SEROPREVALENCE OF *MYCOBACTERIUM TUBERCULOSIS* ANTIBODIES IN APPARENTLY HEALTHY INHABITANTS OF GABIA AND CHINKE COMMUNITIES OF PLATEAU STATE, NIGERIA.

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SEPTEMBER, 2018.

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BEING A THESIS, IN THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF PHYLOSOPHY IN MEDICAL LABORATORY SCIENCE (IMMUNOLOGY/ IMMUNOCHEMISTRY) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SEPTEMBER, 2018.

CERTIFICATION

This is to certify that this work on a Sero Prevalence of *Mycobacterium tuberculosis* Antibodies among Apparently Healthy Inhabitants of Gabia and Chinke communities of Plateau State, Nigeria was carried out by Ashi, Robert Rigye, in the Department of Medical Laboratory Science of University of Benin.

_____	_____
PROF. I.N. IBEH	**DATE**
Supervisor	
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DR. F.O. AKINBO	**DATE**
Head of Department	
_____	_____
EXTERNAL EXAMINER	**DATE**

DEDICATION

This study is dedicated to my family for the prayers, patience and moral support given to me in the course of the study

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I sincerely express my profound gratitude to the Almighty God for His constant grace upon me in the course of this study.

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study. Lastly, my sincere gratitude to the participants for availing themselves for the research.

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ABSTRACT

Tuberculosis continues to be a major public health problem globally. The aim of the study was to detect *Mycobacterium tuberculosis* antibodies in apparently healthy individuals in Gabia and Chike communities of Bassa Local Government Area of Plateau State, Nigeria. Out of the 228 respondents of the questionnaire served, 205 (89.9%) acknowledged that they heard about tuberculosis. Two hundred and twenty eight (228) samples of human serum were examined for *Mycobacterium tuberculosis* antibodies using a one step TB IgG/IgM flow chromatographic immunoassay. The prevalence of *Mycobacterium tuberculosis* in the study areas was 19.7%. The prevalence in the inhabitants of Chinke was 10.5% while that of Gabia was 9.2%. The females in the study population had a prevalence of 12.3% while the males had 7.5%. The prevalence was more in individuals in the age brackets of 40 – 49 years (7.9%) and 50 – 59 years (3.5%), while individuals within the age brackets of 70 – 79 years in Chinke and 80 – 89 years in Gabia did not present with *Mycobacterium tuberculosis* antibodies. Considering active and inactive (Latent) infection, a total of 18 (7.9%) individuals had IgM antibodies, with males constituting 4.7% and females with 3.1%, indicative of recent infection while 27 (11.9%) had IgG antibodies showing previous infection. Considering active and inactive (Latent) infection, a total of 18 (7.9%) individuals had IgM antibodies, with males constituting 4.7% and females with 3.1%, indicative of recent infection while 27 (11.9%) had IgG antibodies showing previous infection. The sputum of IgM positive cases were further examined for acid fast bacilli and the result was statistically significant ($p>0.02$), showing 2.6% with 2+, 5.2% with a +. The high prevalence of *Mycobacterium tuberculosis* in the study areas is worrisome as the positive individuals are not only at risk of having full blown tuberculosis in the future but are also sources of spreading the bacteria to the populace. Though several TB eradication programmes

have been mounted globally, the disease still present serious health challenges especially in developing countries.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Mycobaterium tuberculosis (MTB) causes the disease called tuberculosis (TB). Tuberculosis has plagued mankind for decades yet is classified as an emerging infectious disease because it's prevalence in the human population increases continually. And the problem has been further exacerbated by the increasing prevalence of drug resistant and extensively drug resistant TB strains (Gary *et al*., 2013).

The disease is marked as the leading cause of mortality globally and the infection is most prevalent in developing countries. Tuberculosis is primarily the disease of the lung and dissemination is dependent upon productive infection of this critical organ (Cooper, 2009). Though, it is known to affect majorly the lungs but can also affect other organs like the bone and brain (Jitendra *et al*., 2013).

The World Health Organization (WHO, 2011) estimated that out of the 8.7 million incident cases of tuberculosis, 59% occurred in Asia while 26% occurred in Africa. This implies that only about 15% occur in the developed world. It is undisputable that the mode of transmission of TB is almost exclusively airborne. The aerosol that emanates from an infected individual can easily cross to a susceptible person who is at close range. Some studies have shown that people in the same household

have higher risk of contracting the disease (WHO, 2011). It is important to note that other casual contact due to overcrowding can play a significant role in the transmission of the disease.

Grzyboswski *et al* (1975) observed that the risk of infection with TB depends on factors such as the relative virulence of the strain, the intensity of exposure to the aerosol droplet, the susceptibility and the immune status of the victim as well as dusty environment.

Lifestyle also has been attributed as a factor that can increase the chances of developing the disease. According to Lam *et al* (2004), tobacco/cigarette smoking can lead to four-fold chances of developing clinical TB. This is due to the habit of sharing one stick of cigarette by two or more persons. The air indoor or outdoor surrounding an individual infected with pulmonary TB can also be polluted which can lead to increase rate of transmission. The Sharing of accommodation with an infected person can also lead to serious infection. Out of 65 inmate screened in a prison cell with an infected person, 38 inmates were positive to tuberculin screening test (TST) (Lawrence and Christian, 2010).

The sero prevalence studies carried out by Awujo *et al* (2008) in the South Eastern State of Nigeria gave 6.67% positivity and this was considered a significant outcome. That was no doubt a worrisome outcome that was not only a future trigger to full blown TB to the carrier of the bacteria but a potential source of

infection to the populace. Tuberculosis is generally associated with poverty and social isolation, poor nutritional status and deprivation. They are linked to the increased incidences of the disease in the study group. The study conducted on Tajikistan prison inmates revealed that the increased incidence of TB was due to the poor facilities and low access to supplemental nutrition (Daniel *et al*., 2014). Since poverty and poor nutrition is highly associated with the risk of developing TB, it further explains that unavailability of supplemental nutrition can affect the treatment of TB. The study carried out by Blesson *et al* (2016) revealed that patients provided with adequate nutritional support recorded low unsuccessful treatment outcome of 9% than those not provided nutritional support with 21%.

In view of the fatality of TB, strict measures are required to control the spread. Earlier victims were isolated but not nowadays. Therefore, precautions such as ; covering of mouth and nose during coughing and sneezing, proper disposal of materials used by the victims, avoid sharing beds and room with infected persons and giving vacation to infected person who are public workers are good prescriptions to strictly adhere (Jitendra *et al*., 2013).

Tuberculosis has a ravaging effect on any given community as such it is imperative to create the awareness in order to change the behavior and attitude of individuals. The study conducted in a Saudi University on 530 students revealed that only 46.7% had the knowledge on tuberculosis. This low level of knowledge on the

disease was considered a major public health hazard (Alsalem *et al*., 2015). Tuberculosis is a preventable and curable disease if detected and treated early. It has a low prevalence in developed nations and this means people living in such countries with low prevalence need not take precautions to prevent the infection. However some measures of prevention are suggested for those living or travelling to areas and countries that have a high prevalence of the infection. Recent studies showed that lack of awareness contributes immensely to the transmission of TB. Public awareness or education should be carried out on the epidemiology of the disease, preventive measures and treatment. Ssakher *et al* (2016) reported a high number of "don't know" during cross sectional survey among 1008 residents of Makkah and Jeddah in Saudi Arabia. According to their report within the population across pilgrimage areas of Saudi Arabia, knowledge was lacking concerning TB transmission, the causes of the disease and means of prevention and the success of treatment.

Another survey by Rana *et al* **(**2015) among non-medical university students in Bangladesh revealed an insufficient general knowledge on TB. The recommendation was the need to carry out health education programmes as the panacea to create awareness on the disease.

Ignorance on TB is the major factor militating against the eradication of the disease globally. There is a gap in knowledge and attitude on TB and its transmission and

this was further confirmed in the study carried among high school students in Ethiopia which revealed the lack of knowledge on Tb and its transmission.

Summarily tuberculosis bacteria spread from one person to another through the air and the increase in transmission is facilitated by low level of knowledge about the disease generally. It is important to be note that the air surrounding susceptible individuals must first be contaminated by someone that has tuberculosis of the lung or throat to effect transmission. The bacterial is usually raised into the air when the person coughs, sneezes, speaks or sings. Therefore, tuberculosis is not spread through hand shake, sharing of food and drinks, sharing tooth brushes or kissing and or sharing of bed and clothes.

1.2 Justification

Tuberculosis is a global disease that has affected a large proportion of the world population. This is majorly due to ignorance associated with poverty. Those who suffer from this disease are mostly the economically and socially low class individuals (Awujo *et al*., 2008). Because of the little or no awareness on this disease, the eradication of the disease has become difficult. The two communities under study have about 80% of the population at the low economic and social class. Occasionally cases of death with associated signs and symptoms of tuberculosis were recorded. Thus, investigation became pertinent to detect the

presence of current and previous Mycobacterium tuberculosis infections and to ascertain the level of infection by using a fraction of the population in each case as participants.

1.3 Aim of Study

The aim of the study was to determine the prevalence of *Mycobacterium tuberculosis* antibodies among the inhabitants of the Gabia and Chinke communities.

1.4 Objectives

1. To determine the presence and frequency of *Mycobacterium tuberculosis* antibodies among participants.
2. To determine the influence of age and sex on the prevalence of TB anibodies in the study areas.
3. To determine active and passive infection.
4. To determine the level of knowledge and awareness.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology

Tuberculosis is known to be one the leading causes of mortality and morbidity across age groups globally. The highest occurrence is found in the developing countries. The causative agent, Tubercle bacilli can be transmitted from one individual to another through the air and only those who are immunosuppressed are the chief victims. Studies showed that the household index indicates that closeness and overcrowding living condition increases the infection rate. This was evident in the studied carried out in South Delhi India (Jitendra *at al*., 2013). Active transmission also occurs more frequently in small household as well as crowded places such as markets in countries with high incidence of TB.

Molecular epidemiologic studies have shown that there are distinct differences in the disease presentation and population demographics in low TB incidence and high TB incidence countries. The incidence is highest among young adult with most cases resulting from recent episodes of infection or re-infection in several African and Asian countries. In contrast, the highest proportion of cases occur in older adults or among immigrants from high TB incidence countries in low incidence countries of Western Europe and North America (**Mathema *et al*., 2008**).

An epidemiologic studies carried out in 2004 also revealed that the risk of contracting TB increases with close contact with sputum-smear-positive patient, (Lam *et al*., 2004).

The current TB epidemic is being sustained and fuelled by increasing resistance of MTB strain to the most effective first line anti TB drug and HIV and its association with Active TB disease, (WHO, 2009).

2.2 Transmission

Tuberculosis is transmitted from an infected person to a susceptible person in microscopic airborne particles called droplet nuclei of about 1 – 5micro meter in diameter which contains the bacteria. When an infected person coughs, sneezes, laughs, shouts or sings, the droplets are released and remain suspended in the air for several hours because of their minute size (Hingley-Wilson *et al*., 2003).

When a susceptible person breathes in the bacillary aerosol, the bacteria passes the defenses of the bronchi unharmed and penetrates into the terminal alveoli of the lungs. At that stage, they are engulfed by both immune cell (macrophages and dendritic cells) and non phagocytic cells. In the early phase of the infection, the bacteria cells, internalized by phagocytic immune cells, replicate intracellularly and the affected immune cells may cross the alveolar barrier to cause systemic dissemination. This process occurs prior to the development of the adaptive

immune responses. When this happen the bacteria develops a protective niche to escape elimination by the immune cells, which makes them persist indefinitely (Tufariello *et al*., 2003).

From there, they can move through the blood to other parts of the body. Pulmonary TB is infectious because the bacteria are easily spread to other people while TB in other parts of the body, such as the kidney or spine, is usually not infectious (CDC, 2009). If a person has confirmed TB or is suspected of having TB, the best way to stop transmission is through immediate isolation. The study carried out in Aba Federal Prison on the inmates, showed that out of the 168 diagnosed, 10% revealed that they were infected while in the prison (Lawrence and Christian, 2010). The infection rate was attributed to the overcrowded new environment. The risk of infection is dependent on such factor as the closeness of contact, the bacillary load inhaled and the immune status of the susceptible new host (Ahmad and Mokaddas, 2009).

2.3 Infection and Pathogenesis

The pathogenesis of TB is the product of the interaction of bacterial virulence and host resistance. Infection occurs when a susceptible person inhale a droplet containing a high load of tubercle bacilli. The droplet migrates from point of reception to reach the terminal alveoli of the lungs (Harries and Dye, 2006). While

in the lungs the phagocytic cells mostly the macrophages and dendritic cells ingest the bacteria cells to destroy them. In most infected persons, the response of the immune system destroys most of the bacteria cells. The few cells multiply in the immune cell and are released when the immune cells die and the bacteria cells are disseminated to other tissues through the bloodstream. At this point, latent tuberculosis is created and the bacteria are found in tissues such as kidney, brain, bone, lymph node etc.

However, the dissemination prepares the immune system for further reaction (ALA, 2009). Within weeks after infection, the immune system is usually able to halt the multiplication to mop out the infection thereby preventing further progression. At this point most people usually recover completely from the infection. However, in some people the tubercle bacilli overcome the defenses of the immune system and begin to multiply, resulting in advancement to active TB disease. This process may occur shortly after infection or several years later (Goodman and Lipman, 2008; **NIAID, 2009**).

2.4 Immune Response

When the bacteria bacilli reaches the alveoli, the resident macrophages secretes inflammatory cytokine and chemokines that serves as signal of infection to the body. The substances produced attract other non specific immune cells toward the site of infection. The immune cells include monocytes, neutrophils and lymphocytes. It was also reported that the pathogen in turn engages strategies that tends to dampen the innate immunity of the invaded alveolar macrophages against their elimination (Harding and Boom, 2010). The resistance to bactericidal mechanism prevents phagosome - lysosome fusion of macrophages, leading to multiplication of the pathogen and eventual necrosis of the macrophage (Wolf *et al.,* 2008).

The bacilli released from the damaged macrophage are engulfed by new sets of macrophages but to no avail. The immune cells that often survive the resistance of the pathogens are the dendritic cells. They are able to convey the pathogen to the lymph nodes and prime T – cells (CD4 and CD8) (Flynn and Chun, 2003).

Flynn and Chun (2008), added that the specific immune response produces prime T cells which migrates to the infection site as guided by the chemokines and cytokines produced by the infected macrophages. The accumulation of the immune cells causes the formation of granuloma at the site of infection. The granuloma is a cascade that walls off the pathogen from the rest of the lungs to prevent the spread of the infection. At this point, the CD4 T- cells produce Interferon – Y (IFN- Y)

that recognizes the infected macrophages to destroy them. This is the process that halts the progression of the infection. However, a few bacteria usually survive the harsh condition within the granuloma and remain dormant. This is the onset of Latent tuberculosis, a condition that does produce signs and symptoms of the disease.

2.5 Active Tuberculosis and the Immune System

Tuberculosis becomes active when the immune system becomes weak and cannot annihilate the bacilli. The organism continues to grow and increase in number to overwhelm the system. Active TB can emerge from Latent TB or from a new episode of the disease. Two groups of people are known to be victims of Active TB; babies and young adults. The second group comprises of those already infected with other diseases that have suppressed the immune system. Such diseases include; HIV, Diabetis mellitus, Cancer, Silicosis, kidney disorder etc.

The evidence of Active TB is the expansion of MTB specific Tcell as found in the peripheral blood (Boadella *et al*., 2012).

2.6 Risk Factors

It is important to bear in mind that tuberculosis infection can be acquired through close and casual contact. The major risk factors associated with tuberculosis infection includes;

i. Relative virulence of the strain of the bacteria.
ii. Intensity of exposure to the infectious tuberculosis case.
iii. Susceptibility and immune status of the exposed individual (Grzyboswski *et al*., 1975)

Other findings have shown that cigarette/tobacco smoking has great effect on innate and adaptive immunity, and those with such life style are highly susceptible to tuberculosis infection at the slightest exposure (Lam *et al*., 2004).

The epidemiologic studies carried out by Lam *et al* (2004) revealed that the risk of contracting tuberculosis increases with close contacts of sputum-smear-positive patients and that the prevalence of clinical disease among intimate contact of tuberculosis cases is high. Overcrowding is a major factor that encourages the spread of tuberculosis in any community. The environment in the prisons, police detentions, military camps, Institutions of learning, markets squares etc of developing countries are most favourable for the dissemination of Microbacterium tuberculosis and the progression of tuberculosis.

The study carried out by Blesson *et al* (2016) on the relationship between nutrition and tuberculosis treatment in West Bengal India showed that with adequate

nutritional support to tuberculosis patients living below poverty line, there is great success compared with those without nutritional scale up.

In addition tothe above**,** here is direct relationship between nutrition and susceptibility because according to Samue *et al* (2016) individual with poor nutrition are highly susceptible to tuberculosis and undergoing treatment need constant balance die boost their immunity.

2.7 Tuberculosis Awareness

The awareness on *Mycobacterium tuberculosis* infection has not reached a satisfactory level globally to serve as a mean to reduce spread. Several studies conducted among communities and educated population revealed that a sizeable global population is ignorant of the disease, especially those with little or no knowledge of science. A study carried out among students of King Saud University concluded that the level of awareness was low among the students studying humanity (Sultan et al., 2015).

It was also discovered that there was a gap in knowledge and attitude on tuberculosis and its transmission among high school students in a town located at Southern Ethiopia which further confirmed that public knowledge on tuberculosis and its transmission is very low worldwide. (Hibstu *et al*., 2016).

Nigeria as a nation has a similar situation of lack of adequate information on tuberculosis infection and treatment. Therefore, for the above target to be achievable in Nigeria using the current passive detection strategy, the people at the community level should be empowered with adequate knowledge of the growing burden of the disease and accessible potentials for cure. Furthermore, Nigeria is a very populous nation that is divided into several administrative units (States) with varying ethnicity, socio-economic and health indices. Unfortunately, the public rarely knows the TB burdens from the States of Nigeria, and this may be contributing to the prevailing inappropriate care seeking behaviour and poor awareness of the disease in Nigeria. It thus seems that the TB data of various States' ministries of health are meant only for generating national estimates and reports such that the general public as well as most health workers are not aware or adequately informed of the disease burden and accessibility of effective treatment (Cyril and Ngozi, 2016)

The study in the United States reported 26 tuberculosis outbreaks to be due to certain environmental factors. According to the report, a review of tuberculosis outbreaks from 2002 to 2011 revealed that the outbreaks were characterized by factors such as substance abuse, incarceration and homelessness **(Maryam *et al*., 2015).**

2.7 Diagnosis of *Mycobacterium tuberculosis* infection

The classical methods for the diagnosis of Mycobacterium tuberculosis are microscopy and culture for active TB and Tuberculin Skin Test (TST) for latent TB.

2.7.1 Sputum Smear Technique

This is the gold standard carried out in most conventional medical laboratories. It could be as confirmation technique. However, it is not very suitable to diagnose Latent tuberculosis because the bacilli may not be found in the sputum. Another limitations to the method is that Mycobacterium tuberculin bacilli is usually present in undetectable number, indicating that unless the number of cells amount to 5000 to 50000 bacilli m/L for the bacilli to be seen on smears (Daniel and Debanne, 1987).

The diagnosis of mycobacterial diseases depends upon identifying the infecting organism in the secretion or tissues. However, there are several limitations of this method. One is that *Mycobacterium tuberculosis* is usually present in undetectable numbers unless up to 5×10^3 to 5×10^4 bacilli/mL before it can be seen on smear, so that it is recognized for the most part in advanced cases. Second, most mycobacteria are slow-growing organisms and require long periods of time to culture, even if the most advanced techniques are used. Third, negative smears are

usually obtained until cavities form. Thus, a fast, easy and reliable method was needed for the diagnosis of tuberculosis. Among several serologic techniques, it has been concluded that enzyme immunoassay (EIA) is a sensitive, reliable, simple and rapid method. Several purified antigens, such as 38-kDa protein, 85A antigen, lipoarabinomannan, plasma membrane antigen, antigen 5 and antigen 60 (A60), have been used for the serodiagnosis of tuberculosis. This study was undertaken to evaluate the usefulness of the EIA method using A60 antigen for the diagnosis of different forms of tuberculosis in Turkish patients. (Yucee *et al*., 2001)

2.7.2 Cultural Technique

The bacteria are usually cultured in the laboratory in cases of mild infection. This method is result orienting but time consuming. Mycobacteria are slow growing organisms and require a long period of time to grow in cultures even with advanced techniques (Kalish *et al*., 1983). And for growth to be seen there must be about 10 – 100 bacilli per m/L in the inoculums.

Considering the two methods above negative smears are usually obtained during infectious stage to indicate no infection (Charpin *et al*., 1990).

2.7.3 Serodiagnosis

Because of the limitations of sputum smear and cultural methods, the increasing rate of TB infection which is a health threat globally and to a large extent the scourge of AID in developed and and developing countries, it has become pertinent to come up with a fast, easy and reliable method for the diagnosis of the disease. Immunoassay was discovered to be the simplest, most sensitive reliable and rapid diagnostic method (Daniel and Debanne, 1985; Daniel and Debanne, 1987)

All immunoaasay test kits are antigen or antibody specific and the test kit used in this case is IgG and IgM antibody which are specific for mycobacterial antigen Julian *et al*., 1997).

The research carried out to detect serum antibody by dual path platform in white – tailed deer infected with *Mycobacterium tuberculosis* confirmed that the traditional skin test diagnostic method has serious limitations, whereas, the emerging serological assays showed promising diagnostic performance. The findings demonstrated a relatively high diagnostic accuracy in the detection that separated a naturally infected deer and the infection due to artificial inoculation (Lyashchenko *et al*., 2013).

Serodiagnosis has been reported as the ultimate when classical diagnostic methods give negative result. A typical case was the use of Elispot assay for the rapid diagnosis of Active TB in a patient undergoing immunosuppressive therapy (Christoph *et al*., 2007). The kits of immune-chromatographic test has become

popular for screening large population for the detection of TB among apparently healthy individuals, as applied to fallow deer to screen for antimycobacterial antibodies (Boadella *et al*., 2012). In the same vein Che'Amat *et al* (2015) used rapid immune-chromatographic test kits to screen for serum antibodies against TB among 126 wild piglets, out of which 69.2% were positive.

Walid *et al* (2011) carried out the screening on three groups of patients to evaluate the clinical usefulness of rapid immunodiagnostic by using immunochromatographic test and came up with the conclusion which indicated that the rapid method is simple and a convenient tool for screening against Active pulmonary tuberculosis in poorly equipped laboratories, especially those found in primary care centers in rural areas.

Immunochromatographic test (ICT) is considered to be rapid and easy to perform without requiring special equipment, both on serum, plasma and whole blood samples. The work carried out on blood of sputum smear positive patients and sputum smear negative individuals showed higher positivity for the former than the later as compared (Bartoloni *et al*., 2003). This suggests that ICT can be adopted for rural screening for *Mycobacterium tuberculosis*.

A study in Sub Sahara Africa has shown that a third of the patients with sputum smear-negative TB died within a year of their initial diagnosis, whereas, a third developed recurrent pulmonary TB. This was due to lack of efficient diagnostic

tool for sputum smear-negative TB. Consequently, this may lead to continues transmission of infection in the community (**Ffekadu *et al*., 2015).** All facts are that the early onset of TB may not show signs of infection in the sputum and culture and the only means to detect the infection is the use of serodiagnosis for the presence of antibodies.

The development of simple, rapid and inexpensive diagnostic tool for tuberculosis is an important goal, particularly in view of the global increase in cases of Active Tuberculosis primarily affecting both the developing and developed countries almost at the same rate. The sensitivity of ICT made a convenient method for first line testing for suspected cases in resource-poor countries where access to diagnostic tools is limited and cost efficiency has a high priority (Martin *et al*., 1998).

Indeed commercial serological antibody detection test was found to be a good screening device, considering the study on both sputum smear positive cases and sputum smear negative cases. The finding revealed that antibody detection test was highly positive in the sputum smear positive cases. This finding gives a high confidence level to adopt the rapid test device for the screening during surveillance studies or other forms of screening (Steingart *et al.,* 2007).

Immunochromatographic test was evaluated in Italy for its sensitivity and specificity. This was achieved by using a rapid ICT device on the blood samples of

individuals confirmed with TB by sputum smear method and those with sputum smear negative result. The sputum smear positive cases were positive for ICT and the sputum smear negative were equally negative The ICT tuberculosis test was confirmed to be rapid and easy to perform without requiring special equipment, both on serum and whole blood sample. Our data, in accordance with those obtained in a previous study conducted in extra-European countries, confirmed higher sensitivities for the smear-positive TB patients than for the smear-negative TB patients, and for pulmonary TB patients than for the extrapulmonary TB patients. Data obtained on the quality of antibody response in the ICT positive samples, might be used to improve the performance of the test (Bartoloni *et al.,* 2003).

The ICT was also used to separate Mycobacteria tuberculosis Complex (MTBC) and Non tuberculosis Mycobacteria (NTM). This was with the view to spare the patients from unnecessary treatment in the case of NTM. The presence of the MTB protein 64 (MTB64) in the MTBC was used a marker to separate it from NTM. The fact that culture method is time consuming and that smear techniques is nonspecific gave ICT the acceptability by WHO as a diagnostic and therapeutic monitoring tool for TB. (Anand *et al.,* 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The two communities (Gabia and Chinke) are located close to the Jos metropolis, the capital city of Plateau State.

The study was conducted among apparently healthy individuals randomly selected from two the neighbouring communities (Gabia and Chinke) in Plateau State.

Gabia has a population of 3245 people consisting of 1225 males and 2020 females while Chinke has a population of 3015 people comprising 1,173 males and 1842 females.

3.2 Sample population

The study was carried out between October, 2016 and July, 2017 in the two communities (Gabia and Chinke). A total of two hundred and twenty eight (228) were recruited base on their informed consent. The age of the participants ranged from 10 to 80 years and above.

3.3 Inclusion and Exclusion Criteria

A random selection was carried out with the inclusion criteria being individuals with no active TB symptoms or past history of TB while the exclusion criteria were individuals with active TB symptoms or past history of TB.

3.4 Sample Size

The sample size was determined by the formula;

$N= Z^2P(1-p)/d^2$ (Daniel, 1999)

where;

N= sample size,

p = prevalence of lack of knowledge of TB = 6.67%, (Awujo *et al*., 2008) z is a constant = 1.96,

d = the precision (in proportion of one, if 5%, d= 0,05

P = 0.07, z = 1.96, d = 0.05

$N= 1.96^2 \times 0.067 (1 – 0.067)/0.05^2 = 100$

Two hundred and twenty eight (228) were collected

3.5 Behavioral Demographic Questionnaire

A structured behavioral questionnaire was developed. The questions on the questionnaire included basic demographic data such as age, sex, educational level. Medical history such as HIV status, previous history of Tb, lung disease, coughing, headache, weakness etc. health and risk behavioural factors such as alcohol and tobacco. Structural environmental factors such as number in the family, ventilation of living room,

3.6 Sample collection

3.6.1 Blood sample

2ml of whole blood was collected from the individuals into plain specimen bottle and allowed to stand for the blood to clot undisturbed.

3.6.2 Sputum sample

Sputum samples were collected from the IgM positive individuals for three consecutive days into clean and sterile wide-mouth universal containers.

3.7 Sample preparation

The sera samples were separated using Pastuer pipette and transferred into separate glass tubes and allowed to settle for the immunoassay.

3.7.1 Test procedures

3.7.1.1 Blood sera

Antibodies to *Mycobacterium tuberculosis* in the plasma were detected using the one step TB IgG/IgM rapid diagnostic Test kits, (Micropoint TB IgG/IgM Rapid Diagnostic Test). The test uses the principle flow chromatography.

The test was performed according to the manufacturer's instructions as outlined below;

1. The test cassette was placed on flat and stable surface.
2. Three large drops of serum were released into the test sample well
3. Timing commenced after the last drop, while observing the color line appearance and the result was read after 10 minutes.

Positive result produced three coloured bands;

First coloured band is for IgG

Second coloured band is for IgM

Third coloured band is for control

Negative result; only the control coloured band appeared.

3.7.1.2 Sputum

The sputum samples collected were processed as followed.

1. Portions were picked by using a disposable wooden applicator sticks
2. Smear of dimension 2cm by 1cm were made on a glass slide and fixed b passing it 3 – 4 times through the flame.
3. Place he slide on he staining rack and pour carbol fuchsin over the smear and heat gently underside of the slide with the flame until steam appear avoiding overheating and boiling.
4. Raise smear with water until colour or stain does not appear in he effluent.

5. Decolorize in 3% HCL in 95% alcohol solution and wait for one minute and repeat the process until the smear appear light pink in colour.
6. Wash well with clean water.
7. Cover the smear with methylene blue or malachite green stain for 1 – 2 minutes.
8. Wash off he stain with clean water.
9. Wipe the back of slide and allow the smear o dry.
10. Examine he smear microscopically with oil immersion objective (x100) (Obasanya *et al*., 2013)

Stained smears were examined microscopically for the presence of acid fast bacilli (AFB) with positive and negative slides along. Recommended by Cepheid (2014) Positive result; indicated by plus (+) sign.

3.8 Statistical Analysis

The data obtained was analysed by Simple percentage were statistically calculated using the software Epi Info 7 StatCalc Application.

CHAPTER FOUR

RESULT

In the present study blood sample of 228 apparently individuals from Gabia and Chinke communities were screened for *Mycobacterium tuberculosis* antibodies.

Among the subjects 84(38.9%) were males and 144(63.1%) were females.

Table 4.1: Age Relate Prevalence of *Mycobacterium Tuberculosis* in Gabia and Chinke

Age	Number Examined	Number Infected	Prevalence %

(years)	Gabia	Chinke	Total	Gabia	Chinke	Total	Gabia	Chinke	Total
10-19	18	15	33	1	2	3	0.4	0.9	1.3
20-29	9	18	27	3	2	5	1.3	0.9	2.2
30-39	17	27	44	3	3	6	1.3	1.3	2.6
40-49	26	36	62	7	11	18	3.1	4.8	7.9
50-59	15	12	27	4	4	8	1.8	1.8	3.5
60-69	19	3	22	2	1	3	0.9	0.4	1.3
70-79	6	1	7	1	0	1	0.4	0.0	0.4
80-89	4	2	6	0	1	1	0.0	0.4	0.4
Total	**114**	**114**	**228**	**21**	**24**	**45**	**9.2**	**10.5**	**19.7**

Table 4.1 shows that the *Mycobacterium tuberculosis* antibodies were most prevalent (7.9%) in individuals in the age group of 40 – 49 years. There was no evidence of antibodies in the sera of individuals from Gabia in age group of 80 – 89 years. Comparatively, the prevalence of antibodies was 0.4% in the sera of individuals of the same age group in Chinke.

Table 4.2: Sex-Related Prevalence of *Mycobacterium Tuberculosis* in Gabia and Chinke

Sex	Number Examined			Number Infected		
	Gabia	Chinke	Total	Gabia	Chinke	Total
Male	45	39	84	9(3.9%)	8(3.5%)	17(7.5%)

Female	69	75	144	12(5.3%)	16(7%)	28(12.3%)
Total	**114**	**114**	**228**	**21(9.2%)**	**24(10.5%)**	**45(19.7%)**

$X^2 = 0.0007$

P = 0.5 (P>0.05)

The prevalence of *Mycobacterium tuberculosis* in the study areas was 19.8%. However, the prevalence was higher in sera of inhabitants of Chinke community (10.5%) than in those of Gabia community (9.2%). Generally, in both communities, the females were more seropositive for the bacterial antibodies with 12.3% than the males counterpart with 7.5%.(Table 4.2)

Table 4.3: IgM-Related Prevalence of *Mycobacterium Tuberculosis* in Gabia and Chinke

Sex	**Number Examined**			**IgM antibody**		
	Gabia	**Chinke**	**Total**	**Gabia**	**Chinke**	**Total**
Male	45	39	84	5(2.2%)	6(2.6%)	11(4.8%)
Female	69	75	144	4(1.8%)	3(1.3%)	7(3.1%)

Total	**114**	**114**	**228**	**9(3.9%)**	**9(3.9%)**	**18(7.8%)**

Gabia ; $X^2 = 0.45$; $p = 0.24$ ($P>0.05$)

Chinke; $X^2 = 3.14$; $p = 0.06$ ($P>0.05$)

The prevalence of *Mycobacterium tuberculosis* IgM antibodies in male in the two communities was 4.8% higher than that of the females (3.1%). (Table 4.3)

Table 4.4: IgG-Related Prevalence of *Mycobacterium Tuberculosis* in Gabia and Chinke

Sex	**Number Examined**			**IgG antibody**		
	Gabia	**Chinke**	**Total**	**Gabia**	**Chinke**	**Total**
Male	45	39	84	4(1.8%)	2(0.9%)	6(2.6%)
Female	69	75	144	8(3.5%)	13(5.7%)	21(9.2%)
Total	**114**	**114**	**228**	**12(5.3%)**	**15(6.6%)**	**27(11.9%)**

Gabia ; $X^2 = 0.21$; $p = 0.44$ ($P>0.05$)

Chinke; $X^2 = 2.3$; $p = 0.05$($P>0.05$)

Table 4.4 shows a IgG antibodies (9.2%) in the sera of females than that of the females (2.6%).

Table 4.5: Sputum Smear Prevalence of Tuberculin bacilli

Sex	**number examined**	**IgM Pos**	**Sputum Smear Pos**	
			++	**+ (%)**
Males	84(36.8)	11(4.8)	2(0.8)	9(3.9)
Females	144(63.2)	7(3.0)	4(1.8)	3(1.3)
Total	**228**	**18(7.8%)**	**6(2.6)**	**12(5.2)**

$X^2 = 4.94$; $p = 0.02$ ($P>0.05$).

*Significant

The sputum of IgM positive cases were further examined for acid fast bacilli and the result was significant (p>0.02), showing 2.6% with 2+ and 5.2% with +. (Table 4.5).

Table 4.6: Respondents related knowledge of tuberculosis

Mode of transmission	**No. of Response**				
	Yes (%)				
Eating and drinking	173(75.9)				
Singing and talking	26(11.4)				
Coughing and laughing	59(25.9)				
Sneezing and shouting	46(20.2)				
Alcohol	103(45.2)				
Tobacco	22(9.6)				
Others					

Heard of TB	205(89.9)				
Seen TB patient	175(76.8)				
TB sign and symptoms	23(134.1)				

Out of the two hundred and twenty eight individuals administered the questionnaire 89.9% indicated that they have ever heard of tuberculosis. However, only 13.1% have knowledge of the sign and symptom of the tuberculosis. The knowledge of the mode of transmission considerably (coughing – 25.9%, sneezing – 20.2%, singing – 11.4%. Alcoholism has 45.2% of the respondents against cigarette smoking with 9.6%.(Table 4.6)

CHAPTER FIVE

DISCUSSION

The global estimate of active tuberculosis cases as at the year 2011 was 9million annually. This is a fraction of the about one third of the world population with Latent tuberculosis (*WHO, 2013*). This work was carried at random to detect the *Mycobacterium tuberculosis* antibodies among the residents of the communities. There was indeed a considerable level of MTB antibodies in the sera of the individuals. The prevalence of 19.8% *Mycobacterium tuberculosis* antibody, IgM and IgG, obtained is worrisome since it is known that 5% infection with *Mycobacterium tuberculosis* in any given population at Latent state is enough to progress to Active tuberculosis within two years and another 5% to 10% can develop the disease later in life (Castro and Jaffe, 2002). The result of the study carried out showed that 7.8% of the sample population had IgM antibodies in their blood system indicating recent infection and this is a cause for urge attention. The individuals that tested positive claimed not to have a prolong cough and other signs or symptom that suggested the presence of *Mycobacterium tuberculosis*. This is in

line with the report of *Daniel et al (1987), which revealed* that there is a stage of TB infection that the Mycobacterium bacilli may not be found in the sputum. According to Yucee *et al (2001)*, *Mycobacterium tuberculosis* can only be detected in sputum and culture when the number of bacilli is above 5000 – 50000 bacilli/mL and 10 – 100 bacilli/mL in sputum smear and culture respectively. Secondly, most mycobacteria are slow-growing organisms and require long period of time to culture, even if the most advanced culture techniques are used (Daniel *and* Debanne*, 1987*), thirdly, negative smears are usually obtained until cavities form (Charpin *et al., 1990*). Thus a fast, easy and reliable method was needed for the diagnosis of tuberculosis and immunochromatographic diagnostic method serves as one of the convenient to detect the presence of the disease.

The study carried out by Lawrence and Christianl (2010) revealed that transmission of TB did not require sleeping in the same room with an infected individual. It would occur at other levels of contact and intimacy including sharing work, eating and recreational environment which is a similar scenario with the study areas. Similarly it was reported that the transmission of TB among prison inmates was not closely associated to cell mate relationship but due to other social network within the prison community (Daniel *et al., 2014*). Social network has been defined as “the web of social relationship that surrounds an individual and the characteristics of those ties” and social integration can be seen as encompassing

both the structural aspects such as size and density of an individual's social networks and functional aspects such as frequency, duration and support yielded by relationships.

Therefore, those at risk of infection with *Mycobacterium tuberculosis* are;

- People who have contact with a person with known or suspected TB disease.
- People with weakened immunity such as those with HIV infection, malnourished, young adults and advanced age or substance abuse.
- Those from low income environment with poor nutrition.
- People who live in unclean or crowded environment and /or without a healthy diet.
- Health care workers who handle patients who are at increased risk.
- Laboratory staff who handle specimens that may contain tubercle bacilli. (Grzyboswski *et al.,* 1979)

Therefore, the deliberate attempt for comprehensive poverty reduction intervention and social network control can reduce infection rate in vulnerable population.

In view of the fact that tuberculosis thrive in densely populated communities/areas, the spread from infected to uninfected individuals is eminent in the study communities because of their social lifestyle.

The rate of infection expressed by the presence of *Mycobacterium tuberculosis* antibodies varied among different age groups with the highest prevalence occurring in the age group 40 – 49 years. This is regardless of the social activities associated to the affected group. However, no reason could account for the higher prevalence in female age group with eighteen individuals.

The diagnosis of tuberculosis mainly depends upon clinical suspicion and radiographic findings with subsequent bacteriological confirmation by sputum smears examination (Castro *et al.,* 2002, Bhatia *et al.,* 2003). The result of the finding in this case can be subjected to further investigation to ascertain the level of infection for subsequent treatment. However, the lack of sensitivity in smear examination, due to low count of bacilli number in circulation and non specificity of radiological findings, prolonged culture and difficulties in the diagnosis of extra pulmonary tuberculosis, has made it necessary to use immunodiagnosis as a convenient and time and cost effective test to supplement clinical information for the definite diagnosis (Castro *et al*., 2002, Bhatia *et al*., 2003). Early detection will ensure that the individuals found to be infected are properly isolated and/or treated. This is why it is mandatory to carry out vigorous enlightenment campaign in the study area to educate the people on the risk factors, mode of acquisition of the bacteria and the available control measures.

The rate of prevalence recorded in the study has shown that the spread across the age brackets of 30 – 39 years and 40 – 49 years are quite statistically significant ($p<0.05$). The highest prevalence with female gender cannot be accounted for. Perhaps this can be due to the fact that the affected age bracket are mostly indulging in the social activities peculiar to the communities such as the drinking of the locally brewed alcoholic drink called burukutu. At such environments, people sit in clusters to consume the product and occasionally, two or more individuals usually share a single container (calabash). Ironically too, a consumer of such a product can be a husband to a non consumer who could be a Christian. This can lead to the risk of infection to the entire family.

It is a known fact that the diagnosis of tuberculosis mainly depends on initial clinical signs and subsequent bacteriology sputum smear examination to confirm the presence of the bacteria and the culture. The above methods most often are not sensitive to Latent tuberculosis and even a most recent infection due to inadequate number of the bacilli that can be detectable. This has necessitated the need to use immunodiagnosis as a conventional, convenient, time and cost effective test to complement clinical information (Bhatia *et al.,* 2003). All immunoaasay test kits are antigen or antibody specific and the test kit used in this case is IgG and IgM antibody which are specific for mycobacterial antigen.

The early diagnosis of *Mycobacterium tuberculosis* from asymptomatic individuals will ensure that the individuals found with the antibody are properly given attention to reduce the chances of transmission to vulnerable subjects. The high prevalence of *Mycobacterium tuberculosis* in the study area is worrisome as the sero-positive individuals are not only at risk of having full blown tuberculosis later in life, but are also a source of spreading the bacteria to the populace. Therefore, there is an urgent need for further epidemiology survey to be undertaken to the study area and to initiate appropriate preventive and control measures. Daniel *et al (*2014*)* reported that factors such as HIV infection, injection drug use and low access to supplemental nutrition were associated with prevalent cases of pulmonary TB. Policies that reduce HIV infection, injection drug use and abuse and improve the nutritional status of communities may drastically reduce the spread of the disease. Consequently, TB programs should consider scaling up nutritional support among TB patients living below the poverty line.

The sero prevalence studies carried out by Awujo *et al* (2008) in the South Eastern State of Nigeria gave a prevalence of 6.67%. That was no doubt a worrisome outcome that was not only a future trigger to full blown TB to the carrier of the bacteria but a potential source of infection to the populace. Tuberculosis is generally associated with poverty and social isolation, poor nutritional status and deprivation. They are linked to the increased incidences of the disease in the study

group. The study conducted on Tajikistan prison inmates revealed that the increased incidence of TB was due to the poor facilities and low access to supplemental nutrition (Daniel *et al*., 2014). Since poverty and poor nutrition is highly associated with the risk of developing TB, it further explains that unavailability of supplemental nutrition can affect the treatment of TB. The study carried out by Blesson *et al* (2016) revealed that patients provided with adequate nutritional support recorded low unsuccessful treatment outcome of 9% than those not provided nutritional support with 21%.

The present study is similar to the one conducted among apparently healthy individuals in Imo state of Nigeria, which reported a 6.67% (Chinedu *et al.,* 2008) prevalence of *Mycobacterium tuberculosis* antibodies. The high prevalence of M. tuberculosis in the study population was equally reported as worrisome as the sero-positive individuals were not only at risk of having full blown tuberculosis later on, but were also sources of spreading the bacteria to the populace. Therefore, when revelations of this nature are made, urgent need for further epidemiological survey undertaken in the study area become the panacea for prevention and further control measures.

Another major factor that increases the rate of transmission is the low or poor knowledge of the disease and the mode of transmission. Several studies in some Asian and African countries even among the supposedly educated individuals

showed that the awareness on *Mycobacterium tuberculosis* disease is low (Sultan *et al.,* 2015, Hibstu and Bago, 2016,). The lack of awareness can further exacerbate the spread of the disease. The outcome of our study has tilted to the low level of knowledge by the participants and the harsh environment. This clearly agrees with the findings by Maryan *et al* (2015) which attributed to the 26 outbreaks of tuberculosis in the United States as a result of incarceration and homelessness. It is important to shift attention to prisons to provide healthy environment for the inmate and to provide comfortable accommodation to the homeless in order to stem the tide of the tuberculosis spread.

5.1 CONCLUSION

Mycobacterium tuberculosis prevalence recorded in the study area is at a reasonable level rate and has cut across the sex and age groups. The prevalence can be associated to ignorance and poverty. A good number of the subjects belong to low social and economic class. As such susceptibility can be influence by poor or lack of good nutrition (Samue *et al*., 2016)

The level of ignorance on the signs and symptoms among the subjects was high with only 23 persons having some knowledge on TB out of 228 subjects which is similar to the findings of Sultan *et al* (2015) among students of King Saud University even though the students may belong to a different social class. This has

revealed that the subjects are not knowledgeable enough on TB as such the attitude and practice of preventive measures are lacking.

There was no clear evidence of HIV as a contributing because none of the subjects reported on an implicating opportunistic infection.

The people mostly consume the locally brewed drink call burukutu. Usually they gather in one place to partake in such activities. He clustering can promote infection easily or facilitate the spread of the disease agent from person to person.

5.2 RECOMMENDATION

In view of The lack of sensitivity of sputum smear examination on recent TB infection, non-specificity of radiological findings, prolonged *M. tuberculosis* culture and difficulties in the diagnosis of extrapulmonary tuberculosis,

1. Immunodiagnostics Test should be used as a convenient and time and cost effective test to supplement clinical information for definite diagnosis of tuberculosis.
2. A deliberate proactive enlightenment campaign should be launched in the study area and even across Plateau State to educate the populace on the danger, risk factors, mode of acquisition of the disease and the possible control measures to stem or minimize the spread.

3. Further investigation should be carried out on those sero-positive cases so that proper and adequate medical attention is provided.

4. There is also urgent need for epidemiological survey to be taken in the study area and beyond to initiate appropriate preventive and control measure.

CONTRIBUTION TO KNOWLEDGE

The study has contributed to knowledge in the following ways ;

1. The 7.8% prevalence of *Mycobacterium tuberculosis* antibodies among the subjects will serve as reference for future research.
2. The use of immunoassay as a diagnostic tool for detecting *Mycobacterium tuberculosis* has been substantiated and can be used for general screening because it is antigen/antibody specific.

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APPENDIX

QUESTIONAIRE

1. Name ----------
2. Age ----------

3. Marital status ---------- married/single
4. Number of children ----------
5. Infected with TB ---------- yes/no

   If yes treated ---------- yes/no
6. HIV status ---------- positive/negative

   If positive, treated ---------- yes/no
7. Mode of transmission

   Eating and drinking ---------- yes/no

   Singing and talking ---------- yes/no

   Coughing and laughing---------- yes/no

   Sneezing and shouting---------- yes/no
8. Alcohol consumption ---------- yes/no
9. Cigarette smoking ---------- yes/no
10. Have heard of Tuberculosis ---------- yes/no
11. Have you ever seen a TB infected

    Person---------- yes/no
12. Do you know the sign and

    symptom of tuberculosis ---------- yes/no
13. Do you want to be tested of TB ------- yes/no

Frequency Table for Respondents Demographic data

		Frequency	Percentage (%)
Gender	Male	84	36.8
	Female	144	63.2
	Total	228	100
Marital Status	Single	80	35.1
	Married	148	64.9
	Total	**228**	**100**
Number of Children	0	4	2.7
	1	7	4.7
	2	18	12.2

	3	17	11.5
	4	46	31.1
	5	41	27.7
	6	10	6.8
	>6	5	3.4
	Total	**148**	**100**
Educational Level	Primary	163	71.5
	Secondary	54	23.7
	Tertiary	11	4.8
	Total	**228**	**100**

Tables for other parts of the questionnaire

		Frequency	**Percentage (%)**
Infected with TB?	Yes	0	0
	No	228	100
	Total	**228**	**100**
If yes, Treated?	Yes	0	0
	No	0	0
	Total	**0**	**0**

		Frequency	Percentage (%)
HIV Status	Positive	0	0
	Negative	228	228
	Total	**228**	**100**
If Positive, Treated?	Yes	0	0
	No	0	0
	Total	**0**	**0**

Transmission mode		**Frequency**	**Percentage (%)**
Through eating and drinking	Yes	173	75.9
	No	55	24.1
	Total	**228**	**100**
Through singing and talking	Yes	26	11.4
	No	202	88.6
	Total	**228**	**100**
Coughing and laughing	Yes	59	25.9
	No	169	74.1
	Total	**228**	**100**

		Frequency	**Percentage (%)**
Through sneezing and shouting	Yes	46	20.2
	No	182	79.8
	Total	**228**	**100**

		Frequency	**Percentage (%)**
Alcohol consumption?	Yes	103	45.2
	No	125	54.8
	Total	**228**	**100**

		Frequency	Percentage (%)
Cigarette smoking?	Yes	22	9.6
	No	206	90.4
	Total	**228**	**100**

		Frequency	Percentage (%)
Have you heard of Tuberculosis?	Yes	205	89.9
	No	23	10.1
	Total	**228**	**100**

		Frequency	Percentage (%)
Have you ever seen a TB infected person?	Yes	175	76.8
	No	53	23.2
	Total	**228**	**100**
Do you know the sign and symptom of TB	Yes	23	13.1
	No	152	86.9
	Total	**175**	**100**

		Frequency	Percentage (%)
Do you want to be tested for Tuberculosis?	Yes	228	100
	No	0	0
	Total	**228**	**100**