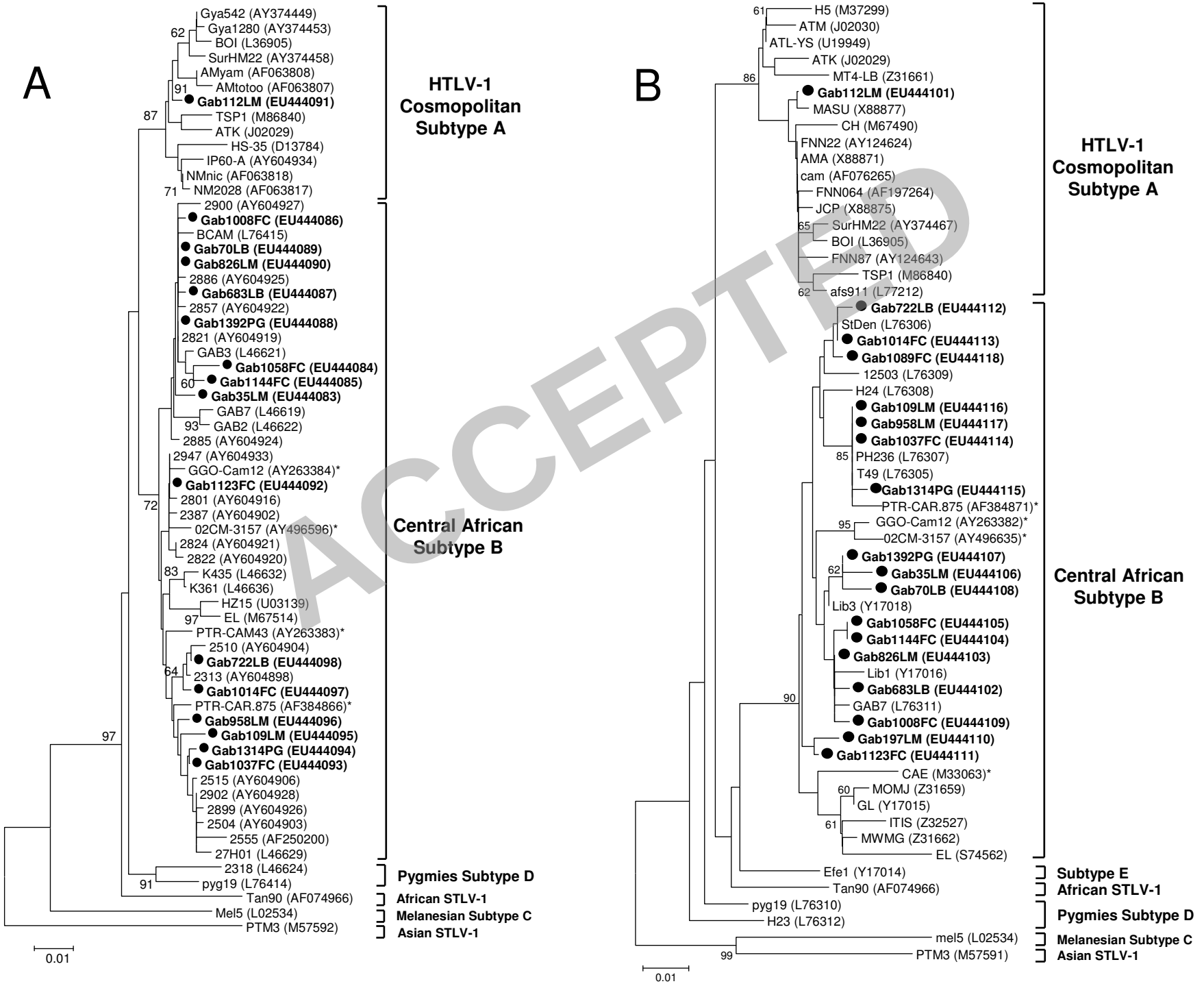

Figure 4