SYNDROMIC MANAGEMENT APPROACH AND LABORATORY DIAGNOSIS OF *TRICHOMELOAS VAGINALIS* IN STI COMPLIANT AND PREGNANT WOMEN ATTENDING MERAWI HEALTH CENTER, MERAWI, AMHARA REGIONAL STATE, ETHIOPIA:

BY MULUNEH ADEME

THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS OF SCIENCE DEGREE IN MEDICAL PARASITOLOGY.

MAY, 2011
MERAWI, ETHIOPIA
Addis Ababa University
Faculty of Medicine
Graduate School
Department of Microbiology, Immunology and Parasitology

**Topic:** - SYNDROMIC MANAGEMENT APPROACH AND LABORATORY DIAGNOSIS OF *TRICHOMONAS VAGINALIS* IN STI COMPLIANT AND PREGNANT WOMEN ATTENDING MERAWI HEALTH CENTER, MERAWI, AMHARA REGIONAL STATE, ETHIOPIA:

**Principal investigator:** - Muluneh Ademe [BSc.]

**Advisors:**

➢ Tadesse Kebede: - [PhD fellow, MSc.]
➢ Nigus Fikrie: - [PhD fellow, MSc.]
Acknowledgment

I would like to express my special gratitude to my advisors: Mr. Tadesse Kebede and Mr. Nigus Fikrie for their constructive and fruitful advices and comments starting from the proposal development to final thesis write up. My sincere acknowledgment goes to Addis Ababa University: Graduate School research program for the provision of financial support to conduct this study. Finally I am very much grateful to Merawi Health Center staffs for their cooperation during data collection.
# Table of Contents

Acknowledgment ........................................................................................................ii

List of tables and figures ............................................................................................v

List of abbreviations ..................................................................................................vi

Abstract .......................................................................................................................vii

1. Introduction ...............................................................................................................1
   1.1. Background information ....................................................................................1
   1.2. Statement of the problem ..................................................................................2
   1.3. Significance of the study ...................................................................................4

2. Literature review .....................................................................................................5
   2.1. Epidemiology .....................................................................................................5
       2.1.1. Global Burden .............................................................................................5
       2.1.2. Magnitude In Africa ...................................................................................5
       2.1.3. Magnitude In Ethiopia ................................................................................6
   2.2. Transmission .....................................................................................................7
   2.3. Pathogenesis ....................................................................................................7
   2.4. Diagnosis .........................................................................................................9
       2.4.1. Syndromic Approach ...............................................................................9
       2.4.2. Laboratory Diagnosis ..............................................................................9
   2.5. Treatment ........................................................................................................11
   2.6. Prevention and Control ....................................................................................11

3. Objectives ...............................................................................................................13

4. Methodology ..........................................................................................................14
   4.1. Study area ........................................................................................................14
   4.2. Study period: ....................................................................................................14
   4.3. Study design .....................................................................................................14
   4.4. Population ........................................................................................................14
   4.5. Study variables.................................................................................................14
   4.6. Sample size determination ..............................................................................15
   4.7. Inclusion criteria: .........................................................................................15
   4.8. Exclusion criteria .............................................................................................15
   4.9. Definition of operational terms ......................................................................16
List of tables and figures

Table 1: distribution of *T. vaginalis* among different age groups, marital status, education status, occupation, sexual partnership, STI awareness and symptoms with respect to *T. vaginalis*.  

<table>
<thead>
<tr>
<th>Table 1:</th>
<th>Page no</th>
</tr>
</thead>
<tbody>
<tr>
<td>distribution of <em>T. vaginalis</em> among different age groups, marital status, education status, occupation, sexual partnership, STI awareness and symptoms with respect to <em>T. vaginalis</em>.</td>
<td>19</td>
</tr>
</tbody>
</table>

Figure 1: percentage of STI awareness for wet mount positive subjects  

<table>
<thead>
<tr>
<th>Figure 1:</th>
<th>Page no</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentage of STI awareness for wet mount positive subjects</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 2: sensitivity and specificity of syndromic management approach with respect to wet mount microscopy of vaginal samples.  

<table>
<thead>
<tr>
<th>Table 2:</th>
<th>Page no</th>
</tr>
</thead>
<tbody>
<tr>
<td>sensitivity and specificity of syndromic management approach with respect to wet mount microscopy of vaginal samples.</td>
<td>21</td>
</tr>
</tbody>
</table>
List of abbreviations

**BV**: Bacterial Vaginosis

**CI**: Confidence Interval

**EIA**: Enzyme Immuno Assay

**FSW**: Female Sex Workers

**HIV**: Human Immunodeficiency Virus

**HSV**: Herpes Simplex Virus

**IRB**: Institution Review Board

**OPD**: Out Patient Diagnosis

**PCR**: Polymerase Chain Reaction

**PMTCT**: Prevention of Mother to Child Transmission

**PR**: Prevalence Rate

**SOP**: Standard Operational Procedures

**STI**: Sexually Transmitted Infection

**TV**: *Trichomonas vaginalis*

**WHO**: World Health Organization
Abstract

Background: *Trichomonas vaginalis* is a flagellated, single cell protozoan parasite. *T. vaginalis* carries the distinction of being the only truly sexually transmitted parasitic infection in humans. It is highly adapted to the human urogenital tract and majority of infections are asymptomatic. Symptomatic trichomoniasis presents with an offensive vaginal discharge and vulval itching in women. *T. vaginalis* infection has been shown to be associated with adverse pregnancy outcomes, particularly premature rupture of membranes, pre-term delivery and low birth weight.

Objective: the aim of this study was to determine the prevalence of *T. vaginalis* among STI compliant and (or) pregnant women attending Merawi Health center. Simultaneous comparison of the laboratory (wet mount microscopy) result with the syndromic management results was performed.

Result: 222 women have participated in this study. 198 were pregnant and 24 were STI complaint. Majority of them were under the age group of 15-25 years (48.6%, 108). 73.9% (164) were illiterate, 94.1% (209) were married, and 86.9% (193) didn’t have awareness to any of STIs. Syndromic management approach has identified 4 women (1.8%) who had self-reported symptoms related to trichomoniasis. However; the laboratory wet mount diagnosis has identified 14 women who were positive for the trophozoites of *T. vaginalis*. As a result, the prevalence of trichomoniasis using the laboratory wet mount result was increased to 6.3% (14/222). The sensitivity of syndromic management approach with respect to the wet microscopy was 21.4%.

Conclusion: As a result of high rates of asymptomatic infections, syndromic management approach should not be the first choice to screen out trichomoniasis. So, this study supports the need for improved diagnostic parameters and disease control activities to reduce adverse trichomoniasis associated reproductive health outcomes such as ectopic pregnancy, low infant birth weight and preterm labor.

Key words: Trichomoniasis, Syndromic Management approach, STI
1. Introduction

1.1. Background information

Sexually transmitted infections (STIs) are caused by micro-organisms that are fastidious in nature and therefore need intimate contact between individuals for transmission. Since these infections have only one host, i.e. man, they are in principle, ideal candidates for elimination (Moodley et al., 2004). However efforts to this end have been largely neglected. This lack of attention stemmed from the premise that only certain, already marginalized population groups, based on behavior, race, social status and occupation, were at risk for contracting STIs. As a result of this social stigmatization, these infections were placed lower on the ladder of health priorities. Curable STIs therefore continue to be a large public health problem especially in resource poor settings (Moodley et al., 2004).

For the want of an appropriate point-of-care test with reasonable sensitivity and specificity to detect the common STI pathogens, the World Health Organization (WHO) introduced various guidelines for the syndromic treatment of symptomatic patients (Cited in Moodley et al., 2004). The syndromic approach uses clinical algorithms so designed that primary health care nurses in resource poor settings may arrive at an appropriate clinical diagnosis based on a patient’s symptoms and clinical signs ((Mullick et al., 2005; Moodley et al., 2004). The clinical diagnosis is then linked to a predefined antimicrobial prescription in which drugs that have shown efficacy against the different STI pathogens in clinical trials are advised (Moodley et al., 2004).

The classic approach to STI case management has been fraught with problems. Studies have repeatedly underscored the inaccuracies associated with linking clinical observations with aetiological diagnosis. This is compounded by the fact that patients often present with multiple infections (Cited in Moodley et al., 2004). In addition, in resource poor settings the diagnosis of STIs has been hampered by the lack of trained health care personnel and appropriate laboratory support. Classic laboratory diagnosis requires staff that is properly trained to collect the required specimens (Cited in Moodley et al., 2004).

A spontaneous complaint of abnormal vaginal discharge (in terms of quantity, color or odor) may be physiological or pathological. Although abnormal vaginal discharge often prompts
women to seek screening for STIs, vaginal discharge is poorly predictive of the presence of an STI (Mitchell, 2004). Vaginal discharge is, however, most commonly a result of a vaginal infection. *T. vaginalis*, *C. albicans* and bacterial vaginosis (BV) are the commonest causes of vaginal infection. The symptom of abnormal vaginal discharge is highly indicative of vaginal infection, but poorly predictive for cervical infection (WHO, 2003).

The flagellated protozoan, *T. vaginalis*, is almost exclusively sexually transmitted in adults. The infection may be asymptomatic. Symptomatic trichomoniasis presents with an offensive vaginal discharge and vulval itching in women, and urethritis in men. *T. vaginalis* infection has been shown to be associated with adverse pregnancy outcomes, particularly premature rupture of membranes, pre-term delivery and low birth weight (Loo *et al*., 2009). This association is particularly important in symptomatic women (WHO, 2003).

Most of the Amhara towns are located along the busiest cross-country road, which links Ethiopia to the port of Djibouti; and the neighboring three regional states (Benshangul Gumuz, Afar, and Oromia). The towns are home to large number of mobile workers such as truckers, intercity bus drivers, and travelling businessmen (Dereje, 2008). Most of the towns have a significant number of rural migrant people who moved to the town in anticipation of getting a job. Most towns are markets for the surrounding rural districts, and a large number of farmers come to town on the weekly market days. Most towns host large numbers of FSWs who operate in hotels, bars, and local brew selling houses. Hence, various cultural and traditional practices exacerbate STI transmission including HIV in the Amhara towns (Dereje, 2008).

### 1.2. Statement of the problem

Globally, sexually transmitted infections constitute to be a major public health problem. The WHO estimates that 75 - 85% of approximately 340 million new cases of the four main curable STIs (gonorrhea, Chlamydia infection, syphilis and trichomoniasis) occur every year in developing countries (Altini *et al*., 2005). Ulcerative and non-ulcerative STIs have emerged as important public health concerns with increasing gynecological morbidity and mortality in women (Ray *et al*., 2009). Among women aged 15-44 years it is estimated that genital tract infections are the second most frequent cause of healthy life lost after maternal mortality and morbidity (Wilkinson *et al*., 1997; Sturm *et al*., 1998).
Ethiopia has one of the highest HIV prevalence’s in sub-Saharan Africa. HIV prevalence is between 14% and 20% among urban pregnant women, 12% in patients treated for STIs, and 74% in commercial sex workers (Hladik et al., 2006). According to Ethiopia’s 2007 single point estimates, the national adult (ages 15-49) HIV prevalence for 2008 is 2.2 percent (male 1.8 percent and female 2.6 percent), with an urban and rural HIV prevalence of 7.7 percent and 0.9 percent, respectively. The same report estimated that there are 1,037,267 people living with HIV in the country, of which 289,734 are in need of antiretroviral treatment (Dereje, 2008).

The Amhara Administrative region, one of the largest regions in the country, has a very high burden of diseases (Fentahun et al., 2003). The adult HIV prevalence for Amhara region is reported to be 2.0 percent, close to the national estimate. The Amhara towns host large numbers of FSWs who operate in hotels, bars, and local brew selling houses. They work as waitresses in these places, and meet clients that way. In 2008, 3930 FSW were found in Debrebrhan, Shoarobit, Ataye, Bati, Dejene, Bure, Chagni, and Estie which could play its part in STI prevalence including HIV (Dereje, 2008).

Trichomonal infection has been associated with adverse reproductive health outcomes including pelvic inflammatory disease, low infant birth weight and premature delivery (cited in Tann et al., 2006). The perceived lack of effect on fertility despite the fact that recent studies have indicated that *T. vaginalis* can cause pelvic inflammatory disease in both HIV infected and uninfected women (cited in Shafir et al., 2009). It is now recognized that, *T. vaginalis* provides pools of leukocytes and macrophages that intensify the shedding of HIV in the genital area (Lemos et al., 2010). Hence, it may play a critical and under recognized role in amplifying HIV transmission (Schwebke et al., 2006; Petrin et al., 1998), and it may have a major impact on the epidemic dynamics of HIV in African American communities (Shafir et al., 2009). *T. vaginalis* infection also doubles the risk of persistent human papilloma virus infection in women (Pattullo et al., 2009).

WHO estimates that, globally 173 million of new cases of *T. vaginalis* occur annually while over 8 million new cases are estimated to occur annually in the United States alone (cited in Shafir et al., 2009). Although the organism appears to be highly prevalent and has a widespread geographic distribution, *T. vaginalis* is not a locally or nationally reportable disease and has not
been the focus of active control programs. This neglect is likely a function of the relatively mild nature of the disease (cited in Leon et al., 2009).

Even though T. vaginalis infection is one of the most common curable sexually transmitted infections, the frequency of infection is not monitored in most countries nor do control programs exist (cited in Leon et al., 2009). Most countries STI guidelines do not include trichomonas diagnostic testing. The syndromic management of suspected vaginal trichomoniasis is the current standard for clinical care (cited in Leon et al., 2009). However, long standing, asymptomatic infections may lead to continued community level transmission. As Trichomonas prevalence depends on many factors such as age, sexual activity, and the number of sex partners, a better understanding of the epidemiology of this infection in women is needed to foster disease control programs in populations at risk for reproductive health complications (Leon et al., 2009).

1.3. Significance of the study

A large burden of gynecological disease exists in developing countries (Rizvi et al., 2006) and complications related to STIs are a major cause of mother and child mortality and morbidity during pregnancy (Menendez et al., 2010). Multiple sexual relationships, low habit of condom use, substance abuse (especially of khat and shisha), and heavy consumption of locally brewed, strong alcoholic drinks such as araki, teji, and tella are common in some Amhara towns (Dereje, 2008). These cultural and traditional practices perpetuate STI transmission (Dereje, 2008). So, this study plays its part in reducing such malpractices by creating awareness on what STIs are, the risks resulted by STIs and the ways how to get prevented from STIs. Family planning clinics are important sites through which to identify, treat, and prevent STI infections as many women in developing countries may attend them (cited in Shafir et al., 2009). Additionally, this study increases the attention given for trichomoniasis by health workers, by showing the current burden of infection.
2. Literature review

2.1. Epidemiology

2.1.1. Global Burden

Nowadays, the advent of HIV has contributed its part for the provision of great attention towards the neglected pathogenic microorganisms which are responsible for complications resulting sexually transmitted infections (Moodley et al., 2004). *T. vaginalis* is one of the most neglected of the neglected pathogenic microorganisms causing STI (Noel et al., 2010). However, the degree of emphasis given for these neglected pathogens also varies depending on different factors that could be highly attributed to the economic capability of the country. (Moodley et al., 2004).

*Trichomonas vaginalis* is the most prevalent non viral sexually transmitted infection worldwide (Mullick et al., 2005). The WHO estimates that *T. vaginalis* accounts for approximately half of all curable sexually transmitted infections worldwide (cited in Mullick et al., 2005). The estimated global incidence of *T. vaginalis* infections is over 170 million cases per year (Shafir et al., 2006). The estimates for North America alone are between 5 and 8 million new infections each year, with an estimated rate of asymptomatic cases as high as 50% (Upcroft et al., 2001).

In coastal Peru, among the 319 women enrolled, the overall prevalence of trichomonal infection was 9.1%. The mean age was 26.3 years, and 35.5% reported having had unprotected intercourse with non primary partners and 19.8% reported two or more sex partners. Trichomonal infection was associated with increased number of sex partners and unprotected sex with non primary partner. Women with *T. vaginalis* infection were more likely to be older; the highest prevalence was found in the oldest age group, 36–40 years. Those with less education were 3 times more likely to have trichomonal infection compared to those who completed high school, and being single was associated with having trichomonal infection (Leon et al., 2009).

2.1.2. Magnitude In Africa

Sexually transmitted infections are common in the developing world (Mullick et al., 2005). STIs are among the first ten causes of unpleasant diseases in young adult males in developing countries and the second major cause of unpleasant diseases in young adult women. Adolescents
and young adults (15–24 years old) make up only 25% of the sexually active population, but represent almost 50% of all new acquired STIs (Ros et al., 2008). However, cross sectional, randomized trials and retrospective cohort studies of antenatal clinic attenders in Africa have found that up to 40% of pregnant women have trichomoniasis (Mullick et al., 2005).

The distribution of trichomoniasis differs in different African countries. In Zimbabwe, the prevalence of trichomoniasis may be as high as 50% in women attending genitourinary clinics while asymptomatic carriage of *T. vaginalis* has been shown in approximately 10% women attending family-planning clinics (Mason et al., 2005). However, the PAP microscopy in Kenya revealed trichomoniasis prevalence of 5.2% (205 out of 3908) (Daly et al., 1994). In rural South Africa, asymptomatic and unrecognized genital tract infections were studied and *T. vaginalis* prevalence among women attending a family planning clinic was 14% (Wilkinson et al., 1997).

On the other hand, pregnant women attending village clinics, South Africa, in the district for prenatal care, in the mean age of 25.2 were enrolled for undetected STIs. Most (246; 75%) were unmarried. In all, 75 (23%) reported having had a previously treated STI within the last year, and many women (153, 47%) described their partner as being a migrant (defined as spending most nights away from home). Prevalence was 41.4% for trichomoniasis (Sturm et al., 1998). In addition, the prevalence of *T. vaginalis* infection, among pregnant women in Entebbe, Uganda was 17.3%. In total, 30.2% of those with *T. vaginalis* were asymptomatic. The sensitivity of syndromic management as applied by health workers in targeting *T. vaginalis* was 66.7%. This would have increased to 69.8%, had the algorithm been followed exactly (Tann et al., 2006).

### 2.1.3. **Magnitude In Ethiopia**

No reliable statistics are presently available on the actual extent of STI in Ethiopia, but it was reported that syphilis was the most frequent disease being treated (Plorde, 1981). Field studies carried out in various provinces showed that psychosocial factors in the Ethiopian culture-related to migration, urbanization, and sexual behavior which might interact in such a way as to result in an increase in STIs (Plorde, 1981).

A total of 106 women symptomatic for STIs were enrolled in Addis Ababa, Ethiopia. The age of the women ranged from 17 to 51 years (median, 27) and 71 (67%) were HIV-1 sero-positive
(Wolday et al., 2004). The most common syndrome was genital discharge and bacterial vaginosis was common in this population. From those who presented genital discharge, the prevalence of *T. vaginalis* (using wet mount and/or culture diagnosis) was found to be 1.2% (1/87). Given the fact that more than 50% of the STIs are misdiagnosed by the syndromic approach, either missing or over diagnosing of infection was common (Wolday et al., 2004).

### 2.2. Transmission

Trichomoniasis is a common, sexually transmitted disease caused by the flagellated protozoan parasite *T. vaginalis*. *T. vaginalis* was first described by Donné in 1836. The trophozoites have four free anterior flagella (one along the outer edge of an undulating membrane) and one recurrent; there is no known cyst form. Trophozoites divide by binary fission (Upcroft et al., 2001). *T. vaginalis* is capable of invading and colonizing the heavily defended host uro-genital mucosa from both sexes, braking through the primary innate defenses and withstanding induced innate and adaptive responses (Noel et al., 2010).

Humans are the sole reservoir of *T. vaginalis*. The parasites are transmitted mainly during sexual intercourse. About 2–17% of female neonates born of infected mothers’ contract, a perinatal infection. *T. vaginalis* is highly labile outside of a host. Nonetheless, a few trophozoites can survive for up to five hours in the water of non-chlorinated thermal baths and for five minutes to 24 hours in tap water with standard chlorination; they are killed within a few minutes in swimming-pool water with high chlorine concentrations (44 mg/l). It is conceivable that infections could be transmitted by wet bathing suits, sponges, towels, etc. as well as acquired from non chlorinated thermal baths and poorly maintained swimming pools, but there is no evidence showing that these are significant sources of infection (Ryu et al., 2006).

### 2.3. Pathogenesis

Notably, *T. vaginalis* infections are often considered non-self limiting in females and recent data even suggest that persistent, undetected infections can persist even after successful treatments (Noel et al., 2010). The factors considered to be involved in the pathogenicity of *T. vaginalis* include; the ability of trichomonads to adhere to vaginal epithelial cells (VECs), the cytotoxic
effect of the pathogen on host cells, trichomonad proteinase activity, and the ability of
trichomonads to produce subcutaneous abscess lesions (Ryu et al., 2006).

The patho-biology of \textit{T. vaginalis} is complex and multifaceted with adhesion to, and alterations of, the various mucosal landmarks (mucus, epithelial cell barrier, extracellular matrix [ECM], innate and adaptive immune cells) thought to be essential to initiate and maintain infections. \textit{T. vaginalis} cells are also known to form large cell aggregates (in a process called swarming or rosetting), which could represent an important process for pathogenesis, suggesting that specific cell-cell interactions also take place between cells of the parasite (Noel et al., 2010).

When the mucosal tissue is damaged the parasite can bind to host ECM proteins and during menstruation or parasite induced micro-haemoragia, \textit{T. vaginalis} also binds to various plasma proteins. Adhesion to host tissue also induces a cellular differentiation of \textit{T. vaginalis} into amoeboid forms (Noel et al., 2010). However, little is known about the molecular and cellular basis of the various processes, with the pathogen lipophosphoglycan (LPG), various adhesions, surface and secreted enzymes and toxins all thought to be involved, but existing data are limited (Noel et al., 2010).

Trichomonal infection in women ranges from an asymptomatic carrier state to profound, acute, inflammatory disease. The parasite principally infects the squamous epithelium of the genital tract but can be recovered from the urethra and has been found in the fallopian tubes and the pelvis. In males, \textit{T. vaginalis} causes urethritis and prostatitis. Respiratory infections are acquired perinatally from infected mothers. In children, trichomonads can infect the urinary tract as well as the vagina. \textit{T. vaginalis} infections have been linked to sterility problems and to adverse pregnancy outcomes (Upcroft et al., 2001).

The most common complaint associated with trichomoniasis infection is a vaginal discharge (Alderete et al., 1984). This discharge is frequently profuse and is often associated with burning, itching, or chafing. When viewed through a speculum, the vaginal mucosa is sometimes diffusely hyperemic, with bright red punctate lesions, although sometimes it is only patchily hyperemic and not infrequently normal in appearance. The frequency of urination and dysuria are the commonest associated symptoms, and urethral involvement is found in a large proportion of
cases. In addition, Trichomoniasis patients show various symptoms and/or signs of vaginitis that included vaginal discharge, pruritus, erythema and vaginal wall edema (Ryu et al., 2006).

2.4. Diagnosis

2.4.1. Syndromic Approach

In the resource constrained settings of many developing countries, laboratory diagnosis to confirm the aetiology of an STI related symptom is not a practical option. Screening using culture, ELISA, and polymerase chain reaction testing are not feasible and are too costly (Mullick et al., 2005). Where services are offered for the treatment of STIs in pregnancy in developing countries, this should rely on syndromic management, based on the provision of treatment for the main organisms that cause specific signs and symptoms (syndromes) associated with certain STIs. Vaginal discharge and genital ulceration are the most frequent syndromes encountered in pregnancy in developing countries (Mullick et al., 2005).

However, this approach still lacks sensitivity and specificity (Mullick et al., 2005). Symptom-based assessments made by physicians were compared with laboratory tests in China. Laboratory test result (culture) was used as the gold standard for the comparisons. The overall sensitivity of physician symptom-based assessment was about 10% (Yin et al., 2008). The specificity was high (>95%) for both males and females. About half of the physician-diagnosed STI cases were actually false-positive according to the laboratory results. The positive predictive value (PPV) for physician diagnosis was only 0.497 (Yin et al., 2008). Therefore, the syndromic approach should not be used as a diagnostic or screening tool (Mullick et al., 2005).

2.4.2. Laboratory Diagnosis

Most trichomonal infections (60%) are asymptomatic. Even, symptomatic women fail to recognize and report their symptoms. As a result the syndromic approach does not detect the infections (Wilkinson et al., 1997). So, the laboratory diagnosis plays significant role in aiding the confirmed investigation of trichomoniasis. The prevalence of trichomonal infection in a population is dependent on the diagnostic techniques employed (Spence et al., 1980). Several techniques such as: culture, direct wet-preparation, Gram stain, Acridine orange, Papanicolaou (Pap) smear, Giemsa stain, and molecular biology based diagnostic methods such as Hybridization assay and PCR have been employed in the diagnosis of T. vaginalis in different
settings (Ojiuromi et al., 2007). The latex agglutination test which can detect antigen in both soluble and insoluble forms can also be used (Carney et al., 1988).

Different samples can be used for the laboratory investigation of trichomoniasis. Vaginal samples (vaginal swab and vaginal secretions), urine and dried blood spot (DBS) are the sample of choices. Vaginal samples have better sensitivity than urine sample to examine the trophozoites which are the diagnostic stages for *T. vaginalis*. Dried blood spot (DBS) specimens can be used to detect anti-trichomonal antibody by enzyme immunoassay (EIA). It detects exposure to trichomonal infection with >90% sensitivity in men and women (Mason et al., 2005). However, semen may be the most sensitive clinical sample for culturing *Trichomonas vaginalis*, though it is not routinely used (Johnson, 2009).

The time of processing of urine specimens and the temperature at which the specimens are held before processing can affect the sensitivity of both culture and molecular techniques in the diagnosis of *T. vaginalis* (Ingersoll et al., 2008). If more than 30 minutes elapsed between collections and testing of urine, then it is likely that the reported prevalence values of trichomoniasis represent an underestimate of the true prevalence values (Shafir et al., 2006). Additionally, whenever possible, the specimen should be kept at 37°C. *T. vaginalis* possesses highly active endo-nucleases that could be responsible for the degradation of DNA, resulting in false negative test results (cited in Shafir et al., 2006). So, if PCR is used, urine should be processed and frozen within 2 hours of collection (Ingersoll et al., 2008).

Currently there is controversy as to the most reliable means of diagnosis. Many investigations of the prevalence of trichomoniasis have employed only one or two of the three common diagnostic modalities: papanicolaou smear, 0.85% NaCl wet mount, and culture (cited in Spence et al., 1980). For example, the prevalence of *T. vaginalis* based on the direct wet smear results in Argentina was 1.8% (Perazzi et al., 2010). Additionally, in Nigeria, total of 3089 men and women (2653 men and 436 women) were examined by saline wet mount of vaginal swabs and 48 women (11%) had trichomoniasis (Bello et al., 1983).

Contradictory findings have also been published in comparisons of culture and microscopic techniques. A report from Loo et al. (2009) showed that, the clinical identification of motile
organisms by microscopic inspection of wet mount preparations had a sensitivity of 40 to 75\% while TV culture had a sensitivity of 86 to 97\%. In Lagos, Nigeria, culturing of the parasite yielded more positive results 85 (29.8\%) when compared to wet mount microscopy 79 (27.7\%) and Giemsa staining 62 (21.7\%) (Ojiuromi et al., 2007). However, there was no statistically significant difference between the wet mount microscopy, staining and culturing. Hence, the study confirmed the reliability of wet microscopy in the diagnosis of \textit{T. vaginalis} when compared with culture (Ojiuromi et al., 2007).

Culture, though considered more sensitive than microscopy, could take up to 3 days (Ojiuromi et al., 2007), 5 days (Johnson, 2009) or 3-7 days (Sahyoun et al., 2004). This makes the culture diagnosis cumbersome and more expensive (Ojiuromi et al., 2007). In addition, the acknowledged insensitivity of the wet mount is in part due to the rapid loss of the characteristic motility of the micro-organisms by which they are readily identified. Microscopy, therefore, has to be performed shortly after obtaining vaginal specimens (Carney et al., 1988). Hence, wet microscopic examination of vaginal samples, which permits quicker intervention, was used in this study.

\section*{2.5. Treatment}

Metronidazole and other 5-Nitroimidazole derivatives such as metronidazole, tinidazole, ornidazole, and secnidazole are effective for treatment of human trichomoniasis and infections caused by other anaerobic protozoa and anaerobic bacteria. Metronidazole is both highly effective and approved by the WHO because of its better curative effect (Ryu et al., 2006; McCutchan et al., 1992). However, clinical trial in Addis Ababa showed that, Nimorazole (Naxogin) has comparable effect with metronidazole in the treatment of vaginal trichomoniasis (Ross, 1973).

\section*{2.6. Prevention and Control}

It is important to control the spread of STIs, and prevention can be the key to this process. Preventive measures taken for trichomoniasis are more or less similar with the other STIs. Abstinence, while not popular, is a reasonable choice for some people and should be encouraged for those who would consider it (Rosen, 2006). Screening should be routine, regardless of
whether the patient is known or suspected to have STI. The most effective method available for protection is condom. The correct and consistent use of condoms is highly effective in preventing sexual transmission of STIs including trichomoniasis (Ros et al., 2008). In addition, prevention should be based on education and counseling of the population, identification of symptomatic and asymptomatic people, effective diagnosis and treatment of patients. It is always necessary for both sexual partners to receive treatment (Ros et al., 2008).
3. Objectives

**General objective:** the general objective of this study is to determine the prevalence of *Trichomonas vaginalis* infection, among STI compliant and/or pregnant women attending Merawi Health center.

**Specific objectives**

i. To determine the prevalence of *T. vaginalis* infection based on the results from the syndromic management and wet microscopy of vaginal samples.

ii. To calculate the sensitivity and specificity of syndromic management approach using wet mount microscopy results as a reference test.

iii. To identify the risk factors associated with *T. vaginalis*. 
4. Methodology

4.1. Study area

The study was carried out in Merawi Health center which is the only health center available to serve for the community in and around Merawi town. Merawi town is located in West Gojjam zone, 530 km northwest of Addis Ababa; just on the main road from Addis Ababa to Bahirdar. Two elementary schools, one high school, and one preparatory school are available. People with different religions are living together friendly. The town has local popularity in exhaustively producing “araki (caticala)” which is the backbone for the economy of most of the inhabitants by making it accessible for sale to local customers and local exporting to different Weredas, Zones and Regions too. Hence the town is where many prostitutes are living, making use of “caticala” for sale to local consumers in the town.

4.2. Study period:

Data collection was taken place from November 05-2010 to January 29-2011.

4.3. Study design

Cross-sectional study design was used to determine the prevalence of *T. vaginalis* infection and the associated risk factors.

4.4. Population

**Source population:** Women complaining for STI and pregnant women attending the antenatal care unit of Merawi Heath center during their follow up were taken as the source of the study.

**Study subjects:** The study subjects were women from the source population who were available during the study period and who fulfilled the eligibility criteria of the study.

4.5. Study variables

**Dependent variables:** The dependent variable of this study was the prevalence of *Trichomonas vaginalis* infection.
Independent variables: Age, Education status, Occupation, number of sexual partnerships, marital status, awareness to STIs and vaginal symptoms were the independent variables.

4.6. Sample size determination

The Sample size was determined based on the statistical formula applied for cross-sectional studies. The minimum sample size was calculated using the 95% confidence interval with 5% margin error. The reference proportion (prevalence) was taken from Uganda (0.173) considering the absence of appropriate reported figure in Ethiopia and taking the advantage of getting representative figure from East Africa.

\[
N = \frac{Z^2 P (1-P)}{d^2}
\]

Where, \( N \) = the minimum sample size required
\( Z = 1.96 \) at 95% confidence interval
\( d^2 = \) is margin of sampling error tolerated (5% marginal error)
\( P = \) an estimate of the prevalence rate for the population.

\[
N = \frac{(1.96)^2 \times 0.173 (1-0.173)}{0.0025}
\]

\[
N = \frac{3.8416 \times 0.173 (0.827)}{0.0025}
\]

N = 220

Therefore, minimum of 220 study subjects have to be enrolled during the study period.

4.7. Inclusion criteria:

Women either with pregnancy or STI complains during the study period, availability in Merawi Health center during the study period and willingness by agreeing with the consent form were inclusion criteria’s.

4.8. Exclusion criteria

Women who had taken anti protozoan treatment within 2 weeks of enrollment were excluded (Pattullo et al., 2009).
4.9. Definition of operational terms

Araki (caticala): Strong locally brewed alcoholic beverages (Dereje, 2008).

Syndromic Management: is identification of consistent groups of symptoms and easily recognized signs (syndromes), and the provision of treatment that will deal with the majority of or the most serious, organisms responsible for producing a syndrome (WHO, 2003).

Woreda: An administrative unit equivalent to a district (Dereje, 2008).

4.10. Sampling technique:

Every eligible study subject attending Merawi Health center during the study period were included in the study through convenient sampling technique.

4.11. Data collection techniques

Well organized questionnaire was prepared by the principal investigator in collaboration with the advisors. The final reviewed and well digested questionnaire was translated in to Amharic language, keeping its understandability to the community where the study has been conducted. Finally, willing women were interviewed based on the questions in the questionnaire paper and the information was registered. The syndromic management results were taken from the health center patient record book and registered too.

Two vaginal swabs were taken from each participant. The samples were prepared for direct wet mount microscopic examination which is the most rapid and cost-effective diagnostic aid in the laboratory. The wet preparation was examined using the 10× and 40× objectives, with the condenser iris diaphragm closed sufficiently to give good contrast. *T. vaginalis* trophozoites are a little larger than pus cells. They are round or oval in shape and move by means of an undulating membrane and flagella. There are 4 anterior flagella and a fifth flagellum forms an undulating membrane. An axostyle protrudes from the end of the organism. Movement is often slight (on the same spot) and not progressional. Hence, a careful search was made to detect the flagellates among the pus cells (Annex 1).
4.12. **Data processing and analysis**

The data obtained from each study subject (interviews, syndromic result and wet mount result) was carefully documented and analyzed using SPSS 16 software application and the final outcomes were presented using tables and figures.

4.13. **Quality control**

Quality control measures were undertaken to increase the reliability of the final outcomes. Primarily; the questionnaire was checked by the investigator for its appropriateness such as typing error, missing questions, and inappropriate translations. Health workers (data collectors) were given detailed explanation on the questionnaire to improve their efficiency in collecting unbiased and consistent data. Secondly; just prior to examination of study samples, the microscope was tested for its appropriate functioning using positive stool and blood film samples. Finally; every 10th samples and every positive samples for trichomoniasis were repeatedly examined by other lab professionals in the health center laboratory to insure the quality of ongoing procedures.

4.14. **Ethical consideration**

This research proposal was ethically cleared by the department ethical review board of the Department of Microbiology, Immunology and Parasitology: Addis Ababa University. Prior to data collection, the medical director of the Merawi Health center was informed on the general overview of the study and permission was asked as per the recommendation letter from the department. Then, eligible study subjects were informed about the aim of the study and asked for their written consent. Data was taken from willing subjects. Finally, the wet microscopy result with respect to trichomoniasis was released to all study participants and the accurate treatment has proceeded according to the laboratory result.
5. Result

A total of 222 women have participated in this study. 198 were pregnant women attending the antenatal clinic unit for their follow up and 24 were women from OPD complaining for symptoms related to STIs. The age of women under study ranged from 15 to 45 years, and majority of them were under the age group of 15-25 years (48.6%, 108). 73.9% (164) were illiterate, 11.7% (26) were elementary students and 7.2% (16) of each were high school and college complete women. Women who were workless (including housewives) possessed the highest percentage 58.6% (130) over the private workers and government workers (34.2% and 7.2% respectively). 94.1% (209) were married while 3.6% (8) were single and 2.3% (5) were divorced. Majority of women didn’t have awareness to any of STIs (86.9%, 193). However; all women who completed college (university) were aware of at least one STI and high school complete women had better awareness (50%, 8/16) than women who were elementary complete (15.5%, 4/26) or illiterate (0.6%, 1/164) (Table 1).

Physician’s symptom-based assessment (syndromic management approach) has identified 4 women who had self-reported symptoms related to trichomoniasis such as; vaginal discharge and pain during urination. Hence, the prevalence of trichomoniasis as per the results of syndromic management was 1.8% (4/222). However; the laboratory wet mount diagnosis has identified 14 women who were positive for the trophozoites of T. vaginalis. As a result, the prevalence of trichomoniasis using the laboratory wet mount result was increased to 6.3% (14/222).

A higher prevalence of T. vaginalis infection was found among young women under the age group of 26 to 35 years (8.9%, 9/101) while women with in the age group of 15-25 years and 36-45 years had lesser prevalence’s of 3.7% (4/108) and 7.7% (1/13) respectively. A slightly higher prevalence was seen on elementary school complete women (7.7%, 2/26) than illiterate (6.7%, 11/164) and high school complete women (6.2%, 1/16). However; No infection of T. vaginalis was detected on women who had completed college/University. In addition, trichomoniasis was more prevalent (10.5%, 8/76) in private worker women (local beer and caticala sellers, café, restaurant, and coffee house workers) than women who were workless (4.6%, 6/130).
Table 1: distribution of *T. vaginalis* among different age groups, marital status, education status, occupation, sexual partnership, STI awareness and symptoms with respect to *T. vaginalis*.

<table>
<thead>
<tr>
<th>Laboratory (wet mount) result</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Trophozoite seen</td>
<td>No trophozoite seen</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

| P value (95% CI) |
|------------------|------------------|
| 0.11 [0.12, 1.26] |
| 0.32 [0.42, 13.27] |
| 0.84 [0.02, 23.8] |
| 0.005 [5.6, 1.257E4] |

<table>
<thead>
<tr>
<th>Age</th>
<th>15-25</th>
<th>26-35</th>
<th>36-45</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>108</td>
</tr>
<tr>
<td>Count</td>
<td>104</td>
<td>92</td>
<td>12</td>
<td>118</td>
</tr>
<tr>
<td>Count</td>
<td>108</td>
<td>101</td>
<td>13</td>
<td>222</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Educational status</th>
<th>Illiterate</th>
<th>Elementary</th>
<th>High school</th>
<th>College</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Count</td>
<td>153</td>
<td>24</td>
<td>15</td>
<td>16</td>
<td>198</td>
</tr>
<tr>
<td>Count</td>
<td>164</td>
<td>26</td>
<td>16</td>
<td>16</td>
<td>196</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Governmental</th>
<th>Private</th>
<th>None</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>0</td>
<td>8</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Count</td>
<td>16</td>
<td>68</td>
<td>124</td>
<td>160</td>
</tr>
<tr>
<td>Count</td>
<td>16</td>
<td>76</td>
<td>130</td>
<td>212</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of sexual partnership</th>
<th>One sexual partner</th>
<th>Have no sexual partner</th>
<th>Have more than one sexual partner</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Count</td>
<td>200</td>
<td>4</td>
<td>4</td>
<td>214</td>
</tr>
<tr>
<td>Count</td>
<td>209</td>
<td>5</td>
<td>8</td>
<td>222</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Marital status</th>
<th>Married</th>
<th>Not married</th>
<th>Divorced</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Count</td>
<td>200</td>
<td>4</td>
<td>4</td>
<td>210</td>
</tr>
<tr>
<td>Count</td>
<td>209</td>
<td>8</td>
<td>5</td>
<td>222</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STI awareness</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>1</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Count</td>
<td>28</td>
<td>180</td>
<td>208</td>
</tr>
<tr>
<td>Count</td>
<td>29</td>
<td>193</td>
<td>222</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptom with respect to trichomoniasis</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Count</td>
<td>1</td>
<td>207</td>
<td>218</td>
</tr>
<tr>
<td>Count</td>
<td>4</td>
<td>218</td>
<td>222</td>
</tr>
</tbody>
</table>

Note: $E = \text{ten the power of...}$
The least prevalence of trichomoniasis was found in married women (4.3%, 9/209) than women who were single (50%, 4/8) and divorced (20%, 1/5). Moreover, trichomoniasis infection was higher in women with more than one sexual partner (50%, 4/8) than women either with one sexual partner (4.3%, 9/209) or without sexual partner (20%, 1/5). In addition, greater trichomoniasis infection was seen in women who didn’t have awareness to any of STIs (6.7%, 13/193) than women who did have awareness (3.4%, 1/29). The finding of this study showed that, most of women with trichomoniasis didn’t have awareness to any of STIs (figure 1).

The current diagnostic approach for STIs (syndromic management approach) was evaluated for its ability in correctly screening trichomoniasis cases. To do this, wet mount results of vaginal samples were taken as reference test to calculate the sensitivity and specificity of syndromic management approach (table 2). The sensitivity and specificity of syndromic management approach were 21.4% and 99.5% respectively. Similarly the positive predictive value and negative predictive values were 75% and 94.9% respectively. This values indicate that; only
21.4% (3/14) of real infection cases were identified by the syndromic approach and about 25% (1/4) of trichomoniasis cases diagnosed by syndromic approach were false positive as per the laboratory wet mount result (PVP = 0.75).

**Table 2:** sensitivity and specificity of syndromic management approach with respect to wet mount examination of vaginal samples

<table>
<thead>
<tr>
<th>Syndromic management result</th>
<th>Laboratory (wet mount) result</th>
<th>Sensitivity = 21.4%</th>
<th>Specificity = 99.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trophozoite seen</td>
<td>No trophozoite seen</td>
<td>Total</td>
</tr>
<tr>
<td>Trichomoniasis suspected</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Trichomoniasis not suspected</td>
<td>11</td>
<td>207</td>
<td>218</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>14</td>
<td>208</td>
<td>222</td>
</tr>
</tbody>
</table>
6. Discussion

There are different laboratory diagnostic tests which help in identifying trichomoniasis. The tests performance range from the identification of the trophozoites to the detection of anti trichomonal antibodies produced against infection. Despite the presence of variety laboratory tests with different sensitivity and specificity, what a test to choose depends on the economic capability of affording the expenses to the test. Currently, no laboratory test is in practice for routine diagnosis of trichomoniasis in most of the health institutions. Instead, the syndromic approach is employed. Hence, the wet microscopy of vaginal samples which has appreciable sensitivity (Ojiuromi et al., 2007) was used to estimate the prevalence of trichomoniasis in this study.

In this study the prevalence of trichomoniasis have been found to be 1.8% based on the syndromic management approach, whereas this prevalence has been found to be 6.3% based on the wet mount microscopy results of vaginal samples. The 1.2% reported trichomoniasis prevalence for Addis Ababa women with genital discharge (Wolday et al., 2004) was lower than the prevalence of this study (6.3%). Even though culture was used for the laboratory diagnosis of T. vaginalis, only 87 Addis Ababa women were included to estimate the prevalence. Indeed, there might be a difference in the education status, awareness to STIs, and occupation of the two populations. For instance, 73.9% (164) of participants in this study were illiterate. 86.9% (193) were not aware of any STIs and 58.6% (130) were workerless. The summative effect, of the above two reasons, might be the reason for the differences in the prevalence of Addis Ababa and this study.

6.3% trichomoniasis prevalence of this study, however, was lower than the result from Uganda (Tann et al., 2006) in which the prevalence was 17.3%. Despite the use of culture for diagnosis, women who have reported vaginal symptoms such as vaginal discharge and vaginal itching were included to estimate trichomoniasis prevalence in Uganda. As shown from the results of this study, the laboratory positivity of women was significantly associated with the vaginal symptoms of women (p value= 0.005, CI= 5.6 to 1.257E4). Hence, the inclusion of symptomatic women who have reported either vaginal discharge or vaginal itching might be the reason for the increase in the trichomoniasis prevalence of Uganda.
The same methodology with this study (wet microscopy) was used in Argentina (Perazzi et al., 2010) to estimate the asymptomatic carriage of trichomoniasis in pregnant women. 1.8% prevalence of *T. vaginalis* was identified. The lower prevalence might be attributed to the inclusion of asymptomatic women who didn’t have self reported symptoms. In addition, a slightly higher prevalence (11%) was found after the saline wet mount of vaginal swabs in Nigeria (Bello et al., 1983). The inclusion of women who visited the STI clinic, who had reported symptoms for STIs, might be the reason for the increase in the prevalence.

In this study, a significant association was found between trichomoniasis and sexual partnership status of women (p value = 0.01, CI= 0.018 to 0.65). Consequently, women who had more than one sexual partner were more affected by trichomoniasis (50%, 4/8) than women who had no sexual partner (20%, 1/5) or with one sexual partner (4.3%, 9/209). This finding agrees with the result from Peru (Leon et al., 2009) which stated that *T. vaginalis* infection occurred more often among women who reported unprotected sex with a non steady partner and women who reported multiple sex partners.

On the other hand: The likelihood of getting trichomoniasis was greater for women who reported vaginal symptoms, such as, vaginal discharge and pain during urination than women who didn’t have reported vaginal symptoms (adjusted OR= 266, CI= 5.6 to 1.257E4, p value= 0.005). Additionally, a positive correlation was found between trichomonal infection and lack of awareness to STIs (r = 0.46). Hence, women who were aware of STIs had a lesser chance of getting trichomonal infection than women who didn’t have the awareness (adjusted OR = 0.7, CI= 0.021 to 23.9), but the association was not statistically significant.

Even though syndromic management approach is currently an accepted diagnostic method applied in most of health institutions, it has its own draw backs. For example, this diagnostic approach cannot be used to detect infections among asymptomatic individuals [cited in Altini et al., 2005]. The results of this study showed that, the syndromic approach was less sensitive to screen out trichomoniasis cases (sensitivity of 24.1%). This finding agrees to the study in China (Yin et al., 2008) which revealed that the algorithm had poor sensitivity of 10% (using culture as reference test) but the syndromic approach identified more cases of trichomoniasis than the other four STIs. The difference between the two sensitivity values might be because the later is the
overall sensitivity value of five STIs (Trichomoniasis, Chlamydia, gonorrhea, HSV and Syphilis).

However, the sensitivity of syndromic management as applied by health workers in Uganda, targeting *T. vaginalis*, was 66.7% (Tann *et al.*, 2006). The inter personnel difference between health workers in strictly following the WHO algorithm might have its own effect to increase or decrease the sensitivity. Indeed women in Uganda, who reported vaginal symptoms, were included. Hence, the chance of identifying trichomoniasis cases by the syndromic approach will be enhanced which in turn increases the sensitivity.

Syndromic management approach is based on self reported symptoms by the clients and physician’s (health personnel) examination for visible signs as shown by strong correlation with the presence of symptoms ($r= 1$ and $p<0.01$). This shows that, only symptomatic patients are benefited from the syndromic approach. In other words; asymptomatic patients who account for the largest proportion (40%-50%) of trichomoniasis infection (Johnson, 2009) will be left undetected. Among the participants who had trichomoniasis based on the laboratory wet mount testing, only 3 (21.4%) were symptomatic (similar to the study by Yin *et al.*, 2008 in which only 16.6% of STI positives reported symptoms). So, the failure in the detection of asymptomatic trichomoniasis patients remains to be one of the limitations of the syndromic approach.

The physician’s symptom based diagnostic approach has another defect because it leads to over diagnosis and over treatment of patients. According to the laboratory wet mount results of this study, 25% (1/4) of trichomoniasis cases diagnosed by the syndromic approach did not have *T. vaginalis* infection ($PVP = 0.75$). This finding supports the result from Addis Ababa (Wolday *et al.*, 2004) and China (Yin *et al.*, 2008), which revealed that 50% of the cases diagnosed by syndromic approach were false positives. Hence, these over diagnosed cases would be unnecessarily over treated that could bring potentially harmful side effects and drug resistance too.
7. Conclusion

*T. vaginalis* infection occurred more often among young women, women who had not completed high school, single (unmarried) women, women who were private workers, women who were not aware of STIs and women who reported multiple sexual partnerships. However, trichomoniasis was significantly associated, only, with multiple sexual partnerships and vaginal symptoms.

A wide variation was observed between the results of syndromic-based diagnosis and laboratory based diagnosis. The majority of trichomoniasis cases in this study (78.6%, 11/14) were left undiagnosed (untreated) by the syndromic management approach. These women will be predisposed to further complications because they didn’t get treated. Untreated women with the infection are also the potential sources to unknowingly transmit the infection and increase the prevalence.

As a result of high rates of asymptomatic infections and the tendency of healthcare workers to deviate from syndromic case management guidelines and rather follow their personal clinical judgment, many trichomonal infections remain untreated. Therefore, syndromic management approach should not be the first choice to screen out trichomoniasis. So, this study supports the need for improved diagnostic parameters and disease control activities to reduce adverse trichomoniasis associated reproductive health outcomes such as ectopic pregnancy, low infant birth weight and preterm labor.
8. Recommendation

- A great attention should be given for the asymptomatic *T. vaginalis* carriers who accounted for the largest proportion of trichomonal infections. Therefore; diagnostic techniques which can identify both symptomatic and asymptomatic cases, should be included in place of the syndromic management approach which only screens symptomatic ones.

- Region to nation-wide awareness creation on the impact of multiple sexual partnerships, modes of transmission, signs and symptoms of *T. vaginalis* and other STIs should be facilitated.

- Since *T. vaginalis* is a common sexually transmitted pathogen, the screening and treatment of sex partners of infected women must be also prioritized as a public health measure to prevent re-infection and reduce infection prevalence.

- Finally, this study calls for an early appraisal and review of the diagnostic policy by national authorities, and the introduction and/or strengthening of laboratory facilities, especially at the peripheral level.
9. Limitation of the study

In this study, laboratory diagnostic tools which have better specificity and sensitivity than the wet mount microscopy of vaginal samples (such as culture, PCR techniques) were not used due to budget constraint. Many STI compliant women from the OPD were not willing to provide vaginal sample. This resulted for the decrease in number of STI complaints in the study.
10. References


Annexes

Annex -1: Laboratory procedure

Sample collection

Prior to data collection, health workers who took part in the data collection have been given detailed explanation on the general objective of the study and what they would be intended to do. They were also briefed on the data collection questionnaire. When the patient came to the health center, health workers (nurses and health officers) in PMTCT and OPDs explained the objective of the study and asked their willingness to take part in the study. Following their consent, they filled the questionnaire and vaginal sample was taken using Sterile cotton wool swab. For women who had vaginal secretion, it was carefully swabbed. However, for women with no secretion, the vaginal vault and walls were swabbed using Sterile cotton wool swab. Two samples (swabs) were taken to get satisfactory amount of sample and to increase the chance of detecting trophozoites of *T. Vaginalis*. The swabs were placed in a tube containing 0.5 ml saline and kept at room temperature for not longer than two hours before examination.

How to take vaginal swab and secretion

- Prepare equipments (Sterile cotton wool swab, glove) and explain the procedure to the patient.
- Wash hands and put on protective gloves.
- The cotton swab (especially the portion to be inserted) shouldn’t be touched with any other contaminant including our hand.
- Holding the vault of vagina and guide the swab in to vagina.
- Gently insert the swab about two inches (5 centimetres) into the vagina and gently swirl the swab in a circular motion for 15-30 seconds (Carefully rotate the swab three times).
- Take away the swab taking care not to contaminate.
- Insert the swab to saline containing test tube labelled with patients ID.
- Vortex it so as to surely take the sample from the swab.
- Prepare wet smear and Examine under microscope.
• For women who had vaginal secretion, the same procedure follows. However, the secretion is carefully taken using the sterile swab instead of inserting to the vaginal vault.

Wet mount preparation

a) The swab (sample) was thoroughly mixed with the saline to get well homogenized sample and the swab was discarded.

b) A drop of homogenized sample was taken using a pipette and put on dry microscope slide for examination. It was repeated for extra two slides, tripling the number of slides to be examined that could enhance the probability of detecting the parasite (trophozoite).

c) The sample was cover-slipped and examined under a microscope.

d) The microscopic examination was begun using the 10x objective noting cellular distribution and the 40x objective was used to identify the presence of motile *T. vaginalis* trophozoites which are oval, flagellated, larger than WBC, and possessing 5 flagella that produce remarkable motility called “cork screw motility”.

e) All the three slides were carefully examined and every positive slide was cross-checked by extra two laboratory professionals for confirmation. For confusing test results the test procedure was repeated accordingly and examined again.

f) The results were reported immediately for treatment corrections, if any.

Finally, health information on the possible sources of infection, mode of transmission, major symptoms and controlling mechanisms of *T. vaginalis* infection was delivered to every study participant. Positive subjects were given the appropriate treatment. Simultaneously, sexual partners of positive women who were available were informed on the case. Taking their oral consent, they received appropriate treatment. However; women who came without their sexual partner were advised to bring them to the health center and get treatment.
Annex –2: Data collection Questionnaire

Addis Ababa University
Faculty of Medicine
Graduate School
Department Of Microbiology, Immunology, and Parasitology
Format for data collection

Direction: Encircle the appropriate choice given under each question and write the appropriate words, numbers or phrases on the corresponding spaces, when needed.

1. Patient code_____________
2. Age ___________________
3. Educational status
   a) Illiterate  b) Elementary  c) High school  d) Collage
   e) Other (specify) ___________________
4. Occupation
   a) Employed  b) Non employed  c) Private
5. Marital status
   a) Married  b) Single  c) Divorced  d) Widowed
6. If you are single, do you have sexual partner?  a) Yes  b) No
7. If yes, how many are they? _________________
8. Have you ever heard about sexually transmitted diseases?  a) Yes  b) No
9. If yes, what diseases do you know________________________

To be filled by the principal investigator

Investigation results for Trichomonas vaginalis infection
10. Clinical diagnosis by syndromic management?  a) Yes  b) No
11. Laboratory diagnosis result
    a) Trophozoites detected
    b) No Trophozoites detected
የመረጃ መሰብሰቢያ መጠይቅ
አዲሰ አበባ የኒቨርሲቲ
ማይክሮባዮሎጂ ኢሚዩኖሎጂ እና ፓራሲቶሎጂ ዲፓርትመንት

ከሚክተሉት ጥያቄዎቹ ግርጌ ክተቀመጡት አማራጭ መካከል ተገቢዉን መልሰ ያክቡ:: በተጨማሪም
ለተቀመጡት ᭋትዎቹ ተገቢዉን ፈጥር ሐረግ ያሰቀምጡ::

1. የሚሰጥር ቁጥር___________________
2. ይወአ______________
3. የማህድር የደረጃ
   ሽ) ይለት የደረጃ
   ለ) 2ይ የደረጃ
   ዓ) ዛን የደረጃ
   ሰ) የሙ የደረጃ
4. ያወአ
   ሽ) የወስክ የደረጃ
   ለ) የማስረጃ የደረጃ
   ዓ) የሴን የደረጃ
5. ያለበት የሸውስ
   ሽ) ይለት የሸውስ
   ለ) የማስረጃ የሸውስ
   ዓ) የሴን የሸውስ
6. የላይወ ለንድ የቆጣጠር የወስክ የደረጃ? ሽ) እም ለ) የማስረጃ
7. የማህድር የሸውስ የሚቀርብ ለንድ የቆጣጠር ይህ ምክንያት?
8. የላይወ ለንድ የቆጣጠር የወስክ የደረጃ (አካባቢ) የሚቀርብ ለንድ የቆጣጠር ይህ ምክንያት?
9. የልወ ለንድ የቆጣጠር ይህ ምክንያት?

የትሪኮሞናስ ያጅናሊስ የማርመራ የወጤቶቹን በተመለከተ

10. የሃኪም የማርመራ ቤት፣ ከሲንድሮሚክ ማኔጅመንት የተወሰደ ለ) አልተገኘበት
11. የላቦራቶሪ የማርመራ ቤት
    ለ) ትሮፎዞይት አልተገኘበትም
Annex-3: Information sheet and consent form

Research title: Syndromic management approach and laboratory diagnosis of *T. vaginalis* on STI compliant and pregnant women who are attending Merawi Health center, Merawi, Amhara Regional State, Ethiopia:

- Principal investigator: Muluneh Ademe
- Address: mobile; +251910272648
  E-mail; mulie2000gc@gmail.com

PART I: Information sheet

Introduction
Here is an invitation requesting you to be part of the research. Prior to your decision you are kindly requested to look for the general overview of the research which is listed below. You have full right to accept or reject yourself from the study. You can ask the principal investigator for confusion.

Purpose of the study
The aim of this study is to estimate the prevalence of *T. vaginalis*, which is the major protozoan parasite causing serious complication of reproductive system of both males and females. However, its risk is with greater magnitude in females that could be further pronounced in pregnant women’s because of its fingerprint in the coming newborn. Furthermore, this study will expand its frame of view to evaluate the effectiveness of syndromic management approach, in diagnosing *T. vaginalis*, which is currently employed in managing STIs in pregnant women.

Procedure
As per your agreement to participate the study, you will be engaged to:

1) Be cooperative during the collection of vaginal swab
2) Feel free by the time you are interviewed for questionnaires keeping in mind for confidentiality every data.

Potential risks
While you are participating in the study, there is no risk you face.
Benefits from the study
The possible benefits you will get from the study will include the following:

- Brief explanation on the possible transmission and prevention of *T. vaginalis* and related STI causing pathogens will be delivered.
- You will know your health status with respect to trichomoniasis.
- The accurate treatment will proceed according to the laboratory result.

Confidentiality
Any information that is obtained in connection with this study and that can be identified with your input will remain confidential and will not be disclosed without your consent. Instead of your name, you will be coded that nobody knows whose result is under the code provided.

Participation and withdrawal
Please know that your participation in this study is entirely voluntary based and you are free to withdraw at any point during the study.

PART II. Consent form
By my signature below, I confirm that I have read and understood this informed consent. I understand this is a research study and my participation is voluntarily. I understand that I may change my mind about participating at any time, without my medical care or legal rights being affected. I have had the opportunity to ask questions and my questions have been answered. I have been given adequate explanation and understand the purpose procedures, risks and benefits of this research study. By signing this form, I give permission for these individuals to have access to my sample as per the above test procedure.

_________________________   ___________   ___________
Code number of patient       date        signature

_________________________   ___________   ___________
Name of principal investigator date        signature
ማወቅ ሰወቻ እና የሱምነት ፎርም

የትርክማስ ቫጅናሊስን ለማዎቅ የሚደረግ ጥናት፣ መርዓዊ፣ አማራ ክልል፣ ኢትዮጵያ::

የጥናቱ ከባለቤት:
• በሆኔ ምር መራ ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻጸር ᬑቅታማነቱን ይፈትሽል::

አድራሻ:
• ሀሳቢ ለማዎቅ የሚደረግ ጥናት፣ መርዓዊ፣ አማራ ክልል፣ ኢትዮጵያ::

የጥናቱ ከባለቤት:
• በሆኔ ምር መራ ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻጸር ᬑቅታማነቱን ይፈትሽል::

ምህክም:
• ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ያሳረደር ደግሞ በነፍሰጡር እናቶች ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

ተጋብቀ ሬልጋ ያለመሆን የተጋብወ የሬክ ከዉሳኔዎ በፊት የሚከተሉትን መረጃዎች ይመልከት:: የጥናቱ አካል የመሆን ወይንም ደግሞ ያለመሆን መብትዎ የተጠበቀ ነዉ:: ለበለጠ መረጃ ይህ ጥናትን ያማክሩ:: ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በነፍሰጡር እናቶች ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

ወደት:
• ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

ገንዘብ ከሆና ከውርስና የሚስጥራዊነት ነገሮች:
• ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

ማገኝ ከሆና ከውርስና የሚስጥራዊነት ነገሮች:
• ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

1) ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

2) ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንድሮሚክ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችልል::

3) ይህ ረቂቅ ተህዋስ (ትርክማስ ቫጅናሊስ) በወንዶችና ሳቶች የመራቢያ አካላት ላይ ይበልጥ ደግሞ በሳስታቸው ነዉ:: በውልት ይህ ጥናት በአሁኔን ወቅት ላይ የዋለዉን የሃኪም ምር መራ (ሲንዲ ማኔጅመንት) ከላቦራቶሪ ምር መራ መስጠት ጋር በማነጻسعد በሳስታቸውን ይችላል::
ክፍል የለት:
የስምምነት ፎርም

የዚህን ጥርት መሰረታዊ ኣላማ እና ሌሎች መረጃዎችን በሚገባ ተገንዝቤያለሁ:: ተሳትፎየ በፈቃደኝነት ቅል ምክንያት ያሏል። ማንኛዉም ሰብዓዊም ይህን ህጋዊ መብቴ ሳይነካ ከጥናቱ የራሴን ማግለል እንደምችልም እንዲሁ:: ሲለ ጥርት የሆነ የጠሪ ከተደጋጋ በበታች መሆኑ መስጥር ሣንቲ ያስቀር ተረድቻለሁ:: ስለ ጥርቱ ምን ይህ ዝር ዝር ጉዳይ በግልጽ ከተረዳሆት ባሻገር ተጨማሪ ምብራሪያ ብፈልጋ መጠየቅ እነደምችልም አዉቄያለሁ:: በመሆኑም በፈትሷ የዚህ ጥናት እንድሆን ሚፈልግ የሚጠበቅብኝን ሁሉ ለማድረግ በመወሰን መሆኑን በፊርማየ አረጋግጣለሁ::

____________________
________________
________________

የሚስጥር ቁጥር
ቀን
ፊር
ማ

________________________
_______________
______________

የጥናቱ ባለቤት ስም
ቀን
ፊር
ማ
Assurance of the Principal Investigator

This thesis is my original work and has not been presented for a degree in any other university, and that all sources of material used for the thesis have been duly acknowledged. The undersigned agrees to accept responsibility for the scientific, ethical and technical conduct of the research project and for the provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this publication.

**Principal investigator:** - Muluneh Ademe [BSc. Addis Ababa University: Faculty of Medicine: Department of Microbiology, Immunology and Parasitology]

Signature-------------------
Date and place of submission------------------

*Addis Ababa, Ethiopia*

**Advisors:**-

I. Tadesse Kebede, [MSc. Lecture and PhD fellow in Addis Ababa University: Faculty of Medicine: Department of Microbiology, Immunology and Parasitology].

Signature -------------------
Date -------------------

*Addis Ababa, Ethiopia*

II. Nigus Fikrie [MSc. Lecture and PhD fellow in Addis Ababa University: Faculty of Medicine: Department of Microbiology, Immunology and Parasitology].

Signature -------------------
Date -------------------

*Addis Ababa, Ethiopia*