OCCURRENCE OF SYPHILIS IN RESIDENTS OF SHER AGENCIES FIRM IN NAIVASHA

PRESENTED BY

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COLLEGE NUMBER 104PO2507

THE KENYA POLYTECHNIC
DEPARTMENT OF HEALTH SCIENCES AND BIOTECHNOLOGY
AUGUST 2005
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THE PROJECT HAS BEEN SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF HIGHER NATIONAL DIPLOMA IN MEDICAL LABORATORY TECHNOLOGY.

THE KENYA POLYTECHNIC
DEPARTMENT OF HEALTH SCIENCES AND BIOTECHNOLOGY

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TABLE OF CONTENTS

Declaration i
Dedication ii
Acknowledgement iii
Abstract iv

CHAPTER ONE

1.0 INTRODUCTION 1
1.1 Aim 2
1.2 Objectives 3
1.3 Statement of Problem 3
1.4 Study Design 4
1.5 Limitation of the Study 4

CHAPTER TWO

2.0 LITERATURE REVIEW
2.1 Pathogenesis of Syphilis 6
2.2 Pathogenesis of Syphilis in HIV Infected Patients 6
2.3 Virulence of Syphilis 11
2.4 Treatment of Syphilis 12
2.5 Control and Prevention 17
2.6 Immunity to Syphilis 19
2.7 Epidemiology 20
CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials, Reagent and Equipments 22
3.2 Reagin Plasma Reagent (RPR) Test 23
3.3 Treponema Pallidum Hamegglutination Assay (TPHA) 26
3.4 Specimen Collection 28
3.5 Interpretation of Cardiolipin and Treponema Seological Test 29
3.6 False Positive Reactions 29

CHAPTER FOUR

4.0 DATA ANALYSIS AND PRESENTATION 31

CHAPTER FIVE

Discussion 35
Conclusion 37
Recommendation 37

REFERENCE 38
DECLARATION

I declare that this project has not been done or produced by anyone in any Institution for an award of Diploma, Higher Diploma or Degree.

Signed .................................................................
Date .................................................................

This Project has been submitted for this examination with approval from supervision.

Supervisor .................................................................
Signed .................................................................
Date .................................................................
DEDICATION

I dedicate this project to my beloved family.
ACKNOWLEDGEMENT

Special thanks go to all the employees of Sher Agencies for making this Project a success. The management of Sher Agencies hospital for their support they gave in enabling me to utilize their laboratory section headed by Phanuel Diang’a.

I will not fail to appreciate the guidance given by Mr. Gerald Murage as the Project Supervisor to see that this Project has been completed. I will not leave behind the neat and presentable typing done by Mrs. Mary Muhonja to make this project to look the way it is.

Finally all thanks goes to Almighty God for making each plan in my course work successful.
ABSTRACT

Sher Agencies is located southern part of the Lake 18 km from Naivasha town.

The study was based in the hospital laboratory for a span of 7 months October 2004 April 2005. 500 employees were screened, 297 families and 253 males 8(3 males and 5 females) tested positive to syphilis (1.6%). RPR test, tested 23 positive (4.6%) which TPHA test 8(2.6%) which TPHA test 8(2.6%) were tested positive. Malaria was the main case of biological false positive reaction 13(2.5%) HIV/AIDS patients were prone to the syphilis, half of syphilis cases were HIV/AIDS positive.

Education and behaviour change were the key factor to be considered in order to prevent the spread of syphilis and other sexual transmitted diseases.
CHAPTER ONE

1.0 INTRODUCTION

History of the disease.

Syphilis is a venereal disease that was named after a mythical shepherded by Francastours in 1530. The origin of the disease is unknown but this illness appears to have been recognized under other names during ancient times. Europe experienced a significant epidemic of syphilis during the sixteenth century and it was at this time that physician recognized it as a venereal disease.

The causative agent was first observed in 1905 when Schaudinn and Hofman saw it exudated from syphilitic lesion since that time many microbiologists have attempted to grow Treponema pallidum in laboratory media all without success. This bacterium can grow in rabbits' testicles where serves as a major larva of treponoma for experiment and diagnostic techniques.

The prevalence of syphilis is not known with certainty. The number of cases reported annually in USA has shown wide fluctuation. It was highest in early 1940s but showed a steady decline until 1955. Coincidence with the application of pencillin therapy. The case rate increased again until it reached a more or less steady. There has been an increase in cases rate each year.

Congenital syphilis has declined markedly since 1950 and reached an all time low in 1978. The trend in congenital syphilis expectedly follows the trend of primary and secondary in women. The first is disproportionally high number of cases in homosexual and bisexual male. In the early 1970 a study in England, Scotland and Wale revealed that 42% of male are reported with primary and secondary syphilis were homosexual. A similar study in USA. In 1974 indicated that 3% of
male reported with syphilis were either homosexual or bisexual. In 1980 this figure had grown to 47%. The second feature of importance and possibly related to the first is predominance of syphilis in urban areas.

In 1981 large cases constituting 26% of the population accounted for more than 61% of reported cases.

Determination of etiology of syphilis took about 20 years from the time of Hoch establishment of germ theory of disease and the etiology of anthrax in 1876. This was not due to lack of effort.

Lassar in 1905 stated that 25 causes of syphilis have been established in the past 25 years. That same year two protozoologist Schaudinn and Hoffman (1905) described the organism that has come to regard as true causes of syphilis ever though it cannot be extracted in its virulent form.

Because of the difficulty of seeing the organism with ordinary light microscopy, it was designated the pale spirochaite and given the name spirochalta pallide or sp – pallidum. Later it was placed in genus Treponema with retentor of species designated I. Pallidum (patholay pathogenic microbiology by Vernon T.sebochid).

Because of the difficulty of seeing the organism with ordinary light microscopy, it was disgnatory the pale spirocheate and given the name spirochaeta pallide or sp – pallidum. Later it was placed in genus treponema with retention of species designation T.pallidrum (pathogenic microbiology by Vernon T. Bchuhurat)

1.1 Aim

To prove that syphilis endemic is facing out with the improved technology, access to medical facilities and change in Morales. To show that syphilis can be
life-threatening in individuals who suffer from HIV/AIDS show that there is need
to use confirmatory tests such as TPHA to avoid biological false positive mainly
(malaria and other chronic sickness).

1.2 Objectives

1. Compare the occurrence of syphilis with the kind of occupation of the
employees.
2. Determine the age group which is mostly affected.
3. To confirm that antenatal clinics for expectant mothers have greatly
reduced congenital syphilis.
4. To study the most common type of syphilis i.e. primary, secondary or
tertiary.
5. If there will be false positive RPR test what is the most cause?
6. The impact of health services toward syphilis prevention and control.

1.3 Statement of Problem

Due to the fact that when syphilis is untreated can cause diverse conditions. It is
important that screening is done and positive individuals be treated. The
population should be educated on how to prevent themselves.

The *treponema pallidum* may move to the central nervous system and finally to
brain leading to mental disability and finally death. Therefore diagnosis and
routine screening is important to avoid loss of man power and profitability of the
farm.
1.4 Study Design

The study was performed at Sher Agencies hospital which is located southern part of the late. It is 18 km from Naivasha town. The study was conducted in the hospital premises with total co-operation from the hospital staffs.

Records for the HIV/AIDS patents were sorted out and the individuals traced in the firm camp. This was the first group of people sorted out.

Expectant mothers attending antenatal clinics were diagnosed for syphilis and result recorded. The three last months the tests were voluntary for the people who visited the lab section. The examined individuals were between the age of 17 – 40 years. The study took seven months. The sexes of the individuals were not put into consideration.

1.5 Limitation of the Study

The study will be limited to presence of Treponema pallidum, the biological factors that cause false positive results, the relationship between HIV/AIDS and syphilis.

Blood samples will be the only sample used and diagnosis of Treponema pallidum will be restricted to employees of Sher Agencies only.
CHAPTER TWO

2.0 LITERATURE REVIEW

*Treponema pallidum*

Spirochaete or slender, undulating, cook screw like, relatively flexible filamentous organism, measuring 2.50 cm in length ubiquitous occurring in nature in soil water decaying organic matter in and upon the body of plants, animals and man. The general which are of material importance.

- Treponema
- Borrelia
- Leptospira

*Treponema/trepo* meaning to turn and *nema* thread are both pathogenic and non pathogenic. The *Treponema pallidum* typical organism are slender spiral measuring about 0.2 um in width (in wet preparation) and 5-15 um in length. The spiral lari are regularly spaced at distance of 1 mm from one another.

The morphology and motility of the organism can be but demonstrated under the dark ground microscopy and features are but observed in exudates taken freshly from the patient. The spirochaetes multiple by transverse binary fusion. The division time is 30 hours.

Drying kills the spirochaetes rapidly as does the elevator of temperature to 42° for about 60 minutes when stored at refrigerator temperature the micro-organism is killed in 1-3 days.
Stored frozen at $70^\circ$ in 10% glycerol or in liquid nitrogen the organism remain viable for 10 – 15 years. They are very susceptible to heat. According to (Boak Carpenter and Warren 1932), saline suspension of infected rabbit testicles are sterilized by exposure to $39^\circ$C for 5 hours, $40^\circ$C for 3 hours, $41^\circ$C for 2 hours and $41.5^\circ$ for 1 hour (M.T. Parker)

2.1 PATHOGENESIS

*T. pallidum* is strict parasite and its life outside the animal body is short. Most cases of syphilis are contracted during sexual intercourse. The treponemes are present in superficial genital lesions and pass from one partner to the other through intact mucous membrane or through minor abrasions. The disease may also be transmitted congenitally by close contact with mucous membrane lesions in kissing and though blood transfusion.

Medical personnel are occasionally infected by an accidental finger prick i.e. an infected needle in venereal syphilis the treponemas penetrate mucosal surface or a broaded skin and multiple at the site of entry and after an incubation period of about a month (range 10-90 days). The clinical manifestations fall into four stages.

i) Primary

ii) Secondary

iii) Latent

iv) Tertiary

i) **Primary Syphilis**

Following contact, viable cells of *T. pallidum* quickly reach the lymph nodes and the bloodstream from here they are disseminated to the remaining part
of the body, primary lesion appears. This usually at the site of initial contact 10-30 days after infection. This lesion is a hard chancre or sore that contain motile spirochetes. The disease at this stage can be diagnosed both serological and microscopic techniques. Patients are contagious during primary stage of syphilis and should be treated. Human defense mechanism do not necessarily cure a patient of syphilis so untreated patients can progress to a more severe form of the disease.

ii) Secondary Syphilis

The chancre usually heals spontaneously and is gone completely within weeks. During this time the *treponomes* infect other parts of the body including the eyes, joints, bones, mouth and the central nervous system. *Treponemes* can also be present in the blood. Between 2 and 12 weeks after the appearance of the chancre a mild generalized body rash develops. This may be accompanied by cutaneous lesion and/or lesions in the mucous membrane of the mouth and the genitalia. Patients with secondary syphilis are also contagious. The symptoms of secondary disappear with or without treatment. Some patients recover but a portion of the untreated patient later develops the symptom of tertiary syphilis.

iii) Tertiary Syphilis

Approximately 1/3 of the patients with secondary syphilis are spontaneously cared; 1/3 had no symptoms and 1/3 displayed the symptoms of tertiary (late) syphilis

iv) Latent Syphilis

In some instances the primary lesion is not noticed or is misdiagnosed and in addition both primary and secondary stage may be over looked. In other
instances, latency develops after unusually severe primary and secondary symptoms that develop at a later time.

Latency may perhaps be regarded as the result of a sustained or temporary biological balance between host and parasite.

Clinical Manifestation

*Treponema pallidum* causes

a) Acquired syphilis

b) Non venereal syphilis

i) Acquired Syphilis

It is transmitted congenitally or by sexual intercourse.

ii) Sexually acquire Syphilis

Infectious Syphilis

These include patients with primary, secondary or early latent infections.

In primary syphilis or ulcer known as the chancre form usually on the genital area. *Treponemes* are present in the chancre fluid. There is enlargement of the nearby dependent lymph glands.

In the secondary syphilis which occurs 6-8 weeks after the primary infection a rash appears corresponding to the spread of the organism in the body by way of circulating blood. *Treponemes* can be found in the red muscular skin
lesions. Because the *treponemes* are also present in the blood, it is possible for persons with secondary syphilis to transmit *T. pallidum* in their blood.

iii) Non Infectious Syphilis

These include patients with late symptoms and latent infectious. Complications of late syphilis include liver damage, bone changes and destructive change in the central nervous system (neuro syphilis), the eye and ear and cardiovascular system.

**Congenital acquired Syphilis**

A mother with untreated infectious syphilis infects her unborn infant. The *treponemes* pass through the placenta in the blood.

When the fetus is infected early in pregnancy spontaneous abortion usually occurs. When infection occurs late in pregnancy the baby usually reaches term but shows signs of infection within the first few weeks or months of life. An infected baby has a rash and may be jaundiced.

It is also known as endemic syphilis **Bejei Njovera** and **Dischaelwa**. The disease occurs mainly in hot dry countries of sub Sahara Africa, near East Africa, Western Asia and parts of Australia. Ulcers form on the skin and muscles membranes. The disease is spread from person to person by contact. All the late complication of syphilis are found in this versions of syphilis.

As stated *Treponema pallidum* in an infected mother can penetrate through the placenta to the fetus in uterus giving rise to congenitally acquired syphilis. About 40% of untreated cases of congenital syphilis lead to death and
abortion of the fetus, of the remaining congenital cases in which a live child is born, the child may show secondary or tertiary symptoms at birth. These usually results in death shortly after birth. Others may show no symptoms at birth but develop secondary or tertiary symptoms any time from the first to the tenth year after birth or occasionally later.

Hutchinson reported a trial of symptoms, one or more of which commonly occur in congenital syphilis who service the infection.

1. **Hutchson Teeth**: In which the central permanent incisors (usually upper) are peg-shaped and passes as crescent notch in the cutting edge.

2. **Interstitial Keratitis**: Resulting in inflammation and cloudiness' of the Cornea leading to blindness

3. Deafness

2.2 **Syphilis in HIV Infected Patient**

Genital sore is an important co-factor for getting HIV infection. In HIV infected patient with syphilis, there are unusual features that need special attention. In some of the patients, RPR, FTA or TPHA may be negative, while some have unusual high RPR titre. Treatment failure to pencillin and early progression to microsyphilis has been reported in the group of patients.

CDC recommendations on treatment of syphilis in HIV infected patients.

a **Early Syphilis**

The same treatment regimen could be used, but careful and frequent follow-up are required after treatment. Quantitative RPR at 1,2 and 3 months and at 3
monthly intervals thereafter should be measured until satisfactory serological response treatment is attained. Patients should be treated and CSF evaluated if:

a. The titre does not declare appropriately (two dilution decrease by three months for primary syphilis or by six months for secondary syphilis.

b. Sustained two dilution or greater increase titre.

b. Latent Syphilis

One year or of unknown duration CSF examination should precede treatment. If lumbar puncture is not possible, the patient should be treated at neurosyphilis.

c. Neurosyphilis

Benzathine penicillin should not be used. Patient should be treated for at least 10 days with either aqueous crystalline penicillin G 2-4 mega until IVI Q4H (12-24 mega unit per day or procaine penicillin 2.4 megaunit IMS daily, plus probencid 500 mg orally gid daily.

2.3 Virulence

The virulence factor of *T. pallidum* is incompletely understood and will probably remain so until the treponemes have been cultivated and can be studied without the infertility presence of host components.

Two virulence factors have been purposed. The first is muco-polybaccharide surface component or capsule of *T. pallidum* that is believed to protect the cell from oxygen taxiary and from the deleterious effects of antibody against duter membrane components. The second is a muco-polysaccaricle that is said to be
the bacteria receptor mediating adherence to host cells by interacting hyaluronic hyduronie acid on the host cell membrane. It also provides the treponeme with N-acetyl – D-glucosamine which is utilized in the synthesis of the capsular substance. The specifivity of the adherence ligand is believed to account for the tissue and organ specifically observed in treponemes infection. Its enzymatic activity would lead to degradation of hyaluronic acid group substance providing structural support for blood vessel and result in pathological of syphilitic lesion.

2.4 Treatment

The chemotherapy of syphilis, like that of TB has been of great interest for many years. The use of arsenical in syphilis was one of the earliest examples of infectious disease chemotherapy.

Penicillin to which the spirochaeres are exquisitely sensitive was used in human infection in 1943 and remains today as treatment of choice. The efficiency of penicillin therapy is however, dependent upon the stage and nature of the disease and the dose/time relationship, primary syphilis in which relatively few treatments are present in most amenable to therapy. In later stage, the disease is more refractory and dose/time relationships are increasingly important.

Penicillin acts upon growing bacterial cells by interference with synthesis of peptidoglycan in cell wall. Thus penicillin therapy in syphilis requires adequate serum level of penicillin for prolonged periods because of long generation time (30 – 33 hours) of spirochaetes. These levels are most easily attached by use of repository or long acting penicillin. Such as procaine penicillin in ageous or oil suspension. In both primary and secondary syphils penicillin the success rate is somewhat diminished and requires increased doses of antibiotics so far as can be established however. _T.pallidum_ has developed resistance to penicillin during several decades of therapeutic use.
More than half of patients in secondary syphilis who are treated with penicillin experience fever chills, myalgias and other influenza like symptoms a few hours after receiving of antibiotic (Warren). This is he to lysis of spirochaetes leading toxicity. The toxic substance possibly lipopolysachandes of the alter sheath. The phenomenal is referred to as Jarich Herxheimer reaction. The reaction is rarely encountered however during treatment with antibiotic of late syphilis.

Jarich – Herxheimer reaction also occurs after treatments of other spirochaetal disease such as lyme disease leptospirosis and relapsing fever. Tumour neurosis factor is an important mediator of this reaction because passive immunization is also against TNF can present the symptoms.

Syphilis in patients allergic to penicillin should be treated with tetracycline or erythromycin: Pregnant women who are not allergic to penicillin should be treated with penicillin exactly the same way as if the patient was not pregnant to prevent the occurrence of congenital syphilis.

**Treatment Dosage**

**Primary, Secondary and Early Latent Syphilis**

1. Procaine penicillin (servigen)
   1.2 megasinit 1 m gd x 10 days
   Before holidays: Benzathine pencilne 1.2 to 2.4 preganat is to be given (i.e. 0.t megnanit/day according to length of holiday) to cover the holiday.
2. Benzathire penciline (penadur)
   2.4 megaunit 1 ml weekly x 3 weeks (half into each slide of buttok).
   For patient who is sensitive to penalin

3. Tetracycline 500 mg qid x 2 weeks.

4. Erythromycin 500 mg qid x 2 weeks

5. Doxycycline 100 mg qid x 2 weeks

**Late Latent Syphilis, Cardioracular Syphilis**

1. Procain pencilin
   1.2 meganite 1m qid x 10 days
   Before holidays: Benzathine pencilin 1.2 to 2.4 megaunit is to be given (i.e. 0.6 megaunit/day according to length of holiday) to cover the holiday.

2. Benzathire penciline (penadur)
   2.4 megaunit 1 ml weekly x 3 weeks (half into each side of buttok)
   For patient who is sensitive to penalin

3. Tetracycline 500 mg qid x 2 weeks
4. Doxycycline 100 mg qid x 2 weeks
5. Doxycycline 100 mg qid x 2 weeks

**Late Latent Syphilis, Cardiovascular Syphilis**

1. Procaine pencilin
   1.2 Megaunit 1 ml qid x 15 days + probeneudl
   0.5 gm orally qid x 15 days
If duration of syphilis is more than one year, try not to use benzathine penicillin because it cannot cross CSF and ocular fluid (except before holiday where the management is the same as above).

For patient who is sensitive to penicillin:

2. Tetracycline 500 mg qid x 4 weeks
3. Erythromycin 500 mg qid x 4 weeks
4. Doxycycline 100 mg bd x 4 weeks

Cardio Vascular Syphilis

Preced belone 20 mg gd x 24 hours preceding first infection and conn side for 2 more days.

Neorosyphilis Ocular Syphilis

1. Admitted into hospital for lumbar puncture soluble penicillin 0.5 megaunit 1MI/IVI PGH + probenecid 0.5 orally Q6H x 20 days + steroid cover.
2. If patient refused admission for treatment procaine penicillin 2.4 megaunit IMI gd x 20 days + probenecid 0.5 gm orally gid x 20 days + steroid cover.

Optic atrophy

Prednisolone 30 mg daily in divided doses x one week, then 20 mg daily for four weeks, then falls off.
Congenital Syphilis

1. Procaine pencillin 50,000 units kg/day 1 ml daily
2. Soluble pencillin 50,000 units kg/day 1 ml/IVI into divided doses x 10-15 days.
3. Benzathire pencillin 50,000 units /kg/dy 1 ml (single) in early congenital syphilis.

Pregnant Women with Syphilis

1. Pencillin or erythromycin, no tetracycline prohibited if used requires special precaution.
2. Reply letter to MCH from SYPSH head office.
3. After treatment, quantitative measure of RPR monthly till delivery.
4. Retreat the patient if the serological evidence of infection or relapse.
5. Follow up three weeks after delivery together with her baby.

Cord Blood Positive Baby

In situations where the mother and the baby could not be followed up a single dose of benzathine pencillin 50,000 units/kg 1 ml should be given to the infant.

Precaution

1. Risk of anaphylaxis
   i) Ask for history of pencillin allergy
   ii) Resuscitation facilities should be available
   iii) Pencillin test
500 ml H.S + one megaunit soluble pencillin inject 0.1 ml hypodermal 200 unit read 15 minutes later wheat 75 mm positive.

Prognosis

The prognosis of treated syphilis depends on the following factors:

1. **Stage of the disease.**

   The cure rate is over 95% if the disease has been adequately treated in primary, secondary and latent stage.

2. **Degree of tissue damage**

   The prognosis in tertiary stage is variable. It depends on the degree of damage in cardiovascular and neurological systems.

**2.5 Control and Prevention**

At present there is not effective vaccine against syphilis. An infected individual may serve as a source of infecter 3-5 years during syphilis as transmission of syphilis is by direct contact. Prevention is by avoiding of sexual promiscuity. Prostitutes both male and female and other promiscuous individuals because they constitute the major source of infection.

Disinfections of external genital or use of vaginal douches after exposure may or may not prevent infection.

Treponemocidal drugs can be used both for prophylaxis and therapeutic.
The use of mechanical barriers such as condoms in which prevention of direct contact between infected mucous membrane is achieved.

Public health procedures for the prevention of syphilis include:

1. Providing laboratory facilities for finding cases including the serodiagnostic "dragnet". In high incidence environment.
2. Clinical facilities for treatment and disinfecting cases.
3. Epidemiological services to trace known contacts and thereby enable early diagnosis and treatment of these potential new sources of infection (verron).
4. Diagnosis of blood for syphilis before transfusion.
5. Expectant mothers to attend antenatal clinic where syphilis tests will be conducted to avoid congenital syphilis or treated in early stages.

➤ Health Education

This is given to patient in the anti-venereal disease office in social hygiene clinic.

➤ Contact Training

Examination and treatment of sexual contact are important steps in the control of spread of sexually transmitted diseases. In early syphilis, contact of preceding three to six months should be traced. Those who have contract within three months should be treated even without symptoms and sign. In late syphilis, spouse or regular sex partner should be screened and in mother, her children as well.
Serological screening for pregnant mothers and early treatment of both partners.

2.6 Immunity to Syphilis

At the present time syphilis is not so severe a disease in man as it was in the early sixteenth century. Whether this is an expression of adaptive response as the part of man in the development of a low degree of natural immunity or a decrease in virulence of spirochaete is not known possibly both may have occurred.

In the immune response to syphilitic infection there is a marked apparent insusceptibility to re-infection. This is illustrated by the fact that a second chancre may be produced by re-infection prior to the appearance of the first chancre but after the first chancre has appeared further re-infection does not produce another initial lesion. It is commonly stated that man once infected, is refractory to infection and that re-infection occurs only very rarely. Immunity therefore superficially resembles that of tuberculosis and is similarly a manifestation of cell-mediated immunity. It is apparent, however that developed immunity is not completely positive in that elimination of the original infiltrating spirochaetes does not always ensure. This may be due to suppressive of cell mediated immunity that is expressed in early stage of Treponema pallidum infection. Thus delayed hypersensitivity manifestation as delayed type skin reaction appears only in later stages of disease.

Infection with Treponeme pallidum also induces a hormonal response with the appearance in serum of anti-lipodial regains as well as antibodies against specific Treponeme pallidum antigen. The protective role of these antibodies is uncertain. Spirochaetes are immobilized and killed by specific abs in the presence of complement, but passive immunization of rabbit with immune serum
does not confer immunity to challenge, although the development of lesion is suppressed or delayed. It has been suggested that muco-polysaccharide capsule which is well developed in vitro interferes with the action of bactericidal antibodies (Warren).

The lack of cultivable strain of *Treponema pallidum* and the incomplete nature of immune response to syphilis have dissuaded health professionals from developing vaccine against syphilis. Nevertheless immune reactions are important for diagnosis of syphilis (Paul Ketcham)

2.7 Epidemiology

The first recognizable epidemic of syphilis occurred in Europe at the end of 15th century since then; there is another epidemic of early syphilis in may countries during and immediately after second world war. Again, there is a rise between 1965 and 1975. It is more prevalent in urban than rural areas. The high risks groups include prostitutes and male homosexuals. Male are more commonly affected than female.

From 1989 – 1998, STD incidence increased significantly in men and women and demonstrated non-linear growth trend with the exception of gonorrhea between 1990 and 1998 the incidence increased more in female (4.20 times) than in males 3.79 times) syphilis incidence increased approximately 20 times during this period at an average annual rate of 52.7%

Between 4-15% of pregnant women are believed to be infected with syphilis in Sub Saharan Africa. Active infection with syphilis in pregnant women results in foetal or infant death or disability for 50-80% of affected pregnancies and is a major course of adult mobility as well.
Syphilis seroprevalence estimated at 38% approximately 640,000 pregnant women with syphilis are undetected annually including 1,030,00 women who attend antenatal care.
CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials, Regent and Equipment

i) Refrigerator
ii) Mechanical rotator
iii) RPR cards test kits
iv) TPHA test kits
v) Plastic microtiter with 'u' shaped wall
vi) Automatic pipette tips
vii) Saline (0.9)%
viii) Test tubes
ix) Cotton wool
x) Gloves
xi) Labels
xii) Syringes 5 ml
xiii) Needle gauge 23
xiv) Methylated spirit
xv) Tonequite
xvi) Pipettes
xvii) Recording book
xviii) Dispenstir
xix) Marker
xx) Disinfectant
3.2 RPR Test

The antigen used in this test is an alcoholic extract of beef heart tissue (cardiolipin) in which lethal and cholesterol are added. The antigen particle is either carbon containing or dyed to enable the relation to be real microscopically on a card.

Principle

RPR card antigen suspension is carbon particle cardiolipin antigen which detects "reagin" an antibody-like substance present in serum or plasma from syphilitic persons and occasionally in serum or plasma of persons with other acute or chronic conditions. The regain binds to the test antigen which consists of cardiolipin – lesion – coated cholesterol particle causing microscopic flocculation.

Procedure

i) Using a new capillary attached rubber bulb to capillary and remove 0.05 ml of specimen from the plasma.

ii) Place measure specimen onto circle of diagnostic test card by compressing rubber bulb, while holding one finger over the hole in the bulb.

iii) Using a new stirrer (broad end) for each specimen spread to fill entire circle. Discard stirrer. Repeat procedure for number of specimen.

iv) Gentle shake antigen dispensing both before use. Holding in vertical position, disperse several drops in dispensing cap to make sure the needle passage is clear.

v) Place one “free-falling” drop onto each test area.

vi) Rotate for 8 minutes under humidifying cover or mechanical rotator at 100 r.p.m.
Report as reactive - showing characteristic clumping ranging from slight but definite.

Non-reactive: - showing no clumping.

18 mm Circle Quantitative Test

For each of the positive quantitative.

i) Place 0.05 ml of 0.992 saline onto circle, number 2-5 a capillary (red line) or serological pie is used 1 ml.

ii) Use a capillary (red line graduated at 0.05 ml) with rubber bulb attached; place 0.05 ml of specimen on circle 1.

iii) Refill capillary to red line with test specimen and hold in a vertical position, prepare serial 2 folds dilutions by drawing saline and test specimen mixture up and down capillary 5-6 times. Avoid formation of bubbles. Transfer 0.05 ml from circle 2 to 3 to 4 to 5. Mixing after each transfer. Discard 0.05 ml after mixing content in circle 5.

iv) Using a new stirrer (broad end) for each specimen test at highest dilution of serum (circle 5) and spread serum filling the entire surface of circle produced to circles 4, 3, 2 and 1.

v) Gently shake antigen dispensing bottle before use. Hold in vertical position; dispense several drops in dispensing bottle cap to make sure needle passage is clear.

vi) Place air “free-falling” drop cup pad test.

vii) Rotate for 8 minutes under humidifying cover or mechanical rotator 100 r.p.m.
Report as

R - Relative
H - Non-Relative
RM - Reactive minimal to moderate

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<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM</td>
<td>H</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td>Report</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td>N</td>
<td></td>
<td>Reactive 1:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>delusion</td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>N</td>
<td></td>
<td>Reactive 1:4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>delusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reactive 1:8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>delusion</td>
</tr>
</tbody>
</table>

**Limitation of Procedure**

The diagnosis of syphilis should not be made on a single reactive result without the support of positive history or clinical evidence. Therefore, or with any serological testing procedure. Reactive cards tests specimen should be subjected to farther serologic study.

Serum which is reactive quantitative testing should be quantities to establish a baseline from which changes can be determined, particularly for evaluating treatment.

False negatives result can occur because of failure to recognize pressure reactions. RPR card test cannot be used for testing spiral fluid.

With cardiolipin type antigen, biological false positive reactions have been reported in degrees such as infectious mononucleosis, leprosy, malaria, lupus rythemature, vaccinia virus pneumonia.
Do not test specimen that are grossly haemolysed contaminated or extremely turbid. "Specimen unsatisfactory testing".

**Advantage**

1. No heating of patient's serum is required for the RPR card.
2. Plasma as well as serum can be used.
3. It is possible to obtain sufficient plasma or serum from capillary blood of this is easier to obtain.
4. RPR can antigen is readily for immediate use.

3.3. *Treponema pallidum* Haemagglutination Assay (TPHA)

**Principle**

The syphilis TPHA test is an indirect haemagglutination test for the detection and titration of specific antibodies against *Treponema pallidum*. Arian erythrocytes are sensitized with antigen of the Nichol's strain of *Treponema pallidum*. In these the presence of syphilis antibodies, there tests cells aggregate to form characteristic patterns on the surface of the microplate walls. Antibodies directed against other non pathogenic treponemes are absorbed by an extract of reactive treponemes present in the cell supervision, thus greatly reducing false positive. Other non specific reactions can be detected and eliminated with the non sensitized control cells.

**Reagent**

i) Test cells (green cap)
   Stabilized suspension of ovary cells sensitized with Nichol's *Treponema pallidum* antigen.

ii) Control cells (white cap).
iii) Diluent Buffer (white cap) phosphate – buffer saline ph 7.2.

iv) Positive control serum pre-diluted 1/20. The titre of this control is 1:2560 and results may vary plus or minus.

v) Negative control (blue cap)
Negative control serum pre-diluted 1/20.

**Procedure TPHA**

i) Bring the test reagents and sample to room temperature.

ii) Dispense into adjacent wall of a micro-titration plate.

<table>
<thead>
<tr>
<th></th>
<th>Control Wall</th>
<th>Test Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1/20 or control</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>Control Cells</td>
<td>75 µl</td>
<td>--</td>
</tr>
<tr>
<td>Test Cells</td>
<td>--</td>
<td>75 µl</td>
</tr>
</tbody>
</table>

iii) Gentle tap all the four balls of the plate to ensure the contents of each wall are thoroughly mixed.

iv) Cover the plate on a flat with surface away from vibration and direct sunlight and leave for 45 – 60 minutes before reading the results.
Interpretation of Results

<table>
<thead>
<tr>
<th>Degree of Haemagglutination</th>
<th>Reading</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniform mat of cells covering entire base of well sometimes with folded</td>
<td>4+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Uniform mat of cells partially covering base of well.</td>
<td>3+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Smaller mat surrounded by a ring of cells</td>
<td>2+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Smaller mats surrounded by a smaller more distinct ring of cells</td>
<td>1+</td>
<td>Reactive</td>
</tr>
<tr>
<td>Well defined dense ring with a hole in the center</td>
<td>+/-</td>
<td>Indeterminate test</td>
</tr>
<tr>
<td>Definite button of non-agglutinated cells. Sometimes with a small hole in the centre</td>
<td>--</td>
<td>Non reactive</td>
</tr>
</tbody>
</table>

The negative control must show a non agglutination pattern with both test and control cell. The positive control must show agglutinins with control cells but not with control.

Sera or plasma sample showing agglutination with control cells indicates the presence of no-specific agglutinins and should be retested after absorption. Positive samples should be retested by the quantitative test i.e. titrated.

3.4 Specimen Collection

i) Blood was collected on the cubical vein of the arm.

ii) The blood was dispensed in containers without anti-coagulant.

iii) The blood was left on the bench for sometime.

iv) The components separated and container components are separated from the serum

v) Serum is placed in clean sterile biojou bottles and serum screened properly.

vi) The samples are labeled at placed or the bench for diagnostic work.
3.5 Interpretation of Cardiolipin and Treponemal Serological Test

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cardiolipin RPR</th>
<th>Treponemal Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early syphilis primary</td>
<td>Relative or non relative</td>
<td>Non-relative or relative</td>
</tr>
<tr>
<td>Secondary</td>
<td>Relative</td>
<td>Relative</td>
</tr>
<tr>
<td>Late syphilis</td>
<td>Relative or</td>
<td>Reactive</td>
</tr>
<tr>
<td>Including</td>
<td>Non reactive</td>
<td></td>
</tr>
<tr>
<td>neuro-syphilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactive or treated</td>
<td>Reactive (non)</td>
<td>Reactive only after many years</td>
</tr>
<tr>
<td></td>
<td>Reactive with reduced</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ab titre</td>
<td></td>
</tr>
<tr>
<td>Congenital syphilis</td>
<td>Reactive or non reactive</td>
<td>Reactive or non reactive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.6 False Positive Reactions

May be as a result of technical error or may be biological in nature. False reactions occur less common with specific test using treponema or treponemal.

Biological False Positive (BEPS) in RPR Testing

The main disadvantage of RPR test is the use non specific antigen which may react with sera of the patient who don't have syphilis. The occurrence of false positive reactions due to these reactions is known as biological false positives. They are classified into acute and chronic.
Acute/Transfer BFP Reaction

They may develop shortly after an acute febrile infections disease and will disappear within a few weeks or months after the illness has subsided.

Chronic BFP Reactions

They persist longer than 6 months. These may occur in a wide variety of infections and non-infections conditions associated with tissue damage.

These include auto-immune diseases particularly lupus erythematosus, leprosy particularly lepromatous leprosy, malaria, relasing fever, infectious mononucleosis, hepatitis conorery allergy disease, repeated blood loss, pregnancy, vaccination, haemolytic anemia and tropical eosinophilia.

Patients should be followed up and investigated for the underlying cause if there is persistent BFP RPR of significant tine (1:8 or greater).
CHAPTER FOUR

Data analysis and presentation.

Table 1: Samples Screened during Study

<table>
<thead>
<tr>
<th>Month</th>
<th>Sample Size</th>
<th>RPR Test</th>
<th>TPHA Test</th>
<th>True Positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>100</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>November</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>January</td>
<td>100</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>February</td>
<td>100</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>March</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>50</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td><strong>23</strong></td>
<td><strong>8</strong></td>
<td><strong>8</strong></td>
</tr>
</tbody>
</table>

Percentages

\[
\text{RPR} \quad \frac{23 \times 100}{500} = 4.6\%
\]

\[
\text{TPHA} \quad \frac{8 \times 100}{500} = 1.6\%
\]

\[
\text{BFP} \quad \frac{13 \times 100}{500} = 2.6\%
\]

\[
\text{True positives} \quad \frac{8 \times 100}{500} = 1.6\%
\]
Comparison of RPR Test and TPHA Test Results

Table 2: Females and Males screened in Relation to the Sample Size

<table>
<thead>
<tr>
<th>Month</th>
<th>Females</th>
<th>Males</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>52</td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td>November</td>
<td>28</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>December</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>January</td>
<td>53</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>February</td>
<td>38</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>March</td>
<td>29</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>April</td>
<td>22</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>247</strong></td>
<td><strong>253</strong></td>
<td><strong>500</strong></td>
</tr>
</tbody>
</table>
Table 3: Infected Males and Females

<table>
<thead>
<tr>
<th>Month</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>December</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>January</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>February</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>March</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Bar Graph of Infected Males and Females
Distribution of Biological False Positive in Males and Females

<table>
<thead>
<tr>
<th></th>
<th>October</th>
<th>January</th>
<th>February</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

Bar Graph showing the Distribution of BFP in Male and Female
CHAPTER FIVE

DISCUSSION

It has been recently reported that the AIDS pandemic has invaded most of the firm’s workers in Naivasha. In Sher Agencies Hospital, cases of AIDS and HIV have been diagnosed at an alarming rate. It is well understood that immune system of HIV/AIDS patients is suppressed and thus they are prone to many infections including opportunistic infections.

Information from camp managers, administration and hospital management said that most of the women their husbands have died of AIDS. They migrate from rural areas to the firm to seek employment. Most of these women convince young men who are also seeking for employment and give them accommodation. In the process they make their young men their sexual partners. This unhealthy behaviour encourages the spread of sexually transmitted diseases including syphilis and HIV/AIDS.

On the other hand, most of the men have left their wives in rural areas in search of employment. The male co-habit up with these single women to satisfy their needs which they are in the residential houses.

Naivasha has the best sceneries for tourists. The sceneries have not only attracted tourists but also prostitutes who believe where tourists are there is money. These prostitutes move around in search of any interested party. Men in the firms with weak morals move around with these ladies and finally spread infectious disease.
The population of Sher workers mainly consists of people from Western and Nyanza Provinces as research which has been carried out earlier show that malaria is rampant in these areas. Thus when their employees travel to their home areas they are bitten by mosquitoes and contact malaria. This is the reason why the months of January and February register high biological false positive i.e. ten and six respectively.

It was found out that syphilis is also associated with HIV/AIDS out of the 8 positive isolated 4 were HIV positive. The syphilis was mostly isolated from farm employees than those who do clerical jobs. It was concluded that the officers who do clerical jobs have a busy schedule and they were well informed about the spread of sexually transmitted diseases.

Congenital syphilis was not reported due to the fact that all mothers in the firm were exposed to free antenatal clinic. Primary and secondary syphilis were the most type of syphilis reported. A total of 500 samples were screened and 8(1.6%) of the samples were positive. It was necessary to use a confirmatory test because some patients may be placed under syphilis therapy while it is not necessary.

The difference between TPHA and RPR test was 3% which showed that there is need for proper examination before being send to the lab and use of confirmatory test.

Females were more affected than males, 3 males and 5 females (37.5% and 62.5% respectively). Free health services in the firm had improved the health of employees of the firm. This is because they had access to free medical treatment. Chronic syphilis was not reported due to yearly screening in the hospital laboratories.
CONCLUSION

Antenatal clinic for pregnant mothers played an importance role to present congenital syphilis. Biological false positive ratio by the use of RPR screening test implied that there is need for such a large firm with many employees to be using TPHA as a confirmatory test so that to avoid giving the wrong chemotherapy.

RECOMMENDATIONS

i) Screening for syphilis should be done twice a year.
ii) All expectant mothers, especially those from rural areas should be informed about the risk that they and their unborn babies may face due to failure of attending antenatal clinic.
iii) Married couples should be faithfully.
iv) HIV/AIDS patients should be diagnosed for syphilis because this disease may be life threatening due to their weakening immune system.
v) People should be educated regularly by holding seminars.
vi) The rate of protective barriers should be encouraged (condom).
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