

https://dspace.org/

Burden and determinants of Bacterial vaginosis in sexually active women aged 18 years and over, enrolled in an HIV prevention trial, in northern KwaZulu Natal

Mutevedzi, Portia Chipo

2009-11-18T09:22:03Z
http://hdl.handle.net/10539/7453

Downloaded from a DSpace repository. https://dspace.org/

Burden and determinants of Bacterial vaginosis in sexually active women aged 18 years and over, enrolled in an HIV prevention trial, in northern KwaZulu Natal.

Portia Chipo Mutevedzi

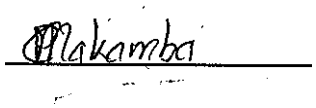
A research report submitted to the faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine: Epidemiology and Biostatistics

2009

Declaration

I, Portia Chipo Mutevedzi declare that this research report is my own work. It is being submitted for the degree of Master of Science (Medicine): Epidemiology and Biostatistics in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature:



6th day of May , 2009

In loving memory of my late mother Charity Wadzanai Makamba.

May your dearly beloved soul rest in peace.

I will forever love and miss you.

Abstract

Background: Bacterial vaginosis (BV) results from a shift in normal vaginal flora and predisposes women to sexually transmitted infections (STI) including HIV. Risk factors for BV are not well understood. This analysis seeks to determine the disease frequency of BV, assess determinants of BV and quantify time to first BV episode in HIV negative women.

Methods: Baseline and follow-up data from 1066 women was analysed in STATA10. Logistic regression was used to determine baseline factors associated with BV and Kaplan Meier survival analysis to estimate time to BV episode.

Results: BV prevalence and incidence was estimated at 48.42% and 81 cases per 100 women years respectively. Controlling for age and education, women with *Trichomonas vaginalis*, *Chlamydia trachomatis*, Herpes Simplex Virus2 and lower socio-economic status were 67%-380%, 31%-472%, 20%-220% and 4%-91% more likely to present with BV respectively. Consistent condom use and being a housewife or student was significantly ($p<0.05$) associated with lower prevalent BV, with a significant interaction between age and education ($p<0.05$). The median time to first BV episode was 9.7 months.

Conclusion: The analysis identifies modifiable risk factors like condom use, injectable contraceptives and treatment of STIs which could potentially decrease the high BV disease burden.

Acknowledgements

Firstly I am greatly indebted to my husband Tinofa Mutevedzi and my daughter Mutsa for bearing my absence for weeks on end while I was at school. For the love, support, encouraging words and editing of the report.

I am truly grateful to my two supervisors, Dr Khin Tint and Professor Marie-Louise Newell for their invaluable input and for the guidance throughout the whole process of compiling this research report. I am deeply thankful to Mitzy Gafos the project leader for the Microbicides trial for unwavering support and guidance and time-off to study.

I thank the Africa Centre for Health and Population Studies for the financial aid and academic access to study materials as well as the Microbicides Development Programme (MDP 301) for data access.

Table of Contents

Declaration ii
Abstract iv
Acknowledgements v
List of figures vii
List of tables viii
Nomenclature ix
CHAPTER ONE 1
Research setting and background 1
Literature Review 2
Statement of the problem and justification for the research 13
Research question 14
Study objectives 14
CHAPTER 2: METHODOLOGY 16
Setting of the primary study 16
Study population 16
Study design 17
Sample size considerations 17
Data collection 18
Data management 19
Data Processing and Data Analysis Methods 20
Data extraction 21
Data cleaning 22
Data analysis 23
Ethical Considerations 27
Chapter 3: Results 28
Chapter 4: Discussion 41
Limitations 48
Strengths 51
Conclusions and Recommendations 52
References 53
Appendices 57

List of figures

Figure	Page
Figure 2.1: Flow of participants from data extraction to final analysis	24
Figure 3.1: Prevalence of BV per BV Ison and Hay diagnosis grade in 1066 HIV negative women at enrolment into an HIV prevention trial in KwaZulu Natal	31
Figure 3.2: Kaplan Meier curve showing time to first BV episode	38
Figure 3.3: Proportion of BV negative and positive women presenting with symptoms at baseline.	40

List of tables

Table	Page
Table 3.1: Description of baseline characteristics and their unadjusted association with prevalent BV	29
Table 3.2: Multivariate analysis of factors associated with BV determined through Logistic regression analysis	34
Table 3.3: Period/Cohort BV incidence rates during follow-up of 526 baseline BV negative women	36
Table 3.4: Survival function/probabilities showing the proportion of BV free women at different time points of follow-up	37
Table 3.5: Proportion of symptoms per BV status at baseline	39
Table C6: BV scoring by Ison and Hay method	63

Nomenclature

ACDIS -------- Africa Centre Demographic Information System

ASC ---------- Atypical Squamous Cells

BV ---------- Bacterial vaginosis

CT ---------- *Chlamydia trachomatis*

CTU ----------Clinical Trials Unit

HIV---------- Human Immunodeficiency Virus

HSIL ---------- High grade Squamous Intra-epithelial Lesion

ICH GCP ----- International Conference for Harmonisation Good Clinical Practices

LSIL ---------- Low grade Squamous Intra-epithelial Lesion

MDP ---------- Microbicides Development Programme

NG ---------- *Neisseria gonorrhoeae*

PID ---------- Pelvic Inflammatory Discharge

STI ---------- Sexually Transmitted Infection

TV ----------*Trichomonas vaginalis*

UK ---------- United Kingdom

CHAPTER ONE

This chapter gives a brief overview of the research setting and the primary study. It also introduces and discusses relevant literature pertaining to Bacterial Vaginosis (BV), and details the epidemiological and public health concerns relating to BV. The chapter thus outlines the motivation for this analysis.

Research setting and background

The Africa Centre for Health and Population Studies is a demographic surveillance site that runs a Demographic Information System (ACDIS), which covers approximately 85,000 people in 11,000 households in an area of about 438 km^2. ACDIS is a longitudinal prospective cohort study of a population within a defined geographic area in rural Umkanyakude district of northern KwaZulu-Natal [1]. It was established to describe the demographic, social and health impact of the HIV (Human Immunodeficiency Virus) epidemic in a population going through a health transition, and to monitor the impact of intervention strategies on the epidemic.

The Africa Centre is one of six partner sites participating in the Microbicides Development Programme (MDP: http://www.mdp.mrc.ac.uk) which is sponsored by the United Kingdom (UK) Medical Research Council (MRC) and funded by the MRC and UK Department for International Development (DfID). The MDP301 randomised, double-blind, placebo controlled, phase III clinical trial aims to evaluate the safety and efficacy of PRO2000/5 microbicide gel in the prevention of vaginally acquired HIV infection.

At the Africa Centre site sexually active women aged 18 years and over who met the eligibility criteria and consented to participate were recruited into the study for a years'

follow-up. Demographic data was collected once at screening, sexual behaviour data was collected once at screening then 4 weekly. Clinical data was collected at baseline and at approximately 12 weekly intervals. Amongst other things, women were assessed for Bacterial vaginosis (BV) at enrolment then 12, 24, 40 and 52 weeks after enrolment. A secondary data analysis of the BV data was conducted to determine the disease burden and assess the factors associated with BV using Africa Centre data only, results are presented in this thesis.

Literature Review

What is Bacterial Vaginosis?

Bacterial vaginosis (BV) is a term reflective of a combination of factors including a shift from the normal vaginal flora which contains predominantly hydrogen peroxide producing Lactobacillus species such as *L. crispatus* and *L. jensensii,* to a polymicrobial flora that includes gram-variable and gram-negative anaerobes such as *Gardnerella vaginalis*, *Prevotella spp*, peptostretococci, *mobiluncus spp* and mycoplasmas [2-14]. BV is highly variable and both the microbiology of the microbes and the products they produce differ considerably between women with BV [13]. BV is often recurrent with the possibility of a woman having three or more confirmed (clinically by Amsel's criteria or microscopically) episodes of BV in 12 months [15].

Clinical features

BV clinically presents as a thin, grey-white, watery, offensive and non-inflammatory discharge with a vaginal pH greater than 4.5. The discharge may or may not be accompanied by vaginal itchiness [16-21]. The odour is due to amines and can be intensified by mixing

vaginal discharge with a drop of potassium hydroxide (positive amine test) [15, 21]. The discharge presents microscopically as clue cells [14-17, 21].

Causes of Bacterial Vaginosis

Microbiological cause

As mentioned above BV is a result of a shift in the normal vaginal microbial flora. Hydrogen peroxide producing strains of lactobacilli (*L. crispatus and L.Jenseii*) produce compounds such as lactic acid, acidolin and hydrogen peroxide, through conversion of glycogen by oestrogenised vaginal epithelial cells [14, 15, 22-24]. This plays an important role in regulating the halide-HPO-myeloperoxidase antimicrobial system and maintaining the acidic pH in the vagina [4, 10, 14, 15, 24, 25]. Wilson [15] reports that at pH of 4.0 or less both the lactobacilli and the myelopreroxidase system becomes less important as the low pH produces an inhibitory effect on bacterial growth such as *G.vaginalis* this inevitably inhibiting BV. Certain risk factors such as sex and menstruation may cause the vaginal pH to rise, facilitating anaerobic bacterial growth, which may result in a shift in the characteristic vaginal flora resulting in BV [14, 15, 22, 23].

Although there seems to be an important interaction between lactobacilli, hydrogen peroxide production, vaginal pH and overgrowth of BV associated bacteria [10, 15, 22, 26, 27], the exact mechanism for the onset of BV is unknown, making it exigent to prevent recurrence. Many attempts have been made to ascertain what causes the reduction of these bacteria with little success [16-22]. Based on in-vitro studies, *G. vaginalis* has been suggested to facilitate the reduction in essential vaginal lactobacilli in at least some cases of non-specific vaginitis in conjunction with anaerobes such as bacteriodes [15, 16, 19-21]. *G. vaginalis* has also been

associated with BV because it has been found in vaginal secretions of 40%-55% of BV asymptomatic women and in vaginal discharge of all patients with BV [16, 18, 19, 21]. Recent studies have suggested an association between metronidazole-resistant anaerobe *Atopobium vaginae* and BV. This bacteria is uncommon in women without BV [5, 28]

Pathology

G. *vaginalis* together with A. *vaginae, Prevotella spp*, peptostretococci *Mobiluncus spp* and mycoplasmas degrade proteins in the vagina to form the foul smelling amines cadaverine, putrescine and triethylamine [21, 29]. The combination of amines and organic acids produced by the anaerobic bacteria causes the exfoliation of the vaginal epithelial cells, resulting in the non-inflammatory vaginal exudates [21]. The disturbed pH allows the gram negative and gram-variable bacilli to adhere to the epithelial cells forming the "clue" cells. . It is important to note that BV is not caused by a single organism but is rather a shift in the normal lactobacillus species to a poly-microbial flora consisting of various anaerobes [16, 19, 21, 30].

Diagnosis

Diagnosis of BV is elusive because BV has not yet been exclusively associated with one or more particular organisms. As a result, microscopy is a better diagnostic tool than culture. Microscopically, BV is diagnosed when "clue" cells are identified after heat fixing of the vaginal smear and staining it by Gram's Method [11, 16-19]. Still the consistency of microscopy diagnoses has been debated [31, 32] because it is subjective to the person reading the microscope slide (inter-observer variation). It is also dependent on good quality standards of specimen collection, preservation and staining before microscopic observation. Clinical

diagnosis does not offer a solution to the diagnosis dilemma either, as it is also subjective to both patient (recall bias) and clinician (observer bias) thus misdiagnosis remains high in both developed and developing countries [11, 16, 18, 19, 21, 28].

Traditionally, the diagnosis of BV based on Amsel composite criteria [11, 32] is made when three of the four following criteria are met: homogenous discharge, pH>4.5, positive amine test and quantitative determination of clue cells representing more than 20% of vaginal epithelial cells [16, 18-20]. This approach was regarded the gold standard until 1983, when a publication by Spiegel et al recommended a new method (Nugent's method) that diagnosed BV by the appearance of a gram stained slide [11, 32]. A scoring system was assigned based on the presence of Lactobacilli *spp*, Gardnerela *spp* and other morphotypes and their relative quantities [7, 11, 32]. The use of scoring systems has allowed recognition of a continuum between normal vaginal flora and BV as opposed to the all or nothing criteria provided by Amsel's method [11, 33]. Several subsequent modifications have been made to the Nugent method finally resulting in the currently used Ison and Hay method [32].

The Ison and Hay method is based on a diagnostic transition from the ideal lactobacilli flora to abnormal flora in five groups ranging from no bacteria (grade 0), normal vaginal bacteria (grade I), intermediate shift from normal vaginal flora resulting in an increase in BV associated bacteria (grade II) and predominantly BV associated bacteria (III) to only gram positive cocci (grade IV) [11, 34]. The grading is based on the relative quantities of the bacteria as defined in Table C6 in Appendix C. However, the sensitivity of Ison and Hay scoring is not well defined and grade 0 (no bacteria) might potentially still be BV cases but with inadequate vaginal specimen, especially in women who insert fingers in the vagina while

washing (finger cleansing). Some authors explain grade 0 as a lack of bacteria after broad spectrum antibiotics [11, 33]. Taylor [33] reports that women with grade II BV (intermediate shift) are more likely to have some of the symptoms of the Amsel's criteria than were women with normal flora [11, 33]. Grade II and III are diagnostic of BV but there is no evidence to confirm that grade 0 and IV are associated with BV [11].

These methods have been validated in genitourinary medicine clinics in the United Kingdom (UK) [11, 33] but extrapolation of results to African settings must be cautionary because little is known about the pattern of vaginal micro-flora associated with BV in Africa [7, 11, 32].

Prevalence of BV

BV is the most common vaginal infection affecting women of child bearing age [14, 23, 30, 31], partly because it is a mixture of unexplained infections. Prevalence rates vary according to the population studied and the geographic location. The reasons for the differences in prevalence are not completely understood but have been attributed to different vaginal hygiene practices associated with culture, ethnicity and socio-economic status [14, 22, 23].

BV prevalence in pregnant women at their first antenatal visit varies from 11% in the United States to 24% in Tanzania. Cross sectional community surveys have reported prevalence rates of 20%-23%, 24%-37%, 48% and 49% in Burkina Faso and Malawi, Tanzania, Gambia and Kenya respectively [7, 14, 25], with higher prevalence in sub-Saharan Africa (20%- >50%) than in industrialized countries (10%-30%) [8, 35]. In South Africa, some studies of antenatal clinic attendees have given prevalence estimates of 25% whilst others gave estimates as high as 52% [12, 25, 31]. In a study of 208 women attending primary health facilities in urban KwaZulu Natal, BV prevalence was 35% overall, but 43% in Sexually Transmitted Infections

(STI) clinics (n=51), 36% in copolscopy clinics (n=50) and 36% in family planning clinics (n= 55) [12].

A situational analysis in 2002-3 in KwaZulu Natal province in South Africa revealed that due to the diagnostic challenges discussed above, few facilities were able to accurately diagnose BV in women [36], thus it is possible that the disease burden is underestimated.

Risk factors for BV

Some demographic, sexual and socio-behavioural factors including black ethnicity, younger age, smoking, contraceptive use and vaginal douching have been associated with an increased the risk of BV [5, 15, 22]. Little progress has been made in identifying and determining the relative significance of factors involved in BV acquisition and recurrence [23, 30].

Vaginal hygiene practices and BV

The use of genital cleansing products and vaginal inserts is common practice in many populations around the world, although they are known to be associated with increased risk of STIs, Pelvic Inflammatory Disease (PID), disturbances of the vaginal flora and ectopic pregnancies [22-24, 37]. With the introduction of vaginal microbicides, interest in the use of vaginal cleansing products and inserts has increased [37], yet the practice is rarely evaluated in studies of women's genital infections and symptoms. Consequently, the role that these may have on BV genital symptoms, clinical findings, laboratory testing results, or the incidence of infection has not been fully explained [37].

Studies have shown a positive association between vaginal douching particularly with antiseptic agents, and susceptibility to BV [7, 22-24, 26]. Authors have speculated that

changes in the vaginal flora due to internal cleansing might allow rapid proliferation of potential pathogens during the altered state, increasing the risk of BV. However, reverse causality could not be ruled out, where BV causes women to douche in an effort to rid themselves of the itchiness, discharge or smell [7]. In contrast, in the Bali STD/AIDS study among 625 commercial sex workers, women who internally cleansed the vagina after every sex act were less likely to report discoloured vaginal discharge and genital odour. There was no significant association between vaginal cleansing and BV in this study [37]. It is noteworthy that frequent vaginal cleansing may lead to inaccurate clinical diagnosis of BV due to the washing out of the vaginal discharge and odour, potentially biasing the association of BV and vaginal cleansing towards the null.

Socio-economic status and BV

o *Education and BV*

There is evidence that women with low educational attainment are less likely to have good reproductive health knowledge. Some studies have also demonstrated an association between lower education attainment and the use vaginal inserts and vaginal cleansing products [22, 37] which are known risk factors for BV. This may influence a positive association between low educational attainment and increased BV cases, as has been observed in some studies [22, 37].

o *Occupation, income and BV*

In a case control study of 200 women in the United States [22] and in a large population based survey of 2,494 women in India [38], a positive association between susceptibility to BV and manual occupations and low income was observed. However, the authors speculated that this may be a confounded association mainly as a result of vaginal douching in gender disadvantaged women in this socio-economic group [22, 38].

Sexual activity and BV

Studies [4, 23, 27, 37] have linked BV with sexual behaviour including a recent change in sexual partner, multiple partners and unprotected sex, although in the study of 250 participants by Schwebke et al [23] associations did not reach statistical significance. Having a new sexual partner has been independently associated with an increase in BV irrespective of the frequency of sexual intercourse suggesting that exposure to a new partner is a more important risk factor than the number of episodes of intercourse [14, 15, 26, 27]. However worth noting is the fact that semen is alkaline and may activate the odour of amines in BV positive women [21]. Thus a woman may have had BV before having a new sexual partner and then becomes aware of the symptoms after sexual intercourse, leading to a spurious relationship between BV and sexual intercourse.

There has been much debate regarding whether or not to classify BV as an STI and this discussion remains controversial partly because causal pathogens are not well understood. *G.vaginalis* is also found in the male urethra [20, 21] thus albeit not scientifically proven, [39] BV infection is probably transmitted sexually although infectivity with G.vaginalis appears to be low [36, 40]. Condom use decreases incidence of BV in women [19, 20], also suggesting the possibility of sexual transmission. In contrast, in five out of six trials, partner treatment had no benefit for the women in terms of reducing recurrent BV episodes [5, 15, 19]. BV has also been detected in women who have never had sex [10, 14]. Consequently BV is not defined as an STI although it is highly likely that sexual activity plays a role in acquisition of BV.

BV and Sexually Transmitted infections (STIs)

Studies including a systematic review by Wilson, have revealed that lactobacilli associated positively with BV are also positively associated with Neisseiria gonorrhea (NG) and Chlamydia trachomatis (CT) infection, but less likely to present with concurrent *Trichomonas vaginalis* (TV) and Herpes Simplex Virus 2 (HSV2) [14, 15, 23, 24]. On the other hand, some researchers have reported a strong association between abnormal vaginal ecology and HSV2 and TV [23, 29, 41] since TV may also be caused by replacement of lactobacilli [24].

It is hypothesized that BV may be a result of endogenous or sexually transmitted infection with an organism that competes or interferes with lactobacilli [41]. On the other hand, disturbances in vaginal flora may lead to increased acquisition of other sexually transmitted infections [24, 29, 41, 42]. One author suggests that an association between BV and other STIs may be a reflection of biological interactions in the vaginal micro flora rather than shared risk factors for their acquisition.[14]

BV and HIV

Cross-sectional studies in North America, Asia and Africa show that women with BV are more likely to have and transmit HIV, due to lack of lactobacilli resulting in increased risk of HIV sero-conversion and STI acquisition [4, 13, 14, 24, 27, 29, 37, 41]. However reverse causality could not be ruled out. In vitro studies have indicated that low vaginal pH reduces HIV infectivity, and hydrogen peroxide producing lactobacilli have a viricidal effect on cell-free HIV-1 whilst G.vaginalis and other BV associated microbes activate HIV resulting in increased HIV expression [13, 24, 41, 43]. In populations with high BV prevalence, BV may be one of the most important risk factors of HIV infection.

Contraception and BV

Results from previous research studies have indicated that some forms of contraception may be associated with BV. Several studies have shown hormonal contraception to be protective against BV [16-21, 23, 24] whilst longitudinal studies of BV incidence have shown an increase in BV in women using Intra Uterine Device (IUD) [15, 44]. The exact physiological mechanism for effect between IUD and BV is unknown but it is speculated that increased oestrogen from hormonal contraceptives may facilitate bacterial lactic acid production by increasing levels of available glycogen in epithelial cells, thereby inhibiting BV-associated organisms [44].

Age and BV

The presence of lactobacilli (L.crispatus and L. jenseii) has been positively associated with being over 20 years of age [15]. Adolescent females represent a menarchal population that is newly influenced by reproductive hormones and is also susceptible to BV due to changes in vaginal tissues [2, 4, 15]. After menopause, the vaginal environment gradually reverts back to the pre-menarchal state. These vaginal physiological variations influence BV prevalence with age [2, 4]. However, in one prospective cross sectional study, BV prevalence peaked at around 30 years, possibility due to infection being incubated and symptoms presenting at a later stage [14].

Sequelae of BV

Irrespective of its status as a Sexually Transmitted Infection (STI), BV is not a minor condition. The sequelae of BV include nonchlamydial and nongonococcal pelvic inflammatory discharge (PID), urinary tract infections, endometritis, preterm labour, membrane rapture, septicaemia, meningitis, postpartum complications in the infant and

neonatal deaths [9, 12-14, 19, 29, 30]. In addition, BV strongly predisposes both pregnant and non-pregnant women to acquisition of STIs such as NG, CT and HIV [12, 30, 40].

The relationship between BV and preterm birth has been demonstrated in at least 12 epidemiologic studies conducted on five continents among pregnant women at different gestational stages [13]. In sub-Saharan Africa, the proportion of pregnancy complications attributed to BV is not well studied, hence not ascertained. In a small study in South Africa, BV was diagnosed in 55 out of 77 women with pregnancy complications and in 32 of 48 women with obstetric complications [8]. Even though the study was small, it is evident that BV is associated with adverse pregnancy outcomes. This raises a need for further studies into the aetiology of BV, its impact on women and ultimately its control, in developing countries.

Treatment of Bacterial vaginosis

Treatment of symptomatic BV is usually by administering high doses of metronidazole [11, 16, 18, 21]. It is worth highlighting this use of antibiotics to treat a condition that is an imbalance rather than an infection. This might be the cause of the high rate of BV relapse [10]. Since vaginal flora can be in a dynamic state, changing day by day, it is recommended that asymptomatic women are not treated [33].

In conclusion, BV is a frequently recurrent, distressing condition mainly prevalent in women of child bearing age. It has a poorly defined aetiology, is difficult to treat and prevalence rates vary significantly across geographic areas. It is associated with a considerable proportion of pregnancy complications and with increased incidence of STIs and HIV.

Statement of the problem and justification for the research

As highlighted in the review above, BV has highly clinically significant sequelae [9, 12-14, 19, 29, 30], yet there are many unanswered questions about its aetiology making management of infection difficult and often idiosyncratic [9, 15-22].

From literature reviews, studies in South Africa have used the Amsel's criteria and Nugent's scoring method but not the Ison and Hay BV scoring method due to it being a relatively new diagnostic scoring method. It is worth while computing prevalence and incidence estimates using new diagnostic technologies especially in the African region were such data is not only lacking but largely variable.

Routinely collected data in rural KwaZulu Natal through the ACDIS has given high HIV prevalence estimates of 27% in women aged 15-49 years, coupled with an incidence rate of 4.0 per 100 person-years (95% confidence interval (CI) 3.3-4.7) [45, 46]. In a microbicide feasibility study of 882 participants conducted in the same area, HIV prevalence and incidence rates in sexually active women aged 16-50 years were approximated at 50% and 12.6 (95% CI 8.2-19.6) per 100 women years of follow-up [47]. BV's positive association with HIV in this area with high HIV disease burden makes further research into the aetiology of BV a public health priority in order to inform prevention strategies.

Reducing BV incident and prevalent cases in this area, where an estimated 9,057 women become pregnant each year in the Hlabisa health sub-district [48] might considerably curb adverse pregnancy outcomes attributable to BV.

Determining the burden of BV and associated factors is also critical in terms of developing effective behavioural change interventions that may be required to curb the disease burden. The health services in this area are already overburdened by HIV and the general community is within the lower economic bracket with the main source of income being social grants [39, 48, 49]. Hence maintaining health in this area is of paramount importance to ensure that limited financial and health resources are not further strained.

This study seeks to determine the disease burden of BV in rural Northern KwaZulu Natal through the use of a large data set of clinical, laboratory and behavioural outcomes. It also aims to investigate the factors associated with BV. This analysis is important from both an epidemiological and public health perspective, and has particular relevance in this part of KwaZulu Natal due to the overall burden of HIV.

Research question

What is the disease burden of BV and what are the factors associated with the disease in sexually active women aged 18 years and over, enrolled in an HIV prevention trial in Northern KwaZulu Natal?

Study objectives

The main objectives of this analysis are as follows:

1. To measure the point prevalence of BV at enrolment into the trial.
2. To determine whether the following factors predispose women to BV at baseline
    - Demographics
    - Vaginal hygiene practices
    - Sexual behaviour practices
    - Socio-economic status
    - Family planning methods
    - Infection with another STI

- An abnormal pap smear

3. To measure the incidence of BV in women followed up in the trial from March 2006 to March 2008 and to quantify the time to first BV episode.
4. To determine the proportion of asymptomatic BV cases and assess the concordance between laboratory diagnosed BV grades and clinical symptoms.

CHAPTER 2: METHODOLOGY

This chapter details the study methodology, including data collection and management, specimen collection and analysis, data extraction cleaning and analysis. Only the relevant methodological issues employed in the primary trial are highlighted in this chapter. Comprehensive details on methodology of the primary trial may be obtained from the Microbicide Development Programme (MDP) 301 Protocol version 1.3 [47].

Data was collected in an international multi-centre, randomized, double blind, placebo-controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO2000/5 gels for the prevention of vaginally acquired HIV infection [47]. Local analysis of data was done to determine the disease burden of BV, its determinants, and the average time to the first BV episode.

Setting of the primary study

The Africa Centre for Health and Population Studies is located in Somkhele in the rural district of Umkhanyakude in northern KwaZulu Natal, South Africa. The study area consists of land under tribal authority and a township under municipal authority [1] .The MDP trial recruited women from the community, using various methods including snowballing and enrolled at 3 primary health care clinics. A total of 1,085 women were enrolled into the primary study by February 2008.

Study population

Women attending the KwaMsane, Mtubatuba and Madwaleni primary health care clinics were enrolled into the primary trial using the following **eligibility criteria**; 18 years and over,

sexually active, HIV negative and willing to undergo HIV tests at 12 weeks interval, not pregnant nor 6 weeks post-partum, willing to undergo genital exams, having no latex allergy, willing to receive condom education and willing and able to give informed consent. Each woman was followed up for a total of 52 weeks.

Study design

For the analysis in this study, a cross-sectional analytical design was used to determine BV point prevalence at enrolment, in the study population of 1,085 women and to establish associations between baseline risk factors and BV at initiation. BV incidence was determined with a prospective cohort design (March 2006 to March 2008) and to compute time to first BV episode in women who were BV negative at baseline.

Sample size considerations

From literature review, BV prevalence has been approximated at around 50%, thus a prediction of between 450 and 500 BV positive women was made from the available sample size. Following precision calculations given below, a sample size of 1085 women would give enough precision to ascertain associations between BV and various risk factors at 5% significance level. With this sample size, the precision (half-width of the 95% confidence interval) of results is approximated at 0.03:

$$\text{Precision} = 1.96\sqrt{(p(1-p)/N)},$$

p is unknown and set at worst case scenario of 0.5, N is sample size (1,085)

The fixed sample size of 1,085 will not bear any limitation on analysis, since it is large enough to detect any associations if they indeed exist.

Data collection

Demographic data was collected at the screening visit which was up to a maximum of 6 weeks prior to enrolment. Sexual behaviour data including number of sexual partners, type of partner and number of sex acts were collected at baseline and at 4 weekly intervals. Clinical data including vaginal specimens, self-reported symptoms and clinical signs observed during pelvic examination were collected at baseline and at approximately every 12 weeks. Data collection methods employed included the use of structured sexual behaviour, clinical and demographic Case Report Forms (CRFs), and laboratory reports generated from testing of biological specimens collected from the participants at baseline and at follow-up visits. Sample CRFs are appended in appendix D.

Specimen collection, transportation and processing

Specimens were collected from all participants regardless of clinical symptoms. Specimens were transported to laboratories located in Durban, about 220kms away from the site and initial processing was done within 24 hours.

Cervical swabs (Gen-Probe Aptima- Gen-Probe Incorporated, San Diego, CA, USA) and vaginal swabs (BD ProbeTec - IVD, Becton, Dickinson and Company, USA) were collected at baseline and at week 24 and inserted in transport media during transportation. These were tested for NG (Gen-Probe Aptima), CT (Gen-Probe Aptima) and TV (PCR). Whole blood was also collected 12 weekly and tested for syphilis (Rapid Plasma Reagin - B&D MacroVue followed by Treponema pallidum hemagglutination assay - Omega Diagnostics Immutrep), and HSV2 (Focus assay). Cervical smears were collected at baseline only, preserved using cytofix and examined microscopically for abnormal cervical cells

BV diagnosis

A high vaginal smear was collected at enrolment and at approximately 12 week intervals.. The slides were transported in a protective jacket to the laboratory. Vaginal flora was characterised by Gram's stain method and slides viewed under a microscope. Presence of "clue" cells was used to determine BV status through the Ison and Hay BV scoring method as indicated in Table C6 (Appendix C). To validate and verify results, 5% of all specimens were read by two individuals who were blinded to each other's result. BV outcome was broadly defined as BV negative and BV positive where grade III was a positive result and Grade 0, I, II and IV were defined as BV negative.

Data management

Data were double entered into an Access front end database in SQL server that was centrally designed by the UK MDP CTU for use by all primary study sites. Data were verified to see if the two entries corresponded before saving. Inconsistencies were addressed at data collection level and data entry and were resolved within 14 days of the visit date. Clinic managers conducted 100% quality control of all data forms at each clinic before the forms were sent for data capture. Quality checks for inconsistencies and missing data were also run fortnightly centrally by the UK MDP CTU. Any inconsistencies were sent to the site to be resolved within 14 days. Quarterly monitoring and yearly audits were conducted on the clinical site and testing laboratories to ensure that the conduct of the study was according to ICH GCP and GCLP guidelines and data obtained was valid and accurate. All participating laboratories were enrolled into Quality Assurance Programs with Contract Laboratory Services (CLS) and National Health Laboratory Services (NHLS), South Africa.

Staff training

To ensure standardised and accurate data collection from the interviewers, extensive training was provided by both the site senior staff as well as by the MDP CTU. Training included interview skills, CRF completion, genital exams and specimen collection, as well as ICH and South African GCP, GCLP and Ethics principles. Training evaluations were conducted and written exams completed to assess comprehension prior to being delegated responsibilities on the trial.

Validity and reliability checks

To assess the validity and reliability of the research methods employed in the primary study, feasibility (2004) and pilot (2005) studies were conducted and these are detailed in the MDP 301 protocol [47].

Data Processing and Data Analysis Methods

Data was analysed using Intercooled Stata 10 (Stata Corporation, College Station, Texas, USA).

Outcome variables

BV outcome: was defined as BV negative or BV positive.

Explanatory variables/ risk factors

Socio-economic demographics

- age* (categorical variable), religion* (categorical- nominal), education* (categorical – ordinal - highest level attained will be considered) and type of employment (categorical- nominal - manual or non manual).

Vaginal cleansing practices

- use of vaginal inserts (binary- yes or no), frequency and time of insertion and type of insert, frequency and timing of washing.

Family planning methods

- (categorical- traditional oral/inserts, barrier, oral, injectables, sterilisation, condom and post menopausal).

Sexual behaviour practices

- Type of partner – long term or any other once off or short term partner number of partner/s (discrete), condom use (categorical – never, always, sometimes), frequency of sex acts in the last week and last month (discrete) and age of sexual debut (categorical).

Infection with another STI

- infection with NG, CT , HSV2 and TV*

Having an abnormal pap smear

- LSIL HSIL and ASC

Clinical symptoms

- Discharge (yes/no); offensive discharge; discharge source (vaginal/cervical/cervico-vaginal) and pH >4.5

*Possible confounding variables are asterisked and assessed further during analysis.

Data extraction

Eight datasets comprising of demographics, condom use, sexual debut, contraception, sex acts, vaginal practices, genital exam and laboratory data were extracted from the main MDP

database. The datasets were saved as text (.txt) files and imported individually into STATA 10.

Data cleaning

Each dataset was individually cleaned. Variables were tabulated, cross tabulated, summarised, graphed and browsed to check for inconsistencies and outliers. Distribution of various variables was assessed. Inconsistent data as well as clinically implausible data was checked against the Case Report Forms (CRF) to verify and correct data. Inconsistent demographic and family planning data was also verified and updated on the dataset. Missing variables were manually checked on the CRF as well as on the laboratory logs to confirm and update data. Specimens which were missed or lost and recollected at a later date were added to the initial time point, by replacing the missing result with the result for the re-analysis/re-collection. A total of 40 individual test results were updated. The age variable was compared to date of birth to verify if age was correct. Variables such as, but not limited to, age, education status, type of employment, frequency of condom use and frequency of sex acts was categorised based on existing literature. Unadjusted Odds Ratios (UOR) and clinical reasoning were also used to inform which categories to combine without distorting results. The various categories were labelled accordingly before analysing them. After categorisation the numbers of participants in each group were assessed to check if they were large enough to determine statistical significance. The best determined categories were then used for analysis. All laboratory tests were categorised as binary (positive or negative). For survival analysis, duplicates were identified and removed before reshaping data into wide format. For the Kaplan Meier analysis, a positive BV laboratory result after enrolment was defined as a failure and data was set to survival data.

Data analysis

Before analysis, the different datasets were merged on trial number which was the participant unique identifier, into one main dataset that was used for analysis.

The flow of participants to the final datasets that were used in the different analysis is shown in Figure 2.1 below. The 68 participants who had missing data on one or more categories in the final model were described and their effect on the final model was assessed by adding them into the final model as a missing category. Log likelihood ratios and change in Odds Ratios, 95% Confidence Intervals and standard errors were assessed to measure the impact of not having these participants on the association between BV and independent explanatory variables.

Descriptive statistics:

The distribution of variables in the population according to BV status was assessed. The distribution of baseline explanatory variables was compared between the BV positive and BV negative categories using the t-test for continuous variables, Unadjusted Odds Ratio (UOR) and Chi-squared and Fischer's exact for categorical variables [50]. Summary statistics including but not limited to means, medians and ranges were computed per BV status. Results were presented in the form of flow charts, pie-charts, bar charts, line graphs and tables. Variables were assessed for colinearity [50] to ensure that correlated variables are not both included in the main regression model.

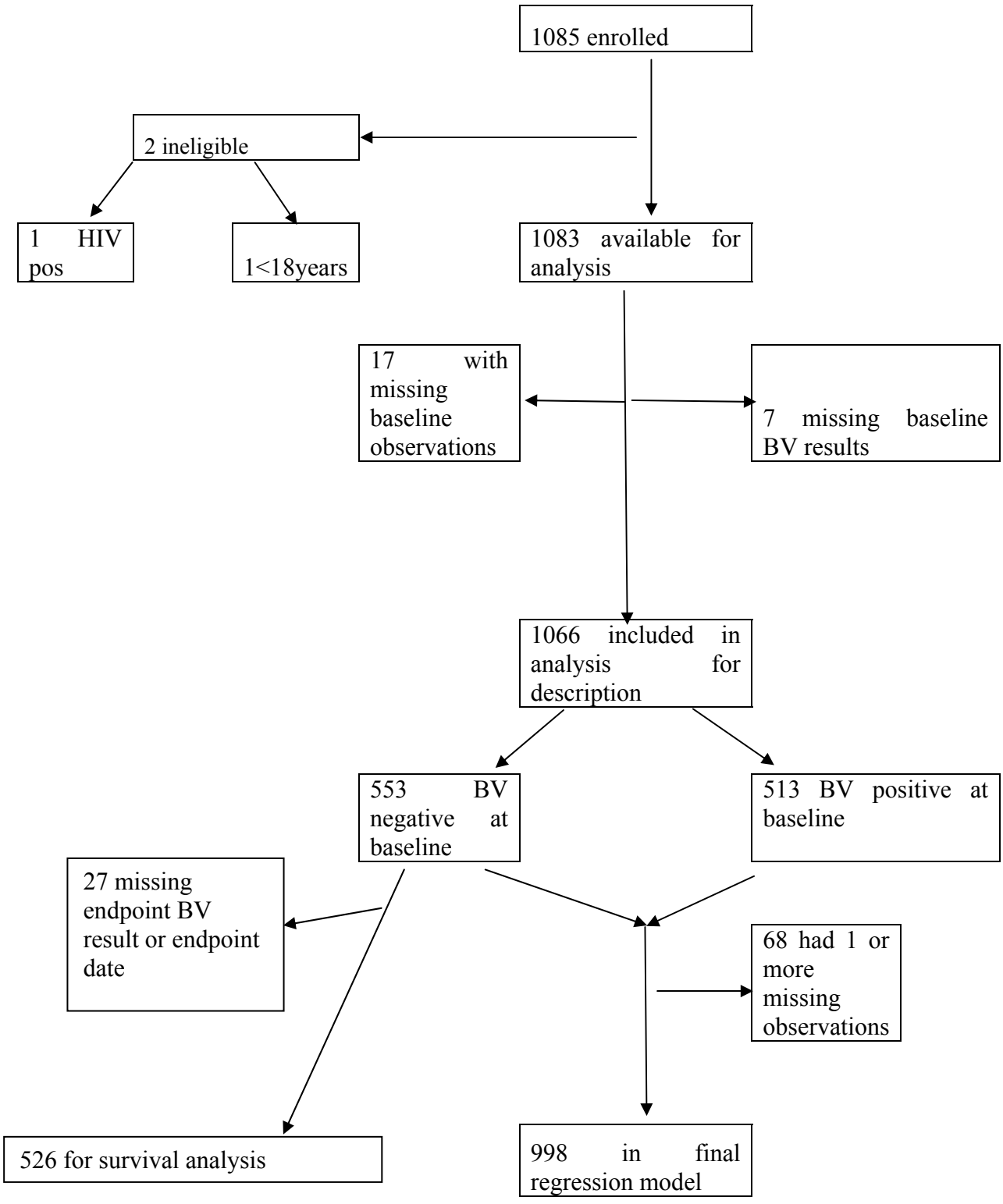


Figure 2.1: Flow of women aged> 18 years enrolled between March 2006 and March 2008 in the MDP 301 in rural KwaZulu Natal, for data extraction and final analysis

Analytical statistics:

All results were presented with significance level and confidence interval. Any result with P<0.05 was considered statistically significant unless otherwise stated.

The study objectives were achieved as detailed below:

1) To measure the point prevalence of BV at enrolment into the trial (week 0).

Prevalence was calculated as the number of BV positive cases/ total study population at enrolment

2) To determine whether the following factors predispose women to BV

- Demographics
- Socio-economic status
- Vaginal hygiene practices
- Family planning methods
- Sexual behaviour practices
- Infection with another STI
- An abnormal pap smear

Null hypothesis: there is no association between the above factors and BV outcome

After univariate analysis, variables that were significant in the univariate model at 15% (p=0.15) were individually added into the final multivariate logistic model, observing change in OR to assess potential confounders and interactions. A less conservative significance level of 15% was used to ensure that any confounded variables in the univariate analysis would not be missed out of the multivariate analysis. Stratification analysis was done to control for potential confounders. Interaction terms were generated and introduced into the model where necessary. Backward elimination and forward selection procedures were employed for comparison with the final model built. Log likelihood ratio tests were also done to determine the best parsimonious model by comparing the log likelihood of the model before and after

addition of a variable. Results from the multivariate model were reported at 5% significance level (p=0.05)

3) To measure the incidence of BV over the 2 year period from March 2006 to March 2008 and to quantify time to first BV episode.

Kaplan Meier Survival analysis [51] was used to compute incidence and to determine average time to first BV episode. Survival probability graphs were computed to estimate the probability of survival as a function of time from entry into the trial. The period between the starting time and the date of the last follow-up observation before the analysis was recorded for each subject. The percentage of women still BV negative at the end of each specified week of visit was used to estimate the probability that a woman will be still BV negative at the end of any given week/visit.

4) To determine the proportion of asymptomatic BV cases and assess the concordance between laboratory diagnosed BV grades and clinical symptoms.

Null hypothesis: there is no difference in symptoms between women with or without BV

Symptoms were categorised into the number of symptoms presenting and vaginal discharge was further assessed to determine the source of discharge as vaginal, cervical, cervico-vaginal and 'not possible to tell'. Descriptive analysis was used to quantify the proportion of asymptomatic and symptomatic cases in the different BV grades. A proportions test was done to assess whether there was a significant difference in the proportion of symptomatic BV positive women compared to BV negative women [50].

The results obtained from this analysis were collated and summarised and are presented in the next chapter.

Ethical Considerations

The primary study documentation was submitted to the UKZN Biomedical Ethics Committee and to the South African Medicines Control Council for approval and approval was granted, approval number T111/05 and N2/19/8/2(1629) respectively. No additional approval was required from this committee for this study as it is a secondary data analysis. The protocol for this secondary data analysis was submitted to the University of Witwatersrand Ethics Committee for educational purposes. Approval was granted, approval number M080332.

Chapter 3: Results

This chapter details the results obtained from the methodology above. Short summaries are included to consolidate the tables and graphs.

Description of the study sample

The mean and median age for the 1,066 participants (as per participant flow) was 34.86 (standard deviation 11.57) and 35.0 years respectively with an inter-quartile range of 24-45 years. There was no significant difference in age between BV positive women (35.41yrs) and BV negative women at baseline (34.35yrs) p=0.13. The age ranges were also similar for BV positive (18-69yrs) and BV negative (18-65yrs) women.

All women were in partnerships with the majority in long term relationships; only 12 women (1%) reported having a casual partner, defined as a sexual encounter with a partner other than the long term partner. Although condom use was relatively low with 568 (56.8%) of women reporting never having used a condom, consistent use was high with 401 (92.8%) of the 432 women reporting condom use, always using a condom. This was well correlated with the women reporting using a condom at the last sex act. Eight hundred and thirty seven (78.6%) of participants had their sexual debut between ages 15-19 yrs. Of the 600 women (56.29%) on contraception, 52% were on injectables. Only 7 women (0.64%) reported ever using vaginal inserts, whilst 207 (19.42%) reported washing the vagina after sexual intercourse.

The number of assets and fuel source used in the household were well correlated, with most people who reported to own no assets using wood as source of fuel. The most prominent

religion was Zionist (46.25%). Fifty one percent (51%) of women had secondary or tertiary education and only 191 (17.93%) of the women were employed.

Table 3.2: Description of baseline characteristics of 1066 women enrolled in the MDP301 trial in rural KwaZulu Natal between March 2006 and March 2008 and their unadjusted association with prevalent BV

Sample characteristics		Total sample	BV Neg (%)	BV Pos (%)	Total Pos/Neg	OR (95% C.I)	p-value
Age	18-34	1066	292 (54.89)	240 (45.11)	532		
	35+		261 (48.88)	273 (51.12)	534	1.27 (1.00-1.62)	0.050
Education	Primary	1066	251 (47.90)	273 (52.10)	524		
	Secondary		302 (55.72)	240 (44.28)	542	0.73 (0.57-0.93)	0.011
Religion	Christian	1066	101 (51.01)	97 (48.99)	198		
	Zionist		252 (51.12)	241 (48.88)	493	1.0 (0.72-1.38)	0.98
	Shembe		134 (51.34)	127 (48.66)	140	0.99 (0.68-1.43)	0.94
	None		11 (50.0)	11 (50.0)	22	1.04 (0.43-2.51)	0.93
	Other		55 (59.78)	37 (40.22)	92	0.70 (0.42-1.56)	0.16
Employment	Unemployed	1065	265 (48.98)	276 (51.02)	541		
	housewife		160 (55.94)	126 (44.06)	286	0.76(0.57-1.01)	0.057
	employed		95 (49.74)	96 (50.26)	191	0.97(0.70-1.35)	0.858
	student		33 (70.21)	14 (29.79)	47	0.41(0.21-0.78)	0.007
Employment type	Unskilled	184	28 (14.66)	24 (12.57)	52		
	Household		19 (9.95)	11 (5.76)	30	0.87 (0.45-1.68)	0.684
	Services		47 (24.61)	55 (28.80)	102	1.00 (0.58-1.72)	0.997
Number of household assets	4	1066	77 (55.40)	62 (44.60)	139		
	3		176 (54.66)	146 (45.34)	322	1.03 (0.69-1.54)	0.884
	2		119 (46.85)	135 (53.15)	254	1.41 (0.93-2.13)	0.106
	1		146 (51.96)	135 (48.04)	281	1.15 (0.76-1.73)	0.507
	0		35 (50.0)	35 (50.0)	70	1.24 (0.70-2.21)	0.461
Fuel source	Electricity	1066	202 (57.55)	149 (42.45)	351		
	Gas		42 (47.73)	46 (52.27)	88	1.48 (0.93-2.37)	0.098
	Paraffin		72 (51.06)	69 (48.94)	141	1.30 (0.88-1.92)	0.191
	Wood		237 (48.77)	249 (51.23)	486	1.42 (1.08-1.88)	0.012
Contraception	No	1066	215 (46.14)	251 (53.86)	466		

Sample characteristics		Total sample	BV Neg (%)	BV Pos (%)	Total Pos/Neg	OR (95% C.I)	p-value
Contraception	Pills	1066	26 (44.07)	33 (55.93)	59	1.09 (0.63-1.88)	0.76
	injectables		186 (59.24)	128 (40.76)	314	0.59 (0.44-0.79)	<0.001
	sterilised		44 (50.57)	43 (49.43)	87	0.84 (0.53-1.32)	0.447
	condom		82 (58.57)	58 (41.43)	140	0.61 (0.41-0.89)	0.010
Sex debut-yrs	<15	1064	33 (47.83)	36 (52.17)	69		
	15-19		448 (53.52)	389 (46.48)	837	0.80 (0.49-1.30)	0.36
	20+		72 (45.57)	86 (54.43)	158	1.09 (0.62-1.92)	0.75
Casual Partner	No	1066	550 (52.18)	504 (47.82)	1054		
	Yes		3 (25.0)	9 (75.0)	12	3.27 (0.88-2.16)	0.076
General condom use	Never	1000	281 (49.47)	287 (50.53)	568		
	Sometimes		15 (48.39)	16 (51.61)	31	1.04 (0.51-2.15)	0.91
	Always		223 (55.61)	178 (44.39)	401	0.78 (0.60-1.01)	0.06
Condom use at last sex act	No	1064	370 (50.75)	359 (49.25)	729		
	Yes		182 (54.33)	153 (45.67)	335	0.87 (0.67-1.23)	0.28
Sex last 7days	Yes	1000	361 (51.28)	343 (48.72)	704		
	No		158 (53.38)	138 (46.62)	296	0.92 (0.70- 1.21)	0.544
Wash after sex	No	1066	454 (52.85)	405 (47.15)	859		
	Yes		99 (47.83)	108 (52.17)	207	1.22 (0.90-1.66)	0.194
Syphilis	Negative	1066	525 (52.24)	480 (47.76)	1005	1	
	Positive		28 (45.90)	33 (54.10)	61	1.29 (0.77-2.16)	0.337
Neisseria gonorrhoea	Negative	1066	510 (51.78)	475 (48.22)	985	1	
	Positive		43 (53.09)	38 (46.91)	81	0.95 (0.60-1.49)	0.821
Chlamydia trachomatis	Negative	1066	533 (52.62)	480 (47.38)	1013	1	
	Positive		20 (37.74)	33 (62.26)	53	1.83 (1.04-3.24)	0.037
Trichomonas vaginalis	Negative	1066	510 (54.78)	421 (45.22)	931		
	Positive		43 (31.85)	92 (68.15)	135	2.59 (1.76-3.81)	<0.001
Herpes Simplex virus 2	Negative	1065	208 (59.77)	140 (40.23)	348		
	Positive		344 (47.98)	373 (52.02)	381	1.61 (1.24-2.09)	<0.001
Pap Smear	Negative	1010	477 (92.08)	41 (7.92)	518		
	Abnormal		444 (90.24)	48 (9.76)	492	1.26 (0.81-1.95)	0.303

1. *Prevalence of BV at enrolment into the trial*

Overall, at enrolment 513 out of 1066 women were diagnosed with BV giving a baseline prevalence of 48.42%, (CI: 45.40% – 51.43%). The pie-chart below shows the prevalence of each BV grade. Of the 51.58% of women who were BV negative, 27.97% had normal vaginal flora, 19.52% had intermediate flora whilst 2.88% had no bacteria.

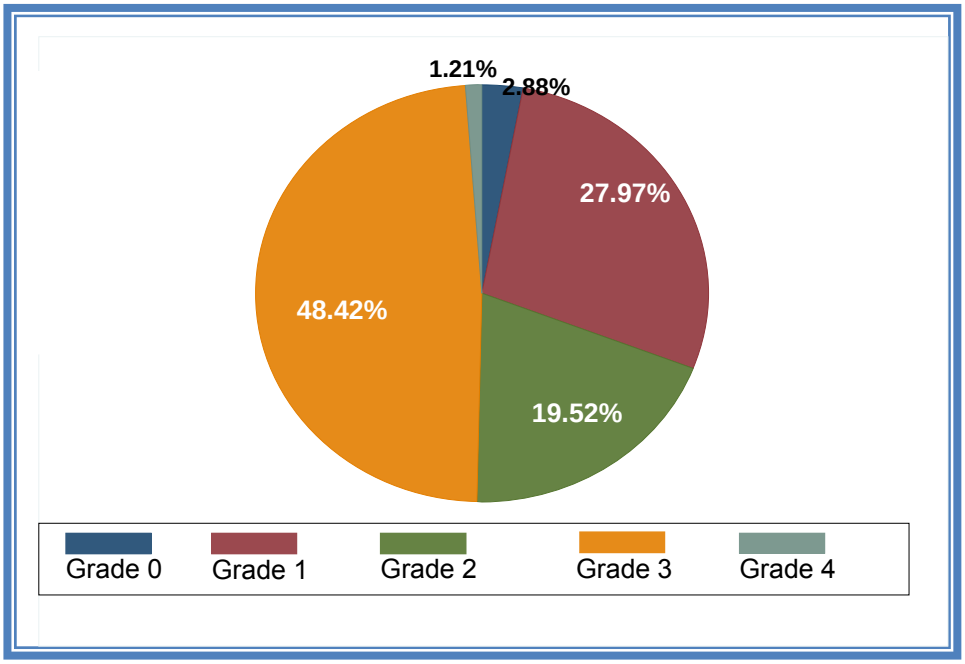


Figure 3.1: Prevalence of BV per BV Ison and Hay diagnosis grade in 1066 HIV negative women at enrolment into an HIV prevention trial in KwaZulu Natal

2. *Determinants of BV*

i. <u>BV and STIs</u>

Table 3.1 shows the prevalence of each STI stratified by baseline BV. The overall baseline prevalence rates were estimated at 5.72% for syphilis, 7.60% for NG, 4.96% for CT, 12.66% for TV and 67.62% for HSV2. Of these four, BV was statistically significantly associated with TV, CT and HSV2, after adjusting for other factors such as age, education, employment and condom use in Table 3.2.

Table 3.2: Multivariate analysis of factors associated with BV determined through Logistic regression analysis, in women aged 18 years and above enrolled in a microbicides trial during March 2006 and March 2008 in rural KwaZulu Natal

Sample characteristics N=998		AOR	95% Confidence Interval	p-value
Age and education	Primary and age<35 years	1		
	Secondary and age>34years	0.49	0.26 - 0.95	0.035
Employment	Unemployed	1		
	housewife	0.65	0.47 - 0.91	0.012
	employed	0.87	0.60 - 1.26	0.462
	student	0.43	0.21- 0.89	0.023
Fuel source	Electricity	1		
	Gas	1.56	0.94 - 2.60	0.084
	Paraffin	1.40	0.91 - 2.17	0.126
	Wood	1.41	1.04 - 1.91	0.026
Contraception	No	1		
	Pills	1.14	0.64 - 2.03	0.668
	injectables	0.66	0.48- 0.91	0.011
	sterilised	0.83	0.51 - 1.35	0.463
Condom use	Never	1		
	Sometimes	1.01	0.47 - 2.15	0.987
	Always	0.68	0.52 - 0.90	0.007
TV	Neg	1		
	Pos	2.52	1.67 - 3.80	<0.001
CT	Neg	1		
	Pos	2.49	1.31 - 4.72	0.005
HSV2	Neg	1		
	Pos	1.63	1.20- 2.20	0.002

Women with TV and CT were more than twice as likely to be infected with BV [Adjusted Odds Ratio (AOR) 2.52 (95% Confidence Interval (C.I.):1.67-3.80) and 2.49 (95 % C.I: 1.31-

4.72) respectively]. Those with HSV2 were 63% [p=0.02 (95 % C.I: 1.20-2.20)] more likely to have BV.

ii. BV and socio-economic demographics

Age was initially assessed assed as a continuous variable, then later categorised into a binary variable (above 34 years and 34 years and below) given that literature has highlighted BV peaking in the thirty year olds and also through stratified analysis of the 5 year interval group categories. At univariate level both age and education were statistically significantly associated with BV but both variables lost significance when introduced into the multivariate model adjusting for presence of an STI. Further analysis revealed a borderline significant interaction (p=0.04) between age and education introduction of which resulted in an improvement in the goodness of fit of the model to explain factors associated with BV. Being both older than 34 years with secondary education [AOR= 0.49 (C.I: 0.26-9.95)] significantly reduced the odds of having BV, compared to women who were younger than 35 years with primary education (Table 3.2).

Being a student AOR= 0.43 p=0.02 or housewife AOR = 0.65 (C.I:0.47-0.91) were statistically significantly associated with a lower prevalence of BV, unemployed respectively. For those employed, the type of employment did not appear to be associated with BV (p>0.05)

In this analysis fuel source was a proxy for socio-economic status [1] and the results showed that women with a lower economic status (using wood for cooking) independently were 41% (AOR= 1.41, C.I: 1.04-1.19) more likely to be BV positive compared to women who used electricity (higher socioeconomic status).

iii. BV and condom use

In the univariate analysis condom use as a form of contraception was highly significantly protective [Unadjusted Odds Ratio (UOR) 0.61 (C.I: 0.41-0.89)] against BV. On the other hand, reportedly using a condom at last sex act and overall condom use categorised as yes or no were not statistically significantly associated with lower BV prevalence. Condom use for contraception was highly correlated with always using a condom. Out of 140 women who reported using a condom for contraception 117 (83.57%) reported always using a condom thus both variables could not be assessed together in the multivariate model. Contraceptive use was further categorised into 4 strata with condom use as no contraception, in light of having a number of women in the other contraceptive categories also reporting sometimes and always using condoms. Subsequently, the 4 strata contraception variable and condom use as never, sometimes, always were introduced into the multivariate model and this significantly increased the model goodness of fit. Consistent condom use had a protective effect of 32% AOR=0.68 (C.I: 0.52- 0.90) whilst inconsistent condom use had no effect on BV after controlling for other variables.

iv. BV and family planning methods

Using injectable hormonal contraception AOR=0.66 (C.I:0.48-0.91), reduced the odds of prevalent BV compared to women who were not on contraception controlling for age, education, employment and condom use.

v. BV and sexual behaviour practices

There was low exposure reporting on casual partners with only 12 women reporting having a casual partner thus effects of this variable on the odds of having BV could not be assessed.

There was no statistically significant association between prevalent BV and sexual debut and having had sex in the last 7 days compared to in the last 4 weeks

vi. BV and vaginal hygiene practices

There was no significant association between presenting with prevalent BV and washing the vagina after sex as well as with religion (Table 3.1). Seven women reported use of vaginal inserts for vaginal cleaning or tightening thus effect of inserts on BV could not be assessed

Missing values in the regression model

Of the 68 participants who did not contribute to the main analysis, 66 of these were missing a condom category, one had a missing HSV2 result and one had a missing employment status. They had a mean age of 31.4 years (S.D 12.3), median age 26.5 years (IQR 21-43). The profiles on sexually transmitted infection showed that 44 (66.67%) had HSV2 while 3 (4.5%) were CT positive 59 (89.4%) and did not have TV. These characteristics were consistent with those of the main cohort used in the final Logistic regression model. Thirty two women [48.48% (95% C.I: 35.99-61.11)] out of 66 had prevalent BV.

There was a 5.38% reduction in the overall model log likelihood value on addition of the category with the individuals missing condom use, into the main model. Despite the decrease in model goodness of fit, all the variables that were significant before adding the missing group remained significant, with a negligible change in the standard errors as well as in the 95% C.I. There was also a vast overlap in the 95% C.I. with and without the missing values. It was concluded that there was no added value in inclusion of these participants in the final

model and based on the log likelihood, the model without the missing values was considered the most parsimonious and informative model of best fit.

3. *Incidence and survival time*

Of the total 553 women who were BV negative at enrolment, 27 had either a missing endpoint date or endpoint BV results, remaining with 526 women available for incidence calculations. A total of 526 baseline BV negative women were followed up providing 313 women years, with a total of 255 incident cases. Kaplan Meier analysis gave an estimated overall follow-up time mean, median and range of 7.2, 5.88 and 1.44 – 15.12 months respectively. The estimated incidence rate was 81 cases per 100 women years (95% C.I: 72 – 92 per 100 women years) with a median time to an incident BV case of 9.72 months, inter-quartile range of 4.56-14.28 months. The incidence rate peaks every subsequent 3 month follow-up period and by 40 weeks just over 40% of the total cohort had had a BV incident case (Table 3.3 and 4).

Table 3.3: Period/Cohort BV incidence rates during follow-up of 526 women who were BV negative at enrolment between March 2006 and March 2008 in rural KwaZulu Natal

Cohort (weeks)	**person-time**	**total failures**	**rate** (per women year)	**95% C. I**	
(0 - 12]	130.01	57	0.44	0.34	0.57
(12 - 24]	88.24	116	1.31	1.10	1.58
(24 - 40]	55.87	21	0.38	0.25	0.58
(40 - 52]	34.13	46	1.35	1.01	1.80
(52-65]	4.76	15	3.15	1.90	5.23
Total	313.01	255	0.815	0.72	0.92

Table 3.4: BV survival function/probabilities of women at different time points of follow-up following enrolment into an HIV prevention trial between March 2006 and March 2008 in rural KwaZulu Natal.

Visit week	Number of women at visit	Number Positive	% of Positives	Survivor function	95 % C.I
0	526			1	
12	495	126	25.45	0.89	0.86 - 0.91
24	342	64	18.71	0.64	0.60 - 0.68
40	204	47	23.04	0.59	0.54 - 0.63
52	124	18	14.52	0.43	0.38 - 0.48

Figure 3.2 shows the survival curve to first BV episode which together with tables 3 and 4 indicate that the percentage of BV positive people tended to have peaks and troughs at alternate 3 monthly visits. The graph also shows that incidence is highest in the first half year illustrated by the steepness of the graph over this time period. The cohort/period incidence rates (Table 3.3), calculated as the incidence rate for a given continuous portion of the total follow-up time, were highly variable with the incidence significantly peaking at period 12-24 and 40-52 weeks [1.31 (95% C.I:1.10 - 1.58) and 1.35 (95% C.I: 1.01 - 1.80)]. The same result is observed in Table 3.4 with the percentage of BV positive people showing a cyclical pattern. Worth noting is the decrease in person time as time progresses, mainly due to data being used before closure of the primary trial. After week 52, only 43% of the total cohort had not experienced a BV episode as indicated by the survivor function which shows the proportion of people who had not had an incident BV case [survivor function: 0.43 (95% C.I: 0.38 - 0.48)].

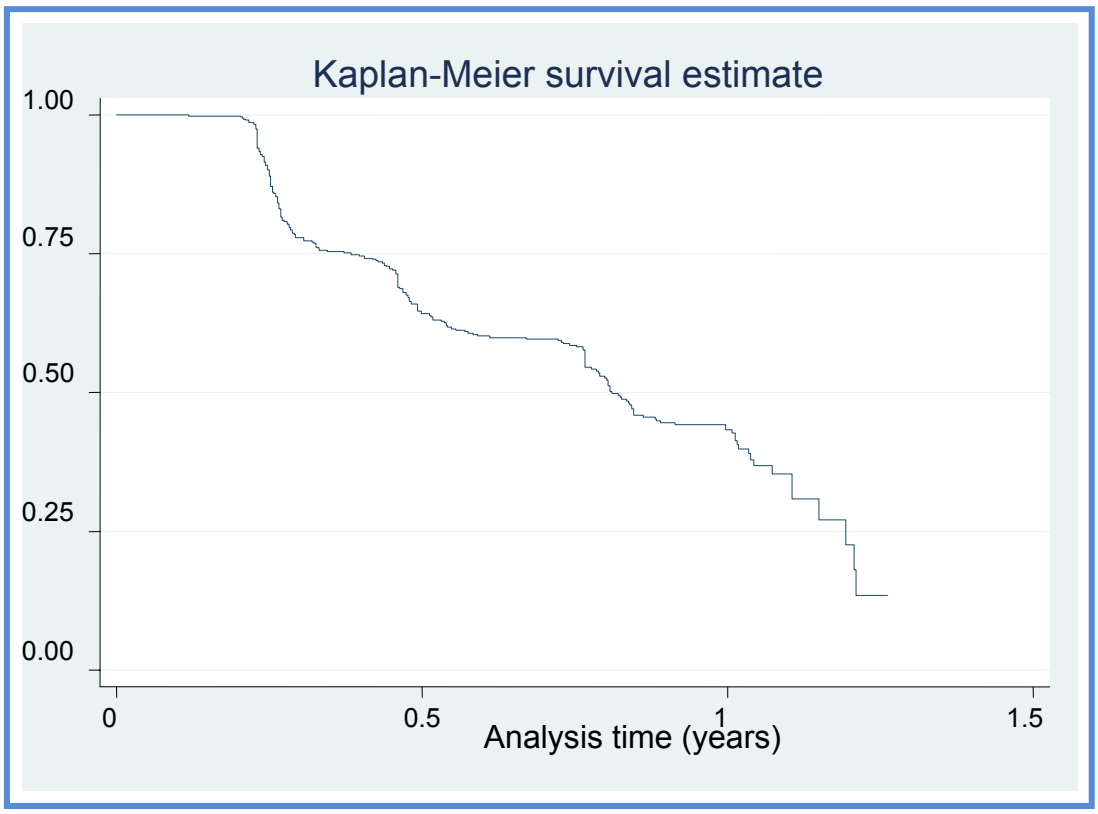


Figure 3.2: Kaplan Meier curve of time to first BV episode in women who were BV negative at enrolment into a microbicides trial in rural KwaZulu Natal.

4. Proportion of asymptomatic BV cases and concordance between laboratory diagnosis and clinical symptoms

BV was diagnosed on the basis of a laboratory Ison and Hay grade III result. Clinical symptoms included vaginal discharge with a fishy smell, alkaline pH <4.5, cervical discharge and discharge from both the vagina and the cervix (cervico-vaginal discharge). In Figure 3.3, any combination of symptoms was considered as the group with two symptoms and with three symptoms. Of the total population, 97 participants presented with symptoms, 60 (61.86%) had vaginal discharge; 45 (46.88) of which was offensive and 61 (67.03%) had a vaginal ph>4.5. Worth noting is that symptoms were experienced in both the BV negative and BV positive groups (Table 3.5).

Table 3.5: Proportion of women with or without BV presenting with symptoms at enrolment during March 2006 to March 2008 in rural KwaZulu Natal.

Symptoms		Baseline BV	Total N in group	n (%)	95% C.I
Discharge	Yes	BV neg	552	49 (8.88)	6.49 - 11.25
		BV pos	512	48 (9.38)	6.84 - 11.91
Ph	>4.5	BV neg	44	29 (65.91)	51.33 - 80.49
		BV pos	47	32 (68.09)	54.25 - 81.92
Offensive discharge	Yes	BV neg	48	24 (50.00)	35.33 - 64.67
		BV pos	48	21 (43.75)	29.19 - 58.31
Source of discharge	Vaginal	BV neg	49	26 (53.06)	38.76 - 67.36
		BV pos	48	34 (70.83)	57.67 - 83.99
	Cervical	BV neg	49	3 (6.12)	-0.75 - 12.99
		BV pos	48	7 (14.58)	4.36 - 24.80
	Cervico-vaginal	BV neg	49	17 (34.69)	21.06 - 48.33
		BV pos	48	6 (12.50)	2.92 - 22.08
	Not possible to tell	BV neg	49	3 (6.12)	-0.75 - 12.99
		BV pos	48	1 (2.08)	-2.05 - 6.21

Of the 513 women who had BV, 465 (90.64%) had no symptoms, 12 (2.34%) had one symptom, 18 (3.51%) had two symptoms and 18 (3.51%) had three symptoms. One or more symptoms were experienced in 49 (8.88%) of women who were BV negative at baseline (Figure 3.3).

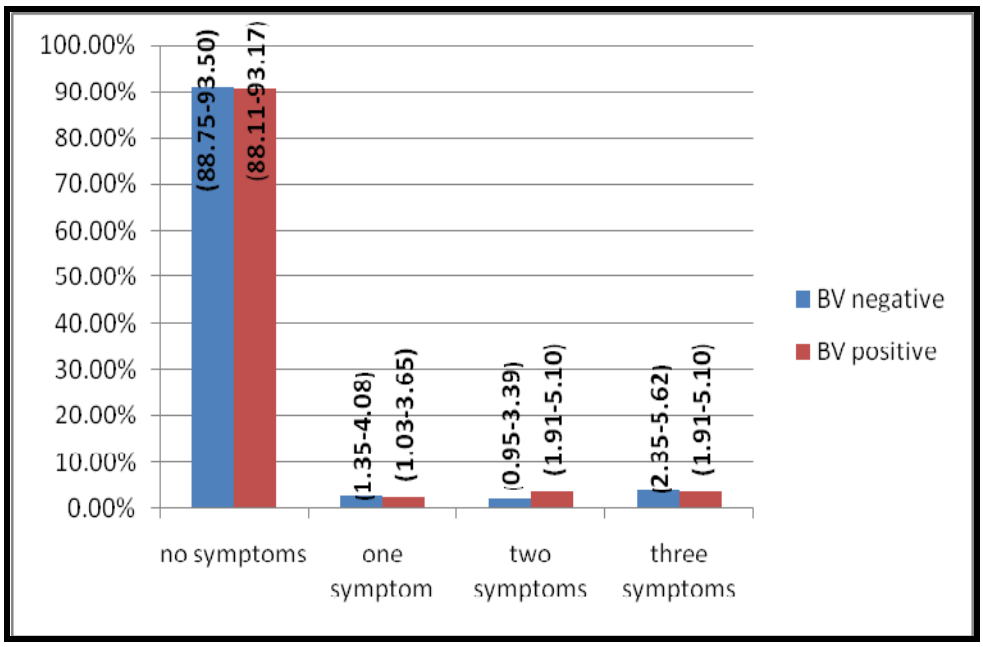


Figure 3.3: Proportion of BV negative and positive women aged 18 years and above in KwaZulu Natal stratified by number of symptoms reported at baseline. 95% Confidence intervals are given in ( ).

Overall, there were no significant differences between the proportion of women BV positive and BV negative women presenting with symptoms p=0.53 [Ho: proportion (negative) – proportion (positive) = 0]. Women with laboratory diagnosed BV did not necessarily present with BV associated symptoms and laboratory diagnosed BV negative women could just as well present with BV associated symptoms, depicting lack of concordance between clinical symptoms and laboratory diagnosis.

A comprehensive discussion of these results and their proposed significance is presented in the next chapter.

Chapter 4: Discussion

The aim of this study was to measure the burden of BV and to determine factors associated with the disease in this area. Better understanding of risk factors will improve understanding of this complex disease which has poorly understood aetiology. This study confirms findings by other studies that there is a high prevalence of largely asymptomatic BV. The prevalence was just under 50% which is comparable with the rates obtained in non-pregnant women in the United States and other developing countries [5, 7, 44] but higher than that obtained in a study in urban KwaZulu Natal [12]. The higher prevalence in this study might have been due to the use of the Ison and Hay diagnostic test with high specificity and sensitivity, compared to the Nugent's criteria used in previously reported studies. This result confirms Ndlovu's [36] concerns that in Africa BV prevalence might be vastly underestimated due to diagnostic challenges.

At baseline, the median and mean ages were similar (34.9 vs. 35.0 years), showing that age distribution was symmetrically normally distributed. This study showed that a number of diverse risk factors including age were associated with prevalent BV. Mean age between BV positive and negative women was similar, however after stratification of age into 35 and above and below 35 years old, BV was more prevalent in the older age group in the univariate analysis; women aged above 35 had a 27% higher odds of having BV compared to women aged 34 and below.. Interestingly this association was depended on education; in univariate analysis This association became statistically insignificant after controlling for education, although the size of the OR did not substantially change. The same loss of statistical significance after adjusting for age was true between BV and education. Interaction was then

further investigated based on the assumption that education is likely to vary depending on the age at initiation of the individual i.e. women below the age of 35 are more likely to have attended school compared to a 50 year old woman. A borderline significant interaction between age and education was detected, the inclusion of which significantly improved the model goodness of fit, with women aged 35 and above with secondary education being 51% (95% C.I: 0.26-0.95) less likely to have prevalent BV compared to younger women with only primary education. This might mean that older educated women lower their odds of having BV by virtue of having more reproductive health knowledge. For younger uneducated people the protective effect offered by age might be outweighed by lack of knowledge predisposing this group to BV. Worth noting is that beyond secondary level, education has no effect on BV. Hypothetically this may be extrapolated to mean that the basics of reproductive health are attained during secondary education after which a threshold on reproductive health improvement is reached. Alternatively this lack of effect may be attributed to the low numbers of people with tertiary education in this study group.

In assessing employment as a binary yes/no variable, being employed had no effect on the odds of having BV compared to those unemployed. For those employed, type of employment was not significantly associated with BV before adjusting for other variables. After stratification of the employment variable into unemployment, housewife, employed and student categories, being a housewife [AOR 0.65 (95% C.I: 0.47-0.90)] or a student [AOR 0.42 (95% C.I: 0.21-0.89)] significantly reduced the odds of having BV at baseline, compared to those unemployed. This protective effect persisted after controlling for factors such as age, education, infection with STIs and contraception use. Notably housewives did not perceive themselves as unemployed but they chose to stay at home ensuring the family's welfare. The

same applied with students who had elders looking after them. However caution must be taken when interpreting the result for students as these were young people in secondary education who might have been more protected against BV by virtue of their age and education [2, 4, 15, 22, 37]. Comparing this group to older women who are presumably unemployed with lower education levels would clearly lead to a biased association between being a student and having BV compared to the unemployed. This is also noted by the decrease in statistical significance and widening of 95% C.I with introduction of age and education into the regression model with employment and BV. Nonetheless, the analysis showed that students had statistically significantly less chance of having prevalent BV after adjusting for other factors.

Women from lower socio-economic status as measured by unemployment and fuel source (wood) for cooking were 41% more likely to present with BV. In the study area households that use wood for cooking are classified as the poorest. This data was also well correlated with data on house structure in terms of type of walls, roof and floor as well as number of assets in the household. These results echo results obtained in previous studies on the association between socio-economic status and BV [52, 53]. Women from disadvantaged socio-economic status are presumably less likely to negotiate condom use [54] as well as seek treatment for STIs leading to more BV cases in this group. In univariate analysis number of household assets was not associated with BV maybe owed to the fact that household assets may explain a household socio-economic status and not necessarily the individual's, especially in adult women who may be living with extended family.

Although it is well documented that a variety of intravaginal practices such as vaginal wiping, vaginal washing and insertion of substances into the vagina are common in several African

countries [24, 55], only 7 women reported using herbs or chemicals for cleaning or drying the vagina in this study group. Thus the data could not be used to assess the effects of these on vaginal flora. One possible explanation to this finding is that women under reported the use of inserts owing to the counselling against use of inserts during the screening visit prior to enrolment. This explanation is plausible taking into account focus group discussions (analysis and results not presented in this report) where there was general consensus that inserts were used in this area with a reported 11% prevalence of inserts [54]. If there was no under reporting then it may be hypothesised that despite not using inserts, general reproductive health might be poor in terms of vaginal hygiene/cleanliness leading to high BV prevalence.

In this study, results in line with previous studies were obtained on associations between BV and CT, TV and HSV2 [41, 52, 56, 57]. Women with TV were more than two and a half times as likely to present with concurrent BV infection. This may be due to TV infection altering the vaginal flora causing it to resemble BV, or BV related flora increasing the chances of acquiring TV. Studies have also reported that CT and NG are associated with BV, where women were less likely to resolve BV if they had NG and CT, hence implying that presence of an STI is causative of BV and not vice versa. [40, 52, 56]. The lactobacilli dominated vaginal ecology may be an important defence against pathogen acquisition by creating an acidic environment that is less hospitable to invading pathogens [57]. Altering of this environment might inevitably lead to BV. One longitudinal study managed to establish the temporal relationship between BV and HSV2 and concluded that BV increases susceptibility to HSV2 although they acknowledged that immunologic responses induced by genital tract infections may produce BV related vaginal environment [58]. Since our study was a cross-sectional

analysis, it was not possible to rule out reverse causality between BV and other STIs, consequently association does not imply causation in this study.

Reported use of contraception was statistically significantly associated with a decrease in prevalent BV at baseline, with those on injectable contraceptives being 44% less likely to have a prevalent BV infection. Previous studies [44, 56, 59] have demonstrated that systemic hormonal contraception protects against BV due to an increase in estrogen, facilitating bacterial lactic acid production thus inhibiting BV associated micro organisms. Changes in vaginal mucosal immune cell populations indicative of altered local immune function may influence the risk of BV [56]. Injectable contraceptives may also modify BV by reducing menstrual frequency and volume, thus minimising vaginal flora disturbances, which are normally experienced during menstruation cycles. Whilst some studies have associated oral contraceptive with BV, in this study contraceptive pills and sterilisation were not significantly associated with BV. Condom use might have partially confounded the protective effect of injectable contraception by making it appear stronger. In univariate analysis with condom use as a type of contraception, women who reported use of injectables had a lower BV prevalence with a narrower OR confidence interval. The OR for injectables slightly increased and the 95 % C.I widening (0.44-0.79 compared to 0.48-0.91), after classifying condom as no contraception, talking into account that even women on other contraceptives did use condoms hence experienced the potentially protective effect conveyed by condoms.

Previous studies have been inconsistent with regard to whether or not condom use is associated with BV. In the univariate analysis, reportedly always using a condom appeared to marginally decrease BV prevalence p=0.06. The association became significant after

controlling for other factors such as STIs and contraception. This may be due to the fact that the relationship between BV and specific risk factors such as sexual risk behaviour, hormonal contraception and condom use may not be entirely explained on the basis of their impact on lactobacilli [56, 60]. Multiple and diverse risk factors can contribute to BV acquisition. Condom use in the presence of an STI may be protective taking into account the association between STIs and BV, assuming that women who use condoms lower their risk of getting an STI and consequently BV. This would also explain the lack of a significant association in the univariate model without STIs. Inconsistent condom use had no effect on BV, which has been observed in previous studies on condom effectiveness [56, 60].

Not only was BV prevalence high in this population but so was the incidence with 81 cases per 100 women years. Incidence in this study population was higher than the 36 cases per 100 women years obtained in a study in Pennsylvania [58] but lower than the 233 per 100 women year obtained in a small study of 96 women in Alabama [61] . About 50% of all women who were BV negative at baseline had at least one BV episode by week 52 and this is consistent with findings in a study in Pennsylvania [60] . The incidence rates remained generally high throughout the follow-up period, with about 20% becoming BV positive at each visit as was observed in a large 3 year follow-up study in Alabama [52]. BV can occur as an incident infection, re-infection, recurrent infection, persistent infection or as natural variability associated with the menstrual cycle [25]. These infections may occur at any given interval thus elevating the incidence of BV at all cohort periods. The proportion of BV positives generally decreased with increase in follow-up time, with a peak at week 40. The reduction may have been due to continual reproductive health counselling offered during the follow-up visits as part of the Microbicides trial [47]. The reasons for the peak are not quite well understood and

future longitudinal studies are required to investigate the possibility of cyclical trends in BV incidence. Interestingly this study shows that although BV prevalence in much higher in Africa compared to America, the incidence regimes mirror each other, which would imply that BV episodes in Africa may take much longer to resolve. Further longitudinal studies are required to assess duration and characteristics of BV episodes.

In spite of the high laboratory BV prevalence, clinical presentation of symptoms was similar in both BV negative and positive women. Only 9% presented with at least one symptom at baseline indicating that BV is largely asymptomatic in this area. Of the 52% who did not have BV, more than 50% had normal vaginal flora. Intemediate flora was quite common (19.5%) in the total population. Transient BV stage was not followed up in this study to see if it reverted back to normal flora or progressed to BV associated flora. The fact that BV is a common condition in Southern Africa, but largely asymptomatic raises concerns especially in pregnant women in resource poor settings where diagnosis is made clinically. Many cases of BV potentially go undetected and untreated thus increasing the risk of pregnancy complications. The high BV prevalence and incidence in this area with a major HIV epidemic is of great concern considering that laboratory and longitudinal studies have ascertained that BV promotes acquisition of HIV [40] thus the HIV infection attributable to BV might be high. In resource poor settings laboratory techniques such as microscopy should be employed for more vigorous BV screening and more accurate detection of asymptomatic BV in pregnant women and high HIV risk groups.

Not only was a large proportion of BV asymptomatic but a fair proportion of BV negative women also presented with either one or more BV symptom. Similar to what was obtained in

a Gambian study [7], cervico-vaginal discharge was high in BV positive women maybe due to concurrent TV and NG infections, causing a medley of symptoms in BV positive women. Twenty four of the 45 participants with offensive discharge were BV negative, which might be attributed to the fact that detecting offensive or discoloured discharge through genital exam is highly subjective. Also worth noting is that it is possible that BV positive women internally washed the vagina in an effort to remove the smell and the discharge before attending clinic visits, resulting in the absence of symptoms on genital examination in this group.

This study's results showed no concordance between clinically diagnosed and laboratory diagnosed BV with the proportion of symptoms in both BV positive and negative women being statistically similar. This might have been due to the high proportion of BV grade II, which was not considered as definitive BV in line with accepted definitions[11]. Further characterisation of BV grade II is required to establish symptoms that are associated with this group, which will further inform if laboratory grade II should be clinically classified as BV negative or if both grades clinically present as BV. This supports the proposition that intermediate flora (BV grade II) precedes the development or follows the resolution of candid BV. It is hoped that diagnosis will improve with the introduction of molecular diagnosis of BV in developed countries [62, 63] and consequently improve BV screening and management.

Limitations

Possible Limitations

The major limitation in determining the incidence of BV over the study period was the presence of the primary study product, 0.5% and 2% PRO2000/5 gels. It is not possible to know who was on the product and who was on placebo before the study ends in 2009 as this

data will not be available for analysis until then. Thus the effect of the product on BV could not be determined. It was assumed that risk factors for BV remain the same for people on product and those on placebo, given that the study was double blinded, so although the overall incidence may be under- or over- estimated, risk factor analysis is unlikely to be affected. Prevalence estimates are not affected.

As discussed in the literature review, diagnosis of BV is difficult, thus prevalence and incidence estimates might not be as accurate as would have been desired, due to non-differential misclassification of diseased cases as non diseased. It was not possible to eliminate this bias.

The effect of BV on HIV incidence during the trial could not be assessed as data on the HIV status was not available for analysis before the closure of the Microbicides trial.

Possible sources of bias

Selection bias might have been introduced since participants volunteered to join the study and those who volunteered might have been systematically different from those who did not and extrapolation might not be justifiable. Selection bias which could have been introduced by virtue of enrolling at the clinics, thus only taking those who are likely to attend at a health facility was controlled for by using snowball recruitment where participants would inform their immediate community about trial involvement. However only those who could access the clinic were enrolled hence still potential recruitment bias. HIV positive women were excluded from the study and this group might be systematically different from the HIV negative women thus making extrapolation to the general population unjustifiable.

Recall bias could not be eliminated since women were asked retrospectively for some data such as sexual behaviour. However the magnitude of this was limited due to the fact that most of the details required were current day to day activities. Reporter bias might have been present especially in terms of sexual behaviour questions with people tending to give socially acceptable answers; hence possibility of residual confounding could not be ruled out. There was also a considerable number of missing pH values for participants who presented with discharge causing non-differential misclassification. There was also possibility of measurement bias in assessment of clinical symptoms which might have led to non-differential misclassification in terms of disease status. This could not be controlled for in analysis thus much emphasis was put in training the clinical staff and laboratory staff and implementing quality control measures.

Loss to follow-up bias might have been introduced during the trial period (prospective cohort) leading to an underestimation of the incidence rate. Pro-active tracking of participants was encouraged to minimise the loss to follow-up. Only a small number of women had reached week 52 when the data was extracted, resulting in an unreliably high period incidence at this time point.

Possible sources of confounding

Possible confounders include age, co-infection with TV, education, and religion. Information on these was collected during the course of the study through the use of structured questionnaires and controlled for during analysis by stratification and regression modelling.

Among the possible confounding variables, data was missing on the STI status in the male partner and therefore we used the STI status of the female as a surrogate marker for the male STI status. Smoking status which has been strongly linked with BV in several studies [59] was not collected, but in this community it is hugely unacceptable for women to smoke (personal communication: Misiwe Mzimela) thus the unmeasured confounding effect of smoking, if at all present, is negligible.

By virtue of the baseline determinants analysis being cross sectional, association does not imply causation in any of these associations since temporal relationship is unknown.

Strengths

Strengths of this study include the study being a large longitudinal and complete dataset of a clearly defined population, with high retention up to week 40 of follow-up. This study looked at women with or without BV symptoms thus increasing generalisability of results for HIV negative women. There were consistent and standardized enrolment and data collection protocols. BV diagnostic techniques used were reliable, accurate and consistently quality controlled. Unlike this study, many studies have argued that levels of education and socio-economic factors normally confound associations between BV and sexual behaviour and vaginal cleansing and African data on such variables has not always been entirely controlled for [7]. Our study included women from urban, peri-urban and rural settings and we could investigate the association between socio-economic, demographic, sexual practices and reproductive health variations with BV. In this epidemiological study, data on several confounding variables was collected and their effect assessed thus reducing the gap in public health knowledge and BV epidemiology.

Conclusions and Recommendations

There is a high asymptomatic disease burden of BV in this area, making it a priority to accurately identify those at risk of BV since cases potentially go undetected thus increasing BV sequelae. Vigorous screening using microscopy in resource poor settings should be done considering that clinical diagnosis through symptoms may lead to under diagnoses of BV.

The results presented here identify modifiable risk factors for BV which could inform interventions to improve vaginal health and decrease BV. These include condom use, injectable contraceptives, vaginal cleanliness and treatment of STIs. However use of injectable contraception should not preclude women from using barrier contraceptives to reduce the risk of acquiring HIV and other STIs. BV treatment during pregnancy, delivering general good vaginal hygiene practices in primary health care facilities and the inclusion of metronidazole in treatment of all women seeking STI treatment may potentially reduce this silent risk factor for HIV in people who are at high risk of HIV. Further studies exploring the usefulness and cost effectiveness of artificial recolonisation of lactobacilli in the vaginal tract [24] are vital.

This study confirms that diverse factors may work separately or in combination resulting in disruption of the vaginal biologic ecosystem. BV related factors must be tackled wholly in order to successfully control BV. Simple inexpensive strategies such as STI treatment and condom use, aimed at reducing BV must be implemented to reduce population attributable risk of BV for HIV and curb adverse pregnancy outcomes in this area of high HIV and BV prevalence and incidence.

References

1. Tanser F, et al., *Cohort Profile: Africa Centre Demographic Information System (ACDIS) and population-based HIV survey.* International Journal of Epidemiology 2008. **37**: p. 956-962.
2. Barousse M M, et al., *Vaginal yeast colonisation, prevalence of vaginitis and associated local immunity in adolescents.* Sexually Transmitted Infections, 2004. **80**: p. 48-53.
3. Boris J, Larsson P.G, and P. C, *Six-year follow-up after successful treatment of bacterial vaginosis.* International Journal of Sexually Transmitted Diseases and AIDS, 1997. **8**(Suppl 1): p. 41.
4. Brabin L, et al., *Factors affecting vaginal pH levels among female adolescents attending genitourinary medicine clinics.* Sexually Transmitted Infections, 2005. **81**: p. 483-487.
5. Bradshaw C S, et al., *Higher-risk behavioural practices associated with bacterial vaginosis compared with vaginal candidiasis.* Obstetrics and Gynecology, 2005. **106**(1): p. 105-114.
6. Callahan D B, Weinberg M, and Gunn R A, *Bacterial vaginosis in pregnancy: Diagnosis and treatment practices of physicians in San Diego, California, 1999.* Sexually Transmitted Diseases, 2003. **30**(8): p. 645-649.
7. Demba E, et al., *Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa.* BMC Infectious Diseases, 2005. **5**(12).
8. Govender L, et al., *Bacterial vaginosis and associated infections in pregnancy.* International Journal of Gynecology and Obstetrics, 1996. **55**: p. 23-28.
9. Hay P, *Bacterial vaginosis and miscarriage.* Current Opinion in Infectious Diseases, 2004. **17**: p. 41-44.
10. Hay P, *Life in the littoral zone: Lactobacilli losing the plot.* Sexually Transmitted Diseases, 2005. **81**: p. 100-102.
11. Ison C A and Hay P E, *Validation of simplified grading of Gram stained vaginal smears for use in genitourinary medicine clinics.* STI online, 2002. **78**: p. 413-415.
12. Kharsany A B M, Hoosen A A, and Moodley J, *Bacterial vaginosis and lower genital tract infections in women attending out-patient clinics at a tertiary institution serving a developing community.* Journal of Obstetrics and Gynaecology, 1997. **17**(2): p. 171-175.
13. Koumans E H and Kendrick J S, *Preventing adverse sequelae of Bacterial vaginosis.* Sexually Transmitted Diseases, 2001. **28**(5): p. 292-296.
14. Morris M C, Rogers P A, and Kinghorn G R, *Is Bacterial vaginosis a sexually transmitted infection?* Sexually Transmitted Infections, 2001. **77**: p. 63-68.
15. Wilson J, *Managing recurrent Bacterial vaginosis.* Sexually Transmitted Infections, 2004. **80**: p. 8-11.
16. Boyd R F and Hoerl B.G, *Basic Medical Microbiology, 4th Edition*, London: Little, Brown and Company.p. 698
17. Collee J G, et al., *Practical Medical Microbiology, 13th Edition*, McCartney, New York: Churchill Livingstone. p.343-46.

18. Ingraham J L and Ingraham C A, *Introduction to Microbiology, 2nd Edition*, USA: Brooks/Cole Thomson Learning. p.653.
19. Mahon R C and Manuselis G, *Textbook of Diagnostic Microbiology, 2nd Edition,* Philadelphia: W.B. Saunders Company. p.1038-39.
20. McKane L and Kandel J, *Microbiology Essentials and Applications, 1st Edition, New York*: McGraw-Hill Book Company. p.571-72.
21. Sleigh J.D and Timbury M C, *Medical Microbiology 4th Edition*. 1994: Churchill Livingstone. p.105, 319-20, 361-2.
22. Rajamanoharan S, et al., *Bacterial vaginosis, ethnicity and the use of genital cleaning agents: A case control study.* Sexually Transmitted Diseases, 1999. **26**(7): p. 404-409.
23. Schwebke J R, Desmond R A, and Oh M K, *Predictors of bacterial vaginosis in adolescent women who douche.* Sexually Transmitted Diseases, July 2004. **31**(7): p. 433-436.
24. Van de Wiljgert J H H M, et al., *Intravaginal practices, vaginal flora disturbances and acquisition of sexually transmitted diseases in Zimbabwean women.* Journal of Infectious Diseases, 2000. **181**: p. 587-94.
25. Morison L, et al., *Bacterial vaginosis in relation to menstrual cycle, menstrual protection method and sexual intercourse in rural Gambian women.* Sexually Transmitted Infection, 2005. **81**: p. 242-247.
26. Hawes S E, Hillier S L, and B. J, *Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections.* Journal of Infectious Diseases, 1996. **174**: p. 1053-63.
27. Nillson U, Hellberg D, and Shoubnikova M, *Sexual risk behaviour associated with Bacterial vaginosis and Chlamydia trachomatis infection.* Sexually Transmitted Infection, 1997. **24**: p. 241-6
28. Schwiertz A, et al., *Throwing the dice for the diagnosis of vaginal complaints.* Annals of Clinical Microbiology and Antimicrobials, 2006. **5**(4).
29. O'Brian R F, *Bacterial vaginosis: many questions - any answers?* Current Opinion in Pediatrics, 2005. **17**(4): p. 473-479.
30. Verstraelen H, et al., *Subclinical iron deficiency is a strong predictor of bacterial vaginosis in early pregnancy.* BMC Infectious Diseases, 2005. **5**(55).
31. Johnson L F, Coetzee D J, and Dorrington R E, *Sentinel surveillance of sexually transmitted infections in South Africa: a review.* Sexually Transmitted Infections, 2005. **81**: p. 287-293.
32. Keane F E A, et al., *Methods employed by genitourinary medicine clinics in the United Kingdom to diagnose bacterial vaginosis.* Sexually transmitted Infections, 2005. **81**(155-157).
33. Taylor R D, et al., *Expert review on: Relation between Gram-stain and clinical criteria for diagnosing bacterial vaginosis with special reference to Gram grade II evaluation* International Journal of Sexually Transmitted Diseases and AIDS, 2003. **14**: p. 6-10.
34. Hay P, *Expert review on "Relation between Gram-stain and clinical criteria for diagnosing bacterial vaginosis with special reference to Gram grade ii evaluation".* Sexually Transmitted Diseases. **Diagnostics Initiative** (4).
35. Taha E, et al., *Bacterial vaginosis and disturbances of vaginal flora: Association with increased acquisition of HIV.* AIDS, 1998. **12**: p. 1699-1706.
36. Ndlovu L and *Reproductive Health Services in KwaZulu Natal; A situation analysis study focusing on HIV/AIDS services*, Department of Health report. Horizons program.2002-2003,

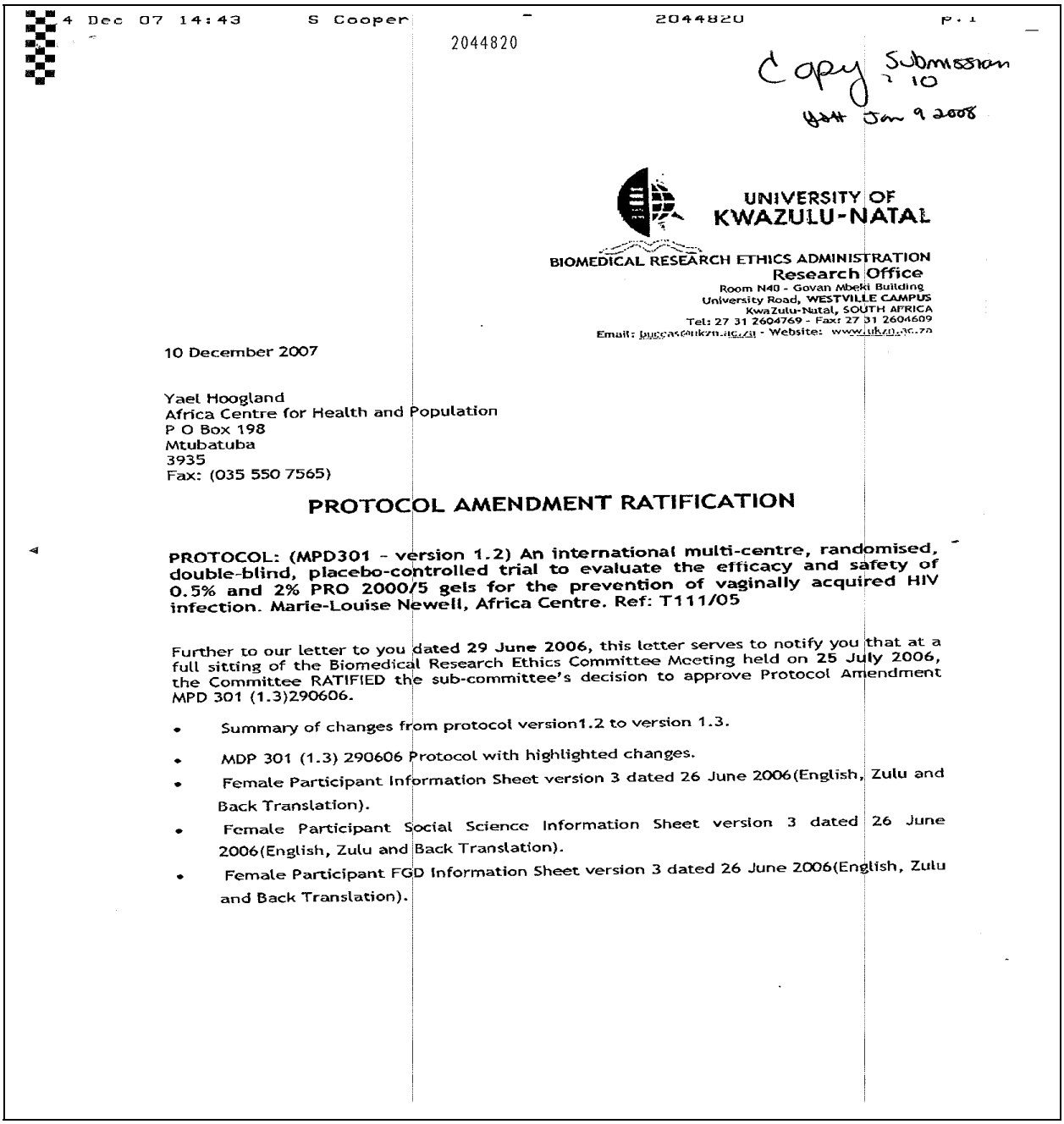
37. Reed B D, Ford K, and Wirawan D N, *The Bali STD/AIDS study: Association between vaginal hygiene practices and sexually transmitted diseases among sex workers.* Sexually transmitted Infections, 2001. **77**: p. 46-52.
38. Patel V, et al., *The burden and determinants of reproductive tract infections in India: a population based study of women in Goa, India.* Sexually transmitted Infections, 2006. **82**: p. 243-249.
39. Zungu S, Moitse S, and Hosegood V, *Social grants in rural areas_Beating a path through a maze of bureaucracy.* ChildrenFIRST2003.
40. Weiesenfeld H C, et al., *Bacterial vaginosis is a strong predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection.* Clinical Infectious Diseases, 2003. **36**: p. 663-8.
41. Moodley P, Conolly C, and Sturm A W, *Interrelationships among Human Immunodeficiency Virus Type 1 Infection, Bacterial Vaginosis, Trichomoniasis and the presence of yeasts.* Journal of Infectious Diseases, 2002. **185**: p. 69-73.
42. Patel V, et al., *The burden and determinants of reproductive tract infections in India: a population based study of women in Goa, India.* Sexually Transmitted Infections 2006. **82**: p. 243-249.
43. Hashemi F B, et al., *Activation of Human Immunodeficiency Virus yype 1 expression by Gardnerella vaginalis.* Journal of Infections Diseases, 1999. **179**: p. 924-930.
44. Riggs M, et al., *Longitudinal association between hormonal contraceptives and Bacterial vaginosis in women of reproductive age.* Sexually Transmitted Diseases, 2007. **34**(12): p. 954-959.
45. Barnighausen T, et al., *High HIV incidence despite high prevalence in rural South Africa: findings from a prospective population based study.*AIDS, 2008, 22(1). p.139-144
46. Welz T, et al., *Continued very high prevalence of HIV infection in rural KwaZulu-Natal, South Africa: a population based longitudinal study.* AIDS, 2007; **21**(11): p. 1467-1472
47. Microbicides Development Programme (MDP301), *An international multi-centre, randomized, double-blind, placebo- controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO2000/5 gels for the prevention of vaginally acquired HIV infection*. 2005 version1.3, UK department for International Development (DFID) and UK Medical Research Council (MRC).
48. Wilkinson D, et al., *Unrecognized sexually transmitted infections in rural South African women: a hidden epidemic.* Bulletin of the World Health Organisation, 1999. **77**(1): p. 22-27.
49. Case A, Hosegood V, and Lund F, *The Child Grant Support in Hlabisa.* ChildrenFIRST 2003, 2003.
50. Fisher A A and F.R. F, *Designing HIV/AIDS Intervention Studies*. 1st edition. 2002, Washington: Population Council.
51. Thomas A Lang and M. Secic, *Reporting survival analyses. How to report statistics in medicine*. 2nd edition. Assessing time-to-event as an endpoint:: Philadelphia. American college of physicians
52. Ness R B, et al., *Variability of Bacterial vaginosis over 6- to 12-month intervals.* Sexually Transmitted Diseases, 2006. **33**(6): p. 381-385.
53. Peipert J F, et al., *Bacterial vaginosis, race and sexually transmitted infections: Does race modify the associations.* Sexually Transmitted Diseases, 2008. **35**(4): p. 363-367.

54. Gafos M, et al. *A preference for hot sex! Similarity of descriptors used to explain local vaginal products and vaginal investigational products in the MDP301 clinical trial in the Umkhanyakude District of northern KwaZulu Natal. International Conference on Microbicides (M2008)*. 2008. New Dehli, India.
55. Hassan W M, et al., *Associations between intravaginal practices and Bacterial vaginosis in Kenyan female sex workers without symptoms of vaginal infections.* Sexually Transmitted Diseases, 2007. **34**(6): p. 384-88.
56. McClelland R S, et al., *A prospective study of risk factors for Bacterial vaginosis in HIV-1-seronegative African women.* Sexually Transmitted Diseases, 2008. **35**(12).
57. Cherpes T L, et al., *Association between acquisition of Herpes Simplex Virus type 2 in women and Bacterial vaginosis.* Clinical Infectious Diseases, 2003. **37**: p. 319-25.
58. Cherpes T L, et al., *A delicate balance: Risk factors for acquisition of Bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race and positive herpes simplex Virus Type 2 serology.* Sexually Transmitted Diseases, 2008. **35**(1): p. 78-83.
59. Smart S, Singal A, and M. A, *Social and sexual risk factors for Bacterial vaginosis.* Sexually Transmitted Diseases, 2004. **80**: p. 58-62.
60. Hutchinson K B, Kip K E, and N.R. B, *condom use and its association with Bacterial vaginosis and Bacterial vaginosis-associated vaginal microflora.* Epidemiology, 2006. **18**(6): p. 702-707.
61. Schwebke J R and Desmond R A, *Risk factors for Bacterial vaginosis in women at high risk for sexually transmitted infections.* Sexually Transmitted Diseases, 2005. **32**(11): p. 654-658.
62. Brotman R M and R. Jacques, *Ready or not: The molecular diagnosis of Bacterial Vaginosis.* Clinical Infectious Diseases, 2008. **47**: p. 44-6.
63. Menard J, et al., *Molecular quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict Bacterial Vaginosis.* Clinical Infectious Diseases, 2008. **47**: p. 33-43.

Appendices

Appendix A: Ethics clearance certificates

University of KwaZulu Natal ethics approval



4 Dec 07 14:43 S Cooper 2044820 P.1

2044820

Copy Submission 2 10

Jan 9 2008

UNIVERSITY OF KWAZULU-NATAL

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office
Room N40 - Govan Mbeki Building
University Road, WESTVILLE CAMPUS
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: bucas@nu.ac.za - Website: www.ukzn.ac.za

10 December 2007

Yael Hoogland
Africa Centre for Health and Population
P O Box 198
Mtubatuba
3935
Fax: (035 550 7565)

PROTOCOL AMENDMENT RATIFICATION

PROTOCOL: (MPD301 - version 1.2) An international multi-centre, randomised, double-blind, placebo-controlled trial to evaluate the efficacy and safety of 0.5% and 2% PRO 2000/5 gels for the prevention of vaginally acquired HIV infection. Marie-Louise Newell, Africa Centre. Ref: T111/05

Further to our letter to you dated 29 June 2006, this letter serves to notify you that at a full sitting of the Biomedical Research Ethics Committee Meeting held on 25 July 2006, the Committee RATIFIED the sub-committee's decision to approve Protocol Amendment MPD 301 (1.3)290606.

- Summary of changes from protocol version1.2 to version 1.3.
- MDP 301 (1.3) 290606 Protocol with highlighted changes.
- Female Participant Information Sheet version 3 dated 26 June 2006(English, Zulu and Back Translation).
- Female Participant Social Science Information Sheet version 3 dated 26 June 2006(English, Zulu and Back Translation).
- Female Participant FGD Information Sheet version 3 dated 26 June 2006(English, Zulu and Back Translation).

University of Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mutevedzi

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080332**
PROJECT	Burden and determinants of bacterial vaginosis in sexually active women aged 18 years and over, enrolled in an HIV prevention trial in N Kwazulu Natal
INVESTIGATORS	Ms PC Mutevedzi
DEPARTMENT	School of Public Health
DATE CONSIDERED	08.03.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.04.25 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr Khin San Tint

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Consent forms

Site name: AFRICA CENTRE – SOUTH AFRICA	Date of visit: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy)		
Screening number: ☐☐☐☐☐☐☐	Initials: ☐☐☐	Year of Birth: ☐☐☐☐	Trial number: ☐☐☐☐☐☐

Microbicides Development Programme Trial 301 (MDP 301)
Protocol version (1.2)
Female Informed Consent Part 2

PLEASE CIRCLE THE CORRECT ANSWER

Print your name and surname:_____

1	Have you received enough information about the study?	YES	NO
2	Have you received your HIV test result?	YES	NO
3	Do you understand that we do not know if the gels prevent HIV and that you may receive a gel that will definitely not prevent HIV?	YES	NO
4	Do you agree to use condoms and gel as much as possible when having sexual intercourse during this study?	YES	NO
5	Do you understand that you will have to stop using gel if you become pregnant?	YES	NO
6	Do you understand that you must report any new symptoms to the study team, even if you think they are not related to the gel?	YES	NO
7	Do you understand that you should give accurate answers to the questions you are asked by the study team and return used and unused applicators to them as requested?	YES	NO
8	Do you understand that you are free to withdraw from the study: - at any time - without having to give a reason for withdrawing - and without affecting future medical care?	YES	NO
9	Do you agree to take part in this study?	YES	NO

If 'NO' to any of the above the volunteer is ineligible for the study

Signature	Print name	Date

Site name: AFRICA CENTRE – SOUTH AFRICA	Date of visit: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy)	
Screening number: ☐☐☐☐☐☐☐	Initials: ☐☐☐	Year of Birth: ☐☐☐☐

Microbicides Development Programme Trial 301 (MDP 301)
Protocol version (1.2)
Female Informed Consent Part 1B

PLEASE CIRCLE THE CORRECT ANSWERS

1	Have you signed part 1A of the informed consent form?	YES	NO
2	Have you been given pre test HIV counselling?	YES	NO
3	Do you agree to your blood being taken, for HIV testing now and stored for possible testing in the future?	YES	NO

If 'NO' to any of the above the volunteer is ineligible for the study

Signature/Thumbprint of volunteer

Signature or thumb-print		Date of signature	
Print name and surname			

Signature of witness (if volunteer illiterate)

Signature		Date of signature	

Tick box if participant is illiterate and refuses to have witness present: ☐

Time of Signature or thumb print (24 hour clock): ☐☐:☐☐

Signature of study staff taking consent

Signature		Date of signature	
Print name and surname			

Signature	Print name	Date

Site name: AFRICA CENTRE – SOUTH AFRICA	Date of visit: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy)

Screening number:	Initials:	Year of Birth:
☐☐☐☐☐☐☐	☐☐☐	☐☐☐☐

Microbicides Development Programme Trial 301 (MDP 301)
Protocol version (1.2)
Female Informed Consent Part 1A

PLEASE CIRCLE THE CORRECT ANSWERS

Print your name and surname:_____

Which member of the study staff have you spoken to about this study:_____
(please print his or her name and surname)

1	Has the MDP 301 trial been explained to you and have you been given a Female Information sheet dated January2006?	YES	NO
2	Have you had an opportunity to ask questions and discuss this study?	YES	NO
3	Have you received satisfactory answers to all of your questions?	YES	NO
4	Do you understand that we do not know if the gels prevent HIV?	YES	NO
5	Do you understand that the correct and consistent use of condoms will prevent HIV?	YES	NO
6	Do you agree to appropriate members of trial staff and the authorities responsible for licensing the gels having access to your medical records?	YES	NO
7	Do you understand that agreeing to be screened does not mean you have to join the trial?	YES	NO
8	Do you agree to be screened for this study?	YES	NO

If 'NO' to any of the above the volunteer is ineligible for the study

Signature	Print name	Date

Site name: AFRICA CENTRE – SOUTH AFRICA	Date of visit: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy)

Screening number:	Initials:	Year of Birth:
☐☐☐☐☐☐☐	☐☐☐	☐☐☐☐

Signature/Thumbprint of volunteer

Signature or thumb-print		Date of signature	
Print name and surname			

Signature of witness (if volunteer illiterate)

Signature		Date of signature	

Tick box if participant is illiterate and refuses to have witness present: ☐

Time of Signature or thumb print (24 hour clock): ☐☐:☐☐

Signature of study staff taking consent

Signature		Date of signature	
Print name and surname			

Signature	Print name	Date

Appendix C: BV diagnosis

Table C6: BV scoring by Ison and Hay method

BV grade	Interpretation	"Clue" cells
Grade 0	No bacteria	Vaginal epithelial cells seen, but no bacteria seen
Grade I	Normal	Predominantly Lactobacilli morphotypes seen. Lactobacillus morphotypes outnumber Gardnerella morphotypes
Grade II	Intermediate	Mixed Lactobacillus and Gardnerella/Mobiluncus morphotypes seen. Usually 1- 15 Lactobacilli per high power field Ratio of Gardnerella/Mobiluncus to Lactobacilli less than 100 to 1
Grade III	BV positive	Few or absent Lactobacillus morphotypes seen, but greatly increased numbers of *Gardnerella vaginalis* and other morphotypes seen. Ratio of Gardnerella/Mobiluncus to Lactobacilli more than 100 to 1
Grade IV	Gram-positive cocci	Vaginal epithelial cells seen. No Lactobacillus or Gardnerella morphotypes seen. Gram positive cocci only

Appendix D: Case report forms for sexual behaviour, clinical and laboratory data respectively

Laboratory Results CRF

MDP 301 –LABORATORY RESULTS Version 1 July 2005 **Site name: AFRICA CENTRE Date of visit:** / / **DD/MM/YYYY Screening number:**

Initials: **Trial number:**

LABORATORY RESULTS – ENROLMENT ?

PROTOCOL PROCEDURES

Pregnancy
QuickVue or list if another test used:
Name of test _____ Reason for use_____
_____
Positive ☐ Negative ☐
Invalid ☐

HSV2

Focus or list if another test used:
Name of test _____ Reason for use_____
_____
Positive ☐ Negative ☐
Equivocal ☐
>3.5 <1.0 1.0-3.5
Index value _____

Syphilis RPR

B&D MacroVue or list if another test used:
Name of test _____ Reason for use_____
_____
Positive ☐ Negative ☐
If positive, titre _____

Syphilis TPHA if RPR positive

Omega Diagnostics Immutrep TPHA or list if another test used:
Name of test _____ Reason for use_____
_____
Positive ☐ Negative ☐

Trichomonas

PCR or list if another test used:
Name of test _____ Reason for use_____
_____
Positive ☐ Negative ☐

Bacterial Vaginosis
Ison and Hay score
0 ☐, I ☐, II ☐, III

☐ , IV ☐

Neisseria gonorrhoea

B & D Probe Tec or list if another test used: Positive ☐ Negative ☐ Equivocal ☐ Inhibition ☐

Name of test _____ Reason for use _____

_____

Chlamydia trachomatis

B & D Probe Tec or list if another test used: Positive ☐ Negative ☐ Equivocal ☐ Inhibition ☐

Name of test _____ Reason for use

Sexual behaviour

Interviewer: read the questions to the volunteer verbatim and allocate the correct answer from the selection, unless it is indicated that you read out each answer to the volunteer as well. Boxed type is the instructions to the interviewer. Type in italics is to be read to the volunteer.

Sicela uphendule lemibuzo ngokucophelela ngoba izimpendulo zayo zibaluleke kakhulu emiphumeleni yalolucwaningo. Khumbula ukuthi ulwazi osinikeza lona luzogcinwa luyimfihlo futhi luyosetshenziselwa kuphela izinjongo eziqondene nalolucwaningo.

Section 1: Family planning

1 Njengamanje zikhona yini izindlela zokuhlela umndeni ozisebenzisayo?
- Yebo → Q. 1a
- Cha → Q. 1b

1a Uma kunguyebo,yiziphi kulezizindlela ezilandelayo ozisebenzisayo? **[Tick all that apply]**
- Natural/rhythm → Q. 2
- Pills → Q. 2
- Foam/jelly/spermicide → Q. 2
- Diaphragm → Q. 2
- Injectable Nur-Isterate → Q. 2
- Injectable Depo-Provera → Q. 2
- Injectable other (specify below if possible) → Q. 2
- IUCD → Q. 2
- Norplant implant → Q. 2
- Condom (male or female) → Q. 2
- Traditional vaginal → Q. 2
- Traditional oral → Q. 2
- Traditional other (specify below if possible) → Q. 2
- Sterilisation → Q. 2
- Other → Q. 2
  - Specify_____

1b Uma kungu cha, kungani ingekho indlela oyisebenzisayo yokuhlela umndeni?
- Uyancelisa
- Ufuna ukukhulelwa
- Awusayi ocansini
- Usukhulile awusayi esikhathini
- Sewavala inzalo (wena noma uphathina wakho)
- Okunye
  - Chaza _____

2 Zingaki izinsuku esezedlulile kusukela osukwini lwakho lokuqala lokuya esikhathini kulokhu kuya kwakho esikhathini okwedlule? [List number, 99 if more than 3 months or 00 if menstruating now] □□

2a Lokhukuya kwakho esikhathini kwakungesikhathi owawusilindele yini?
- Yebo
- Cha

Section 2: Sexual activity and condom use

Interviewer: please spend sometime ensuring that the volunteer understands what is termed by a sex act: one sex act is "penetrative vaginal sex that may or may not end up with ejaculation."
Also- don't forget to probe for EXACT NUMBERS.

"Manje sengizokubuza mayelana nokwenza kwakho ucansi nokusebenzisa kwakho amakhondomu. Sicela uphendule ngokweqiniso ngoba izimpendulo zakho zibaluleke kakhulu emiphumeleni yalolucwaningo."

3	Njengamanje unaye yini uphathina wesilisa oya naye ocansini?	Yebo ☐ Cha ☐	→ Q. 5

Ensure that the volunteer understands what we mean by each category of partner.
1*) Long-term stable partners* include some/most of the following characteristics: official marriage, traditional marriage, bride price paid, man known and accepted by woman's family, have children together, live together, long-term relationship, man provides regular financial/material support, may be cohabiting or non-cohabiting.
2) *Other partners)* includes all partners who do not fit into the first category above.

4	Luhloboluni lukaphathina onaye njengamanje? [**Tick all that apply]**	Osekuqhutshekwe isikhathi eside nizwana naye noma nabo (1 noma ngaphezulu) ☐ Ezinye izinhlobo zomaqondana(1 noma ngaphezulu) ☐	
5	Zingaki izinsuku ezedlulile ugcinile ukuya ocansini?	1 (lokhu kumbandakanya izolo, yilobusuku bayizolo, nanamhlanje) ☐ 2 (kuthangi) ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 1-4 amasonto ngaphezu kwamasonto awu 4	
6	**Uyisebenzisile yini ikhondomu ngesikhathi ogcine ngaso ukwenza ucansi?**	Yebo ☐ Cha ☐	
7	Wawuneminyaka emingaki mhla uya ocansini okokuqala ngqa?	Ngaphansi kweninyaka ewu 15 ☐	

15 iminyaka kuya ku 19 weminyaka angikaze ngiye ocansini

Iminyaka ewu 20 noma ngaphezulu

Uyenqaba ukuphendula

Section 3: Pregnancy test

8 Has urine sample been collected for pregnancy test?

Yes

No

8a Why not?

Not indicated in protocol schedule

Not clinically indicated

Not possible to obtain urine specimen

Other

(specify_____)

Interviewer code □□□

Demographics

Interviewer: read the questions to the volunteers verbatim and allocate the correct answer from the selection, unless it is indicated that you read out each answer to the volunteer as well. Boxed type is instructions to the interviewer. Type in italics is to be read to the volunteer.

Section 1: Personal details

1 Usuku lokuzalwa ☐☐/☐☐/☐☐☐☐ DD / MM / YYYY

Noma iminyaka (uma usuku lokuzalwa lungaziwa) ☐☐

2 Yiluphi ulimi olukhulumayo ekhaya? _____

3 Ukhonza kuliphi isonto/Iyiphi inkonzo ongaphansi kwayo?

UngumKristu (oyiProtestani)	☐	UngumKristu (ongachaziwe)	☐
UngumKristu (oyiKhatholika)	☐	Inkolo yesintu	☐
UyiSeventh day adventist	☐	UngumKristu ozelwe kabusha	☐
UyiMuslim	☐	UyiHindu	☐
UyiSayoni	☐	KwaShembe	☐
KoFakazi bakaJehova	☐	Akukho	☐
Okunye	☐	Chaza _____	

Section 2: Education and sources of income

"Manje ngizokubuza imibuzo ethize emayelana nezinga lemfundo yakho nokuthi imali engena emndenini wakho itholakala kanjani. Lolulwazi aluyukusetshenziselwa okunye ngaphandle kokuba lube nomthelela ekucubunguleni imiphumela yalolucwaningo. Sifuna ukubona ukuthi abantu abayingxenye yalolucwaningo kulelizwe bayafana yini nabangenele lolucwaningo kwamanye amazwe."

.

4	Yiliphi izinga eliphezulu lemfundo owalithola?		Alikho	☐	
	Ngaya esikoleni kodwa angiqedanga izinga eliphansi		Ngaqeda izinga eliphansi	☐	
	Angiqedanga eSekhondari	☐	Ngaqeda eSekhondari	☐	
	Angiqedanga esikhungweni semfundo ephakeme	☐	Ngaqeda imfundo ephakeme	☐	
	Angithandi ukuphendula	☐	Angiqedanga esikhungweni semfundo ephakeme kodwa ngafundela umsebenzi	☐	
5a	Ungasichaza kanjani isimo sakho somsebenzi?		Ngiqashwe ngokugcwele	☐	→Q. 5c
	Ngibambe itoho	☐	→Q. 5c Ngingumfundi	☐	
	Ngisafuna umsebenzi	☐	Angivumi ukuphendula	☐	
	Angiqashiwe	☐	Ngihlala ekhaya	☐	
	Sengampesha	☐			
	Okunye	☐			
			Chaza_____		
5b	Ngonyaka odlule, ukhona umsebenzi owawuwenza?		Yebo	☐	
			Cha	☐	→Q. 6

5c

Chaza kafishane ngomsebenzi okuyiwona owenzayo noma owawuwenza. ***Interviewer tick the answer most relevant to the description – make sure to only tick other if the participant's work does not fit into any of the categories.***

Umsebenzi ongafundelwa wamandla	☐	Ukudayisa/ukwenzela abanye umsebenzi othile	☐
Ukulima izitshalo	☐	Umsebenzi wasekhaya/endlini	☐
Ukudoba	☐	Ukufuya	☐
Okunye	☐	Ukukhiqiza izinto	☐
		Chaza_____	

5d	Ikuphi kulokhu okulandelayo okuwuchaza kangcono umsebenzi wakho?	Ngihola njalo	☐	Ngiyazisebenza	☐
		Ngiyitoho	☐	Ngihola ngesitokwe	☐
		Angiholi nhlobo	☐	Okunye Chaza_____	☐
5e	Wenzeka/wawenzeka kuphi lomsebenz? [beka uphawu kukho konke okuyikho]	Ekhaya	☐	Emzini womqashi	☐
		Emgwaqweni	☐	Esitolo/emakethe/endlwaneni yokuthengisela	☐
		Efemini	☐	Emahlathini/epulazini/ engadini	☐
		Kwezokwakha/ezimayini/ enkwalini	☐	Okunye Chaza_____ _____	☐

Section 3: Housing and household

"Manje sengizokubuza imibuzo ethile mayelana nomndeni wakho. Uma sikhuluma ngomndeni sisho izindlu uqobo lwazo, nabantu bonke abayingxenye yalomuzi, kubala wena nabo bonke abahlala kuwo (abomndeni nezihlobo). Kungaba yindlu eyodwa noma izindlu eziningi kodwa kungezomndeni owodwa, noma ngabe lezizindlu zingasondelene. Umndeni wakhiwe iqembu labantu, imvamisa abayizihlobo, abahlanganyelayo ekucazeleni abanakho futhi bavame nokudla ndawonye".

6	Ubani ongathi uyinhloko yomndeni?			Yimina	☐ →Q. 8
		Uphathina	☐	Ingane yakwethu	☐ →Q. 7a
		Ingane	☐	Umakoti/umkhwenyana	☐ →Q. 7a
		Umzali	☐	Umamezala/ubabezala	☐ →Q. 7a
		Ezinye izihlobo	☐	Umshana (wesilisa/wesi fazane)	☐ →Q. 7a
		Omunye	☐	Chaza_____ _____	→Q. 7a

We would like to ask you some questions about the head of the household you have just identified.

7a	Ungasichaza kanjani isimo somsebenzi wenhloko yomndeni?			uqashwe ngokugcwele	☐ →Q. 7c
		Uyitoho	☐	→Q. 7c Umfundi	☐
		Ufuna umsebenzi	☐	Uyenqaba ukuphendula	☐
		Akaqashiwe	☐	Owesifazane ongasebenzi	☐
		Usewampesha	☐	Angazi	☐
		Okunye	☐	Chaza_____ _____	

7b	Onyakeni odlule ngabe inhloko yekhaya ukhona yini umsebenzi eke yawenza?			Yebo Cha Angazi	☐ ☐ →Q. 8 ☐ →Q. 8
7c	Chaza kafishane okuyiwona msebenzi obalulekile inhloko yekhaya evame ukuwenza noma eyake yawenza. ***Interviewer tick the answer most relevant to the description – make sure to only tick ' other' if the work does not fit into any of the categories***				
		Umsebenzi ongafundelwa wamandla Ukulima izitshalo Ukudoba Angazi Okunye	☐ ☐ ☐ ☐ ☐	Ukudayisa/ukwenzela abanye umsebenzi othile Umsebenzi wasekhaya/endlini Ukufuya Ukukhiqiza izinto (Chaza_____ _____)	☐ ☐ ☐ ☐
7d	Ikuphi kulokhu okulandelayo okuwuchaza kangcono umsebenzi owenziwa inhloko yalomuzi?	Ukukhokhelwa ngazozonke izikhathi itoho Akakhokhelwa Okunye	☐ ☐ ☐ ☐	Uyazisebenza Ukhokhelwa ngesitokwe Angazi (Chaza_____ _____)	☐ ☐ ☐
7e	Ikuphi lapho wawuwenzela khona noma lapho osebenzela khona? [beka uphawu kukho konke okuyikhona]	Endlini yasekhaya Emgwaqweni Embonini/efemini Ezindaweni zezinkontileka/emayini/ enkwalini Okunye	☐ ☐ ☐ ☐ ☐	Emzini womqashi Esitolo/emakethe/endaweni encane yokudayisela Ehlathini lokutshalwa/ epulazini/engadini Angazi (Chaza_____ _____)	☐ ☐ ☐ ☐
8	Zingaki izindlu emzini wakho ezisetshenziselwa ukulala?			Yisho inombolo yazo Noma ubeke uphawu lapha uma ungafuni ukuphendula	☐☐ ☐
9	Bangaki abantu abalala emzini wakho?			Isho inombolo yabo Noma ubeke uphawu lapha uma ungafuni ukuphendula	☐☐ ☐

10	Uhlobo luni lomuzi ohlala kulo?			Endlini yomkhandlu wedolobha	☐
		Indlu osayikhokhela ibhondi	☐	Endlini okhokha kuyo irenti	☐
		Efulethini osalikhokhela ibhondi	☐	Efulethini okhokha kulo irenti	☐
		Ekamelweni elingaphakathi noma elingaphandle kwendlu	☐	Emjondolo	☐
		Okunye	☐	Chaza_____	
10a	Izindonga zendlu yakho zakhiwe ngani?	Ngezitini zikasimende	☐	Ngamasoyi	☐
		Ngothayela	☐	Ngowatela nokuphahlekwa/ngezintingo	☐
		Ngamapulangwe	☐	Notshani	☐
		Amatshe nosimende	☐	Ngama-prefab	☐
		Okunye	☐	Amatshe nodaka	☐
		Chaza_____			
10b	Lwakhiwe ngani uphahla lwakho?	Ukhethe	☐	Utshani/umhlanga/amakhasi kabanana /ngoqwalo	☐
		Ngama-tiles	☐	Chaza_____	
		Ngo-asbestos	☐	_____	
		Okunye	☐		
10c	Lakhiwe ngani iphansi?	Amapulangwe angenziwe lutho	☐	Umhlabathi/udaka/isihlabathi/ubulongwe	☐
		Parquet/ipulangwe elipholishwayo	☐	Usemende/ukhonkolo/isitini	☐
		Umata oyivinyl/imicwana yeasphalt	☐	Amatiles ayiceramic/iterrazo	☐
		U- carpet	☐	Okunye	☐
				Chaza_____	
				_____	
11	Ikuphi lapho nivamise ukuthola khona amanzi okuphuza emndenini njengamanje? _____				
11a	Ikuphi enikusebenzisa kakhulu ukwenza umlilo wokupheka? _____				
12	Ubani ongumnikazi wendlu/wekhaya	Uphathina	☐	Imina	☐

lapho uhlala khona?

Umnikazi ndawo ozimele	☐	Owakwethu	☐
Umzali/abasemzini	☐	Uhulumeni/umkhandlu	☐
Esinye isihlobo	☐	Uyenqaba ukuphendula	☐
Inkampani	☐	Chaza_____	
Okunye	☐	_____	

13 Umndeni wakho unakho yini lokhu okulandelayo: {beka uphawu olulodwa kulokho nalokho okuyimpendulo okusohlwini}

	Umsakazo			
Ugesi	Yebo	☐	Cha	☐
Umabonakude	Yebo	☐	Cha	☐
Ucingo	Yebo	☐	Cha	☐
Isiqandisi/ifiliji	Yebo	☐	Cha	☐
I-personal computer	Yebo	☐	Cha	☐
Umshini wokuwasha	Yebo	☐	Cha	☐

14 Ukhona yini oyilungu lomndeni wakho onalokhu: [beka uphawu olulodwa kuleyo naleyo mpendulo]

Ibhayisikili	Yebo	☐	Cha	☐
Isithuthuthu /noma isikuta	Yebo	☐	Cha	☐
Imoto	Yebo	☐	Cha	☐
Imbongolo noma ihashi	Yebo	☐	Cha	☐
Izimvu noma izinkomo	Yebo	☐	Cha	☐

Section 4- Access to resources

15	Kulezizinyanga eziyi-12 ezedlule, ngabe wena nanoma iliphi ilungu lomndeni wakho nike nanikezwa yini incwajana ngudokotela yokuyothenga umuthi awunqumileyo kodwa anangakwazi ukuwuthola ngoba ningakwazanga ukuwukhokhela?	Yebo	☐
		Cha	☐
		Uyenqaba ukuphendula	☐
16	Imali oyingenisayo emndenini ingakanani uma uyiqhathanisa naleyo umyeni/uphathina wakho ayingenisayo?	Ngingenisa imali enkulu kunaye	☐
		Ungenisa enkulu kuneyami	☐
		Singenisa imali ecishe ilingane	☐

Interviewer code ☐☐☐

Genital Examination

MDP 301 – Genital Examination - E1 Version 1.0 18 August 2005 **Date of visit: Site name: AFRICA CENTRE – SOUTH AFRICA** / / **Screening number:**

Initials: **Trial number:**

WK 0 4 8 12 16 20 24 28

32 36 40 44 48 52 Extra

1. Was contact bleeding observed today (eg from speculum or cervical swab)? Yes ☐ No ☐

2. Was cervicovaginal bleeding observed on exam today? Yes **(complete table below)** ☐ No ☐ **→Qn3a**

Describe **Tick one box only**	Describe the source if this was identified **Tick one box only**	Most likely explanation **Tick one box only**	Relationship to study product **Tick one box only**
Blood tinged discharge ☐	Discharge ☐ Not identified ☐	Menses ☐ Infection ☐	Unrelated ☐
spotting ☐	Cervix (before swabbing) ☐	Hormonal ☐ Pregnancy ☐	Unlikely ☐
bleeding ☐	Cervical polyp ☐	Gel/product ☐ Applicator ☐	Possibly ☐
profuse ☐	Through cervical os ☐	Not sure ☐	Probably ☐
	Other_____ ☐	Other_____ ☐	Definitely ☐

If you observe bleeding and the participant hasn't reported this, check with her and if she says 'yes' complete C1 and correct CS [or at week 0 correct Medical History and Log of Pre-existing conditions]. Remind her that it is important to note any genital symptoms including non-menstrual bleeding.
If the bleeding is profuse (heavier than menses) and non-menstrual, complete a NAE form unless week 0 prior to gel.
Report bleeding that is coming from a lesion under 5 or 6 or 8, not here.
If NMB was reported at interview, but you do not observe any, go back and complete the 'most likely explanation' and 'relationship' on C1

3a) Did you observe any unusual discharge today? Yes ☐ **→Qn3b** No ☐ **→Qn4**

3b) What was the source of the discharge? **Tick one box only** Not possible to tell ☐

Vaginal ☐ Cervical ☐ Cervico-vaginal ☐

3c) What was the pH of the vaginal discharge? ____._____ Not done in error ☐

3d) What were the characteristics of the discharge? Tick yes or no for each

Curdlike Yes ☐ No ☐ Offensive Yes ☐ No ☐ Bloody Yes ☐ No ☐

3e) Could the discharge be gel? Tick one box only Don't know ☐ Yes ☐ No ☐

4 What percentage of the cervical surface area was cervical ectopy? Answer even if no ectopy

0% = no ectopy ☐ 1-25% ☐ 26-50% ☐ 51-100% ☐

Interviewer code ☐☐☐