PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS, SYPHILIS, HEPATITIS B, AND RISK FACTORS AMONG TRUCK DRIVERS IN TEMA, GHANA

BOAHEMAA- ATTA PRISCILLA

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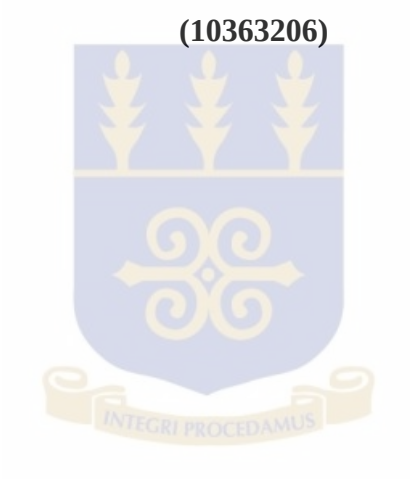

THESIS SUBMITTED TO THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF GHANA MEDICAL SCHOOL (UGMS), IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A MASTER OF PHILOSOPHY (M.PHIL) DEGREE IN IMMUNOLOGY

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DECLARATION

I, PRISCILLA BOAHEMAA-ATTA do hereby declare that with the exception of references to other people's work, which have been duly acknowledged, this project is the outcome of my own research conducted at the Department of Pathology, University of Ghana Medical School, College of Health Sciences under the supervision of Dr. Michael Ofori and Dr. Henry Asare-Anane. Neither all nor part of this project has been presented for another degree elsewhere.

…… Date: ……2015

PRISCILLA BOAHEMAA-ATTA

(Student)

…….. Date: ……..2015

DR. MICHAEL OFORI

(Supervisor)

…….. Date: ……..2015

DR. HENRY ASARE-ANANE

(Supervisor)

DEDICATION

I dedicate this work to the memory of the late Dr. Dr. Daniel Osei, who believed in me and gave me this opportunity to be where I am today. Also to the late Mr. Edward Ashigbi Adjei for his selfless service to ensuring that this work became a reality.

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ABSTRACT

Human immunodeficiency virus (HIV), Hepatitis B virus (HBV) and syphilis infections have now spread to all parts of the world, and the rate of infection is found to be high in the cities with high number of mobile populations. One of such mobile populations is long distance truck drivers. Although studies from Asia, Europe, USA, and elsewhere have documented high prevalence of HBV, HIV and syphilis infections among truck drivers (long and short distance), such data are sparse from Africa. To my knowledge there is no such data from Ghana. This study investigates the prevalence of HIV, HBV, syphilis and the risk factors among truck drivers using the seaport at Tema. The study is a cross sectional study carried out between the months of March, 2013 and June, 2013 among truck drivers at Tema seaport. All the 106 consenting participants completed a structured questionnaire assessing socio-demographic characteristics and risk factor profile for the infections under investigation. Blood samples from consenting participants were tested at the Public Health Reference Laboratory, Korle-Bu Teaching Hospital, Accra, Ghana for the presence of antibodies to HIV-1 and 2, using Genscreen™ ULTRA HIV Ag-Ab (Bio-Rad Laboratories, Hercules, CA). Antibodies to syphilis was tested using [*T. pallidum haema*gglutination assay (TPHA) test; Serodia Fujirebio]. Hepatitis B surface antigen (HBsAg) was tested using Roche COBAS e411 analyzer with elecsys HBsAg II quant test (Roche Diagnostics, Germany). A total of 1250 truck drivers (Ghanaian and non-Ghanaian residents) at the Tema port were eligible for the study, however 106 (8.4%) of the eligible took part in the study. None of the foreign citizens (non-Ghanaian residents) took part in the study. The mean age of the participants was 40.56 ± 11.56 years (range: 18–74 years). For the 106 participants tested, HIV sero-

prevalence was 0.98% (1 out of 106), 14.2% (15 out of 106) tested seropositive for HBsAg and reactive syphilis serology was noted in 3.8% (4 out of 106) of the participants. On multivariant analysis, the independent determinants for HBV infection were being a long distance driver [Odds Ratio (OR): 6.88; 95% CI 0.86-52.89], having multiple sexual partners (OR: 6.36; 95% CI 1.35 - 29.79) and previous visit to commercial sex worker (OR: 6.85; 95% CI: 0.89-52.89). Interestingly, knowledge of HIV, HBV and syphilis infections and preventive measures were high among the truck drivers.The data indicates a low HIV prevalence among truck drivers in Ghana, however the high prevalence of HBV and syphilis coupled with their risky behaviour suggest an increase potential risk of HIV in the truck driving population in Ghana.

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LIST OF ABBREVIATIONS

KBTH ..... Korle-Bu Teaching Hospital

AIDS .....Acquired Immunodeficiency Syndrome

HIV ..... Human Immunodeficiency Virus

RNA ..... Ribonucleic Acid

DNA ..... Deoxyribonucleic Acid

CD4 ..... Cluster of Differentiation 4

MOH ..... Ministry of Health

GHS ..... Ghana Health Service

µ ..... Microlitres

g ..... Acceleration due to Gravity

ml ..... Millilitre

STI ..... Sexually Transmitted Infection

STD ..... Sexually Transmitted Disease

OR ..... Odds Ratio

CI ..... Confidence Interval

HBV ..... Hepatitis B Virus

TPHA ..... *Treponema pallidum* Haemagglution Assay

OD ..... Optical Density

CSW ..... Commercial Sex Worker

HBsAg ..... Hepatitis B surface Antigen

PCR ..... Polymerase Chain Reaction

NGO ..... Non Governmental Organization

EDTA ..... Ethylenediamine tetra-acetic acid

CCR5 ..... Chemokine Co-receptor 5

CXCR4 ..... C-X- C Chemokine receptor 4

MHC ..... Major Histocompatibility Complex

ELISA ..... Enzyme-Linked Immunosorbent Assay

HBcAg ..... Hepatitis B core antigen

HBeAg ..... Hepatitis B e antigen

EIA ..... Enzyme Immunoassay

VDRL ..... Venereal Disease of Reference Laboratory

RPR ..... Rapid plasma reagin

UNAIDS ..... Joint United Nations Program on HIV/AIDS

MARP ..... Most at Risk People

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human immunodeficiency virus (HIV) and sexually transmitted infections (STIs) have now spread to all parts of the world and the rates of infection are found to be particularly high in those cities with a greater number of mobile populations (Richens, 2006). The movement of individuals may be linked to long-term (permanent) stay or short-term (seasonal) migration (Giami and Bail, 2011; Nahmias and Nahmias, 2011) or occupations such as trading and truck driving (Pickering *et al.*, 1996; Bwayo *et al.*, 1994). The role of truck drivers in the spread of HIV, Hepatitis B Virus (HBV) and STIs have been studied world-wide (Stratford *et al.* 2000; Pandey *et al.* 2008). Studies from Africa and Asia have demonstrated a link between long-distance truck drivers and the prevalence of HIV/STIs (Manjunath *et al.*, 2002; Mustikawati *et al.*, 2009; Mbugua*et al.*, 1995). Within these regions, there is growing evidence that the high rates of HIV/STIs among truck drivers largely occur by sexual contact with HIV/STIs-infected women, often commercial sex workers (CSW), whiles away from home. The infected men then transmit the virus to their wives and other sex partners en route and in their place of origin (Pison *et al.*, 1993; Gangakhedkar *et al.*, 1997; Wolffers *et al.*, 2002; Brockerhoff et al., 1999). Consistent with this pattern, there is growing evidence that truck drivers and other mobile individuals may act as bridge populations who spread the infections from high-risk to low-risk populations, and from urban to rural areas (Pison *et al.*, 1993; Morris *et al.*, 1996; Entz *et al.*, 2000; Chandrasekaran *et al.*, 2005; Decosas *et al.*, 1995; Lurie *et al.*,2003; Magis-Rodriguez *et al.*, 2009).

1.2 Problem statement

Ghana, one of the growing economies in sub-Saharan Africa, has one of the largest road networks and sea ports in the sub-Saharan Africa and most of the neighboring land-locked countries such as Niger, Burkina Faso and Mali use the road networks and the sea ports at Tema and Takoradi to convey merchandise, equipment and consumer goods to their respective countries. Apart from truck drivers and mates from the neighboring countries, local companies operating haulage business engage Ghanaians in the truck driving occupation. Undocumented reports estimate that between 5,000 and 100,000 long-distance truck drivers use the road networks in Ghana. These truck drivers and mates are away from home for long periods of time and in the unhealthy environment at the ports and highways, they frequently have sex with commercial sex workers (CSW). During their journeys, long-distance drivers stop at rest houses, guest houses, and roadside hotels that usually provide food, rest, sex workers, alcohol and drugs. They pick up the women at their rest point, have sexual intercourse with them and leave them there. These women are mostly locals so other drivers also come and have sex with them as well as the local men. Long–distance drivers are therefore crucial in spreading HIV, HBV and STIs throughout the country.

Sentinel studies conducted by the Ghana AIDS Commission has included most sub-populations thought to be at high-risk for HIV, HBV and STIs, but has not included long-distance truck drivers (HIV Sentinel Surveillance Report 2012 of the National AIDS/STI Control Programme of the Ghana Health Service). Despite this gap, truck drivers are considered to be at increased risk for HIV and other STIs, of whom some are targeted by state and private organizational programmes (Pickering *et al.,* 1996; Bwayo *et al.,* 1994; Stratford*et al.,* 2000; Pandey *et al.,* 2008; Joint United Nations Programme on

HIV/AIDS, World health Organisation: AIDS Epidemic Update, Geneva: UNAIDS, 2007). However, despite the considerable population of truck drivers who use our numerous road networks and sea ports, to my knowledge, there are no research studies examining HIV, HBV or STIs prevalence and risk factors of truck driving population in the country. Research on the sexual behaviour of truck drivers is sparse and no information exists on their sexual behaviour in the country.

1.3 Aim

This study therefore aimed at investigating the prevalence of HIV, HBV and syphilis and to examine the association of HIV, HBV and syphilis with various suggested risk factors for transmission among truck drivers in Ghana.

1.4 Objectives

The specific objectives are:

- To determine the prevalence of HIV, HBV and Syphilis among truck drivers at Tema rest stops and ports.
- To define the risk factors associated with the transmission of HIV, HBV and Syphilis among the truck drivers.
- To collect and collate useful epidemiological data which will serve as a future reference for health and research purposes on HIV/AIDS risk factors.

1.5 Justification

The relation between truck driving occupation and the high transmission of HIV, HBV and Syphilis is an interesting field of research that has not been explored in Ghana. Research into this area in more detail could provide useful information in the

identification of truck drivers and who are carriers or sero-positives of HIV, HBV andSyphilis. An in-depth study of truck drivers might provide a better understanding of the risk factors for the transmission of HIV, HBV and STIs in the country. This may led to further description of this population as high, medium or low risk population. The results of this study would act as the basis for setting up systems and put in place measures to monitor and control the spread of HIV, HBV syphilis and other STIs among truck drivers. The Ghana AIDS Commission, Ghana Health Service, Ministry of Health and NGO's can use the results in their daily activities. Data from this study would form the basis for vigorous advocacy and hopefully policy formulation to improve living conditions at rest stops and healthcare delivery to truck drivers. It would also galvanize policy on counseling and screening for HIV, HBV and STIs among local and foreign truck drivers and assistants so that the appropriate preventative measures would be instituted to prevent the onward transmission by those with the infection to families and sexual partners. As truck drivers, CSW, local residents and the general population become increasingly aware of the risks of HIV, HBV and Syphilis associated with migration and mobility (Saggurti *et al.*, 2009), it is possible that the risk factor profile of the truck drivers and the residents in the villages along highways and the sea port area would change.

1.6 Expected outcome

The study is expected to provide the following outputs:

- Preliminary data on the prevalence of HBV, HIV and Syphilis among truck drivers.
- A description of the risk factors and the mode of transmission in truck drivers.

- Data that would form a firm basis for advocacy and policy change concerning the improvement of living conditions and health system of local and foreign truck drivers.

1.7 Beneficiaries

The beneficiaries of the study will be primarily the Ghana AIDS Commission, Ghana Health Service, the Ministry of Health, Non-governmental organizations, policy makers and the entire people of the country. The citizens of Ghana will benefit by having more accurate information on the conditions in haulage business and truck driving occupation and the prevalence and transmission of HIV, HBV and Syphilis among local and foreign truck drivers in Ghana.

CHAPTER TWO

2.0 Literature Review

2.1 Human Immunodeficiency Virus and Sexually Transmitted Infections

Sexually Transmitted Diseases (STDs) are caused by Sexually Transmitted Infections (STIs) which are passed on from one person to another by sexual contact or genital mucosa contact (Morse *et al.*, 2004). STIs are associated with HIV since they are mainly transmitted through sexual intercourse although in some cases they may be transmitted vertically from mother to child or through blood products (Mathews et al., 2014). Co-infection of HIV and other STIs are very common, making STI prevention a key step in controlling the spread of HIV/AIDS (Workowski, 2010).

The asymptomatic nature of STIs and HIV infection makes screening an important step in prevention since infected individuals might not know that they have the infection and might spread it unknowingly. Population at risk of HIV and STIs include commercial sex workers, transport workers, prisoners, homosexuals, migrant workers and partners of infected individuals (Adjei *et al.*, 2006; Pandey *et al.*, 2008; McCree *et al*., 2010; Dahal *et al*., 2013).

2.2 Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus (HIV) causes disease which is characterised by profound immunosuppression with associated life threatening–opportunistic infections, malignant tumours, and neurological disorders (Abbas and Lichtman, 2003). The disease which is termed Acquired Immunodeficiency Syndrome (AIDS) was first described in 1983, and since then it has become a worldwide epidemic, expanding in scope and magnitude as

HIV infections have affected different population and geographic regions. A variant of the HIV which is prevalent in West Africa was isolated later and was designated HIV-2, the first isolate became HIV-1 and the later became HIV-2 (Morse *et al*., 2004).

2.2.1 Classification, genome, and virion of Human Immunodeficiency virus

The human immunodeficiency virus is a Lentivirus which belongs to the family *retroviridae*. The virion is icosahedral in shape with an envelope that surrounds a capsid which contains the genome (Cann, 2005). The lipid envelope contains a glycoprotein termed as gp 120 which serve as an antigen that binds the $CD4^+$ receptors on helper T lymphocytes. In addition to the gp 120 the envelope contains a fusion protein called gp 41 which forms a lollipop stick that promotes cell to cell adhesion (Zane, 2001).

The genome contains two single stranded RNA which are positive sense in nature. The genome consist of three major genes that codes for various structural components, The *env* gene codes for the envelope glycoproteins gp120 and gp 41. The *gag* gene codes for the core proteins p24, p40 and p55, and the *pol* gene codes for enzyme proteins such as reverse transcriptase (p51 and p66), integrase (p32), and protease (p11) (Abbas and Lichtman, 2003; Cann, 2005).

In addition to these three structural genes that are common to all retroviruses, lentiviruses have two auxiliary genes called *Tat* and *Rev*. which are involved in transactivation of viral and cellular genes and, the regulation of RNA splicing respectively. *Nef, Vpr, Vif, Vpu* which are accessory proteins regulate viral expression

and play important roles in disease pathogenesis and are unique to the lentiviruses (Zane, 2001; Murray *et al*., 2002).

2.2.2 Replication of Human Immunodeficiency Virus.

During infection the gp120 on the viral envelope binds to the $CD4^+$ receptor and chemokine co-receptor (CCR5) on macrophages and enters by fusion. Later, the virus enters helper T lymphocytes by binding the gp120 to $CD4^+$ receptor and a different chemokine receptor called fusin (CXCR4) on the lymphocytes(Talaro and Talaro, 2002). As the envelope of the virus fuses with the plasma membrane of the host cell, the two single stranded RNA containing the viral genome is released into the cytoplasm of the host cell and the reverse transcriptase makes a DNA copy from the genomic RNA (Zane, 2001). The newly formed viral DNA is integrated into the host cell DNA as a provirus and the process is catalyzed by the enzyme integrase. (Cann, 2005). The proviral DNA is transcribed by the host enzyme known as the RNA polymerase II. The full-length RNA produced from the transcription is cleaved by HIV protease into several mRNAs containing *gag, gag-pol,* or*env* gene sequence. After the cleavage they are assembled and bud from the cell membrane of the host cell forming a mature viral particle that can infect other cells (Murray *et al.,* 2002).

Mutation during HIV replication due to factors such as recombination and lack of proof reading on the part of reverse transcriptase has given rise to four phenotypic groups namely, 'Marjor' (M group), 'New' (N group), the Outlier (O group), and the P group from gorilla simian immunodeficiency virus which was isolated in 2009 from a

Cameroonian woman (Burke, 1997; Hunt, 2009). The M group accounts for about ninety percent (90%) of HIV infection globally, whereas the group O is found in Cameroon and N is found in both Gabon and Cameroon (Plantier *et al.,* 2009). The group M, due to genetical evolution has ten subtypes; A, B, C, D, E, F, G, H, I and J. with A, B, and C being the most prevalent in the world (Gao *et al,* 1996; Buonaguro, 2007).

2.2.3 Pathogenesis of Human Immunodeficiency Syndrome

Primary infections occur when HIV infects dendritic cells which play a key role in the initial dissemination of the virus into the lymphoid tissue. Extensive viral replication occurs within days after the first exposure. This leads to viremia, during which high numbers of viral particles are found in the peripheral blood (Abbas and Lichtman, 2005; Zane, 2001). The viremia allows the virus to disseminate throughout the body to the target cells. During the latent period, HIV replication and destruction by the immune system is at equilibrium. It is estimated that 10 billion HIV particles are produced and destroyed each day (Zane, 2001; Morse *et al*., 2004). At this phase the immune system is competent and can therefore combat most infections and opportunistic microbes. There are few or no clinical manifestations of the HIV infection at this phase (Abbas. and Lichtman, 2005).

Destruction of the $CD4^+$ T cells steadily progresses and the population in the blood declines. Clinical AIDS sets in when the immune system fails to maintain the equilibrium due to continual viral replication and infection of healthy $CD4^+$ T cells. At this lethal

phase, viremia may climb dramatically as viral replication in other reservoirs accelerates unchecked (Murray *et al*., 2002). The consequences of $CD4^+$ T cell dysfunction caused by HIV infection includes the clinical phase of AIDS which is characterised by severe depression of the immune response because of the depletion of the $CD4^+$ helper T cells by the viral infection (Zane, 2001). The immunodeficiency results in increased susceptibility to multiple opportunistic infections. The opportunistic infections include fungal infections such as *Candida albicans* and *Cryptococcus neoformans*, protozoan infections such *Toxoplasma gondii* and *Crytosporidium*, viral infections such as the Herpes simplex and Herpes zoster,and bacterial infections such asthose caused by *Mycobacterium* (Burton and Engelkirk, 2000). The targets of AIDS is not only limited to the immune system, but the central nervous system is also involved. The infected macrophages cross the blood brain barrier and release the viruses which invade the nervous tissues (Morse *et al*., 2004). Some of the clinical symptoms include persistent fever, diarrhoea, chronically swollen lymph nodes, and extensive weight loss. Rate of disease progression differ among the HIV-1 subtypes, people infected with subtype D progress to AIDS faster than the other subtypes (Vasan *et al*., 2006, Baeten *et al*., 2007)

Some individuals have been found to have mutant chemokine receptors especially the CCR5 and the CCR2 on macrophages and activated $CD4^+$ T helper cells. This makes binding of HIV impossible hence the immune cells don't get infected when exposed to HIV (Hunt, 2009). Some individuals have also been identified to produce excessive chemokines which binds and block the chemokine receptors. It therefore takes a longer time for such individuals to progress to AIDS since the blocking of the chemokine

receptors prevents HIV from infecting the immune cells (Hunt, 2009). Some women in Kenya, have unique Human Leucocyte Antigens; Major Histocompatibility Complex I and II (MHC I and II) which are able to process and present epitopes from highly conserved region of the p24 protein of HIV. This conserved region of the p24 protein is key in viral assembly of HIV, therefore epitopes are able to evoke extraordinally strong Cytotoxic T lymphocyte response which prevents complete replication and re-infection of new cells. These women although are exposed to HIV, it has no effect on them due to their unique immunity to HIV (Rowland-Jones *et al.*, 1998).

2.2.4.Transmission of Human Immunodeficiency Virus

The viral particles are found in all body fluids such as blood, semen and vaginal secretions. Breast milk is also considered as a potential source of infection since it contains a significant number of leucocytes. HIV have been isolated from urine, sweat, tears, and saliva but in minute quantities (less than one virus per cubic centimetre) such that it cannot be considered as a potential source of infection (Talaro and Talaro, 2002; Murray *et al*., 2002).

HIV is transmitted predominantly through sexual intercourse, more especially anal sex which lacerates the rectal mucosa, and provides entrance for the virus into the blood from the semen (Wu, 2008; Merrigan *et al.*, 2011).

Transfer of blood or blood product is another mode of transmission. Although, transfer of blood and blood products is an effective route for viral transmission, incidence of HIV infection through blood transfusion is not common in recent times because blood is

routinely screened for antibodies to HIV before transfusion (Hoffbrand and Moss, 2008). Transmission can occur through sharing of needles, and this is common among intravenous drug users. This mode of infection is a significant factor in the spread of HIV because infected intravenous drug users end up infecting their sexual partners (Talaro and Talaro, 2002). Infantscan get infected utero, through the birth process or through breast feeding, and the transmission rates vary from 13% to 40% in untreated women (Morse *et al*., 2004).

2.2.5 Diagnosis of Human Immunodeficiency Virus

There are several methods currently available for diagnosing an HIV infection, which detects HIV antibodies, antigens and nucleic acid.

Antibody Detection technique relies on the host immune response to diagnose HIV infection (Cheesbrough, 2003). Antibodies (IgG) to HIV glycoproteins such as gp41, gp120 and gp36 in the case HIV-2 infection, are detectable three months into the window period and after seroconversion. Enzyme – linked immunosorbent assay (ELISA) also known as Enzyme Immunoassay (EIA) have been the conventional method for detecting HIV antibodies (IgG),both quantitatively and qualitatively (Pagana and Pagana, 2002). However the duration of the test (about six hours) makes emergency diagnoses of HIV difficult especially in labour case when the status of the woman is not known. This led to the development of rapid antibody test for HIV which has been tested to have over 90% sensitivity and specificity. There are rapid test such as the Oral Quick Advance HIV1/2 Antibody Test, Multispot HIV-1/HIV-2 Rapid Test,Uni-Gold Recombigen HIV-1 Test,

Reveal G2 Rapid HIV-1 Antibody Test that are able to detect IgG antibodies qualitatively within twenty (20) minutes using whole blood, serum, plasma or saliva, however confirmatory test such as western blot is required to diagnose positive results (Pesce*et al*., 2006).

Western blot technique detects antibodies specific to viral proteins of HIV (Cheesbrough, 2003). The test utilize immobilized range of viral proteins from the envelop and the core of the virus to detect HIV antibodies in patient's serum. Results are read per manufacturer's instructions. However detection of at least one antibody from the core and envelopeproteins each indicates positive results (Fearon, 2005). Results from Western blot are presented as indeterminate when the antibody detection pattern does not correspond with the manufacturer's description of positive results. Indeterminate results are as a result of seroconversion at the time the sample was taken and retesting is recommended in such case. And where repeatedly indeterminate results is obtained, Polymerase Chain Reaction is recommended to confirm HIV seropositivity or Negativity (Ittiravivongs *et al.,*. 1996). However due to the improved HIV diagnosing techniques that are simpler and faster, Western blot is no more used in clinical laboratory practice (Sato *et al.,* 1994).

Viral nucleic acid can be detected quantitatively and qualitatively in the serum using the Polymerase chain reaction (PCR). The specificity and sensitivity nature of this PCR technique allows HIV to be diagnosed by amplifying minute quantities of HIV nucleic acid (Pagana and Pagana, 2002). The test is used to confirm negative results in newborn babies born to HIV seropositive mothers, due to maternal IgG which would be circulating in the babies blood. HIV antibody test maytherefore give positive results whereas the

baby is not infected (Fearon, 2005). PCR is also used in diagnosing HIV in patients who are extremely immunocompromised and antibodies cannot be detected in their serum. However the specific primers used in PCR makes it not ideal for diagnosing HIV-2 infections hence it can give false HIV negative results if the infection is by HIV-2 (Fearon, 2005). Quantitative detection of HIV RNA is used to detect the baseline viral load (amount of viral particles in the blood) and to monitor disease progression before and after initiation of Antiretroviral therapy (De′sire′, 2001).

Viral antigen (mainly p24) can be detected using immunological techniques such as Radioimmunoassay (RIA) and Enzyme immunoassay (EIA) which utilize antibodies to detect p24 in HIV infected specimen (Murray *et al*., 2002). The antigen p24 can be detected before antibodies development in newly infected individuals. Antigen (p24) detection is used as a confirmatory test when indeterminate result is obtained from western blot. However, when p24 antigen test is reactive and western blot gives negative or indeterminate result, an antibody test is recommended for confirmation (Fearon, 2005).

2.2.6 Treatmentof Human Immunodeficiency Virus

Although all efforts to cure HIV/AIDS have proof futile, the introduction of Antiretroviral drugs have alter the natural history of HIV/AIDS (Weller and Williams, 2001). Before the advent of Highly Active Antiretroviral Therapy (HAART) in 1996, the treatment available targeted at reducing opportunistic infections and prolonging life (Talaro and Talaro, 2002). The HAART reduce viral load and boast the immune system by interrupting different stages in the HIV viral replication (Murray *et al*, 2002, Mills *et*

al., 2011). The first step in viral replication is the entry into the host immune cells, and this step serve as a target for some antiretroviral (ART) agents. The entry inhibitors bind and block the CCR5 receptors on macrophages and the $CD4^+$ helper T cells and prevent HIV from entering the host through fusion (Lobritz *et al*, 2013).

Fusion inhibitors also prevent the release of viral nucleic acids into the host cell by blocking gp41 which is a protein that promotes fusion. Blocking the gp41 protein therefore terminates fusion (Balley and Fisher, 2008). Some of the ART agents are reverse transcriptase inhibitors which prevent reverse transcription of viral ribonucleic acid (RNA) to produce complementary deoxyribonucleic (cDNA). Termination of reverse transcription prevents the viral genome from being integrated into the human genome, making it vulnerable to destruction by host immune system (Weller and Williams, 2001). Antiretriviral agents which are integrase inhibitors binds and prevents integrase from incorporating the viral DNA into the host genome (.Garrido *et al.*, 2010). Another group of ART agents are the Protease inhibitors which disrupt post-translational processing of viral proteins by binding to protease. This results in premature release of viral particles. Protease inhibitors are very potent ART, however poor adherence to therapy containing protease inhibitors have been reported (Lobritz *et al*, 2013).

There have always been controversy about when to initiate antiretroviral therapy due to the long term side effects of ART such as drug resistance (Cater, 2013). World Health Organization (WHO) in 2006 recommended that treatment is initiated when $CD4^+$ count is 200 cells/µL or below (WHO, 2006). However weighing the benefits of early initiation of ART and the side effects, WHO has modified its recommendation by initiating treatment at 350 cells/µL (WHO, 2009), and the International AIDS Society also

recommends that ART starts at 500 cells/μL (Thompson *et al*., 2010). Although early ART initiation give better treatment outcomes (Mills *et al.*, 2011), this treatment strategy recommendations by WHO and International AIDS Society may be limited to the advanced countries (Weller and Williams, 2001). In the developing countries where resources are limited, the new treatment recommendation by WHO is a challenge, as international donor organizations are not willing to support this new treatment strategy due to the cost involved (Ford *et al*., 2010).

Resistance to ART was a bigger challenge just after the advent of HAART which combines three different ART agents. This challenge arose because of previous exposure to some ART agents which could not suppress HIV replication and resulted in drug resistant strains (Balley and Fisher, 2008). The presence of new generation HAART which is easy to adhere and targets drug resistant strains have decrease drug resistance among HIV patients (Cater, 2013).

2.2.7 Epidemiology of Human Immunodeficiency Virus

Human immunodeficiency virus and Acquired Immune Deficiency Syndrome (HIV/ AIDS) is a global problem and millions of lives are lost due to this pandemic. More than thirty-four million (34, 000,000) people are living with HIV with about 2.5 million been new infections (UNAIDS global report, 2012). The prevalence of HIV/AIDS have reduce drastically in the world especially in Sub-Saharan Africa where HIV has had adverse impact (UNAIDS global report, 2012).

Ghana as a sub-Sahara African country has suffered this HIV pandemic, although it is not known when exactly HIV got into the country. In 1986, the first case of HIV/AIDS was recorded, and in an effort to track its prevalence, the Ministry of Health established national HIV Sentinel Surveillance (HSS) system in 1990. HIV sentinel survey has been conducted annually using pregnant women who visit antenatal clinics since 1994 to date. In 2005 Ghana experienced a decrease in prevalence level from 3.1% in 2004 to 2.7% (HIV Sentinel Surveillance Report, 2004; Sentinel Surveillance Report, 2005).Today, an estimated number of 235,982 people are living with HIV in Ghana, 7991 new cases were recorded in 2012 with 852 being children, bringing the prevalence level to 1.37% (HIV Sentinel Surveillance Report, 2012). Although HIV prevalence rate has decreased, there are still reasons to worry since several cases go unreported.

HIV prevalence has exhibited different patterns in the country. High prevalence rate is observed in densely populated areas such as the cities, mining areas, towns along main transportation route and border towns.

Currently, the prevalence rate ranges from 0.2% in Nalerigu to 10% in Agormanya. The Eastern region currently has registered the highest prevalence rate of 3.6% followed by the Greater Accra region which registered 3.5%. The Ashanti has a prevalence rate of 2.6%. The Brong- Ahafo region, volta, western and the upper east have prevalence rates ranging from 2% to 2.6%. The central region has a prevalence of 1.9% and the Upper west region has a prevalence of 1.2%. The northern region has the lowest prevalence rate of 0.9% (HIV Sentinel Surveillance Report, 2012).

HIV-1 infection accounts for 96.4% of all HIV infection cases in Ghana, exclusively HIV-2 accounts for 0.7% and dual infection (HIV-1 and HIV-2) accounts for 2.9% of the cases in Ghana. In Ghana, HIV prevalence is high among the youth. In 2010 the youth between 30-39 years registered the highest prevalence rate of 2.8%. In 2011 prevalence was highest among people aged between 30 years and 34 years (2.8%). In 2010 the prevalence among people aged between 15 and 24 years dropped from 2.1% in 2009 to 1.7% in 2010, and rose marginally to 1.7% in 2011. The prevalence rate among people aged between 15 and 19 years rose sharply to 1.9% in 2011 (HIV Sentinel Surveillance Report, 2011). This report indicates that high HIV prevalence in Ghana is fuel by the youth.

2.3 Syphilis Infection

Syphilis is a disease caused by a spirochete called *Treponema pallidum.* This spirochete differs from other spirochetes by its regular tight spiral and motility nature (Pagana and Pagana, 2002). Humans are the reservoir for this bacteria and transmission is primarily by direct contact with lesions, body secretions, semen, vaginal discharge, blood, mucous membrane, and saliva of infected people usually through sexual contact, blood transfusion and transplancental infection that is from mother to child (Burton and Engelkirk, 2000).The disease occurs in three stages, the primary, the secondary and the tertiary stage. The primary or the initial stage involves painless lesions called chancres at the site where the spirochete penetrated. In the secondary stage, the disease disseminate to all parts of the body with clinical signs such as headache, fever, myalgias, anorexia, lymphadenopathy and prominent skin lesions dispersed all over the body (Cheesbrough,

2003). After the primary or secondary phase, infected persons may go into remission, if not the disease progress to the tertiary phase. Tissue damage occurs which spread to several organs and systems in the body such as the central nervous system, the cardiovascular system, visceral organ, sense organs and bones during the tertiary phase (Murray *et al*., 2002)

Syphilis is found worldwide but its incidence have decreased drastically due to the introduction of penicillin in the 1940s (Burton and Engelkirk, 2000), which has reflected in prevalence of syphilis (0.6%) in Ghana (HIV Sentinel Surveillance Report, 2012).

Primary infection can be diagnosed by examining exudates from lesions for motile T. pallidum using darkfield electron microscope. Immunological techniques have been the main technique for diagnosing syphilis. Tissue destruction during syphilis infection releases cardiolipin which stimulates the production of anti-cardiolipin antibodies. Serological test such as Venereal Disease of Reference Laboratory (VDRL) and Rapid plasma regain (RPR) test detect anti-cardiolipin antibodies, but these antibodies are not specific to Treponema pallidum (Pagana and Pagana, 2002). Positive non-specific Treponemal test are confirmed using specific treponema test such as (*Treponema pallidum* haemagglutination assay (TPHA), *Treponema pallidum* particle agglutination assay (TPPA), Fluorescent Treponemal Antibody absorption Test (FTA-ABS) and Enzyme Immunoassay (EIA) (Cheesbrough, 2003). Syphilis is treated using Benzathine Penicillin (Penicillin G) and people that are sensitive to penicillin are treated with tretracyclin, dodecycline, or ceftriaxone (Burton and Engelkirk, 2000).

2.4 Hepatitis B Infection

Hepatitis B virus (HBV) also known as the Dane's particle is an enveloped hepadnavirus. The virion consist of a genome made of double stranded DNA with single stranded regions**,** surrounded by hepatitis B core antigen (HBcAg). The envelope is made of hepatitis B surface antigen (HBsAg) which contains three glycoproteins HBsAg S, HBsAg M, and HBsAg L (Cheesbbrough, 2003). Hepatitis B e antigen (HBeAg) is also a minor component of the virion.

The virus (HBV) is found in body fluids such as blood and blood components, saliva, semen, vaginal secretion, breast milk and amniotic fluid, and transmission is by sexual contact, transfer of blood and blood products through blood transfusion or during intravenous drug usage (Murray *et al*., 2002). HBV has defined and distinct tropism for hepatocytes where replication takes place. HBV replicates within three days after infection, and symptoms may not be observed within forty-five days depending on the infectious dose, route of entry and the person's immune status. (No cytopathic effect is observed at this stage. During this period the viral genome integrates into the host chromatin and remains latent, and filamenteous forms of HBsAg builds up in the host cytoplasm.

Using MHC class II molecules infected cells displays epitopes of HBsAg, HBeAg and HBcAg which trigger cell-mediated immune response. Lysis of infected cells produces symptoms and the infection is resolved in acute infection. Anti-HBsAg antibodies are produced during the acute face to neutralize the HBsAg. Chronic infection sets in when cell –mediated immune response is not able to resolve infection. As infection progresses the amount of HBsAg becomes more than the generated anti-HBsAg antibodies. HBsAg

binds and block these antibodies so that they are incapable of neutralizing the antigen. Immune complexes are formed as a result which leads to type III hypersensitivity reaction (Morse *et al.,* 2004; Burton and Engelkirk, 2000; Murray*et al*., 2002). HBsAg is the first immunological marker detectable in the serum. It appears weeks to months before the onset of clinical symptoms, but in acute infection with recovery, HBsAg cannot be detected.

Most laboratory investigations are based on detection of HBsAg and HBsAb. HBeAg also appears in the serum soon after HBsAg and disappears before the unset of recovery. Persistence of HBeAg is an indication of increased infectivity but HBeAg detection is done in only specialized laboratories.

HBV can be diagnosed using ELISA, rapid immunochromatographic strips and card test, latex agglutination, reverse passive haemagglutination (Pagana and Pagana, 2002; Cheesbrough, 2003). Vaccination has been the effective way of preventing HBV, using attenuated surface antigen (Beasley, 2009).

2.5 Behavioral factors in the HIV/AIDS and STI epidemic

Sexual intercourse has been the major mode of transmission of HIV infection and STI in Ghana and the youth accounts for majority of the HIV/STI cases (HIV Sentinel Surveillance Report, 2010). Limited job opportunities coupled with high cost of living has contributed significantly to high HIV/STIs prevalence in Ghana. Unemployment and poverty have put pressure on people, causing them to migrate from rural areas to urban areas in search of greener pasture. Some end up stranded and exchange sex for shelter,

food and protection. Others also indulge in paid sex making them vulnerable to HIV infection (Côté, 2004). Due to peer group influence some may indulge in intravenous drug usage and casual sex.

A lot of education and preventive measures have been put in place but Most at Risk People (MARP) such as Commercial sex workers (CSW), Men Sex Men (MSM), and Intravenous Drug Users (IDU) are not able to access this services due to stigmatization, social hostility, legal barriers, fear of losing jobs and families, and many other reasons (Ghana AIDS Commission, Ghana Country Progress Report, 2012).

They hide their identity and infect other sexual partners once infected. They therefore continue to be source of new infections.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design and Site Description

A cross sectional study was conducted between the months of January and June, 2013 among truck drivers at Tema port. Tema city is located in the Greater Accra region, 25 kilometers from the capital city Accra. Tema popularly known as the industrial city is the 5th populated town in Ghana with 402,637 inhabitants (Ghana Statistical Service, 2010 Population and Housing Census). The numerous industries and companies in Tema have attracted lot of migrants into the city. Ghana has two seaports, the Takoradi seaport which is located in the western region and the Tema seaport located in the Tema metropolis. The Tema port which is the larger of the two sea ports spans a land area of 3.9 million square metres, and it is 21 nautical miles off the north-east coast of Accra. The port is flanked by an industrial city and within its environs are Inland Clearance Depots (ICDs), Transport and haulage companies, Warehouses, and related service centres. Tema port is well connected to the hinterland which makes it the preferred and ideal gateway to most of the regions in Ghana and also the neighbouring countries such as Burkina Faso, Niger and Mali. The Port is also serviced by leading shipping companies and clearing companies. In addition, the port is more active than the Takoradi port, and also serves as a common destination for truck drivers, especially the availability of alcohol, drugs and cheap sex made it an ideal site for this study. The protocol of this study was approved by the Ethical and Protocol Review Committee of the University of Ghana Medical School in January, 2013.

3.2 Sampling methodology

3.2.1 Inclusion criteria

- All truck drivers who were in active service were considered eligible for inclusion.

3.2.2 Exclusion criteria

- Retired truck drivers, truck owners, driving assistants and people who hold administrative positions at the study site and are not drivers were considered not eligible for inclusion.

3.2.3 Sample Size Determination

The prevalence of HIV in the Greater Accra region as at the time of the study was 3.6% (HIV Sentinel Surveillance Report, 2012). At an allowable error of 3.5%, and significance level of 95%, the sample size of the study was calculated using this formula:

$$N = \frac{Z^2_{(\alpha/2)}\, P\,(1-P)}{E^2}$$

Where:

N = sample size

Z = significant level

P = estimated prevalence

E = allowable error

A minimum sample size of ninety-nine (99) was obtained which was approximated to the nearest hundred, however total of hundred and six (106) study participants were recruited for the study.

3.3 Study Population

Subjects of this study were male truck drivers (Ghanaians and non-Ghanaians). The truck drivers were stratified into long distance truck drivers and short distance truck drivers. Drivers who travel more than hundred kilometer (100 km) were considered as long distance drivers while those who travel 100 km or less were termed short distance drivers. After an explanation of the purpose of the study, all truck drivers were invited to participate in the study. Truck drivers were informed that the study was confidential and that information provided by them would not affect their trucking business. A total of 106 truck drivers (out of 1,250 eligible truck drivers) showed interest in the study. Written informed consent form was obtained from each participant. Information regarding the protocol and the informed consents were presented at the appropriate literacy level. None of the non-Ghanaians consented to take part in the study. The study was conducted in a confidential manner and random unique study-generated numbers were employed to identify the participants.

3.4 Questionaire

All the 106 participants completed structured questionnaire assessing socio-demographic characteristics, sexual behaviors, and HIV/STI related knowledge. Socio-demographic variables included age, place of origin, educational level, marital status and number of children. History of sexual behaviour included involvement in paid sex, number of sexual partners and condom use. Other risk factors included alcohol and drug usage, smoking history and the presence of tattoo or tribal mark.

3.5 Sample collection, Processing and Storage

Blood sample (10 ml via venepunture) was collected into EDTA tubes from all individuals who consented and completed the questionaire. Samples were transported on ice to Korle-Bu Teaching Hospital (KBTH), and plasma was separated by centrifuging the whole blood at 1600 g for 8 minutes. Plasma (2 ml) was aliqoted into three different apendorf tubes labeled A, B and C, aliquot A and B were stored at -20 ^{O}C for the purpose of this study, while aliquot C was stored at -80 ^{O}C for any future study.

3.5.1 HIV Screening

3.5.1.1 One Step Anti-HIV (1 and 2) Tri-line Test

All samples were initially screened for the presence of antibody to HIV-1 and HIV-2 using One Step Anti-HIV (1 and 2) Tri-line Test. The One Step Anti-HIV (1 and 2) Tri-line Test is a colloidal gold enhanced rapid immunochromatographic assay for detecting antibodies of all isotypes (IgG, IgM, IgA) to HIV (1 and 2). The test card has a sample well which contains a recombinant HIV antigen conjugated to colloidal gold. Upon

application of HIV positive plasma, serum or whole blood to the well, antibodies to HIV binds to the recombinant HIV antigen conjugated to the colloidal gold resulting in the formation of conjugate-HIV antibody complex. Addition of sample diluent helps the conjugate-HIV antibody complex to migrate along the card membrane. The card membrane has three regions; the HIV-1 (T1), HIV-2 (T2) and the control, (C) region. The T1 region has recombinant antigens gp41, p24 and gp120 immobilized to it, and as the conjugate-HIV antibody complex migrates along this region, the conjugated antibodies binds to the immobilized antigens resulting in the formation of colored test line indicating HIV positive test results. The T2 region has a recombinant gp36 antigen which is specific to HIV-2 immobilized in it, and as the conjugate-HIV antibody complex migrates along this region, a colored line develops indicating HIV-2 positive test results. The control region has anti-HIV antibodies immobilized in it and there is a color development irrespective of test results. The color development is an indication that the conjugate (the recombinant HIV antigen in the sample well) is potent, and the color is as a result of the conjugate binding to the immobilized antibodies (One Step Anti-HIV1 and 2 Tri-line Test manual). The test was carried out in accordance with the manufacturer's instructions (Appendix III).

3.5.1.2 OraQuick® ADVANCE Rapid HIV-1/2 antibody test

All reactive samples from the One Step Anti-HIV1 and 2 Tri-line Test (First Response) were retested with OraQuick® ADVANCE Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc. USA) for confirmation. The OraQuick® ADVANCE Rapid HIV-1/2 Antibody Test kit is made up of a test card, developer solution vial, specimen collection loop, and a test stand. The test card has a sample pad for adsorption of specimen, protein A colloidal gold conjugate, and a nitrocellulose strip containing two test regions and a control region. A recombinant HIV-1 glycoprotein gp41 have been immobilized in the Test region one (T1). Test region 2 contains HIV-2 envelope glycoprotein gp36, while the control region contains goat antihuman IgG. The test was carried out by picking a loopful of plasma into the developer solution vial using the sample collection loop. The developer vial solution was shaken gently to obtain a uniform solution. The diluted sample was put into the test stand and the test card was then inserted into the diluted sample using the flat pad, and incubated for 20 minutes. During the incubation period, the sample pad in the test card adsorbs the diluted sample and as it flows through the test device the protein A colloidal gold conjugate becomes hydrated. If HIV antibodies are present in the sample, they bind to the protein A colloidal gold conjugate and the complex formed migrates along the nitrocellulose membrane. The HIV antibodies-protein A colloidal gold conjugate complex binds to the immobilized recombinant antigens in the test region which results in color development. If the sample contains no HIV antibodies no color develops at the test region. Other human IgG (which is not specific to HIV) present in the sample binds to the protein A colloidal gold conjugate as well, and as the complex migrate to the test region, the human IgG binds the goat antihuman antibodies and color develops. Color development at the control region is an

indication that the result is valid. After 20 minutes of incubation, samples that tested negative were considered indeterminate and therefore tested again using the Genscreen™ ULTRA HIV Ag-Ab (Bio-Rad Laboratories, Hercules, CA). The test was carried out using the manufacturer's protocol (Appendix III)

3.5.1.3 The Genscreen™ ULTRA HIV Ag-Ab

The Genscreen™ ULTRA HIV Ag-Ab (Bio-Rad Laboratories, Hercules, CA) detects HIV p24 antigen and antibodies to HIV-1 (groups M and O).

Using the reagents and materials listed in Appendix III, 25µl of conjugate 1 (biotinylated polyclonal antibodies to p24 HIV 1) was first added to each well followed by 75µl of HIV Ag positive control (purified and inactivated HIV- 1 antigen) in well A1. 75µl of HIV Ab positive control (human plasma positive for anti-HIV antibodies) was added in well B1, 75µl of negative control (human plasma negative for HIV antigen, anti-HIV-1 antibodies, and anti-HIV-2 antibodies) in well C1, D1 and E1 and 75µl of specimen (serum from participants) was added in subsequent wells. After adding the samples, the conjugate 1 turned blue. The mixture was homogenized by shaking the microplate after the pipetting steps, and incubated for an hour. The plate was then washed three times with wash buffer (diluted from the concentrated buffer; 1:20 dilution), and blotted dry. 100µl of conjugate 2 (peroxidase labelled Streptavidin and purified HIV1&2 antigens + skimmed milk solution) was added to each microwell, incubated for 30 minutes and washed five times. 80µl of freshly prepared substrate solution (Peroxidase substrate buffer + chromogen) was quickly dispensed into each well, after which the reaction was allowed to develop in the dark for 30 minutes at room temperature (25^{o}C). This resulted

in the development of pink color in some wells that had the samples added to them while the wells without samples remained colorless. The reaction was then stopped by adding 100µl of the stop solution. After the addition of the stopping solution the pink coloration of the substrate disappears (for the negative samples), and wells with positive samples developed a yellow color. The optical density (OD) of the wells were determined at 450/620-700nm using a plate reader (spectrophotometer) within 30minutes of stopping the reaction. The cut off value was used to determine if a sample was positive or negative using the Manufacturer's instruction.

3.5.2 Syphilis Screening

3.5.2.1 Accu-Tell One Step Anti-Treponema Pallidum test

Samples were screened for syphilis using Accu-Tell One Step Anti-Treponema Pallidum test (AccuBio Tech Co., Ltd.,). This rapid test employs the sandwich principle in detecting antibodies to *Treponema pallidum* in serum. The test card has a sample well in which *Treponema pallidum* antigen (TP Ag 1) conjugated with colloidal gold particles has been immobilized. A recombinant *Treponema pallidum*antigens (TP Ag 2) has been immobilized in the test region and anti- *Treponema pallidum* antibodies in the control region. On application of the test sample, the anti- *Treponema pallidum* antibodies bind to the conjugated TP Ag 1 which forms a colored mixture. As the colored mixture migrate chromatographically along the nitrocellulose membrane in the card, if the test sample contains anti- *Treponema pallidum* antibodies, the antibodies bind to the TP Ag 2 in the test region and a color develops indicating a positive results. No color development is an indication of negative results. As the mixture migrate to the control region the TP Ag 1 in the conjugate binds to the immobilized antibodies in the control region and a

color develops. Color development in the control region is an indication that the TP Ag 1/colloidal gold conjugate is potent. Color development at the test and control region is an indication of valid positive results. Color development at the control region alone is an indication of valid negative results, however color development at the test region alone indicates invalid test results.

3.5.2.2 Treponema pallidum haemagglutination assay (TPHA)

All reactive samples were confirmed qualitatively using a Treponema pallidum haemagglutination assay (TPHA, Fujurebio, Tokyo, Japan) which is a specific to *Treponema pallidum*.The TPHA test uses avian erythrocytes that are coated with *Treponema pallidum* antigens which bind to anti *Treponema pallidum* antibodies in the test serum or plasma. The antigen-antibody (Ag-Ab) binding results in agglutination of erythrocytes indicating a positive test results. However if the test sample contains no anti *Treponema pallidum* antibodies, there would be no Ag-Ab reaction, hence no agglutination occurs indicating negative test results.

Before performing the assay reagents, controls and test samples were put on the working bench to attain room temperature. The 96 well microtitration plates were labeled as follows; 3 wells in a column for negative control, and four wells in a column for each test sample. Diluent was dispensed into the wells for the test samples as follows; 25µl into rows 1, 3 and 4, and 100µl into row 2. Tweenty-five microlitres (25 µl) of each test sample was dispensed into the appropriate wells in row 1, and well mixed. Tweenty-five microlitres (25µl) was transferred from row 1 to row 2, and from row 2 to row 3 after

mixing the content well. Tweenty-five microlitres (25µl) was discarded from row 3. Tweenty-five microlitres (25µl) was transferred from row 2 to row 4 and mixed well, after which 25 µl discarded from row 4.

Seventy-five (75 µL) of well mixed test cells was added to row 4 and the plate swirled gently to mix. The test plate was covered and agglutination patterns were examined after 45 to 60 minutes.Positive reactions are showed by agglutination of the cells while negative reaction showed settling of the cells to a button as a small ring.All the sample analysis for syphilis was carried out following the manufacturer's protocol (Appendix V).

3.5.3 Hepatitis B Screening

3.5.3.1 Accu-Tell One Step HBsAg rapid test

Samples (plasma) were screened for HBsAg using the Accu-Tell One Step HBsAg rapid test (AccuBio Tech Co., Ltd.,). The Accu-Tell One Step HBsAg rapid test employs sandwich immunoassays in the detection of HBsAg in serum or plasma. Antigens in sample bind to recombinant anti HBsAg antibodies conjugated to a colloidal gold particles in the sample well and resulting mixture move alone the cellulose membrane which contains the test region and the control region. If the samples contain HBsAg, the HBsAg in the HBsAg-HBsAb-conjugate complex binds to the anti- HBsAg antibodies immobilized in the test region and a color develops which indicates positive results. Color development at both test and control region indicates valid positive test results and color development at the control region alone is an indication of valid negative test

results. All positive samples were confirmed using Roche COBAS e411 analyzer with elecsys HBsAg II quant test (Roche Diagnostics, Germany).

3.5.3.2 Roche COBAS e411 analyzer with elecsys HBsAg II quant test

Roche COBAS e411 analyzer with elecsys HBsAg II quant reagent kit employs the sandwich ELISA principle in quantifying HBsAg in HBV positive samples. The initial step involves incubation of sample, with two biotinylated monoclonal anti-HBsAg antibodies and a mixture of monoclonal and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex as a chemiluminescence molecule. The first incubation results in a sandwich complex formation and in the second incubation, streptavidin-coated microparticles are added to the sandwich complex which causes the complex to be bounded to a solid phase through biotin-streptavidin interaction.

The mixture is aspirated into a measuring cell where the microparticles are captured onto the surface of an electrode with the aid of magnetic force. Unbound substances are removed, and the resulting chemiluminescence reactions are measured and converted to HBsAg concentrations. The total duration of each assay is 18 minutes (Roche COBAS e411 analyzer with elecsys HBsAg II quant reagent kit manual, 2013).

The analytic measurement range (AMR) of Roche COBAS e411 analyzer with elecsys HBsAg II quant is between 5 and 13,000 IU/mL when the samples are 100-fold diluted. The high sentivity and precision coupled with its wide analytical range makes it an ideal in confirming the presence of HBsAg (Roche COBAS e411 analyzer with elecsys HBsAg

II quant reagent kit, manual, 2013).All the sample analysis for HBsAg detection was carried out following the manufacturer's protocol (Appendix IV).

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3.6 Statistical analysis

Data was entered into access database and SPSS version 20 statistical software was used to do the descriptive and inferential statistics. The descriptive statistics included mean, standard deviation, and range. For inferential statistics, the chi-square test was used to test the associations between categorical data, and P value of < 0.05 was considered significant.

The prevalence of HIV, HBV and syphilis was compared between long and short distance truck drivers, religions, age groups, educational level and the defined risk factors.

For each generally accepted risk factor for the diseases under investigation, Odds ratio was determined between those with the disease (cases) and those without the disease (controls).

CHAPTER FOUR

4.0 RESULTS

4.1 Study population

Between the months of March 2013 and June 2013, 106 truck drivers aged 18 – 73 years (mean age: 40.56 ± 11.56 years) were recruited for the study. The consenting participants (106) were only Ghanaians as none of the non-Ghanaian truck drivers consented to take part in the study. All the consented truck drivers completed interviews and blood testing, and the results herein presented are from 106 Ghanaian truck drivers. Out of 106 truck drivers, 84.9% (90 of 106) had been driving for more than five (5) years while 15.1% (16 of 106) had driven for less than 5 years.

Seventy-four (74) of the 106 participants (69.8%) were from the Greater Accra region, thirty (28.3%) originated from the Ashanti region, one (0.95%) was from the Northern and one (0.95%) from the Brong – Ahafo region. Of the 106 participants 11 (10.4%) had no formal education, 80 (75.5%) had Basic education, while those who had Senior Secondary School Certificate education formed 14.1% (15 of 106) of the study participants. Muslims formed 58.5% (62 of 106) of the participants, while Christians were 41.5% (44 0f 106).

Fourteen (14) participants (13.2%) reported signs of sexually transmitted infections (STI) such as genital ulcer, genital itches, painful urination and urethral discharge, with 16% (17 of 106) having history of Gonorrhoea.

4.2 Prevalence of HIV, HBV and syphilis among study participants

The overall prevalence of HIV-1 was 0.94% (1 of 106), with none testing positive for HIV-2. Syphilis infection among truck drivers was 3.8% (4 of 106) and 14.2% (15 of 106) had HBsAg.

Table 1 shows the Odds Ratios (ORs) and the corresponding 95% Confidence Intervals (CIs) according to age for HBV and syphilis. Hepatitis B virus and syphilis seropositivity were not associated with age. Hepatitis B Virus seropositivity was highest (6 of 15 seropositives) among truck drivers aged 31- 40 years, however none of the drivers who were 20 years and below tested positive. Compared to the 31–40 year group, truck drivers aged 21-30 years and those above 50 years were more likely to be at risk of HBV seropositivity (OR:1.3; 95% CI: 0.29-5.88). However drivers aged 41-50 years were slightly at decreased risk of HBV (OR: 0.98; 95% CI: 0.22-4.31).

Syphilis seropositivity was highest among truck drivers that were above 50 years (2 of 4 seropositives), with none of the drivers aged 20 years and below, and 41-50 years testing positive for syphilis. However, drivers aged 21-30 years and 31- 40 years were likely to be at decrease risk of syphilis infection (OR: 0.47; 95% CI: 0.04-5.71) and (OR: 0.18; C1: 0.02-2.41) respectively, compared to drivers who were aged above 50 years.

Table 1: Age distribution, HBV and syphilis sero-positivity among participants. Pos: Positive; Neg: Negative; * : Baseline for

			HBV Status					Syphilis status				
		[N=106]	Pos(15)	Neg (91)	OR	95% CI	p-value	Pos (4)	Neg (102)	OR	95% CI	p-value
Age	≤ 20	2	0	2	-	-	0.81	0	2	-	-	0.81
	21 –30	18	3	15	1.30	0.29-5.88	0.51	1	17	0.47	0.04-5.71	0.50
	31 –40	45	6	39	*			1	45	0.18	0.02-2.14	0.47
	41 –50	23	3	20	0.98	0.22-4.31	0.59	0	23	-	-	0.19
	≥ 50	18	3	15	1.30	0.29-5.88	0.55	2	16	*		

OR calculation; - = Non-estimable.

Table 2:Odd ratio for HBV and syphilis according to religion and education.

		[N=106]	HBV Status		OR	95% CI	p-value	Syphilis status		OR	95% CI	p-value
			Pos(15)	Neg (91)				Pos (4)	Neg (102)			
Religion	Muslim	62	10	52	1.5	0.145-4.74	0.14	3	59	2.19	0.22-21.74	0.45
	Christian	44	5	39	*			1	44	*		
Edu .Level	None	11	3	8	*			0	11	-		
	Basic	80	9	71	0.34	0.08-1.51	0.16	3	77	*		
	SSS	15	3	12	0.67	0.11-4.17	0.51	1	14	1.83	0.18-18.9	0.50

Pos: Positive; Neg: Negative; * : Baseline for OR calculation; - = Non-estimable.s

4.3 Risk Factors and Seropositivity of HBV and Syphilis

Table 2 shows the OR of the educational level and religious background of the truck drivers, and HBV and syphilis seropositivity. Compared to truck drivers who had no formal education, the truck drivers with basic education (OR: 0.34; 95% CI: 0.08 – 1.51) and Senior Secondary School Education (OR: 0.67; 95% CI: 0.11 – 4.17) were at decreased risk of HBV infection. None of the drivers who had no formal education tested positive for syphilis, however drivers with Senior Secondary School Education were more likely to be sero-positive for syphilis (OR: 1.83; 95% CI: 0.18 – 18.91) compared to drivers with basic education. Muslims were more likely to be at risk of syphilis (OR: 2.19; 95% CI: 0.22 – 21.74) and HBV (OR: 1.50; 95% CI: 0.47 – 4.74) compared to Christians.

Table 3 shows the ORs and the corresponding 95% CIs according to driving distance and behaviour characteristics of the truck drivers. Compared to short distance truck drivers, the long distance truck drivers were more likely to be at risk of HBV seropositivity (OR: 6.89; 95% CI 0.86 - 55.55). Truck drivers who spend more than 2 weeks in transit, accounting for 77.4% were more likely (OR: 4.76; 95% CI: 0.59 – 38.02) to be sero-positive for HBV as compared to those who spend less than 2 weeks in transit. Truck drivers who had multiple sexual partners were at 6.36-fold (95% CI: 1.35 – 29.79) more likely to be HBV sero-positive compared to those who had single sexual partners. Similarly, truck drivers who visit commercial sex workers (CSW) were 6.85- fold (95% CI: 0.88 – 52.89) more likely to have HBV infection compared to those who reportedly did not visit CSWs. Truck drivers who had no previous history of condom use accounting for 70.8% (75 of 106) were more likely to be at risk of HBV (OR: 1.33; 95% CI: 0.7 – 15.873), and syphilis (OR: 1.37; 95% CI: 0.14 – 1.37) infection compared to

those who had history of condom use. Interestingly, previous history of alcohol use was associated with decreased HBsAg (OR: 0.24; 95% CI: 0.06 – 1.33) and syphilis (OR: 0.46; 95% CI: 0.06 – 3.34) seropositivity.

		[N=106]	Hepatitis B Status		OR	95% CI	P-value	Syphilis status		OR	95% CI	p-value
			Pos (15)	Neg (91)				Pos (4)	Neg (102)			
Driving distance	Long Distance	75	14	61	6.89	(0.86-55.55)	0.03	1	74	1.25	(0.13-12.00)	0.65
	Short Distance	31	1	30	*			3	28	*		
Duration of Trip	>2 Weeks	82	14	68	4.76	(0.59-38.02)	0.11	4	78	-	-	0.46
	< 2 Weeks	24	1	23	*			0	24			
Marital Status	Yes	83	12	71	1.13	(0.29-4.39)	0.58	3	79	1.11	(0.12-10.50)	0.70
	No	23	3	20	*			1	22	*		
Knowledge of HIV/STI	Average	60	6	54	1.39	(0.15-1.39)	0.16	3	57			
	Good	46	9	37	*			1	45	2.37	(0.24-2.38)	0.41
Multiple sexual partners	Yes	59	13	46	6.36	(1.35-29.79)	0.09	1	58	0.25	(0.02-2.51)	0.21
	No	47	2	45	*			3	44	*		
Visit to CSW	Yes	4	2	2	6.85	(0.88-52.89)	0.04	0	4	-	-	0.83
	No	102	13	89	*			4	98			
Condom Use	No	75	12	73	1.33	(0.7-15.873)	0.12	3	72	1.37	(0.14-1.37)	0.63
	Yes	21	3	18	*			1	20	*		
Alcohol Use	Yes	34	2	32	0.24	(0.06-1.33)	0.07	2	32	0.46	(0.06-3.34)	0.40
	No	72	13	59	*			2	70	*		
Drug Use	Yes	6	0	6	-	-	0.74	0	6	-	-	0.73
	No	100	15	85				4	96			

.Table 3; Odds ratio for HBV and syphilis according to suggested risk factors.

*= OR baseline; Pos: Positive; Neg: Negative; *: Baseline for OR calculation; - = non estimable; N= Number of study participants.

CHAPTER FIVE

5.0 DISCUSSION

Migration and mobility have contributed significantly to the spread of HIV/AIDS, HBV, and syphilis infections due to the risky behaviour adopted by mobile population such as having multiple sexual partners, participation in commercial sex, poor condom use, and illicit drug use (McCree *et al*., 2010; Saggurti*et al.*,2008). Drivers and other mobile individuals act as bridge populations who spread the infections from high-risk to low-risk populations (Baishali *et al*., 2007; Pandey *et al*., 2008; Delany-Moretlwel *et al*., 2013). Knowledge of the prevalence, risk factors and distribution of HIV, HBV, and Syphilis among truck drivers is important for planning preventive measures and for the development of vaccination programmes. Further comparison of prevalence and risk factors among truck drivers and the general population in the same geographical area may provide basis for action, and changes in public health policy, education and in clinical practice. This study aimed at determining prevalence of HIV, HBV and syphilis infections among long distance and short distance truck drivers who use the road networks in Ghana.

This is believed to be the first study to determine the correlates of HIV, HBV and syphilis infections in long distance and short distance truck drivers using the road networks in Ghana; and demonstrates high prevalence of HBV and syphilis infections, and a considerable potential for the transmission of HIV among truck drivers in Ghana. This study found an overall prevalence rate of HBV and syphilis higher than that of the general population [13% for HBV and 0.6% for syphilis] (HIV Sentinel Surveillance Report, 2012 of the National AIDS/STI Control Program, Ghana Health Service). This data of high sero-prevalence of HBV and syphilis infections suggest a catastrophic future burden of disease and healthcare needs due to HBV and

syphilis among truck drivers (Ladep *et al.,* 2013). Both infections have similar route of transmission as HIV, sbut because HBV is more infectious it may act as a biological marker for HIV transmission (Matthews *et al*., 2014). Interestingly the sero-prevalence of HIV among truck drivers was lower than that of the general population(1.37% ; HIV Sentinel Surveillance Report, 2012 of the National AIDS/STI Control Program, Ghana Health Service). The reasons for this low prevalence could not be discerned from this study however, the small sample size may be a contributing factor. Another reason may be that drivers who were at higher risk of HIV due to their risky sexual behaviour did not consent despite the assurance of confidentiality. The sero-prevalence of HBV in this study was higher thanthe results of a similar study in India (5.7%) by Gawande *et al.,* (2000), but comparable to what has been reported in Ghana among prisoners (17.4%) (Adjei *et al*., 2006),15% and 11.59% among blood donors in Korle-Bu Teaching Hospital and Tamale Teaching Hoapital respectively (Ampofo *et al*., 2002; Dongdem *et al.,* 2009). The prevalence of syphilis among truck drivers in China as reported by Zhang *et al.,* (2013) was 0.68%, which was 5.6 folds lower than what was observed in this study. However the prevalence of syphilis reported in India and Burkina Faso among truck drivers were 9.3% and 21.9% respectively, which are higher than what has been reported in this study (Gawande *et al.,*2000; Lankoande *et al*., 1998).

This study therefore adds to the growing evidence that truck drivers represent a high-risk group for HIV, HBV and syphilis infections (Pandey *et al.,*2008; Azuonwu *et al.* 2011*;* Jackson *et al.,* 1997; Ramjee and Gouws, 2002; Gibney *et al*., 2002) however, further studies with a large number of truck drivers including non-Ghanaians will be necessary to draw a definitive conclusion.

On multivariate analysis, the independent determinants for HBV infection were being a long distance driver, having multiple sexual partners and previous visit to commercial sex workers. The higher sero-prevalence of HBV and syphilis among truck drivers is a major public health problem in view of the huge and growing numbers of individuals and co-operate bodies in the haulage and trucking business. The implications of these findings are obvious and raise concern about the need for preventive measures such as educational campaign and vaccination in the haulage and the trucking business. From the study it was also obvious that a great deal of unsafe sexual activity and alcohol use occurs in the trucking and haulage setting, and with the high rates of HBV and syphilis sero-positivity among truck drivers, it is clear that truck drivers have a substantial risk of contracting these infectionsas well as HIV while in the truck driving profession.

The risk of HBV and syphilis infections did not correlate with increasing age, however greater proportion of the drivers who tested positive for HBV were aged 31-40 years and above 50 years (Table 1). The reason for this disparity could not be discerned in this study; further studies with a large number of truck drivers who are in active service in the haulage and truck driving business will be necessary to draw a definitive conclusion.

Growing evidence suggest that long-distance drivers are at an increased risk of HIV, HBV, and syphilis infection (Gawande, *et al*., 2000; McCree *et al*., 2010; Mbugua *et al*., 1995; Stratford *et al*., 2000). Long distance truck drivers as reported by other studies were more likely to be at risk of HBV compared to the short distance truck drivers (table 3) in this study (Arulogun *et al*., 2011, Coffee *et al*., 2007; Pezzoli *et al*., 2009). This may be attributed to the multiple sexual relationship which has been defined as one of the major risk factors of HBV in this study, which is consistent with what has been reported in truck drivers, migrants and mobile population

elsewhere (Saggurti*et al.*, 2011;Coffee *et al.*, 2007; Pezzoli *et al.*, 2009). Interestingly most of the truck drivers who were seropositive for HBV and syphilis had no history of condom use (Table 3). This suggests a low risk perception and poor knowledge about the efficacy of condom in preventing the HIV, HBV, syphilis and other STIs transmission among the truck drivers (Arulogun, *et al.*, 2011; Sunmola, 2005). Based on these findings, it is likely that low risk perception among the truck drivers led to less protected sexual behaviors which render them more vulnerable to HBV and syphilis infections.

Recommendations

- Further studies with a large number of truck drivers including non-Ghanaians will be necessary to draw a definitive conclusion on the prevalence of HIV, HBV, Syphilis and other STIs in Ghana.
- Educational campaign on HIV, HBV, Syphilis and other STIs transmission and prevention is recommended in the truck driving population at their respective literacy level.
- Periodic screening and vaccination (for HBV) programmes is recommended to prevent onward spread of HIV, HBV, syphilis and other STIs in the truck driving population.

Conclusion

Although the results indicates low HIV prevalence among truck drivers in this study, the high prevalence of HBV and syphilis infection, coupled with the risky sexual behaviour of the drivers suggest an increase potential risk of HIV infection among truck drivers especially the long distance truck drivers. The long distance truck drivers are more likely to bridge the broader population through sexual contacts if strong preventive programs are not initiated immediately.

Limitations

The study participants are unlikely to be representative of the general truck drivers as participants were recruited from the Tema port only. None of the non-Ghanaians participated in the study despite the assurance that their participation would not affect their immigration status. The small sample size and inability to collect information on sexual practices prior to the truck driving business, and under reporting of sexual activity, alcohol use and other risky behaviors limited the study. The study was based on self-reporting which could be biased by the truck drivers recall ability.

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APPENDIX I

INFORMED CONSENT FORM

UNIVERSITY OF GHANA MEDICAL SCHOOL

COLLEGE OF HEALTH SCIENCES

Department of Pathology

P. O. BOX 4236

Korle-Bu,

Accra, Ghana

Participant ID Number:

Participant Name:

PARTICIPANT INFORMATION AND CONSENT FORM

This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate.

Title of Research: Prevalence of Human Immunodeficiency Virus and Sexually Transmitted Infections Among Long Distance Truck Drivers in Ghana.

Name(s) and affiliation(s) of researcher(s): This study is being conducted by ; Rev. Israel Nicholas Nii-Trebi Department of Medical Laboratory Sciences, School of Allied Health Sciences Medical School, College of Health Sciences, Korle Bu, Accra.

.

You have been invited to take part in a research study on the commonness of HIV and STI and risk factors among truck drivers in Ghana. The researcher will first explain the study and will ask you to participate by signing this agreement which states that the study has been explained, that your questions have been answered and that you agree to participate. The researcher will explain the purpose of the study. He or she will explain how the study will be carried out and what you will be expected to do.

The researcher will also explain the possible risks and benefits of participating in the study. You should ask the researcher any questions you have about any of these things before you decide whether you wish to take part in the study. Please read the form and talk to the researcher about any questions you may have. Then, if you decide to participate, please sign and date this form in front of the person who explained the study to you. You will be given a copy of this form to keep.

Background: Human immunodeficiency virus (HIV) has now spread to all parts of the world and the rates of infection are found to be particularly high in those cities with a high number of truck driver drivers and migrant workers. The relation between haulage business and truck driving profession and the high transmission of HIV/sexually transmitted infections (STIs) have been known for several years. Studies from Africa and Asia have demonstrated a link between haulage business and truck driving profession and multi-partner sexual networking as well as the prevalence of HIV/STIs. Within these regions, there is growing evidence that the high rates of HIV/STIs among haulage business and truck driving profession largely occur by sexual contact with HIV/STIs-infected women, often sex workers. While away from home; the infected men then transmit the virus to wives and other sex partners in their place of origin. Consistent with this pattern, there is growing evidence that long-and short-distance truck drivers and assistants and other mobile individuals may act as bridge populations who spread the infections from high to low-risk populations and regions and urban to rural areas.

Purpose(s) of research: The purpose of this research is to find out how common HIV/STIs are among truck drivers and assistants at Tema and Takoradi sea ports, guest houses, hotels and rest stops; to describe the reasons or causes for the spread of these infections among the long-and short-disatnce truckdrivers and their assistants; to offer them counseling and testing as well as treatment for these infections; to inform and educate them of the causes of spread of these

infections particularly through unprotected sex with commercial sex workers and sharing of needles and syringes. They shall also be educated on measures which could be taken to prevent the spread of these infections.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research: To find answers to the issues raised above, when the team of researchers come to your "community" on a visit, you will be asked a few questions to obtain information. Some of the questions are educational background, marital status, drug/alcohol use, smoking history, length of stay, place of origin, sexual partners, HIV/STI status and knowledge/attitude towards HIV/AIDs. These are routine questions asked in studies like the one you have been invited to take part.

In addition you will be asked to provide blood sample (about 1 tablespoon). A trained biomedical scientist will insert a needle into your vein in one of your arms and draw some blood. This may cause pain, discomfort and bruising at the site of needle insertion. Your blood sample will be tested for HIV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* organisms and Syphilis. Urine and sputum samples will also be taken from you for diagnosis of the organisms mentioned above. You will be taken through individual pretest counseling sessions before data and specimen collection and you will be requested to return in four (4) weeks to receive testing results. The researchers will notify and advice you on referrals for treatment or management in cases where any of these viruses and bacterial infections are detected. In total we expect to recruit 600 long- and short-distance truck drivers and assistants migrant workers into this study.

Risk(s): By participating in this research, you are likely to have some uneasiness of questioning, physical examination and a slight pain from collection of blood. The procedure of blood drawing for laboratory test sample can be associated with rare risks including bruising, bleeding or skin infection. Before blood collection, your arm will be cleaned and a new hollow needle/plastic tube will be placed in your arm to take the blood samples. When the needle goes into a vein, it hurts for a short time. The study team will try and decrease the chances of those risks/dangers happening, but if an untoward event happens, you will be immediately managed by a study physician and will be provided with free medical care in hospital.

Benefits(s): There are no direct benefits to the study participants. However, as part of the objectives of the goal, we hope that the data generated will form the firm basis to find appropriate social interventions that will influence behavioural change toward the risk of HIV/STIs infection.

Confidentiality: All of your records from this study will be treated as confidential medical records. The medical results with participant's name and identifying information will only be available to me, the principal investigators and the study supervisors. Information collected on study forms and database will be given code numbers. No name will be recorded on the research forms or in the electronic database. The findings of this study may be reported in publications or reports but your name will not be mentioned. However, as part of my responsibility to conduct this research properly, I may allow officials from the ethics committees or the safety committee to have access to your records.

The blood and urine samples will be stored in an ice-chest and transported to the laboratory (Department of Microbiology, University of Ghana Medical School., Korle Bu). The remaining blood samples will be destroyed three (3) years after all study analyses have been completed. All the blood samples will be labeled with a code so that your identity is not revealed to the people who do the tests.

Voluntariness: You do not have to take part in this research if you do not wish to do so, and this will not affect your truck driving and assistantship or mobility status. Taking part in this study should be out of your own free will. You are not under obligation to do so. Research is entirely voluntary.

Alternatives to participation: This study does not involve the administration of investigational drugs or use of new curative procedures.

Withdrawal from the research: You may choose to stop participating in this research at any time that you wish to, without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you before you choose to withdraw may be modified or used in analysis reports and publications without your name being mentioned. These cannot be removed anymore. I do promise to make effort to comply with your wishes as much as practicable.

Contact(s): If you have any questions you may ask those now or later. If you wish to ask questions later, you may contact:

Rev. Israel Nicholas Nii-Trebi,

Department of Med. Lab. Sciences,

School of Allied Health Sciences,

Korle Bu, Accra, Ghana.

Tel: 0249-107-110

Prof. Andrew Anthony Adjei,

Department of Pathology,

University of Ghana Medical School,

Korle Bu, Accra, Ghana.

Tel: 020-813-5979; 0274-430-256

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my migration status in the community.

Signed by……..

Date……..

Place…….

If illiterate

Signed by the investigator…….

In the presence of an independent literate witness……

(where possible this person should be selected by the participant)

Date……..

Place…….

APPENDIX II

SURVEY QUESTIONNAIRE

1. Age:

2. Sex: (a) M (b) F

3. **Educational background:** (a) None (b) Primary (c) J.S.S. (e) S.S.S.

   (f) M.S.L.S. (g) Secondary (h) Tertiary

4. **Religion**

   (i) Christian (ii) Muslim (iii) Other (iv) None

5. **Marital status**: (a) Married (b) Single (c) Widowed (d) Divorced

   (e) Other

   If married, please state the type of marriage

   (a) Monogamous (b) Polygamous (c) Other

   (i) Do you have any sexual partner besides your wife (ves)? (a) Yes (b) No

(ii) If yes state reasons……

(iii) If not married, do you have any sexual partner(s)?

(a) Yes (b) No

(iv) If yes, how many?

(a) One (b) Two (c) Three (d) Four

6. Do you have children? (a) Yes (b) No

(i) If Yes, how many children?

(a) One (b) Two (c) Three (d) Four (e) > Four

7. Place of Origin:

(IF GHANAIAN) Where did you live?

.....

(IF NON-GHANAIAN) Where did you live prior to migrating to this city/town in Ghana?

(i) Mali (ii) Niger (iii) Burkina Faso (iv) L'Cote d'Voire

(v) Togo (vi) Other

8. **Occupation:** (i) Truck driver (ii) Driver Assistant (Mate)

(i) Are you long distance truck driver? (a) Yes (b) No

(ii) Are you short distance truck driver? (a) Yes (b) No

9. At what age did you enter the truck driving profession?

(i) Year(s)

10. How many trips do you make per month?

(i) One (ii) Two (iii) > Two

11. How long do you stay from home in the past year?

(i) Week(s) (ii) < 6 Months (iii) > 6 Months

12. (i) If Driver Assistant

(ii) Are you long distance truck driver assistant? (a) Yes (b) No

(ii) Are you short distance truck driver assistant? (a) Yes (b) No

13. At what age did you enter the truck driving assistant profession?

(i) Years

14. How many trips do you make per month?

(i) One (ii) Two (iii) > Two

15. How long do you stay from home in the past year?

(i) Week(s) (ii) < 6 Months (iii) > 6 Months

16. **Alcohol use:**

(i) Do you drink alcohol?

(a) Yes (b) No (c) Never

If Yes, are you (i) Regular/Active drinker (ii) Binge drinker

(ii) How much do you drink?

(a) Per day……...

(b) Per week……

17. If regular/active, For how long have you been drinking alcohol?

(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years

(e) > 10 years

18. **Smoking History:** Do you smoke cigarettes?

(a) Yes (b) No (c) Never

If Yes, are you (i) Regular/Active smoker (ii) Occasional smoker

19. If regular/active, For how long have you been smoking cigarettes?

(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years

(e) > 10 years

20. How many pack(s) of cigarette do you smoke weekly?

(a) one (b) two (c) three (d) four (e) > five

21. **Tobacco Chewing History:** Do you chew tobacco?

(a) Yes (b) No (c) Never

If Yes, are you (i) Regular/Active (ii) Occasional

22. If regular/active, For how long have you been chewing tobacco?

(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years

(e) > 10 years

23. How many pack(s) of tobacco do you chew weekly?

(a) one (b) two (c) three (d) four (e) > five

24. **Drug use:** Do you use drugs? (a) Yes (b) No

If Yes, are you (i) Regular/Active drug user (ii) Occasional drug user

25. If regular/active, For how long have you been using drugs?

(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years

(e) > 10 years

26. What type of drug do you use? (a) Marijuana (b) Heroin (c) Cocaine

(e) Crack (f) Diazepam/Valium (h) Others (specify)……

27. Mode of intake of drugs; (a) Sniff (b) Smoke (c) IV (d) Oral

28. **Sexual behavior, knowledge and attitudes of HIV/STI**

Do you have a sexual partner besides your wife (ves)?

(a) Yes (b) No

29. If Yes, how many sexual partners do you have?

(a) 1 (b) 2 (c) 3 (d) 4 (5) >5

30. Is/Are your sexual partner(s) a Male or Female? (a) Male (b) Female

31. Have you ever visited a commercial sex worker?

(i) Yes (ii) No

32. Have you visited a commercial sex worker in the past 6 months?

(i) Yes (ii) No

33. If Yes, did you had any sexual contact/intercourse with her?

(a) Yes (b) No

34. How may commercial sex workers have you visited in the past 12 months?

(a) 1 (b) 2 (c) 3 (d) 4 (5) >5

35. Do you often stop for sex with a commercial sex worker?

(a) Yes (b) No

36. Do you use condoms during sex?

(a) Always (b) Sometimes (c) No

37. Have you ever heard of HIV or AIDS? (a) Yes (b) No

38. Are you aware there are some diseases that can be passed through sexual contact/intercourse (a) Yes (b) No

39. Can the correct use of a condom every time a person is engaged in sexual contact or intercourse help protect him/her from HIV infection?

(a) Yes (b) No

40. Can a person contract HIV infection through the sharing of food with an HIV infected person? (a) Yes (b) No

41. Can a person contract HIV infection by shaking the hand of an HIV infected person? (a) Yes (b) No

42. Can a person contract HIV infection through injections with a needle previously used by someone else? (a) Yes (b) No

43. Can a person contract HIV infection through receiving blood (blood transfusion) from HIV infected individual? (a) Yes (b) No

44. Have you received a blood transfusion before? (a) Yes (b) No

45. Can a person protect himself/herself from HIV by abstaining from sexual contact or intercourse especially with multiple partners or commercial sex workers? (a) Yes (b) No

46. Can a healthy apparently healthy looking individual be infected with HIV?

(a) Yes (b) No

47. Based on your current understanding of HIV, is there medication that can cure HIV? (a) Yes (b) No

48. Can the HIV infection in a pregnant woman be passed on to her unborn child?

(a) Yes (b) No

49. Have you ever undergone HIV testing in the past 6 months or since you commenced the truck driving or assistantship profession?

50. If No, Why? Give reason(s)

51. Have you ever heard of STIs? (a) Yes (b) No

52. Have you experienced any of the following symptoms in the past 12 months?

(a) Genital ulcers (b) Swelling in groin (c) Itching in genital area

(d) frequent painful urination

53. Have you ever been treated for STIs diseases?

(a) Yes (b) No (c) N/A

54. Which of following STIs diseases did you receive treatment?

(a) Gonorrhoea (b) Syphilis (c) *Chlamydia trachomatis* infection

55. Have you had a tattoo or tribal mark done on your body?

(a) Yes (b) No

APPENDIX III

REAGENT AND PROCEEDURE FOR HIV TESTING

Genscreen™ULTRA HIV Ag-Ab.

Reagents

- Reagent 1 (R1): Microplate
- Reagent 2 (R2): Washing solution (20x concentrate)
- Reagent 3 (R3): Negative Control
- Reagent 4 (R4): HIV Ab positive control
- Reagent 5 (R5): HIV Ag positive solution
- Reagent 6 (R6): Conjugate 1
- Conjugate 2 working solution: reagent 7a (R7a) [Lyophilised Conjugate vial] + Reagent 7b (R7b)[Conjugate Diluent vial]
- Enzyme development solution: Reagent 8 (R8) [Substrate Buffer] + Reagent 9 (R9) [chromogen]
- Reagent 10 (R10): Stopping solution

3.1.2 Procedure

- The washing solution for use was prepared by doing 1 :20 dilution using distilled water (40ml of R2 to 760ml of distilled water)
- The conjugate 2 working solution was prepared by mixing R7a and R7b.

- The carrier tray and the strips (R1) were unwrapped from the protective pouch and labeled A,B,C,D,E,F, G,H, and I.
- 25ul of conjugate 1 (R6) was pipetted into each well.
- 75ul of HIV Ag positive control (R5) was pipette into well A1.
- 75ul of HIV Ab control (R4) was pipetted into well B1.
- 75ul of negative control (R3) was pipette into well C1, D1 and E1
- 75ul of specimen 1, 2, 3, and 4 was pipetted into well F1,G1, H1, and I1.
- The mixture was homogenised by shaking the microplate after the pipetting step.
- The microplate was covered with adhesive film to ensure a tight seal, after which the mixture was incubated at 37^{o}C for 1 hour in thermostat-controlled water-bath.
- The adhesive film was removed and contents of all the wells were aspirated into a container for biohazardous waste (containing sodium hypochlorite).
- A minimum of 0.370ml of washing solution was added into each well to soak for at least 30 seconds and aspirated again. The washing step was repeated twice. *(The residual volume must be lower than 10ul, if necessary the plate can be dried by turning it upside down on absorbent paper).*
- 100ul of conjugate 2 solution was pipetted into all wells after the washing step. *(The conjugate must be shaken before use.)*
- The plate was covered with new adhesive film and incubated for 30 minutes at room temperature (25^{o}C).
- The adhesive film was removed and, the wells emptied by aspiration, after which washing was done 5 times.

- 80ul of prepared substrate solution was pipette into all wells and incubated in the dark at room temperature for 30 minutes.
- A stopping solution (R10) of 100 µl was added to each well, and the optical density was read at 450/620-700nm using a plate reader within 30minutes of stopping the reaction. *(The stripes must always be kept away from light before reading. The bottom of the plate must be wiped before the reading).*
- The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/or HIV-2 was determined by comparing the absorbance measured for each sample to the calculated cut-off value.

One Step Anti-HIV (1 and 2) Tri-line Test

Proceedure

- Plasma samples were thawed at room temperature for 30-60 minutes.
- Cards were removed from its rapper when the samples attained room temperature.
- Using micropipette provided by the manufacturer, 3 drops of plasma was pipette in the sample well of the test card.
- Results were read after 10 minutes.

OraQuick® ADVANCE Rapid HIV-1/2 antibody test

Procedure

- Places kits on bench to bring to room temperature.
- Samples thawed at room temperature for 30-60 minutes.
- 40µl of plasma was pipetted into test well and left on the work bench for 20 minutes after which the results were read.

APPENDIX IV

Reagents and Procedure for Hepatitis B surface antigen (HBsAg) detection

COBAS Elecsys e411 HBsAg II quant

Reagents

- HBSAG-QN
    - Streptavidin-coated microparticles
    - Anti HBsAg-Ab-biotin
    - Two biotinylated monoclonal anti- HBsAg antibodies
    - Anti- HBsAg-Ab-Ru (bpy) contains;
        - Monoclonal HBsAg antibody (mouse),
        - Polyclonal HBsAg antibodies (sheep) labelled with ruthenium complex.
        - Phosphate buffer

- HBSAG-QN Cal 1
    - Negative calibrator 1
- HBSAG-QN Cal2
    - Positive calibrator 2
    - HBsAg approx, 0.5IU/mL in human serum
- HBSAG-QN Dil HepB
    - Human serum negative for HBsAg and Anti-HBs

Procedure

- Test samples (plasma) were pipette into cuvettes and loaded into the COBAS e411
- The reagents were loaded onto the machine and as the 'start' command was issued the samples were analyzed qualitatively.

Protocol for detecting HBsAg

- Plasma samples were thawed at room temperature for 30-60 minutes.
- Cards were removed from its rapper when the samples attained room temperature.
- Using micropipette provided by the manufacturer, 3 drops of plasma was pipette in the sample well of the test card.
- Results were read after 10 minutes.

APPENDIX V

Procedure Syphilis screening

Qualitative Procedure for SYPHILIS TPHA TEST (DebenDiagnostic Ltd, UK.).

- All reagents and specimens were brought to room temperature prior to use.
- Four wells were used for each specimen
- Diluents were disperse into the microtitration plate as follows
    - 25µl in rows 1,3 and 4 and 100µl in row 2
- 25ul of each sample was dispensed into the wells in row 1and mixed.
- The mixture (25ul) was transferred from row 1 to row 2 mixed well and transferred to row 3.
- 25ul of the mixture was discarded from row 3, and 25ul of the mixture in row 2 was pipette into row 4 and well mixed.
- 25ul of the mixture was discarded from row 4, and 75ul of well mixed test cells was added to row 4 and mixed gently.

- The mixture in the wells were incubated at room temperature for 45 to 60 minutes after which wells were examined for agglutination (The final dilutions in row 3 and 4 are 1/80).

SYPHILIS ANTI-TP CARD (PMC Medical Pty. Ltd)

Procedure

- Samples were thawed at room temperature for 60 minutes
- A drop of serum was put into the test well using the micropipette.
- Test cards were incubated at room temperature for 20 minutes after which results were read.