Molecular Epidemiology of HIV-2 Infection in KwaZulu-Natal Province, South Africa

by

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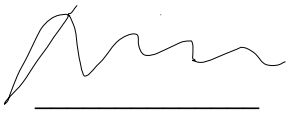
DECLARATION

This study presents original work by the author and has not been submitted to this, or any other University. The research described in this thesis was carried out in the Department of Virology, National Health Laboratory Service / University of KwaZulu-Natal, under the supervision of Professor Tulio de Oliveira. Where use is made of the work of others, it has been duly acknowledged in the text.



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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

1. SINGH, L., PARBOOSING, R., MANASA, J., MOODLEY, P. & de OLIVEIRA , T. (2013) High level of HIV-2 false positivity in KwaZulu-Natal province: a region of South Africa with a very high HIV-1 subtype C prevalence. *J Med Virol*, 85(12): 2065 – 2071.

2. Molecular epidemiology of HIV-2 infection in KwaZulu-Natal province, South Africa (Poster presentation - SA AIDS Conference 2011).

ABSTRACT

Infection with HIV-2 has important implications for the diagnosis, treatment and management of the infection. The objective of this study was to describe the seroprevalence and molecular epidemiology of HIV-2 in KwaZulu-Natal – the province with the highest HIV prevalence in South Africa, which in turn is the country with the highest HIV prevalence in the world. HIV-1 positive samples were screened using a rapid test for HIV-2. Samples showing antibody positivity were subject to molecular confirmation by PCR and / or serological confirmation by Western blot. There was a large difference in results (10.6% by Western blotting versus 0% by PCR). This discrepancy between molecular and serological confirmation was attributed to cross-reacting antibodies. The use of rapid tests and Western blots for HIV-2 diagnosis in South Africa, should, therefore, be interpreted with caution. Based on the results of this study, HIV-2 is most probably not present in KwaZulu-Natal.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	antiretroviral therapy
HIV-1	Human Immunodeficiency Virus type 1
HIV-2	Human Immunodeficiency Virus type 2
INT	integrase
KZN	KwaZulu-Natal
MHC	Major Histocompatibility Complex
NC	nucleocapsid
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PRO	protease
RT	reverse transcriptase
SA	South Africa
SIVcpz	simian immunodeficiency virus – chimpanzee
SIVsm	simian immunodeficiency virus – sooty mangabey
TAR	*tat* activation-response region

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ETHICS

Approval was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee (*BREC 264/09*) and the KwaZulu-Natal Provincial Department of Health.

CHAPTER 1: INTRODUCTION

1.1 Background

The etiological agent of acquired immunodeficiency syndrome (AIDS) is designated human immunodeficiency virus (HIV). This virus is a member of the genus lentivirus and belongs to the Retroviridae family. HIV is highly mutable, producing many different strains. Classification of HIV is based on genetic similarities, and can thus be divided into different types, groups and subtypes. Accordingly, HIV can be separated into types 1 and 2 (Quinn, 1994). HIV-2 can further be divided into seven subtypes (A to G). Subtypes A and B are categorized as epidemic subtypes, while C – G are non-epidemic subtypes. Subtype A is the most predominant and is proposed as the most pathogenic. An intersubtype recombinant involving subtypes A and B has been recently described in Cameroon. (Soriano et al., 2000, Lemey et al., 2003, Ntemgwa et al., 2009). A highly divergent HIV-2 strain was identified in 2004 and Damond *et al* proposed classification of this strain into a new subtype H (Damond et al., 2004).

1.2 Discovery and Origin of HIV-2

In 1986, three years following the discovery of HIV-1, another related immune-deficiency-causing virus was isolated. Sera from a cohort of Senegalese sex workers were found to react preferentially with antigens from simian immunodeficiency virus (SIV) - a related lentivirus. Phylogenenetic analyses confirmed that this virus was more genetically related to SIV from

the sooty mangabey (*Cercocebus atys*) than to HIV-1, which shows more genetic relatedness to SIV from the chimpanzee (*Pan troglodytes troglodytes*) (Figure 1). This virus, which was originally known as lymphadenopathy-associated virus type 2, is presently known as human immunodeficiency virus type 2.

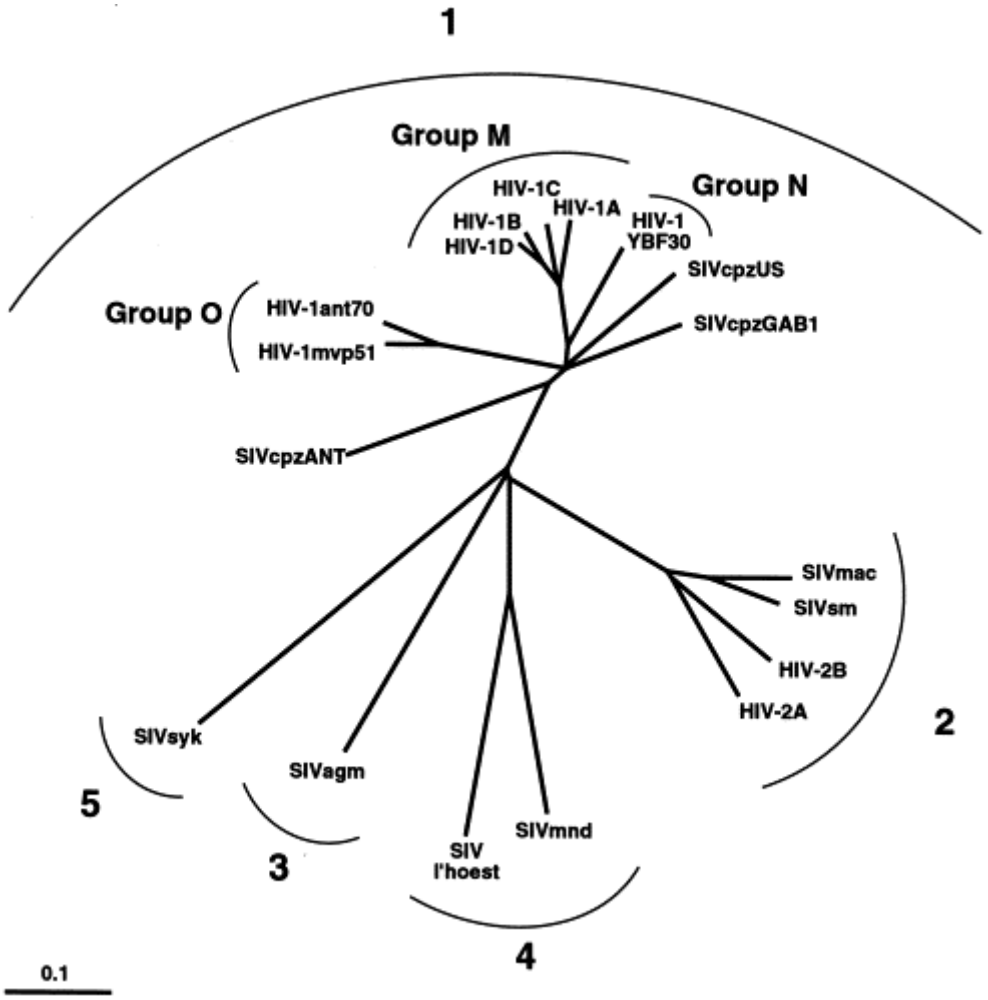

Figure 1: Phylogenetic relationship of *pol* region of primate lentiviruses (McGrath et al., 2001). Letters A – D represent the subtypes of HIV-1 and HIV-2

1.3 HIV-2 Structure

Like HIV-1, HIV-2 is a retrovirus associated with AIDS. The HIV-2 virion consists of a complex genome surrounded by a cone-shaped capsid (Turner and Summers, 1999). A lipid bilayer, derived from the host cell membrane functions as the envelope; surface glycoproteins (gp125) are anchored to the viral surface via the gp36 transmembrane protein. This lipid bilayer also comprises of proteins, including major histocompatibility complex (MHC) antigens, actin and ubiquitin – all of which are host-cell derived. The matrix protein (p17) is found on the inner surface of the virion. This surrounds a conical capsid core (p24) particle comprised of approximately 2000 capsid proteins. This centralized core further encapsidates two copies of the viral RNA genome, which in association with the capsid, is known as ribonucleoprotein complex (Turner and Summers, 1999, Björling et al., 1994). The three structural genes (*gag*, *pol*, and *env*) contain information needed to make structural proteins for new virus particles. A diagrammatic representation of the structure of HIV is shown in Figure 2.

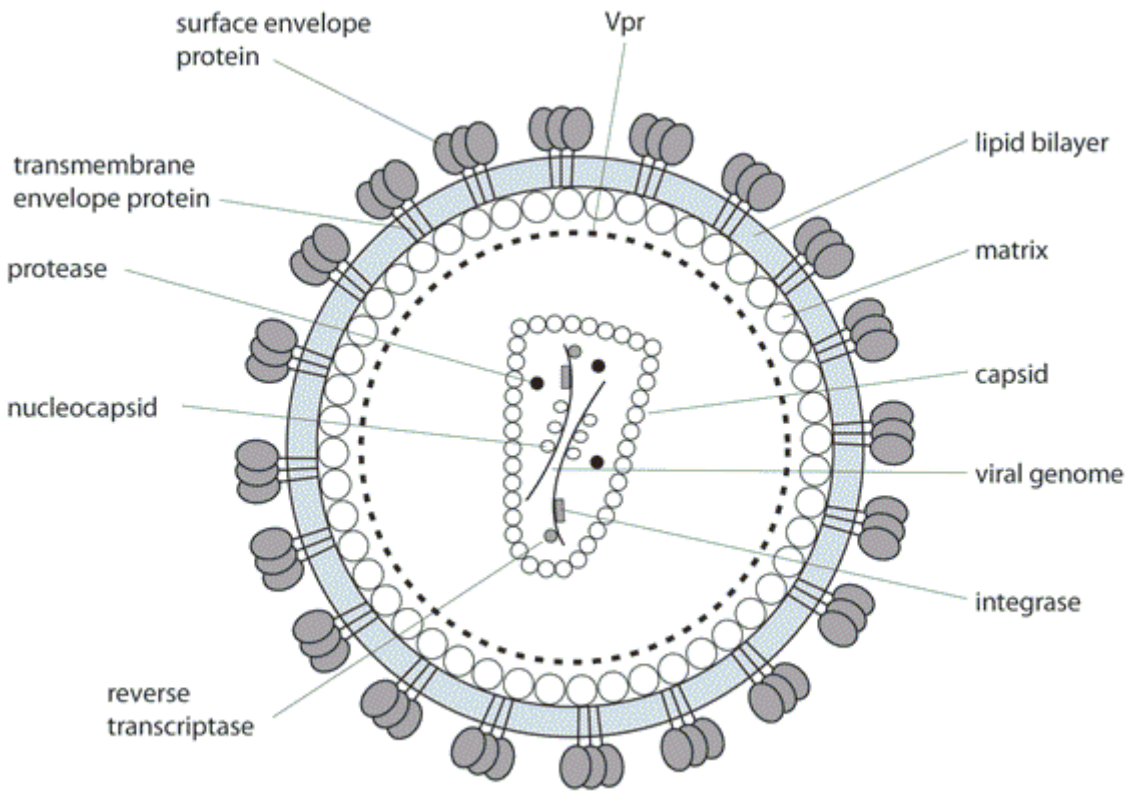

Figure 2: Diagrammatic representation of the HIV virion

(available at: http://idshowcase.lshtm.ac.uk/id501/ID501/S1S2/ID501_S1S2_050_010.html)

1.4 HIV-2 Genetics

While both HIV-1 and HIV-2 belong to the *Lentivirinae* subfamily, there is only approximately 40% genetic similarity between these two viruses (Bock and Markovitz, 2001). HIV-2 consists of two identical copies of single-stranded RNA molecules. The 9.2 kb genome encodes nine open reading frames: *gag, pol, vif, vpr, tat, rev, vpx, env* and *nef,* which is flanked by the 5' and 3' long terminal repeats (Azevedo-Pereira, 2013). Viral

enzymes protease (PRO), reverse transcriptase (RT) and integrase (INT) form part of the polymerase gene, as in HIV-1. The three structural genes (*gag*, *pol* and *env*) are the same for all retroviruses (Fanales-Belasio et al., 2010) although the *vpu* gene of HIV-1 and SIV of chimpanzees is replaced by the *vpx* gene present in HIV-2 and SIV of sooty mangabeys. The function of *vpx* is unclear but it may have functions associated with nuclear import (Reeves and Doms, 2002). The genetic organizations of the two viruses are shown in Figure 3, below.

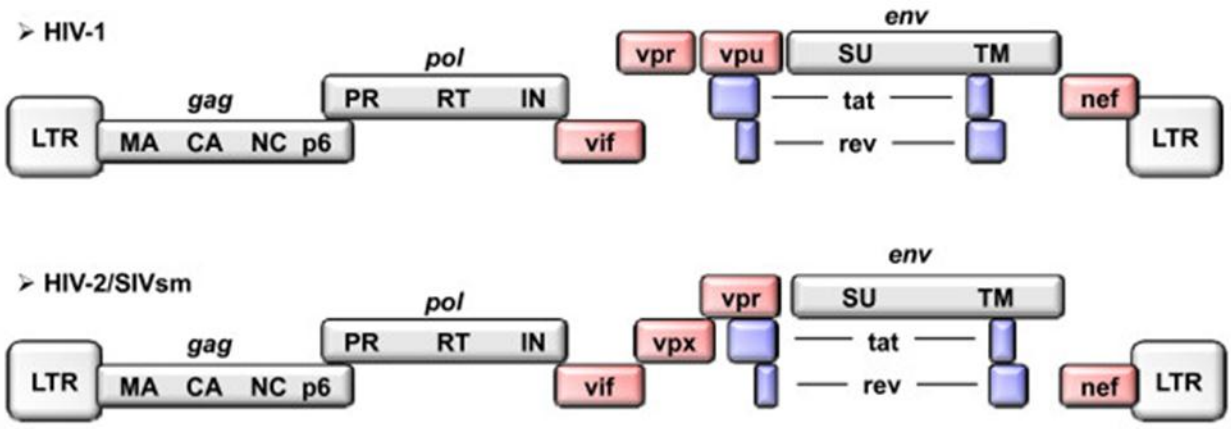

Figure 3: Comparison of genetic organization between (A) HIV-1 and (B) HIV-2 genomes (Ayinde et al., 2010)

1.5 HIV-2 Epidemiology

Further differences exist from a geographic perspective; worldwide, the predominant virus is HIV-1, accounting for approximately 95% of global HIV infections (Figure 4). HIV-2, however, is concentrated mainly in West Africa (Zeh et al., 2005, Nielsen and Bryson, 2000, Kanki and Meloni, 2004).

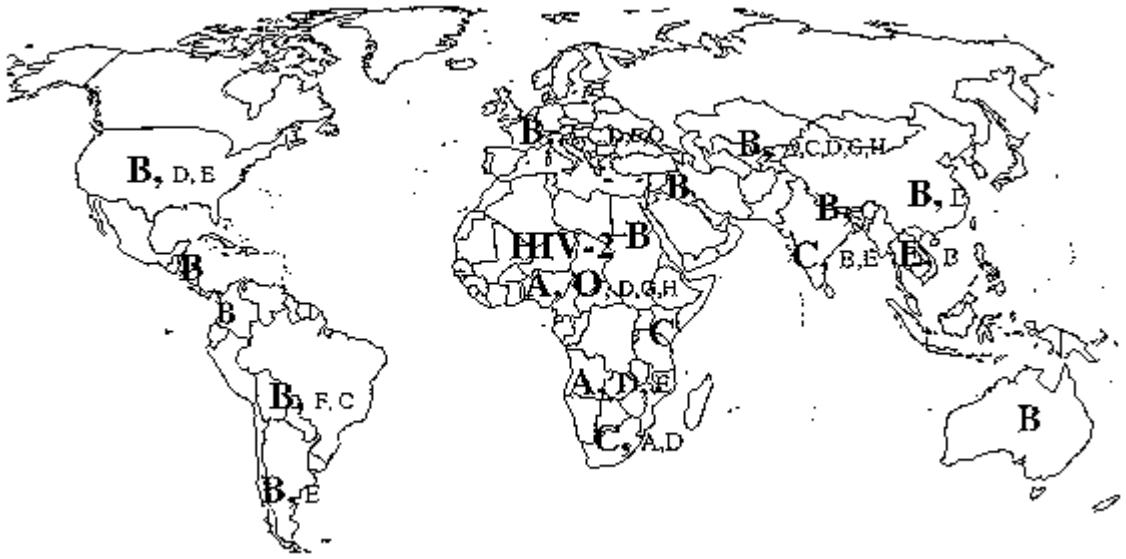

Figure 4: Geographic distribution of HIV-1 subtypes and HIV-2 worldwide (Nielsen and Bryson, 2000)

HIV-2 cases have, however, been reported in Europe, India and the United States, as well as areas with historical and socio-economic ties to West Africa. For instance, in Portugal, HIV-2 is responsible for 4.5% of the AIDS cases, and in France 1.8% of new infections are due to HIV-2 (Campbell-Yesufu and Gandhi, 2011). The predominant genotype is subtype A and it

accounts for the majority of HIV-2 infections in Guinnea-Bissau and Europe. Subtype B appears to have originated from the eastern parts of West Africa. Sierra Leone has the greatest diversity of HIV-2 subtypes (A, B E and F) (Reeves and Doms, 2002). Recently, an intersubtype recombinant involving subtypes A and B was described in Cameroon (Ntemgwa et al., 2009).

1.6 HIV and KwaZulu-Natal Province, South Africa

KwaZulu-Natal (KZN) is the province with the highest HIV prevalence in South Africa (SA), which in turn is the country with the highest HIV prevalence in the world (UNAIDS, 2013). The appearance of HIV-2 in other geographic areas outside its origin has reflected associations with individuals of West African origin, hence the spread of HIV-2 worldwide may be attributed to the migration of these infected individuals (Quinn, 1994, Gao et al., 1994).

It is not unlikely that HIV-2 may be present in SA, following high levels of population immigration to the country (Lehohla, 2011). In addition, KZN hosts the two largest harbours in the country (Durban and Richards Bay), which contribute to the increase in the number of foreign individuals in the province (Young, 2012). A dramatic increase in migration of individuals from African countries into South Africa raises concerns over the prevalence of HIV-2 infection in South Africa (Singh et al., 2013). Moreover, it is virtually impossible to

know how many undocumented migrants reside in South Africa, or where they come from. It is therefore essential for areas with a high immigrant population to consider surveillance for HIV-2.

1.7 HIV-2 Pathogenesis

Differences between HIV-1 and HIV-2 infections remain largely unexplained. In contrast to the wealth of knowledge of HIV-1, there is still a paucity of data regarding crucial aspects of HIV-2.

HIV-2 shares the same transmission routes as HIV-1, although the rates of sexual and perinatal transmission are significantly lower in HIV-2 infections. In contrast to HIV-1, HIV-2 – infected individuals display decreased plasma and semen viral loads, thereby contributing to a slower immune deterioration and reduced transmission rates (Jerome, 2010). Substantial differences in disease development have also been observed in HIV-1 and HIV-2 infections. Both virus types lead to clinically indistinguishable AIDS, although the period between infection and the development of AIDS is substantially longer in HIV-2 – infected individuals (Bock and Markovitz, 2001, Popper et al., 1999, Travers et al., 1995).

This difference in progression to AIDS in HIV-2 infections may be largely due to differences in viral loads. This is true for other lentiviral infections; SIVsm-infected macaques showed correlating levels of plasma viremia and rates of AIDS-associated disease. Similarly, higher

viral loads are associated with a more rapid disease progression in cats infected with feline immunodeficiency virus (Popper et al., 1999).

To further support this, Popper *et al* showed that differences in HIV-1 and -2 viral loads are a key attribute to the differences in pathogenicity between these two viruses, showing that the median viral load was 30 times lower in HIV-2–infected individuals (Popper et al., 1999). While similar proviral loads exist in both types of infection, HIV-2 plasma viral loads are significantly lower than HIV-1 (Singh et al., 2013). This difference may account for the attenuated disease seen in HIV-2 infections. Additional research into the biological differences between these two virus types may provide insight into the pathogenic mechanisms of this class of retroviruses.

1.8 Dual Infection

Many studies have recognized co-infection with HIV types 1 and 2, which are common in areas with a high HIV-2 prevalence, such as West Africa (George et al., 1992, Campbell-Yesufu and Gandhi, 2011, Landman et al., 2009). It has been documented that infection with HIV-2 confers some degree of protection against subsequent infection with the more virulent HIV type 1. Travers *et al* demonstrated this protective effect to be approximately 70% against HIV-1 superinfection in HIV-2 infected high-risk female sex workers in Dakar, Senegal. The mechanism by which HIV-2 alters the natural course of HIV-1 infection

remains unclear. It has been proposed that this protection may be attributed to the robust cross-reactivity between conserved HIV-1 and -2 epitopes (Travers et al., 1995).

In vitro studies show that HIV-1 replication can be inhibited by HIV-2 interference through an unidentified molecular pathway. Other studies revealed that the HIV-2 *tat* activation-response region (TAR) can act as an inhibitor of HIV-1 replication by suppressing replication in cells co-infected by both virus types (Browning et al., 1999, Kokkotou et al., 2000). Other mechanisms such as receptor-mediated viral interference were proposed by Martin and Nayak, who demonstrated that HIV-1 and -2 both compete for the same target cell receptor (CD4) and that infection with one virus type may down-regulate the cell receptor, thereby preventing subsequent infection with another virus type (Martin and Nayak, 1996).

Recombination events between dually infected cells may occur, resulting in progeny virions which are mosaics of the two parent strains. This is associated with the increased diversity and variation seen in HIV. These recombination events between HIV-1 and HIV-2, however, are extremely rare (Blackard et al., 2002), despite their coexistence in certain parts of the world.

1.9 Laboratory Diagnosis

The genetic and antigenic similarity between HIV types 1 and 2 presents an HIV-2 diagnostic dilemma; serological assays that unambiguously differentiate between HIV types 1 and 2 are lacking. In addition, molecular laboratory methods which are used to monitor treatment (viral load quantification) and diagnose HIV infection (PCR) or sequence the virus (to facilitate HIV-2 resistance testing) are currently not available or optimized for HIV-2.

An HIV-2 specific Western blot is done for serological confirmation of infection, where seropositivity requires antibody reactivity to *env* + *gag* + *pol* antigens, or to 2 *env* antigens (Kanki and Meloni, 2004). However, HIV-2 type-specific Western blots also display a high degree of non-specificity (Qiu et al., 2009). The high degree of cross-reactivity between HIV-1 and -2 is also evidenced by the results of this study (Singh et al., 2013).

Consequently, HIV-2 – infected individuals are at risk of misdiagnosis and treatment failure since HIV diagnostic testing and viral load quantification are specific for HIV-1. Resistance testing and vaccine development are also impaired.

1.10 HIV-2 Treatment

In vitro analyses of the efficacy of available antiretroviral therapy (ART) against HIV-2 has not been thoroughly explored. In addition, all approved antiretroviral drugs were designed to specifically target HIV-1 (Witvrouw et al., 2004, Kanki and Meloni, 2004). Since HIV-1 and -2 *RT* and *Pro* are structurally and functionally similar, it is possible that some antiretroviral agents with broad activity may be effective against HIV-2. However, due to the distant genetic makeup between these two viruses (approximately only 60% similarity at nucleotide level in the PRO and RT genes), equal activity is not guaranteed against these two virus types (Witvrouw et al., 2004). In fact, it has been established that certain antiretrovirals work less effectively against HIV-2, compared to HIV-1 (Rodes et al., 2005).

HIV-2 is naturally resistant to all drugs in the non-nucleoside reverse transcriptase inhibitor (NNRTI) group that are presently available: in some instances, there is greater than a 200-fold decrease in susceptibility of HIV-2 to nevirapine, delavirdine and efavirenz (Witvrouw et al., 2004, Rodes et al., 2005). The differences between HIV-1 and -2 at the genomic level of RT are featured in Figure 5, with emphasis on regions associated with HIV-1 NRTI/NNRTI drug resistance. HIV-2 also displays resistance to the fusion inhibitor T-20 (enfuvirtide) (Smith et al., 2008).

Zidovudine and other nucleoside reverse transcriptase inhibitors (NRTI) have demonstrated efficacy against HIV-2 with the development of resistance and their patterns being similar in HIV-1 and HIV-2 infections (Mullins et al., 2004). Treatment regimens with nucleoside analogues directed clinically against HIV-1, may be similarly useful against HIV-2 (Cox et al., 1994).

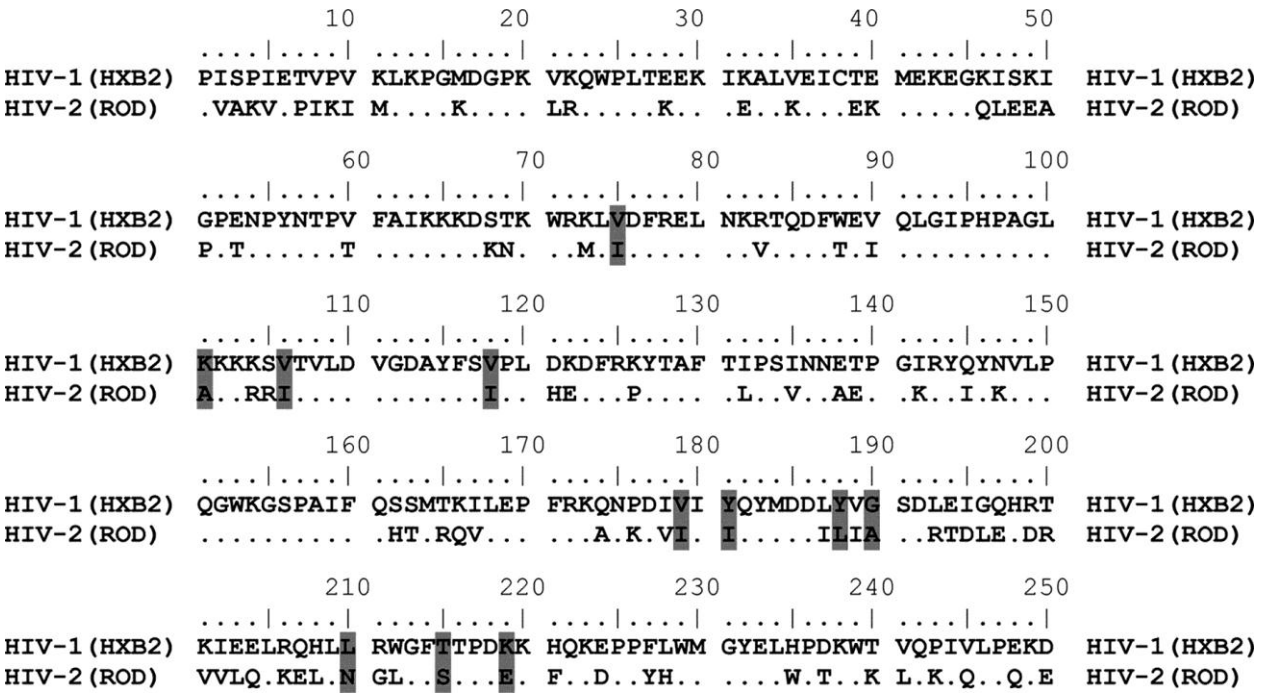

Figure 5: Nucleotide differences between HIV-1 and HIV-2 in the reverse transcriptase region of the genomes (Ntemgwa et al., 2009). Highlighted areas are those with polymorphisms at positions involved in HIV-1 transcriptase resistance. Dots are indicative of sequence similarity.

Weber *et al* showed that differences in the protease genetic sequences reveal that HIV-2 may be constitutively resistant to protease inhibitors (PIs); sites of the genome associated with protease resistance in HIV-1 are similar to wild-type protease sequences of HIV-2 (Weber et al., 1997). This was confirmed by the results of a study by Witvrouw *et al*, who demonstrated a reduced potency of PI's, amprenavir in particular, against HIV-2 *in vitro* (Witvrouw et al., 2004). Hence, PI-containing regimens that are active against HIV-1, may demonstrate reduced potency against HIV-2. Other studies have shown, however, that treatment with dual PI or NRTI-PI combination regimens results in clinical improvement (Ntemgwa et al., 2009).

The genetic differences between HIV-1 and -2 protease region are highlighted in Figure 6, with emphasis on the regions associated with HIV-1 PR drug resistance.

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                    10         20         30         40         50
               ....|....| ....|....| ....|....| ....|....| ....|....|
HIV-1 (HXB2)   PQITLWQRPL VTIKIGGQLK EALLDTGADD TVLEEMNLPG RWKPKMIGGI  HIV-1 (HXB2)
HIV-2 (ROD)    ..FS..K..V ..AY.E..PV .V........ SIVAGIE.GN NYS..IV...  HIV-2 (ROD)

                    60         70         80         90
               ....|....| ....|....| ....|....| ....|....| ....|....
HIV-1 (HXB2)   GGFIKVRQYD QILIEICGHK AIGTVLVGPT PVNIIGRNLL TQIGCTLNF   HIV-1 (HXB2)
HIV-2 (ROD)    ....NTKE.K NVE..VLNK. VRA.IMT.D. .I..F...I. .AL.MS..L   HIV-2 (ROD)
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Figure 6: Nucleotide differences between HIV-1 and HIV-2 in the protease region of the genomes (Ntemgwa et al., 2009). Highlighted areas are those with polymorphisms at positions involved in HIV-1 protease resistance. Dots are indicative of sequence similarity.

1.11 Aims and Objectives

- To determine the prevalence of HIV-2 infection in KZN

- To optimize laboratory methods for HIV-2 diagnosis and confirmation

CHAPTER 2: PUBLICATION

SINGH, L., PARBOOSING, R., MANASA, J., MOODLEY, P. & de OLIVEIRA , T. (2013) High level of HIV-2 false positivity in KwaZulu-Natal province: a region of South Africa with a very high HIV-1 subtype C prevalence. *J Med Virol,* 85(12): 2065 – 2071.

CHAPTER 3: SUMMARY AND CONCLUSIONS

- Vast differences exist between HIV-1 and HIV-2. These include differences in rates of disease transmission, laboratory diagnosis as well as treatment strategies. HIV-2 is generally less pathogenic than HIV-1; disease progression occurs at a slower rate in HIV-2 and the clinical latency period is longer. The HIV-2 attenuated phenotype may be associated with the limited geographic distribution demonstrated by HIV-2 compared to HIV-1. In addition, vaccine responses are likely to differ between HIV-1 and HIV-2.

- Cross-reacting antibodies between HIV-1 and -2 are not uncommon and misclassification of these infections is quite likely. Using serology alone may, therefore, grossly overestimate the true prevalence of HIV-2 infection.

- Serological diagnosis of HIV-2 infection should be interpreted with caution as there is a high level of false-positivity which reduces the specificity of these assays. Western blots, which are capable of HIV type-specific differentiation, can be useful in HIV-2 diagnosis. Results for these assays should be interpreted based on the manufacturer's criteria for interpretation, and also according to WHO guidelines, to decrease the number of indeterminate results which may occur due to cross-reacting or non-specific antibody responses.

- Short peptide-based immunoassays such as the Pepti-LAV assay used in this study proved to be a more reliable indicator of HIV-2 infection than the Western blot. The increased specificity of this assay was comparable to that of the Western blot (seroprevalence of 1.6% versus 10.7%). As a result, a more specific immunoassay will provide more confidence in distinguishing between HIV-1 and -2 infections.

- PCR for molecular detection of HIV-2 provides a much more reliable tool for confirmation of infection. The primers used should be broad enough to cover the genetic diversity of HIV-2 subtypes, but remaining specific to the HIV-2 genome. Of note, HIV-1 and -2 share an approximately 60% homology in conserved genes (*gag* and *pol*) and between 35% - 45% in the *env* genes (Jerome, 2010). Primers used for PCR detection should, therefore, target these envelope proteins, which are more type-specific.

- PCR, however, cannot be used as the ultimate confirmatory assay for HIV-2 diagnosis due to low plasma viral loads, which are common in HIV-2 infections. The lower HIV-2 RNA levels found in the blood plasma compartment may be associated with reduced sensitivity of molecular assays such as PCR, for example.

- The sample type used is also relevant for correct HIV-2 diagnosis. Like other retroviruses, HIV-2 induces a lifelong infection, by integration of the viral genome into

the host cell's DNA. Consequently, the ideal sample type for molecular confirmation of HIV-2 infection is a whole blood sample which has peripheral blood mononuclear cells (PBMCs) to detect this HIV-2 proviral DNA. A limitation of this study is that the only available sample types were serum and plasma samples. These were subsequently used in this study, but were, however, deemed suitable based on sensitivity experiments and HIV-1 PCR experiments conducted.

- HIV-2 is largely confined to West Africa and the spread is mainly attributed to population migration of infected individuals. This study aimed to determine the prevalence of HIV-2 in KZN, a region where HIV-1 subtype C is highly prevalent. The results show that HIV-2 is most likely not prevalent in this province.

- The differences highlighted in this study have important diagnostic, clinical and treatment implications. Reliable methods for differentiating between HIV-1 and -2 infections need to be established and this is an avenue for future research. Continued population surveillance relating to HIV-2 infection is vital.

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