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Original Article

Seroreactivity clarification and viral load quantitation in HIV-1 and HIV-2 infections in Ghana

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In Ghana, West Africa, the prevalence of dual HIV-1 and HIV-2 infections remains to be clarified, and HIV viral load measurement is yet to be established. Conventional assays for HIV-1 RNA measurements have been limited specifically to HIV-1 subtype B, preventing their utilization for Ghana where HIV-1 subtypes A, D and G are prevalent. Therefore, we set out to distinguish the types of HIV infection existing in Ghana so as to determine the extent of actual dual infections, and to measure plasma HIV-1 RNA. Blood samples were collected from 563 sick and healthy Ghanaians who visited hospitals in 1996 and 1997. After T cells were counted, HIV antibody was screened and confirmed by six different commercial assays and one in-house test. Nested PCR was then used to verify HIV-1 and HIV-2 presence by type-specific primers. Plasma HIV-1 RNA was measured by an improved commercial RT-PCR assay, sensitive to all HIV-1 group M subtypes. HIV-1 alone (89%) clearly dominated over HIV-2 alone (2%), and HIV-1 and HIV-2 dual infections were found in 9%. Valid viral load measurements were obtained on test plasma representing the main HIV-1 subtype (A) prevailing in Ghana. A high amount of HIV-1 RNA (5.9 mean $\log_{10}$ RNA copies/ml) was observed in the typical stages of HIV infection represented by groups of $CD4^+$ cell counts. We have clarified the seroprevalence of HIV-1 and HIV-2 amongst HIV seropositives, and the high viral load of HIV-1 reflects its influence on AIDS in Ghana.

Key words: HIV-1; HIV-2; dual infection; serology; high viral load

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Introduction

Human immunodeficiency virus (HIV) infections are sometimes complicated by the co-infection with both HIV-1 and HIV-2, because of the similarities in viral polypeptides and disease presentation[1]. The sensitivity and specificity of HIV-1 serological assays can also be affected by the presence of HIV-2 and vice versa[2].

In Ghana, West Africa, HIV-1 has reportedly become predominant over HIV-2 with the occurrence of dual infections[3]. A serological survey 10 years earlier indicated that HIV-2 prevailed over HIV-1[4]. In such other West African countries as Cote d'Ivoire, Nigeria, and The Gambia, HIV-1 and HIV-2 infections are common, and dual infection has been recently confirmed[5,6]. Interestingly, protection from HIV-1 infection by HIV-2 was reported in some parts of West Africa[7] and has become a controversy[8]. Recent serological studies in Ghana identified dual HIV-1 and HIV-2 infections; however, discordant results prevented the establishment of actual prevalence levels[9,10,11].

Polymerase chain reaction (PCR) has become an established tool for diagnostic confirmation of HIV, and highly specific primers have been developed for African HIV-1 isolates[12]. Analyses of Ghanaian HIV-2 isolates have provided important information about the HIV-2 genome[13,14,15], and this has been utilized in PCR primer design[5].

We therefore set out to establish a clear serological diagnostic system with confirmation by PCR. Our aim was to clarify HIV antibody type reactivity and the actual extent of dual infection in Ghana.

Plasma HIV RNA measurements are now an important diagnostic marker of disease progression and patient management[16] worldwide. However, failure to measure HIV-1 subtypes A and G has been reported for some commercial assays[17,18]. This had serious implications, because in Ghana, HIV-1 subtype A mainly prevails, with subtypes D and G circulating to a lesser extent[19].

We therefore employed an improved version reverse transcriptase PCR (RT-PCR) assay, sensitive to all the major (group M) HIV-1 subtypes, to assess HIV-1 RNA levels and thus provide baseline HIV-1 viral load data for Ghana.

Materials and Methods

Study group

A cross-sectional study was conducted on 563 persons in attendance at 8 hospitals in Southern Ghana from early 1996 to late 1997, after informed consent was obtained. These consisted of patients with acquired immunodeficiency syndrome (AIDS), sexually transmitted diseases, and others with previously detected HIV infection. Healthy individuals requiring HIV testing for various purposes were also included. The expanded WHO case definition for AIDS surveillance[20] was adopted for health status evaluation of the study subjects.

Sample collection & processing

Blood volumes of about 10 ml were collected at the various hospitals with heparin or ethylenediaminetetraacetic acid disodium (EDTA-2Na) as anti-coagulant and transported at 4℃ within 12 hours to the Virology laboratory, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, for processing. Two hundred microliters of whole blood samples were removed for T cell counts. Remaining blood was separated into plasma and peripheral blood lymphocytes (PBL) and then stored at −70℃. Plasma and PBL samples were later transported under dry ice to the Department of Microbiology, Tokyo Medical & Dental University for further analyses.

T cell counts

To determine the immune status of the study subjects, the absolute numbers of $CD4^+$ and $CD8^+$ T lymphocytes were measured by a FACSCount system (Becton Dickinson, CA). One hundred microliters of unlysed whole blood was applied within 24 hours after collection, according to the supplied protocol. This resulted in binding with specific fluorescein isothiocynate (FITC)-labeled antibodies against $CD3^+$, $CD4^+$ and $CD8^+$ (Becton Dickinson, CA). The FACSCount instrument then read absolute counts for these T cell subsets.

Serological analyses

Plasma samples were tested for HIV-1 and HIV-2 antibodies by a three-step serological system that comprised six commercial assays and one in-house assay as indicated in Table 1.

In the first screening step, the samples were titrated on two particle agglutination assays (PA), Serodia and Genedia MIXT PA, HIV-1/-2 (Fujirebio, Tokyo) which employ particles sensitized with whole HIV-1 and HIV-2 lysate. Sample dilution was over one million-fold on the Serodia test with twenty-fold for the Genedia test. The agglutination patterns were interpreted according to the instructions from the manufacturer.

HIV antibody positive plasma by PA were then confirmed by in-house indirect immunofluorescence assays (IFA) adapted from a report by Gallo *et al.*[21] Final observations under UV light with a BX-FLA fluorescent microscope (Olympus, Tokyo) were recorded.

The second step after screening was to separate HIV antibody positive plasma into categories of HIV-1 only or HIV-2 only by immunoblotting against specific viral proteins that constitute Env, Pol, and Gag components. Seropositive plasma were tested on LAV-BLOT 1 and LAV-BLOT 2 (Fujirebio, Tokyo), and bands of specific molecular weights representing bound Env, Pol, and Gag antibodies were displayed by enzyme colorimetry. The strict criteria adopted were the presence of at least two Env protein bands plus any others in accordance with WHO recommendations for HIV Western blots[22]. HIV-1 Env consists of gp160, gp120, and gp41 proteins, whilst HIV-2 has gp140, gp105, and gp36. Immunoblot testing of seropositive plasma was then repeated with PEPTI-LAV 1-2 (Fujirebio, Tokyo), a

Table 1. Characteristics of the three-step serological system comprising seven HIV antibody assays.
HIV-1 and HIV-2 genomes encode, respectively, the following proteins that constitute the envelope (Env), core (Gag), and polymerase (Pol) components.
HIV-1: Env - gp160, gp120, gp41; Gag - p55, p40, p25, p18; Pol - p68, p52, and p34
HIV-2: Env - gp140, gp105, gp36; Gag - p56, p26, p16; Pol - p68, p34
Plasma was screened first by particle agglutination and indirect immunofluorecence methods, followed by confirmation of all the HIV antibody positives with immunoblotting and particle agglutination inhibition.
*Plasma reactive with at least two major Env proteins of HIV-1 (or HIV-2) was regarded as positive for HIV-1 (or HIV-2) antibodies, in accordance with the WHO recommendations for HIV Western blots[22].

STEP	ASSAY	PRINCIPLE
1. Screening:		
HIV-1/-2	Serodia PA (Fujirebio, Japan)	Agglutination by particles sensitised with whole HIV-1 or HIV-2 1ysates
	Genedia Mixt PA (Fujirebio, Japan)	
	Indirect Fluorescene (in-house)	Indirect immunofluorescence staining using cells infected with HIV isolates
2. Separation:		
HIV-1	LAV BLOT 1, (Fujirebio, Japan)	Western blotting of HIV-1 Env, Gag and Pol proteins
HIV-2	LAV BLOT 2 (Fujirebio, Japan)	Western blotting of HIV-2 Env, Gag and Pol proteins
HIV-1 HIV-2	PEPTI-LAV 1,2 (Fujirebio, Japan)	Immunoblots of transmembrane glycoproteins; HIV-1 gp41, HIV-2 gp36
3. Clarification:		
HIV-1 HIV-2	INNO-LIA HIV (Immunogenetics, Belgium)	Immunoblots of HIV-1, HIV-2 Env recombinant and synthetic peptides
Dual HIV-1 & -2	Serodia PA HIV INHIBITION (Fujirebio, Japan)	Inhibition of HIV-1 and HIV-2 Serodia PA by HIV-1gp41 and HIV-2gp36, respectively

mono-epitope assay with two specific synthetic peptides fixed on a membrane. These are transmembrane envelope glycoproteins, gp41 of HIV-1 and gp36 of HIV-2. Plasma samples were incubated as directed by the manufacturer and results recorded. When simultaneous reactivity to both peptides was noticed, however, dual infection was scored only if the two peptides were recognized with the same intensity.

In our final approach, the HIV-1, HIV-2, and dual HIV-1 and HIV-2 categories that had been obtained were verified. INNO-LIA HIV Confirmation (Immunogenetics, Antwerp), another immunoblot assay from a different source, was used to further confirm and differentiate the presence of the HIV-1 and HIV-2 antibodies. This test is based on specific HIV-1 gp120 and gp41 recombinant proteins, plus HIV-2 gp105 and gp36 synthetic peptides, coated as discrete lines on the same strip.

The dual HIV-1 & HIV-2 samples were also re-tested with Serodia PA HIV-1 and HIV-2 Inhibition assays (Fujirebio, Tokyo). Plasma was titrated on HIV-1 and HIV-2 assays simultaneously with and without HIV-1 gp41 and HIV-2 gp36 purified protein as inhibitor. When compared with the controls, significant reduction of antibody titers (usually greater than 500-fold) by the inhibitors confirmed the presence of either HIV-1 or HIV-2 antibodies.

PCR analyses

DNA was extracted from plasma or PBL samples representative of the various categories: HIV-1 only, HIV-2 only, and dual HIV-1 & HIV-2. HIV-1 specific primer pairs from the *pol* genome with a sequence as described by Fransen *et al.*[12] were employed (Table 2). HIV-2 specific LTR region primers (LTRg) with sequences as described by Gao *et al.*[14] and another set (LTRi) recently described by Ishikawa *et al.*[5] were used (Table 2). The PCR reaction was performed on 1 to 2 μg of DNA extract in a 25 μl total reaction volume. The reaction mixture contained 50 mM KCl, 1.5 mM

Table 2. Primer sequences for HIV-1 and HIV-2 nested PCRs.
Primers used in nested PCRs were as reported: HIV-1 *pol* by Fransen *et al.*[12], HIV-1 *pol* by Fransen *et al.*[12], and HIV-2 LTRg by Gao *et al.*[14], and HIV-2 LTRi by Ishikawa *et al.*[5]. Nucleotide numbering was according to published sequences for HIV-LAI(*), and HIV-2 ROD (#). For the first round primer pair, OF = outer forward and OR = outer reverse. For the second round primer, IF = inner forward and IR = inner reverse. Final products with expected basepair (bp) sizes were taken as positive.

Type and genomic region	Primer name	Nucleotide sequence (5' - 3')	Location	Product size (bp)
HIV-1 *pol*	hpol1 OF	CCCTACAATCCCCAAAGTCAAGG	*4235 - 4257	
	hpol2 OR	TACTGCCCCTTCACCTTTCCA	4538 - 4558	324
	hpol3 IF	TAAGACAGCAGTACAAATGGCAG	4327 - 4349	
	hpol4 IR	GCTGTCCCTGTAATAACCG	4481 - 4499	173
HIV-2 LTRg	ltra OF	CTGAGACTGCAGGGACTTTCCAGAAGGG	#9379 - 9406	
	ltrb OR	AAGCAGAAAGGGTCCTAACAGACCAGGGT	9739 - 9767	389
	ltrc IF	AGGCTGGCAGATTGAGCCCTGGGAGGTTC	9513 - 9541	
	ltrd IR	CCAGGCGGCGACTAGGAGAGATGGGAGCAC	9682 - 9711	199
HIV-2 LTRi	ltr1 OF	GCTGGCAGATTGAGCCCTG	#18 - 35	
	ltr2 OR	AAGGGTCCTAACAGACCAGGG	243 - 262	245
	ltr3 IF	CAGCACTAGCAGGTAGAGCCTGGG	49 - 71	
	ltr4 IR	GGCGGCGACTAGGAGAGATGG	191 - 210	162

$MgCl_2$, 10mM Tris-HCl, pH 8.3, 0.2 mM of each dNTP, 0.625 units of AmpliTaq Gold DNA polymerase (Perkin Elmer, New Jersey) and 1uM of each primer. The temperature cycles for the HIV-1 and HIV-2 nested PCRs were set as described previously[12,5,14], with the inclusion of a 95℃ hot start for seven minutes. The second-round reactions (inner primer pair) were performed with 1 μl of amplified DNA from the first round of PCR. The final products were analyzed by electrophoresis with 2% agarose gels containing ethidium bromide.

Viral load

An improved version of the Roche AMPLICOR HIV-1 MONITOR RT-PCR (Roche, New Jersey) including add-in primers sensitive to HIV-1 group M subtypes, was used to measure plasma viral load. According to the manufacturer, nucleotide sequences of the *gag* primers were shifted to a region that is more conserved among all group M subtypes (namely A, B, C, D, E, F and G). Plasma volumes of 200 μl together with assay controls were subjected to sample preparation, reverse transcription, amplification, and final detection and quantitation as described in the assay protocol. The RT-PCR was done in a 96 well format GeneAmp 9600 thermal cycle machine (Perkin Elmer, New Jersey). The optical density readings for final detection and quantitation were done at 450 nm in a microplate reader Model 550 (Bio-Rad, CA). HIV-1 RNA copies/ml in the test plasma were calculated from the formula provided in the assay protocol.

Results

Serological screening for HIV antibodies by PA and IFA

Out of 563 plasma samples screened, 400 samples were found to be positive for HIV-1 or HIV-2 antibodies after screening with Serodia and Genedia Mixt PA HIV-1/-2. Samples positive for HIV antibody on the particle agglutination assays were confirmed by IFA. On the Serodia PA assays, all the HIV-2 reactive samples were also reactive for HIV-1. These dual reactives had a mean antibody titer of 409,600 for HIV-1 and HIV-2, but higher HIV-2 titers (>1,638,400) occurred in some cases. The antibody titers are not shown because of

the large numbers screened. The 400 PA and IFA HIV seropositives were then clarified by immunoblotting. One sample that was positive for HIV-1 antibody by Serodia PA, but negative for anti-HIV antibodies by Genedia PA and IFA, was also separately examined on the immunoblot assays.

Clarification into HIV-1, HIV-2, and dual HIV-1 & -2 groups

All of the 400 HIV seropositive plasma from the screening step were distributed into HIV-1, HIV-2 and dual HIV-1 and HIV-2 categories from the combined results of the immunoblotting and PA inhibition assays. The prevalence levels of each category in the HIV-infected population that we studied are indicated in Table 3. The various immunoblot antibody profiles given in Table 4 include the typical positive, cross-reactive, and indeterminate reactions observed. Indeterminate is the description for a sample that was positive by PA or IFA but did not fulfill the criteria for a positive Western blot.

Samples classified as dual HIV-1 &-2 seropositive displayed the clear appearance of antibodies against all of the Env, Gag, and Pol antigens of HIV-1 and HIV-2. Among the samples positive for HIV-1 antibodies, none of them reacted with two HIV-2 Env proteins (envelope gp140, gp105, and transmembrane gp36); however, antibodies against HIV-2 Gag and Pol proteins were constantly evident. In the HIV-2 antibody-positive samples, no reactivity against HIV-1 gp41 was seen, but other HIV-1 Env (gp160, gp120) plus Gag and Pol proteins were frequently stained. The dual reactive HIV-1 and HIV-2 plasma samples shown in Table 3 were all confirmed by the PA inhibition assays. Addition of the specific inhibitors (purified HIV-1 gp41 and HIV-2 gp36 proteins) caused significant reduction (about 500-fold) in antibody titer and final titers averaged 2048. The discrepant Serodia PA positive sample was negative on the immunoblots and was taken as a false-positive result and excluded from further analyses.

Table 3. Distribution of the study subjects according to type of HIV antibody.

The 400 HIV seropositive samples that were HIV-1 or HIV-2 antibody positive by the Serodia and Genedia Mixt PA HIV-1/-2 and IFA screening assays were discriminated into separate serotypes (indicated in bold type). The combined results of the LAV BLOT, PEPTI-LAV, and INNNO-LIA assays enabled distribution of the seropositive samples into the following groups: HIV-1 alone (356), HIV-2 alone (9), and dual HIV-1 and HIV-2 (35). Prevalence was therefore estimated as HIV-1 (89%), HIV-2 (2%), and dual HIV-1 & -2 (9%).

		HIV-1		
		Positive	Negative	Total
HIV-2	Positive	**35**	**9**	44
	Negative	**356**	163	519
	Total	391	172	563

Verification by nested PCR

The HIV-1 *pol* and HIV-2 LTR primers displayed specific reactivity with HIV-1 and HIV-2 positive controls, respectively, and HIV seronegative PBL were negative as expected (data not shown). To check the quality of the DNA extracted, PCR using the primers for the (β-globin gene were also performed. All the HIV-1 and dual HIV-1&-2 antibody positive plasma samples that were identified by immunoblotting and PA inhibition and subjected to HIV-1 *pol* nested PCR, gave positive products as shown in Table 4. HIV-1 seropositives with non-positive (indeterminate) HIV-2 immunoblot profiles were negative by both HIV-2 LTR nested PCRs. HIV-2 seropositives with indeterminate HIV-1 immunoblot results were also negative by the HIV-1 *pol* nested PCR. The dual HIV-1 &-2 and the HIV-2 seropositives were all PCR positive for HIV-2 by at least one or both LTR nested PCRs.

HIV-1 RNA measurements of plasma

An evaluation of the improved Roche AMPLICOR HIV-1 MONITOR RT-PCR assay on plasma samples from HIV-1 infected Ghanaians with subtype classification already determined[19] is shown in Table 5. Some of the NJ- labeled samples were collected with heparin as anti-coagulant, and approximately 10-fold reduction in HIV-1 RNA quantities were observed. All of these plasma samples from non-subtype B HIV-1 infected persons gave valid results and generally, HIV-1 viral load ranged from 10,000 to over 600,000 copies/ml, with 2 million copies/ml also recorded. When nine HIV-2 antibody-positive plasma samples were similarly tested by the Roche assay to check for cross-reactivity with HIV-1, the results did not seem significant or valid, because the amounts found were close to the minimum detection level of 200 copies/ml (data not shown).

Viral load expressed as $\log_{10}$ HIV-1 RNA copies/ml quantities for fifty-five HIV-1 seropositive plasma samples with EDTA as anti-coagulant are shown according to their $CD4^+$ counts in Figure 1. Eleven of these were also positive for HIV-2 antibody (dual HIV-1 &-2

Table 4. PCR verification of samples representative of the HIV antibody categories.
Representative samples reflecting the typical antibody profiles for HIV-1, and dual HIV-1 and HIV-2 categories that were identified are shown. All of the nine HIV-2 samples are presented. Antibodies against the component proteins (molecular weights shown) of Env, Gag, and Pol regions that did not satisfy the WHO positive cliteria for at least two Env antibodies are indicated. These are usually described as indeterminate, because they are not-positive but also not negative. Cross-reactivity was caused by the presence of single Env antibodies. nd = not done; sympt. = symptomatic for HIV-related disease; asympt. = no signs of HIV-related disease; all present = all the Env, Gag, and Pol antibodies were observed; postive = positive-PCR product was obtained; both positive = both primer sets positive; negative = PCR was negative.

Representative samples	Env, Gag, Pol antibodies		Nested PCR			Immune status cells/ul		Clinical condition
	HIV-1	HIV-2	HIV-1 *pol*	HIV-2 LTRi	LTRg	$CD4^+$	$CD8^+$	
HIV-1								
NJ96-135	all present	all absent	positive	negative		1462	2000	asympt.
NJ96-58	all present	all absent	positive	negative		671	1400	asympt.
NJ96-22	all present	all absent	positive	negative		50	413	AIDS
NJ96-308	all present	p68, p26, p56	positive	negative		216	648	asympt.
NJ96-168	all present	gp36,p68,p26	positive	negative		346	1153	sympt.
NJ96-326	all present	gp36, p26	positive	negative		50	110	AIDS
MH-6	all present	gp105, p26	positive	negative		332	548	asympt.
MH-30	all present	gp36,p68,p16	positive	negative		471	1092	asympt.
SJ-10	all present	gp105,p68,p26	positive	negative		313	1370	sympt.
KS-09	all present	gp36,p68,p26	positive	negative		154	1123	AIDS
DUAL HIV-1 &-2								
NJ96-01	all present	all present	positive	nd	positive	190	1130	AIDS
NJ96-21	all present	all present	positive	nd	positive	240	1356	sympt.
NJ96-99	all present	all present	positive	nd	positive	52	734	AIDS
NJ96-100	all present	all present	positive	nd	positive	499	677	sympt.
NJ96-124	all present	all present	positive	nd	positive	70	299	AIDS
NJ96-147	all present	all present	positive	nd	positive	245	1795	sympt.
NJ96-175	all present	all present	positive	both	positive	50	168	AIDS
MHW-1986	all present	all present	positive	both	positive	nd	nd	asympt.
NJ97-58	all present	all present	positive	both	positive	50	519	AIDS
NJ97-68	all present	all present	positive	both	positive	289	568	sympt.
KU-24	all present	all present	positive	both	positive	251	1024	sympt.
SJ-27	all present	all present	positive	both	positive	191	200	AIDS
SJ-37	all present	all present	positive	both	positive	256	2000	sympt.
HIV-2								
NJ96-146	p68, p55, p25	all present	negative	nd	positive	50	714	AIDS
NJ96-177	gp160,p68,p25	all present	negative	nd	positive	50	470	AIDS
NJ96-246	all present	all present	negative	nd	positive	50	452	AIDS
NJ97-08	gp120,p25,p18	all present	negative	nd	positive	nd	nd	sympt.
W-11	all absent	all present	negative	positive	nd	nd	nd	sympt.
W-9	p25,p40,p68	all present	negative	positive	nd	446	558	sympt.
W-10	p55,p25	all present	negative	positive	nd	78	775	AIDS
SM-01	p25,p55,p40	all present	negative	positive	nd	1396	1158	sympt.
KU-17	gp120,p25,p55	all present	negative	positive	nd	1326	1677	sympt.

seropositive). $CD4^+$ counts of less than 200 cells/μl were associated with the highest HIV-1 RNA levels. $CD4^+$ counts and viral load did not seem to differ significantly between HIV-1 seropositives and dual HIV-1&-2 antibody positive samples (Data not shown because the low number of dual seropositives made application of statistical tests difficult).

Table 5. Plasma HIV-1 viral load in HIV-1 subtype characterized subjects.
Viral load in HIV-1 seropositive samples whose subtype had been determined[17] as indicated are displayed. NJ- samples were collected with heparin, whilst other samples were taken with EDTA-2Na as anti-coagulant. Immune status is indicated by the $CD4^+$ and $CD8^+$ T cell counts. Clinical condition shown by: sympt. = symptomatic for typical HIV related disease; asympt. = no sign of typical HIV related disease.

Sample number	HIV-1 subtype	HIV-1 RNA copies/ml	Clinical status	$CD4^+$ cells/ul	$CD8^+$ cells/ul
NJ-183	A	10,000	AIDS	118	448
NJ-232	A	20,000	asympt.	724	586
NJ-29	A	20,000	AIDS	161	696
NJ-179	A	30,000	AIDS	193	985
NJ-45	A	40,000	AIDS	355	1163
NJ-147	A	40,000	AIDS	245	1795
NJ-247	A	40,000	AIDS	50	452
NJ-173	G	70,000	sympt.	452	793
NJ-174	G	300,000	AIDS	225	1219
MHW-2103	A	400,000	AIDS	233	814
MHW-2175	A	600,000	sympt.	388	734
MHW-2176	A	2,000,000	AIDS	69	1946

Discussion

Our serological antibody detection scheme consisted of screening for HIV antibodies initially with whole HIV particles (PA and IFA) followed by separation with the specific anti-HIV-1 and anti-HIV-2 component proteins by immunoblotting and PA inhibition. The percentage prevalence for positive HIV antibody presence was calculated from Table 3 to give an indication of the distribution of the specific types amongst the HIV seropositives. In Ghana, HIV-1 infections (89%) are clearly dominant with few HIV-2 (2%) infections and some dual infections (9%) present.

Although Serodia PA end-point titration gave indications of the presence of either HIV-1 or HIV-2 antibody, this was an unreliable prediction of dual infection. Comparison of the PA results against PCR indicated that high HIV-1 titers in some dual-reactive samples on the PA were probably due to cross-reactivity, because these were confirmed as exclusive HIV-2 infections by PCR. Relatively high HIV-2 PA antibody titers, however, did indicate the true presence of HIV-2 infection as confirmed by PCR.

In the interpretation of the Western blots, we strictly followed the criteria recommended by the WHO, which describes antibodies against two Env protein bands as the minimum identification requirement[22]. The results for antibodies against the synthetic and recombinant Env proteins of HIV-1 (gp160, 120 and 41) and HIV-2 (gp140, 105, 36) were combined for analyses. This is exemplified by the antibody profiles in Table 4.

The full presence of antibodies against all the Env, Gag, and Pol proteins in both HIV-1 and HIV-2

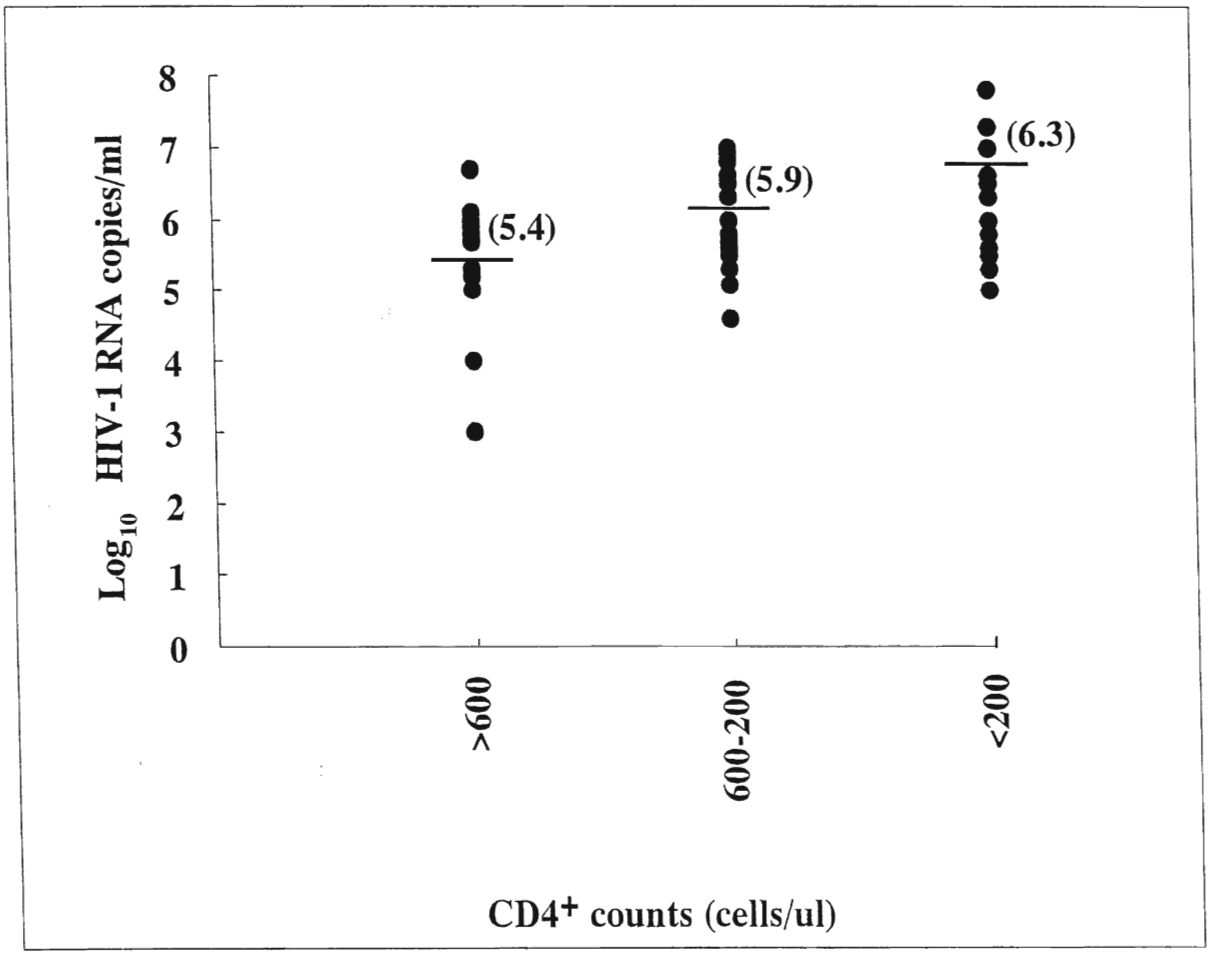

Figure 1. Plasma HIV-1 viral load in HIV-1 seropositives according to groups of $CD4^+$ T cell counts that reflect different stages of HIV infection.

Plasma HIV-1 viral load in seropositive subjects is shown against counts of $CD4^+$ T cells/ul grouped according to levels representative of AIDS disease stages. $CD4^+$ >600 /ul represent HIV infections with no symptoms of HIV related disease; 200 to 600/ul range represent chronic stage of HIV infection with few symptoms; <200/ul indicates late-stage HIV infections with progressive AIDS. Mean log HIV-1 viral load for each of the $CD4^+$ groups is indicated in parentheses.

Western blots implied dual HIV-1 and HIV-2 infection. This was confirmed by the production of bands of equal intensity for antibodies against the transmembrane HIV-1 and HIV-2 specific peptides on the Pepti-Lav assay. The PA inhibition test involving purified proteins for the same epitope (HIV-1 gp41, HIV-2 gp36) and the complete HIV-1 and HIV-2 Env antibody profiles on the INNO-Lia test also supported the dual seropositive classification. Final confirmation of dual infection was demonstrated by the nested PCR with HIV-1 and HIV-2 type-specific primers.

Samples indeterminate for HIV-1 or HIV-2 antibody by Western blot analyses proved negative when tested by nested PCR. A 40–60% homology at both the nucleic acid and amino acid levels in the open reading frame for the structural *env, gag*, and *pol* genes of HIV-1 and HIV-2 has been reported[1]. This could account for the cross-reactivity leading to the indeterminate observations or may represent recombination. Diagnostic confirmation by PCR should eliminate such doubtful antibody observations, as we have shown in accordance with the reports of others[5,23]. The complementary effectiveness of our serological diagnostic system was emphasized by verification with nested PCR, and, consequently, HIV-1 was confirmed as the major dominating type. Because of the recent spread of HIV-2 into Asia, as demonstrated by mixed HIV-1 and HIV-2 infections in India[24], improved HIV-2 PCR detection strategies[5,23] will be useful worldwide and not only in West Africa.

Although HIV-1 viral load has become an important marker in clinical practice especially in the Western world[16,17], it is yet to gain widespread use for AIDS in Africa where subtypes are diverse and dual infections occur. Our HIV-1 RNA measurements indicate the acceptability for use of the improved Roche AMPLICOR HIV-1 MONITOR RT-PCR test in Ghana. HIV-1 RNA was detectable regardless of clinical stage, confirming the suitability of the assay as an indication of virus replication, and there was an inverse correlation between viral load and $CD4^+$ counts.

Interestingly, rather high amounts of HIV-1 RNA were prevalent in the samples presently studied, as compared with those in other reports[16]. The mean amounts of HIV-1 RNA that we observed in the samples categorized by categories of $CD4^+$ counts/μl as being greater than 600, 200 to 600, and less than 200 were 250,000; 800,000 and 2,000,000 copies/ml respectively. For the same $CD4^+$ categories, Saag *et al.* reported 16,000; 63,000 and 1,000,000 copies/ml respectively[25]. As shown in Figure 1, the mean level of plasma viral load was greater than 5 $\log_{10}$ (100,000 HIV-1 RNA copies/ml) across the $CD4^+$ T cell ranges with highest levels appearing in late stages of the disease. Subjects with $CD4^+$ counts greater than 600 cells/ul were mainly without symptoms of AIDS, whilst those having under 200 cells/ ul usually displayed full-blown AIDS. The $CD4^+$ ranges adopted represent the typical stages of HIV infection[16], but, from the clinical histories available, it was impossible for us to establish primary or acute seroconversion stages. Such initial HIV infections usually remain unnoticed because of similarities in symptoms with those of common local infections such as malaria.

Although this was not a stated objective, we tried to compare HIV-1 RNA and $CD4^+$ levels between HIV-1 and dual HIV-1 & -2 infections for healthy and sick subjects in our study group. Those differences seemed insignificant, but the low numbers (less than 10) were not suitable for the application of statistical tests. However, we think that this implies infection with HIV-2 in Ghana is not significantly protective against HIV-1 infection, because mean HIV-1 viral load was greater than 100,000 copies/ml in the group with dual HIV-1 &-2 infection. The current high levels of HIV-1 (89%) could indirectly reflect insignificant cross-protection by HIV-2, since HIV-2 was thought to dominate in Ghana in the past[4]. Our viral load data represent the first processing of such data for Ghana and provide baseline HIV-1 RNA data to facilitate the management of AIDS patients and the application of antiretroviral therapy. High HIV-1 viral load levels demonstrate the clear dominance of HIV-1 infections among AIDS patients in Ghana.

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