




## High prevalence of human T-cell leukemia virus type-1b genotype among blood donors in Gabon, Central Africa

Jill-Léa Ramassamy <sup>1,2</sup> Olivier Cassar <sup>1</sup> Manoushka Toumbiri,<sup>3</sup> Abdoulaye Diané,<sup>3</sup> Antony Idam Mamimandjiami,<sup>1,3,4</sup> Calixte Bengone,<sup>5</sup> Jophrette Mireille Ntsame-Ndong,<sup>5</sup> Augustin Mouinga-Ondémé,<sup>3,†</sup> and Antoine Gessain <sup>1,†</sup>

**BACKGROUND:** The African continent is considered to be the largest endemic area of HTLV-1 infection, with at least several million infected individuals. Systematic screening of blood donors can prevent the transmission of HTLV-1 in blood. Gabon is one of the countries with the highest prevalence of HTLV-1 worldwide, and yet the routine testing of blood donors has still not been introduced.

**METHODS:** All blood donations collected between April and July 2017 at the Centre National de Transfusion Sanguine of Gabon were studied. Plasma samples were screened by ELISA for the presence of HTLV-1/2 antibodies. Western blot (WB) and polymerase chain reaction (PCR) tests were used for confirmation.

**RESULTS:** In total, 3123 blood donors were tested, including 1740 repeat and 1378 first-time blood donors (FTBDs). Of them, 132 samples tested positive for HTLV-1/2 by ELISA (4.2%). WB and PCR confirmed HTLV-1 infection for 23 individuals. The overall prevalence of HTLV-1 was 0.74% [95% CI 0.47%-1.10%], 1% in FTBD, and 0.5% in repeat donors. Age and sex-adjusted prevalence was five-fold lower in FTBD than in the general adult population of rural areas of Gabon. All detected HTLV-1 strains belonged to the central African HTLV-1b genotype but were highly diverse.

**CONCLUSION:** We report an overall prevalence of HTLV-1 of 0.74%, one of the highest values reported for blood donors in Africa. Given the high risk of HTLV-1 transmission in blood, it is necessary to conduct cost-effectiveness studies to determine the need and feasibility of implementing screening of HTLV-1 in blood donors in Gabon.

**H**uman T-cell leukemia virus type 1 (HTLV-1) is an oncogenic retrovirus that infects at least 5 to 10 million people worldwide.<sup>1</sup> HTLV-1 infection can lead to severe diseases, such as adult T-cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM), for which there is currently no effective treatment. HTLV-1

From the <sup>1</sup>Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, Département de Virologie, Institut Pasteur, UMR 3569, CNRS; the <sup>2</sup>Université de Paris, Paris, France; the <sup>3</sup>Unité des infections rétrovirales et pathologies associées, Centre International de Recherches Médicales de Franceville; the <sup>4</sup>Ecole Doctorale Régionale d'Afrique Centrale, Infectiologie Tropicale, Franceville and <sup>5</sup>Centre National de Transfusion sanguine (CNTS), Libreville, Gabon.

*Address reprint requests to:* Jill-Léa Ramassamy, Institut Pasteur, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, 28 Rue du Dr. Roux, F-75015 Paris, France; e-mail: jill.lea.ramassamy@pasteur.fr;

Antoine Gessain, Institut Pasteur, Unité d'Epidémiologie et Physiopathologie des Virus Oncogènes, 28 Rue du Dr. Roux, F-75015 Paris, France; e-mail: agessain@pasteur.fr

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

<sup>†</sup>These authors contributed equally to this work.

This study received funding from the CNRS (UMR 3569) (AG); the Institut Pasteur, France (AG), and through the "Investissement d'Avenir" as part of a French "Laboratoire d'Excellence" (LabEx) research program: Integrative Biology of Emerging Infectious Diseases (ANR10-LBX-62 IBEID) (AG). JLR received financial support from the European Union (FOOD/2016/379-660).

Received for publication August 23, 2019; revision received February 26, 2020, and accepted March 22, 2020.

doi:10.1111/trf.15838

© 2020 The Authors. *Transfusion* published by Wiley Periodicals LLC on behalf of AABB.

TRANSFUSION 2020;60;1483-1491

infected individuals will have a lifetime risk of 2-7% to develop an ATLL<sup>2</sup> and of 0.1-2% for HAM/TSP.<sup>3</sup>

The African continent is considered to be the largest endemic area of HTLV-1 infection, with at least several million infected individuals.<sup>1</sup> In Central Africa, the highest prevalence was reported in Gabon. Indeed, several studies performed since 1986 have reported a high prevalence of HTLV-1 around 5% to 10% in the rural adult population<sup>4-8</sup> and 1% to 5% among pregnant women<sup>9,10</sup> in Gabon. Gessain and Cassar estimated that between 16,000 and 30,000 people are infected with HTLV-1 in Gabon.<sup>1</sup> HTLV-1 can be transmitted through unprotected sexual contact, prolonged breastfeeding, and contaminated blood products, during blood transfusion or intravenous drug injections. However, the relative contribution of the various transmission pathways remains to be determined. A study of hospitalized children in Gabon showed that maternal transmission and blood transfusion were the two principal means of transmission for HTLV-1 infection in children.<sup>11</sup> Contaminated blood products are a major issue, as HTLV-1 infection following transfusion of contaminated blood has often been reported to be associated with progression to HAM/TSP.<sup>3,12,13</sup> However, transmission can easily be prevented by the screening of blood donations before use. Very few well performed studies on HTLV-1 infection in blood donors have been done in Africa with stringent serological and/or molecular criteria.<sup>14-16</sup>

Despite the remarkably high prevalence of HTLV-1 in the general adult population of Gabon, no preventive measures have been implemented, and the prevalence of HTLV-1 infection in blood donors remains to be determined. In this context, we conducted a cross-sectional study to measure the prevalence of HTLV-1 infection among blood donors in Gabon.

## MATERIAL AND METHODS

### Population

This study was conducted at the national blood transfusion center (*Centre National de Transfusion Sanguine*; CNTS) of Gabon, in the city of Libreville. Almost half the Gabonese population is concentrated in Libreville (813,000 of 2,119,000). The CNTS currently recruits unpaid volunteer and family blood donors, on the basis of a risk factor screening questionnaire followed by a health check. The CNTS routinely screened blood donors for HCV, HIV, HBV, and syphilis. Median storage time of blood donations is 2 days at the CNTS and no leukocyte depletion techniques are currently used in Gabon. We included all blood donations from April 20 through July 10, 2017.

### Ethics

A collaboration agreement was signed between the *Centre International de Recherches Médicales de Franceville*

(CIRMF) and the CNTS. Ethics approval was obtained from the *Comité National d'Ethique* of Gabon (Permit number 001/CNE/018/11). All subjects provided written informed consent.

### Serological tests

Plasma samples were subjected to ELISA (HTLV-I/II ELISA 4.0, MP Biomedicals) for the detection of HTLV-1/2 antibodies. Samples testing positive were subjected to a Western blot (WB) assay (HTLV BLOT 2.4, MP Biomedicals) for confirmation. The results were interpreted according to the manufacturer's instructions, which are detailed in Table 2 legend.

### Molecular tests

High-molecular weight DNA was extracted from peripheral blood buffy coats (PB-BC) with the QIAamp blood minikit (Qiagen). Polymerase chain reaction (PCR) was carried out on all samples testing positive by ELISA, to amplify a 522 bp fragment of the *env* gene with the Env11 and Env22 primers, as previously described.<sup>16</sup> The amplicons were visualized by electrophoresis in a 1.5% agarose gel, which was stained with ethidium bromide. Amplicons of the expected size were sent to the Eurofins sequencing platform at Cochin Hospital (Paris, France) for sequencing.

### Phylogenetic analysis

HTLV-1 genotypes were identified by BLAST searches of the sequences obtained, using the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST>). A phylogenetic tree was constructed from the alignment of the 522 bp *env* gene fragment sequences obtained, by the maximum likelihood method, with Seaview software version 4.

### Statistical analysis

Prevalence and 95% confidence intervals (95% CIs) were calculated for the total sample and by sex, age group, and type of donation. The prevalence of HTLV-1 infection was compared between groups using Chi-square tests, and crude odds ratios (OR) were calculated with 95% CIs. We considered p values <0.05 to be statistically significant. No multivariable analysis was performed due to the lack of significant variables in the univariate analysis.

The prevalence of HTLV-1 among blood donors was compared with that in the rural population of Gabon, according to the results of a recently published epidemiological survey.<sup>7</sup> To ensure comparability, we included only participants under the age of 59 years from the rural study (1403 of the 2060 participants) and only first-time donors as to reduce the selection bias effect of blood donors. The same serological and molecular tests were performed and the same definition of positivity was applied in both studies: an individual with a positive serological result confirmed by WB (HTLV-1 or HTLV profile) or PCR was considered to be

infected with HTLV-1. We calculated the crude OR and sex- and age-adjusted OR between first-time blood donors (FTBDs) and the rural population. All analyses were performed with STATA 15.0 software (Stata Corporation).

## RESULTS

### Population

In total, 3123 blood donors (mean age, 31 years; range, 17-59 years) from the CNTS were included in the survey: 2561 men (82%) and 562 women (18%). These donors were comprised of 1740 repeat blood donors (56%) and 1378 new blood donors (44%) (Table 1). Most blood donations came from family donors (62%) and 35% came from volunteers. The type of donor was not recorded for 99 of the donors (3%).

### Serological and molecular results for HTLV-1

We tested 3123 plasma samples by ELISA; 132 of these samples tested positive for HTLV-1/2 (4.2%) and were subjected to a WB assay for confirmation. According to the kit manufacturer's instructions, 17 of these 132 samples were seropositive for HTLV-1 (13%), two were HTLV-seroreactive (2%), 55 were sero-indeterminate (41%), and 58 were seronegative (44%) (Table 2). None of the samples were positive for HTLV-2.

Molecular amplification was performed on DNA extracted from the PB-BC of the 132 ELISA-positive individuals. Positive amplification was achieved for 20 samples (15.2% of the ELISA-positive samples). Of them, 15 had an HTLV-1 WB profile, 4 had an indeterminate profile and one was HTLV (Table 2).

An individual was considered to be infected with HTLV-1 if the WB profile obtained was HTLV-1 ( $n = 17$ ),

HTLV ( $n = 2$ ), or sero-indeterminate but with a positive PCR result ( $n = 4$ ).

### Phylogenetic analysis

An analysis of the 20 characterized strains on the 522 bp segment of the *env* gene, showed that the strains were closely related, with a nucleotide divergence of 0% to 2.9% between them. There were 9 unique sequences, differing by 1 to 15 nucleotides. Two pairs of individuals were infected with identical strains. Three individuals carried identical strain while another strain was found in four individuals. BLAST searches and phylogenetic analyses indicated that all 20 strains belonged to the Central African HTLV-1b genotype. However, phylogenetic analysis showed considerable diversity among the HTLV-1b strains. The strains could be grouped into five groups within the HTLV-1b subclade supported by high maximum posterior probabilities (0.76-0.93) (Fig. 1).

**TABLE 2. PCR results (amplification of the 522 bp fragment of *env* gene) according to WB profiles, for the 132 samples testing positive by ELISA**

WB profiles	N	PCR+	%
HTLV-1	17	15	88%
HTLV	2	1	50%
Indeterminate	55	4	7%
Negative	58	0	0%
<b>Total</b>	<b>132</b>	<b>20</b>	<b>15.2%</b>

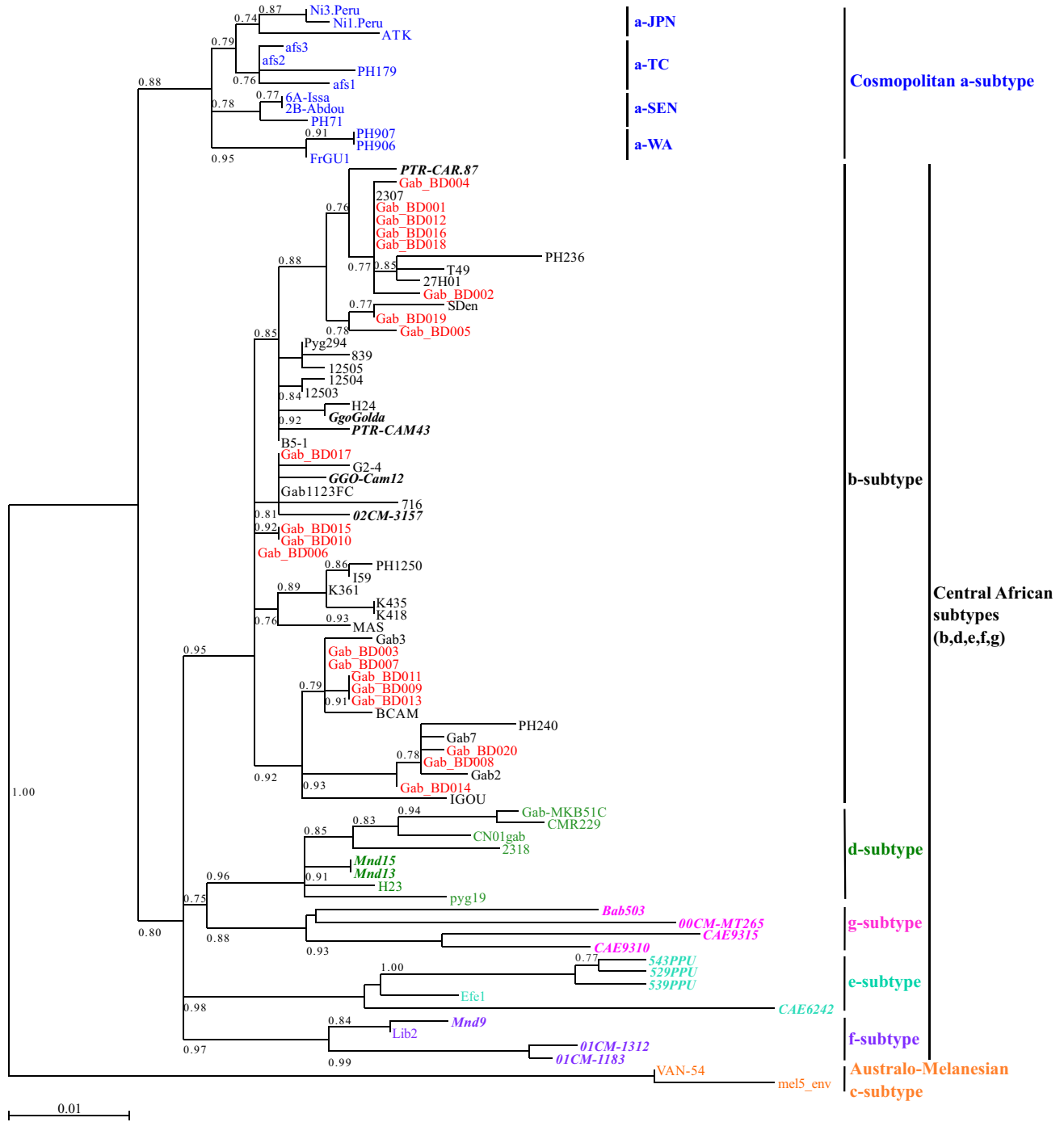
According to the manufacturer's instructions (HTLV BLOT 2.4, MP Biomedicals), the seropositivity criteria for HTLV-1 includes reactivity to p19gag, with or without p24gag, and to GD21 with the presence of the rgp46-I peptide called MTA-1. An HTLV profile has been defined by reactivity to GD21, p19gag, and p24gag in the absence of rgp46-I and rgp46-II peptides. Samples with only partial reactivity to some of these viral proteins were considered indeterminate.

**TABLE 1. Prevalence of HTLV-1 according to demographic characteristics and donor status**

	n+/N	HTLV-1 prevalence (95% CI)	Crude OR (95% CI)	p value
Sex				
M	17/2561	0.7% (0.4-1.1)	1	0.31
F	6/562	1.1% (0.4-2.3)	1.03 (0.6-4.1)	
Age (years)				
17-25	4/778	0.5% (0.1-1.3)	1	0.22
26-35	10/1598	0.6% (0.3-1.1)	1.22 (0.4-3.9)	
36-59	9/747	1.2% (0.6-2.3)	2.36 (0.7-7.7)	
History of blood donation				
Repeat	9/1740	0.5% (0.2-1)	1	0.11
First-time	14/1378	1.0% (0.6-1.7)	1.97 (0.9-4.6)	
Unknown	0/5	0% (0-52)*	-	
Type of blood donor				
Volunteer	5/1083	0.5% (0.2-1.1)	1	0.17
Familial	16/1941	0.8% (0.5-1.3)	1.79 (0.7-4.9)	
Unknown	2/99	2.0% (0.2-7.1)	4.45 (0.9-23.2)	
<b>Total</b>	<b>23/3123</b>	<b>0.7% (0.5-1.1)</b>		

\* One-sided 97.5% confidence interval.

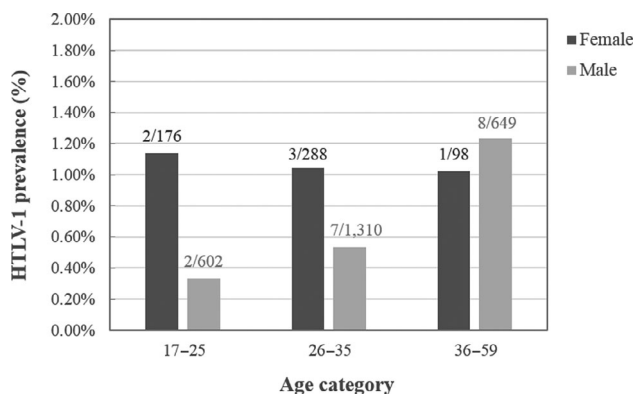
n+ = number of HTLV-1 infected individuals; N total number of individuals tested.



**Fig 1.** Phylogenetic tree generated by the maximum likelihood method with a 522 bp fragment of the *env* gene. Phylogenetic comparisons were performed with the 522-nucleotide *env gp21* gene fragment obtained from 88 HTLV-1 isolates, including the 20 sequences from infected blood donors (in red) and 68 previously published sequences. The GenBank accession numbers of the new sequences from the blood donors are MK949491-MK949510. STLV-1 strains are shown in bold italic typeface. The phylogeny was derived by the maximum likelihood (ML) method with the GTR model. Horizontal branch lengths are drawn to scale, with the bar indicating 0.01 nucleotide replacements per site. Maximum posterior probability were calculated and reported on the maximum likelihood tree (threshold value  $\geq 0.50$ ). a-TC, a-JPN, a-SEN, a-WA, correspond to the Transcontinental, Japanese, Senegalese, and West African clades of the a-genotype respectively. [Color figure can be viewed at wileyonlinelibrary.com]

### Epidemiological analyses

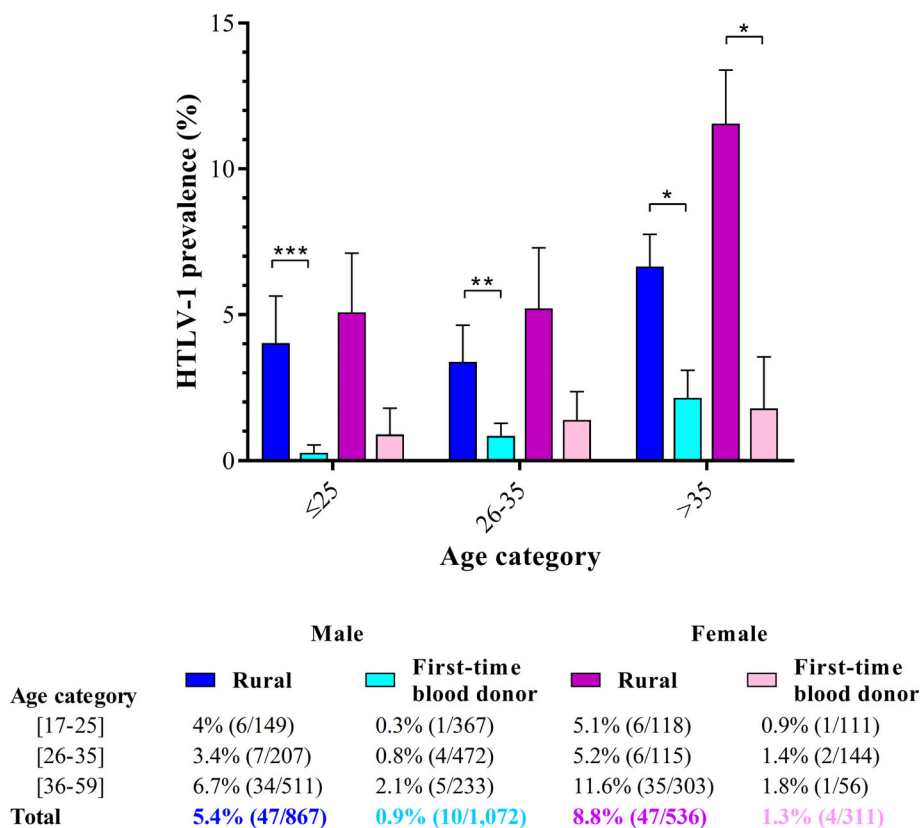
In total, 23 individuals were considered to be infected with HTLV-1, giving an overall prevalence of HTLV-1 among blood donors of 0.74% (95% CI 0.47%-1.10%). There was no significant difference of HTLV-1 prevalence between female and male blood donors (prevalences of HTLV-1 infection



**Fig 2.** HTLV-1 prevalence among Gabonese blood donors, by age and sex. There were no significant differences of HTLV-1 prevalence between sex and age-classes pairwise comparisons.

were 1.1% and 0.7%, respectively) (Table 1). Prevalence increased with age: 0.5% among those under 25 years of age, 0.6% among those aged 26-35 years and 1.2% among those over 35 years of age, although this trend was not statistically significant ( $p$  value 0.22) and was only observed in males (Fig. 2). FTBDs had a higher prevalence of infection (1.0%) than repeat donors (0.5%) and the difference was marginally significant ( $p$  value 0.11). There was no difference in prevalence between volunteers and family blood donors (0.5 and 0.8%, respectively) (Table 1).

We compared these results with the prevalence obtained in a recent population-based survey of the rural population.<sup>7</sup> We included only individuals under the age of 59 years from this study in the analysis (1403 of 2060). The prevalence of HTLV-1 in the rural subgroup was 6.7% (95% CI 5.4%-8.1%). This prevalence was almost 10 times higher than that in all blood donors (crude OR 9.7; 95% CI 6.0-15.5) and was 7 times higher when compared to FTBDs only (crude OR 7.0; 95% CI 4.0-12.5) (Fig. 3). However, demographic characteristics differed significantly between the two populations. Indeed, most of the blood donors were young men. The resulting mean age was 38.4 years for the rural subgroup (range, 15-59 years) and 31.0 years



**Fig 3.** Prevalence of HTLV-1 by age and sex among first-time blood donors and in rural populations. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . The data for the rural subgroup was extracted from a nationwide epidemiological survey in Gabon, previously published.<sup>7</sup> [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 3. Multivariable analysis of the prevalence of HTLV-1 in the rural and first-time blood donor (FTBD) subgroups**

Risk factors	First-time blood donors		Rural population		Adjusted OR	95% CI	p value
	N	n+ (%)	N	n+ (%)			
Sex							
Male	1072	10 (0.9)	867	47 (5.4)	1		
Female	311	4 (1.3)	536	47 (8.8)	1.68	1.13-2.50	0.010 <sup>†</sup>
Age group (years)							
15-25	478	2 (0.4)	267	12 (4.5)	1		0.0014 <sup>†</sup>
26-35	616	6 (1.0)	322	13 (4.0)	1.15	0.57-2.32	
36-59	289	6 (2.1)	814	69 (8.5)	2.41	1.33-4.38	
Survey population							
Blood donors	1383	14 (1.0)	-	-	1		
Rural	-	-	1403	94 (6.7)	4.76	2.63-8.63	<0.001 <sup>‡</sup>

The data for the rural subgroup was extracted from a published nationwide epidemiological survey in Gabon.<sup>7</sup>

<sup>†</sup> p < 0.01.

<sup>‡</sup> p < 0.001.

N = total number of individuals tested; n+ = number of HTLV-1 infected individuals; (%) = prevalence of HTLV-1.

for blood donors (range, 17 to 59 years). The female-to-male ratio was 0.62 for the rural subgroup and 0.22 for the blood donor subgroup. In the rural subgroup, prevalence was significantly higher in women than in men (8.8% [47 of 536] and 5.4% [47 of 867], respectively, p value 0.015), and increased with age (p value 0.007). We therefore conducted a multivariable analysis including age, sex, and survey population (Table 3). After adjustment for age and sex, the risk of a rural individual being infected with HTLV-1 was almost five times higher than that for a FTBD (age- and sex-adjusted OR 4.76; 95% CI 2.63-8.63).

## DISCUSSION

Our findings demonstrate the presence of HTLV-1 infection in blood donors in Gabon. We did not detect infection with HTLV-2. All HTLV-1 strains from infected blood donors belonged to the central African HTLV-1b genotype. This genotype is the most frequent in Gabon, although rare genotypes, such as HTLV-1d, have occasionally been reported.<sup>8,17</sup> Only one published study has reported the seroprevalence of HTLV-1 in blood donors in Gabon. It included 704 blood donors, mostly men, from Franceville between 1989 and 1990 and reported a relatively high seroprevalence of HTLV-1 infection of 6% (42 of 704). However, the results of this previous study should be treated with caution due to the absence of stringent criteria for WB interpretation.<sup>18</sup>

In recent surveys of large rural adult populations in Gabon, the overall prevalence of HTLV-1 was 7.3% in the study by Caron et al. and 8.7% in the study by Djuicy et al., with prevalence values ranging from 5.5% to 14.0% in the different provinces.<sup>7,8</sup> The prevalence of HTLV-1 among blood donors in our study was about seven fold lower than observed in the rural population of Gabon, and was lower by a factor of five after adjustment for age and sex. The difference in prevalence between blood donors and the rural

population can be explained in several ways. The demographic characteristics of blood donors are generally not representative of the general population. Most blood donors in this study were male, as frequently reported for blood banks in Africa, and this is the subgroup of the population for which the prevalence of HTLV-1 is generally the lowest. Nevertheless, even after adjustment for age and sex, the difference remained considerable. Blood donor-based studies include a significant source of selection bias due to donor selection procedures or self-selection. Blood donors are usually one of the healthier subgroups of the population. Furthermore, as HTLV-1 has risk factors in common with HIV, there may be a preselection of uninfected individuals due to screening for specific risk factors by questionnaire and for HIV and hepatitis B infections, resulting in an underestimation of HTLV-1 prevalence in repeat blood donors. In our study, the prevalence in first-time donors was double that of repeat donors, although this difference was not significant. In countries in which blood donation is remunerated, blood donors are more likely to come from poorer populations, which is not the case in Gabon where blood donors are not paid. Most of the blood donations studied here came from the relatives of hospitalized patients, as often reported in other blood banks in Africa. This difference in prevalence probably results from different socio-economic contexts and environments and therefore suggests the existence of risk factors that are specific to a rural setting.<sup>7,8</sup>

Most studies on the detection of HTLV-1 infection in populations of blood donors in Africa have performed serological screening without confirmatory WB and/or specific PCR testing.<sup>19</sup> Seroprevalence is undoubtedly overestimated in these studies, due to the high rates of false positives obtained with ELISA and EIA. Indeed, in our study, only 17% of the samples that tested positive by ELISA (23 of 132) were confirmed positive by PCR or WB. Very few studies on blood donors in Africa have included robust confirmatory tests (Table 4). In West Africa, Gessain et al. reported an HTLV-1

**TABLE 4. Seroprevalence of HTLV-1 among blood donors in Africa**

Country	Male	n HTLV-1/N tested	Prevalence	Confirmatory assay	Reference
Ethiopia	NA	3/1600	0.19	WB (DBL HTLV blot 2.3)	Vrieling et al. <sup>21</sup>
Tunisia	NA	0/500	0.00	WB (Genelabs HTLV blot 2.4)	Mojaat et al. <sup>42</sup>
Mozambique	81	18/2019	0.89	WB (Genelabs HTLV blot 2.4) + PCR	Gudo et al. <sup>15</sup>
South Africa	57	57/46,752	0.13* 0.06†	Inno-LIA	Vermeulen et al. <sup>16</sup>
Guinea	93	22/1785	1.2	WB (Ortho Diagnostic)	Gessain et al. <sup>20</sup>
Senegal	73	7/4900	0.14	WB (Diagnostic Biotechnology HTLV-1/2 blot 2.4)	Diop et al. <sup>14</sup>
Gabon	82	23/3123	0.74	WB (MP Biomedical HTLV blot 2.4) + PCR	Current study

Only large studies of more than 500 blood donors and with confirmation, either by WB or by PCR, are included in this table. Several other studies on the seroprevalence of HTLV-1 in blood donors in Africa have been performed but are not included in this table due to the lack of confirmatory testing.

\* 0.13% was the calculated prevalence in first-time donors.

† 0.06% was the estimated overall prevalence, weighted to annual blood donations in South Africa.

n = number of HTLV-1 infection detected; N = total number of individuals tested; NA = not available; WB = Western blot.

seroprevalence of 1.23% (22 of 1785) in Guinea,<sup>20</sup> whereas Diop et al. reported an overall HTLV-1 & -2 seroprevalence of 0.16% (8 of 4900) at the CNTS of Dakar in Senegal (0.14 and 0.02%, respectively).<sup>14</sup> The seroprevalences of HTLV-1 and HTLV-2 in the Ethiopian blood bank were 0.19% (3 of 1600) and 0.25% (4 of 1600), respectively,<sup>21</sup> and Gudo et al. reported a seroprevalence of HTLV-1 of 0.89% (18 of 2019) in Mozambique.<sup>15</sup> The largest study among African blood donors was recently performed in South Africa; the prevalence of HTLV-1 was found to be 0.12% among both first-time and repeat blood donors (57 of 46,752) and was 0.06% when weighted to annual blood donations, with the highest prevalence among black donors.<sup>16</sup> The situation in Guinea and Mozambique is similar to that reported here.

The prevalence of HTLV-1 infection is lower in blood donors than in the general population, but the risk of contamination due to the transfusion of infected blood remains high. Several countries have implemented routine testing of blood donors to reduce this risk.<sup>22,23</sup> Murphy and Marano et al. pointed out the following paradox: several high-income countries in which this virus is not endemic have introduced the systematic screening of blood donors, whereas most of the endemic countries in which HTLV-1 prevalence is high do not perform any screening tests, mostly due to cost considerations.<sup>22,23</sup> In particular, none of the African countries screens blood donations for HTLV-1. Several studies have raised questions about the cost-effectiveness of the systematic screening of blood donors in different contexts, but most of these studies were performed in high-income countries.<sup>24-27</sup>

In South Africa, where HTLV-1 prevalence is elevated among black population of blood donors, Vermeulen et al. analyzed the different cost-effectiveness of implementing either a systematic or a first-time donor only strategy for HTLV-1 screening.<sup>27</sup> Considering the available resources and the probability of HTLV-1 transmission and the consecutive risk of developing an ATLL or TSP/HAM, the authors advocated to not implement any of the HTLV-1 screening strategy in South Africa.

Some countries, such as Brazil, Canada, France, Australia, and UK, have tried to increase cost-effectiveness by screening only first-time donors.<sup>26,28-30</sup> This strategy is based on the markedly low incidence rate of HTLV-1 infection in repeat blood donors in these countries.<sup>28-30</sup> However, care is required in the implication of such results, as the incidence of infection in blood donors varies considerably between countries and is expected to be much higher in Gabon.

Commercial confirmatory WB assays are expensive. One possible alternative would be the use of two different screening immunoassays without confirmatory tests. In this strategy, samples repeatedly testing positive in the first EIA used for screening are then tested with a second, different EIA. Even without confirmation, this strategy greatly decreases the number of false positives.<sup>31,32</sup> This dual-EIA algorithm had a positive predictive value of 92.8% in a blood donor study in Brazil<sup>28</sup> and of 89.7% in Sweden.<sup>32</sup>

Other alternatives include leukocyte reduction by filtration and freezing blood products, although there are not yet enough evidences to support the efficacy of such measures. Some studies have reported that leukocyte reduction by filtration can effectively reduce HTLV-1 transmission.<sup>22,23,33</sup>

Murphy et al. also discussed the relevance of freezing blood products as a potential strategy for reducing the risk of transmission. This approach has been reported in various studies.<sup>34-36</sup>

Transfusion has been shown to be a major risk factor for HTLV-1 infection in some studies in Gabon, particularly in hospitalized children.<sup>11</sup> However, this finding was not consistent in all studies, and similar surveys conducted in rural populations of Gabon have reported contrary results. Transfusion was significantly associated with HTLV-1 infection in the univariate analysis of Caron et al.,<sup>8</sup> but this association was not significant in the multivariate analysis performed by Djuicy et al.<sup>7</sup> Nevertheless, hospitalization was clearly associated with HTLV-1 infection in both studies, with a risk of HTLV-1 infection increasing with the number of hospitalizations.<sup>7,8</sup> This association could suggest

possible nosocomial transmission of HTLV-1, for example through contact with contaminated sharp objects. Such horizontal transmission of HTLV-1 could easily be prevented with standard precautions and compliance with hygiene rules. Sexual transmission can also be prevented through awareness campaigns and educational programs on sexually transmitted diseases and the importance of condom use. Sexual transmission, particularly from men to women, is assumed to be the main mode of HTLV-1 transmission in a number of countries.

Delaporte et al. reported that almost half the children infected with HTLV-1 hospitalized in a specific hospital in Gabon had an HTLV-1-positive mother, whereas the others probably acquired the infection through blood transfusion.<sup>11</sup> Acquisition of HTLV-1 infection during childhood is serious as it may lead to a higher risk of developing an ATLL<sup>37</sup> while transfusion acquired infection predisposes to TSP/HAM.<sup>3,12</sup> In Gabon, the attributable risk of maternal transmission was estimated at 0.55.<sup>11</sup> This maternal transmission of HTLV-1 infection is very important in countries in which the prevalence of infection is high in pregnant women. In Gabon, the prevalence of HTLV-1 among pregnant women has been reported to be 2.1%-2.9%, but can reach values as high as 5%.<sup>9,10</sup> The efficiency of mother-to-child transmission depends on the duration of breast feeding with an upper limit of 30%<sup>38</sup> and has been estimated at 15% in the Franceville area of Gabon.<sup>39</sup> Transmission occurs during prolonged breastfeeding, through the transfer of infected cells in breast milk. Reducing the duration of breastfeeding or replacing breastfeeding by bottle-feeding has repeatedly been shown to decrease the HTLV-1 transmission rate significantly.<sup>40</sup> For this reason, Japan and HTLV-1 endemic overseas departments of France have implemented systematic prenatal screening and recommend that women with HTLV-1 should avoid breastfeeding, or at least shorten its duration. However, such measures are relevant only in developed countries where alternative feeding methods are readily available. In developing countries, in which child survival is closely linked to breastfeeding, the benefits of breastfeeding can outweigh the risk of viral transmission.<sup>41</sup> In this context, the screening of blood donors seems to be one of the most feasible measures for ensuring transfusion safety and effectively reducing HTLV-1 transmission in the Gabonese population. The various modes of HTLV-1 transmission are all preventable, but the relative importance of each of these modes of transmission remains unclear. Further studies are therefore required to determine the impact of the various risk factors on the occurrence of HTLV-1 transmission in the population.

## CONCLUSION

Our findings demonstrate the presence of HTLV-1 in blood donations in Gabon, with a high prevalence. The

demographic associations of prevalence in blood donors resemble those in the general population but the overall prevalence of infection is, as expected, lower. However, the risk of HTLV-1 transmission in blood remains significant. It is therefore necessary to conduct cost-effectiveness studies to determine the need and feasibility of implementing screening for HTLV-1 infection in blood donors in Gabon, to ensure transfusion safety.

## ACKNOWLEDGMENTS

We thank the Centre international de Recherches Médicales de Franceville in Gabon.

## CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

## REFERENCES

- Gessain A, Cassar O. Epidemiological Aspects and World Distribution of HTLV-1 Infection. *Front Microbiol* 2012;3:388.
- Iwanaga M, Watanabe T, Yamaguchi K. Adult T-cell leukemia: a review of epidemiological evidence. *Front Microbiol* 2012; 3:322.
- Bangham CRM, Araujo A, Yamano Y, et al. HTLV-1-associated myelopathy/tropical spastic paraparesis. *Nat Rev Dis Primer* 2015;1:15012.
- Delaporte E, Dupont A, Peeters M, et al. Epidemiology of HTLV-I in Gabon (Western Equatorial Africa). *Int J Cancer* 1988;42:687-9.
- Hesran JYL, Delaporte E, Gaudebout C, et al. Demographic factors associated with HTLV-1 infection in a Gabonese community. *Int J Epidemiol* 1994;23:812-7.
- Delaporte E, Monplaisir N, Louwagie J, et al. Prevalence of HTLV-I and HTLV-II infection in Gabon, Africa: comparison of the serological and PCR results. *Int J Cancer* 1991;49:373-6.
- Djuicy DD, Mouinga-Ondémé A, Cassar O, et al. Risk factors for HTLV-1 infection in Central Africa: A rural population-based survey in Gabon. *PLoS Negl Trop Dis* 2018;12:e0006832.
- Caron M, Besson G, Padilla C, et al. Revisiting human T-cell lymphotropic virus types 1 and 2 infections among rural population in Gabon, central Africa thirty years after the first analysis. *PLoS Negl Trop Dis* 2018;12:e0006833.
- Etenna SL-D, Caron M, Besson G, et al. New Insights into prevalence, genetic diversity, and proviral load of human T-cell leukemia virus types 1 and 2 in pregnant women in Gabon in Equatorial Central Africa. *J Clin Microbiol* 2008;46:3607-14.
- Pegha Moukandja I, Ngoungou EB, Lemamy GJ, et al. Non-malarial infectious diseases of antenatal care in pregnant women in Franceville, Gabon. *BMC Pregnancy Childbirth* 2017;17:185.
- Delaporte E, Peeters M, Bardy JL, et al. Blood transfusion as a major risk factor for HTLV-I infection among hospitalized children in Gabon (Equatorial Africa). *J Acquir Immune Defic Syndr* 1993;6:424-8.



12. Gout O, Baulac M, Gessain A, et al. Rapid development of myelopathy after HTLV-I infection acquired by transfusion during cardiac transplantation. *N Engl J Med* 1990;322:383-8.
13. Taylor GP. Human T-lymphotropic virus type 1 infection and solid organ transplantation. *Rev Med Virol* 2018;28:e1970.
14. Diop S, Calattini S, Abah-Dakou J, et al. Seroprevalence and molecular epidemiology of human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2 in blood donors from Dakar. *Senegal J Clin Microbiol* 2006;44:1550-4.
15. Gudo ES, Abreu CM, Mussá T, et al. Serologic and molecular typing of human T-lymphotropic virus among blood donors in Maputo City, Mozambique. *Transfusion (Paris)* 2009;49:1146-50.
16. Vermeulen M, Sykes W, Coleman C, et al. The prevalence of human T-lymphotropic virus type 1 & 2 (HTLV-1/2) in South African blood donors. *Vox Sang* 2019;114:451-8.
17. Mahieux R, Ibrahim F, Mauclere P, et al. 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* 1997;71:1317-33.
18. Berteau F, Mention J, Tisedre J, et al. 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* 1993;86:12-5.
19. Ngoma AM, Omokoko MD, Mutombo PB, et al. Seroprevalence of human T-lymphotropic virus (HTLV) in blood donors in sub-Saharan Africa: a systematic review and meta-analysis. *Vox Sang* 2019;114:413-25.
20. Gessain A, Fretz C, Koulibaly M, et al. Evidence of HTLV-II infection in Guinea, West Africa. *J Acquir Immune Defic Syndr* 1993;6:324-5.
21. Vrieliink H, Sisay Y, Reesink HW, et al. Evaluation of a combined lysate/recombinant antigen anti-HTLV-I/II ELISA in high and low endemic areas of HTLV-I/II infection. *Transfus Med* 1995;5:135-7.
22. Murphy EL. Infection with human T-lymphotropic virus types-1 and -2 (HTLV-1 and -2): Implications for blood transfusion safety. *Transfus Clin Biol J Soc Francaise Transfus Sang* 2016;23:13-9.
23. Marano G, Vaglio S, Pupella S, et al. Human T-lymphotropic virus and transfusion safety: does one size fit all? *Transfusion (Paris)* 2016;56:249-60.
24. Stigum H, Magnus P, Samdal HH, et al. Human T-cell lymphotropic virus testing of blood donors in Norway: a cost-effect model. *Int J Epidemiol* 2000;29:1076-84.
25. Zou S, Stramer SL, Dodd RY. Donor testing and risk: current prevalence, incidence, and residual risk of transfusion-transmissible agents in US allogeneic donations. *Transfus Med Rev* 2012;26:119-28.
26. Styles CE, Seed CR, Hoad VC, et al. Reconsideration of blood donation testing strategy for human T-cell lymphotropic virus in Australia. *Vox Sang* 2017;112:723-32.
27. Vermeulen M, van den Berg K, Sykes W, et al. Health economic implications of testing blood donors in South Africa for HTLV 1 & 2 infection. *Vox Sang* 2019;114:467-77.
28. Carneiro-Proietti ABF, Sabino EC, Leão S, et al. Human T-lymphotropic virus type 1 and type 2 seroprevalence, incidence, and residual transfusion risk among blood donors in Brazil during 2007-2009. *AIDS Res Hum Retroviruses* 2012;28:1265-72.
29. O'Brien SF, Goldman M, Scalia V, et al. The epidemiology of human T-cell lymphotropic virus types I and II in Canadian blood donors. *Transfus Med* 2013;23:358-66.
30. Laperche S, Pillonel J. Relevance of safety measures to avoid HTLV transmission by transfusion in 2014. *Transfus. Clin. Biol. J. Soc. Francaise Transfus. Sang* 2014;21:167-72.
31. Seed CR, Margaritis AR, Bolton WV, et al. Improved efficiency of national HIV, HCV, and HTLV antibody testing algorithms based on sequential screening immunoassays. *Transfusion (Paris)* 2003;43:226-34.
32. Thorstensson R, Albert J, Andersson S. Strategies for diagnosis of HTLV-I and -II. *Transfusion (Paris)* 2002;42:780-91.
33. Hewitt PE, Davison K, Howell DR, et al. Human T-lymphotropic virus lookback in NHS Blood and Transplant (England) reveals the efficacy of leukoreduction. *Transfusion (Paris)* 2013;53:2168-75.
34. Manns A, Wilks RJ, Murphy EL, et al. A prospective study of transmission by transfusion of HTLV-I and risk factors associated with seroconversion. *Int J Cancer* 1992;51:886-91.
35. Kleinman S, Swanson P, Allain JP, et al. Transfusion transmission of human T-lymphotropic virus types I and II: serologic and polymerase chain reaction results in recipients identified through look-back investigations. *Transfusion* 1993;33:14-8.
36. Donegan E, Lee H, Operskalski EA, et al. Transfusion transmission of retroviruses: human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1. *Transfusion* 1994;34:478-83.
37. Bartholomew C, Jack N, Edwards J, et al. HTLV-I serostatus of mothers of patients with adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Hum Virol* 1998;1:302-5.
38. Percher F, Jeannin P, Martin-Latil S, et al. Mother-to-child transmission of HTLV-1 epidemiological aspects, mechanisms and determinants of mother-to-child transmission. *Viruses* 2016;8:40.
39. Nyambi PN, Ville Y, Louwagie J, et al. 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* 1996;12:187-92.
40. Hino S. Establishment of the milk-borne transmission as a key factor for the peculiar endemicity of human T-lymphotropic virus type 1 (HTLV-1): the ATL Prevention Program Nagasaki. *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87:152-66.
41. Prendergast AJ, Goga AE, Waitt C, et al. Transmission of CMV, HTLV-1, and HIV through breastmilk. *Lancet Child Adolesc Health* 2019;3:264-73.
42. Mojaat N, Kaabi H, Hmida S, et al. Seroprevalence of HTLV-I/II antibodies in blood donors and different groups at risk in Tunisia. *J Acquir Immune Defic Syndr* 1999;22:14-315. ■