

Systematic Review

Seroprevalence of HTLV-1 and HTLV-2 amongst mothers and children in Malawi within the context of a systematic review and meta-analysis of HTLV seroprevalence in Africa

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Abstract

OBJECTIVES Human T-lymphotropic virus (HTLV)-1 causes T-cell leukaemia and myelopathy. Together with HTLV-2, it is endemic in some African nations. Seroprevalence data from Malawi are scarce, with no reports on associated disease incidence. HTLV seroprevalence and type were tested in 418 healthy mothers from Malawi. In addition, we tested the sera of 534 children to investigate mother-to-child transmission. To provide context, we conducted a systematic review and meta-analysis of HTLV seroprevalence in African women and children.

METHODS Stored samples from a previous childhood cancer and BBV study were analysed. ELISA was used for HTLV screening followed by immunoblot for confirmation and typing. Standard methods were used for the systematic review.

RESULTS HTLV seroprevalence was 2.6% (11/418) in mothers and 2.2% (12/534) in children. Three mothers carried HTLV-1 alone, seven had HTLV-2 and one was dually infected. Three children carried HTLV-1 alone, seven had HTLV-2 and two were dually infected. Only two corresponding mothers of the 12 HTLV-positive children were HTLV positive. The systematic review included 66 studies of women and 13 of children conducted in 25 African countries. Seroprevalence of HTLV-1 varied from 0 to 17% and of HTLV-2 from 0 to 4%.

CONCLUSIONS In contrast to findings from other studies in Africa, the seroprevalence of HTLV-2 was higher than that of HTLV-1 in Malawi and one of the highest for the African region. The lack of mother-child concordance suggests alternative sources of infection among children. Our data and analyses contribute to HTLV prevalence mapping in Africa.

keywords Africa, Human T-lymphotropic virus, Malawi, prevalence, seroprevalence, mother-to-child transmission, MTCT, HTLV-1, HTLV-2, systematic review, meta-analysis, healthy women

Introduction

HTLV-1 is the causative agent of aggressive adult T-cell leukaemia/lymphoma (ATLL) and the progressive,

chronic, disabling HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) as well as other inflammatory conditions such as infective dermatitis and uveitis [1]. Surveillance data for prevalence of these two conditions are scarce and virtually unavailable for large areas such as China, India and many African countries. Even fewer data are available on prevalence and pathogenicity of HTLV-2, which is known to be endemic

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in African Pygmies [2] and Native Americans [3]. In the United States, HTLV-2 is more common than HTLV-1 and is associated with female sex, older age, Asian, Hispanic and African ethnicity, low level of education, a history of injecting drug use in the 1960s/1970s (a result of birth cohort effects) and residence in western and south-western USA, where HTLV-2 clusters are found [4]. There are case reports describing associations between HTLV-2 and a small increased risk of bacterial infections, particularly of the chest and bladder [5], increased cancer risk [6] and rare reports of HAM/TSP [7].

HTLV-1, an oncogenic human RNA retrovirus, was discovered 35 years ago [8], followed by the identification of HTLV-2, HTLV-3 and HTLV-4 [9–11]. These human viruses have arisen through inter-species transmission of simian T-lymphotropic viruses (STLV) [12]. HTLV-1 is found in endemic clusters and an estimated 10–20 million individuals, worldwide, are infected [2, 13]. Virus is transmitted from human-to-human through infected lymphocytes and may be acquired through mother-to-child transmission (MTCT): at birth and more commonly through breastfeeding, sexual intercourse, blood transfusions, organ transplantations and contaminated needle reuse.

The African continent has a population over 1 billion and represents the largest endemic area for HTLV infection but with many data gaps. HTLV seroprevalence has been reported only once in Malawi, at 2.5%, when 159 blood donor sera were screened by ELISA, without typing or confirmatory testing [14]. HTLV associated diseases have not been reported in Malawi, which may be due to limited diagnosis, lack of surveillance and poor survival.

This study investigated the prevalence of anti-HTLV-1 and HTLV-2 antibodies in stored sera from a previous childhood cancer study. We had access to the sera of 534 children diagnosed with cancer in Blantyre, Malawi and 418 paired healthy mothers. ELISA positive tests were confirmed and typed by immunoblotting. In addition, we applied context to our results by conducting a systematic review and meta-analysis of published HTLV seroprevalence data of African women and children.

Methodology

Study population

Anonymised stored serum samples from Malawian children ($n = 534$) and, where available, from their biological mothers ($n = 418$), were tested. Samples had been originally collected as part of a childhood malignancy and blood-borne virus (BBV) study at the Queen

Elizabeth Central Hospital in Blantyre, Malawi between 2006 and 2010 [15]. Their mothers were the children's healthy controls as part of this original study. Ethical approval for the study was obtained from the Oxford Tropical Research Ethics Committee and the Malawian College of Medicine Research and Ethics Committee. Written informed consent was obtained from mothers and the guardians of the children. Details of the original study are published elsewhere [15]. Demographic data were not available for all children (minimum $n = 174$, maximum $n = 276$) or mothers (minimum $n = 140$, maximum $n = 209$), and data were not available for all data points. Results are, therefore, presented as % of data available.

Screening, confirmation and typing of HTLV

Replicating clinical diagnostic algorithms, the study protocol required that all ELISA reactive samples underwent immunoblot (IB) confirmatory testing. Only indeterminate IBs were tested further with polymerase chain reaction (PCR), where whole blood was available. All sera were screened in duplicate by ELISA (MP Diagnostics HTLV-1/2 ELISA v4, antibodies against gp46-I, gp46-II, GD21) following the manufacturers' instructions (MP Biomedicals, Cambridge, Cambridgeshire, UK). ELISA reactive or borderline reactive sera were tested and typed as HTLV-1, HTLV-2, HTLV-1 and HTLV-2, negative and indeterminate using an FDA approved, confirmatory, qualitative enzyme immunoblot assay (IB), (MP Diagnostics HTLV blot v2.4), which were interpreted using the HTLV European Research Network guidelines [16, 17]. Both assays included HTLV-positive serum controls provided by the manufacturer. Where frozen whole blood was available, samples typed as indeterminate were further tested for the presence of HTLV DNA ($n = 18$). DNA was isolated from frozen whole blood of serologically indeterminate samples and its integrity was confirmed by optimised PCR amplification of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using commercially available primers. Samples were tested for the presence of HTLV-1 DNA by PCR as previously described [18].

Systematic review

PRISMA guidelines for systematic reviews and meta-analyses were adhered to during protocol design and analyses. The NCBI PubMed database was searched using the search terms 'HTLV', 'human T-lymphotropic virus', 'mother', 'child', 'transmission' plus each respective African nation to 31 December 2014.

The inclusion criteria were cross-sectional seroprevalence studies; healthy women (to match our cohort,

including blood donors, ante-natal clinic attendees, healthy volunteers); sex workers; children; living in Africa; opt-in, opt-out or anonymous un-linked (blood tests/other body fluid); approved by ethics; screened for HTLV; and confirmed with a confirmatory test (CT), and studies with zero seroprevalence by ELISA were included (Figure 1). The exclusion criteria were case reports; co-infection studies in an already known infected population; ill patients (hospitalised, patients with underlying diagnosis); no CT; men; seroprevalence by gender unspecified; patient self-reporting; and not in English language (Figure 1).

Applying the above criteria at stage 1 all potential papers' titles and abstracts were screened, and at stage 2 duplicates and papers not fitting all criteria were removed (Figure 1). Data were collected in Excel: location, year of publication, authors, study title, study characteristics, cohort gender, sample size, seroprevalence using ELISA, seroprevalence using confirmatory test and PCR confirmed. All citations on included papers were checked for any additional studies with one additional study identified not in the original PubMed searches (Figure 1). Data were screened and extracted by JF and checked by FM and MB. Any discordance was resolved through discussion.

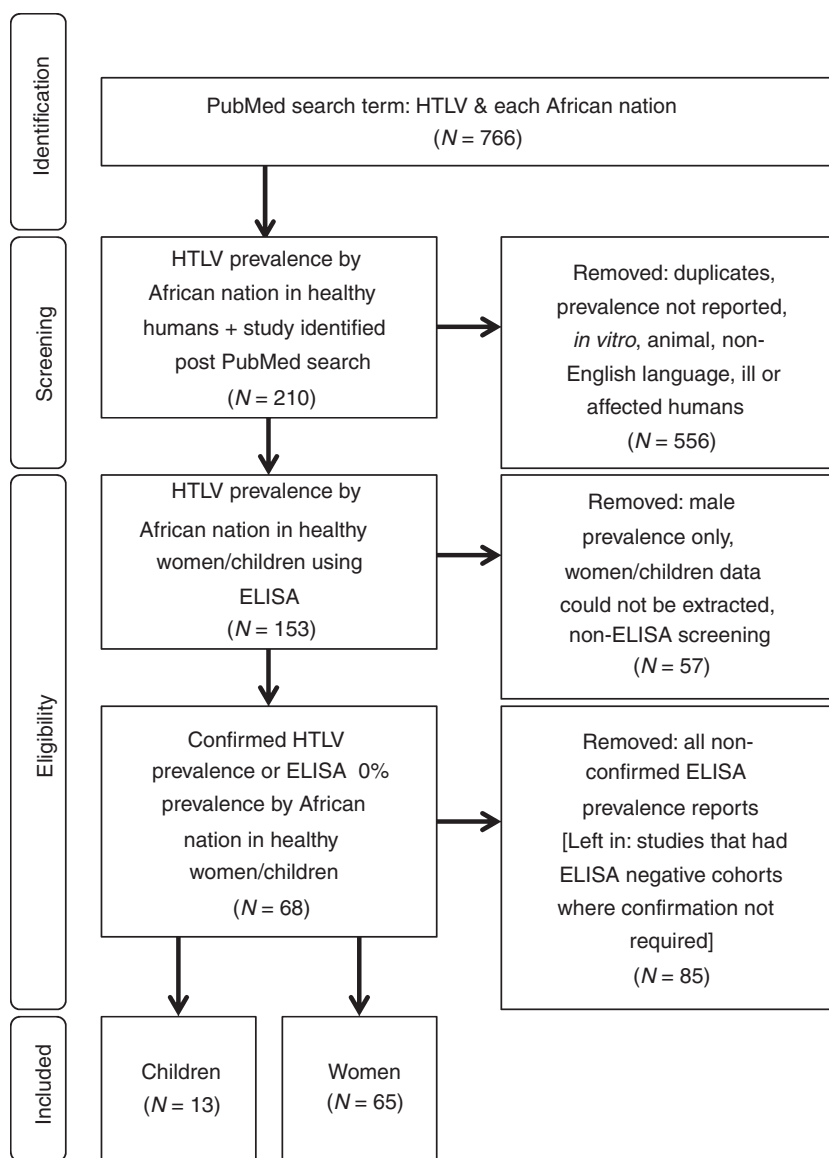


Figure 1 Flow chart of all manuscripts screened for prevalence of HTLV in healthy women and children living in Africa. Ten publications had data for both women and children.

Meta-analysis

The confidence intervals for individual studies were calculated by the exact binomial method and the combined estimate by random effects meta-analysis using the logistic transformation. Where there were no observed HTLV cases, giving zero seroprevalence, 0.5 was added to frequencies for the meta-analysis. There were much variability between studies; therefore, they all received similar weights in random effects meta-analysis. Forest plots were created from the results of the meta-analysis: squares represent the prevalence's reported for the individual studies, square areas being proportional to the weight the study received in the meta-analysis. Horizontal lines show the 95% confidence intervals for the prevalence. The diamond shapes represent the combined estimates for each region, the deepest point being the estimated combined prevalence and the width of the diamond representing the 95% confidence interval. The confidence intervals were calculated on the log odds scale and transformed back to the proportion scale, and so they are not symmetrical. The data presented here were incorporated into the final analyses to give regional HTLV seroprevalence estimates.

Results

Demographics

Several data points were collected as part of the original publication [15], and we summarised it to have an overall picture of the cohorts' socio-economical and health status; universal data were not available. 40% of children were female; mean age was 7.3 years (range 0.2–16). A total of 48% of children had Burkitt's, 12% neuroblastoma, none had ATLL. Only 12% of the mothers of the children had an illness during pregnancy, only 5.6% needed an intervention during delivery (caesarean section, stitches or assistance), 68% were delivered at home, only 4% were preterm deliveries. After birth, 98% were breastfed, 34% ≥ 2 years, 11% had blood transfusions, 51% had ever received an injection and 18% had a hospital admission before sampling; 81% had malaria in the past and 9% were HIV positive.

A total of 76% of mothers were married, 48% had >5 pregnancies, 45% had received injectable contraception, 36% reported to have had >1 partner, 9% reported having their first sexual intercourse during primary schooling and 34% during secondary school, 99% reported never having engaged in sex work, 94% never used condoms and 26% were HIV positive. A total of 81% of households had no electricity and 79% of children had to share plates with family members.

Screening for HTLV prevalence in Malawian women and children

Twenty-seven mothers' sera samples were repeatedly ELISA reactive and one was borderline reactive. Ten of 28 were HTLV-positive by IB of which two were HTLV-1 positive, seven HTLV-2 and one was positive for both anti-HTLV-1 and anti-HTLV-2 antibodies (Table 1). HTLV-1-specific DNA was amplified by PCR from whole blood from 1/18 IB indeterminate participants; no HTLV-1 or HTLV-2-specific DNA could be amplified from the remaining 17 indeterminate samples.

Therefore, the final HTLV prevalence in 418 healthy women was 2.6%: three mothers were typed HTLV-1 (0.7%) and seven HTLV-2 (1.7%) with one (0.2%) mother being dually infected (Table 1).

Of 534 children, 82 sera were ELISA reactive and 12 by IB; three were typed HTLV-1, seven HTLV-2 and two HTLV-1 and HTLV-2 (Table 1). Only two children of HTLV-positive mothers were also confirmed to be HTLV positive: the child of the HTLV-1 and HTLV-2 mother was HTLV-2 positive and the child of a mother with HTLV-1 was HTLV-1 and HTLV-2 positive. The mothers of the remaining 10 HTLV-positive children were all HTLV seronegative. Whole blood was not available for any child.

In summary, the HTLV seroprevalence in children was 2.2%: 0.6% for HTLV-1, 1.3% for HTLV-2 and 0.4% for HTLV-1/2 infection (Table 1).

Demographic data were not available for all the HTLV-positive cases. However, where data were available, none of the HTLV-positive children or mothers were HIV coinfecting. Otherwise they resembled the rest of the HTLV-negative cohort. Mothers were married, had never used condoms, had never engaged in sex work, had >5 pregnancies and only one had received injectable contraception. Children's mean age was 8 years (range: 2.5–13.4 years), had been born vaginally, had not been born prematurely, had been breastfed for >2 years. Only one child had received injections in the past but none had had a blood transfusion and only three had a history of malaria.

Table 1 HTLV seroprevalence in Malawi: screening of 418 mothers and 534 children and results of confirmatory testing of ELISA-positive samples

	N	HTLV-1 (%)	HTLV-2 (%)	HTLV-1 and HTLV-2 (%)	Total (%)
Mothers	418	3 (0.7)	7 (1.7)	1 (0.2)	11 (2.6)
Children	534	3 (0.6)	7 (1.3)	2 (0.4)	12 (2.2)

Systematic review and meta-analysis of HTLV prevalence in Africa

At stage 1, 766 PubMed publications on HTLV in African countries were identified. At stage 2, all publications that met the outlined inclusion/exclusion criteria were collected. Specific effort was made to extract data on women and children only from the manuscripts. Data were available for 24 countries for women and eight for children and were published between 1988 and 2014 for women and between 1988 and 1999 for children. Thirty-four studies had data for cohorts of healthy volunteers, three for blood donors, twenty-two for antenatal care (ANC) attendees and fifteen for sex workers. Mean sample size for women was 609 (range 26–2070) and 562 for children (range 46–1323). Some manuscripts gave data on multiple subgroups of study population therefore additional data sets were created. In the first instance, analysis was conducted on 74 data sets for women (Table 3, Figure 2) and 13 for children (Table 4, Figure 3) from a total of 68 publications, three of which contained data for children only (Figure 1, Table 2). Malawi findings were included in final analyses (Tables 3/4, Figures 2/3).

We analysed data by country and region (North, West and Central, East and Southern Africa) but deliberately refrained from calculating a prevalence estimate for the African continent (Table 3, Figure 2). For women, the highest seroprevalence of HTLV was reported in West and Central Africa (3.2%, 95%CI 2.6–4.0), which was also the source of the most studies (54/74 data sets, 13 countries). Limited data were available for children but West and Central Africa was again the most commonly studied region (10 of 13 data sets, 5 countries) with an estimated HTLV seroprevalence of 2.0% (95%CI 1.2–3.4) (Table 4, Figure 3). Both for women and children, the most commonly reported subtype was HTLV-1. HTLV-1 and HTLV-2 dual infections in healthy women and children were only reported in Nigeria, at 2.2% and 0.2%, respectively, and in sex workers at 1.67% (Table 2).

Assuming that sex workers may have an unusually high exposure risk we compared them to other women using logistic regression with robust standard errors allowing for the clustering in studies and adjusting for region. The odds ratio of HTLV for sex workers compared to all other women was 1.34 (95% CI 0.80–2.23, $P = 0.3$). Therefore, although they had a slightly higher risk of being positive for HTLV, this may have been due to chance variation; therefore, sex workers were not excluded from the analysis.

HTLV is more prevalent in sexually active and older women [2]. Analysis of HTLV by age stratification could

not be conducted as age was not available on all the mothers in our study; however, they were all sexually active and of reproductive age.

In light of the HTLV-2 seroprevalence in our Malawi study, we were interested to know the seroprevalence of HTLV-2 in Africa. HTLV-2 had been observed in 11 of 74 data sets for women with stark regional variability: Eritrea 2.1%, Cameroon 0.3% and 0.8%, Gabon 1.1% and 0.1%, Ghana 0.1%, Guinea-Bissau 0.1% (on two occasions), Nigeria 1.1% and 3.9% (Table 2). HTLV-2 was concentrated in women of Pygmy origin. Two of 13 studies reported HTLV-2 in children: Nigeria 0.5% and Gabon 0.3% (Table 2).

Focusing on countries neighbouring Malawi, HTLV-2 was not observed in four studies originating from Mozambique (combined total sample size: 1564) [19–22] nor in the only Zambian study [23]; publications from Tanzania did not report confirmatory testing and were excluded from the meta-analysis. Our observation of HTLV-2 at 1.7% in women and 1.3% in children was high in comparison with the rest of Africa as well as neighbouring countries, but similar to Nigeria and Eritrea.

Discussion

To our knowledge, this is the first report on the seroprevalence and type of HTLV in Malawi combining screening with robust confirmatory testing. We had access to stored sera of children with childhood cancers and their mothers as their controls who had taken part in a previous study [15]. HTLV seroprevalence was 2.6% in mothers and 2.2% in children. HTLV-2 was seen more commonly at 1.7% and 1.3% for mothers and children compared to 0.7% and 0.6% for HTLV-1, respectively. None of the HTLV-positive cases were hepatitis C [24] or HIV positive. High levels of HTLV intermediate screening results are commonly seen in African sera [25]; only one of the 18 tested indeterminate samples could be resolved by DNA testing. Ten of 12 HTLV-positive children had HTLV-negative mothers.

In this retrospective study, the mothers were of child-bearing age with known multiple pregnancies but we can only make assumptions about their HTLV acquisition. They could have acquired these viruses through: MTCT from their own mothers; unprotected sex; contact with non-human primates (bites/hunting/butchering, pets), use of unsterile instrumentation (female genital mutilation [FGM], tattooing/piercing) or contaminated needles; unscreened blood products or other means not yet characterised. Bush-meat hunting, heroin trafficking and FGM are prevalent in Malawi although not

Table 2 Systematic review of all included manuscripts reporting African HTLV prevalence in healthy women and children

Reference	Year	Nation	Region	Cohort	Size	Prevalence %			
						HTLV-1	HTLV-2	Dual	Total
Larouze <i>et al.</i> [42]	1985	Algeria	North	SW	140	0.00	0.00	0.00	0.00
Constantine <i>et al.</i> [43]	1991	Egypt	North	SW	158	0.00	0.00	0.00	0.00
Larouze <i>et al.</i> [42]	1985	Tunisia	North	HV	442	0.00	0.00	0.00	0.00
Fox <i>et al.</i> [44]	1988	Djibouti	East	SW	327	1.22	0.00	0.00	1.22
Constantine <i>et al.</i> [45]	1992	Djibouti	East	ANC	33	6.06	0.00	0.00	6.06
Andersson <i>et al.</i> [46]	1999	Eritrea	East	SW	97	0.00	2.06	0.00	2.06
				ANC	113	0.00	0.00	0.00	0.00
Ramos <i>et al.</i> [47]	2011	Ethiopia	East	ANC	165	0.00	0.00	0.00	0.00
Ramos <i>et al.</i> [48]	2012	Ethiopia	East	ANC	328	0.00	0.00	0.00	0.00
Scott <i>et al.</i> [49]	1991	Somalia	East	SW	57	0.00	0.00	0.00	0.00
Bayley <i>et al.</i> [23]	1985	Zambia	East	HV	132	0.00	0.00	0.00	0.00
Collenberg <i>et al.</i> [50]	2006	Burkina Faso	West	ANC	492	1.02	0.00	0.00	1.02
Dumas <i>et al.</i> [51]	1991	Benin	West	HV	1329	1.96	0.00	0.00	1.96
Houinato <i>et al.</i> [52]	1996	Benin	West	HV	853	1.29	0.00	0.00	1.29
Ndumbe <i>et al.</i> [53]	1992	Cameroon	West	ANC	170	0.59	0.00	0.00	0.59
Mauclere <i>et al.</i> [54]	1993	Cameroon	West	SW	391	0.26	0.77	0.00	1.02
Mauclere <i>et al.</i> [55]	1995	Cameroon	West	SW	332	0.90	0.30	0.00	1.20
Mauclere <i>et al.</i> [56]	1997	Cameroon	West	HV	1977	1.52	0.00	0.00	1.52
Filippone <i>et al.</i> [33]	2012	Cameroon	West	HV*	978	2.04	0.00	0.00	2.04
Gessain <i>et al.</i> [28]	1993	CAR	West	HV	689	0.29	0.00	0.00	0.29
Wiktor <i>et al.</i> [57]	1990	DR Congo	West	SW	377	3.18	0.00	0.00	3.18
Goubau <i>et al.</i> [58]	1993	DR Congo	West	HV	1956	2.15	0.00	0.00	2.15
Jeannel <i>et al.</i> [59]	1993	DR Congo	West	HV	352	0.85	0.00	0.00	0.85
Delaporte <i>et al.</i> [60]	1995	DR Congo	West	ANC	1160	3.71	0.00	0.00	3.71
				SW	1183	7.27	0.00	0.00	7.27
Tuppin <i>et al.</i> [61]	1996	DR Congo	West	ANC	2070	0.68	0.00	0.00	0.68
Schrijvers <i>et al.</i> [62]	1991	Gabon	West	HV	651	6.76	0.00	0.00	6.76
Delaporte <i>et al.</i> [63]	1993	Gabon	West	HV	30	16.67	0.00	0.00	16.67
Delaporte <i>et al.</i> [40]	1993	Gabon	West	ANC	434	7.14	0.00	0.00	7.14
Le Hesran <i>et al.</i> [64]	1994	Gabon	West	HV	505	13.07	0.00	0.00	13.07
Bertherat <i>et al.</i> [65]	1998	Gabon	West	HV	275	7.27	1.09	0.00	8.36
Moynet <i>et al.</i> [66]	2001	Gabon	West	HV	311	7.90	0.00	0.00	7.90
Etenna <i>et al.</i> [67]	2008	Gabon	West	ANC	907	2.09	0.11	0.00	2.21
Biggar <i>et al.</i> [68]	1993	Ghana	West	HV	1242	1.29	0.00	0.00	1.29
Apea-Kubi <i>et al.</i> [69]	2006	Ghana	West	ANC	294	2.72	0.00	0.00	2.72
Armah <i>et al.</i> [70]	2006	Ghana	West	ANC	960	2.08	0.10	0.00	2.19
Jeannel <i>et al.</i> [71]	1995	Guinea	West	HV	718	2.23	0.00	0.00	2.23
Naucler <i>et al.</i> [72]	1992	Guinea-Bissau	West	ANC	272	3.31	0.00	0.00	3.31
Norrgren <i>et al.</i> [73]	1995	Guinea-Bissau	West	HV	143	4.90	0.00	0.00	4.90
Andersson <i>et al.</i> [74]	1997	Guinea-Bissau	West	ANC	1231	2.19	0.08	0.00	2.27
Melbye <i>et al.</i> [75]	1998	Guinea-Bissau	West	HV	193	12.44	0.00	0.00	12.44
Larsen <i>et al.</i> [76]	2000	Guinea-Bissau	West	HV	1183	4.73	0.08	0.00	4.82
Holmgren <i>et al.</i> [77]	2002	Guinea-Bissau	West	HV	1605	5.79	0.00	0.00	5.79
Holmgren <i>et al.</i> [78]	2003	Guinea-Bissau	West	HV	816	10.17	0.00	0.00	10.17
Ariyoshi <i>et al.</i> [79]	2003	Guinea-Bissau	West	HV	105	10.48	0.00	0.00	10.48
Norrgren <i>et al.</i> [80]	2008	Guinea-Bissau	West	HV	1050	3.24	0.00	0.00	3.24
da Silva <i>et al.</i> [81]	2009	Guinea-Bissau	West	HV	1507	2.92	0.00	0.00	2.90
Ouattara <i>et al.</i> [82]	1989	Ivory Coast	West	HV	594	2.02	0.00	0.00	2.02
				SW	149	2.68	0.00	0.00	2.68
Verdier <i>et al.</i> [83]	1989	Ivory Coast	West	ANC	513	1.95	0.00	0.00	1.95
				SW	390	7.44	0.00	0.00	7.44
Dada <i>et al.</i> [84]	1993	Nigeria	West	SW	885	2.82	0.00	0.00	2.82

Table 2 (Continued)

Reference	Year	Nation	Region	Cohort	Size	Prevalence %			
						HTLV-1	HTLV-2	Dual	Total
Olaleye <i>et al.</i> [85]	1994	Nigeria	West	SW	60	3.33	3.33	1.67	8.33
Olaleye <i>et al.</i> [86]	1995	Nigeria	West	ANC	364	5.50	3.85	2.20	11.54
Olaleye <i>et al.</i> [39]	1999	Nigeria	West	HV	460	4.35	1.09	0.00	5.43
Eltom <i>et al.</i> [87]	2002	Nigeria	West	SW	863	3.24	0.00	0.00	3.24
Durojaiye <i>et al.</i> [88]	2014	Nigeria	West	HV	26	0.00	0.00	0.00	0.00
Okoye <i>et al.</i> [89]	2014	Nigeria	West	ANC	200	0.50	0.00	0.00	0.50
Larouze <i>et al.</i> [42]	1985	Senegal	West	HV	237	0.00	0.00	0.00	0.00
Diop <i>et al.</i> [90]	2006	Senegal	West	BD	1315	0.08	0.00	0.00	0.08
				ANC	178	1.78	0.00	0.00	1.78
Pepin <i>et al.</i> [91]	1991	The Gambia	West	SW	354	10.45	0.00	0.00	10.45
Del Mistro <i>et al.</i> [37]	1994	The Gambia	West	HV	909	1.21	0.00	0.00	1.21
Melo <i>et al.</i> [19]	2000	Mozambique	South	ANC	132	0.76	0.00	0.00	0.76
Cunha <i>et al.</i> [20]	2007	Mozambique	South	BD	576	1.22	0.00	0.00	1.22
Gudo <i>et al.</i> [21]	2009	Mozambique	South	BD	373	0.80	0.00	0.00	0.80
Caterino-de-Araujo <i>et al.</i> [22]	2010	Mozambique	South	HV	483	2.90	0.00	0.00	2.90
Steele <i>et al.</i> [92]	1994	Namibia	South	HV*	30	3.33	0.00	0.00	3.33
Botha <i>et al.</i> [93]	1985	South Africa	South	HV	211	5.21	0.00	0.00	5.21
				ANC	911	1.32	0.00	0.00	1.32
Bhigjee <i>et al.</i> [94]	1993	South Africa	South	HV	527	1.90	0.00	0.00	1.90
Goubau <i>et al.</i> [58]	1993	South Africa	South	ANC	428	0.23	0.00	0.00	0.23
Bhigjee <i>et al.</i> [95]	1994	South Africa	South	HV	197	2.54	0.00	0.00	2.54
Taylor <i>et al.</i> [96]	1996	South Africa	South	ANC	1259	0.56	0.00	0.00	0.56
Andersson <i>et al.</i> [46]	1999	Eritrea	East	C	161	0.00	0.00	0.00	0.00
Steele <i>et al.</i> [92]	1994	Namibia	South	C	244	0.82	0.00	0.00	0.82
Taylor <i>et al.</i> [96]	1996	South Africa	South	C*	1323	0.38	0.00	0.00	0.38
Jeannel <i>et al.</i> [59]	1993	DR Congo	West	C	715	1.40	0.00	0.00	1.40
Delaporte <i>et al.</i> [97]	1988	Gabon	West	C	684	2.19	0.00	0.00	2.19
Delaporte <i>et al.</i> [40]	1993	Gabon	West	C	610	2.79	0.00	0.00	2.79
Le Hesran <i>et al.</i> [64]	1994	Gabon	West	C	378	3.70	0.00	0.00	3.70
Nyambi <i>et al.</i> [38]	1996	Gabon	West	C	309	6.80	0.32	0.00	7.12
Verdier <i>et al.</i> [83]	1989	Ivory Coast	West	C	364	1.37	0.00	0.00	0.00
Williams <i>et al.</i> [98]	1993	Nigeria	West	C	46	6.52	0.00	0.00	6.52
Olaleye <i>et al.</i> [85]	1994	Nigeria	West	C	1081	0.74	0.46	0.19	1.39
Olaleye <i>et al.</i> [39]	1999	Nigeria	West	C	476	1.05	0.00	0.00	1.05
Del Mistro <i>et al.</i> [37]	1994	The Gambia	West	C	916	0.11	0.00	0.00	0.11

DR Congo, Democratic Republic of Congo; HV, healthy volunteers; HV*, bushwomen; BD, blood donor; ANC, antenatal clinic; SW, sex worker; C, children, Children*, younger than 72 months of age.

sufficiently surveyed [26]. To our knowledge, no publications examining IVDU in Malawi are available, but a systematic review of data on IVDU in African populations showed that IVDU was more prevalent in adult males (66–94% of IVDU use) and almost exclusively seen in female sex workers [26]. In our cohort, none of the HTLV-positive mothers reported sex work or drug usage or a partner who had engaged in IVDU. The Malawian blood transfusion service (MBTS) tests blood donations for HIV, HBV, HCV, syphilis and malaria but not for HTLV (personal communication with Malawian blood transfusion services). Prior to the establishment of the MBTS, one study

assessed the safety of blood donors in Malawi in 2001 [14]. Among 159 blood donations, the prevalence of HIV-1 infection was 10.7%, 8.1% for HBV carriage, 6.8% for anti-HCV and 2.5% for anti-HTLV-1.

The lack of concordance between mothers and their children remains unexplained; it might be an indication of horizontal transmission or the possibility that the child had been breastfed by someone other than their mother. The origin and route of transmission of the unusually high HTLV-2 seroprevalence found in our study participants remains unknown and prospective study would allow verification and identification of infection source.

Table 3 Prevalence of HTLV-1 and HTLV-2 in healthy adult women in four geographically defined African regions determined through meta-analysis of eligible publications defined by the systematic review

Region (sample number)	Countries (sample number)	Without Malawi data		With Malawi data	
		% seroprevalence	95% CI	% seroprevalence	95% CI
North (3)	Algeria (1), Egypt (1), Tunisia (1)	0.0	0.0–1.1		
West and Central (52)	Benin (2), Burkina Faso (1), Cameroon (5), Central Africa Republic (1), DR Congo (6), Gabon (7), Gambia (2), Ghana (3), Guinea-Bissau (10), Guinea (1), Ivory Coast (4), Nigeria (7), Senegal (3)	3.2	2.6–4.0		
East (8)	Djibouti (2), Eritrea (2), Ethiopia (2), Zambia (1), Somalia (1)	1.0	0.5–2.0		
East (9)	Djibouti (2), Eritrea (2), Ethiopia (2), Malawi (1), Zambia (1), Somalia (1)			1.2	0.6–2.3
Southern (11)	Mozambique (4), Namibia (1), South Africa (6)	1.6	1.0–2.5		

DR Congo, Democratic Republic of Congo.

Our systematic review of HTLV in women and children confirmed a distribution of HTLV in large areas of Africa (West, Central, East and Southern Africa). HTLV was not detected in healthy women in the three eligible studies from North Africa ($n = 740$); this region seems to have a low HTLV seroprevalence, although this should be verified with additional larger prevalence studies.

The prevalence of HTLV-1 in many areas of the world is poorly mapped and incidence of other subtypes is even

less well known. Africa is thought to be endemic for HTLV [2], but prevalence data are only partially available or studies have only been conducted using ELISA without confirmation or typing. East Africa is especially under surveyed and HTLV untyped. Malawi is known for its high HIV-1 prevalence (~11%) [27], but HTLV has only been reported in a single study with a reported seroprevalence of 2.5% in blood donors [14]. The overall HTLV seroprevalence in our study was thus very similar to that found in this previous report. As typing was not

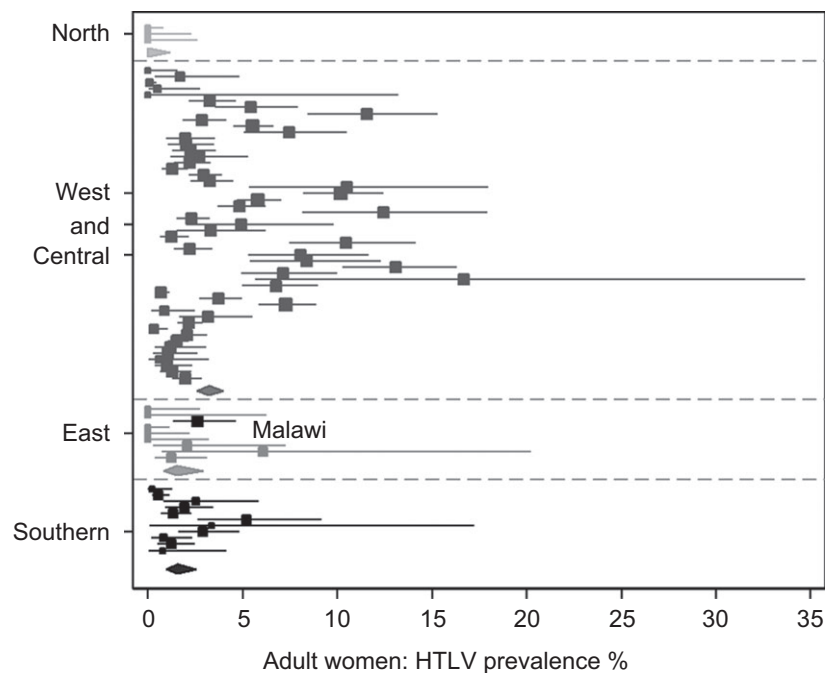


Figure 2 Forest plot showing prevalence of HTLV-1 and HTLV-2 among women, as reported in four regions of Africa, including data from Malawi. Squares represent each individual study prevalence with its area being proportional to the weight the study received in the meta-analysis and horizontal lines showing the 95% CI's. Diamonds represent the combined estimates for each region with its width representing the 95% CI.

Table 4 Prevalence of HTLV-1 and HTLV-2 in children in four geographically defined African regions determined through meta-analysis of eligible publications defined by the systematic review

Region (sample number)	Countries (sample number)	Without Malawi data		With Malawi data	
		% seroprevalence	95% CI	% seroprevalence	95% CI
North (0)					
West and Central (10)	DR Congo (1), Gabon (4), Gambia (1), Ivory Coast (1), Nigeria (3)	2.0	1.2–3.4		
East (1)	Eritrea (1)	0.3	0.0–4.7		
East (2)	Eritrea (1), Malawi (1)			1.3	0.2–7.1
Southern (2)	Namibia (1), South Africa (1)	0.5	0.2–1.0		

DR Congo, Democratic Republic of Congo.

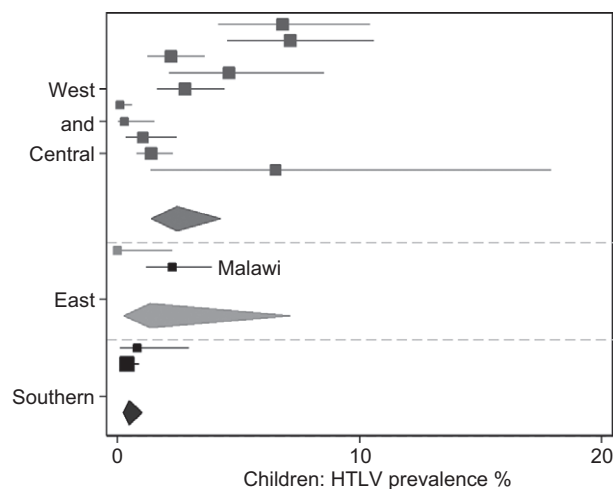


Figure 3 Forest plot showing prevalence of HTLV-1 and HTLV-2 among children, as reported in three regions of Africa, including data from Malawi. Squares represent each individual study prevalence with its area being proportional to the weight the study received in the meta-analysis and horizontal lines showing the 95% CIs. Diamonds represent the combined estimates for each region with its width representing the 95% CI.

performed in the study by Candotti *et al.*, it is possible that HTLV-2 was present in these blood donors.

In our systematic review only two other African countries reported high rates of HTLV-2 seroprevalence: Nigeria and Eritrea, about 2000 miles apart. HTLV-2 was not detected in Mozambique or Zambia, neighbouring countries of Malawi (Table 2). Worldwide, HTLV-2 is endemic in the Americas and associated with IVDU [4], but in Africa it is endemic in West and Central Africa, as confirmed by our systematic review [28–36]. None of the reviewed studies reported IVDU but mostly unprotected sex as the risk factor for HTLV acquisition.

Three manuscripts in our systematic review addressed HTLV MTCT [37–39], two of which also reported serodiscordance between mothers and their children. In the first of these studies, Del Mistro *et al.*, identified an HTLV-1 infected child, from a total of 916 children screened, whose mother had an indeterminate serological pattern and a negative HTLV PCR result [37]. Secondly, Olaleye *et al.*, reported five HTLV-1-positive children of 476 children from a total of 460 mothers in their study; none of the mothers of the infected children had HTLV [39]. The other interesting feature of this study was that none of the 20 HTLV-positive mothers had HTLV-positive children. Our hypotheses as to how these childhood infections could have occurred align with those of the authors of these reports: infection of children with negative mothers may possibly be a result of repeat blood transfusions for a variety of conditions, including sickle cell disease and malaria as well as usage of unsterilised body scarification instruments [39, 40]. We are aware that 18% of all the children in our study had historical hospital admission, in addition approximately 50% were hospital-born and 11% had received blood transfusions. However, to our knowledge, none of the HTLV-positive children in our study had received a blood transfusion. Breastfeeding is a potential virus transmission route if the children had been breastfed by an infected wet nurse other than their HTLV-discordant or HTLV-negative mother. We had no data on the sexual activity of the children but only one child was older than 13 years. Exposure to wildlife and primate bites are additional risk factors [41].

The strengths of this study were the large total and matched sample sizes, the combination of highly sensitive and specific screening and confirmation testing and the HTLV mapping of Malawi, an under surveyed country. The study has several limitations. We tested stored samples and, therefore, were unable to prospectively collect

demographic and risk factor data specific to HTLV seroprevalence studies. None of the HTLV-positive cases were HIV positive, which is interesting in itself considering that both share the same route of transmission. Samples were also a few years old which might have affected their reactivity. The cohort of children had cancer and antibody cross reactivity might have led to false positive results, although the highly specific confirmatory testing should negate this risk and HTLV infections were also found in the healthy women. Additional molecular testing of seropositive samples would have been ideal but we did not have access to the required DNA.

In our systematic review, we included data only on healthy women to best match our cohort of women and reduce co-infection/co-morbidity bias. There were very few published reports of childhood HTLV and we excluded those who were known HIV positive. We believe data from the 1980s are valuable, although an overestimation of HTLV prevalence is possible. Furthermore, because African HTLV seroprevalence studies are limited in their number, we did not exclude any samples based on study size but calculated regional estimates rather than national estimates in an attempt to reduce dependence upon studies with small cohorts. Inherent bias from small studies and those from the 1980s was reduced by including only publications that had used serological confirmatory testing. We used appropriate study weighting based on cohort size in our analyses and regional seroprevalence estimates.

In summary, our study highlights that HTLV is prevalent in Malawi. However, neither ATLL nor HAM/TSP has been reported so far. These HTLV-1-associated diseases are difficult to diagnose with high risk of mortality from ATLL before diagnosis has been made. They are, therefore, notoriously underreported unless specifically looked for. In addition, HTLV-2 is not as pathogenic as HTLV-1 and will be transmitted unknowingly from generation to generation.

Prospective field studies of HTLV as well as its associated diseases are needed in Malawi to allow health policy makers to develop practical and sustainable health policies with the long-term aim of eradicating HTLV.

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References

1. Martin F, Taylor GP, Jacobson S. Inflammatory manifestations of HTLV-1 and their therapeutic options. *Exp Rev Clin Immunol* 2014; **10**: 1531–1546.
2. Gessain A, Cassar O. Epidemiological Aspects and World Distribution of HTLV-1 Infection. *Front Microbiol* 2012; **3**: 388.
3. Roucoux DF, Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus type II. *AIDS Rev* 2004; **6**: 144–154.
4. Chang YB, Kaidarova Z, Hinds D *et al.* Seroprevalence and demographic determinants of human T-lymphotropic virus type 1 and 2 infections among first-time blood donors—United States, 2000–2009. *J Infect Dis* 2014; **209**: 523–531.
5. Murphy EL, Wang B, Sacher RA *et al.* Respiratory and urinary tract infections, arthritis, and asthma associated with HTLV-I and HTLV-II infection. *Emerg Infect Dis* 2004; **10**: 109–116.
6. Biswas HH, Kaidarova Z, Garratty G *et al.* Increased all-cause and cancer mortality in HTLV-II infection. *J Acquir Immune Defic Syndr* 2010; **54**: 290–296.
7. Araujo A, Hall WW. Human T-lymphotropic virus type II and neurological disease. *Ann Neurol* 2004; **56**: 10–19.
8. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. 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* 1980; **77**: 7415–7419.
9. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 1982; **218**: 571–573.
10. Calattini S, Chevalier SA, Duprez R *et al.* Discovery of a new human T-cell lymphotropic virus (HTLV-3) in Central Africa. *Retrovirology* 2005; **2**: 30.
11. Wolfe ND, Heneine W, Carr JK *et al.* Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. *Proc Natl Acad Sci U S A* 2005; **102**: 7994–7999.
12. Mahieux R, Gessain A. HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. *Viruses* 2011; **3**: 1074–1090.
13. de-The G, Bomford R. An HTLV-I vaccine: why, how, for whom? *AIDS Res Hum Retroviruses* 1993; **9**: 381–386.
14. Candotti D, Mundy C, Kadeweile G, Nkhoma W, Bates I, Allain JP. Serological and molecular screening for viruses in blood donors from Ntcheu, Malawi: high prevalence of HIV-1 subtype C and of markers of hepatitis B and C viruses. *J Med Virol* 2001; **65**: 1–5.
15. Mutalima N, Molyneux E, Jaffe H *et al.* Associations between Burkitt lymphoma among children in Malawi and infection with HIV, EBV and malaria: results from a case-control study. *PLoS ONE* 2008; **3**: e2505.

16. Seroepidemiology of the human T-cell leukaemia/lymphoma viruses in Europe. THER Network. *J Acquir Immune Defic Syndr* 1996;13:68–77.
17. Food and Drug Administration US. FDA approves first test to confirm the presence of Human T-cell Lymphotropic Virus-I/II antibodies, 2014 [cited 2015 22-April-2015]. (Available from: <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm426605.htm>) [11-Dec-2014].
18. Vandamme AM, Van Laethem K, Liu HF *et al.* Use of a generic polymerase chain reaction assay detecting human T-lymphotropic virus (HTLV) types I, II and divergent simian strains in the evaluation of individuals with indeterminate HTLV serology. *J Med Virol* 1997; 52: 1–7.
19. Melo J, Beby-Defaux A, Faria C *et al.* HIV and HTLV prevalences among women seen for sexually transmitted diseases or pregnancy follow-up in Maputo, Mozambique. *J Acquir Immune Defic Syndr* 2000; 23: 203–204.
20. Cunha L, Plouzeau C, Ingrand P *et al.* Use of replacement blood donors to study the epidemiology of major blood-borne viruses in the general population of Maputo, Mozambique. *J Med Virol* 2007; 79: 1832–1840.
21. Gudo ES, Abreu CM, Mussa T *et al.* Serologic and molecular typing of human T-lymphotropic virus among blood donors in Maputo City, Mozambique. *Transfusion* 2009; 49: 1146–1150.
22. Caterino-de-Araujo A, Magri MC, Costa EA, Manuel RC. Prevalence of human T-cell lymphotropic virus (HTLV-1/2) in individuals from public health centers in Mozambique. *AIDS Res Hum Retroviruses* 2010; 26: 559–561.
23. Bayley AC, Downing RG, Cheingsong-Popov R, Tedder RS, Dalgleish AG, Weiss RA. HTLV-III serology distinguishes atypical and endemic Kaposi's sarcoma in Africa. *Lancet* 1985; 1: 359–361.
24. Fox JM, Newton R, Bedaj M *et al.* Prevalence of hepatitis C virus in mothers and their children in Malawi. *Trop Med Int Health* 2015; 20: 638–642.
25. Mahieux R, Horal P, Mauclere P *et al.* Human T-cell lymphotropic virus type 1 gag indeterminate western blot patterns in Central Africa: relationship to *Plasmodium falciparum* infection. *J Clin Microbiol* 2000; 38: 4049–4057.
26. Reid SR. Injection drug use, unsafe medical injections, and HIV in Africa: a systematic review. *Harm Reduct J* 2009; 6: 24.
27. National Statistical Office. Malawi Demographic and Health Survey 2010. NSO and ICF Macro, Zomba, Malawi, and Calverton, Maryland, USA (2011). 2010: 2011.
28. Gessain A, Herve V, Jeannel D, Garin B, Mathiot C & de-The G. HTLV-1 but not HTLV-2 found in pygmies from Central African Republic. *J Acquir Immune Defic Syndr* 1993;6:1373–1374.
29. Goubau P, Liu HF, De Lange GG, Vandamme AM, Desmyter J. HTLV-II seroprevalence in pygmies across Africa since 1970. *AIDS Res Hum Retroviruses* 1993; 9: 709–713.
30. Froment A, Delaporte E, Dazza MC, Larouze B. HTLV-II among pygmies from Cameroon. *AIDS Res Hum Retroviruses* 1993; 9: 707.
31. Ndumbe PM, Atchou G, Biwole M, Lobe V, Ayuk-Takem J. Infections among pygmies in the Eastern Province of Cameroon. *Med Microbiol Immunol* 1993; 182: 281–284.
32. Mauclere P, Afonso PV, Meertens L *et al.* HTLV-2B strains, similar to those found in several Amerindian tribes, are endemic in central African Bakola Pygmies. *J Infect Dis* 2011; 203: 1316–1323.
33. Filippone C, Bassot S, Betsem E *et al.* A new and frequent human T-cell leukemia virus indeterminate Western blot pattern: epidemiological determinants and PCR results in central African inhabitants. *J Clin Microbiol* 2012; 50: 1663–1672.
34. Goubau P, Desmyter J, Ghesquiere J, Kasereka B. HTLV-II among pygmies. *Nature* 1992; 359: 201.
35. Goubau P, Vandamme A, Beuselinc K, Desmyter J. Proviral HTLV-I and HTLV-II in the Efe pygmies of northeastern Zaire. *J Acquir Immune Defic Syndr* 1996; 12: 208–209.
36. Gessain A, Fretz C, Koulibaly M *et al.* Evidence of HTLV-II infection in Guinea, West Africa. *J Acquir Immune Defic Syndr* 1993; 6: 324–325.
37. Del Mistro A, Chotard J, Hall AJ *et al.* HTLV-I/II seroprevalence in The Gambia: a study of mother-child pairs. *AIDS Res Hum Retroviruses* 1994; 10: 617–620.
38. 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* 1996; 12: 187–192.
39. Olaleye DO, Omotade OO, Sheng Z, Adeyemo AA, Odaibo GN. Human T-cell lymphotropic virus types I and II infections in mother-child pairs in Nigeria. *J Trop Pediatr* 1999; 45: 66–70.
40. 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: 424–428.
41. Kazanji M, Mouinga-Ondeme A, Lekana-Douki-Etenna S, Caron M, Makuwa M, Mahieux R *et al.* Origin of HTLV-1 in hunters of nonhuman primates in Central Africa. *J Infect Dis* 2015; 211: 361–365.
42. Larouze B, Schaffar-Deshayes L, Blesonski S *et al.* Antibodies to HTLV-I p24 in African and Portuguese populations. *Cancer Res* 1985; 45 (Suppl.): 4630s–4632s.
43. Constantine NT, Fathi SM, Corwin AL, Danahy RS, Callahan JD & Watts DM. A serosurvey for HTLV-I among high-risk populations and normal adults in Egypt. *Epidemiol Infect* 1991;107:429–433.
44. Fox E, Constantine NT, Abbatte EA, Said S, Rodier G. Low prevalence of infection by HTLV-I in populations at risk for HIV in Djibouti. *Ann Inst Pasteur Virol* 1988; 139: 443–447.
45. Constantine NT, Fox E, Rodier G, Abbatte EA. Monitoring for HIV-1, HIV-2, HTLV-I sero-progression and sero-conversion in a population at risk in east Africa. *J Egypt Public Health Assoc* 1992; 67: 535–547.
46. Andersson S, Tessema HG, Wahren B. Is there a focus of HTLV-II infections in the Horn of Africa? *J Acquir Immune Defic Syndr* 1999; 21: 353–354.

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47. Ramos JM, Toro C, Reyes F, Amor A, Gutierrez F. Seroprevalence of HIV-1, HBV, HTLV-1 and *Treponema pallidum* among pregnant women in a rural hospital in Southern Ethiopia. *J Clin Virol* 2011; 51: 83–85.
48. Ramos JM, Belda S, Reyes F, Rodriguez JC, Royo G, Gutierrez F. Prevalence of HIV, HBV, HCV, HTLV and *Treponema pallidum* among patients attending a rural hospital in Southern Ethiopia. *J Clin Virol* 2012; 53: 268–269.
49. Scott DA, Corwin AL, Constantine NT *et al.* Low prevalence of human immunodeficiency virus-1 (HIV-1), HIV-2, and human T cell lymphotropic virus-1 infection in Somalia. *Am J Trop Med Hyg* 1991; 45: 653–659.
50. Collenberg E, Ouedraogo T, Ganame J *et al.* Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: A comparative analysis. *J Med Virol* 2006; 78: 683–692.
51. Dumas M, Houinato D, Verdier M *et al.* Seroepidemiology of human T-cell lymphotropic virus type III in Benin (West Africa). *AIDS Res Hum Retroviruses* 1991; 7: 447–451.
52. Houinato D, Verdier M, Josse R *et al.* Seroepidemiological study of retroviruses (HTLV-I/II, HIV-1, HIV-2) in the Department of Atacora, northern Benin. *Trop Med Int Health* 1996; 1: 205–209.
53. Ndumbe PM, Okie F, Nyambi P, Delaporte E, de The G. Retrovirus infections in the south of Cameroon. *Ann Soc Belg Med Trop* 1992; 72: 141–144.
54. Mauclere P, Gessain A, Garcia-Calleja JM *et al.* HTLV-II in African prostitutes from Cameroon. *Aids* 1993; 7: 1394–1395.
55. Mauclere P, Mahieux R, Garcia-Calleja JM *et al.* A new HTLV type II subtype A isolate in an HIV type 1-infected prostitute from Cameroon. *Central Africa. AIDS Res Hum Retroviruses* 1995; 11: 989–993.
56. Mauclere P, Le Hesran JY, Mahieux R *et al.* 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* 1997; 176: 505–509.
57. Wiktor SZ, Piot P, Mann JM *et al.* Human T cell lymphotropic virus type I (HTLV-I) among female prostitutes in Kinshasa, Zaire. *J Infect Dis* 1990; 161: 1073–1077.
58. Goubau P, Desmyter J, Swanson P *et al.* Detection of HTLV-I and HTLV-II infection in Africans using type-specific envelope peptides. *J Med Virol* 1993; 39: 28–32.
59. Jeannel D, Garin B, Kazadi K, Singa L, de The G. The risk of tropical spastic paraparesis differs according to ethnic group among HTLV-I carriers in Inongo, Zaire. *J Acquir Immune Defic Syndr* 1993; 6: 840–844.
60. Delaporte E, Buve A, Nzila N *et al.* HTLV-I infection among prostitutes and pregnant women in Kinshasa, Zaire: how important is high-risk sexual behavior? *J Acquir Immune Defic Syndr* 1995; 8: 511–515.
61. Tuppin P, Makuwa M, Guerma T *et al.* Low HTLV-I/II seroprevalence in pregnant women in Congo and a geographic cluster of an HTLV-like indeterminate western blot pattern. *J Acquir Immune Defic Syndr* 1996; 11: 105–107.
62. Schrijvers D, Delaporte E, Peeters M, Dupont A, Meheus A. Seroprevalence of retroviral infection in women with different fertility statuses in Gabon, western equatorial Africa. *J Acquir Immune Defic Syndr* 1991; 4: 468–470.
63. Delaporte E, Klotz F, Peeters M *et al.* Non-Hodgkin lymphoma in Gabon and its relation to HTLV-I. *Int J Cancer* 1993; 53: 48–50.
64. Le Hesran JY, Delaporte E, Gaudebout C *et al.* Demographic factors associated with HTLV-1 infection in a Gabonese community. *Int J Epidemiol* 1994; 23: 812–817.
65. Bertherat E, Makuwa M, Renaut A, Nabias R, Georges-Courbot MC. HIV-1, HTLV-I, and HTLV-II in a semiurban population in East Gabon. *J Acquir Immune Defic Syndr* 1998; 19: 430–432.
66. Moynet D, Pouliquen JF, Londres-Gagliardi D *et al.* High variability of HTLV-I in a remote population of Gabon as compared to that of a similar population of French Guiana. *Virus Genes* 2001; 23: 257–261.
67. Etenna SL, Caron M, Besson G, Makuwa M, Gessain A, Mahe A *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: 3607–3614.
68. Biggar RJ, Neequaye JE, Neequaye AR *et al.* The prevalence of antibodies to the human T lymphotropic virus (HTLV) in Ghana, West Africa. *AIDS Res Hum Retroviruses* 1993; 9: 505–511.
69. Apea-Kubi KA, Yamaguchi S, Sakyi B, Ofori-Adjei D. HTLV-1 and other viral sexually transmitted infections in antenatal and gynaecological patients in Ghana. *West Afr J Med* 2006; 25: 17–21.
70. Armah HB, Narter-Olaga EG, Adjei AA, Asomaning K, Gyasi RK, Tettey Y. Seroprevalence of human T-cell lymphotropic virus type I among pregnant women in Accra, Ghana. *J Med Microbiol* 2006; 55 (Pt 6): 765–770.
71. Jeannel D, Kourouma K, Fretz C *et al.* Regional differences in human retroviral infections HIV-1, HIV-2, and HTLV-I/II in rural Guinea (West Africa). *J Acquir Immune Defic Syndr* 1995; 8: 315–318.
72. Naucner A, Andersson S, Albino P, Paolo DSA, Andreasson PA & Biberfeld G. Association between HTLV-1 and HIV-2 infections in Bissau, Guinea-Bissau. *Aids* 1992;6:510–511.
73. Norrgren H, Andersson S, Naucner A, Dias F, Johansson I, Biberfeld G. HIV-1, HIV-2, HTLV-I/II and *Treponema pallidum* infections: incidence, prevalence, and HIV-2-associated mortality in an occupational cohort in Guinea-Bissau. *J Acquir Immune Defic Syndr* 1995; 9: 422–428.
74. Andersson S, Dias F, Mendez PJ, Rodrigues A, Biberfeld G. HTLV-I and -II infections in a nationwide survey of pregnant women in Guinea-Bissau, West Africa. *J Acquir Immune Defic Syndr* 1997; 15: 320–322.
75. Melbye M, Poulsen AG, Gallo D *et al.* HTLV-1 infection in a population-based cohort of older persons in Guinea-Bis-

- sau, West Africa: risk factors and impact on survival. *Int J Cancer* 1998; **76**: 293–298.
76. Larsen C, Pialoux G, Salmon D *et al.* Prevalence of hepatitis C and hepatitis B infection in the HIV-infected population of France, 2004. *Euro Surveill* 2008; **13**: pii: 18888.
 77. Holmgren B, Andersson S, Harding E *et al.* Increased prevalence of HTLV-1 among HIV-2-infected women but not HIV-2-infected men in rural Guinea-Bissau. *J Acquir Immune Defic Syndr* 2002; **30**: 342–350.
 78. Holmgren B, da Silva Z, Larsen O, Vastrup P, Andersson S, Aaby P. Dual infections with HIV-1, HIV-2 and HTLV-I are more common in older women than in men in Guinea-Bissau. *Aids* 2003; **17**: 241–253.
 79. Ariyoshi K, Berry N, Cham F *et al.* Quantification of Human T-lymphotropic virus type I (HTLV-I) provirus load in a rural West African population: no enhancement of human immunodeficiency virus type 2 pathogenesis, but HTLV-I provirus load relates to mortality. *J Infect Dis* 2003; **188**: 1648–1651.
 80. Norrgren HR, Bamba S, Larsen O *et al.* Increased prevalence of HTLV-1 in patients with pulmonary tuberculosis coinfecting with HIV, but not in HIV-negative patients with tuberculosis. *J Acquir Immune Defic Syndr* 2008; **48**: 607–610.
 81. da Silva ZJ, Nielsen J, Andersen A *et al.* Decline in human T-cell lymphotropic virus-1 prevalence in urban areas of Bissau, Guinea-Bissau: exploring the association with HIV infections. *Aids* 2009; **23**: 637–639.
 82. Ouattara SA, Gody M & de-The G. Prevalence of HTLV-I compared to HIV-1 and HIV-2 antibodies in different groups in the Ivory Coast (West Africa). *J Acquir Immune Defic Syndr*. 1989; **2**: 481–485.
 83. Verdier M, Denis F, Sangare A *et al.* Prevalence of antibody to human T cell leukemia virus type 1 (HTLV-1) in populations of Ivory Coast, West Africa. *J Infect Dis* 1989; **160**: 363–370.
 84. Dada AJ, Oyewole F, Onofowokan R *et al.* Demographic characteristics of retroviral infections (HIV-1, HIV-2, and HTLV-I) among female professional sex workers in Lagos, Nigeria. *J Acquir Immune Defic Syndr* 1993; **6**: 1358–1363.
 85. Olaleye DO, Bernstein L, Sheng Z *et al.* Type-specific immune response to human T cell lymphotropic virus (HTLV) type I and type II infections in Nigeria. *Am J Trop Med Hyg* 1994; **50**: 479–486.
 86. Olaleye DO, Ekweozor CC, Sheng Z, Rasheed S. Evidence of serological cross-reactivities with human immunodeficiency virus types 1 and 2 and human T-lymphotropic virus types I and II in sera of pregnant women in Ibadan, Nigeria. *Int J Epidemiol* 1995; **24**: 198–203.
 87. Eltom MA, Mbulaiteye SM, Dada AJ, Whitby D, Biggar RJ. Transmission of human herpesvirus 8 by sexual activity among adults in Lagos, Nigeria. *Aids* 2002; **16**: 2473–2478.
 88. Durojaiye I, Akinbami A, Dosunmu A *et al.* Seroprevalence of human T lymphotropic virus antibodies among healthy blood donors at a tertiary centre in Lagos, Nigeria. *Pan Afr Med J* 2014; **17**: 301.
 89. Okoye AE, Ibegbulam OG, Onoh RC *et al.* Seroprevalence and correlates of human T-cell lymphoma/leukemia virus type 1 antibodies among pregnant women at the University of Nigeria Teaching Hospital, Enugu, Nigeria. *Int J Womens Health* 2014; **6**: 849–855.
 90. Diop S, Calattini S, Abah-Dakou J, Thiam D, Diakhate L, Gessain A. 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**: 1550–1554.
 91. Pepin J, Dunn D, Gaye I *et al.* HIV-2 infection among prostitutes working in The Gambia: association with serological evidence of genital ulcer diseases and with generalized lymphadenopathy. *Aids* 1991; **5**: 69–75.
 92. Steele AD, Bos P, Joubert JJ *et al.* Low prevalence of human T lymphotropic virus type I in !Kung San in Bushmanland, Namibia. *Am J Trop Med Hyg* 1994; **51**: 460–465.
 93. Botha MC, Jones M, de Klerk WA, Yamamoto N. Distribution and possible spread of human T-cell leukaemia virus type I in human communities in the northern and eastern Transvaal. *S Afr Med J* 1985; **67**: 668–671.
 94. Bhigjee AI, Vinsen C, Windsor IM, Gouws E, Bill PL, Tait D. Prevalence and transmission of HTLV-I infection in Natal/KwaZulu. *S Afr Med J* 1993; **83**: 665–667.
 95. Bhigjee AI, Thaler D, Madurai S, Gouws E, Bill PL. Seroprevalence of HTLV-I in Natal/KwaZulu. *S Afr Med J* 1994; **84**: 368.
 96. Taylor MB, Parker SP, Crewe-Brown HH, McIntyre J, Cubitt WD. Seroprevalence of HTLV-I in relation to that of HIV-1 in the Gauteng region, South Africa, using dried blood spots on filter papers. *Epidemiol Infect* 1996; **117**: 343–348.
 97. Delaporte E, Dupont A, Peeters M *et al.* Epidemiology of HTLV-I in Gabon (Western Equatorial Africa). *Int J Cancer* 1988; **42**: 687–689.
 98. Williams CK, Alexander SS, Bodner A *et al.* Frequency of adult T-cell leukaemia/lymphoma and HTLV-I in Ibadan, Nigeria. *Br J Cancer* 1993; **67**: 783–786.

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