DETECTION OF *Mycobacterium bovis* ANTIBODIES USING THE LATERAL-FLOW TECHNIQUE IN SMALL RUMINANTS IN KADUNA METROPOLIS, NIGERIA

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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FEBRUARY, 2013

DECLARATION

I hereby declare that the work in this thesis titled "DETECTION OF *Mycobacterium bovis* ANTIBODIES USING THE LATERAL-FLOW TECHNIQUE IN SMALL RUMINANTS IN KADUNA METROPOLIS, NIGERIA" has been performed by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Professor C.A. Kudi, Professor I. Ajogi and Dr. M. Bello.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma in any institution.

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Name of student	Signature	Date

CERTIFICATION

The thesis titled "DETECTION OF *Mycobacterium bovis* ANTIBODIES USING THE LATERAL-FLOW TECHNIQUE IN SMALL RUMINANTS IN KADUNA METROPOLIS, NIGERIA", by Esther Godiya HARUNA meets the regulations governing the award of the degree of Master of Science, in Veterinary Public Health and Preventive Medicine of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The study was designed to determine the prevalence of *Mycobacterium bovis* (*M. bovis*) antibodies in sheep and goats in slaughter houses and livestock markets, and the risk of infection and level of awareness of animal handlers/owners to bovine tuberculosis within Kaduna metropolis. A total of 1010 sera samples from small ruminants, consisting of 498 sheep and 512 goats were collected at random from 4 abattoirs during slaughter and from livestock markets within the metropolis. The Anigen® Rapid Bovine TB Antibody Test which is specific for *M. bovis* was used to determine the presence of bovine tuberculosis antibodies in the serum samples. Structured questionnaires were distributed to animal handlers/owners after being pilot tested and adjusted. The results showed that out of the total 1010 sera samples taken, 137 (13.56%). tested positive to bovine tuberculosis. Species specific prevalence rates of 10.84% (54/498) for sheep and 16.41% (84/512) for goat were found. There was significant association between detection of *M. bovis* antibodies and species sampled, with goats having a significantly higher prevalence than sheep ($P<0.05$; $\chi^2 = 7.153$, $P= 0.0075$). Females of both species had sero-prevalence of 14.98% (89/594) while males had 11.54% (48/416). The sero-prevalence amongst age groups was 12%, 15%, 7% and 14% for 1-12months, 13-24months, 25-36months and ≥36months respectively. There was significance occurrence of reactors to bovine tuberculosis amongst the age group greater than 24 months ($P< 0.05$). Sero-prevalence of 13.36% (68/509) and 13.77% (69/501) for slaughter houses and livestock markets respectively was recorded. Sixty-five percent of the respondents were aware of the disease tuberculosis (TB), 41.9% were aware of bovine TB while 39.7% were aware that bovine TB is zoonotic. Level of education was found to be associated with the degree of awareness of bovine tuberculosis as zoonoses among the respondents ($P = 0.0001$). Out of the 72.5% respondents that keep livestock, 55% of them do so

in close proximity with the humans and 52% have a preference for small ruminant as source of meat. The detection of *Mycobacterium bovis* infection in apparently healthy small ruminants is of public health significant, considering their close proximity to humans. There is therefore the need to educate the populace on zoonotic implication of bovine tuberculosis and the relevant authorities should put in place control measures against the disease.

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ABBREVIATIONS

BTB:	Bovine Tuberculosis
TB:	Tuberculosis
PPD:	Purified Protein Derivative
IQRT:	Immunochromatography Rapid Test
AUIBAR:	African Union Inter African Bureau for Animal Resources
CIDT:	Cervical Intra dermal Tuberculin Test
TST:	Tuberculin Skin Test
HIV/AIDS:	Human Immunodeficiency Virus- Acquired Immune Deficiency Syndrome
FAO:	Food and Agriculture Organization
WHO:	World Health Organization
WAD:	West African Dwarf
OIE:	Office of International Epizootics
DEFRA:	Department of Environment Food and Rural Affairs
SIT:	Single Intradermal Tuberculin Test
SICCT:	Single Intradermal Comparative Cervical Tuberculin Test
NTBLCP:	National Tuberculosis Leprosy Control Program
ILCA:	International Livestock Centre in Africa

CHAPTER 1

INTRODUCTION

1.1 GENERAL OVERVIEW

Tuberculosis is an important zoonosis causing chronic, infectious, contagious, debilitating disease which has become an increasing problem in animals and humans worldwide, especially in developing countries like Nigeria (WHO, 1994; Cadmus, 2003; Ofukwu *et al*., 2008). Tuberculosis is said to be the ninth most frequent cause of death in Africa (WHO, 1994). The disease is caused by a *Mycobacterim* organism; it is an acid-fast organism which is almost ubiquitous. *Mycobacterium bovis* (*M. bovis*) a member of the *Mycobacterium tuberculosis* complex (MTBC) is the most universal pathogen among Mycobacteria species, causing tuberculosis in a wide variety of domestic animals, wild animals and humans (Collins and Grange, 1983; Wayne and Kubica, 1986; O'Reilly and Daborn, 1995). Other members of the *Mycobacterium tuberculosis* complex (MTBC) comprise of closely related species *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti* (Wayne and Kubica 1986; Van Soolingen *et al*., 1997). Route of transmission is via inhalation of aerosol facilitated by close contact between infected and susceptible host. Transmission can however occur via ingestion of contaminated materials e.g. feed, milk and water.

Mycobacterium bovis which is the cause of bovine tuberculosis and *Mycobacterium tuberculosis* the cause of classical human tuberculosis are genetically and antigenically very similar and cause identical clinical disease in humans (Cosivi *et al*., 1998). Throughout the world, the most commonly recognized hosts for *M. bovis* are domesticated bovids. However, in recent years,

several wildlife reservoir hosts have been identified, including brushtail possums in New Zealand (Morris and Pfeiffer, 1995), European badgers in the United Kingdom (Clifton-Hadley *et al*., 1993), white-tailed deer in Michigan (O'Brien *et al*., 2002) and Cape buffalo and several antelope species in South Africa (WHO, 1994). Other species, such as elephants and rhinoceros (Dalovisio *et al*., 1992) have been identified as hosts for *M. bovis* infection in captivity.

Tuberculosis in goats is widespread in the Mediterranean countries (Bernabe *et al.*, 1991; Gutierrez and Garcia, 1993). Strains of *Mycobacterium tuberculosis* complex were isolated from caprine in different geographical areas in Spain and because of their genetic, biochemical and epidemiology difference, was coined *Mycobacterium tuberculosis* subsp. *caprine* (Aranaz *et al*., 1999). Gutierrez *et al*. (1997) reported that three human isolates displayed caprine spoligotyping patterns and it was found that there was close contact with the animals. The prevalence study of *Mycobacterium bovis* subsp. *caprae* and *Mycobacterium bovis* subsp. *bovis* from 1999 to 2001 was conducted among German tuberculosis cases. It showed that *M. bovis* subsp. *caprae* accounted for a high ratio of human *M. bovis* associated cases in southern part of the country (Tanja *et al.*, 2003). However, only few cases of tuberculosis have been reported in goats in countries with high incidence of *M. bovis* in cattle (Aranaz *et al.*, 1999).

The extensive production system under which livestock are managed in tropical animal production system of Africa may have a significant influence on the distribution of animal tuberculosis. Since contaminated pasture is a means of transmission of the organism, sheep and goats may become infected with *M. bovis* when sharing pasture with infected cattle, at watering ponds, market places and sharing the same shelter at night (Ayele *et al.,* 2004).

In Nigeria, sheep are kept predominantly by Fulani pastoralists, who manage them semi-intensively. Goats are kept mainly by crop farmers and low income earners, whose management practices include housing overnight and tethering by day (Bayer, 1984). As a result of these practice, bovine tuberculosis in humans is becoming increasingly important in developing countries like Nigeria where humans and animals share the same microenvironment and dwelling premises especially in the rural areas (Abubakar *et al.*, 2011). Kidane *et al.* (2002) reported that about 17% of lymphadenitis cases were caused by *M. bovis* in Ethiopia. A possible transmission of *Mycobacterium* from humans to animals cannot be overruled as Hiko and Agga (2010) identified two *M. tuberculosis* isolates from tuberculous lesions of goats in their study.

The free movement within country and trans-border smuggling of animals contribute significantly to inter and intra-species transmission and spread of *M. bovis* infection and thus contributes to the global upsurge in the prevalence of bovine tuberculosis in animals and humans especially in developing countries (Shitaye *et al.*, 2007).

1.2 STATEMENT OF THE RESEARCH PROBLEM

Bovine tuberculosis caused by *Mycobacterium bovis* is an important zoonosis and has remained a major cause of morbidity and mortality worldwide (WHO, 2004). It has been implicated in both human and animal extra-pulmonary and pulmonary tuberculosis either through ingestion of unpasteurized contaminated milk/milk products, infected meat/meat products or through aerogenous route facilitated by close contact (Cotter *et al.*, 1996; Cosivi *et al.*, 1998; Cousins and Dawson, 1999; Radostits *et al.*, 2000; Cadmus, 2003; Ojo *et al.*, 2008; Bello *et al.*, 2009).

An estimate of 98.9% tuberculosis cases is reported in cattle in most African countries (AUIBAR, 2006). The prevalence of bovine tuberculosis in Nigeria has increased overtime from 3.4% in 1976 to 14.6% in 2007 (Abubakar, 2007). The widespread distribution of *M. bovis* in farm and wild animal populations represents a large reservoir of this micro-organism worldwide. Human cases of tuberculosis associated with *M bovis* infection have also been described in Nigeria (Idrisu and Schnurrenberger, 1977; Abubakar *et al*., 2005; Ofukwu *et al*., 2008). The problem of *M. bovis* in Nigeria may persist because the control policy stated in the Animal Disease (Control) Decree of 1988 is poorly or inadequately implemented in recent years (Abubakar *et al.*, 2011).

Tuberculosis, being a major opportunistic infection can further compromise the immune status of some already immunocompromised persons living with HIV/AIDS, and other immunosuppressive and debilitating disorders such as diabetes, chronic renal disease and cancer. (Harries, 1990; Hamburg and Freiden, 1994; Cosivi *et al.*, 1995; Pablos-Mendez *et al.*, 1997; Perez *et al.*, 2006). In 2008, there was an estimated 1.4million new case of tuberculosis among persons with HIV infection and tuberculosis accounted for 25% of AIDS related deaths (WHO, 2010).

There are a lot of challenges associated with diagnosis of tuberculosis in most developing countries, such as: limited use of intradermal tuberculin skin testing in the field due to repeat visits and inadequate handling facilities, in addition to the potential for cross reactivity with environmental Mycobacteria (Kaneene and Theon, 2004; Cadmus *et al*., 2006; Michel *et al*., 2006). Gathering of cattle or small ruminants together can be quite challenging in most pastoral settlements due to obvious lack of adequate handling facilities. Other factors such as specific training of the Veterinarians or inoculation sites, proper keeping of syringes in working order,

refrigeration and appropriate storage of samples and reagents can be difficult due to unreliable power supplies. These can compromise the correct diagnosis of bovine tuberculosis. In Nigeria, common diagnostic test for human TB in primary health centers and hospitals is microscopic examination and culture and speciation are not routinely done (NTBLCP, 2008). The epidemiology and public health significance of bovine tuberculosis has not been fully recognized as few laboratories in the country are capable of differentiating *M. bovis* from *M. tuberculosis* and other members of the *M. tuberculosis* complex (Cadmus *et al.*, 2004).

Although the standardized purified protein derivative (PPD) antigen has been used for many years as a predictor of active or latent tuberculosis infection in cattle, it has not been fully exploited in small ruminants in Nigeria. Other factors such as susceptible populations, Bacillus Calmette-Gue□rin (BCG) vaccination and cross-sensitization with other atypical *Mycobacterium* species have all affected the accuracy of the tuberculin skin test (Koo *et al.*, 2005).

1.3 JUSTIFICATION

Bovine tuberculosis being a zoonotic disease has a direct impact on public health and livestock production and therefore is a major threat to humanity. The roles of meat and milk the commonest source of protein to man in the transmission of the disease remains significant. Small ruminants constitute a major source of meat consumed in Nigeria with about 35% of total meat supply (Bayer, 1984).

Nigeria has one of the largest livestock populations in Africa and Kaduna state is the center of trade in livestock and livestock feed, attracting investors from neighboring states and countries. The state currently has 11 large livestock markets (Anon, 2010). Close physical contact between

humans and potentially infected animals is present in most developing countries and even though proper food hygiene practice could play a role in controlling this disease, such practices are difficult to institute in developing countries. Hence, there is the need for regular surveillance and determination of health status of the animals.

Infections due to *M. bovis* is widespread, nevertheless, few cases of tuberculosis in goats have been reported in countries with high incidence of *M. bovis* in cattle such as Nigeria. The possible presence of tuberculosis in goats in Nigeria was reported by Ojo (1994) on the basis of gross lesion only and Cadmus *et al*. (2006) through molecular identification.

Due to limitations associated with routine tuberculin skin test, it is essential to use one of the several new in-vitro assays that appear to be more reliable in predicting active tuberculosis in BCG vaccinated animals or when cross-sensitivity to atypical Mycobacteria coexists (Koo *et al.*, 2005). The lateral-flow technology format is easy to use, non-invasive, and adaptable for multi-species use. Animals can be retested immediately as no antigen is injected into the animal, and the animal requires only one handling as compared to the tuberculin test.

The need to also check the prevalence of the disease in small ruminants in Kaduna State is apparent. Since, a well coordinated report on any disease is the foundation of epidemiological data, within which lies the knowledge of the disease and control strategy that can be employed either in the treatment or in its control.

1.4 AIM OF THE STUDY

The aim of the study is to determine the prevalence of *Mycobacterium bovis* in small ruminants and the awareness that small ruminants can be possible source of the disease in humans in Kaduna metropolis.

1.5 OBJECTIVES

i. To determine the prevalence of *Mycobacterium bovis* in small ruminant flocks and those at slaughter using the Lateral flow technique.

ii. To assess the risk factors (such as close physical contact, food hygiene practices e.g. consumption of animal products) and level of awareness of *Mycobacterium bovis* infection in small ruminants among small ruminant handlers/owners in Kaduna metropolis, through administration of questionnaires.

1.6 RESEARCH QUESTION

1. Are small ruminants infected with *Mycobacterium bovis*?

2. Are small ruminant handlers/owners in Kaduna metropolis informed about bovine tuberculosis in sheep and goats?

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORICAL PERSPECTIVE OF BOVINE TUBERCULOSIS

Tuberculosis (TB) is believed to have been present in humans for thousands of years with skeletal remains showing that prehistoric humans (4000BC) had tuberculosis (Pritchard, 1988). The earliest references to the infectious nature of tuberculosis appeared in 17th century Italian medical literature. Due to the variety of its symptoms, TB was not recognized as a unified disease until the 1820s, and was not named TB until 1839 by J.L. Schonlein (en.wikipedia.org/wiki/tuberculosis).

Bovine tuberculosis has also been identified as an infectious disease of cattle for many years; it is perceived that bovine tuberculosis had its origins in cattle of India at the beginning of Christian's era during the nineteenth century (Francis, 1947).

In 1882, after Koch isolated the tubercle bacilli, (*Mycobacterium tuberculosis*) the disease was first described in horse in 1884 and then in man, subsequently in cattle and other animals around 1888 (Villaescusa, 2007). Human, avian and bovine types of tubercle were first described in the USA by Theobald Smith and *M. bovis* was first named by Karlson and Lessel in 1970 (Pritchard, 1988). Koch did not believe that bovine and human tuberculosis were similar, therefore wrongly assumed that human type of the organism had low pathogenicity for cattle, that the reverse was also the case and that *M. bovis* posed little danger to human health (de la Rua-Domenech, 2006a). This perception delayed the recognition of infected milk as a source of infection. Following a debate that arose about the zoonotic implication of TB, a committee set up by the British Royal Commission to investigate the zoonotic implication of TB submitted a final report

published in 1911 which concluded that *M. bovis* can infect man resulting in clinical disease, and cow's milk was recognized as a substantial source of infection particularly for children (Grange and Yates, 1994; de la Rua-Domenech, 2006a).

It was earlier postulated that TB originally evolved from animals then to humans (Diamond, 2002). New findings however indicate that TB emerged first in humans then later transmitted to animals (Writh *et al*., 2008).

Recent findings suggest that the common ancestor of the *M. tuberculosis* complex emerged from its progenitor perhaps 40,000 years ago in East Africa. Some 10,000 to 20,000 years later, 2 independent species evolved one resulting in *M. tuberculosis* lineage in humans while the other spread from humans to animals, resulting in the modification of its host spectrum and formation of other *M. tuberculosis* complex member species including *M. bovis* (Gutierrez *et al*., 2005; Writh *et al*., 2008; Michel *et al*., 2010). This alteration to animal host probably corresponds with the domestication of livestock approximately 13,000 years ago (Diamond, 2002).

In recent history, cattle served as principal reservoir species for *M. bovis* thus the name bovine tuberculosis. This term is also used to describe *M. bovis* infection in other bovine species including wildlife and humans to show the bovine source of the infection (Hewinson *et al*., 2006). Although the ultimate origin of *M. bovis* is unknown, historical data could suggest that bovine tuberculosis actually emerged in Europe and was distributed throughout the world mostly during the colonial period (de la Rua-Domenech, 2006a). Meyers and Steele (1969) suggested that *M. bovis* emerged in Europe and spread from northern Italy to Western Europe and the UK (de la Rua-Domenech, 2006a). *Mycobacterium bovis* was subsequently distributed throughout

the world through exportation of infected cattle from the UK and the Netherlands to their former colonies (Renwick *et al*., 2007).

In Africa, *M. bovis* was reported at the beginning of the 20th century (Michel *et al*., 2010). During colonial times, the emigration of settlers and their livestock facilitated the large scale intercontinental movement of infected cattle. In the post-colonial period, cattle were exported from Europe into many African countries mainly to improve the dairy production in these countries. As a result, strains representative of *M. bovis* species which had evolved through colonial expansion in a restricted geographical location were eventually shared between geographically distinct countries with political and economical ties (Muller *et al*., 2009). Intensification of the dairy industry in combination with movement of cattle has contributed to the transmission of *M. bovis* particularly in the absence of suitable control measures (Michel *et al*., 2010).

Cattle trade between neighboring countries and trading partners probably lead to the regional distribution of *M. bovis* and to the dominance of strains in large areas (Cadmus *et al*., 2006; Muller *et al*., 2008).

2.2 THE IMPORTANCE OF BOVINE TUBERCULOSIS

2.2.1 General Overview

Bovine tuberculosis is categorized as a list B disease by the Office of International Epizootics (OIE) due to the potential public health, socio-economic and trade implications in countries where the disease is present (OIE, 2009).

As a zoonotic disease, bovine TB poses greater problem in developing countries because of deficiency in preventive and/or control measures (Abubakar *et al.,* 2011). Of all African nations, only seven countries apply control and preventive measures, while the remaining 48 countries control the disease inadequately or not at all. It is estimated that 15% of the cattle population are in countries where bovine TB control measures are undertaken, however, approximately 85% of the cattle population and 82% of the human population of Africans are in areas where bovine TB surveillance and control activities are often inadequate or unavailable (Cosivi *et al*., 1998).

Bovine tuberculosis has a major impact on the farming industry. In 1999/2000, UK Government spent approximately £38 million out of which £17 million was used for cattle testing, while 2004/2005 fiscal year revealed an estimated £91 million spent on bovine tuberculosis program out of which £36 million was devoted for cattle testing (DEFRA 2005a). These shows an increase incurred in monetary terms over the years in the control of the disease. Other major disruptions such as movement restrictions, handling of cattle for testing, drop in milk and production in general are unavoidably experienced by farmers (Bennett and Cooke, 2006). In most developing countries like Nigeria, the economic impact cannot be quantified in monetary terms because of poor or inadequate implementation of control policies in recent years and also due to inadequate record keeping as it relates to animal production.

Compared to human tuberculosis, bovine tuberculosis has a much wider host range. Natural *M. bovis* infections have been described in most domestic animal species and in many wild mammals in UK and other places. (Morris *et al*., 1994; Cousins, 2001; Delahay *et al.,* 2002). The persistence of the disease in these countries associated with presence of these wildlife reservoirs present a great problem.

2.2.2 Bovine Tuberculosis as a Zoonosis

The epidemiological link between tuberculosis in cattle and in humans especially children has been long recognized (Michel *et al*., 2010). Globally, one third of the world population is infected with the tubercle bacillus but the number of cases owing to *M. bovis* has been difficult to establish (Cosivi *et al*., 1998; de la Rua-Domenech *et al.*, 2006a; Theon *et al.*, 2006). In 1998, WHO, however reported that 3.1% of tuberculous cases in humans worldwide are attributable to *M. bovis* and that 0.4%-10% of sputum isolates from patients in African countries could be *M. bovis* (Cosivi *et al.*, 1998; Theon *et al*., 2006).

Tuberculosis in humans due to *M. bovis* is clinically, radiologically and pathologically indistinguishable from cases caused by *M. tuberculosis* (Wedlock *et al.*, 2002; Ayele *et al.*, 2004). In addition, culture identification of human tuberculosis cases which might have been caused by *M. bovis* is not routinely done in many laboratories in developing countries consequently making it difficult to differentiate the two species (Cosivi *et al*., 1998; Thoen *et al*., 2006). Thus, the magnitude of human tuberculosis caused by *M. bovis* is unknown.

Human tuberculosis due to *M. bovis* was conventionally due to ingestion of contaminated milk resulting in non-pulmonary TB often presenting as cervical lymphadenopathy (Grange and Yates, 1994). Direct aerogenous infection of *M bovis* occurs in humans leading to pulmonary TB and agricultural workers are individuals at risk (Smith *et al*., 2004).

Mycobacterium bovis can also be transmitted directly by aerogenous route and through direct contact with material contaminated with nose and mouth secretions from an infected herd of cattle (Francis, 1972; Collins and Grange, 1980). Agricultural workers, Veterinarians, abattoir workers, autopsy personnel, farmers, milkers, and animal keepers are all potentially individuals

at risk (Smith *et al.,* 2004; Ofukwu, 2006). Two cases of pulmonary tuberculosis infection by *M. bovis* from a farming family were reported in the UK suggesting that there is a risk to humans from *M. bovis* sourced in the respiratory tract of cattle (Smith *et al*., 2004). Chronic skin tuberculosis (*lupus vulgaris*) and genitourinary TB caused by *M. bovis* are also reported (Grange, 2001). An estimated prevalence of the infection in cattle was 15-20% with about 2500 people dying annually from zoonotic TB in Great Britain, representing 6% of total human deaths due to tuberculosis (de la Rua-Domenech, 2006a).

In most developed countries, the disease in farmed animals and the risk of human infection due to *M. bovis* is relatively low due to regulated meat inspection and pasteurization of milk (Neill *et al*., 2005). Human TB caused by *M. bovis* in developed countries has been estimated to be around <1% of all TB cases (Grange, 2001; Neill *et al*., 2005). In such cases, the transmissible post primary (reactivation) form of the disease is encountered mostly in elderly persons who have or have had agricultural association with livestock and acquired the infection before the bovine eradication schemes were completed (WHO. 1994; Neill *et al.,* 2005).

The reemergence of *M. bovis* infection in captive and free-ranging wild animals, with subsequent transmission of infection to domestic animals is of concern to most developed countries (Michel, 2002; Kaneene and Theon, 2004). Another concern to developed countries now are the reemergence of infections due to the introduction of *M. bovis* by immigrants from countries with high TB prevalence and reactivation of the disease in HIV-infected patients (Pavlik *et al*., 2003; Theon *et al*., 2006).

The disease in developing countries is a major problem because transmission to humans not only exists unchanged but the human population has a greater vulnerability due to poverty, HIV/AIDS

and reduced access to health care and lack of information (Ayele *et al*., 2004; WHO. 2009; Michel *et al*., 2010). There is limited data available on the exact percentage of *M bovis* infection in humans even though it was reported that in African countries, 85% of cattle and 82% of the human population live in areas where the disease is prevalent or only partially controlled or no control measures, the exact percentage of *M. bovis* infection in human TB cases is often difficult to determine (de la Rua-Domenech, 2006a). This can be attributed to many factors such as inadequate diagnostic methods, politico-economic reasons (Abubakar *et al*., 2011).

2.2.3. Bovine Tuberculosis in Cattle

Bovine tuberculosis is one of the most devastating diseases of cattle, having great impact on animal health and farming industry in many countries (Fetene and Kebede, 2009). The disease caused more losses among U.S. farm animals in the early part of the century than all other infectious diseases combined (McGuiness, 1998). In England more than 40% of cows in dairy herds were infected and 0.5% had tuberculous mastitis (Collins and Grange, 1983). This led to establishment of compulsory TB eradication scheme with annual herd testing, compensation for reactors and restriction of herd movement (Hardie and Watson, 1992). By the end of 1970, the herd incidence reached the historical minimum; from 3.5% in 1961 to 0.49% in 1979 (de la Rua-Domenech *et al*., 2006b). Bovine tuberculosis in most African countries has had its detrimental impact on livestock population, however the extent, quantity and qualitative effect is not readily available because Government resources for monitoring animal diseases are poor and the capacity of the private sector to assume such responsibility is also lacking. In Senegal and Gambia previous studies on prevalence of bovine tuberculosis have shown a relatively low occurrence (Zinsstag *et al*., 2006), prevalence rates of 2 to 19% has been reported in Mali, 4 to

28% in Burkina Faso, 13% in Niger, 2 to 19% in Ghana (Delafosse *et al*., 1995; Zinsstag *et al*., 2006; Muller *et al*., 2008). In Nigeria, prevalence rates of 0.4%, 8.2%, 8.8%, 12.5%, 14% (Dusai and Abdullahi, 1994; Cadmus *et al*., 2006; Abubakar, 2007; Danbirni *et al*., 2010).

2.2.4. *Mycobacterium bovis* Infection in Goats and Sheep

Mycobacterium bovis has a broad host range and is the principal agent responsible for tuberculosis in domestic and wild animals in which it causes chronic, progressive and principally respiratory diseases (O'Reilly and Daborn, 1995; Cousins, 2001; de la Rua-Domenech, 2006a).

Among domestic animals, captive (ferrel) deer and occasionally goats act as maintenance host of *M. bovis*. Goats are very susceptible to infection with *M. bovis* and if they are kept with infected cattle herd may have incidence reaching 70% (Radostits *et al*., 2000; Hiko and Agga, 2010). Goats can develop both pulmonary TB and tuberculous mastitis, with resultant shedding of the bacillus in the milk of lactating goats (O'Reilly and Daborn, 1995). Clinical TB due to *M. bovis* infection has been reported in person's who are in-contact with domestic goat herd in Spain, with mortality rates ranging from 20% to 50% per year in different milking flocks (Hikko and Agga, 2010). A goat-adapted strain of *M. bovis* referred to as *M. bovis* subsp *caprae* by certain authors was responsible for one-third of all zoonotic TB cases reported in Germany between 1991 and 2001 (Tanja *et al.*, 2003). Clinically, caprine TB is characterized by bronchopneumonia manifested by cough and terminal dyspnea. Intestinal ulcerations with diarrhea and enlargement of lymph nodes of the alimentary tract may occur. All these lesions are similar to those seen in cattle (Radostits *et al*., 2000; Ayele *et al*., 2004; Hikko and Agga, 2010).

Individual cases of *M. bovis* infections in sheep have also been reported, however, ovine diseases is rare and usually associated with cattle cases (Humblet *et al*., 2009). In some study, the caprine Mycobacterial isolate was also found in a sheep and was attributed to close contact with goats (Aranaz *et al*., 1999). Gutierrez *et al*. (1997) reported three human isolates of a goat-adapted strain of *M. bovis* linked with goat farming. One of the patients was resident in a rural area where goat farming was common, the second worked in an abattoir, and the third was a Veterinary practitioner with history of contact with infected goat herds (Aranaz *et al*., 1999; de la Rua-Domenech, 2006a). Despite high incidence of tuberculosis in cattle, only few cases of TB have been reported in goats (Aranaz *et al*., 1999).

M. bovis and *M. caprae* are usually the cause of TB in goats (Alvarez *et al*., 2008; Crawshaw *et al*., 2008; Daniel *et al*., 2009). However, *M. tuberculosis* infection in goats has also been reported by Cadmus *et al*. (2009).

2.3. AETIOLOGY AND PATHOGENESIS OF BOVINE TUBERCULOSIS

2.3.1 Aetiology

Tuberculosis is caused by *Mycobacterium* species; it is an acid-fast organism which is almost ubiquitous. Bovine tuberculosis is caused by *M. bovis*, it belongs to a group of Mycobacteria known as *Mycobacterium tuberculosis* complex (MTBC), family, Mycobacteriaceae which comprises four main species; *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microtii* as well as *M. canetti* and *M. caprae* (Van Soolingen *et* al., 1997; Neill *et al*., 2005).

M. bovis is the most universal pathogen among the MTBC causing tuberculosis in a wide variety of domestic and wild animals including humans (Collins and Grange, 1983; Wayne and Kubica,

1986; O'Reilly and Daborn, 1995). Susceptible species include cattle, goats, cats, dogs, pigs, buffalo, badgers, possums, deer and bison, non-primates and humans (O'Reilly and Daborn, 1995). *Mycobacterium bovis* which is the cause of bovine tuberculosis and *Mycobacterium tuberculosis* the cause of classical human tuberculosis are genetically and antigenically very similar and cause identical clinical disease in humans (Cosivi *et al*., 2008). Certain animals are considered 'spill-over' host, while others are said to be 'maintenance' or 'reservoir' host and transmit the infection (de la Rua-Domenech, 2006a).

M. bovis is a small aerobic, non-motile bacillus, the high lipid content of this pathogen accounts for lot of its unique clinical characteristics. It divides every 16 to 20 hour, an extremely slow rate compared with other bacteria (Radostits *et al*., 2000). It is classified as a Gram-positive bacterium because its cell wall lacks a phospholipid outer membrane and if Gram stain is carried out, *M. bovis* either stains very weakly Gram-positive or does not retain dye as a result of high lipid and mycolic acid contents of its cell wall. Mycobacterium can withstand weak disinfectant and in a dry state for weeks. In nature, it grows within the cells of host organism however; mycobacterium can be cultured in the laboratory.

2.3.2. Clinical Signs and Lesions

Infection with *M. bovis* is usually chronic and can remain subclinical for a long time (Radostits *et al*., 2000). Clinical signs of tuberculosis vary depending on the extent and location of the lesions. Bovine TB is mainly a disease of the respiratory tract however, generalized disease can occur, but it is rare in countries where eradication control program is in place (Radostits *et al*., 2000).

A useful diagnostic sign is detection of enlarged superficial lymph nodes. The principal sign of tuberculosis is wasting or emaciation that arises regardless of good nutrition; other general signs include weakness, anorexia, dyspnea and low grade fluctuating fever. In mammals, the organs of the thoracic cavity are normally involved (Radostits *et al*., 2000). When the lungs are extensively affected, an intermittent hacking cough is usually detected mostly after exercise (Kaneene and Theon, 2004).

The cell-mediated (T-lymphocytes) is the first and most predominant immune response and is the cause of the chronic characteristic caseous granuloma (Ritacco *et al*., 1991; Monaghan *et al*., 1994). *M bovis* infection first results in formation of a primary focus, usually located in the lungs and thoracic lymph nodes (Neill *et al*., 2001; Kaneene and Theon, 2004). In mammals, lymphatic drainage from the primary focus leads to formation of caseous lesions in an adjacent lymph node, this lymph node lesion together with the primary focus is called the 'primary complex'. This 'primary complex' seldom heals in animals, the disease then progresses over a number of years during which the humoral (antibody production) immune response then appears in the more advanced stages of the disease (Kaneene and Theon, 2004). Infected cattle can become infectious long before they show any clinical signs or lesions typical of the disease (de la Rua-Domenech *et al*., 2006b). The natural and acquired immune response mechanism of a host is often successful in limiting proliferation of tubercule bacilli and the development of progressive disease (de la Rua-Domenech, 2006b).

A great number of tuberculous cattle were previously reported to have lung tissue lesions (Corner, 1994; Neill *et al*., 2005), however, studies have shown that lung lesions are more common when no control measures were in place (Menzies and Neill, 2000). Tuberculous lesions are found most frequently in the bronchial and or mediastinal lymph nodes, the second

most affected nodes are those of the head, the retropharyngeal and sub-maxillary lymph nodes in the absence of lesions in the lungs (Neill *et al*., 2001). *M. bovis* has been isolated within the upper respiratory tract, the tonsils, nasal pharynx, trachea and nasal mucus contributes to the spread (Neill *et al*., 1994).

2.3.3. Route of Transmission and Infective Dose

Several routes of transmission for *M. bovis* infection exist. Transmission can be either direct, through contact or indirect from exposure to viable bacteria in a contaminated environment (e.g. pasture and feed). However, the primary routes of infection are through the respiratory and gastrointestinal tracts (Kaneene and Theon, 2004). Infection by inhalation is the most efficient form of transmission as it requires low number of organisms as infective dose and majority of the lesions seen are in the respiratory tract (Collins and Grange, 1983; Pritchard, 1988; Neill *et al*., 1994).

Infection via respiratory route has been identified in herding animals such as domestic cattle, feral water buffalo and African Cape buffalo and captive herds of various cervid species (Kaneene and Theon, 2004). Among the wildlife species that are kept in confinement in zoos and free-ranging wildlife that maintain social groups in underground dens such as, European badgers, brushtail possum, transmission of *M. bovis* via respiratory route appears to be effective (Morris and Pfeiffer, 1995; Kaneene and Theon, 2004).

Infection by ingestion, the second most common route of infection is more likely to occur in calves suckling tuberculous cows or by swallowing infected sputum or consumption of feed or water contaminated with mucous or nasal secretions, feces, or urine containing infective

organism (Palmer *et al*., 2002). Oral transmission of *M. bovis* has been detected in several species in cattle that graze pastures contaminated with the organism (Benham and Broom, 1991; Hutchings and Harris, 1997; Kaneene and Theon, 2004) and also in feral swine that scavenge among infected animal carcasses in wild carnivores in Africa and in dogs and cats that consume contaminated milk or scavenge contaminated carcasses (Kaneene and Theon, 2004). The consumption of unpasteurized milk or dairy products from infected cows was an important cause of children tuberculosis in the UK until pasteurization became widespread in the mid 20^{th} century (DEFRA, 2005a, 2006, 2007).

In general, the infective dose required via respiratory tract is much lower (with minimum infective dose of five bacilli or less if delivered in an aerosol to alveolar spaces) than that of oral route (with minimum infective dose of 10^7 bacilli) (Neill *et al*., 1991; Corner, 2005), hence requiring a very high oral dose (Corner, 2005). It has been reported that the size of inhaled droplet containing Mycobacterium and the dose are important in establishment of the disease via aerosol (Neill *et al*., 1991; Menzies and Neill, 2000).

Experimental investigation involving exposure of animals to *M. bovis* through intravenous, intratracheal, intraperitoneal and via oral route has indicated that the nature and extent of resultant disease vary with route of exposure (Kaneene and Theon, 2004). Generalized systemic lesions were reported when infection route used was IV, SC or oral route and with doses between 10^6 and 10^8cfu (Palmer *et al*., 1999). Lesions were mainly found in upper respiratory tract then lower respiratory tract when intra-tonsillar route was challenged with doses between 10^6 and 10^8cfu (Palmer *et al*., 1999). With doses between 10^3 and 10^5cfu, generalized lesions were found when intra-tracheal route was undertaken (Buddle *et al*., 1994).

The interrelationship between the immune response of the host and the proliferation of the bacilli in macrophages plays a role in the progression of the disease from early infection of macrophages to the development of caseous nodules that undergo calcification and liquefaction, as well as the regression, progression or generalized spread of lesions (Kaneene and Theon, 2004).

2.3.4. Excretion of *M bovis* and Survival in the Environment

Following experimental infection in calves, excretion of *M. bovis* in nasal mucus is a coherent feature in all infected cattle which can continue for weeks and even months (Neill *et al*., 1988; 1989). In addition, shedding rates ranging from 6% and 20% were found among naturally infected, tuberculous cattle in different countries, whereby the occurrence of lung lesions maybe very low (Neill *et al*., 1988). *M. bovis* was detected in the nasal mucous and faeces of calves in contact with cattle infected intra-nasally thirty-five days after exposure, this implied that excretion by recently infected animals may occur (Cassidy *et al*., 1999).

M. bovis maybe excreted in mucus, milk, urine and faeces in cases where widespread bovine TB occurs (Scanlon and Quinn, 2000). These routes nevertheless are relatively insignificant in developed countries because of regular surveillance (Neill *et al*., 2005). Data on environmental survival of *M. bovis* is contentious (Humblet *et al*., 2009). Menzies and Neill (2000), in their review stated that *M. bovis* is able to survive in the environment for only a few weeks under natural conditions. Since environmental contamination is less effective means of disease transmission to cattle (Humblet *et al*., 2009), infection of cattle through environment is most likely insignificant as it requires a high dose of 10^7 bacilli (O'Reilly and Daborn, 1995). As a

result of the high infective dose required for oral transmission, and survival rate of *M. bovis*, Morris *et al*. (1994), also concluded that the role of contaminated feed and pasture is negligible.

The survival of *M. bovis* in liquid cattle slurry protected from the direct sunlight has been estimated in previous studies (Menzies and Neil, 2000; Scanlon and Quinn, 2000). According to Menzies and Neill (2000), *M. bovis* can survive for months in soil and slurry (from 18 to 332 days) at temperatures between 12-24°C and up to 700 days when buried in soil protected from sunlight and available nutrients such as feces, blood and urine (Humblet *et al*., 2009).

Other studies suggested the following as major factors influencing survival of *M. bovis* in soil and pastures; temperature, moisture and pH, exposure to sunlight, dissolved oxygen, presence of naturally occurring antibodies in the soil, natural micro-flora and types of micro-floral associations and soil ecology (Duffield and Young, 1985; Jackson *et al*., 1995; Tanner and Micheal, 1999; Scanlon and Quinn, 2000; Kanene and theon, 2004). Water points are to be considered potential risk factors because of the moist environment and great amount of shade; these two are favorable conditions for *M. bovis* survival as identified by experiments (Humblet *et al*., 2009).

The importance of environmental contamination to the spread of bovine TB to healthy cattle might differ between developed and developing countries (Humblet *et al*., 2009). Environmental sources could play a significant role in their spread to wide range of animal species from different habitats and ecological niches, leading to spillovers (Michel *et al*., 2007). In Africa, most herds share water points, therefore an excreting animal with high infective potential may present a high risk (Humblet *et al*., 2009).

2.3.5. Understanding How Cattle to Cattle Transmission Occurs in the Field

Transmission of *M. bovis* between animals can be through inhalation of aerosol, ingestion or through breaks in skin, however, the importance of these routes differ between species (Kanene and Theon, 2004). In cattle, aerosol transmission is mostly encountered; hence, close contact between animals is a major risk (Ameni *et al*., 2006; Humblet *et al*., 2009). Certain animals become infected after ingesting the organism; this is seen mostly in calves nursed by infected lactating cows (Wilsmore and Taylor, 2008). Study conducted has shown that the severity of bovine TB was significantly greater in cattle kept indoors at a higher population density than in cattle kept on pasture (Ameni *et al*., 2006). Innate immunity, host defense, latency and reactivation could play an important role in understanding why some animals do not become infected and why so few in herd react positively to the skin test (Villaescusa, 2007).

2.4 EPIDEMIOLOGY/ DISTRIBUTION OF BOVINE TUBERCULOSIS

M. bovis has an exceptionally wide host range with virtually all mammals susceptible to the infection. The infection has been sporadically identified in several domestic species other than cattle including pigs, cats, dogs, alpacas, llamas, goats, sheep, deer and farmed deer (Wilsmore and Taylor, 2008). Among wildlife species other than the badgers (*Meles meles*) that serves as reservoir/maintenance host are the feral pig (*Suis scrofa*) and feral Asian water buffalo (*Bubalis bubalis*) in Australia, white tailed deer (*Odocoilens virginalis*) in Michigan, the bushtail possum (*Trichosurus vulpecula*), the ferret (*Mustela furo*) in New Zealand (Corner, 2005; Delahay *et al*., 2007).

2.4.1 Burden of Bovine Tuberculosis in the World

Tuberculosis remains an important worldwide health problem and currently one of the leading causes of death in humans due to infectious diseases globally with an estimated total number of 9.4 million incident cases worldwide in 2009 and over 1.7 million deaths reported in 2007 (WHO, 2010). In 2004, TB incidence rates were estimated to have peaked but subsequently reduced at a rate of less than 1% per year (WHO, 2010). However, overall worldwide burden keeps rising as a result of the rapid world population growth (WHO, 2010), with the majority of cases in Asia and Africa while a lesser proportion of cases are in the Eastern Mediterranean region, European region and the Americas (WHO, 2010). Twenty-two (22) countries constitute 80% of the worldwide burden and India, China, South Africa, Nigeria and Indonesia are the first five countries in the world in terms of total numbers of incident cases in 2009 (WHO, 2010).

Bovine tuberculosis is widespread in cattle throughout the globe. As stated by the worldwide animal health information database of the OIE (Michel *et al*., 2010), 128 out of 155 countries reported the presence of *M. bovis* infection and/or clinical disease in their cattle population during the period between 2005 to 2008.

2.4.2 Burden of Bovine Tuberculosis in Africa

Bovine tuberculosis is widespread throughout Africa and is thought to have been brought into Africa by European settlers in the early 1800s (Cosivi *et* al., 1998; Ayele *et al*., 2004; de la Rua-Domenech, 2006a). The prevalence of BTB has however reached very low level in most developed countries (Michel *et al*., 2010) due to policies put in place which was aimed at complete eradication of the disease from its livestock populations in order to ensure food safety.

On the other hand, the situation is greatly different in developing countries because they are unable to employ adequate control measures for the control or eradication of bovine tuberculosis (Michel *et al*., 2010). The prevalence of the disease was reported in 33 (80%) out of 41 African countries (Daborn and Grange, 1993). In the African continent, it was reported that nearly 85% of cattle and 82% of the human population coexist in areas where the disease is prevalent and/or partially controlled (Cosivi *et al*., 1998), and only a relatively few countries consider bovine TB as a notifiable disease (Neill *et al*., 2005) and therefore the presence and extent of bovine TB in developing countries has been poorly studied in the past. In recent times, studies have revealed new data confirming the presence of *M. bovis* in cattle in different African regions (Cadmus *et al*., 2006; Diguimbaye *et al*., 2004; 2006; Abubakar, 2007; Okaiyeto *et al*., 2008; Michel *et al*., 2010).

In Africa, high prevalence rates of bovine TB (up to 50% at herd level) were reported in areas of Zambia where cattle and antelopes share grazing areas and water points, and also areas where the traditional management of livestock in transhuman herds prevailed (Oloya *et al*., 2007; Munyeme *et al*., 2009). In Tanzania, 88% of villages screened had at least one animal that tested positive for bovine TB and 10.5% of people with stomach or lymph gland tuberculosis were infected by *M. bovis* (Bolognesi, 2007). Ethiopia is among the 3 African countries with the highest burden of human TB cases (WHO, 2008) with the prevalence of bovine TB ranging from 3.4% in small holder production systems that keep zebu cattle to 50% in peri-urban (intensive) dairy production systems (Ameni and Roger, 1998; Ameni *et al*., 2003; Regassa, *et al*., 2008). In Tanzania, 14% of cattle were found to be infected with bovine TB following tuberculin testing of about 10,000 cattle over the course of 3-years showing that the disease is widespread.

In Nigeria, the prevalence of bovine TB over the last 30years has been reported to be between 2.5% in 1976 to 14% in 2007 (Idrisu and Schnurrenberger, 1977; Cadmus *et al*., 2006; 2010a; Abubakar *et al*., 2011).

Worldwide more than 2 million deaths occur each year as a result of tuberculosis regardless of which tuberculous agent is involved (Neill *et al*., 2005), with the highest number per capita occurring in Africa. This is due to high prevalence of HIV/AIDS in African countries with the combine effect of multi-drug resistant strains of mycobacterium (Neill *et al*., 2005). WHO (1998), reported an estimated global prevalence of human TB cases and *M. bovis* as reaching 3.1% of all human TB cases and 0.4-10% of sputum isolates from patients in African countries could be *M. bovis* , however possible underestimation should be considered (Neill *et al*., 2005; Michel *et al*., 2010). Isolation rate of *M. bovis* from symptomatic human patients in specific studies was 13.8% in Mexico, 6.9% in Uganda, 5% in Nigeria, 0.5% in Taiwan, between 0 and 2.5% in Latin American countries (Cadmus *et al*., 2006; Oloya *et al*., 2008; Jou *et al*., 2008; de Kantar *et al*., 2008; Michel *et al*., 2010).

2.4.3. Epidemiology/ Burden of *M. bovis* Infection in Sheep and Goat

Tuberculosis in goats has been recognized since the early 1900s mostly as a rare disease; in 1958 Francis reported that 1% of domestic goats in some European countries were affected when they co-exist with cattle (Alvarez *et al*., 2008). In India, goats are sometimes tied in a room where TB patients reside with the belief that the goaty smell from the urine and breadth of these animals is helpful to the patient (Tenguria *et al*., 2011).

M. bovis and *M. bovis* subsp. *caprae* is mostly the causative agent of tuberculosis in goats (Alvarez *et al*., 2008; Crawshaw *et al*., 2008; Daniel *et al*., 2009; Alvarez *et al*., 2008), however, *M. tuberculosis* infection has also been reported by Cadmus *et al*. (2009). Outbreaks of tuberculosis in caprine species caused by *M. bovis* have recently been reported in the UK, Italy and Portugal (Crawshaw *et al*., 2008; Daniel *et al*., 2009; Zanardi *et al*., 2009; Quintas *et al*., 2010; Alvarez *et al*., 2008). In Spain, outbreaks in goats caused by *M. bovis* has been reported (Gutierrez *et al*., 1998; Liebana *et al*., 1998), however more recently *M. bovis* subsp. *caprae* is the main agent isolated from caprine outbreaks (Alvarez *et al*., 2008).

In Germany, epidemiological studies carried out between 1999 and 2001 showed that *M. bovis* subsp *caprae* was the causative agent of one-third (31%) of human *M. bovis* associated tuberculosis cases analyzed (Kubica *et al*., 2003).

2.4.4. Burden and Risk of Zoonotic Tuberculosis due to *Mycobacterium bovis*

Mycobacterium bovis is a relevant zoonotic pathogen that can be transmitted between animals, from animals to humans and vice-versa (Theon *et al*., 2006). *M. bovis* has been classified by OIE as risk 3 pathogen for public health (OIE, 2005; Theon *et al*., 2006). It has been documented that *M. bovis* has been associated with extrapulmonary TB in infants and children as a result of drinking unpasteurized milk from infected cattle (Theon *et al*., 2006).

Although varying portions of pulmonary TB occur, almost all cases of non-pulmonary type of TB in humans have been due to *M. bovis* (Schwabe, 1984, Nwanta *et al*., 2010). From a review of a number of zoonotic TB studies from 1954 to 1970, it was established that proportion of

human cases due to *M. bovis* accounted for 3.1% of all forms of TB; 2.1% of pulmonary forms and 9.4% of extra-pulmonary forms (Cosivi *et al*., 1998).

A broad survey of human TB due to *M. bovis* in a number of regions of the world has been documented:- in Latin America, a moderate estimate of 2% of total pulmonary TB cases and 8% of extra-pulmonary TB cases are caused by *M. bovis* (Cosivi *et al*., 1998). In East Germany, 4.6% of all newly diagnosed pulmonary cases in 1982 were due to *M. bovis* the percentage however decreased from 3.5% to 3.1% in 1983 and 1984 respectively (Theon *et al*., 2006). During the period of 1990-2003 in the UK, 0.5-1.5% of an average of 7,000 human cases of TB were confirmed to be caused by *M. bovis* (De La Rua-Domenech, 2006b). In Argentina, 8% of mycobacterial isolates from extra-pulmonary specimens were typed as *M. bovis* in 1982 (Peluffo and Kantor, 1982; Theon *et al*., 2006). In France, in the year 1995 cases due to *M. bovis* accounted for 0.5% of all mycobacterium isolates (Robert *et al*., 1999 and Theon *et al*., 2006).

As stated by Cosivi *et al*. (1998), preliminary studies conducted in Africa show that (5-7%) of human TB cases is caused by *M. bovis*. A study in Egypt revealed that nine (9) out of 20 randomly selected patients with TB peritonitis were infected with *M. bovis* (Nafeh *et al*., 1992). Similar studies have been reported in parts of Africa such as Tanzania, Zaire (Congo Democratic Republic), Ethiopia and Cameroon. In Nigeria, reports on isolation of *M. bovis* in 5 sputum positive cultures has been made since 1977 by Idrisu and Schnurrenberger (1977), Zoonotic TB due to *M. bovis* in Nigeria is said to account for 5% of all cases of TB in humans and up to 3% of cases in children less than 5years (Ofukwu, 2006). Recently Abubakar *et al*. (2007) showed a high prevalence of both bovine and human TB amongst herds' men in Federal Capital Territory, Abuja.

Reliable information is usually not available or very low as regards to cases of human TB caused by *M. bovis*, even though it is of public health concern giving the global upsurge in prevalence of TB especially in developing countries (Nwanta *et al*., 2010). Tuberculosis due to *M. bovis* may occur in humans through direct aerogenous route or through direct contact with material contaminated with nose or mouth secretions from an infected herd of cattle and indirectly through ingestion of unpasteurized milk or contaminated meat (WHO, 1994). Individuals at risk include Veterinarians, abattoir workers, meat inspectors, autopsy personnel, farmers, milkers, animal keepers, animal dealers, laboratory personnel and pet owners.

Presently the problem of *M. bovis* in developing countries may to some extent mimic the pre-eradication era in Europe where the prevalence of bovine tuberculosis in human population was relatively high (Neill *et al*., 2005).

An important risk factor is co-infection with HIV (Theon *et al*., 2006) considering the prevalence of bovine tuberculosis in cattle and HIV positive individuals in Africa, the close contact between cattle holders and animals are of major public health concern as well as the inadequate implementation of regular pasteurization of milk in most developing countries (Ayele *et al*., 2004; Awah-Ndukum *et al*., 2010; Abubakar *et al*., 2011). The contribution of *M. bovis* infections to tuberculosis cases in humans in Africa is not well known. This is primarily due to lack of diagnostic laboratories with the means to distinguish strains of *M. tuberculosis* and *M. bovis* (Ayele *et al*., 2004).

2.4.6 Current Status of Bovine Tuberculosis in Nigeria

Nigeria is one of the African countries where bovine tuberculosis is lingering (Okaiyeto *et al*., 2008; Cadmus *et al*., 2010b and Abubakar *et al*., 2011). Nigeria has the fourth highest burden of

human tuberculosis in the world, with an incidence of 304 cases per 100,000 and mortality of 89 per 100,000 in 2002 (WHO, 2004). The state of animal tuberculosis and the degree of zoonotic transmission of TB from animals to human is not well known as there is no known national surveillance and control policy that exists (Cadmus *et al*., 2006).

The presence of bovine tuberculosis in Nigeria can be traced back to 1932 (Alhaji, 1976; Ayanwale, 1984; Du'sai and Abdullahi, 1994; Cadmus *et al*., 1999, 2003) have all pointed to the endemic nature of bovine tuberculosis in different parts of Nigeria. Wekhe and Berepubo (1989) reported a prevalence of 8.2% in the study carried out in an eastern abattoir of the country; while Cadmus (2006), confirmed the prevalence of 8.8% in Bodija abattoir in the western Nigeria. Though other workers like Dusai and Abdullahi (1994) had presented a prevalence of 0.49% between 1989 and 1993 in a northern abattoir in Kaduna, this report was however based on abattoir records. Retrospective studies conducted by Nwanta, *et al*. (2011) reported a prevalence rate of 1.4% for cattle in Enugu State. Other studies conducted in other parts of the country revealed prevalence rates of 2.1% in Maiduguri (Aliyu and Kalra, 1993), 1.44% and 2.80% based on abattoir records (Cadmus *et al*., 1999), 0.8% in Sokoto (Garba, 2001), 1.1% in Cross River (Bikom and Oboegbulem, 2007) and 0.55% in Ogbomosho areas (Ameen *et al*., 2008). Bovine TB lesions in Northeastern Nigeria ranged from 3.19 to 5.16% as reported by Aliyu *et al.* (2009)

Reports of bovine tuberculosis in both organized resident farms and nomadic herds have been made in different parts of Nigeria (Cadmus *et al*., 2004; Okaiyeto *et al*., 2008, Danbirni *et al* 2010). Okaiyeto *et al*. (2008) reported 5% prevalence of BTB in a study in Kaduna state using tuberculin test, while a prevalence of 12.5% was reported in 2010 using Immunochromatography rapid test (IQRT) (Danbirni *et al*., 2010). A study conducted by Danbirni *et al*. (2010) reported

prevalence of 17.5% in 3 dairy farms sampled in Kaduna state and 17.1% prevalence from milk obtained from cows positive to IQRT and packed yoghurt made from milk obtained from those positive and negative cows. This is of serious public health significance in Nigeria as control measures are seldom applied (Danbirni *et al*., 2010, Abubakar *et al*., 2011). A significant percentage of 82% in a survey amongst pastoralists in Kaduna say they consume unpasteurized milk (Onoja *et al*., 2010).

Tuberculosis has been described as a reemerging disease at the interface of domestic animals and wildlife by Palmer (2007). Recent studies by Joseph *et al*. (2012) revealed a prevalence of 12% for *M*. *bovis* in wildlife in Yankari Game Reserve Bauchi State.

With an estimated population of 14 million cattle and over 140 million human population, Nigeria is faced with a high burden of tuberculosis and ranking 4th among the tuberculosis-burdened nations of the world with a prevalence of 521 per 100,000 population in the year 2007 (WHO, 2009). The prevalence of human tuberculosis has increased over time due to the HIV-AIDS epidemic (Ayele *et al*., 2004) and a strong positive linear relationship is said to exist between tuberculosis and HIV-AIDS persons.

The burden of TB in Nigeria is not precisely known but the steady rise of the number of case notifications since 1996 is evidence that the case burden of TB disease on the Nigerian population is expanding and substantial with time (Annual report of NTBLCP, 2008). The growing incidence rate of TB is doubtlessly/almost certain that transmission of the deadly disease is continuing in Nigeria.

2.5. DIAGNOSIS AND CONTROL OF BOVINE TUBERCULOSIS

2.5.1. General Overview

Effective surveillance of bovine tuberculosis in developing countries where reliable data on disease prevalence is limited or even lacking is a prerequisite for control measures (Borna Muller, 2010). Tuberculosis lesions may be found in any organ or body cavity of diseased animals. The lesions are however difficult to find even during postmortem examination in early stages of the disease (Wilsmore and Taylor, 2008), but in later stages, the granumatous nodules caused by bovine tuberculosis becomes very evident in the lungs and associated lymph nodes and in lymph nodes of the head, intestinal tract, abdominal and reproductive organs, nervous system, superficial body lymph node and bones (Wilsmore and Taylor, 2008).

Human and animals with tuberculosis develop a primary immune response which is cell-mediated (CMI) (Neill *et al*., 2005). Different techniques to establish cellular immunity for the diagnosis of tuberculosis have been described. The tuberculin skin test is the internationally accepted standard method for detection of bovine tuberculosis which is widely used by Europe and other countries in the world (Costello *et al*., 1997; de la Rua-Domenech *et al*., 2006b; Mitchel *et al*., 2006).

Antemortem surveillance for bovine tuberculosis most primarily relies on detection of infected cattle at an early stage by use of sensitive cell mediated immune- based immunodiagnostic test. Tuberculin skin test has been used for many years in test and slaughter programs (Wilsmore and Taylor, 2008). More recently, the gamma interferon and lymphocyte transformation assays have been used as an additional test to tuberculin skin test to increase the sensitivity of testing

programs (Ameni *et al*., 2000; de la Rau-Domenech *et al*., 2006b; Wilsmore and Taylor 2008; Awah-Ndukum *et al*., 2010).

2.5.2. Detection of Cellular Immunity using Tuberculin Skin Test

The skin test measures a delayed hypersensitivity reaction mediated by sensitized T-cells after the intra-dermal injection with tuberculin (Monaghan *et al*., 1994). Hypersensitivity to tuberculin usually occurs in cattle between three and six weeks after infection with tubercle bacilli (Francis, 1947). Therefore there is possibility that recently infected animals do not react to the tuberculin test, though the test detect subclinical infection (de la Rau-Domenech *et al*., 2006b).

There are two types of skin test: the single intra dermal test (SIT) using the bovine tuberculin alone is usually performed at the caudal fold of the base of the tail, and also on the side of the neck, then the single comparative cervical intra-dermal test (SICCT) using avian and bovine tuberculin; is usually performed on the side of the neck (OIE, 2009). The skin of the neck is more sensitive to tuberculin than the skin of the caudal fold (Monaghen *et al*., 1994). The comparative intra-dermal tuberculin test with bovine and avian tuberculin is used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacterium or relate genera (OIE, 2009). The decision to use the single or comparative test generally depends on the prevalence of tuberculosis infection and on the level of environmental exposure to the other sensitizing organisms (Monagahen *et al*., 1994; OIE, 2009).

Different sensitivity and specificity results have been reported for SICCT and SIT. A sensitivity of SICCT test of roughly 74% and 95% and specificity of at least 99% (Monaghen *et al*., 1994;

Costello *et al*., 1997) and a sensitivity of 83.9% with a specificity between 75.5% and 99% for the SIT could be considered as references after evaluation of various studies (de la Rua-Domenech *et al*., 2006b).

More recently, the γ-IFN test has been proposed as a supplementary test that can be used to increase particularly the sensitivity of testing programs (Wilsmore and Taylor, 2008). As the γ-IFN test is able to detect a substantial proportion of infected cattle that elude detection by the tuberculin skin test, most likely because it identifies animals at a slightly earlier stage of infection than the skin test does (de la Rau-Domenech *et al*., 2006b). From studies carried out by the DEFRA founded projects γ-IFN assay is said to enhance detection with possibly greater sensitivity than the skin test, differentiate vaccinated from infected animals and from environmental exposure to non-pathogenic Mycobacteria, and detect disease earlier than skin test reagents (Wilsmore and Taylor, 2008).

The lymphocyte proliferation test is not commonly used in cattle, but may be useful in wildlife and zoo animals (OIE, 2009).

A potential problem with using the skin test and the γ-IFN test is that the skin test which relies on inoculation of Mycobacterial antigen can itself stimulate immune responses and affect the performance of other diagnostic test performed afterward (Wilsmore and Taylor, 2008). The performances and sensitivity of the two tests can also be affected by co-infecting agents such as the presence of paratuberculosis (Alicia *et al*., 2006; Awah-Ndukum *et al*., 2010). False positive reactors with the tuberculin skin test and/ or γ-IFN test most likely as a result of cross reactivity with *M. avium* subsp. *paratuberculosis* has been documented (Biet *et al*., 2005*;* Alicia *et al*., 2006*; de la* Rau-Domenech *et al*., 2006b). Therefore, the TST even though internationally

recognized as a field diagnosis for bovine TB in live animals (Neill *et al*., 2005) may not be suitable to eradicate bovine TB in herds with dual Mycobacterial infection.

2.5 3. Detection of Humoral Immunity

Anergic response (or failure to react) from some cattle to the tuberculin injection is of concern (Pritchard, 1988; Monaghan *et al*., 1994). Though the reason for this is not fully clarified, it could be linked to advance stage of the disease, generalized disease or pregnancy (Lepper *et al*., 1977). Test of humoral immunity using the Enzme-linked immunosorbent assay (ELISA), immunochromatography (lateral flow) assay and other serologic based test may complement test of cellular immunity in anergic hosts (Awah-Ndukum, 2010). ELISA techniques could be used to screen disease at a herd level or to detect the disease at a more advanced stage (De Kantor and Ritacco, 2006). Other cocktail of selected Mycobacterium antigen employed to detect circulating antibodies include ESAT-6, MTSA-10, MPTSI, MPT63, MPB59, MPB70, MPB83 (Lyashchenko *et al*., 1998; Pollock *et al*., 2001; Banerjee *et al*., 2003; Cousins and Florisson, 2005; Lyaschenko *et al*., 2007, 2008).

The aim of diagnostic technique development is to achieve a sensitive and specific test that is able to detect cattle that have been exposed to *M. bovis* but have not developed the disease, identifying those that are at risk of spreading the disease and also to distinguish vaccinated ones from infected ones (Neill *et al*., 2005). Therefore, it is beneficial to use multiple diagnostic tests to detect Mycobacteria infection in animals and humans.

2.5.4. Post Mortem Examination

Tuberculosis can also be diagnosed based on post mortem findings (OIE, 2009). Tuberculous lesions such as abscess with yellowish pus, and tubercles which maybe caseaous or occasionally 'gritty' calcification seen in carcasses, during slaughter/meat inspection are diagnostic (Gracey and Collins, 1992; FAO, 1994). Failure to detect visible lesions should not necessarily be interpreted as absence of infection; gross visible lesions suggest that the disease is at an advanced or late stage (Corner, 1994; Shitaye *et al*., 2006). Most African countries detect bovine TB based on post mortem findings of tuberculous lesions during meat inspection (Shitaye *et al*., 2006).

2.5.5 Demonstration of Tubercle Bacilli/ Identification of Tubercle

The presence of *M. bovis* in clinical (e.g. sputum) and postmortem specimens may be demonstrated by examination of stained smears or tissue sections and confirmed by cultivation of organism on primary isolation medium (OIE, 2009).

2.5.5.1 Direct Smears with the Ziehl-Neelson Stain

The acid fastness of *M. bovis* is normally demonstrated in the classic Ziehl-Neelsen stain, but a fluorescent acid-fast stain maybe used (OIE, 2009). A presumptive diagnosis of Mycobacteriosis can be made if the tissue has characteristic histological lesions such as caseous necrosis, mineralization, epitheloid cells, multinucleated giant cells and macrophages (Strong and Kubica, 1985; WHO, 1998; OIE, 2009).

Diagnosis can be confirmed by growth and isolation of the organism on a variety of selective media (Lowenstein-Jesen media, Middlebrook broth). Cultures are incubated for a minimum of 8 weeks (preferably 10-12 weeks) at 37°C with or without CO_2 (OIE, 2009). The organism can further be characterized by carrying out various biochemical tests (Nitrate reduction, Niacin and Catalase test), Morphological characteristic of the colonies (smooth and off-white (buff) in color) and Nucleic acid recognitions using the Direct Polymerase Chain Reaction (PCR) or alternatively Molecular Typing Techniques such as Spoligotyping which will identify *M. bovis* isolates and provide some molecular typing information on the isolate that is of epidemiological value (Van Soolingen *et al*., 1998; Warren *et al*., 2006; OIE, 2009).

2.5.6. Lateral Flow Technique and Development of other Diagnostic Methods

It is apparent that no single screening test meets all the requirements for surveillance and diagnosis of tuberculosis. Therefore a combination of several tests increases the overall sensitivity and specificity of screening and surveillance programs for tuberculosis in both animals and humans (Greenwald *et al*., 2007). It is of necessity to be able to detect both active and latent infections in animals (Greenwald *et al*., 2007). Recent technological advances have enabled the development of new rapid in-vitro techniques for tuberculosis surveillance and diagnosis, including cell-mediated immunity antibody, and nucleic-acid-based methods (Greenwald *et al*., 2007). The complete sequencing of the *M. tuberculosis* and *M. bovis* genome has made possible the identification of proteins unique to the *M. tuberculosis* complex (Cole *et al*., 1998; Garnier *et al*., 2003).

The lateral flow assay utilizes a cocktail of selected *M. bovis* antigens as both qualitative capture and detectors of specific antibodies against *M. bovis* in plasma, serum and whole blood, combined with a sample pad, a conjugated pad, and a sink pad in individual plastic cassettes. The results can be obtained in 20 minutes and require a small volume (30µl) of serum, plasma or whole blood. The bound antibodies are seen with the naked eyes as a colored band within the test window. The rapid test is portable and does not require electricity, laboratory equipment or specific technical training (Anigen Animal Genetics Incorporation, 2005; Greenwald *et al*., 2007). Commercially available rapid test include; Prima-TB.STAT PAK® (ChemBio Diagnostic Systems Inc. Medfort, NY) and Anigen® Rapid Bovine Tuberculosis Antibodies Test (IQRT) (Anigen Animal Genetics Incorporation, 2005). The IQRT is specific and sensitive to *M. bovis*.

2.5.6.1 Principle of the Lateral Flow Test

The immunochromatographic (lateral-flow) technology employs a unique cocktail of recombinant *M. bovis* proteins that are bound to the membrane solid phase. Blue latex particles conjugated with protein (e.g. MPB70) are used as the detection system. A typical test strip has the following components: a sample pad, conjugate or reagent pad, reaction membrane and a wick or reservoir absorbent pad. The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid spontaneously. Once a test sample is applied to the sample (s) well followed by the addition of a diluents, it flows laterally through the membrane strip. When it reaches the conjugate pad, antibodies, if present, bind to protein-latex conjugate and then the migrating immune complex binds to the antigens on the solid phase in the test (T) area producing a blue line. In the absence of antibodies there is no line in the test (T) area. The sample continues to migrate along the

membrane and produces a blue line in the control (C) area demonstrating that the reagents are functioning properly (Chembio diagnostic system, incorporate, 2007).

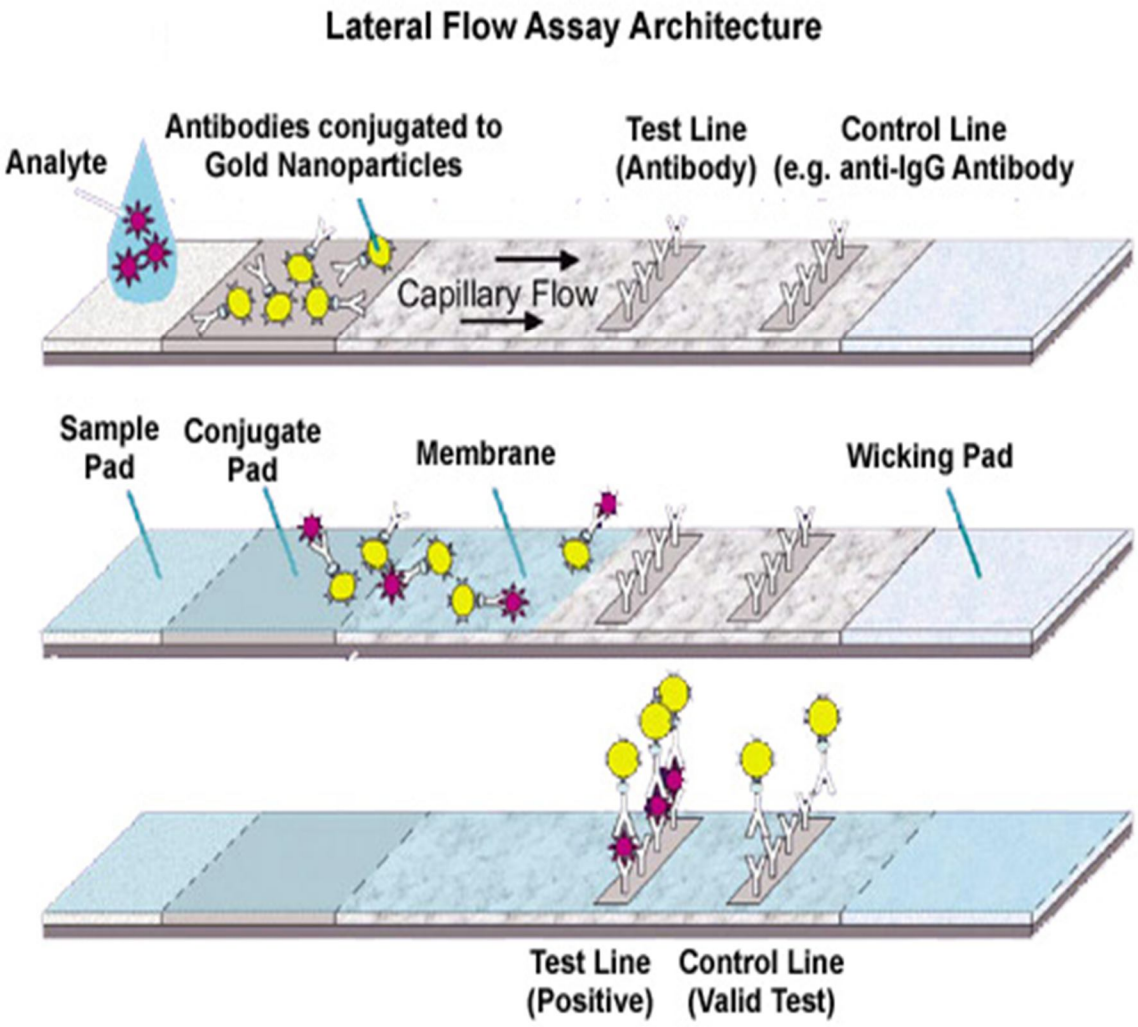


Figure 2.1: Lateral flow assay architecture (www.cytodiagnostics.com/lateral-flow-immunoassays.php)

2.5.7 Control and Eradication of Bovine Tuberculosis

Control and eradication of bovine tuberculosis is a desirable objective both from an animal health perspective and because of the zoonotic implications of *M. bovis*. Control and eradication have been achieved in many countries through test-and-slaughter policy combined with abattoir surveillance (FAO, 2012).

Identification of bovine tuberculosis by meat inspection at slaughter houses is an important surveillance tool, though its sensitivity is rather low (de la Rua-Domenech *et al*., 2006b). Depending on the prevalence of the disease in the country, abattoir surveillance can be used as a cost efficient method alone or combined with routine cattle testing (FAO, 2012). This however can be true following reliable inspection practices at slaughter houses, supported by an efficient animal identification system and adequate record keeping at both the farmer and the slaughter houses level (FAO, 2012).

Test and slaughter has been used by many countries to eradicate or markedly reduced the prevalence of bovine tuberculosis in cattle. Herds are tested using tuberculin skin test (TST) and reactors are immediately removed for slaughter. The herds are then retested after a 60 to 90 days until no further reactors are detected and there is no evidence of tubercules in reactors at slaughter (OIE, 2005).

Control and eradication program are targeting cattle population mostly. The World Organization for animal health (OIE) published guidelines and standards for bovine tuberculosis testing in cattle but does not have specific guidelines for small ruminants (sheep and goats) (Tschopp *et al*., 2011). There is therefore no official standard for skin testing in small ruminants, testing and

result interpretation follows very closely the cattle OIE standards combined with individual author adaptations as it relates to cut-off point used (Tschopp *et al*., 2011).

Successful control programs based on test-and-slaughter depends on institutional and technical requirements such as; an efficient cattle identification system that allows for effective trace-back to the herds of origin of tuberculous animals detected at slaughter houses and high standard of meat inspection practices. Others include a legal frame work for enforcing control measures and compensating the farmers that are affected, diagnostic capability of laboratories that are used for tuberculosis diagnosis based on the isolation and species identification of the bacterium from tuberculous lesion on organs (FAO, 2012).

In developing countries, the policy of test-and-slaughter is not always vigorously pursued; positive reactor animals are not effectively quarantined or culled and limited laboratory diagnostic capacity amongst other things makes it difficult to control the disease in livestock (Abubakar *et al*., 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

This research was conducted in Kaduna state with an estimated land area of about 46,063 km², the state lies between longititude of 30'' east of the Greenwich meridian and also between latitude 0900 and 11 30'' North of the equator. Kaduna state occupies part of the Central position of the Northern part of Nigeria (with Kaduna as its capital) and shares border with Zamfara, Katsina, Niger, Kano, Bauchi and Plateau, Nasarawa States. To the South-West, the state shares a border with the Federal Capital Territory, Abuja. According to the National Population Census (2006), Kaduna state has a human population of more than 6 million and from National Livestock Reports (1992), 1.2million goats and 850,000 sheep.

Kaduna state has a total amount of annual rainfall varying between 1,000mm and 1,500mm and a rainy season, which is between120 and 150 days long. The northern part of the state is semi-arid, but further south, as the rain levels increase, the climate becomes sub-humid. The extreme south of the state is marked by a series of rocky hills, which are responsible for the island of rainfall (National Livestock Reports, 1992).

Kaduna state is essentially an agrarian society with about 75% engaging in farming and it also has potentials for livestock industry.

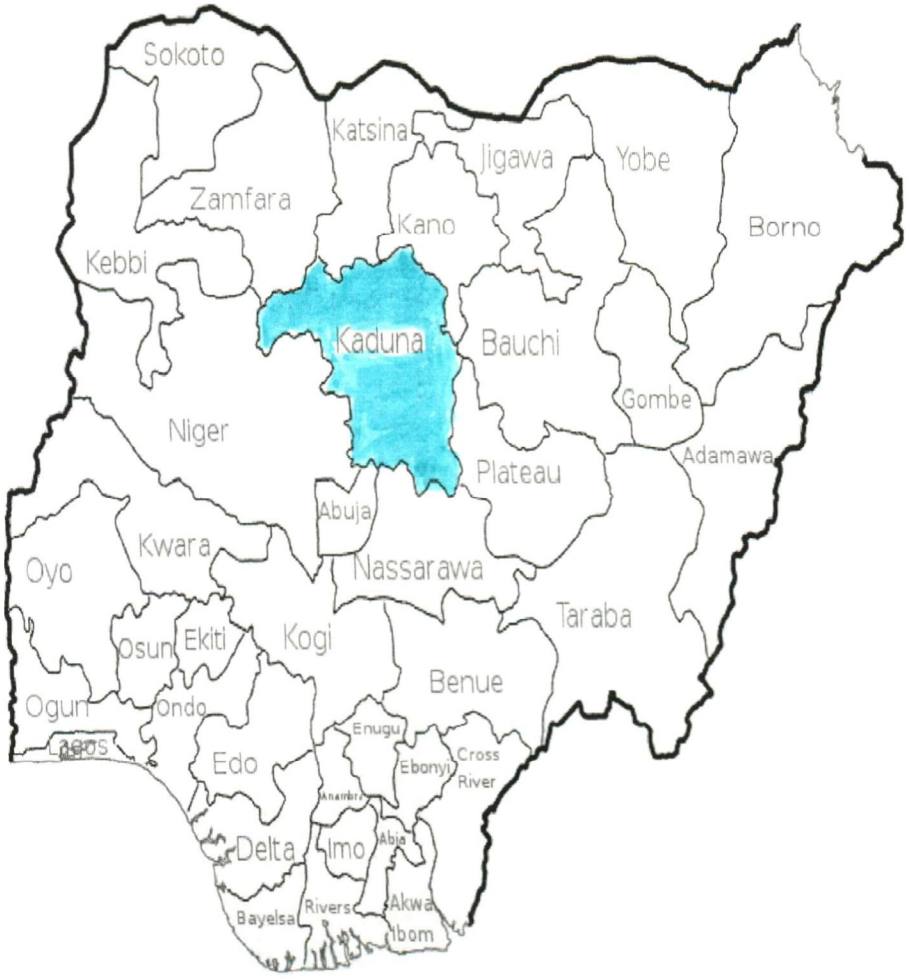


Figure 3.1: Map of Kaduna state and the various states that border it (http:/mapsof.net/uploads/static-maps/Nigeria_states.png)

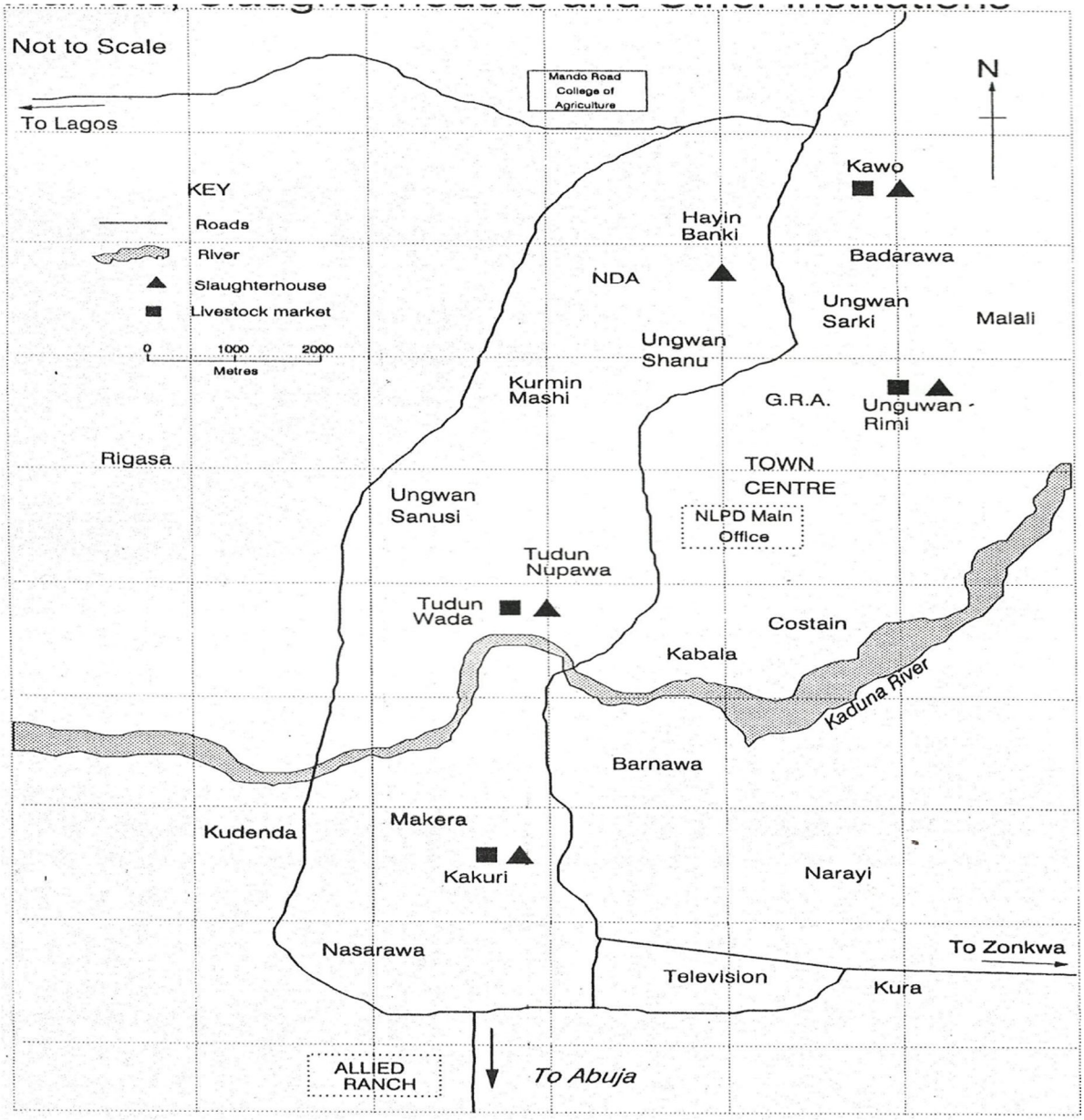


Figure 3.2: Map of Kaduna metropolis and the distribution of slaughter houses and livestock markets (National Livestock Report, 1992)

3.2. SAMPLING

3.2.1 Sample Size

Sampling was randomly carried out and the size determined as described by Mugo, (2008). Hiko and Agga (2010) reported a prevalence rate of 4.2% in Ethiopia.

$$n = \frac{Z^2 Pq}{d^2}$$

Where:

n = sample size

q = 1-P

P = prevalence = 4.2%.

d = desired absolute precision= 0.0125

Z = appropriate value for the standard normal deviate for the desired confidence= 1.96

Therefore,

$$n = \frac{1.96^2 \times 0.042(1 - 0.042)}{0.0125^2} = 989$$

This sample size shows the minimum that can be collected. However, for effective investigation, a total of one thousand and ten (1010) serum samples were collected, i.e. five hundred and one (501) from live flocks and five hundred and nine (509) from the abattoirs/slaughter houses.

3.2.2 Sampling Procedure

A total of 498 and 512 blood samples from sheep and goats were collected respectively from the four (4) major slaughter houses and livestock markets within Kaduna metropolis. These include Tudun-wada, Kawo, Kakuri and Ungwar-rimi. Based on the small ruminant population in the various location and convenience; 171 sheep samples and 177 goat samples were obtained from Tudun-wada, 117 sheep samples and 126 goat samples from Kawo, 140 sheep samples and 140 goat samples from Kakuri and 70 sheep samples and 69 goat samples from Ungwar-rimi were obtained respectively. A total of 509 from slaughter houses and 501 from livestock markets were collected. Flocks sampled were based on the willingness of the owners to cooperate. From each of the locations, animals meant for slaughter were first before those at the livestock markets.

3.3 COLLECTION OF SERA

Blood sample from abattoir-: The following data were collected from the animals intended for slaughter, viz; the breed, sex and ages. Five milliliters (5mls) of blood was aseptically collected from the severed jugular vein using clean labeled sample bottle devoid of anticoagulant and allowed to stand at room temperature for sera separation from cellular component. Samples were then transported to the laboratory in cooler boxes. The resultant sera was transferred into clean labeled serum sample bottles and stored at -20°C. The sera were allowed to return to room temperature (15-30°C) before use.

Blood sample from flocks-: The animals were properly restrained; the following parameters were obtained; age, sex and breed. Five milliliters (5mls) of blood was collected aseptically using sterile hypodermic 23G needles and 10ml syringe via the jugular vein. The blood collected

was transferred into clean labeled sample bottle devoid of anticoagulant and allowed to stand at room temperature for sera separation from cellular component. Samples were then transported to the laboratory in cooler boxes. The resultant sera was then transferred into clean labeled serum sample bottles and stored at -20°C. The sera were allowed to return to room temperature (15-30°C) before use.

The age of sheep and goats were estimated by looking at their teeth. Baby teeth (milk teeth)- under 1 year of age; first pair permanent tooth- 1-1½ years; two pairs permanent teeth- 1½ to 2 years; three pairs permanent teeth- 2½ to 3 years; four pairs (full mouth)- more than 3 years while broken mouth- aged (Vatta *et al*., 2006).

The sera were analyzed in the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria.

3.4 ANALYSIS OF SERA

Serum samples were analyzed using Anigen® rapid bovine tuberculosis test kit obtained from Anigen Animal Genetic Inc. in South Korea. The test kit contained Anigen® rapid bovine tuberculosis antigen test devices and specimen droppers. The rapid test is based upon the detection in infected animals, of antibodies to a set of recombinant tuberculosis antigens. It is easy to use, non-invasive and adaptable for multi-species use. The principle is based on immunochromatography assay for detection of antibodies (IgM and IgG) against *M bovis*. The MPB70 is a highly specie-specific protein secreted by *M bovis* and was identified as a major antigen from culture filtrate protein of *M bovis*. It uses a drop of serum or plasma and gives a

visual result within 15-20 minutes (Greenwald *et al*, 2003). The test was carried out according to manufacturer's instructions as follows.

- The test kit was removed from the foil pouch and placed on a flat dry surface.
- Four drops of the test serum was added slowly to the sample hole using the specimen dropper. If the migration did not appear after one minute, one more drop of the test serum was added to the sample hole.
- A test result would be seen as a purple band in the result window of the kit.
- The test was then interpreted within 15-20 minutes. Result interpreted beyond 20 minutes was considered invalid.
- Even if the intensity of the purple band was faint, it was interpreted as positive if it appears within 20 minutes.

Interpretation

- A color band appearing in the left section of the result window showed that the test kit was working properly. The band is the control line (C).
- The right section of the result window indicates the test results. The appearance of another color band in the right section of the result window indicated the test line (T).
- The presence of only one purple color band within the result window indicated a negative result.
- The presence of two color bands (T and C) within the result windows no matter which band appeared first was an indication of a positive result.
- If the purple color band was not visible within the result window after performing the test, the result was considered invalid.

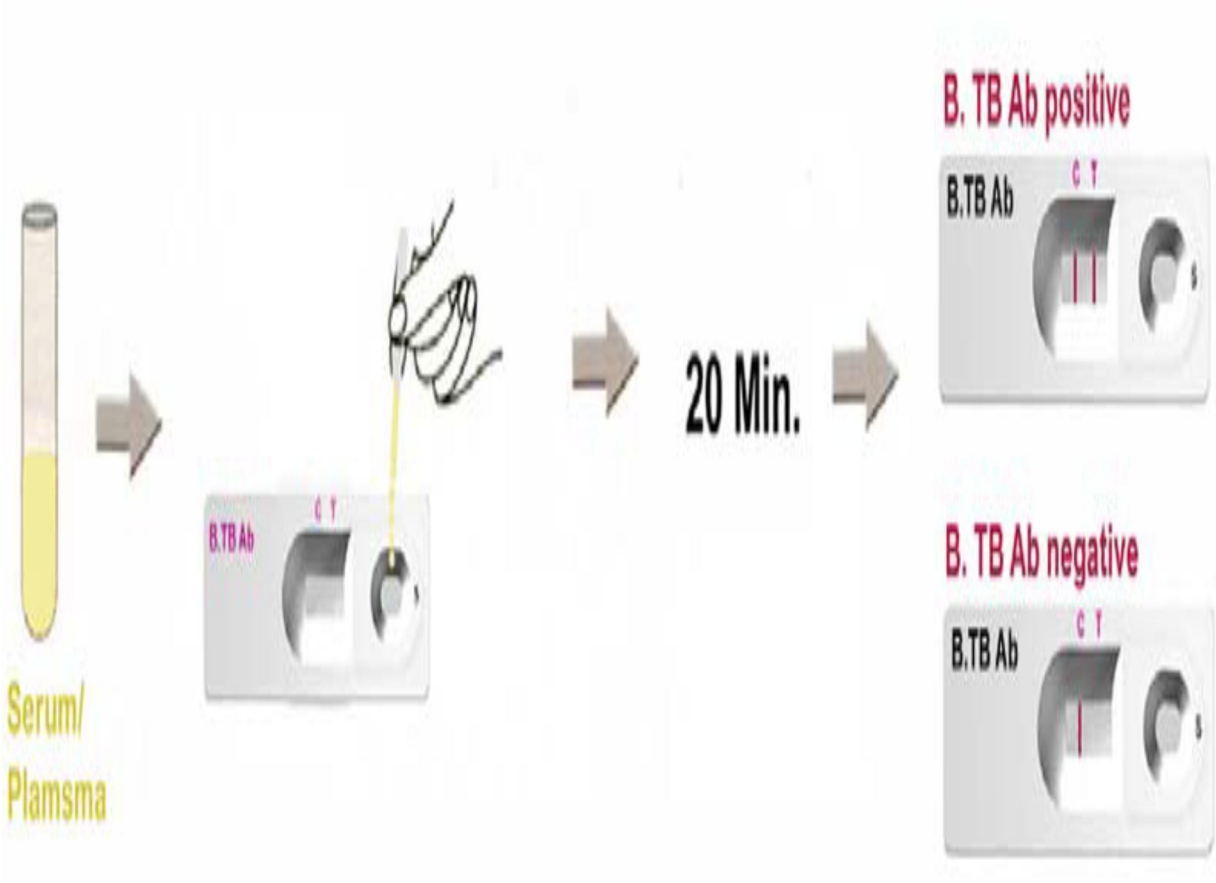


Figure 3.3: Illustration of test result, extract from Anigen Animal Inc. Manual (Anigen Rapid Bovine TB AB Test Kit, 2005).

3.5 QUESTIONNAIRE

Structured questionnaires were administered to small ruminant handlers and a willing sampled populace. The sampled populace comprised of willing butchers, livestock marketers and consumers that buy meat at slaughter houses. Some of the respondents were interviewed by the researcher. This was done in order to obtain information on a range of variables relating to management practices, level of awareness about tuberculosis and eating habits from the willing participants. A total of 300 open and close ended structured questionnaires were administered.

3.6 DATA ANALYSIS

Descriptive charts were constructed with Microsoft Excel® 2007. GraphPad Prism version 4.0 for Windows was used for data analysis. Chi square (χ^2) was used to compare categorical variables, Odds ratio (OR) and 95% confidence interval on the ORs were calculated to measure strengths and statistical significance of associations between some variables and sero-positivity to *Mycobacterium bovis*. A value of $P<0.05$ was considered significant. The prevalence of *Mycobacterium bovis* infection in small ruminants was determined from the data obtained using the formula:

$$\text{Prevalence} = \frac{\text{positive samples}}{\text{total samples analysed}} \times 100$$

CHAPTER 4

RESULTS

4.1 PREVALENCE OF BOVINE TUBERCULOSIS IN SMALL RUMINANTS IN KADUNA METROPOLIS

The result showed an overall prevalence of 13.56% (137/1010) for small ruminants sampled. Specie-specific prevalence rate of 10.84% (54/498) for sheep and 16.41% (84/512) for goats were recorded (Table 4.1). The females of both species sampled had a higher prevalence of 14.98% (89/594), while the males of both species had prevalence rate of 11.54% (48/416). Based on age, the prevalence rates for small ruminants sampled were as follows: 12%, 15.64%, 7.78% and 14.81% for 1-12months, 13-24months, 25-36months and ≥36months respectively.

Table 4.1 Sero-prevalence of *M. bovis* in small ruminants based on species

Species	No of animals tested	No of animals positive (%)	χ^2	P- value	OR at 95% CI
Sheep	498	54 (10.84)	7.153	0.0075	0.607(0.4197-0.8774)
Goat	512	84 (16.41)			
Total	1010	137 (13.56)			

4.2 SERO-PREVALENCE OF *MYCOBACTERIUM BOVIS* IN SMALL RUMINANTS SAMPLED IN SLAUGHTER HOUSES AND LIVESTOCK MARKETS

The sero-prevalence rate of *M. bovis* in small ruminants sampled showed 13.36% (68/509) from slaughter houses and 13.77% (69/501) from livestock markets (fig. 4.1).

4.3 SEX-BASED SEROPREVALENCE RATE OF *MYCOBACTERIUM BOVIS* IN SHEEP AND GOATS

The sex-based prevalence rate of the small ruminants sampled showed 48 (11.54%) out of 416 males were positive to *M. bovis* and 89 (14.98%) out of 594 females were positive (figure 4.2). The species sex-based prevalence rate was as follows: 6.49% (27/416) for male goats, 5.05% (21/416) for male sheep, 9.60% (57/594) female goats and 5.39% (32/594) female sheep respectively. The infection rate was higher in females than in males. The result obtained was not statistically significant ($\chi^2 = 2.476$, P value = 0.1156, OR at 95% CI = 0.7401 (0.5082-1.078).

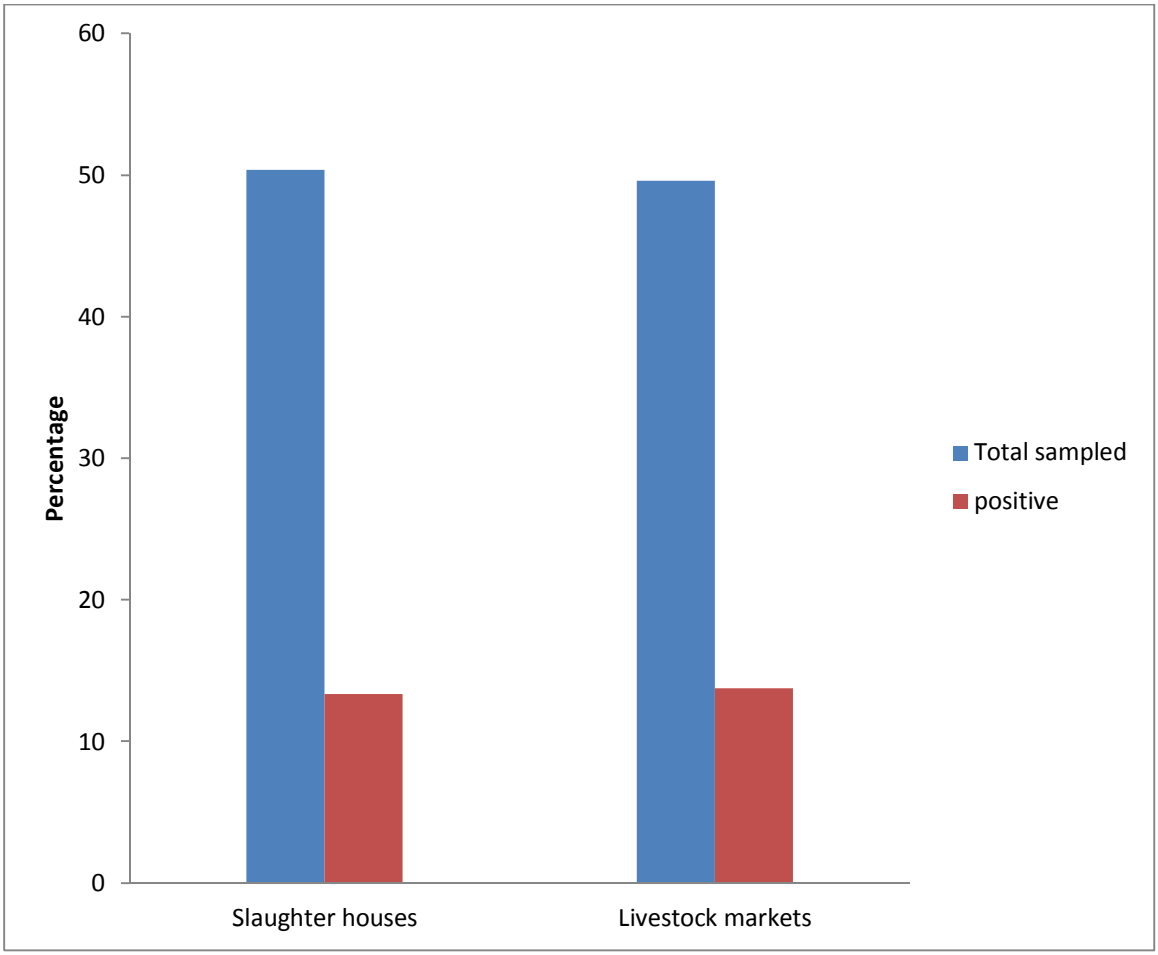


Figure 4.1: Percentage positive for *M. bovis* antibodies in small ruminants sampled at slaughter houses and livestock markets

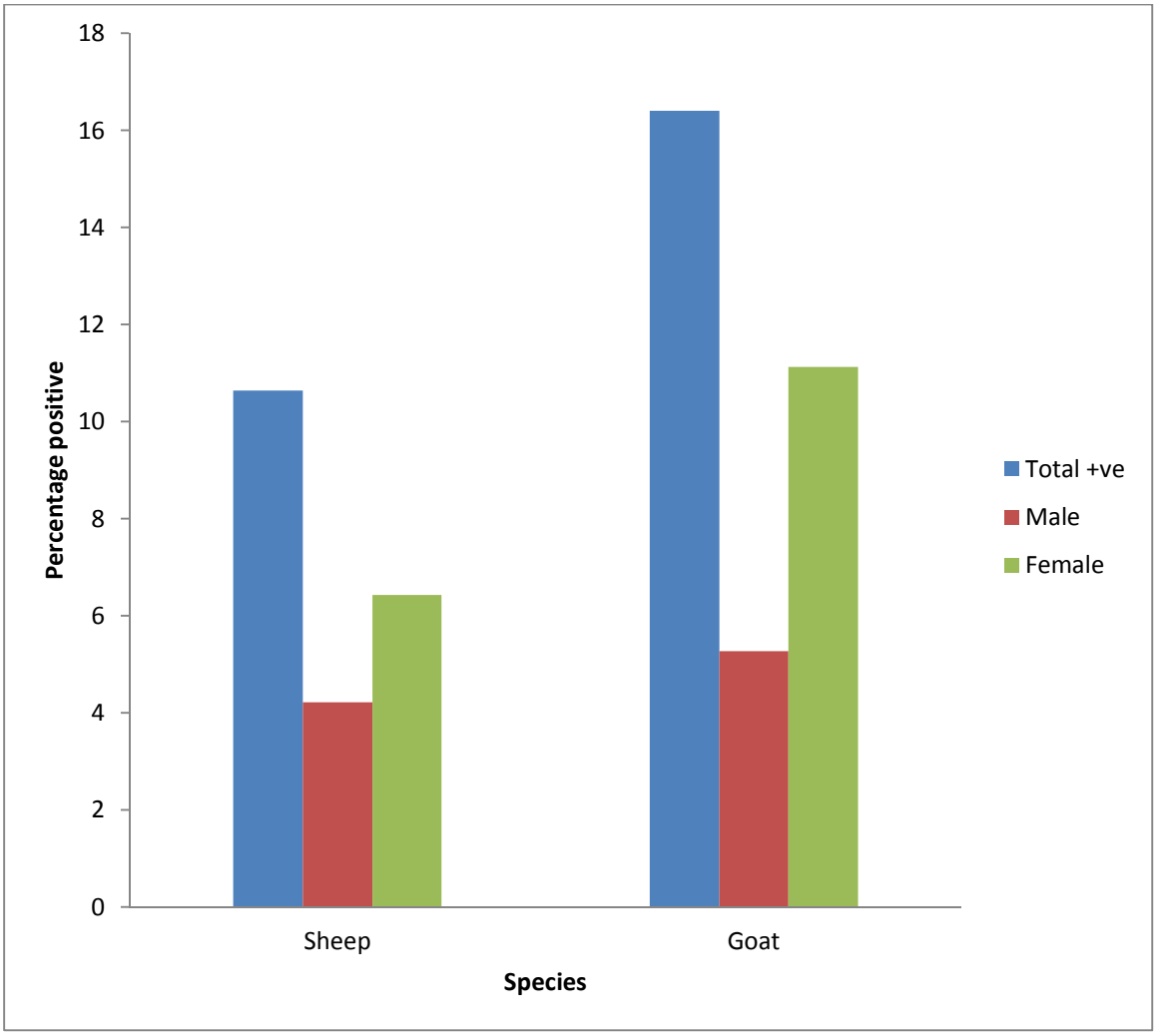


Figure 4.2: Percentage positive for *M. bovis* antibodies in male and female small ruminants sampled

4.4 AGE-BASED SERO-PREVALENCE RATE OF *MYCOBACTERIUM BOVIS* IN SHEEP AND GOATS

There were differences in prevalence rates among the different age groups of small ruminants sampled. Small ruminants, 1-12months had a prevalence of 12% (21/175), 13-24months 15.64% (94/601), 25-36months 7.78% (14/180) and ≥36months 14.81% (8/54) respectively (Table 4.2). The result obtained were statistically significant ($\chi^2 = 7.788$, P value = 0.0506).

Table 4.2: Sero-prevalence of *M. bovis* in small ruminants based on age.

Age	Species	No. tested	No. positive (%)	χ^2	P- value
1-12 months		175	21 (12.00)		
	Sheep	33	2		
	Goat	142	19	7.788	0.0506
13-24 months		601	94 (15.64)		
	Sheep	278	34		
	Goat	323	60		
25-36 months		180	14 (7.78)		
	Sheep	138	11		
	Goat	42	3		
≥ 36 months		54	8 (14.81)		
	Sheep	49	6		
	Goat	5	2		

4.5 BREED-BASED SERO-PREVALENCE RATE OF *MYCOBACTERIUM BOVIS* IN SHEEP

Out of the three breed sampled, Balami had a prevalence rate of 8.11% (6/74), Yankasa had 10.11% (38/376) and Ouda 18.75% (9/48) respectively (Fig 4.3).

4.6 BREED-BASED SERO-PREVALENCE RATE OF *MYCOBACTERIUM BOVIS* IN GOATS SAMPLED

The goats sampled had the following percentage positive for the various breeds; West African Dwarf (WAD), 12.24% (6/49), Kano brown 15.03% (29/193), red Sokoto17.94% (47/262) and Sahel/desert 25% (2/8) respectively (fig 4.3).

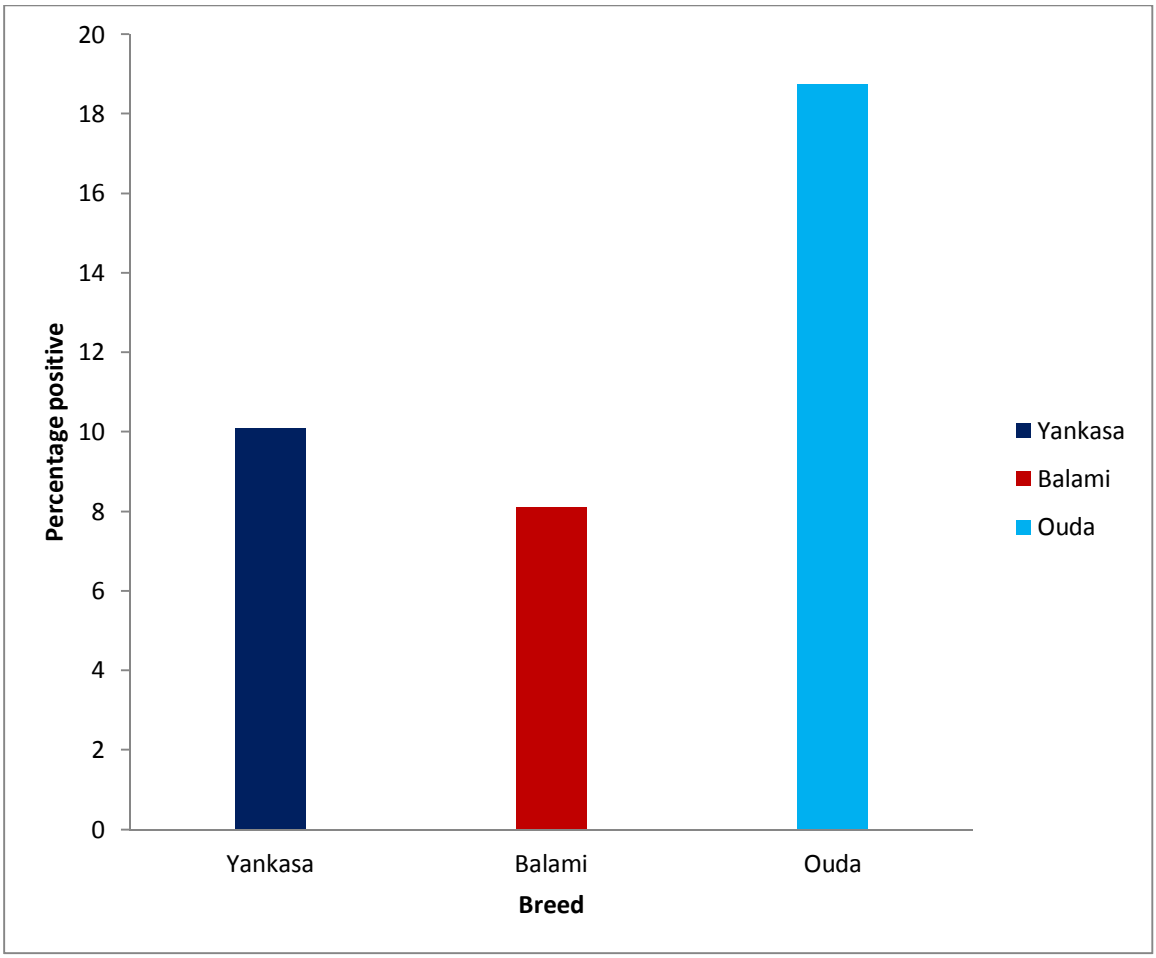


Figure 4.3: Percentage positive for *M. bovis* antibodies in sheep based on breed

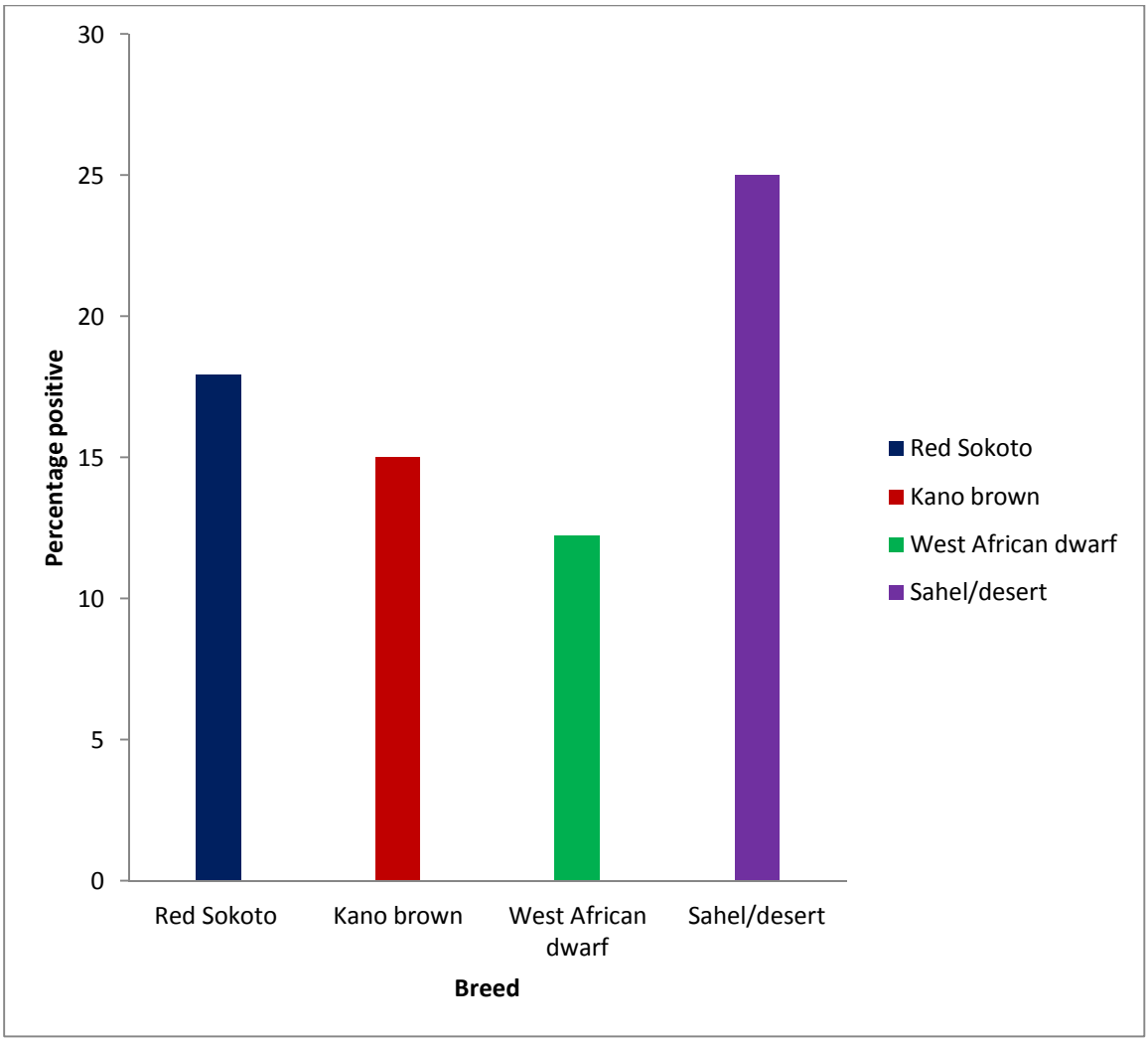


Figure 4.4: Percentage positive for *M. bovis* antibodies in goats based on breed

4.7 ASSESSMENT OF AWARENESS

Out of 300 questionnaires distributed, 76.33% (229/300) return was achieved. The questionnaire survey showed that, 64.63% were aware of bovine TB, 41.92% were aware of bovine TB in sheep and goats, while 39.74% were aware of its transmission to man (fig 4.5). Knowledge of bovine TB and level of education was statistically significant with a P value of 0.0001 ($P \geq 0.05$). A total of 72.49% of the respondents keep livestock, 55% of them keep these animals at close proximity to themselves (fig 4.6). Fifty-two (52) percent of the respondents have a preference for sheep and goat as source of meat (fig 4.7).

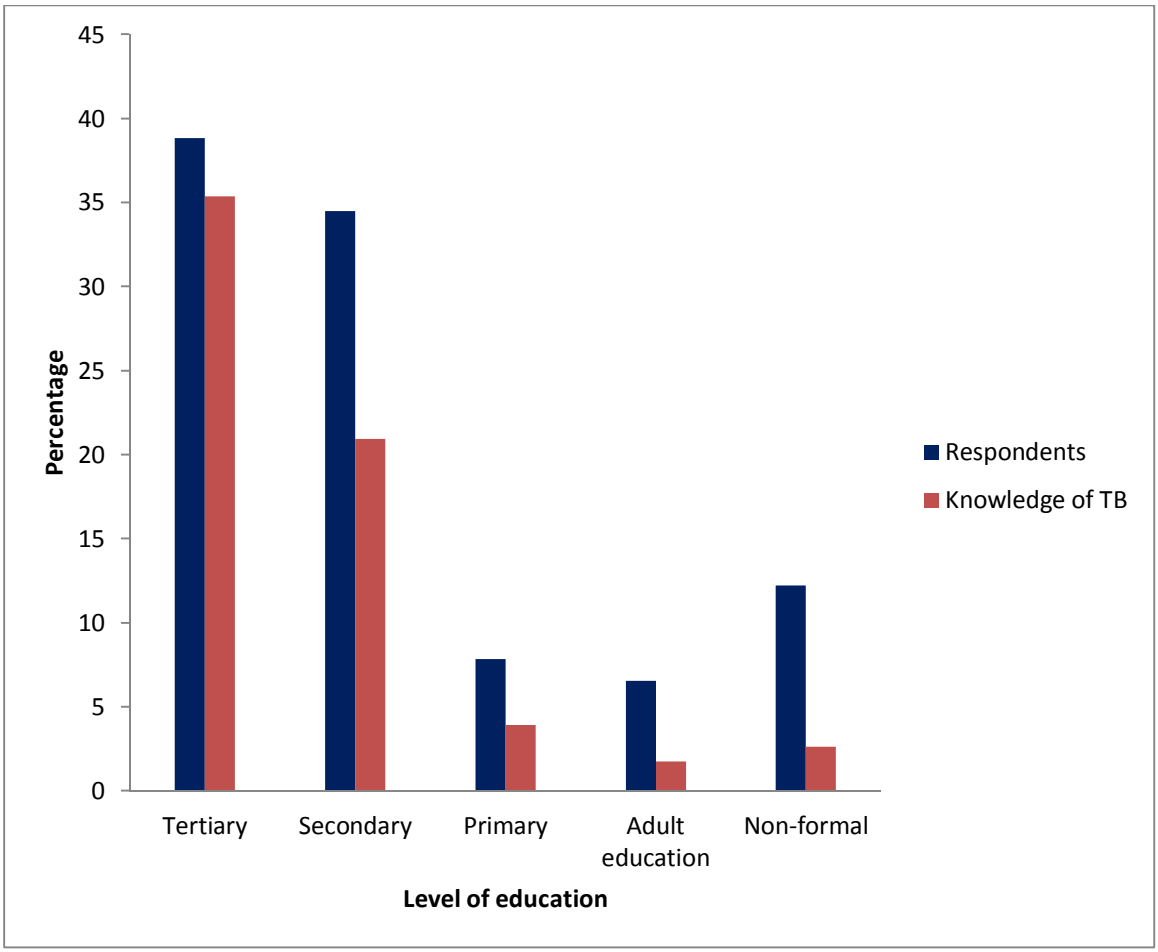
4.8 LEVEL OF EDUCATION AND KNOWLEDGE OF BOVINE TUBERCULOSIS

The percentages of respondents with tertiary education were 38.86% (89/229) and out of those with tertiary education, 35.37% (81/229) knew about TB, 20.96% (48/229) of those with secondary education knew about TB while 3.93% (9/229) and 1.7% (4/229) of respondents with primary education and non-formal education respectively knew about TB (fig. 4.4).

A total of 96 respondents out of 229 (41.92%) say TB affects sheep and goats while 9.61% (22/229) and 48.47% (11/229) do not know or have no idea of TB affecting small ruminants respectively. Ninety-one out of two hundred and twenty-nine (39.74%) say TB can be transmitted to man while 10.04% (23/229) and 50.22% (115/229) do not know or have no idea about TB transmission to man (fig. 4.5).

Figure 4.6 show that 35.37% (81/229) of the respondents have a history of cough and out of the 81 respondents, 66 (81.48%) of them keep livestock. Fifty-seven respondents out of the eighty-one (70.37%) with history of cough, keep these livestock in close proximity with humans.

The percentage of respondents that prefer cattle as source of meat were 47.60% (109/229), while 13.97% (32/229) and 37.55% (86/229) preferred sheep and goat respectively (fig. 4.7). Daily meat consumption rate by the respondents was 38.68% (89/229), weekly was 24.02% (55/229), monthly was 11.79% (27/229) and not specific rate was 25.45% (56/229) (fig. 4.8).



$X^2 = 61.62$, P-value = 0.0001

Figure 4.5: Level of education and knowledge of bovine tuberculosis

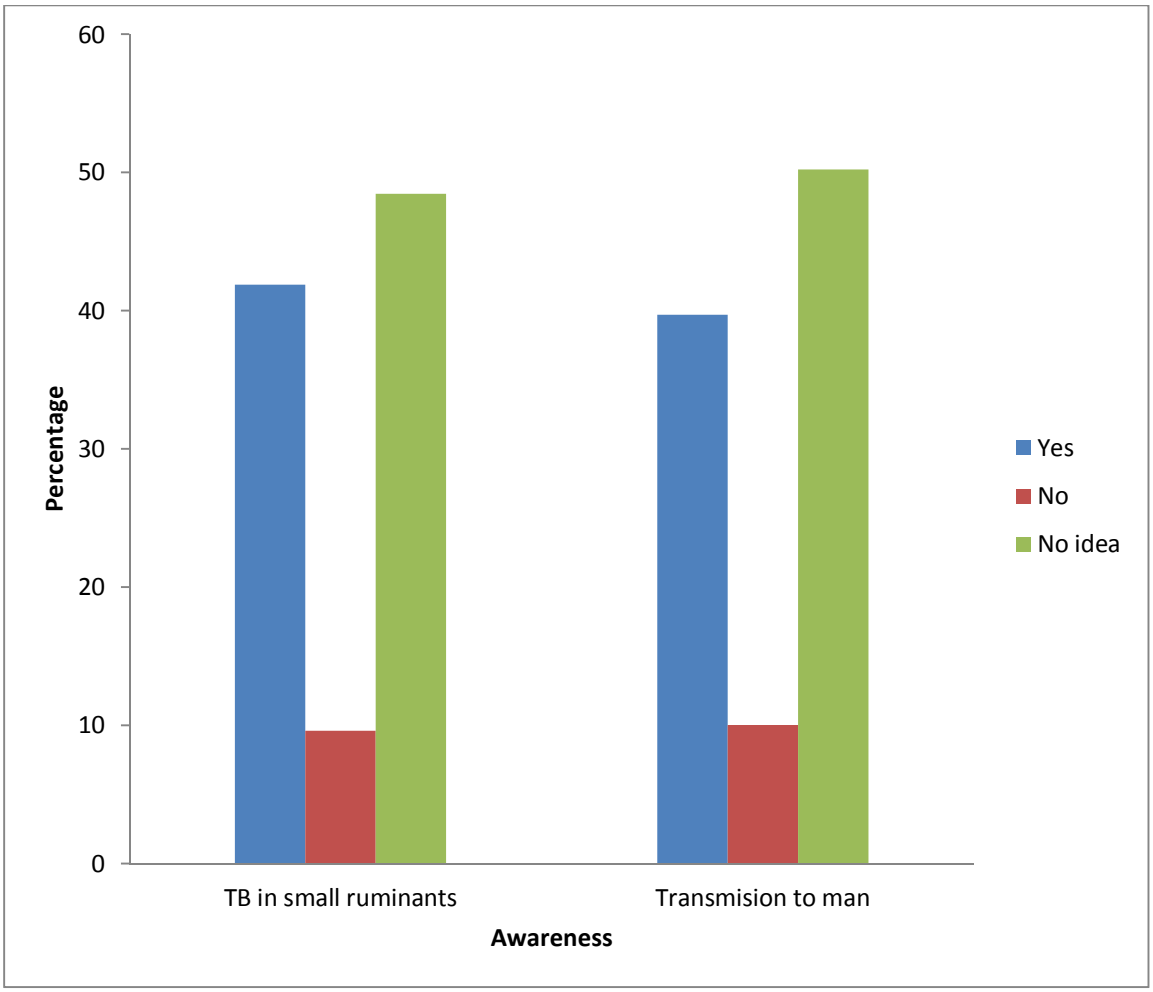


Figure 4.6: Level of awareness of tuberculosis in sheep and goats and as a zoonosis

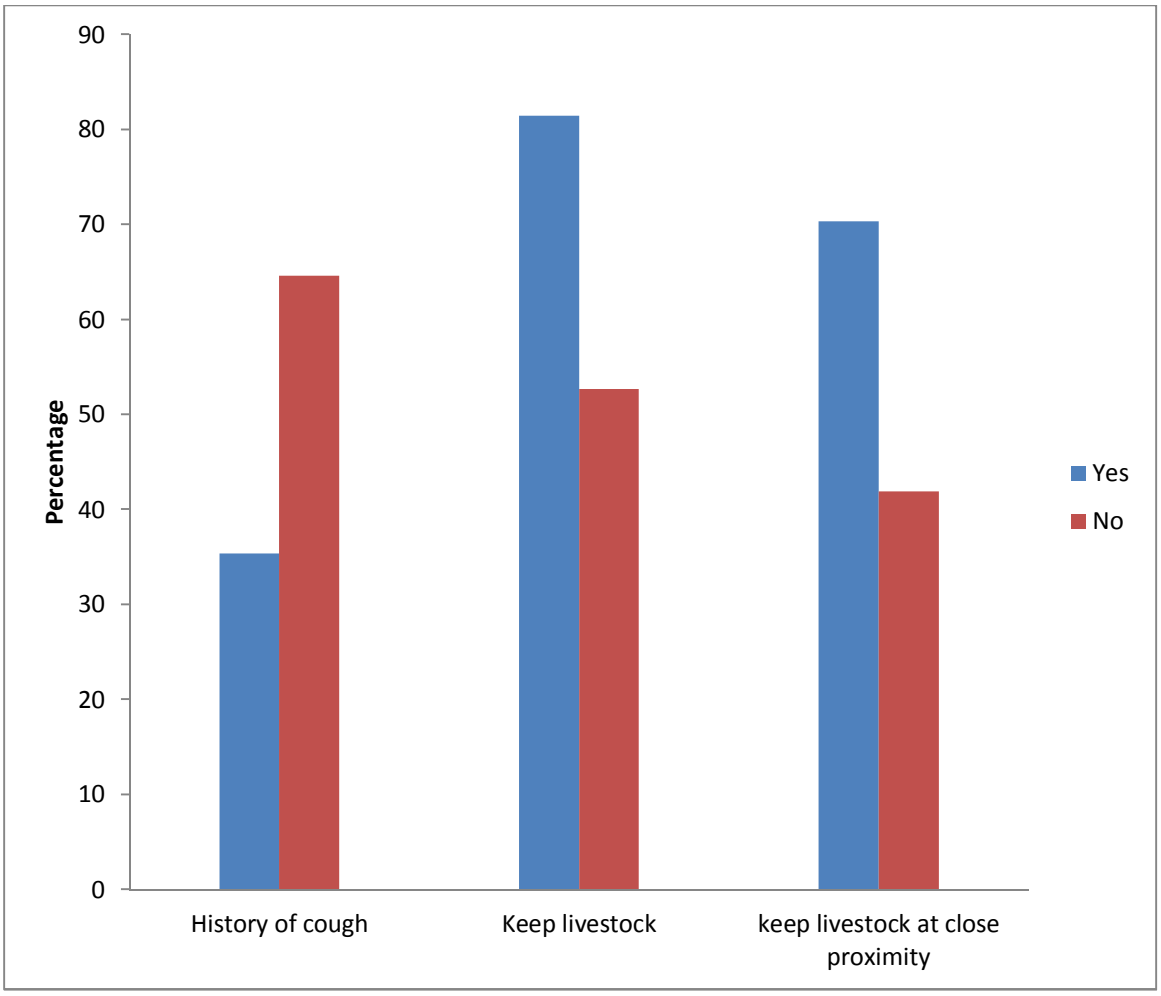


Figure 4.7: Respondents with a history of cough, keep livestock and those that keep the livestock at close proximity (i.e. within the same compound in human dwellings) to themselves

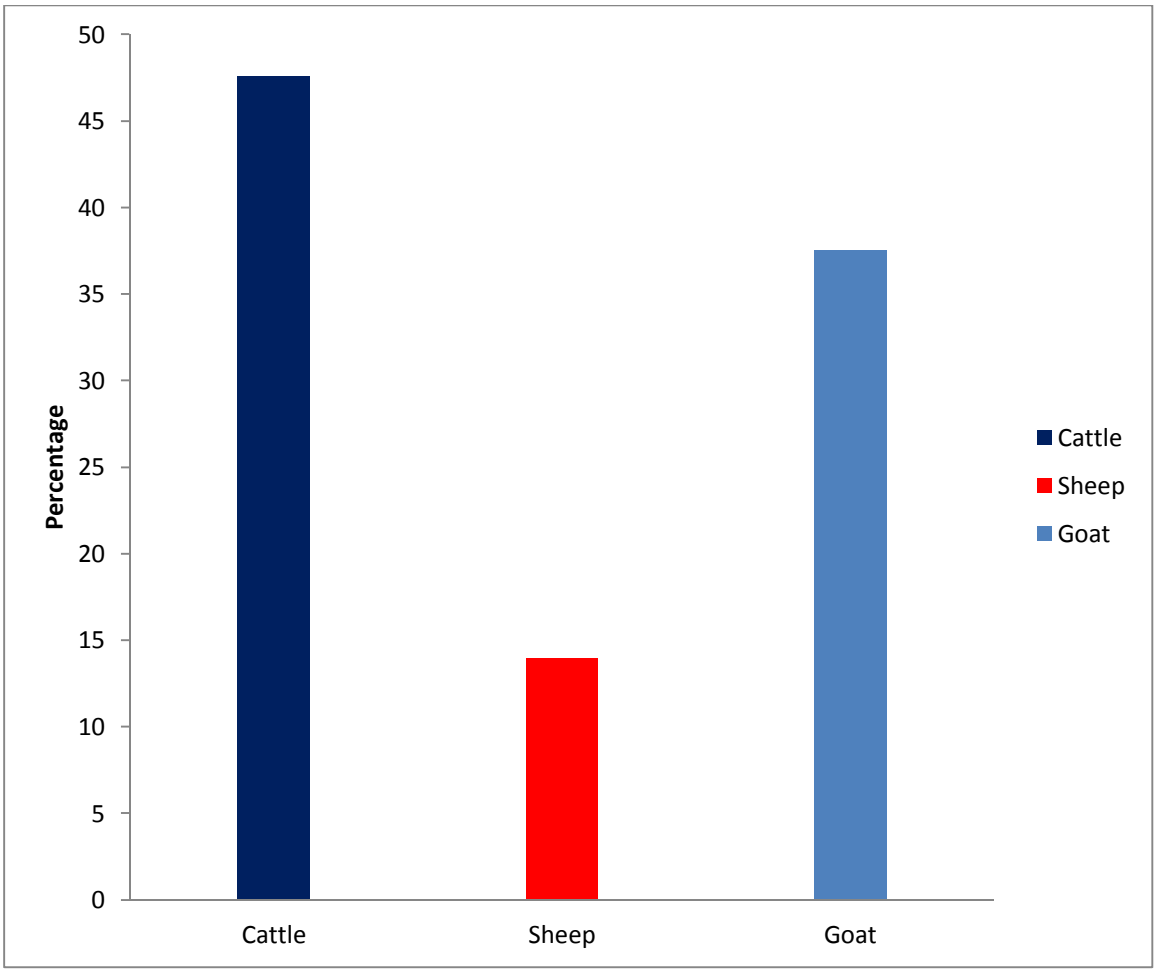


Figure 4.8: Percentage of respondents that prefer cattle, sheep or goat as source of meat

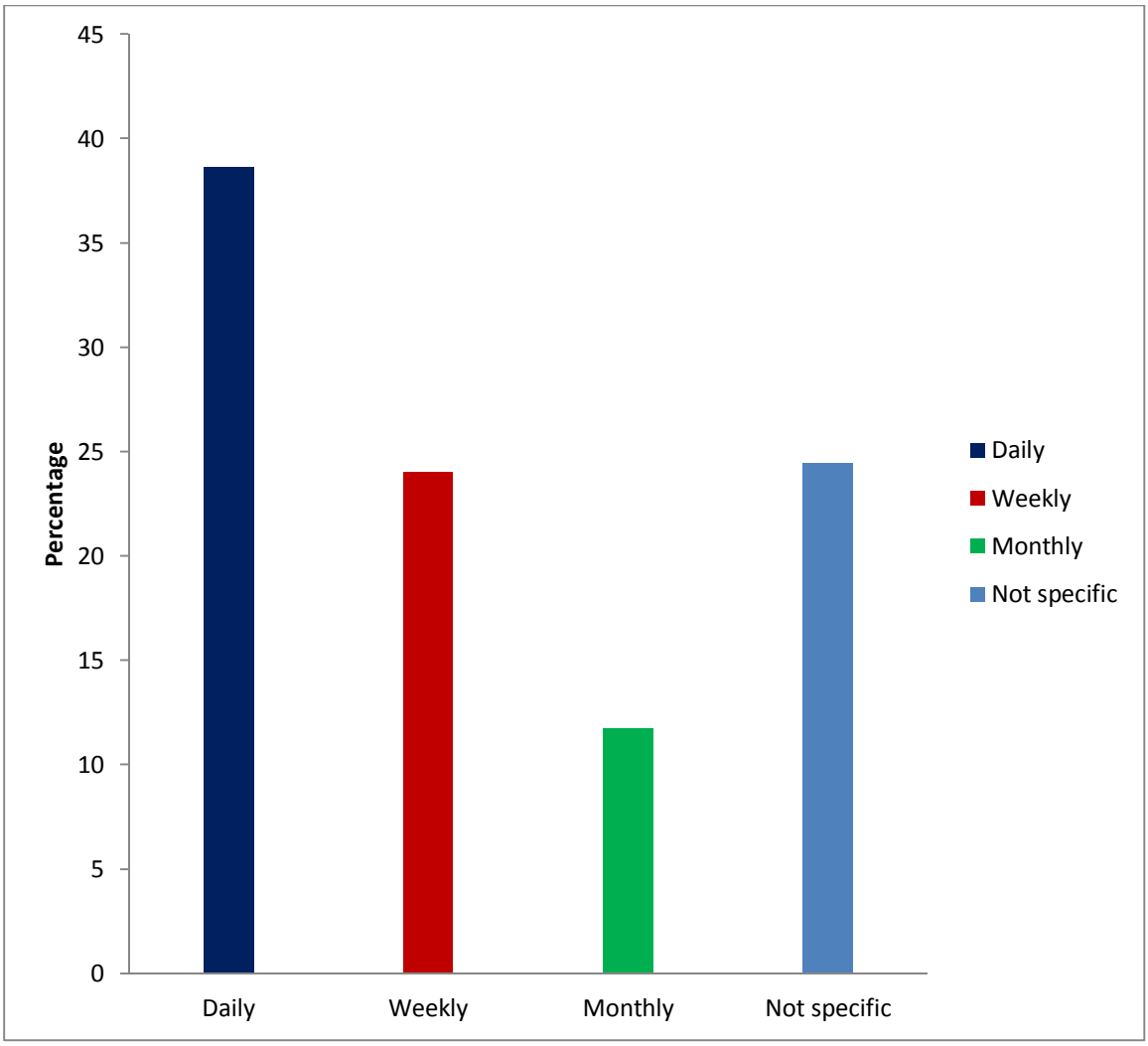


Figure 4.9: Percentage daily consumption rate of meat

CHAPTER 5

DISCUSSION

Small ruminants are not routinely screened for *Mycobacterium bovis* in Nigeria, reports on prevalence of *M. bovis* are mostly in cattle following a research design. Thus, the real epidemiology and zoonotic potential of bovine tuberculosis in the country is largely unknown. In this study, the high detection rate of *M. bovis* antibodies in small ruminants shows potential exposure to reactors. The sero-prevalence of 16.41% in goat recorded in this study was higher than the 4.3% (n=1387) reported by Cadmus *et al*. (2009). The difference in prevalence may be due to the different type of diagnostic method used because in the previous report, it was based on postmortem findings. Postmortem examinations in slaughter houses are far less sensitive than immunological test which detect an often subclinical infection (de la Rua-Domenech *et al*., 2006b). Sensitivity to postmortem surveillance for the detection of tuberculous lesions depends on a work load time and diligence of the inspector conducting the examination (Shitaye *et al*., 2006) and this has been found to be grossly inadequate. Thus the 16.41% sero-prevalence obtained in this study was most likely a proper representation of the flock prevalence status to *M bovis* because the antibovine TB antibody test kit has a specificity of 98.6% and sensitivity of 85.1% against TST (Anigen Genetic Inc. 2005). Studies in different countries have reported the following prevalence in goats. Javed *et al*. (2010) reported a prevalence of 2.4% in goat farms using tuberculin skin test from Pakistan; Hiko and Agga (2010) reported a prevalence of 4.2% following postmortem examination; Tafess *et al*. (2011) also recorded 3.1% using single intradermal tuberculin skin test and Kassa *et al*. (2012) recorded 4.3% using comparative intradermal test (CIDT).

The antibody prevalence of 10.84% recorded in sheep in this study is higher than the 1.44% reported by Kassa *et al*. (2012) in Ethopia. Occasionally, though prevalence of 18% has been observed in sheep flock in New Zealand (Davidson *et al*., 1981). In Nigeria reports on *M. bovis* infection in sheep was not available prior to this study. The variation in the prevalence obtained in this study and others can again be attributed to the type of test used. The PPD has been known to cross react with environmental Mycobacteria (Buddle *et al*., 2002) which may likely affect the result, as compared to the IQRT assay which is said to have a sensitivity of 85.1% and specificity of 98.6% (Anigen Genetic Inc., 2005). The high prevalence recorded in this study may also be attributed to the consistent exposure and closeness of small ruminants, cattle and humans, lack of/or competition on the insufficient grazing areas. In addition, the unchecked prevalence of *M. bovis* in small ruminants has perpetuated the disease resulting in higher prevalence. Therefore, the 10.84% prevalence obtained in sheep gives a more likely true picture of the flock prevalence status to *M. bovis*.

The overall sero-prevalence of *M. bovis* in small ruminant was 13.56%. Though prior to this study, no report has been made on the prevalence of small ruminants tuberculosis either at abattoir or by tuberculin test in Nigeria, few studies have been conducted on bovine tuberculosis in cattle. The observed antibody prevalence of 13.56% is closely related to the 14.3% reported by Abubakar (2007) in lactating cattle in Kaduna using the PPD, but it is however lower than the 17.5% reported by Danbirni *et al*. (2010) in a similar study using IQRT. The disparity was thought to be due to the different diagnostic test used, giving credence to the use of lateral flow assay for bovine TB screening. Small ruminants are important livestock component and are herded together with cattle, Tschopp *et al*. (2009) showed that keeping small ruminants with cattle herds was associated with higher numbers of positive bovine tuberculosis reactors in cattle.

Small ruminants have daily close and intensive contact with cattle in communal herding practice or often overstocked during transportation and at livestock markets. This husbandry system practiced in the country and in most parts of Africa gives plenty opportunity for disease transmission, probably giving rise to the high prevalence rate in small ruminants in this studies.

Gender has been cited as a risk factor in various studies carried out in Africa (Humblet *et al*., 2009). There is however different views regarding the influence of sex in the susceptibility to *M. bovis* infection. Some opined that male cattle were significantly more affected by bovine TB than female animals (Humblet *et al*., 2009). Cross-sectional studies in Tanzania and Malawi revealed that male animals were significantly affected by *M. bovis* than female animals. Whereas a cross-sectional study in 1470 animals in Uganda revealed significantly more female animals affected by bovine TB (Humblet *et al*., 2009) and Kudi *et al*. (2012) also reported more female camels been susceptible to *M. bovis* infection than male animals.

In this study, the prevalence of *M. bovis* in female small ruminants was higher (14.98%) than in males (11.54%), however, there was no statistically significant difference observed at 95% CI 0.7401 (0.5082-1.078). The females may have a higher prevalence rate because of their role in lambing/kidding and milk production, and as a result tend to be more exposed to the infection than the males (Kudi *et al*., 2012 and Kassa *et al*., 2012). Also, the females stay longer in the flocks than males as they are sent to slaughter and the longer the animal stays in a flock the more likely it is exposed to the disease (Kudi *et al*., 2012)..

Age of animals is one of the main individual risk factors identified by numerous studies showing that exposure increases with age. This has been seen in various cross-sectional studies carried out in Tanzania, Zambia, Chad, Nigeria, Ethopia (Cook *et al*., 1996; Cleaveland *et al*., 2007;

Munyeme *et al*., 2008; Cadmus *et al*., 2010a; Hiko and Agga, 2010; Kassa *et al*., 2012 and Kudi *et al*., 2012).

In this study the age prevalence was higher for animals between 13 to 24 months and ≥36 months. This agrees with the study of Kassa *et al*. (2012) which reported a higher prevalence of *M. bovis* among small ruminants 2 to 5 years of age. Various studies suggest that old animals were more susceptible for *M*. bovis infection (Cleaveland *et al*., 2007; Philips *et al*., 2002 and Kassa *et al*., 2012), this correspond with finding of this study where age prevalence to *M. bovis* infection in small ruminants was statistically significant (P = 0.0506). It suggest that animals might get infected at a young age but express the disease clinically when they are adult (Griffin *et al*., 1996), due to the long incubation period of tuberculosis. The older the animal and the longer it stays in a flock, the more likely it is exposed to disease.

There is no conclusive evidence that sex, age and reproductive stage of the host have a direct influence in the transmission of bovine tuberculosis (Morris *et al*., 1994), other factors such as the immune status of individual animals compromised by the presence of other infection and nutritional status may affect susceptibility to infection with *M. bovis* (Humblet *et al*., 2009).

The small ruminants are reared under the traditional extensive and semi-extensive management system where animals are kept together irrespective of age, sex, species, and health status, grazing from the same natural pasture with common drinking spots and confined by herders or group of herders with their livestock within a limited enclosure for shelter or houses at night (Bayer, 1984). There is high possibility of long and close proximity during grazing and drinking between small ruminants and other animals (e.g. cattle). Consequently, management practices

may have played a vital role in the high prevalence rate observed in small ruminants in this study.

Nigerians are continuously faced with the risk of infection with bovine TB directly or indirectly through the food chain. Lack of awareness of the zoonotic nature of bovine TB is one of the major problems related to the control of tuberculosis (Abubakar *et al*., 2011)

From the questionnaire survey, level of education was statistically significant (P= 0.0001) to the knowledge and level of awareness of bovine tuberculosis. Those that have attained tertiary and secondary school education were more aware of tuberculosis than those with adult or non-formal education. Different studies have shown that most livestock handlers are unaware of the disease and its public health risk (Cadmus *et al*., 2010a; Onoja *et al*., 2010). This may be attributed to the level of education of these group of people, sheep flock are known to be kept mostly by Fulani pastoralist while goats are mainly kept by crop farmers (Bayer, 1984). These groups of people are said to be less privileged with inability to meet the cost of formal education therefore, literacy level is low amongst them.

Most of the small ruminant handlers were also not conversant with the modes of transmission of the disease. And from the study butchers and livestock marketers with low literacy levels were the ones mostly at risk of being exposed to the *M. bovis* infection. Reports showed that small ruminants are susceptible to bovine tuberculosis, the respiratory tract is often affected and as such they remain a potential source of infection for other animals and humans (Bernabe *et al*., 1991; Hiko and Agga, 2010). In this study, out of the 166 (73%) of the respondent that keep livestock, 126 (55%) of them keep their sheep and goats at close proximity inside their houses at night. This highlights the potential for zoonotic transmission. *M. bovis* and *M. tuberculosis* has

been isolated from goats in various studies (Cadmus *et al*., 2009 and Kassa *et al*., 2012). The increased physical contact and closeness between animal handlers and potentially infected animals represent the population at risk for *M. bovis* (Cosivi *et al*., 1998; Okaiyeto *et al*., 2008; Onoja *et al*., 2010).

Consumption of unpasteurized milk is still a common practice amongst animal handlers, as the butchers even try to milk the ewes and doe before slaughter for drinking oblivious of the tuberculosis status of the animals. Approximately 52% of the respondents preferred sheep and goat as source of meat, and meat has been known to be source of infection (Jose and Gutierrez, 2006). Poor implementation of control measures such as rigorous meat inspection at abattoirs has been identified as a factor responsible for the persistence of bovine tuberculosis in Nigeria (Abubakar *et al*., 2011).

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

This study showed that sheep and goats in Kaduna state are exposed to bovine tuberculosis. This is the first time small ruminants are being screened for *M. bovis* in Nigeria. There is sparse information on magnitude and distribution, risk of exposure, transmission and public health importance of *M. bovis* infection in small ruminants in Nigeria. The high detection rates of circulating anti-bovine tuberculosis antibodies in small ruminants in the study shows that small ruminants were highly exposed to *M. bovis* and the risk of transmission to humans and other animals cannot be overemphasized owing to the close proximity of humans to these animals. The high prevalence of bovine tuberculosis in small ruminant meant for human consumption is of serious public health concern not just for Kaduna state but the country at large because a reasonable number of sheep and goats slaughtered at the various slaughter houses are imported into Kaduna from neighboring states and countries. Therefore, both national and international multi-sectorial initiatives must be put in place to review the problem of bovine tuberculosis in Nigeria. Objective controlled measures should be put in place such as targeted control movement of animals, combined Veterinary and Medical efforts to enhance tuberculosis diagnosis, active sensitization of the populace at risk of exposure on the transmission and prevention of the disease, and intensification of meat inspection in small ruminants should be employed.

6.2 CONCLUSION

This study has established a sero-prevalence of 10.84% for sheep and 16.41% for goats. The high sero-prevalence in these small ruminants is an indication of possible disease transmission at the animal-human-interface owing to the husbandry practices in Nigeria. Where small ruminants are herded together with cattle during the day and at night, kept inside inadequately aerated farmer's houses for protection. The sero-prevalence of 13.8% and 13.4% recorded in livestock markets and slaughter houses respectively, were rather high and therefore constitute potential risk to consumers. Especially to the pastoral communities where goat's milk is usually consumed unpasteurized and the meat considered a poor man's cow. The awareness and risk factors associated with the transmission of bovine tuberculosis is limited amongst the populace, however in this study, education has been shown to significantly affect the level of awareness. Hence, risk of zoonotic transmission should be addressed through education and preventive programs, improved hygienic practices and pasteurization of milk.

6.3 RECOMMENDATION

1. The small ruminant handlers and general public should be enlightened continuously on the zoonotic implication of bovine tuberculosis, proper food handling, personal hygienic practices, animal husbandry practices and associated risks, in order to achieve good human health, improved livestock health and productivity in Nigeria.
2. Routine and effective meat inspection should be intensified for small ruminants and livestock in general.

3. Further studies should be carried out to understand the epidemiology of bovine tuberculosis in small ruminant and develop control measures suitable to check the spread of the disease in Nigeria.

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APPENDIX I



Plate I: A single line showing negative test result for *M. bovis* in a goat serum sample



Plate II: A double line showing a positive test result for *M. bovis* in a goat serum sample

APPENDIX II

QUESTIONNAIRE ON AWARENESS OF TUBERCULOSIS (A)

This questionnaire is part of a research that is aimed at assessing the level of awareness of tuberculosis. All information given would be used solely for academic purposes and treated confidentially. Thank you.

SIGN……. DATE……..

SERIAL NUMBER……

LIVESTOCK MARKET (LSM)/ABATTOIR

Please kindly fill and tick (√) where appropriate

1. Location
   a. Tudun/Wada [ ]
   b. Kakuri [ ]
   c. Ungwar/Rimi [ ]
   d. Kawo [ ]
   e. Others (specify) ……..

2. Sex Male [ ] Female [ ]

3. Age
   a. 10-20 [ ]
   b. 20-30 [ ]
   c. 30-40 [ ]
   d. 40- and above [ ]

4. Educational qualification
   a. Non- formal [ ]
   b. Adult education [ ]
   c. Primary [ ]
   d. Secondary [ ]
   e. Tertiary [ ]

5. How long have you been in the profession?
   a. 1-5 years [ ]
   b. 5-10 years [ ]
   c. 10 years and above [ ]

6. Sources of livestock

S/N	STATE	TICK	S/N	STATE	TICK	S/N	STATE	TICK
a.	Adamawa		h.	Kano		o.	Plateau	
b.	Bauchi		i.	Katsina		p.	Sokoto	
c.	Benue		j.	Kebbi		q.	Taraba	
d.	Borno		k.	Kogi		r.	Yobe	
e.	Gombe		l.	Kwara		s.	Zamfara	
f.	Jigawa		m.	Nasarawa		t.	Others	
g.	Kaduna		n.	Niger				

7. Have you or any house member suffered from persistent cough?
   Yes [ ] No [ ]

8. If yes, did you seek medical help?
   Yes [ ] No [ ]

SECTION B

9. Do you keep livestock in your house?
   Yes [ ] No [ ]

10. What type and number do you have?

Animal	Number		Total
	Male	Female	
Sheep			
Goat			
Pig			
Cattle			
Chicken			
Ducks			
Others			

11. Where do you keep your animals? ……..

12. Do the animals go out to graze and feed?
    Yes [ ] No [ ]

13. If not, what is the source of feed…….

14. Do you know the breed of your goat?
    Yes [ ] No [ ]

15. If yes, what breed of goat do you have?
    a. Red sokoto [ ]
    b. West African dwarf [ ]
    c. Red kano [ ]
    d. Others (specify) ……

16. Do you know the breed of your sheep?
    Yes [ ] No [ ]

17. If yes, what breed of sheep do you have?
    a. Yankasa [ ]
    b. Ouda [ ]
    c. Balami [ ]
    d. Others (specify) ……

SECTION C

18. List diseases that you know affects sheep and goats……..
    ……

19. Which ones amongst them can infect humans ……..
    ……

20. Do you know what condition is called tuberculosis?
    Yes [ ] No [ ] No idea [ ]

21. If yes, what is it associated with?
    ……

22. Does tuberculosis affect sheep and goats?

Yes [ ] No [ ] No idea [ ]

23. If yes, how do you think sheep and goats get infected?
……

24. Do you know if sheep and goats can pass the infection to man?
Yes [ ] No [ ] No idea [ ]

25. If yes, how? …….

26. Where is the source of your meat?
a. Slaughter house/abattoir [ ]
b. Market [ ]
c. Residential houses [ ]
d. Not specific [ ]

27. Have you been to the abattoir/slaughter house before?
Yes [ ] No [ ] No idea [ ]

28. Is routine meat inspection carried out in the slaughter house by veterinarians and/or other animal health workers?
Yes [ ] No [ ] No idea [ ]

29. Which of this meat do you prefer and patronize?
Cattle [ ] Sheep [ ] Goat [ ]

30. How often do you consume products (meat, milk) from sheep and goats?
Daily [ ] Weekly [ ]
Monthly [ ] Not specific [ ]

Appendix II

QUESTIONNAIRE ON AWARENESS OF TUBERCULOSIS (B)

This questionnaire is part of a research that is aimed at assessing the level of awareness of tuberculosis. All information given would be used solely for academic purposes and treated confidentially. Thank you.

SIGN……. DATE……..

SERIAL NUMBER……

Please kindly fill and tick (√) where appropriate

DEMOGRAPHIC INFORMATION

31. House no/ address:
……..

32. Sex Male [ ] Female [ ]

33. Household size
    a. 1-5 persons [ ]
    b. 6-10 persons [ ]
    c. 11-15 persons [ ]
    d. 16-20 persons [ ]

34. Educational qualification
    No formal [ ] Primary [ ] Secondary [ ]
    Tertiary [ ] Others Specify: …….

35. Occupation
    Student [ ] Farmer [ ] Civil servant [ ]
    Business [ ] Others Specify: ……

36. Have you or any house member suffered from persistent cough?
    Yes [ ] No [ ]

37. If yes, did you seek medical help?
    Yes [ ] No [ ]

SECTION B

38. Do you keep livestock in your house?
    Yes [ ] No [ ]

39. What type and number do you have?

Animal	Number		Total
	Male	Female	
Sheep			
Goat			
Pig			
Cattle			
Chicken			
Ducks			
Others			
Total			

40. Where do you keep your animals? ……..

41. Do the animals go out to graze and feed?
    Yes [ ] No [ ]

42. If not, what is the source of feed…….

43. Do you know the breed of your goat?
    Yes [ ] No [ ]

44. If yes, what breed of goat do you have?
    e. Red Sokoto [ ]
    f. West African dwarf [ ]

g. Red Kano [ ]
h. Others (specify) ……

45. Do you know the breed of your sheep?
Yes [ ] No [ ]

46. If yes, what breed of sheep do you have?
e. Yankasa [ ]
f. Ouda [ ]
g. Balami [ ]
h. Others (specify) ……

47. Where did you obtain your sheep/goats from?
……

SECTION C

48. List diseases that you know affects sheep and goats……..
……

49. Which ones amongst them can infect humans ……..
……

50. Do you know what condition is called tuberculosis?
Yes [ ] No [ ] No idea [ ]

51. If yes, what is it associated with?
……

52. Does tuberculosis affect sheep and goats?
Yes [ ] No [ ] No idea [ ]

53. If yes, how do you think sheep and goats get infected?
……

54. Do you know if sheep and goats can pass the infection to man?
Yes [ ] No [ ] No idea [ ]

55. If yes, how? …….

56. Where is the source of your meat?
    e. Slaughter house/abattoir [ ]
    f. Market [ ]
    g. Residential houses [ ]
    h. Not specific [ ]

57. Have you been to the abattoir/slaughter house before?
    Yes [ ] No [ ] No idea [ ]

58. Is routine meat inspection carried out in the slaughter house by veterinarians and/or other animal health workers?
    Yes [ ] No [ ] No idea [ ]

59. Which of this meat do you prefer and patronize?
    Cattle [ ] Sheep [ ] Goat [ ]

60. How often do you consume products (meat, milk) from sheep and goats?
    Daily [ ] Weekly [ ]
    Monthly [ ] Not specific [ ]