CHAPTER ONE

1.0: INTRODUCTION

1.1: Background to the study

Syphilis is an acute and chronic infectious disease caused by the bacterium *Treponema pallidum* subspecies *pallidum* (*T. pallidum*) and transmitted either by direct contact, sexual intercourse or congenitally from a pregnant mother to her unborn foetus (Hook et al, 1992 and Brooks et al, 2004). In pregnancy, a woman who has syphilis infection can transmit the infection to the foetus through the placenta beginning in the 10th to the 15th weeks of her gestation period. This condition can lead to congenital infection and if not treated in its early stages, can lead to perinatal death, abortion, stillbirth and premature delivery. Others born alive may develop congenital syphilis in childhood, exhibiting interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, bone defects, joint swellings and a variety of central nervous system abnormalities (Stuart, 2004 and Chaudhary et al, 2007).

The risk of transmission of syphilis in pregnancy diminishes as gestational age of the woman advances. Vertical transmission in an untreated syphilitic mother ranges from 70% to 100% for primary syphilis, 40% for early latent syphilis and 10% for late latent syphilis (Singh et al, 1999). While sexual transmission is almost exclusively described in the first two years of infection, transmission from mothers with late latent syphilis to their infants can also occur with an estimated rate of 10% (Singh et al, 1999 and Fumara, 1975). The majority of infants with congenital syphilis are infected in utero after the fourth month of gestation (Tramont 2005), but infection can occur as early as nine weeks' gestation or via contact with an active genital lesion at the time of delivery (Harter et al, 1976 and Singh et al, 1999). Breast feeding

is not associated with syphilis transmission unless an infectious lesion is present on the breast (Genc et al, 2000).

Just as syphilis is classified into early and late depending on the time of acquisition and diagnosis, congenital syphilis has also been divided into two clinical syndromes: early and late congenital syphilis. The early congenital syphilis is diagnosed in the first two years of life which includes stillbirths while the late congenital syphilis refers to cases that present after two years of age, and usually manifest near puberty (Egglestone et al, 2000).

In the laboratory, lack of methods for demonstrating the presence of *Treponema pallidum* by growth necessitates the use of alternative methods which are traditionally divided into direct detection of the organism and serologically for the presence of patient's antibody against the organism, *T. pallidum.* The serological methods are further divided into two classes. One class is the non-treponemal tests that detect antibodies to calipoidal antigens present in either the host or *T. pallidum*; examples are the Rapid Plasma Reagin (RPR) test, Venereal Disease Research Laboratory (VDRL) test, Toluidine red unheated serum test (TRUST) and the Unheated Serum Reagin (USR) test. Reactivity to any of these tests generally indicates host tissue damage that may not be specific for syphilis. The other class of test is the treponemal tests which uses specific treponemal antigens. Confirmation of infection therefore requires reactive non-treponemal and specific treponemal one. Examples of the specific treponemal tests are *Treponemal pallidum* haemagglutination assay (TPHA), *Treponemal pallidum* particle agglutination assay (TPPA), Immunochromatographic (IC), *Rapid Strip Test,* Latex agglutination *Syphilis Fast* test, FTA-ABS (Fluorescent treponemal antibody absorption) and Various enzyme immunoassays.

The epidemiology of both syphilis in pregnancy and congenital syphilis varies from country to country. The World Health Organization (WHO) estimates that there are about 12 million new cases of syphilis worldwide each year. According to WHO, many of these cases occur in resource constraint countries including Ghana. Out of the 12 million new cases, an estimated 5.8 million occur in Southeast Asia, 3.5 million cases in sub-Saharan Africa and 1.3 million in Latin America and the Caribbean (WHO/CDS/CDR/EDC/2001.10, Cheesbrough, 2003).

WHO also estimates that out of a million pregnant women with syphilis infection, 460,000 of such pregnancies result in abortions or perinatal deaths, 270,000 of such cases give birth to premature babies or babies with low birth weights with 270,000 babies being born with congenital syphilitic infection (Dobson, 2004 and Walker et al, 2002). An estimated two million pregnancies are affected annually; approximately 25% of these pregnancies end in stillbirths or spontaneous abortions, and a further 25% of the newborns have low birth weights or serious infections, both of which are associated with increased risk of perinatal deaths (Watson-Jones et al, 2002 and WHO, 2007).

A study conducted by Genc and colleagues in 2000 showed that the prevalence of syphilis seropositivity in pregnancy, after accounting for biologically false positive tests, is between 0.02% and 4.5% in Northern Europe and the USA. According to them, only a small proportion of these pregnancies resulted in congenital syphilis (Genc et al, 2000).

In Ghana, the HIV Sentinel Survey (HSS) Report reported the median syphilis prevalence for 2009 as 3.7% having recorded 3.8% and 5.1% in 2008 and 2007 respectively. In 2006, 2005 and 2004, the median syphilis prevalencies were 3.1%, 3.6% and 5.6% respectively. In 2009, the prevalence in Ashanti region and Kumasi were 7.0% and 3.0% respectively (GHS HSS reports, 2006, 2007, 2008 and 2009).

1.2: Justification

Syphilis in pregnancy can lead to adverse pregnancy outcomes, this can be averted if detected and treated with a cheap antibiotic, penicillin (Watson-Jones et al, 2002, Terris-Prestholt, et al, 2003 and Doroshenko et al, 2006). Hence screening and treatment of syphilis infected pregnant mothers will help decrease the adverse pregnancy outcomes like perinatal deaths, abortions, stillbirths or premature deliveries, and also prevent neonates born from mothers with syphilis from developing congenital syphilis which may manifest as interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, bone defects, joint swellings and a variety of central nervous system abnormalities (Cheesbrough, 2003 and Brooks et al, 2004, WHO, 2001, 2001 and 2004).

Given the adverse effects of undiagnosed or untreated maternal syphilis, on the developing foetus coupled with the cost effectiveness of screening and treating maternal syphilis (Terris-Prestholt et al, 2003), many countries have adopted the policy of universal antenatal syphilis screening to identify asymptomatic pregnant women with congenitally transmissible syphilis for treatment (Doroshenko et al, 2006). However, there is still a general under appreciation of the burden of congenital syphilis. A large reduction in congenital syphilis is feasible with relatively simple interventions focused on maternal and newborn care (WHO, 2007a and 2007b).

Since the cost of screening and treating maternal syphilis is cheap (Watson-Jones et al, 2002 and Terris-Prestholt et al, 2003), WHO recommends that all pregnant women should be screened for syphilis and treated if infected. In Ghana, the national reproductive health policy document recommends that all pregnant women should be screened for syphilis and treated accordingly. But unfortunately, available evidence indicates that, apart from the yearly HIV

sentinel survey which is carried out in some selected sentinel sites by using a simple and rapid *Treponema*-specific diagnostic point-of-care (POC) test which cannot differentiate between current infections from past treated or untreated infections, this policy is not effectively carried out nationwide (GHS HSS, 2007 and 2008, Dassah et al, 2010). At the Komfo Anokye Teaching Hospital (KATH) and other health facilities in the Ashanti region, syphilis screening is not routinely carried out. The test is only carried out upon a Clinician's request. In that case, it is only the non-specific treponemal Venereal Disease Research Laboratory (VDRL) test that is done without confirming with any of the specific treponemal assays. It is therefore possible that some of the abortions, stillbirths, premature deliveries and low birth weights encountered in pregnant women at KATH and surrounding health facilities could be due to syphilis. This study therefore seeks to study the extent of syphilis in pregnant women attending ANC at KATH and risk factors associated with the transmission of infection. This would enable effective planning to control syphilis in pregnancy.

1.3: Aim of Study

The aim of this study is to determine the prevalence of syphilis and associated risk factors among pregnant women attending the Komfo Anokye Teaching Hospital (KATH) for antenatal care (ANC) services.

1.4: Study Objectives.

1. To determine the prevalence of syphilis among pregnant women who visit KATH for their antenatal care.
2. To determine risk(s) factors associated with syphilis infection in pregnant women.
3. To generate data for planning and control of syphilis in pregnancy.

CHAPTER TWO

2.0: LITERATURE REVIEW

2.1: HISTORICAL BACKGROUND

Authorities believe that syphilis was introduced into Europe in 1493 by crew members returning from Christopher Columbus's first expedition to America. The first well-recorded European outbreak of what is now known as syphilis occurred in 1494 when it broke out among French troops besieging Naples (Oriel, 1994). The French might have been infected via Spanish mercenaries serving King Charles of France in that siege (Lobdell et al, 1974). From that centre, the disease swept across Europe. As Jared Diamond describes it, "when syphilis was first definitely recorded in Europe in 1495, its pustules often covered the body from the head to the knees, caused flesh to fall from people's faces, and led to death within a few months". In addition, the disease was more frequently fatal than it is today. Diamond concluded, "by 1546, the disease had evolved into the disease with the symptoms so well known to us".

By the 16th century, syphilis had become a major public disease. However, the spirochete responsible for syphilis was discovered only in 1905 by a German zoologist Fritz Schaudinn. In 1906 a German bacteriologist August von Wassermann developed the first blood reaction test for the diagnosis of the disease (Brath, 2006 and Kohl et al, 2005).

In 1913, a Japanese scientist, Hideyo Noguchi, demonstrated the presence of the spirochete *T. pallidum* in the brain of a progressive paralytic patient while working at the Rockefeller University, (then called the Rockefeller Institute for Medical Research). He proved through his demonstration that *Treponema pallidum* was the cause of the disease by successfully

culturing the germ (*T. pallidum*) responsible for syphilis in sections made from the brains of patients who had died from general paralysis (Hideyo et al, 1913).

Prior to Noguchi's discovery, syphilis had been a burden to humanity in many lands. Without its cause being understood, it was sometimes misdiagnosed and often misattributed to damage by political enemies. In 1909, another German bacteriologist; Paul Ehrlich discovered the first effective treatment, an arsenic-containing compound, Salvarsan (a historic proprietary name for asphenamine). The antibiotic penicillin, which is still the preferred treatment, was shown to be highly effective against the disease since 1943 (Herman, 1912 and Kelly, 2009).

2.2: THEORIES ON THE ORIGIN OF SYPHILIS

Three theories on the origin of syphilis have been subjected to debate in both the anthropological and historical fields. The "pre-Columbian theory" holds that syphilis was present in Europe before the discovery by the Americans. Some scholars believed that its symptoms were described by Hippocrates in Classical Greece in its venereal/tertiary form. There were other suspected syphilitic findings before the European contact; these included the 13–14th century Augustinian friary in the Northeastern English port of Kingston upon Hull. This city's maritime history, and the continual arrival of sailors from distant places, is thought to have been a key factor in the transmission of syphilis into the Northeastern English port (Keys, 2000). Carbon-dated skeletons of monks who lived in the friary showed bone lesions typical of venereal syphilis.

Another theory, the "Columbian Exchange theory" holds that syphilis was a New World disease brought back by Columbus and Martin Alonso Pinzon (Lobdell et al, 1974).

According to Debora MacKenzie, supporters of the Columbian theory found syphilis lesions in pre-contact Indigenous people of the Americans and cited documentary evidence linking crewmen of Columbus's voyages to the Naples syphilis outbreak of 1494 (Debora, 2008). A recent study of the genes of venereal syphilis and related bacteria has supported this theory, by locating an intermediate disease between yaws and syphilis in Guyana, South America (Harper et al, 2008).

A historian, Alfred Crosby, suggested that both theories are correct in a third theory called "Combination theory". Crosby's argument was built on the similarities of the species of bacteria which cause yaws and syphilis. The bacterium that causes syphilis belongs to the same phylogenic family as the bacteria which cause yaws and several other diseases. Despite the tradition of assigning the homeland of yaws to sub-Saharan Africa, Crosby noted that there is no unequivocal evidence of any related disease having been present in pre-Columbian Europe, Africa, or Asia. There is undisputable evidence of syphilis having existed in the pre-Columbian Americans. Crosby writes, "It is not impossible that the organisms causing *treponematosis* arrived from America in the 1490 and evolved into both venereal and non-venereal syphilis and yaws" (Debora, 2008).

2.3: CLASSIFICATION OF THE TREPONEMES

The spirochete bacterium (*Treponema pallidum* subspecies *pallidum*), which causes syphilis belongs to the genus *Treponema*, one of the 3 human pathogenic genera, *Borrelia* and *Leptospira* all of the family *Treponemataceae* under the order *Spirochaetales*. Other species of the genus *Treponema* include; *Treponema pallidum* subspecies *pertenue,* which causes yaws, *Treponema pallidum* subspecies *endemicum,* the causative organism of endemic

syphilis (also called bejel) and *Treponema carateum,* which also causes pinta. (Brooks et al, 2004).

A apart from their morphology which is now possible, the pathogenic treponemes cannot be distinguished by antigenic, biochemical, or genetic criteria. Differentiation of the treponematosis however is based on their geographical location, modes of transmission, and clinical manifestations. Similarities in treponemal infections include their generalized nature, regional and general lymphadenopathy, chronicity, spontaneous healing, asymptomatic periods, and relatively painless symptoms. Although specific strain differentiation is not available, different human isolates have been characterized. These isolates exhibit various degrees of virulence as determined by animal inoculation studies. During blood transfusion, infected blood infected with the bacteria can also transmit the infection to the one receiving the donated blood (Albrecht et al, 1996).

2.4: ACQUIRED SYPHILIS

Natural infection of *T. pallidum* is limited to the human host. Acquired human infection is usually transmitted by sexual contact with a very low infective dose of less than 100 organisms. The infectious lesion is on the skin or the mucous membranes of the genitalia of an infected person. In 10-20% of cases, the primary lesion is intrarectal, perianal or oral. It may however be anywhere in the body. *T. pallidum* can probably penetrate through intact mucous membranes or any break in the epidermis of the skin (Albrecht et al, 1996).

Acquired syphilis has an early infectious stage occurring within the first 2 years of infection and a late non-infectious stage. The early stage syphilis includes primary syphilis, secondary

syphilis and early latent syphilis. The late stage on the other hand includes late latent syphilis, benign late syphilis, cardiovascular syphilis and neurosyphilis (Albrecht et al, 1996).

2.5: MATERNAL SYPHILIS AND THE NEONATAL PERIOD

In an antenatal condition, a pregnant mother who has syphilitic infection can transmit the infection in her blood to the foetus through the placenta beginning in the 10th to the 15th weeks of her gestation period. This condition can lead to congenital infection and if not treated in its early stages, can lead to perinatal deaths, miscarriages, stillbirths or preterm deliveries. Others born alive are likely to develop signs of congenital syphilis like interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, bone defects, joint swellings and a variety of central nervous system abnormalities in childhood (Tramont et al, 2005).

2.6: MORPHOLOGY AND IDENTIFICATION OF *TREPONEMA PALLIDUM*

2.6.1: Structure of *T. pallidum*

Treponemes are helically coiled, corkscrew-shaped organisms 6 to 15 µm long and 0.1 to 0.2 µm wide (figures 2.1 and 2.2). Since the organisms stain poorly with aniline dyes, their presence in tissues can be visualized by silver impregnation methods. Live treponemes, which are too slender to be seen by conventional light microscopy, can be visualized by using dark-field microscopy. *Treponema pallidum* subsp *pallidum* exhibits characteristic motility that consists of rapid rotation about its longitudinal axis and bending, flexing, and snapping about its full length (Albrecht et al, 1996).

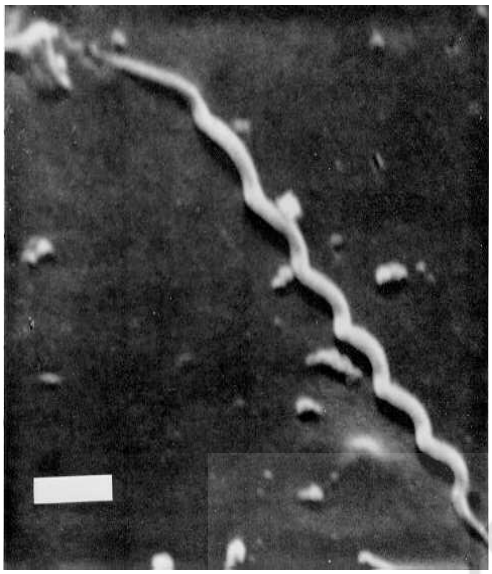


Figure 2.1: Scanned electron micrograph of *T. pallidum.* (Fitzgerald TJ, Cleveland P, Johnson RC et al): Scanning electron microscopy of *T. pallidum* (Nicholes strain) attached to cultured mammalian cells (From J Bacteriol, 1977).

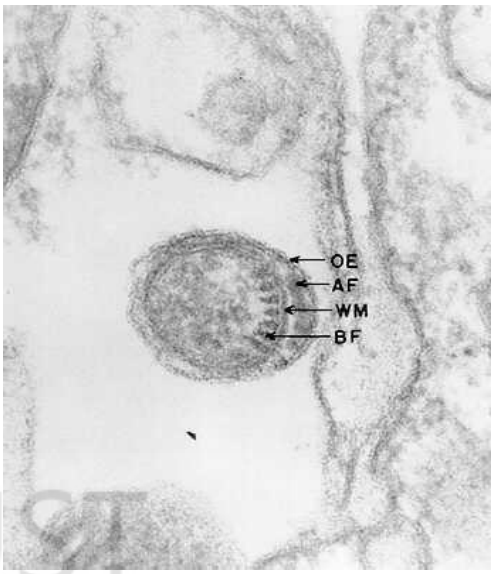


Figure 2.2: Transmission electron micrograph cross-section of *T.* (From ***pallidum*.** Abbreviations: OE, outer envelope (membrane); AF, axial filament; WM, cell wall membrane BF, body fibrils. (From Johnson RC, Ritzi DM, Levermore BP et al, 1973).

2.6.2: Cultural Characteristics of *T. pallidum*

T. pallidum, is a helical microorganism with a thin peptidoglycan coat and a flagellum that resides within the periplasmic space. It is a fastidious organism that exhibits narrow optimal pH range of 7.2 to 7.4 within a temperature range of 30 to 37°C. It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants. Traditionally this organism has been considered a strict anaerobe, but it is now known to be microaerophilic (Miller, 1975). In a tissue culture, it requires only glucose, maltose and mannose for multiplication. *T. pallidum* is

an obligate parasite and hence derives many of its nutrients from its host. Although it lacks respiratory electron transports chain in its system, it accomplishes the production of ATP by the substrate-level phosphorylation (Fraser et al, 1998).

Treponemes multiply by binary transverse fission. The in vivo generation time is relatively long (30 hours). Despite intense efforts over the past 75 years, *T pallidum* subsp *pallidum* has not been successfully cultured in vitro. While limited replication has been obtained by co-cultivation with tissue culture cells, viable organisms can be maintained for 18 to 21 days in complex media. The other three pathogenic treponemes also have not been successfully grown in vitro (Musher, 1990).

2.7: GENOME SEQUENCE OF *TREPONEMA PALLIDUM*

Like gram negative bacteria, *T. pallidum* sp. *pallidum* has an outer membrane, but its inner membrane contains only real integral membrane proteins, some of which are surface exposed (Weinsrock et al, 1998). This characteristic may provide some understanding of how the organism elicits such a vigorous inflammatory and immunological clearance, although paucity of surface protein means that its cell surface presents few targets for a host immune response. The organism has genes for 22 different lipoproteins, which may elite strong inflammatory response (Pennisi, 1998).

Fraser and colleagues reported the complete genome sequence of *Treponema pallidum.* According to them, the *T. pallidum* genome which consists of 1,138,006 base pairs contained 1041 predicted open reading frames (ORF) of which 476 of the ORFs had orthologs in *B. burgdorferi,* the causative organism for Lyme disease. More than 40% of the orthologous genes in the *B. burgdorferi* bacteria were highly conserved and in other bacteria. 150 of the

ORFs shared in the two bacteria, encoded proteins of unknown biological function and almost 50% of these were unique to the spirochetes (Fraser et al, 1998).

T. pallidum has 44 tRNA species. These are organized into eight clusters of 25 genes as well as 19 single genes and two rRNA operons. The average G and C content of the genome is 52.8% and the average size of the open reading frames is 1023 base pairs. The average size of bacterial proteins is 37,771 Daltons. It is apparent that potential virulence factors exist in the *T. pallidum* genome which includes a family of 12 potential membrane proteins and several putative haemolysins. The sequences that drive the replication, transcription, translation, and repair of DNA in the genome of *T. pallidum* were present with a small number of identifiable transporters apart from phosphoenolpyruvate phosphotransferase carbohydrate transporters. Due to the absence of genes responsible for the encoding of superoxide dismutase, catalase or peroxidase, *T. pallidum* can be said to be an organism which possesses common genes as well as a considerable diversity among pathogenic spirochetes (Fraser et al, 1998).

2.8: ANTIGENIC PROPERTIES OF *TREPONEMA PALLIDUM*

Although *T. pallidum* subsp *pallidum* cannot be cultured in vitro, it can be cultured in vivo for routine research for characterization of its antigens. It has hyaluronidase that breaks down the hyaluronic acid in tissue thereby enhancing its invasiveness. More than 100 noted protein antigen profiles of all subspecies of *T. pallidum* are indistinguishable. The endoflagella are composed of three core proteins. Humans with syphilis develop antibodies capable of staining *T. pallidum* by indirect immunofluorescence, immobilizing and killing motile *T. pallidum* and fixing complement in the presence of a suspension of *T. pallidum* or related spirochetes. The spirochetes also cause the development of a distinct antibody-like substance,

reagin, which gives positive complement fixation (CF) and flocculation tests with aqueous suspensions of cardiolipin, an important component of the treponemal antigens, extracted from normal mammalian tissues. Both reagin and anti-treponemal antibody can be used for the serological diagnosis of syphilis (Albrecht et al, 1996 and Brooks et al, 2004).

2.9: EPIDEMIOLOGY OF MATERNAL AND CONGENITAL SYPHILIS INFECTION

2.9.1: Prevalence of Syphilis

Data on global prevalence of sexually transmitted infections (STIs) are limited because STI surveillance has been largely neglected and funding for surveillance remains inadequate. The best available estimates indicate that each year some 340 million new cases of syphilis, gonorrhea, chlamydia and trichomoniasis occur in men and women aged 15–49; overall, STI prevalence continue to rise in most countries, including developed countries. Venereal syphilis is distributed worldwide. Over the past several decades, it has become a significant public health problem in many underdeveloped countries including Ghana (WHO, 2005, 2007, 2009). The same WHO estimates that there are about 12 million new cases of syphilis worldwide each year. According to WHO, many of these cases occur in resource poor countries. For example, out of the 12 million new cases recorded each year, an estimated 5.8 million of them occur in Southeast Asia, 3.5 million cases in sub-Saharan Africa and 1.3 million in Latin America and the Caribbean (WHO, 2001, 2005 and Cheesbrough, 2003). The 2001 WHO's (Geneva) report on Global Prevalence and Incidence of selected curable Sexually Transmitted Infections also indicate that out of the same 12 million new cases of syphilis reported every year, areas like Eastern Europe and Central Asia normally have an estimation of 100 000 new cases, 140 000 for Western Europe and 240 000 for both the

Eastern Asia and the Pacific. 370 000 are also estimated to occur in the North Africa and the Middle East while Australia and the New Zealand have 10 000 every year (WHO, 2001).

The magnitude of the problem of maternal syphilis is not fully recognized worldwide because prevalence of syphilis estimates depend on the prevalence in women attending antenatal clinics, a population which may not be a good representation of the general population. In antenatal populations, the epidemiology of both maternal and congenital syphilis differs from country to country, sub-region to sub-region and continent to continent. For example the estimated prevalence of maternal and congenital syphilis varies between 0.2% in developed countries to 13% in developing countries (Marie-Louise et al, 2000). The 2009 WHO report from the Regional Office of South-East Asia (SEARO) reported variations in the seroprevalence of maternal syphilis among the regions of the World Health Organization. According to WHO, these regional variations are distributed as 3.90% for the American Region, 1.98% for the African Region, 1.50% for the European Region 1.50%, 1.48% for the South-East Asia Region, 1.11% for the Eastern Mediterranean Region and 0.70% for the Western Pacific Region (WHO/SEARO report, 2009).

Other countries, particularly Eastern Europe, experienced a dramatic increase in cases of maternal syphilis during the 1990's. For example, in the Russian Federation, the incidence of syphilis in pregnancy in Moscow had increased to an estimated prevalence of 1.1% (Tikhonova et al, 2003).

In the eastern Mediterranean Region, the highest syphilis prevalence rate amongst pregnant women was reported by Djibouti (3.1%), followed by Morocco (3.0%) and Sudan (2.4%). In Africa, syphilis prevalence rates amongst pregnant women vary from 2.5% in Burkina Faso

to 17.4% in Cameroon (Myer et al, 2003). A research conducted by Myer and friends in 2003 on the impact of on-site testing for maternal syphilis in rural South Africa showed that out of a population size of 7134 women seeking antenatal care, 793 (11.1%) tested positive for syphilis (Myer et al, 2003). Another research conducted by Khadija I. Yahya-Malima and colleagues in 2008 on HIV-1, HSV-2 and syphilis among pregnant women in rural area Tanzania: Prevalence and risk factors showed a syphilis seropositivity of 1.6% **(**Khadija, et al, 2008**)**.

The incidence of congenital syphilis also differs from country to country, sub-region to sub-region and continent to continent. For example, a report received in 2003 from some Latin American countries with information on maternal and congenital syphilis showed that the prevalence of maternal and congenital syphilis fluctuated between 0.4% in Panama and 6.2% in El Salvador. According to the same report, the incidence of congenital syphilis as reported by the countries ranged between 0.0 cases per 1,000 live births in Cuba and 4.0 cases per 1,000 live births in Brazil (WHO, 2001).

In a developed country like the USA, the prevalence of congenital syphilis which peaked at 107 per 100,000 live births in 1991 declined to 11.2 cases per 100,000 live births by 2002. The Centre for Disease Control (CDC), Atlanta USA, reported in 2004 that this decline in the rates closely paralleled the decrease in the rates of primary and secondary syphilis in women from 1991 to 2002 (Dobson, 2004 and CDC, 2004). A study conducted by Genc and Ledger in 2000 showed that the prevalence of syphilis seropositivity in pregnant women attending ANC in Northern Europe and the USA, after accounting for biologically false positive (BFP) tests, is between 0.02% and 4.5%. According to them, only a small proportion of these pregnancies resulted in congenital syphilis (Genc et al, 2000).

A release issued by WHO, estimated that out of a million pregnant women with syphilis infection worldwide, 460,000 of such pregnancies resulted in abortions or perinatal deaths, 270,000 of such cases gave birth to premature babies or babies with low birth weights (LBW) with 270,000 babies being born with congenital syphilitic infection (WHO, 2001, 2005, Dobson, 2004 and Walker et al, 2002).

In parts of the world where "venereal diseases" have not been controlled, such as sub-Saharan Africa, the magnitude of the problems associated with congenital syphilis is reminiscent of those faced in the West during early 1900s. In Ethiopia, an estimated 5% of all pregnancies are lost each year through syphilis induced abortions (Schulz et al, 1987). In Zambia, 24% of all stillbirths and adverse pregnancy outcomes could be attributed to syphilis (Schulz et al, 1987). In the same country, Zambia, it was published that almost 1% of infants born in a major university hospital in Lusaka had signs of congenital syphilis at delivery (Hira et al, 1982). Also, in 1993, a population-based study in Malawi showed that 26% of stillbirths, 11% of neonatal deaths, 5% of post-neonatal deaths, and 8% of infant deaths were attributable to active maternal syphilis infection (McDermott et al, 1993).

In Ghana, the HIV Sentinel Survey (HSS) Report reported the median syphilis prevalence for 2009 as 3.7%, having recorded 3.8% and 5.1% in 2008 and 2007 respectively. In 2006, 2005 and 2004, the median syphilis prevalencies were 3.1%, 3.6% and 5.6% respectively. In 2009, the prevalence in Ashanti region and Kumasi were 7.0% and 3.0% respectively (GHS HSS, 2007, 2008, 2009 and 2010).

2.9.2: Transmission of syphilis

Since humans are the only source of treponemal infection, there are no known nonhuman reservoirs. Although transmission at the time of delivery can also result from direct contact with infectious genital lesions of the mother, foetal infection is as a result of haematogenous spread from an infected mother. Haematogenous spread is dependent upon the occurrence of maternal spirochaetaemia. Since the earlier stage of syphilis is characterized by the spirochaetaemia, the probability of transmission to the foetus is nearly 100% if the mother has early syphilis (Ingraham, 1951).

Although the probability of transmission to the foetus can be up to 70% four years after acquisition of the disease by the mother (Zenker et al, 1991), most infants born to mothers with late latent syphilis are uninfected (Riley et al, 1992 and Radolf et al, 1999). The main factors that determine the probability of foetal infection are the stage of maternal syphilis and duration of exposure in utero.

2.10: CLINICAL PRESENTATIONS OF MATERNAL AND CONGENITAL SYPHILIS

Syphilis is a complex systemic disease with protean manifestations and virtually any organ in the body can be involved. It has been described in medicine as the great imitator or the great imposter in view of its multiple clinical manifestations (Tramont, 2005). Clinically, a pregnant syphilitic woman may present with the various stages of syphilis infection in congenital infection; symptoms may include rhinorrhoea, obstructed nasal respiration plus oedema of the face and ulceration of uro-genital tract, mouth or rectum. If untreated, this can be followed by a more generalized infection usually characterized by disseminated muco-

cutaneous lesions. There may be fever and general malaise, as well as hair loss and mild hepatitis (WHO, 2001).

Complications include pregnancy wastages (abortions, premature deliveries, and stillbirths). Neonatal or congenital syphilis may occur in about one-third of new-born babies born from women with untreated syphilis. Disorders of the musculo-skeletal, cardiovascular and nervous systems may be the final stages of the disease (tertiary syphilis) (WHO, 2001).

Congenital syphilis may be asymptomatic, especially in the first weeks of life, in about 50% of cases. Usually, symptoms appear in the first months but the clinical manifestations may be delayed until the second year of life. The most frequent clinical signs of congenital syphilis at birth are hepatosplenomegaly (33–100%), bone changes seen on X-ray (75–100%), blistering skin rash (40%), fever (16%), low birth weight (10–40%), bleeding (10%), swelling of the joints, abnormal facies, oedema, abdominal distension, pallor, respiratory distress and pseudoparalysis (Brion et al 1991, Davanzo et al, 1992 and Watson-Jones et al, 2002). Infants born with congenital syphilis may have rashes on their skin and mucous membrane lesions. They often fail to gain weight, becoming marasmic. Infected infants develop interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, bone defects, joint swellings and a variety of central nervous system abnormalities Case fatality rates for symptomatic congenital syphilis have been reported to vary between 15% and 38% in sub-Saharan Africa (Watson-Jones et al, 2002 and Saloojee et al 2004).

2.11: DIAGNOSIS OF MATERNAL SYPHILIS

A number of serological tests are available for the diagnosis of syphilis. Traditionally, the tests are done in two steps. First, a non-specific treponemal/syphilis test is done to detect reaginic antigen that stimulate the production of cardiolipin antibodies. Examples of such

tests are Venereal Diseases Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests. If the non-treponemal test is reactive (positive), a second (confirmatory) specific treponemal test is then carried out using an antigen of *T. pallidum* subs. *pallidum*. Examples of such specific tests are the *Treponema pallidum* haemagglutination assay (TPHA) and *Treponema pallidum* particle agglutination assay (TPPA). A combination of both non-specific and specific treponemal tests is recommended in the maternal diagnosis of syphilis (Cheesbrough, 2003). Apart from their non-specificity which may result in about 28% BFP, the non-treponemal tests are inexpensive, sensitive and can differentiate between recent, past and treated infections (Romanowski et al, 1991). Although specific to *T. pallidum* subs. *pallidum*, specific treponemal tests cannot differentiate between active, present and past treated infections. Hence, where feasible, a reactive non-treponemal test should be confirmed by a specific treponemal test to diagnose maternal syphilis. The traditional treponemal tests like FTA-ABS (Fluorescent treponemal antibody absorption) and some EIA (enzyme immunoassays) require laboratories equipped with expensive equipment and technical expertise, which are not usually available in primary healthcare facilities. However, rapid and easy to use specific treponemal tests like TPPA, IC Rapid Test Syphilis and modified TPHA tests use immunochromatographic (IC) strips that can use serum, plasma or whole blood. They do not require sophisticated equipment and special storage conditions. They have sensitiviy and specificity ranges of 85–98% and 92–98% respectively (Romanowski et al, 1991).

2.11.1: Serological Identification of *T. pallidum* in Pregnant Women

A person infected with *T. pallidum* produces two types of antibodies, a non-specific antibody that reacts with cardiolipin antigen in non-specific syphilis test to form a cardiolipin-

cholesterol-lecithin complex, and a specific treponemal antibody that reacts with treponemal antigen in specific treponemal tests (Brooks et al, 2004).

Examples of such tests include:

1. RPR (Rapid plasma reagin) test where choline chloride is added to the cardiolipin cholesterol-lecithin antigen to remove the necessitation for heat-inactivation, enabling both plasma and serum to be used in the test. The addition of carbon enables macroscopic viewing of results.
2. VDRL (Venereal Disease Research Laboratory) test where heat-inactivated serum is reacted with freshly prepared cardiolipin-cholesterol-lecithin antigen.
3. TRUST (Toluidine red unheated serum test). Also, the choline chloride is added to the cardiolipin-cholesterol-lecithin antigen to remove the necessitation for heat-inactivation, enabling both plasma and serum to be used in the test. Red particles are added to enable macroscopic viewing of particles.
4. USR (Unheated Serum Reagin) test.

Prenatal screening using non-specific treponemal serological tests is feasible and affordable in most developing countries. Biologically false positive (BFP) results are usually associated with low titres. Hence, in high-prevalence areas where there is limited or no access to confirmatory tests, a woman with a non-specific treponemal titre of $\geq$ 1:8 should be treated (Watson-Jones et al, 2002 and Wendel et al, 2002). Also, given the serious mobidity and potential mortality associated with congenital infection and the lack of quantitative RPR testing in many settings, all pregnant women with a positive non-specific treponemal or specific treponemal test results should be treated immediately (Watson-Jones et al, 2002 and Wendel et al, 2002).

2.12: THE COST-EFFECTIVENESS OF MANAGING MATERNAL SYPHILIS

Although there are challenges to eliminating maternal and congenital syphilis, it has been shown that its management is feasible and cost-effective (Terris-Prestholt et al, 2003). A number of studies have been carried out to assess the cost-effectiveness of screening pregnant women for syphilis (Hira et al, 1990, Fonck et al, 2001, Terris-Prestholt et al, 2003 and Blandford et al, 2007). These studies indicate that screening is cost-effective and cost saving even when the prevalence of syphilis is considerably lower than 1%. This is true for developed countries as well, where the cost of providing care for congenital syphilis is very high. Economic analysis carried out in sub-Saharan Africa indicated that screening is highly cost-effective even at a relatively low level of prevalence, e.g. 1% (Hira et al, 1990, and Terris-Prestholt et al, 2003), the cost of averting a case of congenital syphilis ranged from US$ 50 to US$ 177 (Terris-Prestholt et al, 2003). In Thailand, a cost–benefit ratio of 2.8 has been estimated even at a prevalence of 0.07% (Phaosavasdi et al, 1987). The tests for syphilis are inexpensive and affordable in most countries. The new rapid tests have made screening even more cost effective and cost-efficient. For example, in South Africa where the prevalence of syphilis is 6.3%, the cost of averting a case of congenital syphilis was US$ 37 with the immunochromatographic strip (ICS) test, US$ 43 with on-site rapid plasma reagin (RPR) test and US$ 111 with referral of specimens to the laboratory (Broznan, et al, 2002.). The ICS test was the most preferred by both staff and patients. In settings with a high prevalence of maternal syphilis, on-site screening with the ICS test is a cost-effective approach to reduce the incidence of congenital syphilis (WHO, 2009).

Screening for syphilis could be combined with screening for other infections such as HIV and malaria, thus complementing each other and reducing the cost of counseling, drawing blood and performing tests. In particular, programmes for the prevention of maternal and congenital

syphilis could be integrated into the prevention of mother to child transmission (PMTCT) of HIV. As long as syphilis is prevalent among adults, the potential for congenital transmission remains high. Therefore, attention should also be paid to the prevention and control of syphilis in adults.

2.13: TREATMENT OF MATERNAL SYPHILIS

All women who test positive for syphilis should be treated to avoid any cases of congenital syphilis, although it may result in some overtreatment because of false-positive results. If a quantitative test is done, using RPR, a titre that is ≥1:8 is a definite indication for treatment. Treatment of syphilis in adults is simple and effective. It depends on the stage of the infection. In the early stages, a single intramuscular injection of 2.4 million IU benzathine benzylpenicillin is sufficient (Watson-Jones et al, 2002). Allergy to penicillin should be excluded before giving the full dose. In case of allergy, patients should be referred to an appropriate centre for desensitization. In the later stages, three weekly doses may be required. Whatever the stage of infection, even a single dose of penicillin will prevent infection in the foetus. Adequate treatment with penicillin will end infectivity within 24–48 hours. According to Brion and colleagues, the RPR/VDRL titre decreases fourfold within six months after treatment hence treatment should be provided early in pregnancy, preferably during the first trimester but definitely before the third trimester (Brion et al, 1991).

The rate of sero-reversion in the titer value after commencement of treatment depends on the pretreatment titre and stage of the disease (Brion et al, 1991 and WHO, 2009). The earlier the treatment is started, the better it is. Hence, new rapid tests that can be performed on-site and treatment started immediately are more effective than earlier tests whose results were made available on subsequent visits only. Presumptive treatment should be provided to all women

who test positive with the rapid tests so that no case of maternal infection is missed. It is important to retest pregnant women in their third trimesters to detect reinfection. Partners of seropositive women should also be tested and treated in order to prevent reinfection. Modification of risk behaviour is also important for the prevention of reinfection. Seropositive women and their partners should be educated and counseled on how to prevent transmission and reinfection, including the use of condoms and the need to get their partners and newborn babies treated (WHO, 2009).

Recommendations by health agencies for the treatment of syphilis vary from agency to agency. The Centers for Disease Control (CDC) and Prevention, Atlanta USA, recommended in 2002 that the treatment of early syphilis (primary, secondary or early latent) with a single dose of 2.4 million units of Benzathine penicillin G is very effective (CDC, 2002). In the United Kingdom, single dose 2.4 maga units of benzathine penicillin is first-line treatment and single dose oral azithromycin is second-line treatment are recommended (BASSH, 2008)

Unfortunately, there is few data available from randomized clinical trials that directly compare the effectiveness of the various therapeutic regiments. The point was underlined in a recent review addressing the efficacy of penicillin for treating maternal syphilis. Under the review, 26 studies met the criteria for detailed scrutiny but none met the criteria that permitted comparison of treatment regimens. Authors concluded that although there was no doubt that penicillin was effective in treating maternal syphilis and in preventing congenital syphilis, there was uncertainty about the optimal treatment regimen (Walker, 2001).

A recently published paper described the considerations behind the CDC recommendations; an expert panel concluded that the available evidence did not indicate that any regimen was more effective than 2.4 million units of benzathine penicillin G for treating early syphilis

(Wendel et al, 2002). Recently-published data describe the effectiveness of the regimen as recommended by the CDC and WHO.

Watson-Jones and colleagues, working in the Mwanza region of the United Republic of Tanzania, studied a cohort of 1688 pregnant women, 133 of whom had high titre (i.e. ≥ 1:8) active syphilis, shown in that population to be associated with adverse outcomes of pregnancy and with stillbirth (Watson-Jones et al, 2002). When the pregnant women with syphilis infection were treated with single dose benzathine penicillin in the study, there was no increase of adverse pregnancy outcome in these women. Only 2.3% of them had stillbirth and 6.3% had low-birth-weight infants as compared with the syphilis seronegative women who still had 2.5% stillbirths and 9.2% low-birth-weight infants (Watson-Jones et al, 2002). This was an important observation, since other studies had suggested that pregnant women with syphilis who received more than 2 or 3 doses of benzathine penicillin had better pregnancy outcome than women who received only one such dose (Donders et al, 1997, Wilkinson et al, 1997). Alexander and colleagues also evaluated the CDC/WHO regimen on maternal syphilis. Their study on 340 pregnant women with syphilis reported treatment effectiveness at 100% (27 out of 27) on pregnant women with primary syphilis, 95% (71 out 75) on mothers with secondary syphilis, 98% (100 out 102) on those with early latent syphilis and 100% (136 out of 136) on women with late latent syphilis (Alexander et al, 1999).

In 2007, Walker reviewed and identified penicillin as the most effective antibiotic treatment regimen (in terms of dose, length of course and mode of administration) of syphilis with and without concomitant infection with HIV for pregnant women infected with syphilis. According to Walker, while there is no doubt that penicillin is effective in the treatment of syphilis in pregnancy and the prevention of congenital syphilis, uncertainty remains about the optimal treatment regimens (Walker, 2007).

2.14: PROBLEMS ASSOCIATED WITH THE TREATMENT OF MATERNAL SYPHILIS

In terms of safety, allergy to penicillin poses the greatest risk associated with maternal treatment. Although 5-20% of patients may consider themselves allergic (Robinson et al, 2002), many of the reactions recalled are localized rashes; only 1% of reactions are type -1 hypersensitivity reactions with generalized itching rush associated with breathing difficulties (Kerr, 1994). Treatment of maternal syphilis may also be complicated by the Jarisch-Herxheimer reaction, an acute febrile reaction frequently accompanied by headache and myalgia that usually occurs within 24 hours after any therapy for syphilis. This can affect approximately 40% pregnant women treated for syphilis and is associated with uterine contraction and variable decelerations in foetal heart rate, but usually resolves without incident (Wendel et al, 2002). Nevertheless, women treated for syphilis in early pregnancy should stay well hydrated and rest; acetaminophen may help with uterine cramping, pelvic pain and fever (Wendel et al, 2002).

2.15: PREVENTION OF MATERNAL AND CONGENITAL SYPHILIS

Maternal and congenital syphilis can be prevented by testing women for syphilis early in pregnancy, treating those who are seropositive, and preventing re-infection. Treating the mother with a single dose of penicillin is nearly always effective in preventing or treating infection in the foetus. Thus, congenital syphilis can be eliminated as a public health problem by strengthening antenatal care programmes to ensure three main things:

- Early antenatal care for all women, with universal syphilis screening and prompt treatment.

- Ensuring that re-infection does not occur by treating all sexual partners and promoting the use of condoms during pregnancy.
- Educating all women on the dangers of the disease and how to prevent its infection.

A research conducted by Heidi and colleagues in 1996 recommended a 'routine serological screening for syphilis for all pregnant women and persons at increased risk of infection as part of preventing maternal and congenital syphilis. Their clinical considerations included the screening of all pregnant women at their first prenatal visit. For women in high-risk groups, repeated serological testing was recommended for them in their third trimester and at delivery. Again, follow-up serological tests should be performed to ascertain the effectiveness of treatment in seropositive cases (Heidi et al, 1996).

2.16: WHY WE CAN PREVENT MATERNAL AND CONGENITAL SYPHILIS

Prevention of congenital syphilis is at least cost-effective (Terris-Prestholt et al, 2003) and in many cases cost saving. It is affordable in countries with limited health budgets. Compared to the prevention of mother-to-child transmission (PMTCT) of HIV, prevention of congenital syphilis is inexpensive, simple, and highly cost effective. However, lack of interest in neonatal and maternal health policies, political will and the absence of modest funding hinder the elimination of maternal and congenital syphilis (Schmid et al, 2007).

2.17: ASSOCIATED RISK FACTORS OF MATERNAL SYPHILIS

Although not everyone is at equal risk of acquiring the infection, all sexually active individuals stand the risk of contracting the disease. This is so because different people have

different sexual live styles which contain different levels of associated sexually transmitted diseases (STDs). Any individual who is capable of engaging in active sex stands a risk of contracting the disease especially those who have multiple sex partners usually engage in unprotected sex. Multivariate analysis showed that women with live-born infants who had less than secondary-level education, who did not watch television during the week before delivery, who had a previous history of syphilis, or who had more than one partner during the pregnancy were at increased risk of syphilis (Karen et al, 2001).

Living in communities with high rates of syphilis prevalence put a pregnant woman at high risk of acquiring the infection. For example, in Ghana, the highest prevalence rates of 24.2% in 2005, 20.5% in 2006, 29.3% in 2007, 30.5% in 2008 and 30.4%% in 2009 in the Asikuma Odoben Brakwa district in the Central region puts all of its inhabitants including pregnant women at high risk of acquiring the infection (HSS, 2005, 2006, 2007, 2008, 2009).

Hence, sexually active individuals, individuals with low educational backgrounds, the unemployed, low income workers, rural dwellers, those who engage in sex at early age in life, women with histories of spontaneous or induced abortions, drug addicts, people with STIs, homosexual men, and those with multiple sexual partners stand a high risk of acquiring syphilis. Other socio-demographic and behavioral risk factors are also associated with syphilis among pregnant women (WHO, 2005).

2.18: MATERNAL AND CONGENITAL SYPHILIS AS A PUBLIC HEALTH PROBLEM

Because few countries have statistics on the numbers of syphilis infected mothers and infants, a full picture of the problem is not easily seen. In addition, many of the affected pregnancies end up in miscarriages in the first or second trimesters, before they are screened for syphilis

infection. This means that those who report for ANC even do so after the first or second trimesters of their pregnancies where the disease might have already infected the developing foetus (Stuart, 2004).

Research conducted by Ratnam and colleagues in Lusaka, Zambia at the University Teaching Hospital summarised reactive TPHA test result in 12.5% of antenatal patients and in 42% of women who had spontaneous abortions the latter half of their pregnancies. The same study showed that among 469 consecutive babies delivered at the University Teaching Hospital, 30 had reactive results to the TPHA test; of these two were stillborn and four had signs of congenital syphilis at birth. Thus, syphilis appears to affect adversely an appreciably high number of pregnant women in Lusaka, Zambia (Ratnam et al, 1982).

Despite the paucity of data, it is still possible to estimate the size of the problem. For example, it is estimated that in sub-Saharan Africa, 2 million women with syphilis become pregnant each year. An estimated 80% of the 2 million remain undetected during pregnancy (Aiken, 1992). In Zimbabwe, studies have shown that syphilis is responsible for 21% of perinatal deaths which is ahead of any other cause of perinatal deaths. In some African countries, the combined loss of life due to syphilis-related stillbirths and infant deaths is thought to be equivalent to the loss from mother-to-child transmission of human immunodeficiency virus (Aiken, 1992).

2.19: IMPACT OF MATERNAL SYPHILIS ON PREGNANCY OUTCOME

The infective organisms (*T. pallidum* subs. *pallidum*) in the blood of a pregnant woman can be transmitted to the foetus, particularly in the early stage of the infection. Most women with syphilis of less than one year's duration will transmit the infection to their unborn child

(Stuart, 2004). The likelihood of transmission is directly related to the stage of maternal syphilis during pregnancy, or the stage of pregnancy when infection is acquired. In early maternal syphilis the maternal–fetal transmission rate can be up to 80%, whereas in late syphilis, infectivity decreases (Stuart, 2004). The concentration of spirochetes in the blood is highest during the first two years after infection and decreases slowly thereafter as a result of acquired immunity. Thus the risk of infecting sexual partners is highest during the first two years, then virtually ceases, although the risk of maternal-fetal transmission continues. The course of maternal infection does not seem to be altered by pregnancy (Stuart, 2004). Although there is considerable variation in the way the adverse outcomes of pregnancies of women infected with syphilis are reported, it is generally accepted that they include spontaneous abortions, perinatal deaths, low birth weights and neonatal infection with syphilis. Several models have been proposed to estimate adverse pregnancy outcomes in women infected with syphilis, with resulting estimates ranging from 50% to 80% (Schulz et al, 1987, Hira et al, 1990 and Watson-Jones et al, 2002).

2.20: WHO's GLOBAL ELIMINATION/ERADICATION OF MATERNAL SYPHILIS

According to WHO's 2005 Geneva report from the Department of Reproductive Health and Research, out of the 12 million new cases of syphilis estimated each year, 2 million of them are among pregnant women. 80% of these can result in adverse outcomes such as stillbirths and spontaneous abortions (40%), perinatal deaths (20%), low-birth weight babies (20%) and serious neonatal infections (WHO, 2005). The annual global number of cases of congenital syphilis is estimated to be between 713 600 and 1 575 000 (Davanzo et al, 1992). More newborn infants are affected by congenital syphilis than any other infection including HIV and tetanus (WHO, 2009). The morbidity and mortality due to congenital syphilis is much

higher than that due to mother-to-child transmission (MTCT) of HIV, yet syphilis has not received the same attention as HIV/AIDS. This is mainly due to inadequate political commitment and insufficient national and international awareness of the burden of maternal syphilis (WHO, 2009). Global or Regional strategy for the elimination of maternal and congenital syphilis is aimed at various stakeholders concerned with the problem including national policy-makers, programme officials, non-governmental organizations, international non-governmental organizations, community based organizations, multilateral and bilateral donor agencies, and United Nation agencies (WHO, 2009).

WHO has also promised its member countries that the organization will support them to plan, implement, monitor and evaluate programmes for the elimination of congenital syphilis. According to WHO, this is feasible if member countries provide antenatal care (ANC) services to all pregnant women, screen them for syphilis and treat all seroreactive women, their partners and their newborn infants (WHO, 2009). It is believed that this eradication programme will reduce spontaneous abortions, miscarriages, stillbirths, pre-term deliveries, low-birth-weight of infants and perinatal deaths, and will thus reduce morbidity and mortality in women and children thereby contributing to the achievement of the following three Millennium Development Goals (MDG Indicators, 2002):

- MDG 4—REDUCE CHILD MORTALITY

  Reduced incidence of low birth weights, perinatal deaths and congenital syphilis will help reduce the mortality rates among children below five years of age.

- MDG 5—IMPROVE MATERNAL HEALTH

  A decrease in the number of stillbirths and spontaneous abortions will help improve maternal health. Increase in access to ANC will also improve maternal health in general. Education and counseling will help in controlling other STI's including HIV.

Treatment of partners will prevent reinfection, thus leading to improved maternal health.

- MDG 6—COMBAT HIV/AIDS, MALARIA AND OTHER DISEASES

  Women screened for syphilis can be counseled and tested for HIV and treated as necessary, thus helping in the prevention of mother-to-child transmission (PMTCT) of HIV. Treatment of syphilis and other STI in women will also reduce the risk of HIV transmission.

2.21: WHO's GLOBAL STRATEGIES FOR THE ELIMINATION OF MATERNAL AND CONGENITAL SYPHILIS

The WHO 2009 Regional strategy issued by the South East Asia for the elimination of maternal and congenital syphilis builds on the 2007 Global elimination of congenital syphilis: rationale and strategy for action. It outlines the guiding principles, key strategies and interventions to achieve the goal of eliminating congenital syphilis. The Regional strategy also proposes initial targets and indicators both at the regional and country levels. It is possible that some targets and indicators may need revision as countries are due to launch elimination programmes at different times (WHO, 2009).

Aside the MDG's, the overall goal of the Global eradication initiative is to ensure that congenital syphilis is no longer a public health problem. According to the 2009 WHO Regional Office for South East-Asia (SEARO) report, this can be achieved by observing the following:

1. Early ANC and universal screening of all pregnant women and prompt treatment of all seropositive women.
2. Treatment of partners of seropositive women, promotion of condom use, education and counseling to prevent infection/reinfection.

3. Prophylactic treatment of all infants born to seropositive women.
4. Screening is more effective if it is performed on-site early in pregnancy and treatment provided immediately to seropositive women. There is also the need to prevent reinfection by treating sexual partners and re-screening in late pregnancy.

The proposed interventions for achieving the goals and objectives of the elimination of maternal and congenital syphilis are grouped under the following four key strategies (WHO, 2009):

1. Ensuring high-level commitment and advocacy which deals with the commitment of health policy decision-makers and other key stakeholders, such as programme managers, is necessary to ensure that the programme receives adequate political, financial and logistical support.
2. Increasing access to, and improving the quality of maternal and child health by providing the only opportunity to screen pregnant women for syphilis.
3. Screening pregnant women and treating seropositive women, their partners and newborn infants where emphasis is laid on the early detection and treatment of syphilis among pregnant women, their sexual partners and babies.
4. Establishing surveillance, monitoring and evaluation systems where surveillance and monitoring are essential to accurately assess the magnitude of maternal and congenital syphilis, plan and evaluate the effectiveness of interventions.

2.22: THE ROLES OF WHO IN THE ELIMINATION/ERADICATION PROGRMME

2.22.1: The Role of WHO at Regional Level in the Elimination Process (WHO, 2009)

At the Regional level, WHO will strengthen links within the Organization and coordinate with other UN agencies, donors, NGOs and other partners. WHO will take a leading role in providing technical support to member countries, WHO will:

- Advocate for high-level commitment and allocation of priority to the elimination of maternal and congenital syphilis, and adequate resources by raising awareness and highlighting the cost-effectiveness of the elimination of maternal and congenital syphilis.
- Develop guidelines, protocols and tools for implementing, monitoring and evaluating the programme.
- Build the capacity of countries to launch the eradication programme
- Provide technical support to countries to implement, monitor and evaluate the programme.
- Promote integration of the elimination programme with PMTCT of HIV and STI prevention and control programmes.
- Mobilize resources.
- Form a Technical Working Group to review technical issues, provide updates and recommend certification of elimination.

2.22.2: The Role of WHO at Country Level in the Elimination Process (WHO, 2009)

At the country level, WHO will provide support to countries:

1. To raise awareness about the problem of congenital syphilis and promote early seeking of ANC by pregnant women.

2. To conduct an assessment of the situation, determine the burden of maternal and congenital syphilis and identify needs and gaps.
3. To identify populations and areas that needs greater attention.
4. To prepare plans and strategies and implement programmes for the eradication process.
5. To provide training to health workers in implementing the elimination programme.
6. To develop an effective management system including human resources, logistics and financial management.
7. To ensure integration of the eradication programme into the MCH services, PMTCT of HIV infection, prevention and control programmes.
8. To develop and implement a system for surveillance, supervision, monitoring and evaluation of the programme.
9. To encourage collaboration among various partners.
10. To mobilize resources.

2.22.3: The Role of Member Countries in the Elimination Process (WHO, 2009)

It is the responsibility of countries to plan, implement, monitor and evaluate the programme for the elimination process. In this context, countries will:

1. Formulate a policy on priority basis for and allocate adequate resources.
2. Include the elimination programme in the national health plan.
3. Secure sustained commitment at all levels to ensure that the required resources are allocated for eradication.
4. Raise awareness in the community about congenital syphilis and encourage all pregnant women to seek early ANC.

5. Integrate the elimination process into the maternal and child health (MCH) services, PMTCT of HIV infection, STI prevention and control programmes.
6. Conduct an assessment of the situation to obtain baseline epidemiological data and identify needs and gaps in order to prepare plans, monitor and evaluate the programme.
7. Manage the programme effectively by addressing issues related to advocacy at all levels, availability of human resources, an efficient logistics system and financial sustainability.
8. Through training, provision of guidelines and supervision, build the capacity of health workers to diagnose maternal and congenital syphilis, treat positive women, their partners and newborn infants, treat babies with congenital syphilis, and provide education and counseling.
9. Mobilize communities including health volunteers and traditional birth attendants (wherever they exist) to refer pregnant women to antenatal clinics for care and syphilis screening.
10. Develop a surveillance system including recording and reporting of cases.
11. Incorporate monitoring and evaluation as an integral part of the programme.

2.22.4: The Role of the Community in the Elimination Process (WHO, 2009)

Communities have an important role to play in the successful implementation of the eradication programme. Without their participation, elimination will be difficult to achieve. Efforts should be made:

1. To raise awareness and understanding of maternal syphilis and its adverse outcomes in the community.
2. To encourage pregnant women to seek for early ANC.

3. To ensure treatment of positive women, their partners and newborn infants.
4. To reduce the stigma and discrimination associated with syphilis.
5. To promote primary prevention of syphilis and other STI's among adults.
6. To address issues related to the beliefs and practices of women and the community about mother and child health (MCH), particularly maternal syphilis, the stigma associated with STI's, role of men as motivators or barriers to seeking health care and the dynamics of household decision-making and preferences for ANC.
7. To build on already existing community participation efforts such as those for HIV/AIDS and expand the focus to enhance the eradication process. Participation of the community is important for compliance with the recommended behavioural changes and acceptance of health programmes.

2.23: FACTORS THAT MAY HINDER THE PREVENTION OF MATERNAL AND CONGENITAL SYPHILIS

Although diagnostic tests are available to screen women for syphilis, and an effective and inexpensive drug for treatment, adequate attention has not been paid to the global eradication/elimination of maternal syphilis despite WHO's recommended routine testing and treatment of pregnant women for syphilis (Hira, 1991 and WHO, 2009). As a consequence, the disease still remains a public health problem (WHO, 2009). The major factors contributing to maternal and congenital syphilis are lack of antenatal care, lack of screening of pregnant women, negative test in the first trimester and test not being repeated, delayed treatment or failure of antenatal treatment (Davanzo, et al 1992). The following factors may also hinder the eradication process:

1. In resource-constrained countries, there is usually very low or no priority accorded to surveillance for maternal and congenital syphilis, leading to under recognition of the magnitude of the problem and consequent lack of policy and inadequate allocation of resources for the eradication programme.
2. There is lack of adherence to policy even where it exists, leading to poor coverage of pregnant women for screening. This has led to the inadequate placement of emphasis on the importance of the disease during pregnancy and, hence, there is little demand for its services.
3. There is a lack of clarity regarding roles, responsibilities and accountability for the elimination of maternal syphilis among various programmes such as STI, HIV/AIDS and Maternal and Child Health (MCH).
4. There is often lack of monitoring and evaluation, which are essential for planning and implementation of interventions.
5. Lack of awareness on the part of service providers on the extent of the problem in their various communities.
6. Inadequate training and logistical support for service providers since the classical tests for syphilis which are not suitable for many primary care settings are difficult to perform, and their results not immediately available for interpretations. Hence, they are not widely used by most maternal service providers but are largely limited to only referral or reference laboratories
7. Lack of research on the prevalence and effects of maternal syphilis has meant that the scale of the problem has often not been appreciated. Mostly, the causes of stillbirths and miscarriages are often not investigated in our hospitals.
8. There is poor coverage and late attendance of pregnant women for ANC and poor coverage of newborn care in many developing countries. Only about 68% of

pregnant women attend antenatal clinics in developing countries and many of them seek care late in pregnancy, the average time at first attendance being five to six months (WHO, 1997). Lack of resources for screening and treatment, and lack of awareness among the community about the need for screening contribute to non-seeking or delayed seeking of ANC by pregnant women.

9. Many seropositive women are reluctant to divulge the names of their partners for fear of retribution. Even when the partners are known, there is usually no system in place to trace and treat them. In many cultures, women do not like to talk about pregnancy, especially during the early period. Stigma attached to STI's and lack of confidentiality in the clinics further compound the issue.
10. There is a lack of male participation in improving MCH.
11. The health system is not well developed at the primary health-care level in many countries, leading to poor quality of services in health-care facilities. Lack of trained staff, guidelines, drugs and equipment, and awareness among health-care providers about the importance of routine screening of pregnant women and the consequences of maternal syphilis are factors that contribute to the poor coverage.
12. Many antenatal care providers are uncomfortable with counseling on Sexually Transmitted Diseases (STI) risk reduction especially the use of condoms.
13. In Ghana, especially the Komfo Anokye Teaching Hospital (KATH) and other health facilities in the Ashanti region do not routinely screen and offer treatment for syphilis to maternal mothers who visit the hospital for ANC services.
14. In some communities in the sub-region, concerns about stigma and possible breaches of confidentiality have inhibited demand for services like these.

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1: STUDY SITES AND SAMPLING STRATEGY

3.1.1: Study Site

The study was conducted in the antenatal clinic (ANC) and the Microbiology laboratory of the Komfo Anokye Teaching hospital, Kumasi. The samples were collected at the clinic and analyzed at the Microbiology laboratory.

3.1.2: Sampling Period

The study took place from September, 2008 to June, 2009. Ethical clearance from the Committee on Human Research, Publications and Ethics (CHRPE) at the Komfo Anokye Teaching Hospital, and the School of Medical Sciences, KNUST was obtained.

3.1.3: Study Population

The study population was pregnant women attending the antenatal clinic (ANC) at the Komfo Anokye Teaching Hospital (KATH), Kumasi.

3.1.4: Eligibility Criteria

3.1.4.1: Inclusion Criteria.

All pregnant women attending antenatal care and consenting to participate in the study

3.1.4.2: Exclusion Criteria.

1. Pregnant women in need of emergency care or having an at-risk pregnancy.
2. Antenatal clients reporting for repeat visits during the study period.

3.1.5: Sampling Strategy

Clients were sampled consecutively. Every client attending antenatal care who met the eligibility criteria was sampled until the required sample size of 845 was obtained.

3.1.6: Sample Size

The expected sample size was based on the estimated syphilis prevalence in Kumasi, thus 5.2% by the 2008 HIV Sentinel Survey report. Based on a confidence interval of 95%, a 5% acceptable margin of error and the need for a sample size large enough to enable analysis by age groups, a total of 845 pregnant women were enrolled.

3.1.7: Contact Process

Women attending KATH ANC were approached and the rational of the study explained to them. Signed informed consent was then sought from those willing to participate. Using a structured questionnaire, their sociodemographic data, history of pregnancy and birth outcomes, and knowledge of syphilis were obtained (Appendix 1).

3.1.8: Sample Collection, Labeling and Confidentiality

3-5mls of participants blood was collected into a BD Vacutainer with SST II Advance Semi-separator gel (BD, Belliver Industrial Estate, Plymouth, PL6 7BP, United Kingdom), mixed by turning it 5 times upside-down and coded with unique identifiers (known to the study investigators only). This identifier was written in the patients antenatal care cards to enable them to be traced for future interventions.

3.1.9: Transfer of Samples

The collected samples were transferred to the Microbiology laboratory where they were screened for syphilis using non treponemal (RPR) and treponemal (modified TPHA) tests.

3.2: TESTING FOR SYPHILIS

3.2.1: Non-specific Treponemal Assay (RPR)

The non-specific treponemal test was performed by using an RPR (Rapid Plasma Reagin) carbon antigen test kit (Fortress Diagnostics Limited, BT41 1QS, United Kingdom).

3.2.1.1: Test Procedure for Non-specific Treponemal RPR Assay

After bringing both the reagents and the samples to be screened to room temperature and re-suspending the prepared antigen, a drop each of both the positive and negative controls and 50ul each of the study participant's sample/serum were placed onto separate circles on the test card in the kit. A single use disposable stirrer/pipette was used to mix and spread the mixture over the entire area enclosed by the ring of the circle. The card was then rotated on a mechanical rotator set at 100 revolutions per minute (r.p.m), circumscribing a circle 2.0 cm in diameter for 8 minutes. Each test circle on the test card was then examined macroscopically in good light for the presence or absence of clumps (aggregates) (Fortress Diagnostics Limited).

Each RPR reactive sample was quantitated. The quantitative test was done by adding 50ul of saline solution to 50ul of the test sample (serum). The mixture was then serially diluted to obtain a 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions. 50ul of each diluted sera was placed onto separate circles on the test card in the kit. The prepared antigen was re-suspended and 1 drop added to each of the diluted samples in the test circles. Single use disposable stirrer/pipette was used to mix and spread the mixture over the entire area enclosed by the ring of the circle. The card was then rotated on a mechanical rotator set at 100 revolutions per minute (r.p.m), circumscribing a circle 2.0 cm in diameter for 8 minutes. Each test circle on the test card was then examined macroscopically in good light for the presence or absence of clumps (aggregates). The interpretation of the quantitative test was done by reporting on the highest

dilution to be reactive among the various dilutions. For example, a reactive RPR serum which gave clumps or aggregates in 1:1, 1:2, 1:4, 1:8 and 1:16 dilutions but not in 1:32 dilutions had a titre value of 1:16 (the highest titre to react).

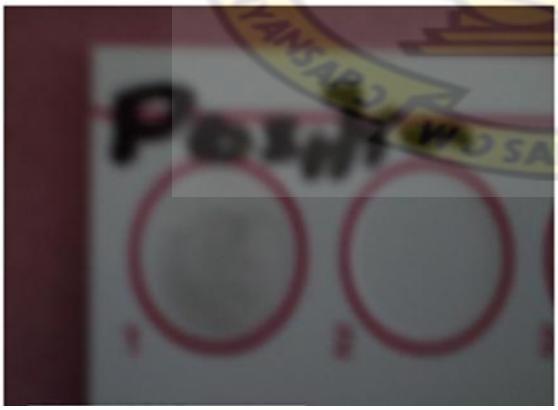
3.2.1.2: Interpretation of RPR Testing.

3.2.1.2a: Positive RPR Tests

RPR reactive samples displayed characteristic agglutinations ranging from slight (weakly-reactive) to intense (strongly reactive). A strong positive reaction was seen as large aggregates in the centre of the test circle while weakly positive reactions demonstrated small aggregates around the edge of the test circle. A positive result of this test was reported as **Reactive.**

3.2.1.2b: Negative RPR Tests

The negative results showed no aggregates. The carbon antigen either remained in a smooth suspension or formed a distinct button. This was reported as **non-Reactive.** Figures 3.1a and 3.1b below show the appearance of reactive and non-reactive RPR test results respectively.



1a

Figure 3.1a: Demonstration of a Reactive (Positive) RPR test.

1b

Figure 3.1b: Demonstration of Non-reactive (Negative) RPR test

3.2.1.2c: Invalid RPR Tests

An RPR test run was invalid if the positive control provided by the reagent manufacture did not react with the carbon antigen to give visible agglutination that could be read macroscopically.

3.2.2: Serial Dilution (Quantitative Test)

All RPR reactive sera were diluted with 0.9% physiological saline into concentrations of 1:2, 1:4, 1:8, 1:16 and 1:32 for titrations.

3.2.3: Specific Treponemal Assay (Modified TPHA)

Again, all RPR reactive sera were tested by specific treponemal tests, TPHA (*Treponemal pallidum* haemagglutination assay). The TPHA employed in this study was Global – Syphilis WB Rapid test for Syphilis (Modified TPHA), (Global invitro LLP, London HA1 3NA, United Kingdom) test kits. It is a modified TPHA test because unlike the traditional antigen-antibody agglutination reaction observed from the normal TPHA reactive tests, here the antibody antigen reaction is seen as a band with a dye to show both patient (Test) and control bands as shown in figure as shown in figure 3.2a and 3.2b. This test was employed because it qualitatively detects the presence of IgM and IgG class of *Treponema* specific antibodies during syphilis infection in whole blood, serum or plasma.

3.2.3.1: Testing Procedure for Specific Treponemal (modified TPHA) tests.

The test kit was brought to room temperature and foil pouches opened to remove test devices (cassettes) and sample droppers. One drop each of test sera was dispensed into the square/rectangular-like port "A" using the droppers provided. Four drops of diluent buffer was dispensed into the round/well-like port "B" close to the port "A" in figure 3.2a and 3.2b. The results were read after 15 minutes (Global invitro LLP).

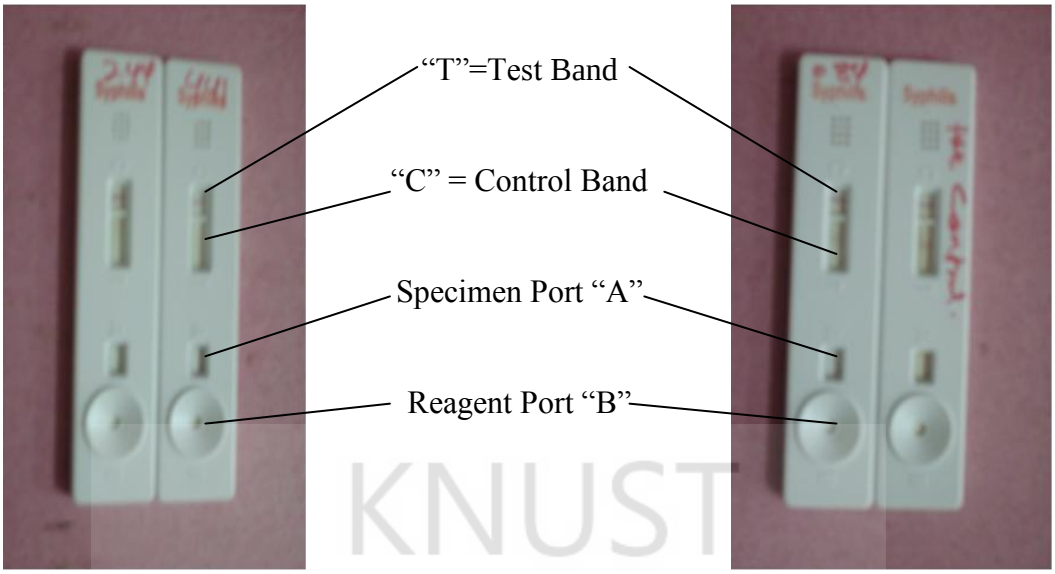


Figure 3.2a: Demonstration of a Negative TPHA (Modified) Test

Figure 3.2b: Demonstration of a Positive TPHA (Modified) Test

3.3.2.2: Interpretation of TPHA (modified) Results

3.3.2.2a: Positive TPHA Results

The modified TPHA test device had a result window which had points labeled "**C**" and "**T**" indicating the control and the test zones respectively. For a result to be positive, two distinct pink to deep purple colours should appear at both the control, "C" and the test, "T" bands, irrespective of how faint or dark the pink to deep purple lines appear at the zones.

3.3.2.2b: Negative TPHA Results

For a result to be negative, only one pink to deep purple line appeared on the control, "C" band with no line appearing at the test, "T" zone. Figures 3.2a and 3.2b below show how positive and negative TPHA test results appeared on the modified TPHA test kits.

3.3.2.2c: Invalid TPHA (modified) Results

A test was invalid if no red line appeared at the control, "C" zone or the background of the bands turned red making it difficult to distinguish between the bands of control "C" or test "T" or both.

3.3: DETERMINATION OF SYPHILIS STATUS OF PATIENTS

Only pregnant mothers who tested positive to both the non-specific treponemal RPR and the specific treponemal (modified TPHA) tests were considered to have syphilis. Those who were reactive to the non treponemal RPR but negative to the treponemal TPHA (modified) were not considered to be syphilis infected mothers but rather had anti cardiolipin antibodies in their body that reacted to the RPR carbon antigens in the test kit to give biologically false positive (BFP) results where positive result is read from a subject who is known to be a true negative for the substance being measured. In the screening of maternal syphilis, Biological false positives (BFPs) may be registered due the following reasons" (Cheesbrough, 2003):

1. Prozone effect, where there is high production of anti-cardiolipin antibody especially in secondary syphilis. In this case, the excess antibody prevents normal antibody-antigen reaction between the antibody from the serum and the antigen in the RPR. This may result in a test being falsely reported as "non-reactive
2. Production of low titre anti-cardiolipin antibodies in other infections like that result from immune disorders and narcotic drug abuse may also be read as a BFP RPR reactive.
3. Abnormal reactions due to HIV infection. Immune suppression due to HIV infection
4. Incorrect performance of test may also contribute to biologically false positive results

3.4: QUALITY CONTROL FOR BOTH RPR AND TPHA TESTS

The following steps were taken to ensure that the results were valid and reliable:

- Test kits from refrigerators were allowed to reach room temperatures before usage. According to the manufacturer, the best results are obtained between 23 and 29°C.
- Stored test kits and their storage facilities were monitored regularly.
- Test kits were stored at 2-8°C
- 2 aliquots (1.5mls each) were prepared from each sample. This prevented repeated freezing and thawing in RPR quantitative tests and TPHA tests.
- Haemolized, lipaemic and turbid serum/plasma samples were not used.
- Each run of tests were validated with positive and negative controls from both the manufacturer and those kept in the laboratory for control purposes.
- In order to deliver the exact quantity of the antigen, the dispensing needle was vertically positioned to the reaction card to prevent prozone effect.

3.5: DATA ANALYSIS AND MANAGEMENT

Data collected on each specimen was recorded in a site note book (register) which had columns for date, serial (code) numbers, age, RPR results for both qualitative and quantitative (titre values) and TPHA results. Results for each specimen were also recorded on their corresponding questionnaires and subsequently entered into the prepared EPI Info 2000 (CDC, Atlanta USA) database.

Data validation was done by manually inspecting the register and crosschecking the entries in the database with the information on each questionnaire to ensure that the correct responses

for each code had been entered into their appropriate places. Data cleaning was done by running frequencies, identifying missing and duplicated records, entering missing records, deleting duplicated records and filling in missing data.

Percentage Prevalence of Syphilis (RPR positive and TPHA positive) and Biologically False Positive (RPR positive and TPHA negative) were determined using the EPI Info software. RPR and TPHA results of each patient were cross tabulated with their sociodemographic information, number of sexual partners, history of pregnancy and birth outcomes and knowledge on the disease especially knowledge on any previous infection and modes of transmission to examine the association between syphilis infection and its associated risk factors. Risk factors and their degree of exposure were determined using the EPI Info 2000 to calculate for probabilities, odds-ratios (OR), relative risks (RR), Confidence Interval (CI) and P-values.

CHAPTER FOUR

4.0: RESULTS

4.1: Recruitment of subjects and their characteristics

A total of 845 pregnant women were recruited into the study between January and June 2009. Results are presented for 841 subjects. 4 were not included in the entry due to incomplete data.

4.2: Sociodemographic characteristics of patients

The age of study subjects ranged between 11 and 44 years with a mean and modal age of 30.05±5.29 and 30 years respectively. Their age and gestational age distribution are as shown in figures 4.1 and 4.2 in pages 49 and 50 respectively.

Figure 4.1: Age distribution of study subjects

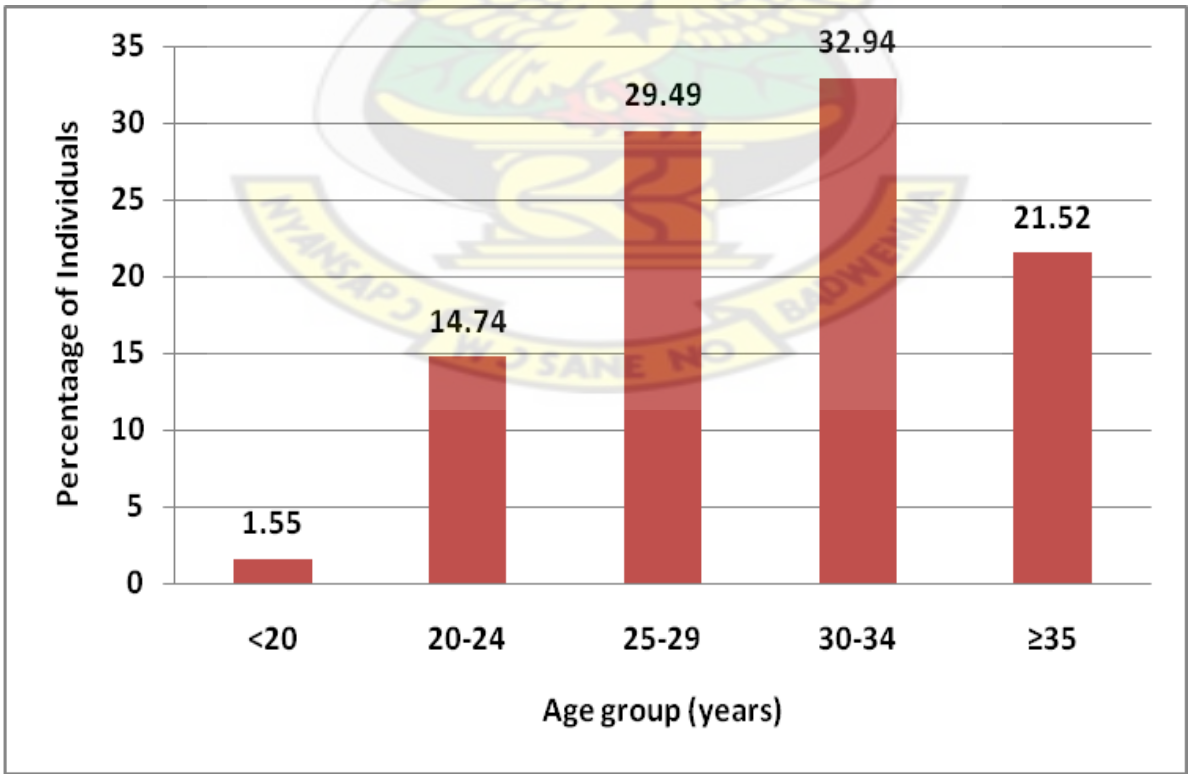
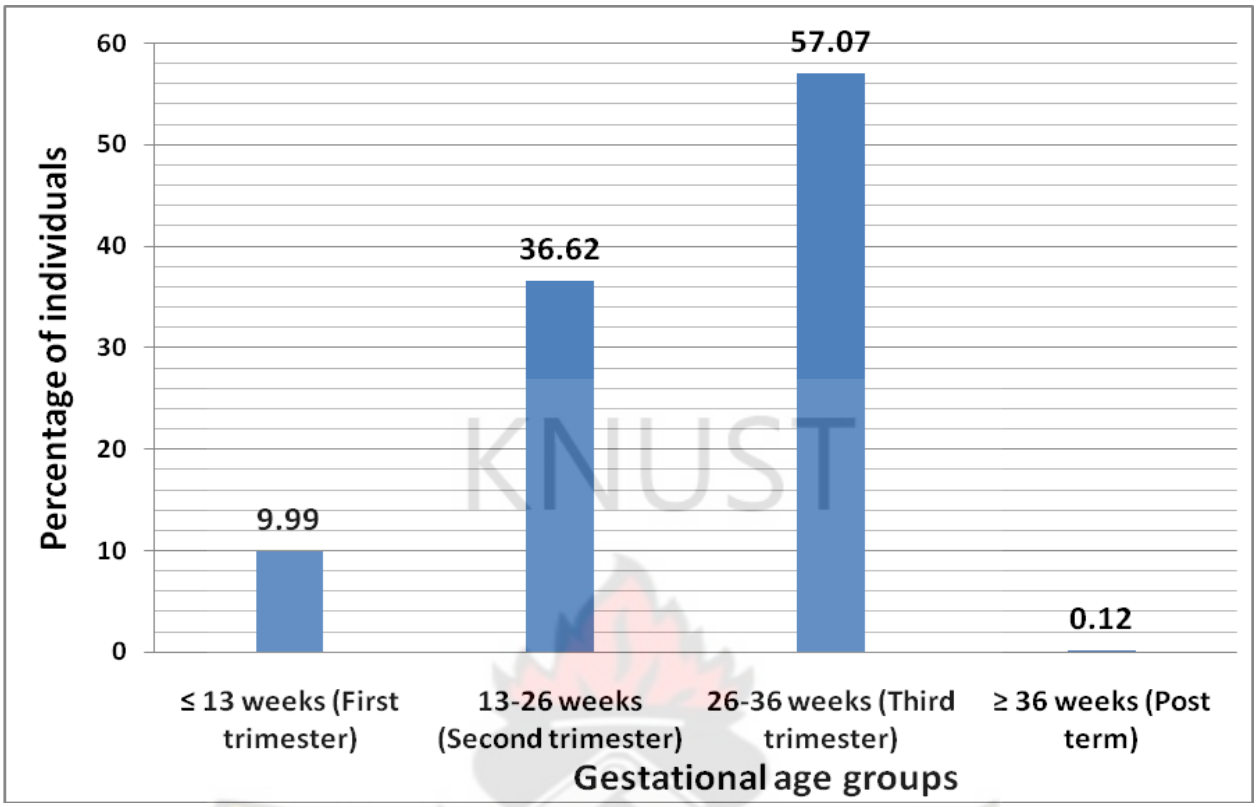


Figure 4.2: Gestational age distribution of study subjects.



From the data obtained, 81.8% of the patients resided in the Kumasi metropolis while the rest, 18.2%, stayed outside the Kumasi metropolis. 21.3% worked in the formal sector (Government workers) while 70.3% were in the informal sector (non-Government workers) and 8.4% unemployed. Of the same 841 patients, 9.3% had not had any form of formal education, 57.3% had schooled only up to the basic level while 15.1% and 18.3% had had secondary and tertiary educations respectively.

Table 4.1: Marital status of women

Marital Status	**Number of women (%)**
Unmarried	96 (11.4)
Married	745 (88.6)
Total	841 (100)

Tables 4.1 above and 4.2 below, respectively show the marital status and the number of sexual partners for the unmarried women within the last 12 months

Table 4.2: Number of sexual partners for the unmarried women

Number of Sexual partners within the last 12 months for the unmarried	**Number of women (%)**
1	74 (77.1)
2	11 (11.5)
3	9 (9.4)
5	2 (2.1)
Total	96 (100)

4.3: History of pregnancy and birth outcomes.

On history of pregnancy and birth outcomes, 62.5% of the women had experienced one or more adverse pregnancy outcomes. Of the 35 women who had had an intra-uterine death (IUD) and still births, 64.3% (22) had lost only one child while the remaining 35.7% (13) had lost two. Table 4.3 below shows the outcomes of previous pregnancies and causes of adverse outcomes.

Table 4.3: History of pregnancy and birth outcomes.

Outcome of Pregnancies	**Number of women (%)**
Live term delivery	315 (37.5)
Pregnancies with adverse outcomes	526 (62.5)
Total	841 (100)
Causes of Adverse Outcomes	
IUD and Stillbirths	35 (6.7)
Miscarriage	261 (49.6)
Unknown Causes	230 (43.7)
Total	526 (100)

4.4: Patients' knowledge on syphilis.

Of the 841 pregnant women interviewed, 422 (50.2%) said they had heard about syphilis whereas 419 (49.8%) said they had never heard about the disease. Of those (422) who had heard about the disease, their sources of information were: mass media (87.9%), health workers (10.0%) and friends (2.1%). Of the 422, only 1 (1.2%) said they have been screened and tested positive for syphilis previously whereas 98.8% said they tested negative at syphilis screening. On knowledge of clinical manifestation of syphilis, their answers were: genital sores (50.7%), skin lesions (2.6%), other forms of manifestations such as itching, discharge etc. (0.7%). The remaining 46.0% of them did not know any clinical manifestation. On how syphilis is transmitted, 84.6% of those who had heard about syphilis (422) said it is transmitted through sexual contact, 0.5% said through blood contact and 0.7% from mother to child. 13.7% were unaware of how syphilis is transmitted, although they had heard about the disease before. On mother to child transmission (MTCT) of syphilis, 54.5% of those who had heard about syphilis (422) said it is possible but can be prevented while 39.8% said it is not possible. The remaining 5.2% did not have any idea about MTCT and its prevention.

4.5: Prevalence of syphilis

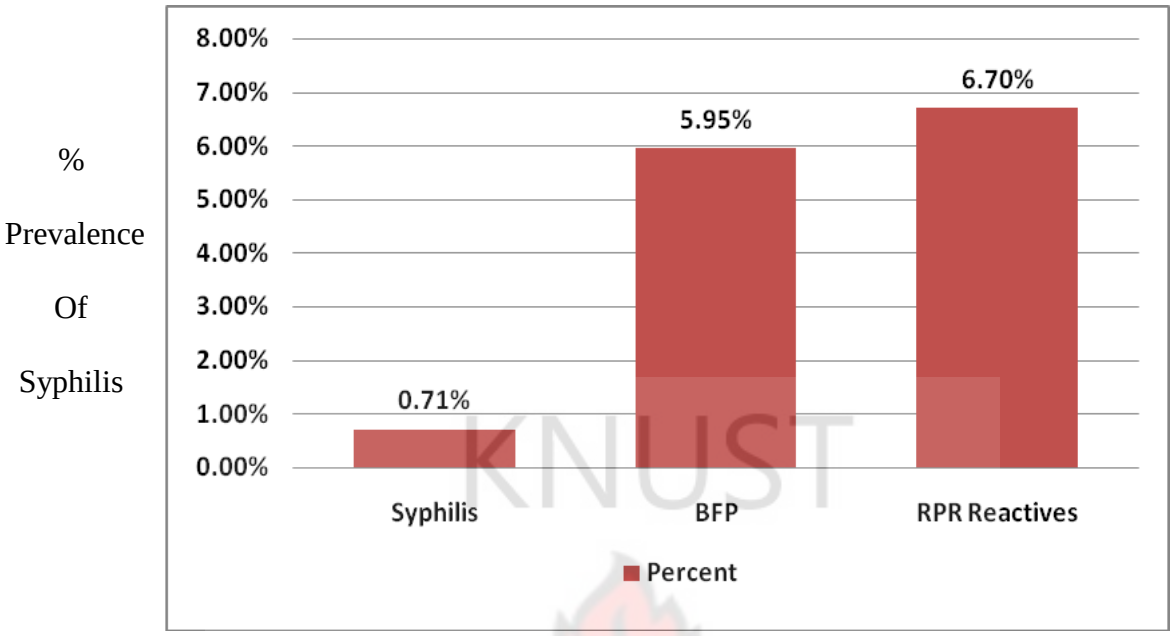
Out of the total number of 841 pregnant women interviewed and screened, 6.7% (56) of them were RPR reactive while 93.3% (789) were non-reactive. 10.7% (6), of the RPR reactives, had titres of 1:8, while 14.3%, 44.6% and 30.4% had titres of 1:4, 1:2 and neat respectively. Table 4.4 below shows the titre distribution for RPR and TPHA reactive samples.

Table 4.4: Titre distribution for RPR reactive and TPHA (modified) positive Samples

Titre Values	No: of RPR Reactives (%)	No: of TPHA Positives
Neat	17 (30.36)	0
1:2	25 (44.64)	4 (0.48%)
1:4	8 (14.29)	1 (0.12%)
1:8	6 (10.71)	1 (0.12%)
Total	56 (100)	6 (0.72%)

Of the 56 RPR reactive women, only 6 were positive for the TPHA (modified) test. Thus, the prevalence of syphilis among ANC clients at KATH was (6/841) 0.71% with Fifty (5.96%) of the samples being biologically false positive (BFP) (RPR reactive and TPHA negative). Figure 4.3 below shows the prevalence of syphilis, BFP and RPR reactives.

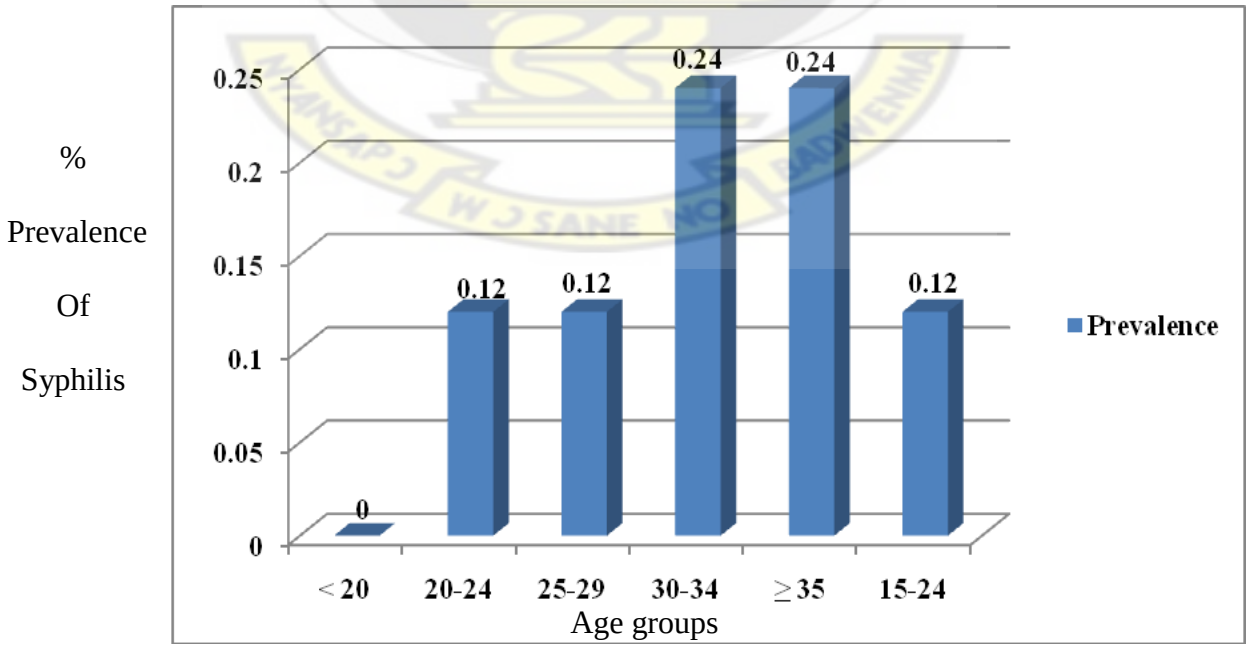
Figure 4.3: Prevalence of syphilis, BFP (RPR Reactive Only) and all RPR reactives



4.5.1: Syphilis prevalence by age distribution

Figure 4.4 below shows how syphilis prevalence was distributed among the various age groups.

Figure 4.4: Age Distribution of Patients with Syphilis



The Odds-Ratio (OR) of acquiring syphilis among the various age groups is shown in table 4.5 below.

Table 4.5: Odds-Ratio (OR) for syphilis among age groups

Age Group	Syphilis Prevalence	Probability (P) (+/Total) (%)	Odds = (+/─)	Odds Ratio(OR)	95% Confidence Interval (CI)	p-Value
≤ 20	0	0/13	0/13	0.00	-	-
20-24	1 (0.12%)	1/125(0.8%)	1/124	1.98	0.12-32.62	0.56
25-29	1 (0.12%)	1/246(0.4%)	1/245	0.51	0.03-8.22	0.56
30-34	2 (0.24%)	2/275(0.7%)	2/273	0.91	0.08-10.07	0.68
≥ 35	2 (0.24%)	2/182(1.1%)	2/180	1.38	0.09-15.39	0.64

Comparing the various age groups to age group 20-24, it could be seen from table 4.5 above that apart from age group 20-24 (OR=1.98, 95% CI=0.12-32.62, p=0.56), the risk of acquiring maternal syphilis increased from age group 25-29 upwards. Since no case was identified in the <20 year group, the risk of being infected with syphilis at age 20 and below is very minimal as compared to age group 20-24 years and above. However, the results cannot be classified as statistically significant (95% CI: 0.12-32.62, p=0.56).

4.5.2: Syphilis prevalence by place of residence and educational background

Although results in table 4.6 below are not statistically significant (95% CI: 0.10-10.0, p=0.69), the ORs show that the risk of acquiring syphilis in Kumasi (OR=1.16, CI=0.12-10.0, p=0.69%) is higher as compared to outside the Kumasi metropolis (OR=0.86, CI=0.10-7.41, p=0.69%). Again, the risk of acquiring syphilis in mothers with only basic education (OR=1.59, CI=5.43-13.72, p=0.56%) is higher as compared to those with tertiary education (OR=0.63, CI=0.07-5.43, p=0.56%).

Table 4.6: Odds-Ratio (OR) for Syphilis by residence and educational background

	Syphilis Prevalence	Probability (+/Total) (%)	Odds =(+/─)	Odds Ratio(OR)	95% Confidence Interval (CI)	P-Value
Place of Residence						
Inside Kumasi	5 (0.59%)	5/683 (0.7%)	5/678	1.16	0.12-10.00	0.69
Outside Kumasi	1 (0.12%)	1/158 (0.6%)	1/157	0.86	0.10-7.41	0.69
Educational Background						
Non-Formal	0	0/79	0/79	0.00	-	0.47
Basic	5 (0.59%)	5/482 (1.0%)	5/477	1.59	5.43-13.72	0.56
Secondary	0	0/127	0/127	0.00	-	0.31
Tertiary	1 (0.12%)	1/153 (0.7%)	1/152	0.63	0.07-5.43	0.56

4.5.3: Syphilis Prevalence by marital status and history of pregnancy and birth outcomes

Table 4.7 below shows that there is a higher risk of the unmarried women acquiring maternal syphilis as compared with the married. The same table also shows that having a previous adverse pregnancy effect puts a mother at 1.20 times risk of syphilis infection as compared to those without any previous adverse pregnancy or birth effects (0.83 times). However looking at the possible causes of the adverse effects, as reported by the women in their responses, coupled with their corresponding p-values, there is no significant relationship between the adverse pregnancy and birth efects and the risk of syphilis acquisition, making the results statistically insignificant.

Table 4.7: Odds-Ratio (OR) by marital status, history of pregnancy and birth outcomes

	Syphilis Prevalence	Probability (+/Total) (%)	Odds = (+/─)	Odds Ratio(OR)	95% C I	P-Value
Marital Status						
Married	5 (0.59%)	5/745 (0.7%)	5/740	0.64	0.07-5.54	0.52
Unmarried	1 (0.12%)	1/96 (1.0%)	1/95	1.56	0.18-13.50	0.52
History of Pregnancy and Birth Outcome						
Adverse Effect	4 (0.48%)	4/526 (0.76%)	4/522	1.20	0.22-6.59	0.60
No Effect	2 (0.24%)	2/315 (0.63%)	2/313	0.83	0.15-4.56	0.60

4.5.4: Syphilis prevalence by knowledge of syphilis and PMTCT

Table 4.8: Odds-Ratio (OR) for Syphilis on Knowledge of Syphilis and PMTCT

Knowledge	Syphilis Prevalence	Odds Ratio(OR)	95% C I	p-Value
Knowledge on Syphilis	2 (0.24%)	0.49	0.09-2.69	0.34
No knowledge on Syphilis	4 (0.48%)	2.02	0.37-11.09	0.34
Knowledge on (P)MTCT	1 (0.12%)	0.16	0.02-1.38	0.07
No knowledge on (P)MTCT	5 (0.59%)	6.12	0.71-52.84	0.07

Table 4.8 above, shows that having knowledge on syphilis, mother to child transmission (MTCT) of syphilis and prevention of mother to child transmission (PMTCT) of syphilis are not predictive enough for syphilis acquisition, hence the results are not statistically significant (95% CI: 0.02-52.84, p>0.07). It however indicates that the risk of syphilis acquisition in women with no knowledge of syphilis, MTCT of syphilis and PMTCT of syphilis is higher as compared to those who knew about syphilis, its MTCT and PMTCT.

Altough the data obtained indicated that women with no knowledge on MTCT and PMTCT were at the greatest risk factors of acquiring the infection (OR=6.12, CI=0.71-52.84, p=0.07), followed by women within age group ≥35 years (OR=1.38, CI=0.09-15.39, p=0.64), the unmarried (OR=1.56, CI=0.18-13.50, p=0.52) and those with no knowledge on syphilis (OR=2.02, Ci=0.37-11.09, p=0.34), followed by those within age group 20-24 years (OR=1.98, CI=0.12-32.62, p=0.56), those with previous adverse pregnancy effects (OR=1.20, CI=0.22-6.59, p=0.60) and the those within the Kumasi metropolis (OR=1.16, CI=0.12-10.0, p=0.69%), none of these risk factors was statistically significant enough to predict the possibility of syphilis infection.

CHAPTER FIVE

5.0: DISCUSSION

Syphilis in pregnancy can lead to adverse pregnancy outcomes like perinatal deaths, miscarriages, stillbirths, premature deliveries or low birth weights. This can be averted if detected and treated with a cheap, safe and readily available antibiotic, penicillin (Watson Jones, et al, 2002 and Doroshenko et al, 2006). Neonates born from mothers with syphilis may develop congenital syphilis which may manifest as interstitial keratitis, Hutchinson's teeth, saddle nose, periostitis, bone defects, joint swellings and a variety of central nervous system abnormalities (Stuart et al, 2004). It is possible that some of the spontaneous abortions, stillbirths, premature deliveries and low birth weights encountered in pregnant women in hospitals and clinics where ANC attendants are not routinely screened for syphilis infection and treated accordingly may be due to syphilis infection in the women. Hence the screening and treatment of pregnant women infected with syphilis will help decrease the adverse pregnancy outcomes like perinatal deaths, abortions (or miscarriages), stillbirths, premature deliveries and neonatal (congenital) syphilis (Terris-Prestholt et al, 2003).

This study sought to assess the extent of syphilis in pregnant women attending ANC at KATH and factors that put them at risk of infection. This will inform authorities in their planning and control strategies for syphilis elimination as directed by WHO in its strategy to eliminate maternal and congenital syphilis (WHO, 2007). According to WHO, the global goal in the elimination of congenital syphilis as a public health problem could be achieved through the implementation of the present elimination strategies which focus on the reduction of the

prevalence of syphilis in pregnant women and the prevention of mother-to-child transmission (PMTCT) of syphilis. These strategies rest on the following four pillars:

1. To ensure sustained political commitment and advocacy.
2. To increase access to and quality of maternal and newborn health care services.
3. To screen and treat pregnant women and their partners for syphilis.
4. To establish surveillance, monitoring and evaluation systems.

Many of the observed cohort characteristics studied in this study, including sociodemographic and economic status, history of previous pregnancy and birth outcomes, educational level, knowledge on syphilis and knowledge on PMTCT are also found in pregnant women from other parts of the African sub-region (Steketee et al, 1996). Hence the findings of this study could be generalized to many other regions in the continent that share similar population characteristics.

The study recorded a syphilis prevalence of 0.71%, a lower value compared to those reported in the Ghana HIV Sentinel Survey Reports and other African countries. The results of this study may confirm that maternal syphilis is not necessarily associated with the extremely high risk of adverse pregnancy outcomes at KATH, especially, stillbirths and premature deliveries. Widespread use of the broad spectrum antibiotic, penicillin might have partially been treating and ameliorating the sequelae of syphilis in the hospital.

The prevalence of syphilis in the present study (0.71%) is much lower than previously reported in the Ashanti region (6.5% for 2008 and 7.0% for 2009) and Kumasi (3.1% for

2008 and 3.0% for 2009) (GHS 2008, 2009). The findings may partly reflect the effect of syndromic management of sexually transmitted infections (STIs) especially those evidenced by genital irritations and sores that have gained much attention lately. The findings (0.71%) though suggestive of the relatively low syphilis prevalence at KATH (Kumasi) compared to other places in Ghana, point out a potential opportunity for indirect syphilis prevention by controlling and preventing sexually transmitted infections (STIs).

With less than 5 years to the deadline to the achievement of the Millennium Development Goals (MDG's) (2015), such a lower prevalence of 0.71% may indicate the closeness to the achievement of the MDG's 4, 5 and 6 which focuses on the reduction of child mortality (MDG 4), improve on maternal health (MDG 5) and combat HIV/AIDS, malaria and other diseases (MDG 6). The MDG 4 targets at reducing by two-thirds, (between 1990 and 2015), the under-five mortality rate, infant mortality rate and immunizing a proportion of one-year-old children against measles. The MDG 5 also targets at reducing by three-quarters, (between 1990 and 2015), the maternal mortality ratio and proportion of births attended by skilled health personnel. MDG 6 also focuses on combating HIV/AIDS, malaria and other diseases, targets at halting and beginning the reverse of the spread of HIV/AIDS, malaria, and other major diseases such as malaria and tuberculosis by 2015. With the current campaign on combating HIV prevalence among pregnant women aged 15-24 in Ghana, coupled with the intensive promotion of condom usage to increase contraceptive prevalence and the efforts of decreasing the number of children orphaned by HIV/AIDS to achieve the MDG's 4, 5 and 6 (MDG Indicators, 2002), such a prevalence is an indication that Ghana has achieved a lot in her bid to achieve MDG's 4, 5 and 6.

The WHO Regional Office for South-East Asia (SEARO) report on the regional strategy for the elimination of maternal and congenital syphilis builds on the WHO's 2007 Global elimination of congenital syphilis: rationale and strategy for action. It outlines the guiding principles, key strategies and interventions to achieve the goal of eliminating congenital syphilis by 2015 as stated in the MDGs 4, 5 and 6. It also proposes initial targets and indicators which may need revision as countries may launch the elimination programmes at different times (WHO, 2009). Aside the MDG's, the overall goal of the Global eradication initiative is to ensure that congenital syphilis is no longer a public health problem. Thus specifically, the prevention of vertical transmission of syphilis from mother to child and subsequent reduction in the incidence of congenital syphilis can be achieved by observing the following (WHO, 2009):

1. Early ANC services for and universal screening of all pregnant women, and prompt treatment of all seropositive women;
2. Treatment of partners of seropositive women, promotion of condom use, education and counseling to prevent infection/reinfection;
3. Prophylactic treatment of all infants born to seropositive women.
4. Screening is more effective if it is performed on-site early in pregnancy and treatment provided immediately to seropositive women.

Stakeholders including policy makers, policy implementers and beneficiaries are therefore advised to work towards the achievement of the goals and objectives of the Global eradication initiative which are grouped under the following four key strategies (WHO, 2009):

1. Ensuring sustained high-level commitment and advocacy which deals with the commitment of health policy decision-makers and other key stakeholders, such as programme managers, is necessary to ensure that the programme receives adequate political, financial and logistical support
2. Increasing access to, and improving the quality of maternal and child health by providing the opportunity to screen pregnant women for syphilis.
3. Screening pregnant women and treating seropositive women, their partners and newborn infants where emphasis is laid on the early detection and treatment of syphilis among pregnant women, their sexual partners and babies.
4. Establishing surveillance, monitoring and evaluation systems where surveillance and monitoring are essential to accurately assess the magnitude of maternal and congenital syphilis, plan and evaluate the effectiveness of interventions.

As demonstrated by Terris-Prestholt and colleagues in sub-Saharan Africa, the cost effectiveness of antenatal syphilis screening and treatment in intervention programmes is influenced by the prevalence of syphilis irrespective of how low or high it may be (Terris-Prestholt et al, 2003). According to them, the cost per DALY (Disability Adjusted Life Years) saved decreases substantially as prevalence increases. Hence the 0.71% prevalence in this study signifies the need for the intervention programmes to be followed in order to achieve the MDG's 4, 5, and 6 for complete eradication before the 2015 deadline.

Observations made at the study site indicates that screening for syphilis could be combined with screening for other infections such as HIV and malaria, thus complementing each other

and reducing the cost of counseling, drawing blood and test performance. In particular, programmes for the prevention of congenital syphilis should be integrated with those for PMTCT of HIV. As long as syphilis is prevalent among adults, the potential for congenital transmission remains high. Therefore, attention should also be paid to the prevention and control of syphilis in adults. Synergy between programmes would consolidate resources, strengthen and improve ANC services as well. These practices will not only reduce the cost involved in organizing two or more tests separately but will relatively make the performance of the tests simple, easy and fast. A study conducted in Haiti on the Cost-Effectiveness of Rapid Syphilis Screening in Prenatal HIV Testing Programs demonstrated that integrating rapid syphilis tests into prenatal care and HIV testing would not only make it cost effective, but will speed up the prevention of congenital syphilis (Bruce et al, 2007). A similar approach may be beneficial in other resource-poor countries like Ghana that are scaling up prenatal HIV testing. This model therefore suggests that including rapid syphilis testing as part of current global initiatives for preventing mother-to-child transmission of HIV could substantially reduce maternal syphilis and infant mortality as well.

Many African countries such as Madagascar, Nigeria, Botswana and Zambia have reported high prevalence of maternal syphilis. For example, in Lusaka, Zambia, syphilis screening in pregnant women demonstrated 12.5% prevalence (Ratnam et al, 1982). According to Ratnam and colleagues, this 12.5% prevalence in Lusaka was due to the high incidence of maternal and congenital syphilis at the University Teaching Hospital, Lusaka, Zambia. In Tanzania, the reason behind a dramatic decrease in syphilis prevalence from 3.0% in 2006 to 0.9% in 2009 (Swai et al, 2006 and Sia, et al, 2009), was due to the commitment demonstrated by authorities in the implementation of WHO's elimination strategies to achieve the MDG's 4, 5 and 6.

Available evidence indicates that there is no routine syphilis screening and treatment at KATH, therefore, this low prevalence (0.71%) of syphilis may not be due to the fact that health professionals in Ghana especially KATH have been implementing the global strategies for the elimination of congenital syphilis as directed by WHO, but may indicate that the prevalence of maternal syphilis in Kumasi are not as high as 7.2%, 3.1% and 3.0% as reported in the 2007, 2008 and 2009 HIV sentinel surveillance reports (HSS, 2007, 2008 and 2009).

In Botswana, maternal syphilis prevalence of 5% recorded in 2006 was as a result of late screening of ante-natal syphilis, delay in treatment and high rate of seroconversion (Romoren et al, 2006). Similar problems of late ante-natal syphilis screening, delay in treatment and high rate of seroconversion were exhibited in the rural Eastern Cape Province, Republic of South Africa where a prevalence of 6.3% was recorded for maternal syphilis (Blandford et al, 2007). These reasons of early ante-natal syphilis screening and treatment and low rate of seroconversion could however not be the reasons behind this lower prevalence of 0.71% at KATH, because KATH does not routinely and regularly screen and treat its ANC clients. Although, evidence was not obtained in the study on the widespread use of the antibiotic, penicillin, it could however be assumed that this lower prevalence could be due to the frequent usage of penicillin and its derivatives, which are available over the counter, by most people for the treatment of most bacterial infections.

It may even be due to the demographic position of KATH where the study was conducted. Apart from its status as the only teaching hospital in the region, it is also the second largest hospital in the country; hence most of the pregnant women who visit the hospital for ANC

services might have already visited one of the numerous maternity clinics or hospitals in and around the metropolis for screening and treatment. Pregnant women normally come to KATH when they are in their 3rd trimesters or when they need emergency care. This was evidenced in the study where 57.0% of the subjects first reported in their 3rd trimester. 36.62% reported in their 2nd trimester while only 9.9% reported in their 1st trimester.

The differences in prevalence obtained in this study and those reported in other studies may also be attributed to the differences in the test algorithms employed in the screening procedures. For example, in Ghana, the HIV Sentinel Survey Report from 2000-2003 registered small mean syphilis prevalence rates between 0.3-0.5% when the VDRL/RPR and TPHA tests were employed. This prevalence however shot up to 5.6%, 3.6%, 3.1%, 5.1, 3.8 and 3.9 from 2004-2009 respectively (GHS 2003-2009) when the initial testing algorithm of RPR/VDRL and TPHA was replaced with a simple and rapid *Treponema*-specific diagnostic point-of-care (POC) test which cannot differentiate between current infection from past treated or untreated infections (Dassah et al, 2010). The results (0.71%) of this study (where the RPR and modified TPHA used can differentiate between current and past infections) may suggest that the change in testing algorithm from RPR/VDRL and TPHA before 2004 to the POC test from 2004 may be the cause of the recent high prevalence of syphilis in Ghana since 2004. Hence the fear of unfolding epidemic of venereal syphilis in parts of Ghana like the Odoben Brakwa district, Assin Fosu, Akim Oda, Sefwi Asafo, Cape Coast and the Amansie West districts (GHS, 2007, 2008 and 2009) may be anti-factual. The 5.95% Biologically False Positives (RPR reactive and TPHA negative) results obtained indicated a prevalence of 6.70% if only the non-specific treponemal RPR test algorithm had been employed.

Previous researchers on maternal syphilis argue that the rapid (example POC) tests which are simple to use and interpret do not necessarily require electricity, sophisticated equipment and very skilled professionals. They can be performed outside the laboratory for instant results and subsequent treatment (Phaosavasdi et al, 1987, Larsen, et al, 1995, Marie-Louise et al, 2000, Karen, 2001 Myer et al, 2003, Watson-Jones, et al, 2002). However, most of these rapid tests cannot distinguish between current infection and past treated infections (Luger, 1988, Egglestone et al, 2000 and Dassah et al, 2010). Similar situation was witnessed in Nigeria where a change from VDRL test only to both VDRL and TPHA/FTA-ABS tests changed the prevalence rates from 4.5% and 14.6% to 1.6% and 9.8% respectively (Nnorom et al, 1996).

5.1: STUDIED RISK FACTORS

The study indicates that though not statistically significant, the risk of acquiring maternal syphilis increases with age, with age group ≥35 at highest risk as reported in other African countries like Tanzania where age groups 35-40 and 41-49 years registered the highest prevalence of 10.4% ($p < 0.001$) (Swai et al, 2006). High sexual activity of this age group could account for this.

An individual's place of residence was not a risk factor for maternal syphilis, hence cannot be used to predict possible infection. However, the study put Kumasi residents (OR=1.16, CI=0.12-10.0, p=0.69) at high risk of syphilis infection compared to those living outside the Kumasi metropolis (OR=0.86, CI=0.10-7.41 p=0.69). In Zambia, syphilis surveillance report identified roadside residents with 16.0% prevalence (95% CI = 13.3–18.9) as people with the highest risk followed by rural dwellers 10.5% (95% CI = 9.5–11.5) and 5.8% (95% CI = 5.4–6.3) for urban citizens (Swai et al, 2006). In Ghana, the HSS also reports that people who stay in rural areas are at high risk of syphilis infection compared to those in urban districts (GHS 2008, 2009). A possible reason may be that frequent migration of people especially the youth from rural areas (outside Kumasi) into the Kumasi metropolis (which is considered as an urban city) to seek for greener pastures may expose the Kumasi residents to high risk of syphilis and other STIs.

People with education up to the basic school level in this study were at highest risk of syphilis infection than others, though not statistically significant. This result is in agreement

with other studies from Africa and Asia where high prevalencies of maternal syphilis were demonstrated among women with no education than those with some education (Swai et al, 2006 and Cheng et al, 2007). Women who have access to higher education may have access to sex education including STIs like syphilis and HIV/AIDS and may therefore observe some precautions before, during and after their sexual activities thereby putting them at lower risk of infection.

Marital status was also not a predictive factor for syphilis infection. However, the unmarried women had a higher risk of syphilis infection compared to the married. Research from India also put unmarried women (49.7%) at higher risk of infection compared to the married women (44.5%) (Reynolds et al, 2006). This risk of exposure may be attributed to the fact that most married women had only one sexual partner (husband) while the unmarried have more than one partner, hence are exposed to STI's including syphilis.

There was no significant relationship between an individual's history of adverse pregnancy or birth outcomes and risk of maternal syphilis. This lack of association could be, partly due to the lower number of syphilis cases registered in this study and other confounding effects of birth related problems such as asphyxia, trauma, hypothermia and others beyond the scope of this study. This may explain that most previous adverse pregnancy outcomes experienced in our hospitals and clinics might be due to other pregnancy/birth related problems.

5.2: CONCLUSION

Syphilis remains a significant public health problem in many resource-poor settings. Lack of adequate antenatal care has been described as an important obstacle to the global prevention of congenital syphilis. Innovative approaches for diagnosis and treatment of maternal syphilis, such as early diagnosis and treatment within the first trimester of each pregnancy, on-site screening and subsequent treatment of both mothers and sexual partners have been documented in several resource-poor settings like Tanzania, Zambia, Kenya and Botswana (Hira et al, 1990, Jenniskens et al, 1995 and Romoren et al, 2006) to be cost effective.

It must however be noted that the lower prevalence of 0.71% found in this study compared to other studies from Africa in the sub-region, is no indication for complacency in the eradication strategies against maternal and congenital syphilis. However as demonstrated by Terris-Prestholt and colleagues on the cost effectiveness of antenatal syphilis screening and treatment in Africa (Terris-Prestholt et al, 2003), the 0.71% prevalence in this study signifies the need for the intervention programmes to be followed as directed by WHO irrespective of how low or high.

None of the studied risk factors like age, place of residence, occupational status, educational background, marital status, number of sexual partners, knowledge on syphilis, knowledge on vertical transmission of syphilis and knowledge on prevention of mother to child transmission of syphilis was predictive for syphilis infection, This may be attributed to the lower number of syphilis cases (6 out of 841) registered.

5.3: RECOMMENDATIONS

In order to achieve the Millennium Development Goals (MDGs) related to the reduction of child mortality (MDG4), the improvement of maternal health (MDG5), and to combat HIV/AIDS, malaria and other diseases, it is recommended to authorities that based on this study:

1. All pregnant women be first screened with non-specific treponemal RPR test followed by specific treponemal test like the TPHA at their first antenatal visits preferably before the 16th week of gestation and again in late pregnancy visits to detect infection acquired subsequently.
2. Women who for some reasons do not get tested or do not have the results of first visit tests should be screened or rescreened and treated accordingly.
3. Specifically, prevention of syphilis from mother to child could be achieved through the incorporation of syphilis screening into antenatal care programmes and prompt treatment of the infected as practiced in the PMTCT of HIV/AIDS.
4. Treatment of all sexual partners of infected women, promotion of condom use during pregnancy and regular counseling of all women on how to prevent infection.

REFERENCES

1. Aiken C G. (1992). The causes of perinatal mortality in Bulawayo, Zimbabwe. Central African Journal of Medicine; 38:263–281.
2. Albrecht T, Jeffrey WA, Michelle JA, Alton GG, Raza A, David M. Asher EJ. And Baron SB. (1996). Medical Microbiology. The University of Texas Medical Branch at Galveston; 4th ed.
3. Alexander JM, Sheffield JS, Sanchez PJ, Mayfield J and Wendel GD. (1999). Efficacy of treatment for syphilis in pregnancy. Obstetrics and Gynecology; 93:5-8.
4. Blandford JM, Gift TL, Vasaika S, Mwesigwa-Kayongo D, Dlali P and Bronzan RN. (2007). Cost-effectiveness of on-site antenatal screening to prevent congenital syphilis in rural Eastern Cape Province, Republic of South Africa. Sexually Transmitted Diseases, 34:S61–S66.
5. Brath Klaus. (2006). "100 years ago died Fritz Schaudinn, discoverer of the syphilis agent: unrecognized in his own country". MMW Fortschritte der Medizin 148 (23): 68. Pubmed 16826746.
6. Brion LP, Brion LP, Manuli M, Rai B, Kresch MJ, Pavlov H, Glaser J. (1991). Long bone radiographic abnormalities as a sign of active congenital syphilis in asymptomatic newborns. Department of Pediatrics, Albert Einstein College of Medicine, Bronx, New York, 88:1037–1040.
7. Brooks GF, Butel JS. and Morse S. A. (2004). Jawerts, Melnick & Alberg's Medical Microbiology 23rd ed. International Edition, 2004, McGraw-Hill Companies, Inc.; 331-342.
8. Broznan R, Mwesgwa-Kayongo D, Narkunas D. (2002). Report to the South African Ministry of Health. Atlanta, Centers for Disease Control and Prevention.

http://203.90.70.117/catalogue/2006-2010/pdf/aids/Regional_strategy_elimination_congenital_syphilis.pdf (Assessed: January, 2010)

9. Bruce R.S, Christopher PN, Sandy N, Nerette F, Claudine N, Patrice J, Jean WP and Daniel WF. (2007). Cost-Effectiveness of Rapid Syphilis Screening in Prenatal HIV Testing Programs in Haiti Pub Lib of Sci May 29, 2007, Volume 4 issue 5 page 183
10. Centre for Disease Control and Prevention (CDC). (2004) Congenital syphilis-United States. Morbidity and Mortality Weekly Report 2004; 53:716-19
11. Chaudhary M, Bineeta K, and Preena B. (2007). Congenital syphilis, still a reality in 21st century: a case report. Journal of Medical Case Reports; 1-90.
12. Cheesbrough Monica. (2003). District Laboratory Practice in Tropical Countries, Part 2; Cambridge University Press, Edinburgh Building, Cambridge CB2 2RU, United Kingdom. Pp. 218-225.
13. Cheng J Q, Zhou H, F C Hong, D Zhang, Y J Zhang, P Pan and Y M Cai. (2007). Syphilis screening and intervention in 500 000 pregnant women in Shenzhen, the People's Republic of China Sexually Transmitted Infection; 83;347-350
14. Crowe G, Theodore C, Forster GE, Goh BT. (1997). Acceptability and compliance with daily injections of procaine penicillin in the outpatient treatment of syphilis – treponemal infection. Sexually Transmission Diseases; 24:127-30.
15. Dassah Edward, Baafuor Kofi Opoku, Yaw Adu-Sarkodie. (2010). Screening for syphilis during pregnancy in Ghana: the role of new rapid point-of-care (POC) diagnostic tests. KNUST, Kumasi, Ghana: No 3, January 2010 Research briefing, News letter available at

http://www.dfid.gov.uk/r4d/PDF/Outputs/ReproHealthHIV_RPC/srhhiv-researchbriefing3-syphilisscreeningghana.pdf (Assessed: February, 2010)

16. Davanzo R, Antonio C, Pulella A, Lincetto O, Schierano S. (1992). Neonatal and post-neonatal onset of early congenital syphilis: a report from Mozambique. Annals of Tropical Paediatrics; 12:445-450.
17. Debora MacKenzie (2008). "Columbus blamed for spread of syphilis". New Scientist. http://www.newscientist.com/article/dn13186-columbus-blamed-for-preadof-syphilis-.html (Assessed: March, 2008)
18. Dobson S. (2004). Congenital Syphilis Resurgent. Advances in Experimental Medicine and Biology; 549:35-40
19. Donders GGG, Desmyter J, Hooft P, Dewet HG. (1997). Apparent failure of one injection of benzathine penicillin G for syphilis during pregnancy in human immunodeficiency virus-seronegative African woman. Sexually Transmitted Diseases; 24:94-101.
20. Doroshenko A, Sherrard J and Pollard A. J. (2006). Syphilis in pregnancy and the neonatal period. International Journal of STD & AIDS: 17: 221-227.
21. Egglestone SI, Turneer AJL. (2000). Serological diagnosis of syphilis. PHLS syphilis serology working group. Communicable Disease and Public Health; Vol: 3 No 3:158-62
22. Fegan G, Dean AG, Shah SP. (1996). Epidemiology Program Office, Centers for Disease Control and Prevention. Atlanta, USA. http://www.cdc.gov/epo/epi/epiinfo.htm. (Assessed: January, 2009)
23. Fitzgerald TJ, Cleveland P, Johnson RC, Miller JN, Sykes JA. (1997): Scanning electron microscopy of attached to cultured mammalian cells *Treponema pallidum* (Nichols strain), Journal of Clinical Immunology; 47(1): 228–233

24. Fonck K, Claeys P, Bashir F, Bwayo J, Fransen L, and Temmerman M. (2001). Syphilis control during pregnancy: effectiveness and sustainability of a decentralized program. Am Jour of Pub Health; 91:705–707Fumara NJ. Syphilis in newborn children. (1975). Clininical Obstetrics and Gynecology. 18:183-9
25. Genc M, Ledger WJ. (2000). Syphilis in pregnancy. Sexually Transmitted Infections; 76:73-9
26. Ghana Health Service (GHS). (2007). HIV Sentinel Survey Report 2006. National AIDS/STI Control Programme. Accra, Ghana: Ghana Health Service.
27. Ghana Health Service (GHS). (2008). HIV Sentinel Survey Report 2007. National AIDS/STI Control Programme. Accra, Ghana: Ghana Health Service.
28. Ghana Health Service (GHS). (2009). HIV Sentinel Survey Report 2008. National AIDS/STI Control Programme. Accra, Ghana: Ghana Health Service.
29. Ghana Health Service (GHS). (2010). HIV Sentinel Survey Report 2009. National AIDS/STI Control Programme. Accra, Ghana: Ghana Health Service.
30. Gloyd Stephen, Sanders Chai and Mary Anne Mercer. (2001). Antenatal syphilis in sub-Saharan Africa: missed opportunities for mortality reduction Health Policy and Planning; 16(1): 29-34
31. Harper KN, Ocampo PS, Steiner BM, George RW, Silverman MS, Bolotin S, Pillay A, Saunders NJ, Armelagos GJ. (2008). "On the origin of the treponematosis: a phylogenetic approach" PloS Negleted Tropical Diseases; 2 (1): e148.
32. Harter CA, Benirschke K. (1976). Fetal syphilis in the first trimester. American Journal of Obstetrics and Gynacology; 128:705-711.
33. Heidi D. Nelson, Nancy Glass, Laurie Huffman, Kim Villemyer; Andrew Hamilton, Paul Frame, Alfred O. Berg. (1996). US Prevevtive Task Force U.S. Preventive Services Task Force. Screening for Syphilis: Annals of Family Medicene

http://www.uspreventiveservicestaskforce.org/3rduspstf/syphilis/syphilup.htm (Assessed: October 2008)

34. Herman A. Metz. (1912). Solving medical mysteries by help of animals. The New York Times Newspaper, 28 January Edition of the 1912 batch.
35. Hideyo Noguchi, Moore JW. (1913). A Demonstration of *Treponema pallidum* in the brain in cases of general paralysis. Journal of experimental medicine 1;17(2):232–238. http://jem.rupress.org/content/17/2/232.full.pdf (Assessed: September, 2008)
36. Hira S. K, G J Bhat, D M Chikamata, B Nkowane, G Tembo, P L Perine, and A Meheus. (1990). Syphilis intervention in pregnancy: Zambian demonstration project. Genitourinary Medical Journal, 1990 June; 66(3): 159–164.
37. Hira SK, Ratnam AV, Sehgal D, Bhat GJ, Chintu C, Lulenga RC. (1982). Congenital syphilis in Lusaka. I. Incidence in a general nursery ward. East African Medical Journal. April; 59(4):241–246.
38. Hook, EW III, Marra, CM. (1992). Acquired syphilis in adults. The New England Journal of Medicine; 326:1060.
39. Ingraham NR. (1951). The value of penicillin alone in the prevention and treatment of congenital syphilis. Acta Dermato-venereologica; 31 Suppl 24:60.
40. Jenniskens F, Obwaka E, Kirisuah *S*, Moses *S*, Yusufali FM, Achola JO. (1995). Syphilis control in pregnancy: decentralization of screening facilities to primary care level, a demonstration project in Nairobi, Kenya. International Journal of Gynaecology and Obstetrics; *1995* Jun; *48* Suppl*:*S121-8.
41. Johnson RC, Ritzi DM and Levermore. (1973).Transmission electron micrograph *T pallidum*. American Society for Microbiology; 42(1): 114–160.
42. Karen L. Southwick, Stanley Blanco, Ana Santander, Miguel Estenssoro, Faustino Torrico, Guillermo Seoane, William Brady, Martha Fears, Joel Lewis, Victoria Pope,

Jeannette Guarner, & William C. Levine. (2001). Maternal and congenital syphilis in Bolivia. Bulletin of the World Health Organization. 79 (1): 33-42

43. Kelly BD. (2009) Syphilis, psychiatry and offending behaviour: clinical cases from nineteenth-century in Ireland. Irish Journal of Medical Science 178:1, 73-77. Online publication date: 1-Apr-2009.
44. Kerr JR. (1994). Penicillin allergy: a study of incidence as reported by patients. British Journal of Clinical Practice; 48:5-7.
45. Keys D (2000). "English syphilis epidemic pre-dated European outbreaks by 150 years". Independent News and Media Limited. Retrieved on 2007-09-22.
46. Kohl, P K and Winzer I (2005). "The 100 years since discovery of Spirochaeta pallida". Der Hautarzt; Zeitschrift für Dermatologie, Venerologie, und verwandte Gebiete 56 (2): 112–5.
47. Larsen SA, BM Steiner and AH Rudolph. (1995). Laboratory diagnosis and interpretation of tests for syphilis. American Society for Microbiology. Clinical Microbiology Reviews; 8:1-21.
48. Lobdell J, Owsley D (1974). "The origin of syphilis". Journal of Sex Research 10 (1): 76–79.
49. Luger AFH. (1988). Serological diagnosis of syphilis: current methods. In: Young H, McMillan A, editors. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Decker; 249-74.
50. Romoren Maria and Mafizur Rahman. (2006). Syphilis screening in the antenatal care: a cross sectional study from Botswana BMC International Health and Human Rights 1186/1472-698
51. Marie-Louise Newell and James Mclntyre. (2000). Congenital and prenatal infections: Prevention, diagnosis and treatment. Cambridge University press, UK; pp. 258-75

52. McDermott J, Steketee R, Larsen S and Wirima J. (1993). Syphilis-associated perinatal and infant mortality in rural Malawi. Bulletin of the World Health Organization; 71: 773–780.
53. Millennium Development Goal Indicators (September, 2002). Report on the Secretary General on the official United Nations site for the MDG Indicators. New York, United Nations Statistics Division, Department of Economic and Social Affairs. Available from: http://millenniumindicators.un.org/unsd/mdg/Default.aspx (Assessed, October, 2008)
54. Miller JN. (1975). Value and Limitations of Non-treponemal and Treponemal tests in the laboratory diagnosis of syphilis. Clinical Obstetrics and Gynecology; 18: 191.
55. Monif GR. (1994). Is current therapy for maternal syphilis inadequate for established fetal infection? American Journal of Obstetrics and Gynecology; 170:705.
56. Musher DM. (1990). Biology of Treponema pallidum. In: Sexually Transmitted Diseases, Second Edition, McGraw-Hill Book Company, New York. Pp 124-143
57. Myer L, Wilkinson D, Lombard C, Zuma K, Rotchford K, Abdool Karim SS. (2003). Impact of on-site testing for maternal syphilis on treatment delays, treatment rates, and perinatal mortality in rural South Africa: a randomised controlled trial. Sexually Transmitted Infections; 2003;79:208–13
58. Nnorom JA, Esu-Williams E, Tilley-Gyado A; (1996). HIV, tuberculosis and syphilis in Nigeria: a descriptive study. International Conference on AIDS: 7-12; 11: 138
59. Oriel J.D. (1994). The Scars of Venus: A History of Venereology. London: Springer-Verlag.
60. Pennisi E. (1998). Genome reveals wiles and weak points of syphilis. Science; 281:324-5.

61. Phaosavasdi S, Snidvongs W, Thasanapradit P, Asavapiriyanon *S*, Ungthavorn P,. Bhongsvej S. (1987). Cost–benefit analysis of diagnosis and treatment of syphilis in pregnant women. Journal of the Medical Association of Thailand. 70:90–95.

62. Radolf JD, Sanchez PJ, Schulz KF, Murphy FK., Holmes KK, Sparling PF, Mardh PA, Lemon SM, Stamm WE, Piot P, Wasserheit JN. (1999). Sexually Transmitted diseases. 3rd ed. New York: McGraw-Hill; p. 1165-89.

63. Ratnam A V, S N Din, S K Hira, G J Bhat, D S Wacha, A Rukmini, and R C Mulenga. (1982). Syphilis in pregnant women in Zambia. British Journal of Venereal Diseases on Sexually Transmitted Infections; 58(6): 355–358.

64. Reynolds SJ, A R Risbud, M E Shepherd, A M Rompalo, M V Ghate, S V Godbole, S N Joshi, A D Divekar, R R Gangakhedkar, R C Bollinger and S M Mehendale. (2006). High rates of syphilis among STI patients are contributing to the spread of HIV-1 in India Sexually Transmitted Infections; 82;121-126

65. Riley BS, Oppenheimer-Marls N, Hansen EJ, Radolf JD, Norgard MV. (1992). Virulent Treponema pallidum activates human vascular endothelial cells. Journal of Infectious Diseases; 165:484-93.

66. Robinson JL, Hameed T, Carr S. (2002). Practical aspects of choosing an antibiotic for patients with a reported allergy to an antibiotic. Journal of Clinical Infectious Diseases; 35:26-31

67. Romanowski B, Sutherland R, Fick GH, Mooney D, Love EJ. (1991). Serologic response to treatment of infectious syphilis. Annals of International Medicine; 114:1005-9

68. Saloojee H, Velaphi S, Goga Y, Afadapa N, Steen R, Lincetto O. (2004). The prevention and management of congenital syphilis: an overview and recommendations. Bulletin of the World Health Organization; 82:424-30.

69. Schmid George P, Bradley P. Stoner, Sarah Hawkes and Nathalie Broutet. (2007). The Need and Plan for Global Elimination of Congenital Syphilis. Sexually Transmitted diseases, July Supplement, Vol. 34, No. 7, p.S5-S10
70. Schulz KF, Cates W Jr, OMara PR. (1987). Pregnancy loss, infant death, and suffering: legacy of syphilis and gonorrhea in Africa. Journal of Genitourinary Medicine, 63: 320–325.
71. Sia E Msuya, Jacqueline Uriyo, Akhtar Hussain, Elizabeth M Mbizvo, Stig Jeansson, Noel E Sam and Babill Stray-Pedersen. (2009). Prevalence of sexually transmitted infections among pregnant women with known HIV status in northern Tanzania. Reproductive Health February, 2009, 6:4doi:10.1186/1742-4755-6-4
72. Singh AE, Romanowski B. (1999). Syphilis: review with emphasis on clinical, epidemiologic and some biologic features. Clinical Microbiology Review; 12:187-209
73. Steketee RW, Wirima JJ, Slutsker L, Breman JG, Heymann DL; (1996). Comparability of treatment groups and risk factors for parasitemia at the first antenatal clinic visit in a study of malaria treatment and prevention in pregnancy in rural Malawi. American Journal of Tropical Medicine and Hygiene; 55: 17-23
74. Stuart M. Berman. (2004). Maternal syphilis: pathophysiology and treatment. Bulletin of the World Health Organization; 82:433-8.
75. Swai O Roland, Geofrey R Somi G, Mecky IN Matee, Japhet Killewo, Eligius F Lyamuya, Gideon Kwesigabo, Tuhuma Tulli, Titus K Kabalimu, Lucy Ng'ang'a, Raphael Isingo and Joel Ndayongeje. (2006). Surveillance of HIV and syphilis infections among antenatal clinic attendees in Tanzania-2003/2004 BMC Public Health. 2006; 6: 91.
76. Terris-Prestholt F, Watson-Jones D, K Mugeye, L Kumaranayake, L. Ndeki, H Weiss, J. Changalucha, J Todd, F. Lisekie, B. Gumodoka, D. Mabey, R. Hayes. (2003). Is

antenatal syphilis screening still cost effective in sub-Saharan Africa. Journal Sexually Transmitted Infections; 79: 375-81.

77. Tikhonova L, Salakhov E, Southwick K, Shakarishvili A, Ryan C, Hillis S (2003). Congenital Syphilis Investigation Team. Congenital syphilis in the Rusian Federation: magnitude, determinants and consequencies. Journal Sexually Transmitted Infections; 79:106-10
78. Tramont EC. (2005). Treponema pallidum (Syphilis). In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Disease, 6th edn, Volumes 1 and 2. Philadelphia: Elsevier Churchill Livingstone: 2768-85.
79. Walker DG, Walker GJ. (2002). Forgotten but not gone: the continuing scourge of congenital syphilis. Lancet Infectious Diseases; 2:432-6
80. Walker GJA. (2001). Antibiotics for syphilis diagnosed during pregnancy. Cochrane Database of Systematic Reviews 2001, Issue 3. Art. No.: CD001143.
81. Walker GJA. (2007). Antibiotics for syphilis diagnosed during pregnancy. Cochrane Database of Systematic Reviews 2007, Issue 3. Art. No.: CD001143.
82. Watson-Jones D, Gumodoka B, Weiss H, Changalucha J, Todd J, Mugeye K, Buve A, Kanga Z, Ndeki L, Rusizoka M, Ross D, Marealle J, Whitehouse A, Balira R, Ngeleja D, Hayes R & Mabey D. (2002). Syphilis in pregnancy in Tanzania. I. Impact of maternal syphilis on outcome of pregnancy. Journal of Infections diseases; 186:940-7.
83. Watson-Jones D, Gumodoka B, Weiss H, Changalucha J, Todd J, Mugeye K, Buve A, Kanga Z, Ndeki L, Rusizoka M, Ross D, Marealle J, Whitehouse A, Balira R, Ngeleja D, Hayes R & Mabey D (2002). Syphilis in pregnancy in Tanzania. II. The effectiveness of antenatal syphilis screening and single-dose benzathine penicillin

treatment for the prevention of adverse pregnancy outcomes. Journal Infectious diseases; 186:948-57.

84. Weinsrock GM, Hardham JM, Mcleod MP, Sodergren EJ, Norri SJ. (1998). The genome of Treponema pallidum: new light on the agents of syphilis. FEMS Microbiology Reviews; 22:323-32.
85. Wendel GD, Sheffield JS, Hollier LM, Hill JB, Ramsey S, Sanchez PJ. (2002). Treatment of syphilis in pregnancy and prevention of congenital syphilis. Clinical Infectious Diseases; 35(Suppl 2):S200–S209.
86. WHO, 2007 policy paper. (2007). Action for the Global Elimination of congenital syphilis: Rationale and Strategy for action. http://whqlibdoc.who.int/publications/2007/9789241595858/eng.pdf (Assessed: May, 2009)
87. WHO, 2009 policy paper. (2009). Action for the Regional Strategy for the Elimination of congenital syphilis: Rationale and Strategy, p.1-47
88. WHO. (2001). Global prevalence and incidence of selected curable sexually transmitted infections. Geneva, World Health Organization, (WHO/CDS/CDR/EDC/2001.10).
89. WHO. (2001). Guidelines for the management of sexually transmitted infections. Geneva: World Health Organization; WHO document WHO/RHR/01.10.
90. WHO. (2001). Prevalence and risk factors of Syphilis: Bulletin of the World Health Organization: Bulletin of the World Health Organization 79 (1): 33-42
91. WHO. (2004) Reproductive health strategy to accelerate progress towards the attainment of international development goals and targets. Geneva,
92. WHO. (2004). Prevention of congenital syphilis-Time for action. Bulletin of the World Health Organization; 82:401-438.

93. WHO. (2009). Regional strategy for the elimination of congenital syphilis (RSECS): Regional Office for South-East Asia (SEARO); pp 1-59
94. WHO/PAHO, 1995. Pan American Health Organization. Plan of action for the elimination of congenital syphilis. Washington, DC, unpublished document (PAHO/WHO/CE116/14).
95. WHO/UNAIDS, 2004. National Maternal and Child Health Surveillance System.
96. Wilkinson D, Sach M, Connolly C. (1997). Epidemiology of syphilis in pregnancy in rural South Africa: opportunities for control. Journal of Tropical Medicine and International Health; 2:57-62
97. Zenker PN, Berman SM. (1991). Congenital syphilis: trends and recommendations for evaluation and management. Pediatrician Infectious Diseases Journal; 10:516-22.a

APPENDICES

APPENDIX 1: Structured Questionnaire

1.A: Copy of Questionnaire

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY AND THE KOMFO ANOKYE TEACHING HOSPITAL, SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

QUESTIONNAIRE ON THE PREVALENCE OF SYPHILIS INFECTION AND ASSOCIATED RISK FACTORS AMONG THE PREGNANT WOMEN WHO VISIT KATH FOR ANTENATAL CARE (ANC)

1. ID Number: …… 2. Age: ……...yrs. 3. Gestational age: ……weeks

4. Residential location: [ ]Kumasi Metro [ ]Outside Kumasi Metro 5.Occupation:……..

6. Educational Background: [ ] Non-formal [ ] Basic [ ] Secondary [ ] Tertiary

7. Are you married? [ ] Yes [ ] No 8. If No, how many sexual partners have you had in the last 12 months? [ ] 1 [ ] 2-3 [ ] 4-5 [ ] More than 5

9. No of Children:[ ] 0 [ ] 1-2 [ ] 3-4 [ ] 5 and above (If answer is none, go to question 10)

10. How many of the children are alive? [ ] 0 [ ] 1-2 [ ] 3-4 [ ] 5 and above

11. Has any pregnancy not resulted in a live term delivery? [ ] Yes [ ] No

12. If Yes, what happened? [ ] Miscarriage [ ] TOP [ ] Preterm [ ] IUD

13. Has any of your babies died on delivery? [ ] Yes [ ] No

14. If Yes, how many? [ ] 1-2 [ ] 3-4 [ ] ≥5

15. Has any of your children died within one week of delivery? [ ] Yes [ ] No

16. If Yes, how many died? [ ] 1-2 [ ] 3-4 [ ] ≥5 17. What was the cause of death?.....

18. Did any baby look abnormal at birth? [ ] Yes [ ] No 19. If Yes, describe abnormality...

20.Have you heard about the disease called syphilis?[]Yes []No(If No, end questionnaire)

21. How did you learn about the disease? [ ] Health Personnel [ ] Media [ ] Family [ ]

Friends [ ]Other……

22. Have you been diagnosed of syphilis before? [ ] Yes [ ] No [ ] Don't know 23. Mention any clinical presentation of the disease. [ ] Genital sores [ ] Skin

Lesions [ ] Don't know [ ] Other……..…...

24. How does one get infected? [ ] Through Sex [ ] Mother to child [ ]

Through Contact [ ] Other…….....

25. Can an infected pregnant mother give the disease to her baby? [ ]Yes [ ]N

(If Yes, go to 26)

26. Can this be prevented? [ ]Yes [ ]No 27. RPR Results. [ ] Reactive [ ] Non-reactive.

28. If Reactive, what is the RPR Titre value?

[ ]1:1(Neat) [ ]1:2 [ ]1:4 [ ]1:8 [ ]1:16 [ ]1:32

29. If RPR is Reactive, What is the TPHA Results. [ ] Positive [ ] Negative

1.B: Key Of Questionnaire:

- Answers to question 5 were grouped into Formal workers, Non-formal workers and the Unemployed.
- Live term delivery = The normal delivery of an infant with gestational age between 37 completed weeks (259 completed days) and 42 completed weeks (294 completed days).
- Miscarriage = The spontaneous expulsion of the products of pregnancy before the middle of the second trimester.
- Stillbirth = The birth of an infant who has died prior to delivery especially after 22 weeks of conception.
- Termination of Pregnancy (TOP) = Any other form of termination that cannot be explained by the patient.
- Preterm delivery = The delivery of an infant with gestational age of more than 20 weeks and less than 37 completed weeks (259 completed days).
- Intrauterine death (IUD)/Intrauterine foetal death (IUFD) = The death of a foetus at ≤ 22 weeks of gestation.

APPENDIX 2: TEST PROCEDURES EMPLOYED

2.A: RPR TEST

2.A.i: Principle of RPR test

The RPR test is a manually performed, visually read, 8 minutes rapid test for the detection of both qualitative and quantitative detection of ant-cardiolipin antibodies. Here, choline chloride is added to the cardiolipin cholesterol-lecithin antigen which removes the need for heat-inactivation and enables both plasma and serum to be used in the test. Carbon is then added to enable macroscopic viewing of results. It is a non specific treponemal method for the serological detection of syphilis. The antigen, a particulate carbon suspension coated with lipid complexes, agglutinates in the presence of serum reagins. Visible agglutination in the form of black clumps which can be viewed macroscopically, indicate the presence of cardiolipin antibodies in the sample tested (Fortress Diagnostic Limited, BT41 1QS, United Kingdom).

2.A.ii: Components of RPR Test kit

The following are the Composition of the RPR test kits (Fortress Diagnostic Limited, United Kingdom).

RPR Carbon Antigen:	Stabilized Carbon Suspension Coated with a lipid complex Sodium Azide 0.95g/L
Positive Control:	Human Serum Sodium Azide 0.95g/L
Negative Control:	Animal Serum Sodium Azide 0.95g/L

2.A.iii: RPR Reagent Preparation

The Carbon Antigen was gently resuspended to ensure thorough mixing. After which it was transferred into a dispensing bottle provided in the test kit. The dispensing bottle was then labeled with the lot number of the antigen, its expiring date and date it was transferred into the bottle. This was very important because according to the manufacturer, once the transfer of antigen was done into the dispensing bottle, it would be stable for 3 months or up to the expiry date, whichever comes first.

2.A.iv: Additional Equipment Used in the RPR Screening Process

1. Mechanical Rotator set at 100 revolutions per minute (r.p.m), circumscribing a circle 2.0cm in diameter.
2. Pasture pipette for dispensing serum samples.
3. A Centrifuge set at 3000 revolution per minute (r.p.m)
4. A Laboratory Refrigerator regulated between 2-8°C
5. A Laboratory Deep freezer regulated between -58°C and -52°C

2.B: TPHA TEST

2.B.i: Principle of TPHA (modified) test

The modified TPHA (*Treponemal pallidum* haemagglutination assay) test is a manually performed, visually read, 15 minutes rapid test for the detection of specific antibodies to the *T. pallidum.* Specifically, Global Invitro LLP rapid modified TPHA test cassette for syphilis employed in this study utilizes the principle of immunochromatography, a unique two-site immunoassay on a membrane. As the test sample flows through the membrane assembly of the test device, the cocktail of recombinant *Treponema* antigens-colloidal gold conjugate

forms a complex with *Treponema* specific antibodies in the sample. This complex moves further on the membrane to the test region where it is immobilized by the cocktail of recombinant *Treponema pallidum* antigens coated on the membrane leading to the formation of a pink to deep purple coloured band at the test region "T", to confirm a positive test result. Absence of this coloured band in the test region "T" indicates a negative test result. The unreacted conjugate and the unbound complex, if any, along with rabbit IgG gold conjugate move further on the membrane and are subsequently immobilized by the goat anti-rabbit antibodies coated on the control region "C" of the membrane assembly, forming a pink to deep purple band. The control band serves to validate the test results (Global Invitro LLP).

2.B.ii: Reagents and Materials in the modified TPHA Test Kit

Each individual pouch contains:

1. Test device: Membrane assembly predisposed with coaktail of recombinant *Treponema pallidum* antigens-colloidal gold conjugate, cocktail of recombinant *Treponema pallidum* antigen and goat anti-rabbit antiserum coated at the respective regions.
2. Disposable plastic dropper
3. Desiccant pouch
4. Diluent Buffer

APPENDIX 3: Results of Tests Performed

CODE NO	Patients Age/years	Gestational age in weeks	RPR Test Results	RPR Titre Values	TPHA Test Results
1	30	16	Non-reactive		
2	29	8	Non-reactive		
3	30	6	Non-reactive		
4	31	24	Non-reactive		
5	24	21	Non-reactive		
6	36	24	Non-reactive		
7	31	32	Non-reactive		
8	24	20	Non-reactive		
9	21	32	Non-reactive		
10	32	32	Non-reactive		
11	26	28	Non-reactive		
12	30	32	Non-reactive		
13	33	32	Non-reactive		
14	28	20	Non-reactive		
15	29	28	Non-reactive		
16	26	16	Non-reactive		
17	28	28	Non-reactive		
18	43	32	Non-reactive		
19	28	28	Non-reactive		
20	34	32	Non-reactive		
21	30	28	Non-reactive		
22	22	28	Non-reactive		
23	24	8	Non-reactive		
24	40	24	Non-reactive		
25	28	32	Non-reactive		
26	36	32	Non-reactive		
27	22	28	Non-reactive		
28	24	28	Non-reactive		
29	32	24	Non-reactive		
30	24	28	Non-reactive		
31	32	20	Non-reactive		
32	29	20	Non-reactive		
33	28	28	Non-reactive		
34	30	26	Non-reactive		
35	34	36	Non-reactive		
36	25	35	Non-reactive		
37	33	24	Non-reactive		
38	33	35	Non-reactive		
39	32	35	Non-reactive		
40	31	24	Non-reactive		

41	39	32	Non-reactive		
42	28	32	Non-reactive		
43	32	30	Non-reactive		
44	24	30	Non-reactive		
45	39	24	Non-reactive		
46	28	24	Non-reactive		
47	40	32	Non-reactive		
48	24	28	Non-reactive		
49	33	29	Non-reactive		
50	37	28	Non-reactive		
51	30	18	Non-reactive		
52	30	30	Non-reactive		
53	33	24	Non-reactive		
54	36	30	Non-reactive		
55	23	32	Non-reactive		
56	28	30	Non-reactive		
57	30	11	Non-reactive		
58	39	28	Non-reactive		
59	31	32	Non-reactive		
60	32	32	Non-reactive		
61	21	20	Non-reactive		
62	31	30	Non-reactive		
63	36	26	Non-reactive		
64	30	28	Non-reactive		
65	27	28	Non-reactive		
66	31	32	Non-reactive		
67	38	12	Non-reactive		
68	30	20	Non-reactive		
69	28	8	Non-reactive		
70	28	28	Non-reactive		
71	36	30	Non-reactive		
73	28	32	Non-reactive		
74	31	30	Non-reactive		
75	26	32	Non-reactive		
76	34	30	Non-reactive		
77	32	12	**Reactive**	1:4	Negative
78	24	26	Non-reactive		
79	24	24	Non-reactive		
80	27	28	Non-reactive		
81	30	28	Non-reactive		
82	20	30	Non-reactive		
83	24	20	Non-reactive		
84	32	20	**Reactive**	1:2	**Positive**

85	31	18	Non-reactive		
86	39	16	Non-reactive		
87	24	32	Non-reactive		
88	39	30	Non-reactive		
89	32	30	Non-reactive		
90	22	20	Non-reactive		
91	28	14	Non-reactive		
92	36	18	Non-reactive		
93	30	28	Non-reactive		
94	30	12	Non-reactive		
95	28	25	Non-reactive		
96	26	24	Non-reactive		
97	37	12	Non-reactive		
98	23	12	Non-reactive		
99	29	30	**Reactive**	1:2	Negative
100	23	30	Non-reactive		
101	24	20	Non-reactive		
102	35	30	Non-reactive		
103	33	24	Non-reactive		
104	30	28	Non-reactive		
105	32	28	Non-reactive		
106	36	28	Non-reactive		
107	26	24	Non-reactive		
108	30	26	**Reactive**	1:2	Negative
109	39	16	Non-reactive		
110	40	28	Non-reactive		
111	27	8	**Reactive**	1:4	Negative
112	28	20	Non-reactive		
113	34	24	Non-reactive		
114	27	32	Non-reactive		
115	36	28	Non-reactive		
116	25	26	Non-reactive		
117	26	20	Non-reactive		
118	34	28	Non-reactive		
119	29	20	Non-reactive		
120	28	20	Non-reactive		
121	26	28	Non-reactive		
122	24	16	**Reactive**	1:8	Negative
123	35	16	Non-reactive		
124	33	24	**Reactive**	1:2	Negative
125	29	22	Non-reactive		
126	28	14	**Reactive**	1:4	Negative
127	30	28	Non-reactive		

128	34	30	Non-reactive		
129	38	28	Non-reactive		
130	30	12	**Reactive**	1:2	Negative
131	28	10	Non-reactive		
132	25	8	Non-reactive		
133	33	28	Non-reactive		
134	38	28	Non-reactive		
135	27	6	Non-reactive		
136	30	12	**Reactive**	1:2	Negative
137	26	23	Non-reactive		
138	22	24	Non-reactive		
139	28	30	Non-reactive		
140	40	14	Non-reactive		
141	35	28	**Reactive**	Neat	Negative
142	30	28	Non-reactive		
143	30	28	Non-reactive		
144	34	32	Non-reactive		
145	22	28	Non-reactive		
146	28	20	**Reactive**	Neat	Negative
147	25	24	Non-reactive		
148	28	28	Non-reactive		
149	35	28	Non-reactive		
150	26	20	Non-reactive		
151	39	16	Non-reactive		
152	31	26	Non-reactive		
153	20	28	Non-reactive		
154	27	20	Non-reactive		
155	29	12	Non-reactive		
156	28	24	Non-reactive		
157	34	23	Non-reactive		
158	37	27	Non-reactive		
159	34	28	Non-reactive		
160	33	24	Non-reactive		
161	16	20	Non-reactive		
162	34	24	Non-reactive		
163	30	20	Non-reactive		
164	29	28	Non-reactive		
165	40	12	Non-reactive		
166	37	28	Non-reactive		
167	24	20	Non-reactive		
168	43	28	**Reactive**	1:2	**Positive**
169	24	14	**Reactive**	1:2	Negative
170	43	26	Non-reactive		

171	31	20	Non-reactive		
172	31	16	Non-reactive		
173	32	28	Non-reactive		
174	34	21	**Reactive**	1:4	Negative
175	27	22	**Reactive**	1:2	Negative
176	27	21	Non-reactive		
177	32	22	Non-reactive		
178	32	20	Non-reactive		
179	29	15	**Reactive**	Neat	Negative
180	27	16	Non-reactive		
181	32	28	Non-reactive		
182	28	24	Non-reactive		
183	26	23	Non-reactive		
184	30	28	Non-reactive		
185	34	16	Non-reactive		
186	34	26	Non-reactive		
187	42	28	Non-reactive		
188	28	24	Non-reactive		
189	38	20	Non-reactive		
190	40	24	Non-reactive		
191	43	8	Non-reactive		
192	22	26	Non-reactive		
193	33	26	Non-reactive		
194	29	28	Non-reactive		
195	24	20	Non-reactive		
196	26	12	Non-reactive		
197	29	38	Non-reactive		
198	34	35	Non-reactive		
199	30	28	Non-reactive		
200	29	24	**Reactive**	Neat	Negative
201	34	30	Non-reactive		
202	21	30	Non-reactive		
203	25	32	Non-reactive		
204	30	34	Non-reactive		
205	33	31	Non-reactive		
206	30	39	Non-reactive		
207	31	36	Non-reactive		
208	20	22	Non-reactive		
209	26	36	Non-reactive		
210	23	30	Non-reactive		
211	23	20	Non-reactive		
212	35	32	Non-reactive		
213	30	27	Non-reactive		

214	26	24	Non-reactive		
215	35	16	Non-reactive		
216	32	36	Non-reactive		
217	32	32	Non-reactive		
218	24	24	Non-reactive		
219	29	32	Non-reactive		
220	21	12	Non-reactive		
221	35	22	Non-reactive		
222	30	30	Non-reactive		
223	25	28	Non-reactive		
224	20	28	Non-reactive		
225	35	12	Non-reactive		
226	30	32	Non-reactive		
227	35	16	Non-reactive		
228	35	11	Non-reactive		
229	29	14	Non-reactive		
230	32	18	Non-reactive		
231	23	24	Non-reactive		
232	28	18	Non-reactive		
233	30	22	**Reactive**	1:2	Negative
234	22	6	Non-reactive		
235	27	28	Non-reactive		
236	30	12	Non-reactive		
237	35	8	Non-reactive		
238	33	12	Non-reactive		
239	35	32	Non-reactive		
240	32	24	Non-reactive		
241	30	12	Non-reactive		
242	29	28	Non-reactive		
243	42	12	Non-reactive		
244	23	36	Non-reactive		
245	29	30	Non-reactive		
246	23	34	Non-reactive		
247	22	16	Non-reactive		
248	23	36	Non-reactive		
249	33	35	Non-reactive		
250	24	34	Non-reactive		
251	35	24	Non-reactive		
252	27	16	Non-reactive		
253	27	16	**Reactive**	1:2	Negative
254	30	36	Non-reactive		
255	32	26	**Reactive**	Neat	Negative
256	37	28	Non-reactive		

257	32	40	Non-reactive		
258	27	30	Non-reactive		
259	32	26	Non-reactive		
260	28	16	Non-reactive		
261	30	28	Non-reactive		
262	25	24	Non-reactive		
263	33	4	Non-reactive		
264	23	22	Non-reactive		
265	38	28	Non-reactive		
266	14	12	Non-reactive		
267	21	28	**Reactive**	1:4	Negative
268	40	16	Non-reactive		
269	29	32	Non-reactive		
270	29	11	Non-reactive		
271	32	24	Non-reactive		
272	27	24	Non-reactive		
273	37	8	Non-reactive		
274	29	12	Non-reactive		
275	36	20	Non-reactive		
276	36	24	**Reactive**	1:2	Negative
277	35	14	Non-reactive		
278	26	36	Non-reactive		
279	35	30	Non-reactive		
280	24	28	Non-reactive		
281	27	22	Non-reactive		
282	39	30	Non-reactive		
283	36	21	Non-reactive		
284	22	28	Non-reactive		
285	35	34	Non-reactive		
286	36	30	Non-reactive		
287	33	16	Non-reactive		
288	26	20	Non-reactive		
289	36	36	Non-reactive		
290	20	24	Non-reactive		
291	24	30	Non-reactive		
292	26	38	Non-reactive		
293	35	11	Non-reactive		
294	31	36	Non-reactive		
295	39	32	**Reactive**	1:8	Negative
296	36	36	Non-reactive		
297	28	23	Non-reactive		
298	33	30	Non-reactive		
299	33	12	Non-reactive		

300	38	28	Non-reactive		
301	25	32	Non-reactive		
302	29	11	Non-reactive		
303	23	28	Non-reactive		
304	27	38	Non-reactive		
305	33	16	Non-reactive		
306	27	30	Non-reactive		
307	35	12	Non-reactive		
308	26	36	Non-reactive		
309	30	12	Non-reactive		
310	25	36	**Reactive**	1:2	Negative
311	38	30	Non-reactive		
312	28	32	Non-reactive		
313	27	32	Non-reactive		
314	31	36	Non-reactive		
315	29	36	Non-reactive		
316	26	32	**Reactive**	1:4	Negative
317	28	35	Non-reactive		
318	25	36	Non-reactive		
319	28	14	Non-reactive		
320	33	24	Non-reactive		
321	35	32	Non-reactive		
322	26	36	Non-reactive		
323	37	32	Non-reactive		
324	44	22	Non-reactive		
325	27	26	Non-reactive		
326	28	28	Non-reactive		
327	21	20	Non-reactive		
328	30	15	Non-reactive		
329	34	24	Non-reactive		
330	30	33	Non-reactive		
331	40	26	**Reactive**	1:8	**Positive**
332	23	32	Non-reactive		
333	22	36	Non-reactive		
334	30	32	Non-reactive		
335	33	30	Non-reactive		
336	36	36	Non-reactive		
337	28	12	Non-reactive		
338	30	24	Non-reactive		
339	37	34	Non-reactive		
340	28	29	Non-reactive		
341	33	7	Non-reactive		
342	36	32	Non-reactive		

343	36	32	Non-reactive		
344	29	36	Non-reactive		
345	27	32	Non-reactive		
346	33	24	Non-reactive		
347	28	24	Non-reactive		
348	30	15	Non-reactive		
349	29	16	Non-reactive		
350	36	12	Non-reactive		
351	32	36	Non-reactive		
352	27	28	Non-reactive		
353	26	28	Non-reactive		
354	31	28	Non-reactive		
355	26	23	Non-reactive		
356	25	36	Non-reactive		
357	35	28	Non-reactive		
358	31	32	Non-reactive		
359	26	22	Non-reactive		
360	30	36	Non-reactive		
361	30	28	Non-reactive		
362	33	20	Non-reactive		
363	37	24	Non-reactive		
364	25	36	Non-reactive		
365	30	12	Non-reactive		
366	33	11	Non-reactive		
367	32	32	Non-reactive		
369	35	14	Non-reactive		
370	35	24	Non-reactive		
371	31	28	Non-reactive		
372	35	28	Non-reactive		
373	30	36	Non-reactive		
374	26	26	Non-reactive		
375	27	31	Non-reactive		
376	24	15	Non-reactive		
377	28	23	Non-reactive		
378	22	36	Non-reactive		
379	27	20	Non-reactive		
380	31	8	Non-reactive		
381	26	20	Non-reactive		
382	17	28	Non-reactive		
383	32	16	Non-reactive		
384	36	12	Non-reactive		
385	37	18	Non-reactive		
386	28	32	Non-reactive		

387	32	20	Non-reactive		
388	24	22	Non-reactive		
389	38	20	Non-reactive		
390	36	20	Non-reactive		
391	33	34	Non-reactive		
392	21	32	Non-reactive		
393	29	28	Non-reactive		
394	21	34	Non-reactive		
395	23	34	Non-reactive		
396	41	23	Non-reactive		
397	27	29	Non-reactive		
398	33	30	Non-reactive		
399	27	34	Non-reactive		
400	34	32	Non-reactive		
401	31	34	Non-reactive		
402	30	24	**Reactive**	Neat	Negative
403	34	16	Non-reactive		
404	33	14	Non-reactive		
405	28	16	Non-reactive		
406	27	26	Non-reactive		
407	26	42	Non-reactive		
408	28	26	Non-reactive		
409	37	32	Non-reactive		
410	28	36	Non-reactive		
411	30	20	Non-reactive		
412	30	32	Non-reactive		
413	28	32	Non-reactive		
414	28	28	Non-reactive		
415	28	36	Non-reactive		
416	33	36	Non-reactive		
417	37	34	Non-reactive		
418	32	30	Non-reactive		
419	28	24	Non-reactive		
420	36	28	Non-reactive		
421	34	32	Non-reactive		
422	33	20	Non-reactive		
423	40	38	Non-reactive		
424	36	39	Non-reactive		
425	24	16	Non-reactive		
426	31	28	Non-reactive		
427	21	20	Non-reactive		
428	30	32	Non-reactive		
429	27	37	Non-reactive		

430	30	36	Non-reactive		
431	37	7	Non-reactive		
432	35	18	Non-reactive		
433	30	28	Non-reactive		
434	20	36	Non-reactive		
435	43	24	Non-reactive		
436	33	19	Non-reactive		
437	32	34	Non-reactive		
438	19	32	Non-reactive		
439	29	36	Non-reactive		
440	24	36	Non-reactive		
441	26	34	Non-reactive		
442	30	28	Non-reactive		
443	32	12	Non-reactive		
444	26	20	Non-reactive		
445	30	5	Non-reactive		
446	23	36	Non-reactive		
447	28	32	**Reactive**	1:4	**Positive**
448	29	11	Non-reactive		
449	33	32	Non-reactive		
450	32	28	Non-reactive		
451	30	20	Non-reactive		
452	26	28	Non-reactive		
453	32	8	Non-reactive		
454	28	18	Non-reactive		
455	11	30	Non-reactive		
456	28	33	Non-reactive		
457	32	32	**Reactive**	1:8	Negative
458	33	28	Non-reactive		
459	19	16	Non-reactive		
460	31	32	Non-reactive		
461	26	28	Non-reactive		
462	18	28	Non-reactive		
463	29	28	Non-reactive		
464	30	36	Non-reactive		
465	27	34	Non-reactive		
466	34	19	Non-reactive		
467	24	20	Non-reactive		
468	37	32	Non-reactive		
469	33	32	Non-reactive		
470	32	28	Non-reactive		
471	42	23	Non-reactive		
472	31	32	Non-reactive		

473	28	32	Non-reactive		
474	26	16	Non-reactive		
475	30	36	Non-reactive		
476	24	24	Non-reactive		
477	25	32	Non-reactive		
478	25	28	Non-reactive		
479	40	24	Non-reactive		
480	28	26	Non-reactive		
481	23	28	Non-reactive		
482	23	36	Non-reactive		
483	32	28	**Reactive**	Neat	Negative
484	35	36	Non-reactive		
485	25	36	Non-reactive		
486	20	28	Non-reactive		
487	32	36	Non-reactive		
488	20	30	Non-reactive		
489	34	24	Non-reactive		
490	28	32	Non-reactive		
491	25	32	Non-reactive		
492	28	40	Non-reactive		
493	25	24	Non-reactive		
494	35	36	Non-reactive		
495	31	35	Non-reactive		
496	35	32	Non-reactive		
497	36	6	Non-reactive		
498	30	20	Non-reactive		
499	23	28	Non-reactive		
500	25	36	Non-reactive		
501	26	36	Non-reactive		
502	36	36	Non-reactive		
503	24	20	Non-reactive		
504	18	32	Non-reactive		
505	27	34	Non-reactive		
506	22	4	Non-reactive		
507	30	16	Non-reactive		
508	35	30	Non-reactive		
509	23	27	Non-reactive		
510	27	20	Non-reactive		
511	28	20	Non-reactive		
512	25	34	Non-reactive		
513	27	36	Non-reactive		
514	28	35	Non-reactive		
515	25	35	Non-reactive		

516	36	9	Non-reactive		
517	35	13	Non-reactive		
518	26	16	Non-reactive		
519	28	16	Non-reactive		
520	34	32	Non-reactive		
521	33	36	Non-reactive		
522	25	20	Non-reactive		
523	35	28	Non-reactive		
525	28	34	Non-reactive		
526	28	26	Non-reactive		
527	29	32	Non-reactive		
528	27	32	Non-reactive		
529	26	34	Non-reactive		
530	39	12	Non-reactive		
531	19	36	Non-reactive		
532	35	24	Non-reactive		
533	30	20	Non-reactive		
534	26	13	Non-reactive		
535	37	3	Non-reactive		
536	30	6	Non-reactive		
537	20	16	Non-reactive		
538	31	33	Non-reactive		
539	32	20	Non-reactive		
540	34	20	Non-reactive		
541	37	26	Non-reactive		
542	23	36	Non-reactive		
543	26	22	Non-reactive		
544	28	12	Non-reactive		
545	31	23	Non-reactive		
546	31	36	Non-reactive		
547	38	24	Non-reactive		
548	37	16	Non-reactive		
549	40	36	Non-reactive		
550	30	16	Non-reactive		
551	28	34	Non-reactive		
552	33	27	Non-reactive		
553	21	36	Non-reactive		
554	40	33	**Reactive**	Neat	Negative
555	39	10	Non-reactive		
556	35	26	Non-reactive		
557	32	36	Non-reactive		
558	34	22	Non-reactive		
559	33	27	Non-reactive		

560	36	16	Non-reactive		
561	27	28	Non-reactive		
562	39	38	**Reactive**	Neat	Negative
563	36	16	Non-reactive		
564	28	16	Non-reactive		
565	28	24	Non-reactive		
566	35	36	Non-reactive		
567	25	34	Non-reactive		
568	21	33	Non-reactive		
570	29	31	Non-reactive		
571	30	16	Non-reactive		
572	31	32	Non-reactive		
573	33	24	Non-reactive		
574	33	25	Non-reactive		
575	36	34	Non-reactive		
576	30	30	Non-reactive		
577	38	28	Non-reactive		
578	40	24	Non-reactive		
579	27	32	Non-reactive		
580	30	36	Non-reactive		
581	30	32	Non-reactive		
582	33	36	Non-reactive		
583	34	28	Non-reactive		
584	30	32	Non-reactive		
585	28	24	Non-reactive		
586	36	8	Non-reactive		
587	28	18	Non-reactive		
588	30	28	Non-reactive		
589	32	26	Non-reactive		
590	23	36	Non-reactive		
591	36	40	Non-reactive		
592	20	24	Non-reactive		
593	31	36	**Reactive**	1:2	Negative
594	33	22	Non-reactive		
595	27	16	Non-reactive		
596	32	30	Non-reactive		
597	34	28	Non-reactive		
598	31	14	Non-reactive		
599	36	32	Non-reactive		
600	24	32	Non-reactive		
601	31	32	**Reactive**	1:2	Negative
602	27	32	Non-reactive		
603	37	36	Non-reactive		

604	24	20	Non-reactive		
605	28	24	Non-reactive		
606	27	40	Non-reactive		
607	32	29	Non-reactive		
608	29	28	Non-reactive		
609	32	40	Non-reactive		
610	26	32	Non-reactive		
611	29	32	Non-reactive		
612	35	36	Non-reactive		
613	24	36	Non-reactive		
614	40	32	Non-reactive		
615	34	15	**Reactive**	1:2	**Positive**
616	32	28	Non-reactive		
617	35	11	Non-reactive		
618	28	36	**Reactive**	1:8	Negative
619	32	36	Non-reactive		
620	20	32	Non-reactive		
621	32	32	Non-reactive		
622	27	32	Non-reactive		
623	32	32	**Reactive**	1:8	Negative
624	27	32	Non-reactive		
625	22	16	Non-reactive		
626	21	16	Non-reactive		
627	24	20	Non-reactive		
628	31	14	Non-reactive		
629	29	11	Non-reactive		
630	42	22	Non-reactive		
631	28	28	Non-reactive		
632	25	20	Non-reactive		
633	25	32	Non-reactive		
634	38	32	Non-reactive		
635	27	36	Non-reactive		
636	39	32	Non-reactive		
637	25	24	Non-reactive		
638	24	32	Non-reactive		
639	35	12	Non-reactive		
640	26	36	Non-reactive		
641	36	26	Non-reactive		
642	26	30	Non-reactive		
643	34	24	Non-reactive		
644	36	24	Non-reactive		
645	42	20	Non-reactive		
646	33	24	Non-reactive		

647	27	35	**Reactive**	Neat	Negative
648	31	35	Non-reactive		
649	30	29	Non-reactive		
650	45	59	Non-reactive		
651	29	28	Non-reactive		
652	37	28	Non-reactive		
653	34	27	Non-reactive		
654	29	36	Non-reactive		
655	36	32	Non-reactive		
656	30	24	Non-reactive		
657	25	11	Non-reactive		
658	30	32	Non-reactive		
659	33	36	Non-reactive		
660	34	26	Non-reactive		
661	20	20	Non-reactive		
662	36	10	Non-reactive		
663	19	32	Non-reactive		
664	40	8	Non-reactive		
665	24	12	Non-reactive		
666	26	30	Non-reactive		
667	30	32	Non-reactive		
668	26	12	Non-reactive		
669	24	20	Non-reactive		
670	30	37	Non-reactive		
671	23	32	Non-reactive		
672	33	31	Non-reactive		
673	32	14	Non-reactive		
674	33	36	Non-reactive		
675	29	15	Non-reactive		
676	41	10	Non-reactive		
677	36	20	Non-reactive		
678	30	12	Non-reactive		
679	23	29	Non-reactive		
680	36	36	Non-reactive		
681	35	16	Non-reactive		
682	33	8	Non-reactive		
683	30	8	Non-reactive		
684	34	36	Non-reactive		
685	30	20	**Reactive**	Neat	Negative
686	38	28	Non-reactive		
687	33	18	Non-reactive		
688	19	24	Non-reactive		
689	27	32	Non-reactive		

690	24	15	Non-reactive		
691	25	32	Non-reactive		
692	36	32	Non-reactive		
693	33	26	Non-reactive		
694	33	23	Non-reactive		
695	34	23	Non-reactive		
696	26	30	**Reactive**	Neat	Negative
697	42	36	Non-reactive		
698	40	34	Non-reactive		
699	35	36	Non-reactive		
700	33	24	Non-reactive		
701	33	36	Non-reactive		
702	43	33	Non-reactive		
703	35	20	Non-reactive		
704	25	16	Non-reactive		
705	40	30	Non-reactive		
706	26	20	Non-reactive		
707	28	16	Non-reactive		
708	33	20	Non-reactive		
709	24	35	Non-reactive		
710	35	18	Non-reactive		
711	22	36	Non-reactive		
712	24	36	Non-reactive		
713	35	19	Non-reactive		
714	22	37	Non-reactive		
715	32	28	Non-reactive		
716	26	32	Non-reactive		
717	35	26	Non-reactive		
718	34	13	Non-reactive		
719	41	16	Non-reactive		
720	25	32	Non-reactive		
721	22	20	**Reactive**	1:2	**Positive**
722	27	16	Non-reactive		
723	26	32	Non-reactive		
724	32	22	Non-reactive		
725	26	32	Non-reactive		
726	22	36	Non-reactive		
727	37	24	Non-reactive		
728	28	36	Non-reactive		
729	37	36	**Reactive**	Neat	Negative
730	32	24	Non-reactive		
731	30	16	Non-reactive		
732	23	38	Non-reactive		

733	24	34	Non-reactive		
734	39	18	Non-reactive		
735	26	35	Non-reactive		
736	28	36	Non-reactive		
737	27	32	Non-reactive		
738	32	20	Non-reactive		
739	35	36	Non-reactive		
740	34	36	**Reactive**	Neat	Negative
741	32	36	**Reactive**	1:2	Negative
742	31	12	**Reactive**	1:4	Negative
743	24	28	Non-reactive		
744	33	32	Non-reactive		
745	19	40	**Reactive**	1:2	Negative
746	30	38	Non-reactive		
747	36	11	Non-reactive		
748	23	38	Non-reactive		
749	37	16	Non-reactive		
750	32	28	Non-reactive		
751	25	29	Non-reactive		
752	27	28	Non-reactive		
753	24	31	Non-reactive		
754	27	16	Non-reactive		
755	30	12	Non-reactive		
756	35	32	**Reactive**	Neat	Negative
757	28	25	Non-reactive		
758	33	16	Non-reactive		
759	30	28	Non-reactive		
760	38	27	Non-reactive		
761	27	17	Non-reactive		
762	18	36	Non-reactive		
763	26	16	Non-reactive		
764	28	30	Non-reactive		
765	27	32	Non-reactive		
766	40	10	Non-reactive		
767	37	15	Non-reactive		
768	25	24	Non-reactive		
769	30	17	Non-reactive		
770	24	32	Non-reactive		
771	32	36	Non-reactive		
772	32	16	Non-reactive		
773	29	16	Non-reactive		
774	23	28	Non-reactive		
775	26	22	Non-reactive		

776	28	31	Non-reactive		
777	23	32	Non-reactive		
778	29	16	Non-reactive		
779	34	20	Non-reactive		
780	39	28	Non-reactive		
781	21	32	Non-reactive		
782	36	20	Non-reactive		
783	32	36	Non-reactive		
784	27	20	Non-reactive		
785	36	34	Non-reactive		
786	24	15	Non-reactive		
787	29	20	**Reactive**	1:2	Negative
788	30	15	Non-reactive		
789	36	24	Non-reactive		
790	22	36	Non-reactive		
791	22	35	Non-reactive		
792	21	28	Non-reactive		
793	32	23	Non-reactive		
794	34	24	Non-reactive		
795	38	12	Non-reactive		
796	22	32	Non-reactive		
797	33	36	Non-reactive		
798	32	12	Non-reactive		
799	20	12	Non-reactive		
800	33	36	Non-reactive		
801	34	13	Non-reactive		
802	35	20	Non-reactive		
803	28	36	Non-reactive		
804	28	30	Non-reactive		
805	35	36	Non-reactive		
806	24	24	Non-reactive		
807	24	40	Non-reactive		
808	40	36	Non-reactive		
809	34	24	Non-reactive		
810	32	36	Non-reactive		
811	34	32	Non-reactive		
812	25	32	Non-reactive		
813	35	36	Non-reactive		
814	30	20	Non-reactive		
815	36	8	Non-reactive		
816	33	28	**Reactive**	1:2	Negative
817	32	12	Non-reactive		
818	35	14	Non-reactive		

819	24	36	Non-reactive		
820	34	18	Reactive	1:2	Negative
821	23	28	Non-reactive		
822	27	34	Non-reactive		
823	20	14	Non-reactive		
824	27	36	Non-reactive		
825	39	24	**Reactive**	Neat	Negative
826	30	27	Non-reactive		
827	22	12	**Reactive**	Neat	Negative
828	32	20	**Reactive**	1:2	Negative
829	29	36	**Reactive**	1:2	Negative
830	27	15	Non-reactive		
831	32	36	Non-reactive		
832	39	15	Non-reactive		
833	28	24	Non-reactive		
834	40	36	Non-reactive		
835	29	37	Non-reactive		
836	25	36	Non-reactive		
837	24	23	Non-reactive		
838	32	16	**Reactive**	1:2	Negative
839	34	18	Non-reactive		
840	24	36	Non-reactive		
841	33	36	Non-reactive		

APPENDIX 4: EPI Info Analysis and Odds Ratio Calculations

Calculation of Odds-Ratio (OR)

	Positive Subjects	Negative Subjects	Total Subjects	Rate of Events
Dependant Variable	A	B	A+B	A/B
Independent Variable	C	D	C+D	C/D
Total	A+C	B+D	(A+B)+(C+D)	A/B+C/D

Using the table above and its variables:

The Odds of Exposure in the positive and negative Cases = A x (A + B)/ B x (A + B) = A / B

Odds of Exposure in positive and negative Controls = C x (C + D) / D x (C + D) = C / D

Hence, the Odds-Ratio (OR) (Odds of exposure) is as shown below:

$$\textbf{Odds Ratio (OR)} = {}^{(A / B)}/_{(C / D)} = \textbf{(A x D) / (B x C)}$$