INVESTIGATION OF TUBERCULOSIS IN SHEEP AT SADAR AND PARBATIPUR UPAZILA UNDER DINAJPUR DISTRICT IN BANGLADESH

A THESIS

BY

AHMED ALI OMAR MUSA
SEMESTER: JANUARY-JUNE, 2018
REGISTRATION NO.: 1705174
SESSION: 2017-2018

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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Submitted to the
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JUNE, 2018

DEDICATED

TO MY

BELOVED Brothers

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The author *June 2018*

ABSTRACT

Tuberculosis is highly zoonotic contagious diseases caused by *Mycobacterium tuberculosis* complex (MTC or MTBC) occurs both human and animal. Sheep are considered susceptible to *M. bovis* infection. The duration of the study was from January to June, 2018. Out of 140 sheep 7 were found positive and overall prevalence was 5% positive, 15% were doubtful cases and 80% were negative. According to this study the highest number of positive TB was found in female sheep that are equivalent to 6.4%, while doubtful cases were 14.9% and the negative cases were 78.7%. In terms of males the positive cases were 2.2%, the doubtful cases were 15.2% whereas the negative cases were 82.6%. In this study the high prevalence 23.5% was recorded 4 and above years while no positive animal was detected in lambs. In this study highest positive rate 14.8% was recorded in the crossbreed followed by non-descript Deshi 2.7%. On the other hand according Body Condition Score group poor body condition showed high prevalence 15% as compared with medium body condition score was 2.7 %, no positive reactor was recorded in sheep having good body condition score. And 10.2% Positive cases were found in lactating females and 3.6 % was found in non-lactating females. The study also recorded that 7.7% positive in pregnant ewes and 6.9% positive in non-pregnant ewes. In the study area, there was no previous history of TB and PPD Skin Test for diagnosis of TB by using TST (tuberculin skin test).

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MTC	-	*Mycobacterium tuberculosis* complex
TST	-	Tuberculin skin test
G+C	-	guanine plus cytosine
BTB	-	Bovine tuberculosis
OIE	-	Office International des Epizooties
HSTU	-	Hajee Mohammed Danesh science and technology
ELISA	-	Enzyme Linked Immunosorbent Assay
IFN-Y	-	Interferon gamma
ITT	-	Intradermal tuberculin test
M	-	Mycobacterium
MS	-	Master of science
MTC	-	Mycobacterial tuberculosis complex
DTH	-	Delayed hypersensitivity
CD	-	Cluster designation
MAb	-	Monoclonal antibody
PPD	-	Purified protein derivative
iNOS	-	inducible nitric oxide syntheses
IFN	-	Interferon
TLRs	-	Toll-like receptors
PCR	-	polymerase chain reaction
SICCT	-	single intradermal cervical comparative tuberculin
SIT	-	single intradermal tuberculin -
PPD	-	purified protein derivative
TBLN	-	Tuberculous lymphadenitis

CHAPTER I

INTRODUCTION

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INTRODUCTION

Bangladesh is located in South East Asia and it is one of the most densely populated countries in the world with an estimated 1,033 people/km^2 (United Nations, 2011). In Bangladesh, similar to human population density, livestock density is also highest (cattle, goats, sheep and buffaloes) in the world with an estimated 145 large ruminants/km^2 compared with 90 for India and 20 for Brazil (BARC Bangladesh, 2010). It is estimated that 52.8 million livestock animals are present in Bangladesh, most of which are food producing cattle and goats (DLS, 2012). About 20% of the human population is directly and 50% is partly dependent on the livestock sector (Bangladesh Economic Review, 2009).

The sheep is one of important ruminant species in the world. Of the total sheep population, 64.43% of sheep are found in Asia and Africa. There are about 920 breeds, of which 233 are found in Asia (FAO, 2003). Sheep stand third in number among the ruminant species in Bangladesh and are used primarily for meat production. Bangladesh possesses 1.69 million sheep at present (BBS, 2004). Though the sheep was localized in particular areas of the country, they are now found all over the country. Most of the sheep of Bangladesh are indigenous in nature with few crossbreeds (Bhuiyan 2006).

Tuberculosis (TB) is a chronic bacterial disease caused by *Mycobacterium tuberculosis* complex (MTC) leading to decreased productivity, economic losses and poses a significant threat to human health. Among MTC organisms, the major agents are *M. tuberculosis* and *M. bovis*. The primary host for *M. bovis* is cattle and *M. tuberculosis* is human. However, occurrence of *M. tuberculosis* in animals and *M. bovis* infection in humans has been reported previously (Ocepek *et al.,* 2005).

Tuberculosis (TB) in sheep, caused by members of the Mycobacterium tuberculosis complex (MTC), has been reported in New Zealand, Sudan, Italy, Ireland and the United Kingdom (Cordes *et al.,* 1981).

Mycobacterium belongs to the Kingdom of Bacteria; Phylum of Actinobacteria; Order of Actinomycetals; Family of *Mycobacteria*ceae (Seifert, 1996; Quinn *et al.,* 2004). They are grouped in the suprageneric rank of actinomycetes that, usually, have a high content (61–71%) of guanine plus cytosine (G+C) in the genomic deoxyribonucleic acid (DNA), and a high lipid content in the wall, probably the highest among all bacteria (Palomino *et al.,* 2007). The *Mycobacteria* comprise more than 80 species, within the complex of related and poorly studied organisms (Rainy *et al.,* 1995). Most of them live and replicate freely in natural ecosystems and seldom, if ever, cause disease. Only a few *Mycobacteria* become successful pathogen of higher vertebrates, preferentially inhabiting the intracellular environment of mononuclear phagocytes. The host-dependent *Mycobacteria* that cannot replicate in the environment are *M. leprae, M. lepraemurium, M. avium subsp. paratuberclosis*, and the members of the *M. tuberculosis* complex (Palomino *et al.,* 2007). Tuberculosis is an infectious disease with distinctive clinical and pathological features. Tuberculosis occurs in humans and many animal species including species of animals used for production of food (milk or meat) for human consumption (cattle, sheep, goats and deer). The principal microorganism associated with human tuberculosis is *M. tuberculosis. M. bovis* is the causative agent of tuberculosis in animals used for production of food and accounts for a relatively small proportion of human cases. In a proportion of human or animal hosts infected with these microorganisms, the infection may ultimately progress to severe systemic illness. Pulmonary disease is the classical feature and ultimately the disease may progress to death of the host if untreated. The classical pathological feature of the disease in humans is the caseating granuloma.

This is an organized aggregation of macrophages surrounding an area of caseous necrosis (Food Safety Authority of Ireland (FSAI, 2008).

In ovine, the occurrence of tuberculosis is very rare although there are few reports indicating the presence of *M. bovis* in sheep and goat (Kassa *et al.,* 2012; Marianelli *et al.,* 2010). This primarily occurs in areas with high intensity sheep population and when there exists close contact between infected cattle and sheep facilitating transmission between these species. India accounts for one fourth of the global TB burden (Central TB division, GOI, 2017).

Tuberculosis is often unnoticed in animals and the infected animals continue to spread the disease to other susceptible animals and human by excreting the organisms through milk, faeces and respiratory droplets. Hence to control tuberculosis both animals and human has to be monitored for disease prevalence (Arunmozhivarman K. *et al.,* 2018). The World Organization for Animal Health OIE published guidelines and standards for BTB testing in cattle, but does not have specific guidelines for small ruminants (sheep and goat). BTB is increasingly reported in small ruminants in European countries, and although usually low in prevalence it is nonetheless associated with severe pathology. This disease has socio-economic and public health importance and is of great significance to international trade of animals and animal products (OIE, 2004).

Tuberculosis in sheep is caused by members of *Mycobacterium tuberculosis* complex predominantly by *Mycobacterium bovis* and *Mycobacterium caprae,* Cordes *et al.* (1981); and few caused by *Mycobacterium tuberculosis* Tschopp and Bobosha (2011), Cadmus *et al.* (2009). Epidemiological studies indicated that tuberculosis in goat and sheep has a wide global distribution and has been reported in various countries of the world including New Zealand, Sudan, Spain, Nigeria, the United Kingdom, Italy, Algeria, Ethiopia Cordes *et al.* (1981); (Gezahegne M. K. *et al.,* 2012)

Tuberculosis is an important disease in many countries including Bangladesh. Tuberculosis in cattle and other domestic animals is caused by two members of *Mycobacterium tuberculosis* complex (*MTC*): *M. bovis* and *M. caprae* (Pavlik *et al.,* 2002; Prodinger *et al.,* 2002; Erler *et al.,* 2004).

However, the present study was conduct with aim and fulfillment of the following objectives:

- To investigate the prevalence of TB in sheep in Sadar and Parbatipur Upazila under Dinajpur District in Bangladesh.
- To determine the risk factors affecting the sheep such as: age, sex, breed, body condition score and production status.
- To forward recommendation

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Sheep tuberculosis

In sheep, the occurrence of tuberculosis is very rare although there are few reports indicating the presence of *M. bovis* in sheep and goat (Kassa *et al.,* 2012; Marianelli *et al.,* 2010). This primarily occurs in areas with high intensity sheep population and when there is close contact between infected cattle and sheep facilitating transmission between these species. India accounts for one fourth of the global TB burden (Central TB division, GOI, 2017).

2.2 History of tuberculosis

It has been hypothesized that the genus *Mycobacterium* originated more than 150 million years ago, *Mycobacterium ulcerans,* causing infections since ancient times, requires specific environmental conditions as reflected nowadays in its distribution worldwide (Hayman J, 1984).

The famous scientist Robert Koch was able to isolate the tubercle bacillus. Using the methylene blue staining recommended by Paul Ehrlich, he identified, isolated and cultivated the bacillus in animal serum. Finally he reproduced the disease by inoculating the bacillus into laboratory animals (Gradmann C, 2001). Robert Koch presented this extraordinary result to the Society of Physiology in Berlin on 24 March 1882, determining a milestone in the fight against TB (Bartolozzi G.2012).

1898: Theobald Smith identifies differences between tuberculosis species infecting cattle (*M. bovis)* and humans (*M. tuberculosis)*, 1901-1911: A British Royal Commission conducts extensive research, Demonstrates cow's milk infected with *M. bovis* caused extra pulmonary TB in people increased risk of cervical lymphadenitis in children who drank cow's milk, 1908 first compulsory pasteurization law, 1917 US State-Federal bovine TB eradication program whole herd testing & culling of reactors Prior to pasteurization estimated 10-30% of human TB cases due to *M. bovis* (CDC, 2015).

2.3 Epidemiology

2.3.1 Etiology

Bovine tuberculosis (TB) infection is infrequently diagnosed in sheep. Most reports are from single individual cases or flock outbreaks. However, in Spain several outbreaks have been reported recently, all of which had epidemiological links with TB-infected cattle herds (Mendoza *et al.,* 2015).

Mycobacterium bovis, the etiological agent of bovine-type tuberculosis (TB), has an exceptionally wide host range and a complex epidemiological pattern of infection, involving interaction between humans and domestic and wild animals. Information on TB in sheep is scarce, and the general picture that emerges is far from conclusive. In a previous study, postmortem examinations on 70 tuberculin reacting sheep were carried out (Marianelli *et al.,* 2010).

Members of the Mycobacterium genus widely occur in natural ecosystems. Most members of mycobacteria are decomposing organic matter and due to the nitrogen binding activity they are useful and essential inhabitants of the soil and surface waters. Some Mycobacterium species in the course of evolution has become pathogenic. Among the pathogenic ones the most important are the members of Mycobacterium tuberculosis complex (MTBC), especially *M. tuberculosis* and *M. bovis*. There are significant differences in the virulence and the host specificity among the complex members (*M. tuberculosis*, *M. bovis*, *M. bovis* bacillus Calmette-Guerin BCG, *M. africanum*, *M. microti*, *M. canettii*, *M. bovis* subsp. caprae), *M. tuberculosis*, *M. africanum* and *M. canettii* are primarily human pathogens, *M. microti* mainly causes disease in rodents, although a few human cases have been reported as well. *M. bovis* can cause disease in both humans and animals (cattle, goats, elephants, deer, cats, seals, etc.), however the infection is more frequent in animals (Csaba Ködmön, 2011).

2.3.2 Geographical distribution

Tuberculosis was once found worldwide; however, control programs have eliminated or markedly reduced this disease from domesticated animals in many countries (OIE, 2009). Nations currently classified as tuberculosis-free include Israel, Iceland, Estonia, Switzerland, Denmark, Australia, Sweden, Norway, Finland, Singapore, Austria, Latvia, Luxembourg, Slovakia, Lithuania, Canada, Barbados, Jamaica and the Czech Republic. This status is mainly confirmed after countries achieving prevalencies of less than 0.1% for a period of six years (OIE, 2012). In other countries active Eradication programs are in progress, these include; some European countries, Japan, New Zealand, the United States, Mexico and some countries of Central and South America (Reviriego *et al.,* 2006; Ryan *et al.,* 2006).

Although bovine tuberculosis has been eradicated from the majority of U.S.A. States, a few infected herds continue to be reported, and a number of states periodically lose their disease-free status. In particular, a focus of infection in wild white-tailed deer has complicated eradication efforts in Michigan. Similar problems exist with infected badgers in the U.K. and Ireland, and infected brush-tailed opossums in New Zealand.

Bovine tuberculosis is still widespread in Africa, parts of Asia and some Middle Eastern countries (Ayele *et al.,* 2004).

2.3.3. Host range of Tuberculosis

Tuberculosis caused by *M. bovis* is rare in sheep, mainly because of the nature of sheep husbandry and the fact that sheep are rarely exposed to infectious material (Francis J 1958). The relatively high incidence of tuberculosis (0.14%-0.22%) in sheep killed in abattoirs in Germany between 1904 and 1918, compared with only eight confirmed cases in Great Britain between 1900 to 1980, adds weight to the theory of husbandry playing an important role, since the sheep in

Germany was housed. Although Chausse considered that sheep were relatively immune to experimental infection with *M. bovis* (Chausse P. 1913). Several authors consider sheep to be highly susceptible to infection (Francis J 1958, Wilson G.S, & Miles S, 1975). Prevalence of up to 18% has been observed in New Zealand where sheep were grazing heavily contaminated pastures (Davidson R.M *et al.,* 1981). The distribution of lesions in this case suggested that ingestion was the cause of infection in 5 1% of animals and inhalation in 48%. Cordes *et al.* tested 281 sheep in an infected flock of 15,000, and found thirty-two animals to be skin test positive (Cordes D.O *et al.,* 1981). Thirty-two of forty-three animals with gross lesions were smear positive or culture positive for *M. bovis*. Gross lesions were generally large and numerous, but varied from being extensive to few. Histopathology revealed that in most cases only a few acid-fast organisms were present. Cordes *et al.* reviewed the literature on susceptibility of sheep to infection with *M. bovis* and considered these animals to be fairly susceptible to infection with *M. bovis* via the oral route (Cordes D.O *et al.,* 1981).

The disease is caused by the members of the *Mycobacterium* family that mainly affects the respiratory system. Three main types of TB and their causative agents are human TB, caused by *M. tuberculosis*, avian TB, caused by *M. avium* and bovine TB, caused by *M. bovis*. Human TB is rarely transferable to non-human species. Avian TB is typically restricted to birds. Bovine or cattle TB, presently known as zoonotic TB, is a highly infectious disease, caused by *M. bovis* having no geographical boundaries and infection occurs in diverse group of animals, which includes farm animals of economic importance, wildlife and humans (Grange JM. 2001, Pavlik I, *et al.,* 1990-1999).

Mycobacterium bovis is the primary cause of tuberculosis in cattle and swamp or Asiatic water buffalo (*Bubalus bubalis*). All species of cattle are affected, although. Bos *indicus* (large horned cattle with a hump such as zebu and Brahman) may be more resistant to the disease than Bos *taunts* (small homed cattle without a hump, e.g. European breeds) (O'Reilly *et al.,* 1995). *Mycobacterium bovis* causes tuberculosis in cattle in almost every country of the world (OIE, 2000). Infection has been reported in 69% of countries in the tropics and in 80% of countries in Africa. Tuberculosis is considered to be one of the most important diseases of cattle in the People's Republic of China, despite the absence of official prevalence figures. *Mycobacterium bovis* causes a spectrum of disease in cattle, ranging from generalized tuberculosis that affects almost every organ of the body, to cases in which the organism infects a single lymph node, causing a single tubercle which may or may not cause further disease.

Water buffalo in northern Australia were originally imported from Indonesia, but were probably infected by the first dairy cattle brought to the Northern Territory in the 1920s. Domestic buffalo in India are known to be infected with *M. bovis*. The overall prevalence of infection in buffalo was almost three times that in cattle when testing was conducted in four states of India (Lall J.M, 1969). The first isolation of *M. bovis* from water buffalo in the Indochina region was performed in two emaciated swamp buffalo that died in north-eastern Thailand in 1992 (Kanameda M. *et al.,* 1995). In a follow-up study of diseased animals in Thailand, the most common lesions in buffalo were caseous granulomas in the thoracic lymph nodes; localized and generalised infections were found depending on the progression of the disease (Kanameda M. *et al.,* 1997). Tuberculosis has also been confirmed in buffalo in Egypt (Nazzar A. *et al.,* 1993). Farmed water buffalo in Egypt were infected at almost three times the rate of cattle (El Taweel A. 1992).

Pigs are susceptible to *M. bovis* infection and *M. bovis* was a common cause of tuberculosis in pigs in the early to mid-1900s. Disease levels in pigs usually reflect those in cattle, and prevalence in the order of 20% have been recorded in some pig populations. The oral route is the most important route of infection in domestic pigs, most frequently caused by feeding milk, milk products or offal from infected cows. Generalized infection was common, but following the implementation of measures to reduce tuberculosis in dairy cattle, the incidence of tuberculosis in pigs has declined; for example, in Australia, incidence was reduced from 17% in 1913 to 0.84% in 1928 (Cousins D.V *et al.,* 1998). In the USA, the rate of tuberculous lesions due to *M. bovis* in slaughtered pigs declined from 15.2% in. 1924 to 1.09% in 1970 (Acha P.N, 1987). Tuberculosis in domestic pigs is now rare in countries that have successfully implemented tuberculosis control programmes, although 3.8% of tissues from suspect tuberculous pigs in New Zealand between 1987 and 1993 were found to be infected with *M. bovis* (De Lisle G.W, 1994).

New Zealand, with its emphasis on extensive pastoral farming of ruminants, and with comparatively low levels of TB in the human and bovine populations, has a correspondingly low prevalence of the disease in pigs. Porcine TB of human origin has not been recorded. Porcine TB of bovine origin is found most frequently in feral pigs coming from areas where there is still a *M. bovis* problem in local cattle and possum populations. Most cases found in farmed pigs are due to the *M. tuberculosis* complex (W.O. Nuttall, 1986).

Mycobacterium bovis has been known to cause tuberculosis in goats, although the importance of the disease varies depending on the country and the type of enterprise in question. In most cases, infection occurs as a direct result of contact with infected cattle. Infection in several herds of goats in India between 1941 and 1942 was associated with common grazing of goats and heavily-infected cattle (Lall J.M 1969). In one case where common grazing had occurred for four years, 24.7% of 417 goats gave a positive result to the tuberculin test and 90% of these had tuberculous lesions. In Australia, where goats are rarely kept with cattle, a single case of *M. bovis* infection in a goat has been reported (Cousin D.V. *et al.,* 1993). In this case, goats were grazing with infected cattle (with a prevalence of 35%) at abnormally high stocking rates. Although the goats appeared malnourished, infection was only detected in a single animal after skin testing and subsequent culture of reactor lymph nodes. No lesions were detected at necropsy of the reactor goats, suggesting that the goats were not as susceptible to the infection as the cattle. A low prevalence of tuberculosis in goats has also been reported in Taipei China (LuY. *et al.,* 1992). Tuberculosis in goats appears to be widespread in Mediterranean countries and is a serious disease (Gutierrez M. *et al.,* 1993). While mixed farming of cattle and goats is not common practice in these countries, common grazing areas sometimes provide close contact. In Spain, tuberculosis in goats is a comparatively common occurrence. Recent reports have suggested that the histopathological responses to infection in goats and cattle may be different. The Caprine lesions, although more severe than bovine lesions, appeared to contain fewer organisms; this was attributed to a difference in either the immune response of the host or the strains infecting cattle and goats (Gutierrez M. *et al.,* 1995). Until recently, tuberculosis in goats in Spain was considered to be due to *M. bovis*. However, strain differences were detected in the isolates from goats and sheep using DNA fingerprinting techniques (Aranaz A. *et al.,* 1996, Gutierrez M. *et al.,* 1993). The zoonotic aspects of this strain have been confirmed by evidence of transmission to humans (Gutierrez M. *et al.,* 1997) and a recent study suggests that the organism responsible for tuberculosis in goats in Spain is a variant of *M. bovis* (Niemann S. *et al.,* 2000). This strain has genetic and phenotypic differences from classical *M. bovis* and has been named *M. tuberculosis* subsp. *caprae* (Aranaz A. *et al.,* 1999). This strain has been detected in both goats and sheep in Spain, but not in cattle.

The subspecies *M. caprae* has probably evolved in goats and is now maintained in that host in Spain.

Tuberculosis was known to occur in dromedary camels *(Camelus dromedarius)* in Egypt in the early 1900s. Between 1910 and 1916, 2.9% of camels slaughtered in Cairo were Tuberculous and the bovine strain was implicated. Camels in Egypt were often kept in close association with cattle and the route of infection was usually the respiratory tract (Mason F.E., 1917). The lungs and bronchial lymph nodes were always affected and lesions were restricted to these organs in 60% of cases. Generalised tuberculosis, with numerous lesions in the principal organs and carcass lymph nodes, was observed in 7% of cases. In 1983, tuberculosis was diagnosed in two of nineteen Bactrian camels (Camelus *bactrianus)* in the USA (Bush M. *et al.,* 1990); pulmonary infection due to *M. bovis* has also been reported in camels in Mauritania (Chatier F. *et al.,* 1991). Alpacas *(Lama Pacos)* are known to be susceptible to M, *bovis* infection (Anon, 1989, Dinkla E.T.B., 1991), although reports of infection in their natural habitat in South America are few. Recently, a case was diagnosed in an alpaca in New Zealand (G.W. de Lisle, personal communication). *Mycobacterium bovis* infection has been reported in llamas *(Lama glama)* in zoological collections in the USA (84) and in a small herd of llamas in the United Kingdom (UK), where infection was linked to cattle and badger populations in the surrounding area (Barlow A.M, *et al.,* 1999).

Mycobacterium bovis was isolated from lung and lymph nodes of two llamas, one of which had 'cluster of grapes' lesions scattered over the parietal pleura, lungs and pericardial sac. The authors concluded that tuberculosis should be considered in the differential diagnosis in cases of ill thrift with or without obvious respiratory signs. Most of the reports of tuberculosis in llamas and alpacas are associated with groups of animals in zoological collections, suggesting that exposure in a confined area and the stress of an artificial environment may lead to increased disease transmission in these species.

Mycobacterium bovis infection in horses is uncommon; when disease occurs, the primary lesions are found in the abdomen, suggesting ingestion as the cause of infection (Wilson G.S & Miles S. 1975). The incidence of tuberculosis in horses is very low in countries with a national programme to eradicate tuberculosis. Information from experimental infection suggests the horse is relatively resistant to infection with *M. bovis* (Francis J. 1958). The course of disease appears to be chronic, with the first symptoms being loss of body condition, despite normal appetite. Lesions are often found in the mesenteric lymph node and liver and lung lesions are normally present.

Although experimental studies have revealed that dogs are equally susceptible to *M. bovis* and *M. tuberculosis* infection (Mills M.A. *et al.,* 1940), summaries of bacteriological studies have indicated that tuberculosis in dogs is more frequently caused by *M. tuberculosis.* In a study detailing 592 cases of tuberculosis in dogs up to 1951, *M.*

tuberculosis caused more than twice as many cases as *M. bovis* (Cheyrolles J. 1951). An extensive review by Snider reported tuberculous lesions in 0.1%-6.7% of dogs subjected to necropsy between 1930 and 1970 (Snider W.R., 1971). Pulmonary tuberculosis is more likely to occur in the dog than the cat, and disseminated disease can occur in both species.

Cats are considered to be more susceptible to *M. bovis* than to *M. tuberculosis* (Chausse P., 1910, Chausse P., 1913, Snider W.R., 1971) and infection generally results from exposure to infectious material from tuberculous cattle.

In a review of published papers in 1945, 96% of cases (n = 147) were due to *M. bovis* and the remainder to *M. tuberculosis* (Verge M.J, 1958). Infection with *M. bovis* in cats was quite common in Europe before bovine tuberculosis was effectively controlled; Snider reported tuberculous lesions in 2%-13% of cats subjected to necropsy between 1930 and 1970 (Snider W.R., 1971).

Tuberculous lesions were seen most frequently in the alimentary tract and mesenteric lymph nodes, indicating that infection was most likely to be acquired by ingestion of infected unpasteurized cow's milk.

Non-healing skin lesions similar to those of cat leprosy, but caused by *M. bovis* have been reported in New Zealand. In a study of fifty-seven *M. bovis*-infected cats between 1974 and 1986, 58% had skin lesions (De Lisle G.W., 1994). Although the skin lesions could have occurred as a result of bite or scratch wounds inflicted by infected possums or cats, this could not be substantiated. Lesions in the head and mesenteric lymph nodes in 11% of the cats suggested infection occurred as a result of cats eating tuberculous feral animals or licking skin lesions rather than as a result of drinking infected milk. The majority of the infected cats were from rural or suburban areas where *M. bovis* was also present in feral and wild animals. Despite the large number of acid-fast organisms in some of the skin lesions, no evidence was found of spread of infection to any of the owners. Restriction endonuclease typing identified a cluster of twelve cats possessing the same type. The cats were assumed to have been infected from a single point source.

Tuberculosis caused by *M. bovis* has been reported to be associated with feline immunodeficiency virus (Monies R.J., 2000).

In a study of twenty-seven cases of tuberculosis in cats in Spain from 1984 to 1994, a cause was determined in eighteen of the cases and 61.2% of these were due to *M. bovis* (Novoa C., *et al.,* 1995).

Tuberculous lesions were observed in the abdominal cavity (22.3%), thoracic cavity (22.2%), or in both locations (55.5%). Lungs and mediastinal lymph nodes were always affected and most cases had miliary tuberculosis.

2.3.4. Sources of infection

Tuberculosis is often unnoticed in animals and the infected animals continue to spread the disease to other susceptible animals and human by excreting the organisms through milk, faeces and respiratory droplets (Arunmozhivarman K. *et al.,* 2018).

People can get infected through consumption of raw animal products and/or close contact with infected animals, particularly livestock (Ayele W.Y., *et al.,* 2004, Wilkins M.J., *et al.,* 2008).

2.3.5 Risk factors

The factor that played an important role in epidemiology of *Mycobacterium bovis* subs. Caprae is its ability to infect human (Gutierrez *et al.,* 1997), domesticated animal and wild life. Twelve cases of *Mycobacterium bovis* subsp. caprae infection have occurred in four humans, three cattle, and five red deer in western Austria since 1994. DNA-fingerprinting of the isolates suggested transmission in and between these species over several years (Prodinger *et al.,* 2002).

In the autumn of 2004, tuberculosis caused by *Mycobacterium caprae* occurred in a zoo in Slovenia. Genotyping results revealed that the dromedary camel and the two bison were infected by the same *M. caprae* (Pate *et al.,* 2006).

The occurrence of TB is linked to both management and ecological factors (population density of various host species, interaction, habitat difference etc) (Morris *et al.,* 1994). Goats may become infected with *M. bovis* when sharing pastures with infected cattle, at watering points, market places and shared night shelters (Naima *et al.,* 2011). In rural traditional smallholders in Ethiopia, small ruminants are important livestock component and are herded together with cattle during the day, whereas at night they are usually kept inside poorly ventilated farmers' house (round hut made of mud and timber and thatched roof) for protection, thus having daily contact to cattle and human. Such husbandry practices are epidemiologically important for potential disease transmission at human-animal interface (Tschopp *et al.,* 2010). Wildlife plays an important role in tuberculosis (TB), some wildlife species have long been known to be maintenance hosts (i. e, wildlife species that can maintain the disease in the absence of infected cattle). Classic examples of maintenance host include the brush tail possum (Trichosurus vulpecula) in New Zealand (Coleman and Cooke, 2001), the white-tailed deer (Odocoileus virginianus) in the USA, the Eurasian badger (Melesmeles) in the UK, Ireland, the African buffalo (Synceruscaffer) in South Africa (De Vos *et al.*, 2001; Rodwell *et al.,* 2001), and more recently described, the wild boar (Susscrofa) in Spain (Naranjo *et al.,* 2008) and wood bison (Bison bison) in Canada. *M. caprae* was also isolated from wildlife species such as red deer (Prodinger *et al.*, 2002) or wild boar (Erler *et al.*, 2004, Machackova *et al.,* 2004). Furthermore, an *M. bovis* subsp. caprae strain was found in wild and livestock animals in Western Austria (Prodinger *et al.,* 2002). In Africa, *M. bovis* was reported in buffaloes in Uganda, in baboons in Kenya (Tarara *et al.,* 1985; Sapolsky and Else, 1987) and in Serengeti-Ngorongoro ecosystem in Tanzania, affecting buffaloes, lions and wildebeest.

In Ethiopia no bovine tuberculosis (BTB) cases have been reported in the wildlife population (Tschopp *et al.,* 2010). The major factors among which contribute to the acquisition of the infection in both urban and rural populations are family ownership of cattle or small ruminants, previous livestock ownership, sharing of the house with animals, consumption of non-pasteurised milk (raw milk) or poorly cooked meat (WHO, 1994; Ayele *et al.*, 2004). All these causalities and/or habits are the daily practices most notably of rural communities in Ethiopia.

Professional occupation or workers such as, abattoir workers, veterinarians and laboratory technicians, animal care taker in zoos and those who are working in animals reservations and at national parks can also acquire the infection in due course of regular work (Grange *et al.,* 1994).

In maintenance, or reservoir, hosts such as wildlife species, cattle, buffalo, and goats, infection can persist through horizontal transfer in the absence of any other source of *M. bovis* and may be transmitted to other susceptible hosts as well. Spillover hosts, such as sheep (Biet *et al.,* 2005), on the other hand, can become infected with *M. bovis* when the challenge level is relatively high, although sheep do not seem to maintain infection within the species in the absence of continuing acquisition of infection from maintenance hosts (Morris *et al.,* 1994). Also play important roles in small ruminant tuberculosis.

Multivariable logistic regression analysis showed that older small ruminants (5 years and above) had 13 times the odds of being tuberculin reactors compared with age category less than 2 years old.

Female small ruminants with parity number greater than 4 had more chance of being bovine tuberculin positivity in relative to those with less than 2 parity numbers (Mamo *et al.* 2012). Javed *et al.,* (2010) showed a breed related bovine tuberculosis (BTB) prevalence in sheep in Pakistan. Thus some breed might be naturally more resistant to the disease or less reacting to PPD (Javed *et al.* 2010).

2.3.6. Routes of transmission of tubercle bacilli

In many developing countries, small ruminants are the "poor man's cow", providing milk but also meat, hides and wool (Javed T.M., *et al.,* 2009). In rural traditional small holders in Ethiopia, small ruminants are important livestock components and are herded together with cattle during the day, whereas at night they are usually kept inside poorly ventilated farmer's houses (round hut made of mud and timber and thatched roof) for protection, thus having daily close contact to cattle and humans (Tschopp R., *et al.,* 2009). Such husbandry practices are epidemiologically important for potential disease transmission at the human-animal interface.

Afar pastoralists in Ethiopia consume both goat and sheep milk very commonly, and to protect these small ruminants from predators, the pastoralists keep these animals in very close proximity to their houses. These conditions are potential risk factors for transmission of zoonotic diseases such as TB of animal origin to human or vice versa.

Although the role of meat in the spread of tuberculosis had not yet been elucidated, (Gutierrez Garcia, 2006) recently invoked a historical perspective, emphasizing meat as a vehicle for the zoonotic transmission of the causal agent of bovine tuberculosis and highlighting its importance and the implications for the health inspections of meat in abattoirs.

Animals transmit infection to each other through ingestion of urine, faeces and lymph, wound discharge, infected milk along with food and water. TB elimination programs in domesticated herds together with milk pasteurization have successfully reduced the incidence rate of TB caused by *M. bovis* among cattle and humans alike in developed countries (Tylor G.M. *et al.,* 2007).

Transmission could have been by aerosols or contamination of fodder due to indiscriminate spitting. Milk and meat are one of the most important links between bovine tuberculosis and human beings especially children (Leite C.Q. *et al.,* 2003, Cosivi O. *et al.,* 1998). *M. bovis* infection in humans can occur through inhalation of infectious droplets from a live or slaughtered animal or by consumption of unpasteurized dairy or meat products from infected animals (Grange J.M. *et al.,* 2001, LoBue P., 2006). Vertical transmission can also occur in infected females. The uterus may serve as a portal for foetal infection and surviving calves commonly develop liver and spleen lesions (Biberstein D.V. *et al.,* 1999). People working in animal husbandry, slaughterhouse workers, veterinarians, and people in close contact with possibly-infected animals are at a higher risk for *M. bovis* infection (Cousin D.V. *et al.,* 1999).

2.3.7 Incidence and prevalence

In an extensive slaughterhouse survey undertaken by MAFQual Meat Services in the 1986/87 slaughter season, a total of 9 905 270 lambs (under 1 year) and **1**976 617 adult sheep from all geographic regions of New Zealand were surveyed. The 17 export slaughterhouses and 13 abattoirs participating reported a total of 35 grossly-identifiable tuberculosis-like lesions. Samples taken from these lesions showed that only two of the 35 were histologically suggestive of tuberculosis (TB), and there was no isolation of *M. bovis.* This represents a sample prevalence of less than 0.00002%, or a factor of 2500 less than that expected (Allen G.M., 1988).

Few studies carried out so far in central highland Ethiopia indicated the existence of TB in small ruminants with low level of prevalence (4.2%) based on abattoir examination results and 3.1% using single intradermal tuberculin test (Gezahegne *et al.,* 2012).

In a previous study, postmortem examinations on 70 tuberculin reacting sheep were carried out (Davidson *et al.,* 1981). Tuberculous lesions similar to those of cattle were found in 61% of cases. *Mycobacterium bovis* was also recovered from 32 of 43 sheep with tuberculous lesions in a previous study (Cordes *et al.,* 1981). These animals belonged to a flock numbering 15,000 head, grazing on a farm with a history of widespread occurrence of TB in both cattle and possums. In another study, an outbreak of *M. bovis* in a flock of sheep housed in close contact with Tuberculous cattle was described (Malone *et al.,* 2003) Gross TB lesions were present in 4 of the 6 tuberculin-reacting sheep, and *M. bovis* was isolated from the lesions. Clinically, however, all affected sheep were healthy. The isolation of *M. bovis* from a sheep with tuberculous lesions, belonging to a flock of 200 ewes, has also been previously reported (Houlihan *et al.,* 2008).

The overall percentages of reactor animals in CCT test were 1.46 and 1.29 in sheep and goat respectively. The percentages of reactors were 0.36 for bovine TB, 1.09 for avian TB in sheep. In goat the percentage of avian TB and bovine TB infection was 1.29 %. The overall percentages of male reactors were 1.72, 3.38 and that of female reactors were 1.39, 0.00 in sheep and goat respectively. The overall percentage of reactor animals in sheep were 1.33, 3.12, 0.00 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years respectively. On the other hand, the overall percentage of reactor animals in goats were 1.36, 0.00, 3.22 and 0.00 for the age group <3 years, 3-4 years, 4.1-6 years and >6 years, respectively. The Jamunapari breed showed maximum (2.85) and the Black Bengal breed showed minimum (0.83) percentage of infection for both types of TB infection.

In general the female, the younger and the older animals were found to be more infected than the male and adult animals in both sheep and goats as he mentioned (Rahman M.M. *et al.,* 2013).

In ovine, the occurrence of tuberculosis is very rare although there are few reports indicating the presence of *M. bovis* in sheep and goat (Kassa *et al.,* 2012; Marianelli *et al.,* 2010). This primarily occurs in areas with high intensity sheep population and when there exists close contact between infected cattle and sheep facilitating transmission between these species. India accounts for one fourth of the global TB burden (Central TB division, GOI, 2017).

Study revealed a moderately low prevalence of TB in goats and sheep of Afar Pastoral Region of Ethiopia (Gezahegne M. Kassa, *et al.,* 2012).

I some 16 areas of New Zealand it is considered that the feral possum (*Trichosurus vulpecula*) is endemically infected with *Mycobacterium bovis,* The presence of infected possums has been shown to cause persistent levels of infection in both farmed deer and cattle, giving the cattle within these areas a 20 times greater chance of reacting to a tuberculin test than cattle from outside these areas. The nationwide incidence of infection in cattle was 0.05% in the year ending 31 March 1987. Farmed deer in New Zealand showed a nationwide prevalence over that same period of O.6%. If sheep were equally susceptible to infection, and were exposed to the same risk of infection as the deer and cattle populations, then a similar prevalence (order of magnitude) of infection would be expected in the sheep population. Given that there are 67.2 million sheep in New Zealand,' then even at a 0.05% prevalence level there would be expected to be about 30 000 infected sheep (Allen G.M., 1988).

Mycobacteria of the *Mycobacterium tuberculosis* complex (MTBC) greatly affect humans and animals worldwide. The life cycle of mycobacteria is complex and the mechanisms resulting in pathogen infection and survival in host cells are not fully understood. Recently, comparative genomics analyses have provided new insights into the evolution and adaptation of the MTBC to survive inside the host. However, most of this information has been obtained using *M. tuberculosis* but not other members of the MTBC such as *M. bovis* and *M. caprae*. In this study, the genome of three *M. bovis* (MB1, MB3, MB4) and one *M. caprae* (MB2) field isolates with different lesion score, prevalence and host distribution phenotypes were sequenced. Genome sequence information was used for whole-genome and protein-targeted comparative genomics analysis with the aim of finding correlates with phenotypic variation with potential implications for tuberculosis (TB) disease risk assessment and control. At the whole-genome level the results of the first comparative genomics study of field isolates of *M. bovis* including *M. caprae* showed that as previously reported for *M. tuberculosis*, sequential chromosomal nucleotide substitutions were the main driver of the *M. bovis* genome evolution. The phylogenetic analysis provided a strong support for the *M. bovis*/*M. caprae* clade, but supported *M. caprae* as a separate species. The comparison of the MB1 and MB4 isolates revealed differences in genome sequence, including gene families that are important for bacterial infection and transmission, thus highlighting differences with functional implications between isolates otherwise classified with the same spoligotype. Strategic protein-targeted analysis using the ESX or type VII secretion system, proteins linking stress response with lipid metabolism, host T cell epitopes of mycobacteria, antigens and peptidoglycan assembly protein identified new genetic markers and candidate vaccine antigens that warrant further study to develop tools to evaluate risks for TB disease caused by *M. bovis*/*M. caprae* and for TB control in humans and animals reported by (Orcau *et al.,* 2011).

The circulation TB in the sheep farm was identified by using ELISA to estimate sero prevalence. Generally humans are the maintenance hosts for *M. tuberculsois*. The sheep is considered to be the spill-over hosts for *M. bovis*, can maintain the organism only when its population density is high and is generally considered very rare in small ruminants (Tschopp *et al.,* 2011).

However, presence of MTB in sheep indicates a possible transmission of infection from human to animal. In this study out of 205 sheep 3 were sero-positives and indicates 1.5% sero-prevalence of TB was observed in study population. Lack of a robust animal TB surveillance system and vaccine use in animals aids in the transmission of TB between animals and from animals to human or *vice versa*. Thus there is an urgent and unmet need for implementation of animal TB control programs in developing countries through extensive surveillance (Arunmozhivarman K. *et al.,* 2018).

2.4 Pathogenesis and pathology of tuberculosis

The pathogenesis of TB, host immune response to tubercle bacilli, dissemination and combination of tuberculous lesions in the initial focus of infection and regional (i.e. draining) lymph nodes have been documented (Griffin and Buchan, 1994: Neill *et al.,* 1994; Dannenberg, 2001; Neill *et al.,* 2001; Smith, 2003; Gupta and Katoch, 2005;

Cassidy, 2006; de la Rua-Dom enech,2006b; Palmer and Waters, 2006; Pollock *et al.,* 2006; Thoen and Barletta, 2006).

TB is characterized by progressive development of granulomatous lesions or tubercles in affected tissues / organs (Blood and Radostits, 1989; McAdams *et al.,* 1995; Cassidy, 2006; Liebana *et al.,* 2008). Predominant findings of lesions in the retropharyngeal, submandibular and parotid lymph nodes also exist in a considerable proportion of animals, suggesting potential foci of excretion on the upper respiratory tract surface (Corner, 1994; Neill *et al.,* 1994).

Macroscopic lesions of tuberculosis in cattle are typically caseous, yellow and mineralized (Dungworth, 1993), and 95% of lesions are located in the lungs and lymph nodes of the head, thorax and abdomen (Corner, 1994). The lesions caused by *M. bovis* and *M. tuberculosis* in cattle are similar in appearance, but *M. tuberculosis* infection usually does not progress beyond the development of small granulomas in the pharyngeal, thoracic and mesenteric lymph nodes (Cousins, Huchzermeyer, Griffin, Bruckner, Van Rensburg & Kriek, 2004).

Microscopic lesions of bovine tuberculosis are typically characterized by the presence of tubercles with central caseation and calcification. In the early stages of infection, these lesions are presence of epitheloid and giant cells at the center of the tubercle, and, as the disease progress, they are surrounded by lymphocytes, plasma cells and monocytes, developing a peripheral fibroplasia and central caseous necrosis (Neill *et al.,* 1994).

2.5 Immune response against mycobacterial infections

There is an initial interaction between macrophages and mycobacteria after infection which defines subsequent events and the consequences of exposure to tubercle bacilli

(Pollock and Neill, 2002). Bacteria can be killed and eliminated from the host, lie dormant, lead to development of active tuberculosis, or reactivate from dormancy at some stage in the future (Welsh *et al.,* 2005).

Apparently, members of this genus may produce spores, as (Ghosh *et al.,* 2009) recently demonstrated with *Mycobacterium marinum*. However, the role of that characteristic on the development of the disease has not been elucidated.

It is well established that. *M. bovis* causes a delayed hypersensitivity type (DTH) reaction; T-cell recognition of *Mycobacterium* antigens may be the major immune response to tuberculosis (Alit *et al.,* 2003; Pollock *et al.,* 2005; Welsh *et al.,* 2005). The immune response against mycobacterial infections is dependent upon a complex interaction between *T. lymphocytes* and macrophages in the context of the granuloma (Liebana *et al.,* 2007).

CD8+ T cells (CD8 cells) have been shown to respond to mycobacterial antigens in humans, cattle, and mice. To determine the role of CD8 cells in bovine TB in vivo, two groups of calves were infected with the virulent *M. bovis* strain AP112/97. After infection, one group was injected with a CD8 cell-depleting monoclonal antibody (MAb), and the other group was injected with an isotype control MAb.

The initial response to an infection is mediated by components of the innate immunity that serves primarily to restrict the multiplication and dissemination of the pathogens, as well as to initiate the ensuing adaptive response. In addition to macrophages, *M. tuberculosis* also interacts with epithelial cells in the alveolar space of the lung and is able to invade and replicate in this cell type (Bermudez and Goodman, 1996; Garcia-Perez *et al.,* 2003). However, the role of alveolar epithelium in mycobacterial infections has not been fully elucidated. In addition to forming a physical barrier, alveolar epithelial cells can express adhesion molecules and release cytokines and chemokines, such as IL-8 and monocyte chemotactic protein-1, and thereby modulate the local immune response (Lin *et al.,* 1998). *M. tuberculosis* infection has also been shown to induce the expression of inducible nitric oxide 15 synthase (iNOS) mRNA by epithelial cells and the production of nitric oxide (NO) (Roy *et al.,* 2004), and, more recently, interferon (IFN)-γ (Sharma *et al.,* 2007). Through the presentation of mycobacterial antigens, and the expression of costimulatory molecules and cytokines, phagocytic cells play an important role in the initiation and direction of the adaptive immunity.

Macrophages are regarded as phagocytic cells that initially ingest *M. tuberculosis*. Thus, they provide an important cellular niche during infection. The macrophages are considered to be the main cellular host for mycobacteria, and their major role is the rapid killing of the invading organism through the release of toxic reactive oxygen and nitrogen intermediates, or killing by lysosomal enzymes following fusion with the bacterial phagosome. The receptors that have been implicated in the uptake of mycobacteria include, the mannose receptor (MR), that recognizes mannose residues on mycobacteria (Schlesinger, 1993; Schlesinger *et al.,* 1996), Fc receptors (FcRs) binding antibody-coated bacteria, CR1, CR3, and CR4, which bind complement factor C3-opsonized bacilli (Schlesinger *et al.,* 1990; Hirsch *et al.,* 1994; Aderem and Underhill, 1999), surfactant receptors (Downing *et al.,* 1995), and scavenger receptors (Zimmerli *et al.,* 1996). Upon infection, macrophages have been shown to secrete proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1, and IL-6, which are believed to be important for the recruitment of cells to the site of infection (Giacomini *et al.,* 2001). Furthermore, the secretion of TNF-α may also aid in the activation of macrophages to produce reactive oxygen and nitrogen intermediates, and help granuloma formation (Flynn *et al.,* 1995; Roach *et al.,* 2002). The mechanisms by which NO and other RNIs may affect antimicrobial activity, could be through the modification of bacterial DNA, proteins and lipids (reviewed in Chan *et al.,* 2001). NO can deaminate, as well as directly damage bacterial DNA, and has been demonstrated to induce apoptosis. RNIs also have the potential to disrupt signalling pathways.

It has been suggested that neutrophils participate in the host defense against mycobacterial infections since circulating neutrophils become activated and are recruited to the lungs early in infection. They can be found at the infection nidus at the onset of infection, as well as several days after the initial reponse (Pedrosa *et al.,* 2000; Fulton *et al.,* 2002). In vivo depletion of neutrophils prior to mycobacterial infection enhances bacterial growth in the lungs of infected mice, whereas local treatment with the neutrophil chemo-attractant macrophage-inflammatory protein-2 enhances neutrophil recruitment and decreases mycobacterial growth (Appelberg *et al.,* 1995; Fulton *et al.,* 2002). The mechanisms by which neutrophils exert their anti-mycobacterial function are not completely resolved, although several hypotheses have been proposed. These include the secretion of chemokines (Riedel and Kaufmann, 1997; Seiler *et al.,* 2003), the induction of granuloma formation (Seiler *et al.,* 2003), and macrophage uptake of neutrophil-specific molecules such as myeloperoxidase (Hanker and Giammara, 1983), and lactoferrin (Silva *et al.,* 1989). Another mechanism whereby neutrophils indirectly contribute to the killing of mycobacteria was recently demonstrated by Tan *et al.* Mycobacteria-infected macrophages acquire the contents of neutrophil granules and their anti-microbial molecules by the uptake of apoptotic neutrophil debris, which is trafficked to endosomes and colocalize with the intracellular bacteria (Tan *et al.,* 2006).

Neutrophils are clearly important in the early immunity to bacterial infections. They respond rapidly to chemotactic stimuli released by the bacteria or inflamed epithelium and, thus, arrive early at the site of infection. Besides their anti-microbial role, neutrophils have been implicated in the modulation of the adaptive immune response by the release of chemo-attractants, which recruit other immune cells, such as T cells, monocytes, macrophages and DCs to the site of infection (Kasama *et al.,* 1993; Yang *et al.,* 2000; Scapini *et al.,* 2001). It was recently demonstrated that neutrophils and DCs interact physically through DC-SIGN expressed on DCs and Mac-1 expressed on neutrophils (van Gisbergen *et al.,* 2005; Megiovanni *et al.,* 2006). This interaction enables neutrophils to induce maturation of DCs via TNF-α secretion and a preferential production of IL-12 by the matured DCs (van Gisbergen *et al.,* 2005), which in turn results in an enhanced activation of T cells (Megiovanni *et al.,* 2006).

Besides phagocytosis, the recognition of *M. tuberculosis* or mycobacterial products is also crucial for an effective host response. Central to the immune defense against microbial pathogens are pattern recognition receptors, such as the toll-like receptors (TLRs). There are 13 members of the TLR family known today, of which TLR1-10 are found in humans (Ulevitch, 2004). Besides microbial products, TLRs also recognize endogenous ligands, such as heat shock proteins (Ohashi *et al.,* 2000; Vabulas *et al.,* 2002), extracellular matrix breakdown products (Termeer *et al.,* 2002; Guillot *et al.,* 2002), and intracellular contents from necrotic cells (Gallucci *et al.,* 1999; Li *et al.,* 2001). Ligation of TLRs initiates a signal transduction pathway that culminates in the activation of NF-κB and induction of several immuno-related genes, including cytokines and chemokines (Hoffmann *et al.,* 1999; Aderem and Ulevitch, 2000). TLR activation is therefore an important link between innate cellular responses and the subsequent activation of adaptive immune response to microbial pathogens. DCs express the broadest repertoire of TLRs through which they can recognize a plethora of microbial compounds. Upon TLR triggering, immature DCs, apart from cytokine secretion, undergo the process of maturation, resulting in an augmented expression of T-cell costimulatory molecules, such as CD80 and CD86, along with antigen-presentation molecules, such as MHC class II (Tsuji *et al.,* 2000; Hertz *et al.,* 2001; Michelsen *et al.,* 2001).

The initial interaction in the lungs is with alveolar macrophages, but after this first encounter DCs and monocyte-derived macrophages recruited to the site of infection, also takes part in the phagocytic process (Henderson *et al.,* 1997; Thurnher *et al.,* 1997). Infected DCs mature and migrate to draining lymph nodes to prime naïve T cells via processed antigens. Inflammation in the lungs provides the signals that direct the effector T lymphocytes back to the site of infection where granulomas are formed. The anatomic affinity of these cells appears to be mainly determined by site-specific integrins, "homing receptors", on their surface and complementary mucosal tissue-specific receptors, addressing", on vascular endothelial cells (Kunkel and Butcher, 2003). In addition, chemokines produced in the local microenvironment promote chemotaxis toward mucosal tissues and regulate integrin expression on mucosal lymphocytes, thereby controlling cell migration (Champbell *et al.,* 2003).

2.6 Clinical findings of tuberculosis

TB In cattle is a chronic and wasting (weight loss) disease and other non-specific clinical signs include anorexia, drop in production (eg: drop in milk yield), chronic intermittent cough (may be productive), dyspnoea and enlarged regional lymph nodes in advanced cases which may rupture (Blood and Radostits, 1989; OIE, 2009).

Lesions in cattle are most often found in organs rich in reticuloendothelial tissue, particularly the lungs and associated lymph nodes (Carter *et al.,* 1993). Lesions are most commonly present in the lower respiratory tract, however the upper respiratory tract and its associated tissues also displays disease in many cases.

Bovine tuberculosis (BTB) is a disease characterized by formation of granulomatous nodules called tubercles whose locations depend largely on the route of infection. In calves, the necropsy procedure performed after slaughtering of animals allows for the detection of gross lesions suggestive of BTB, even in apparently healthy cattle. Veterinary inspection is established as a routine procedure in most of the slaughterhouses in developing countries. The detection of infected animals is mainly restricted to the routine slaughterhouse inspection (Corner *et al.,* 1994).

2.7 Diagnosis of tuberculosis

The diagnosis of tuberculosis in the living animal may be based on clinical findings, the tuberculin test (TB screening test), histopathological examination and demonstration of the organisms in exudates or excretions (Jones *et al.,* 1997). There are different methods used for the diagnosis of tuberculosis in animals and man (Raval *et al.,* 2006).

Tuberculosis was diagnosed in three flocks of sheep in Galicia, Spain, in 2009 and 2010. Two flocks were infected with *Mycobacterium bovis* and one flock was infected with *Mycobacterium caprae*. Infection was confirmed by the comparative intradermal tuberculin test, bacteriology, molecular analysis and histopathology. Sheep have the potential to act as a reservoir for tuberculosis noted by (Prodinger *et al.,* 2014).

Cross-sectional study *Caprine* TB was conducted on 1990 randomly selected male goats that were slaughtered at Luna Export Abattoir of central Ethiopia. Postmortem examination, mycobacterial culturing and molecular typing techniques like genus typing, deletion typing and spoligotyping were used. The overall prevalence of *caprine* TB-like lesions was 3.5%. The lesion prevalence increased significantly with increasing age. Mycobacteria were found by culture and seen as acid fast bacilli in 12% of the goats with TB-like lesions. Characterization of the eight isolates using multiplex polymerase chain reaction (PCR) indicated that five of them belonged to the genus *Mycobacterium*. Four of the latter were confirmed to be members of the *M. tuberculosis* complex. Further characterization of the three *M. tuberculosis* isolates by spoligotyping identified them as type SIT53 and two new spoligotypes by (Deresa *et al.,* 2013).

Young goats were inoculated intratracheally with a low dose of *Mycobacterium bovis* to determine if they develop lesions similar to those seen in the natural disease in cattle. After 3 months, the challenge induced small lesions (<1 cm diameter) localized in the lungs and pulmonary lymph nodes, similar to those seen in the natural cattle disease. All of the *M. bovis*-inoculated young goats showed strong cellular immune responses to bovine PPD. Results of the present study suggest that young goats can be used as animal models since a low dose challenge mimics the natural pathogenesis and pathology processes caused by *M. bovis* in cattle. γ 2002 Elsevier Science B.V. All rights reserved. Studied by (Ramirez *et al.,* 2003).

A tuberculosis (TB) outbreak caused by *Mycobacterium bovis* occurred in a mixed herd of three cattle and eighteen goats in Northern Italy in 2005. All the cattle were removed, as opposed to the co-existing goats, who remained in the farm and were not subsequently tested by the official intradermal tuberculin test. At the beginning of May 2006, a 7-day old calf was introduced into the herd from an officially TB-free (OTF) farm. On October 2006, tuberculous lesions were detected at the slaughterhouse in the same animal. The following epidemiological investigation on the herd highlighted a clinical suspicion of TB in one goat out of 35, and visible lesions were found at necropsy in the respiratory and intestinal tracts. Bacteriological culture and molecular tests confirmed the presence of *M. bovis* in both animals. Spoligotyping and Mycobacterial Interspersed Repetitive Units - Variable Number of Tandem Repeats (MIRU-VNTR) showed the same genomic profile of the previous breakdown occurred in 2005. Since this profile has never been described in Italy, these findings suggest the probable transmission of TB within the farm among cattle and goats. The remaining 34 goats were also tested by single intradermal cervical comparative tuberculin (SICCT) test, Interferon (IFN)-γ assay and ELISA for antibody to *M. bovis*. The SICCT test and the IFN-γ showed a good concordance with 20 and 19 positive reactors, respectively. By ELISA we found 12 Ab-positive animals, seven of which had not been detected by the tests for cell-mediated immunity. Finally, 15 goats were found positive for gross lesions at necropsy. The in vivo tests revealed a total of 27 positive animals out of 35, which highlights the usefulness of the serology in parallel with SICCT and IFN-γ when an advanced stage of infection is suspected. Moreover, our results confirm the necessity for adopting the official tuberculin test on goats co-existing with cattle reported by Zanardi *et al.* (2013).

2.8 PPD skin test

A cross sectional study was conducted on 2231 small ruminants in four districts of the Afar Pastoral Region of Ethiopia to investigate the epidemiology of tuberculosis in goats and sheep using comparative intradermal tuberculin skin test, postmortem examination, mycobacteriological culture and molecular typing methods. Mycobacteriological culture and molecular characterization of isolates from tissue lesions of tuberculin reactor goats resulted in isolation of *Mycobacterium tuberculosis* (SIT149) and non-tuberculosis mycobacteria as causative agents of tuberculosis and tuberculosis-like diseases in goats, respectively. The isolation of *Mycobacterium tuberculosis* in goat suggests a potential transmission of the causative agent from human and warrants further investigation in the role of small ruminants in epidemiology of human tuberculosis in the region. Studied Kassa *et al.* (2012).

Detection of infected animals is a key step in eradication programs of tuberculosis. *Paratuberculosis* infection has been demonstrated to compromise the specificity of the diagnostic tests. However, its effect on their sensitivity has not been clarified. In the present study, skin tests and the interferon-gamma (IFN-γ) assay were evaluated in a goat flock (n = 177) with a mixed tuberculosis-*paratuberculosis* infection in order to assess the possible effect of *paratuberculosis* on their sensitivity. Culture of mycobacteria was performed as the gold standard to determine the true infection status. All techniques showed lower sensitivities than previously described; the single intradermal tuberculin (SIT) test and the IFN-γ assay detected 71% (62.4-78.6, 95% C.I.) of the infected animals; the single intradermal cervical comparative tuberculin (SICCT) test detected only 42.7% (34.1-51.7, 95% C.I.) of infected animals. The highest level of sensitivity was obtained when SIT test and IFN-γ assay were combined in parallel (90.8%, 84.5-95.2, 95% C.I.). Sensitivities of the tests were also assessed by comparing animals suffering tuberculosis and animals with a mixed infection; tests were found to be more effective in the former group. *Paratuberculosis* seems to have a major effect in the sensitivity of the diagnostic tests under study, and therefore must be taken into account; in particular, the use of the SICCT test should be questioned when both tuberculosis and *paratuberculosis* are present. γ 2007 Elsevier B.V. All rights reserved mentioned by Alvarez *et al.* (2008).

The effect of an inactivated *paratuberculosis* vaccine on the diagnosis of tuberculosis (TB) in goats was investigated in a herd with a history of clinical *paratuberculosis* but which was free of TB. Cohorts of animals in 2006, 2008 and 2009, were vaccinated once at 1 month of age, and 50% of the 2006 cohort served as unvaccinated controls. The goats were aged 8 months, 20 months and 3.5 years old at the time of the survey. All animals were assessed using a single intradermal injection of bovine tuberculin purified protein derivative (PPD) (SID test), or using both bovine and avian PPD (CID test). An interferon (IFN)-γ assay using both bovine and avian PPD was carried out on the 2006 cohort and was interpreted according to three different 'cut-off' points. No unvaccinated (control) animals tested positive to any of the assays, confirming that the herd was TB-free. The SID test had a low specificity in vaccinated animals at 8 and 20 months of age, whereas the CID test demonstrated 100% specificity in animals ≥20 months-old. The specificity of IFN-γ assay was less than maximal for vaccinated animals 3.5 years old as small numbers of false positives were detected, although this depended on the chosen cut-off point. The study findings demonstrate that the use of an inactivated *paratuberculosis* vaccine in goats <1 month-old in a TB-free herd does not result in false positives to a CID test for TB when performed in animals ≥20 months-old by Chartier *et al.* (2011).

On this study found that the number of infections detected by the gamma-interferon test was considerably greater than the number detected by the single intradermal tuberculin test. A group of 10 animals was negative to both tests in two consecutive rounds and three kids were negative in the last round of testing. Gamma-interferon assay is appropriate for diagnosis and eradication of tuberculosis in goats. This test is able to detect early *Mycobacterium bovis* infection. Avian reactors with simultaneous increased reaction to bovine PPD in the gamma-interferon assay (designated as avian reactors) should be considered test positive for *M. bovis*. By serial testing with the gamma-interferon and the single intradermal tuberculin tests, and a policy of segregation of kids at birth, it is possible to achieve a group of animals test negative for tuberculosis from a herd of goats with high immunoreactivity to this infection, reported by Liébana *et al.* (1998).

2.9 Zoonotic finding

Some authors suggest that small ruminants act only as amplifier hosts and cannot maintain the disease in a herd (Coleman and Cooke, 2001). Bovine tuberculosis (BTB) in small ruminant would become a problem only when they are in close contact with cattle with high disease prevalence (Malone 2003). However, reports show that they are susceptible to BTB, that the respiratory tract is often affected and that therefore they remain a potential source of infection for other animals or humans. In Ethiopia, 83% of the farmers kept their sheep and goat inside their house at night and 5% drank regularly goat milk (Tschopp *et al*., 2011). This highlights the potential of zoonotic transmission. Tuberculous lymphadenitis (TBLN) in humans was shown to be a major problem in Meskan Woreda, south Ethiopia and was reported in 40% of all diagnosed tuberculosis patients in Butajira hospital (Yassin *et al*., 2003). Of all farmer families whose livestock were tested, 9% were diagnosed with lymphadenitis in the last five years, suggesting a possible animal source of infection through consumption of infected raw animal product. TBLN can be caused by ingestion of raw animal products containing *M. bovis* (Cosivi *et al*., 1998).

However, no *M. bovis* was isolated from the TBLN patients (Tschopp *et al*., 2011).

In Germany, *M. bovis* subsp. *caprae* has been described as the causative agent of almost one-third (31 %) of the human *M. bovis* -associated TB cases analyzed. This proportion was surprisingly high, especially when compared with the prevalence of *M. bovis* subsp *caprae* strains in human or animal isolates in other countries. Thus, it might be assumed that *M. bovis subsp. caprae* represents a newly emerging genotype in Germany and is now spreading to other European countries. Outside Germany, small numbers of *M. bovis* subsp. *caprae* strains have been identified only in Spain (Gutierrez *et al*., 1997), and in Austria (Prodinger *et al*., 2002). In Croatia, A 13-year-old boy clinically presented enlargement of cervical lymph node with consecutive isolation of *M. caprae* (Cvetnic *et al*., 2007).

Mycobacterium caprae, a member of the *Mycobacterium tuberculosis* complex, causes tuberculosis (TB) in man and animals. Some features distinguish *M. caprae* from its epidemiological twin, *Mycobacterium bovis*: *M. caprae* is evolutionarily older, accounts for a smaller burden of zoonotic TB and is not globally distributed, but primarily restricted to European countries. *M. caprae* occurs only in a low proportion of human TB cases and this proportion may even decrease, if progress toward eradication of animal TB in Europe continues. So why bother, if *M. caprae* is not an enigma for diagnostic TB tests and if resistance against first-line drugs is a rarity with *M. caprae*? This 'European' pathogen of zoonotic TB asks interesting questions regarding the definition of a species. The latter, seemingly only an academic question, particularly requires and challenges the collaboration between human and veterinary medicine reported by (Prodinger *et al.,* 2014).

Reverse zoonosis is a disease of human that are transferred to other animals such as goat. In the study conducted on small ruminants in Afar Pastoral Region, *Mycobacterium tuberculosis* strain SIT149 was isolated from a goat suggesting the possibility of its transmission from human to goat (Mamo *et al*., 2012). The SIT149 strain of *Mycobacterium tuberculosis* is a dominant strain in Ethiopia (Brudey *et al*., 2006), and it was a common isolate in human pulmonary TB patients from the same Afar Pastoral Region indicating that the isolate has been circulating in the area.

Afar pastoralists have close contact with goats and sheep and often keep young goats and sheep in their house at night which might be a potential factor for transmission from human patient to animals (Mamo *et al*., 2012).

Similarly other study conducted by Benti *et al.,* 2013, isolated three *M. tuberculosis* strains from goat at Luna Export Abattoir of central Ethiopia which was unexpected as a previous study has reported that the causative agent of caprine TB in some European countries was *M. bovis* (O'Reilly and Daborn, 1995; Prodinger *et al*., 2005) and *M. caprae* in several European countries (Ayele *et al*., 2004). Even recently, outbreaks of TB in goats in the United Kingdom, Italy and Portugal were reported to be caused by *M. bovis* (Daniel *et al*., 2009; Quintas *et al.,* 2010; Tafess *et al*., 2011). However, *M. tuberculosis* has previously been isolated from goats in Nigeria (Cadamus *et al*., 2009). The isolation of *M. tuberculosis* from goats in this study is likely to be due to transmission of the bacterium from TB infected people to goats. Transmission of *M. tuberculosis* from man to cattle has been reported from Slovenia (Ocepek *et al*., 2005) and in Ethiopia; cattle owned by farmers with active TB were four times more likely to have TB than cattle owned by those farmers without active TB (Regassa *et al*., 2008). Furthermore, study done on small ruminants of central Ethiopia reported isolation of *Mycobacterium tuberculosis* from goat (Tschopp *et al.,* 2011).

According to this study covered 110 isolates (89 *M. bovis* and 21 *M. caprae*) that accounted for respectively 1.9% and 0.3% of the *M. tuberculosis* complex isolates available at the NRL. Data on risk of exposure to *M. bovis* or to *M. caprae* were available in 82 (74%) of the 110 cases, was studied by (Rodríguez *et al.,* 2007).

The agent of bovine tuberculosis, *Mycobacterium bovis*, is a zoonosis which can be transmitted to human beings. In France, the prevalence of tuberculosis due to *M. bovis* has drastically decreased, both for animals and humans, since public health measures were introduced to prevent its transmission. However, a new outbreak of the disease is noted among cattle in several French areas and more particularly in Aquitaine. In 2008, 40% of bovine tuberculosis French cases provided from Aquitaine. From November 2004 to October 2008, 15 cases were registered at Bordeaux's academic hospital (CHU). Thirteen of them were due to *M. bovis* and two to *Mycobacterium caprae*. It represents 2.9% of tuberculosis due to tuberculosis complex. An analysis of the 15 patients' medical files showed that it occurred either to old people who reactivated a former infection, or to younger ones who were born in countries with a strong *M. bovis* endemic disease. Extra-pulmonary forms and especially ganglionics ones are the most frequent. *M. caprae* seems to be an emergent species among animal mycobacteries transmissible to human being. An epidemiological monitoring seems to be necessary to establish a relation between the regional outbreak of bovine tuberculosis and human tuberculosis by (Aimé *et al.,* 2012).

2.10 Treatment and Control

In developed countries, TB has been localized in more vulnerable populations, such as immigrants and persons with social contention. There is an increase of extra-pulmonary presentation in this context, related to non-European ethnicity, HIV infection, and younger age. In Spain, the increasing immigrant population has presented a need to improve coordination between territories and strengthen surveillance. The global control plan is based on the DOTS strategy, although the objectives and activities were redefined in 2006 to incorporate the measurement of global development, and community and healthcare strengthening. Adequate control measures in a more local context and continual activity evaluation are necessary to decrease the burden of suffering and economic loss that causes this ancient disease.

Most countries carrying out campaigns of bovine tuberculosis (TB) eradication impose a ban on the use of mycobacterial vaccines in cattle. However, vaccination against *paratuberculosis* (PTB) in goats is often allowed even when its effect on TB diagnosis has not been fully evaluated. To address this issue, goat kids previously vaccinated against PTB were experimentally infected with TB. Evaluation of interferon-γ (IFN-γ) secretion induced by avian and bovine tuberculins (PPD) showed a predominant avian PPD-biased response in the vaccinated group from week 4 post-vaccination onward. Although 60% of the animals were bovine reactors at week 14, avian PPD-biased responses returned at week 16. After challenge with *M. caprae*, the IFN-γ responses radically changed to show predominant bovine PPD-biased responses from week 18 onward. In addition, cross-reactions with bovine PPD that had been observed in the vaccinated group at week 14 were reduced when using the *M. tuberculosis* complex-specific antigens ESAT-6/CFP-10 and Rv3615c as new DIVA (differentiation of infected and vaccinated animals) reagents, which further maintained sensitivity post-challenge. Ninety percent of the animals reacted positively to the tuberculin cervical comparative intradermal test performed at 12 weeks post-infection. Furthermore, post-mortem analysis showed reductions in tuberculous lesions and bacterial burden in some vaccinated animals, particularly expressed in terms of the degree of extra-pulmonary dissemination of TB infection. Our results suggest a degree of interference of PTB vaccination with current TB diagnostics that can be fully mitigated when using new DIVA reagents. A partial protective effect associated with vaccination was also observed in some vaccinated animals (Pérez *et al.,* 2012).

Tuberculosis (TB) has affected humanity since the beginning of the recorded time and is associated with poverty, malnutrition, overcrowding, and immunosuppression. Since Koch discovered the infectious nature of the disease in 1882, knowledge about its history and physiopathology has advanced, but it continues to be a global public health problem. More than 9 million new cases occurred in 2008 worldwide (with an incidence of 139/100,000 inhabitants), of whom more than one million died. Over half million of the cases presented with multidrug resistant-TB. Africa represents the continent with the highest incidence and the most HIV co-infection. The situation in Eastern Europe is also worrisome because of the high incidence and frequency drug resistance.

On 1 May 2004, 10 new States joined the European Union, including Cyprus (CY), the Czech Republic (CR), Estonia (ES), Hungary (HU), Latvia (LA), Lithuania (LI), Malta (MA), Poland (PO), Slovakia (SK), and Slovenia (SN). Using OIE and published data, this paper summarizes the status of bovine and human tuberculosis in animals in these countries between 1996 and 2003. National control programmes against bovine tuberculosis in cattle have been successful: the current herd incidence of this disease in cattle is currently lower than 0.2%, so all countries meet the OIE requirements for freedom from the disease. Furthermore, two countries have already been officially declared bovine tuberculosis-free EU States: the CR on 31 March 2004 (European Commission Decision No. 2004/320/EC) and SK on 4 March 2005 (Commission Decision No. 2005/179/EC). The last outbreak of bovine tuberculosis was diagnosed in cattle in CY (1928), ES (1986), LA (1989), SK (1993), CR (1995), and MA (2001). However, several issues of concern remain including the potential existence of a wildlife reservoir, the presence of *Mycobacterium bovis*, *M. caprae*, and other members of the *M. tuberculosis* complex (particularly *M. tuberculosis* or *M. microti*) in imported domestic or wild animals, and the potential for delayed detection of bovine tuberculosis in those States where annual tuberculin testing is no longer performed on cattle older than 24 months. γ 2005 Elsevier B.V. All rights reserved this study conducted by (Pavlik, 2006).

Progressing development of tuberculosis (TB) vaccines largely depends on the availability of animal models to test their safety and efficacy before starting with expensive clinical trials. The present study provides a comprehensive evaluation of bacillus Calmette-Guerin (BCG) effects on clinical, immunological, pathological and bacteriological parameters in goats after an experimental challenge with *Mycobacterium caprae*. Vaccination of goats with BCG reduced the volume of lung gross lesions, the bacterial load in pulmonary lymph nodes and increased the weight gain when compared to unvaccinated animals. Differences in post-challenge IFN-γ responses to ESAT-6/CFP-10 were found to be a useful follow-up biomarker of disease progression and vaccine efficacy. Our results endorse this animal model for further TB vaccine trials reported by (Val *et al.,* 2014).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Area of the study

This study was design to focus on the investigation of sheep tuberculosis in Sadar and Parbatipur Upazila under Dinajpur District, the experiment plan was prepared in the Department of Pathology and Parasitology Faculty of Veterinary and Animal Sciences, Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Figure 1: Map of the Area study

3.2 Duration of the study

Duration of the study was January to June, 2018.

3.3 The major work of the present study

- Neck region injected the tuberculin test in selected sheep in the area of study.
- To investigate the prevalence of sheep tuberculosis based on risk factors related such as: Age**,** Sex, type of Breeds, lactation stage and production status.

3.4 Test material

- Tuberculin gun
- bPPD (bovine purified protein derivative)
- Calipers
- Mask
- Ice carrier with ice pack
- Cloves
- Cotton
- Providone iodine
- Scissors/blade
- Water
- Mark pen
- Gumboot and apron etc.

Figure 2: Tuberculin gum

Figure 3: PPD Tuberculin

Figure 4: Mammalian tuberculin PPD

Figure 5: Calipers

Figure 6: preparing the site of injection (Clipping and shaving)

3.5 Selection and grouping of animal

To investigate the prevalence of sheep tuberculosis infection in 140 sheep at Sadar and Parbatipur in Dinajpur district was selected using single interdermal tuberculin skin test. Considering the Age, sex, type of breed, lactation stage , body condition score and production status as risk factors.

3.6 Experimental layout

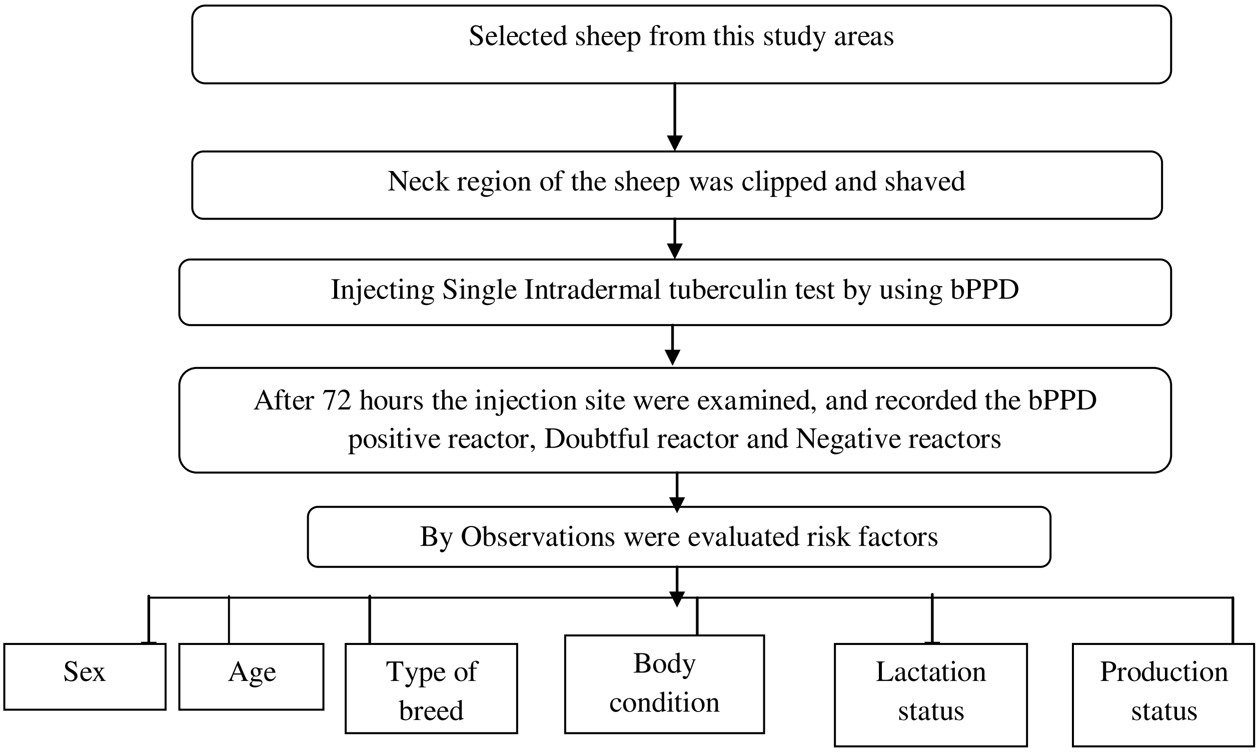


Figure 7: Experimental layout

3.7 Test procedure

The test used is the single intradermal injection of bovine tuberculin purified protein derivative (PPD), commonly known as the tuberculin skin test (TST), which is also used to screen cattle for TB.

Tuberculin is a mixture of proteins extracted from cultures of the bacterium that causes TB. The skin test involves injecting a small amount of 'bovine' tuberculin (derived from *M. bovis*) into the deep layer of the skin.

The skin is first clipped, shaved and fold thickness of skin were measured in millimeters using special calipers before injecting tuberculin in the middle of the neck region. After 24, 48 or 72 hours, measured the skin thickness at the site of the injections to determine if the animal is classified as a positive reactor, doubtful or negative reactor.

Table 1: Schedule and procedure of inoculation:

Test	Reagent	Site	Rout	Dose	Time of observation post incubation
Single intradermal tuberculin test	bPPD	Neck region (skin fold)	i/d	0.1 ml	24, 48, and 72 Hrs

3.8 Reading of the results of inoculations

The reading was taking 72 hours after the tuberculin injection. If an animal's immune system has previously been 'sensitized' (primed) by infection with *M. bovis* then the injection of tuberculin triggers an inflammatory response and a reaction (swelling or 'lump') develops at the injection site.

The swelling was either soft and edematous or somewhat hard in nature.
The results were interpreted according to OIE standards (OIE, 2009):
a. If the reaction is ≥ 4.0 mm greater than the test is considered positive reactor.
b. If the reaction is between 2.0 and < 4.0 mm than the test is considered doubtful.
c. If the reaction is < 2.0 mm than the test is considered negative.
The swelling was felt by palpation at the site of injection and also by observation.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 Tuberculosis in sheep

During the present study the prevalence of Sheep tuberculosis was investigated in Sheep population of Sadar and Parbutapur Upazila under Dinajpur District, for this purpose a total of 140 samples were investigated for sheep TB through TST (Tuberculin Skin Test). Breed of investigated sheep shown here, during tuberculin skin test (TST), body condition score (Bcs) on the basis of observation of anatomical parts such as vertebral column, ribs, and spines, the study animals were classified as poor (score 1 to 2), medium (3 to 4), or good (greater than 5) were examined. Clinical examination also carried out for dyspnea, nasal discharge, diarrhea and constipation were recorded but in most cases these signs were absent. The tuberculin skin test (TST) was conduct from January to June 2018, out of 140 sheep sample, seven were positive, twenty one were doubtful cases and hundred twelve cases were negative.

4.2 Tuberculin skin testing (TST)

The primary screening test for TB single intradermal tuberculin skin test (TST) with bovine PPD in the middle neck region which is used throughout the world in its various formats to screen cattle, buffalo, bison, goat, sheep and people for TB, it is the internationally accepted standard for detecting *M. bovis* infection in live animals, out of 140 sheep, 7 positive were found then examined average thickness day zero to third day and average skin thickness response of tuberculin skin test.

Table 2: Results of tuberculin skin test (TST) based on thickness of skin before and after injection

A	B	C	D	E	F
Sample No.	**Area**	**Day (0)(mm)**	**Day 3 (mm)**	**D-C (mm)**	**Result**
1	Sadar	1.53mm	7mm	5.47mm	Positive
2	Parbutapur	2.74mm	8mm	5.26mm	Positive
3	Parbutapur	2.07mm	7.87mm	5.8mm	Positive
4	Sadar	1.02mm	8mm	6.98mm	Positive
5	Parbutapur	2.02mm	7.85mm	5.83mm	Positive
6	Sadar	1mm	6.54mm	5.54mm	Positive
7	Parbutapur	2mm	9mm	7mm	Positive

C= Skin thickness before injection

D= Skin thickness after injection

E= Skin thickness response

4.3 Results of tuberculin skin testing based on using bPPD

In the present study, the overall sheep samples were categorized in two category i.e. numbers of sheep had previous history of TB and PPD skin test performed by using TST (tuberculin skin test) 100% there were not any cases, this study was first time performed in sheep population of Sadar and Parbatipur Upazila under Dinajpur District.

Table 3: Results of tuberculin skin testing based on using bPPD

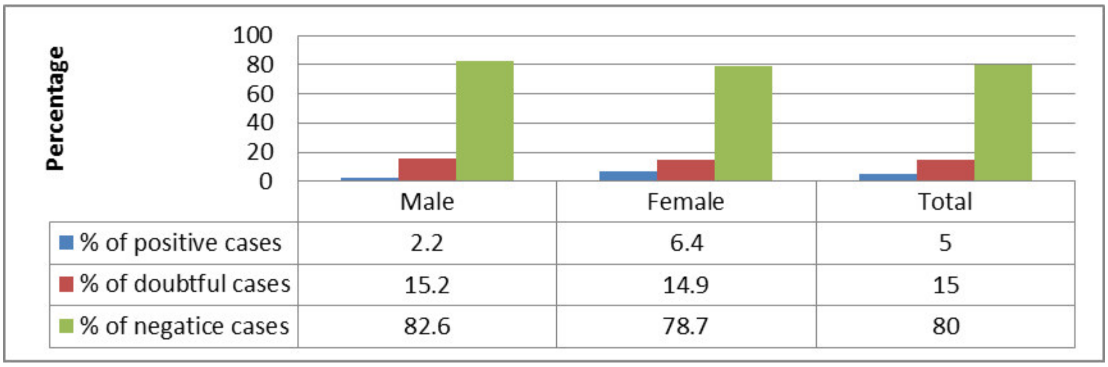
Farmers respondents	No. of TB In Previous History	Percentage (%)	PPD Skin Test Performed Previous History	Percentage (%)
YES	0	0	0	0
NO	140	100	100	100
Total	140	100	100	100

4.4 Results of tuberculin skin test based on sex

In the present study the highest number of positive sheep TB was found in female sheep that are equivalent to 6.4%, while doubtful cases were 14.9% and the negative cases were 78.7%. In terms of males the positive cases were 2.2%, the doubtful cases were 15.2% whereas the negative cases were 82.6%.

Table 4: Results of tuberculin skin test based on sex

Sex	No of observed	Positive Cases		Doubtful Cases		Negative Cases	
		No	%	No	%	No	%
Male	46	1	2.2	7	15.2	38	82.6
Female	94	6	6.4	14	14.9	74	78.7
Total	140	7	5%	21	15%	112	80%



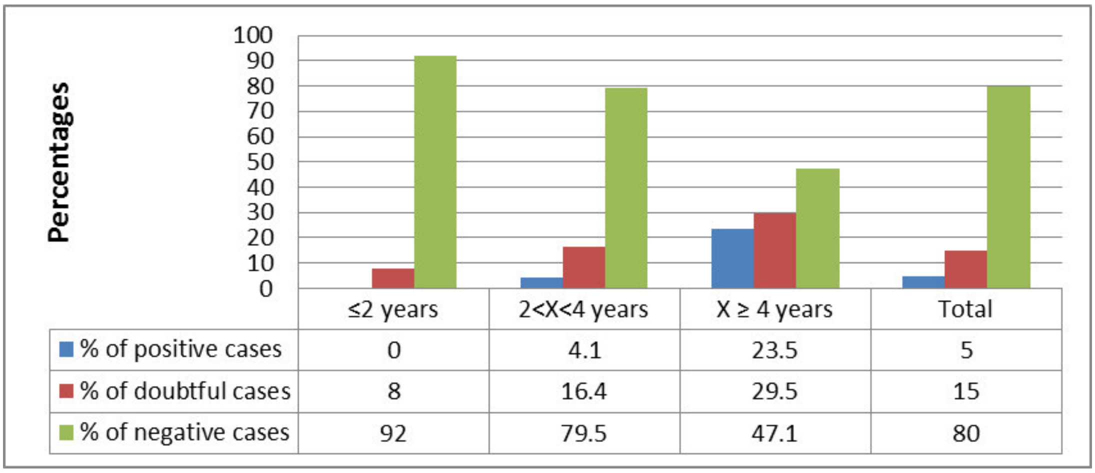
Graph 1: Results of tuberculin skin test based on sex

4.5 Results of tuberculin skin test based on age

All the sheep samples were categorized in three age groups i.e. ≤ 2 years, in between 2 to 4 years and of more than 4 years of age. The high prevalence was recorded to be 23.5% in animals of more than 4 years and 4.1% in animals between 2 to 4 years of age while no positive animal was detected in animals of less than 2 years of age, while highest doubtful cases were 29.5% in sheep of more than 4 years, secondly 16.4% in sheep between 2 to 4 years of age least in sheep less than 2 years of age was 8%, the negative cases were 92%, 79.5% and 47.1% in sheep less than 2 years, between 2 to 4 years of age and more than 4 years respectively.

Table 5: Results of tuberculin skin test based on age

Age	No of observed	Positive Cases		Doubtful Cases		Negative Cases	
		No	%	No	%	No	%
≤2 years	**50**	**0**	**0**	**4**	**8**	**46**	**92**
2<X<4 years	**73**	**3**	**4.1**	**12**	**16.4**	**58**	**79.5**
X ≥ 4 years	**17**	**4**	**23.5**	**5**	**29.5**	**8**	**47.1**
Total	140	7	5	21	15	112	80



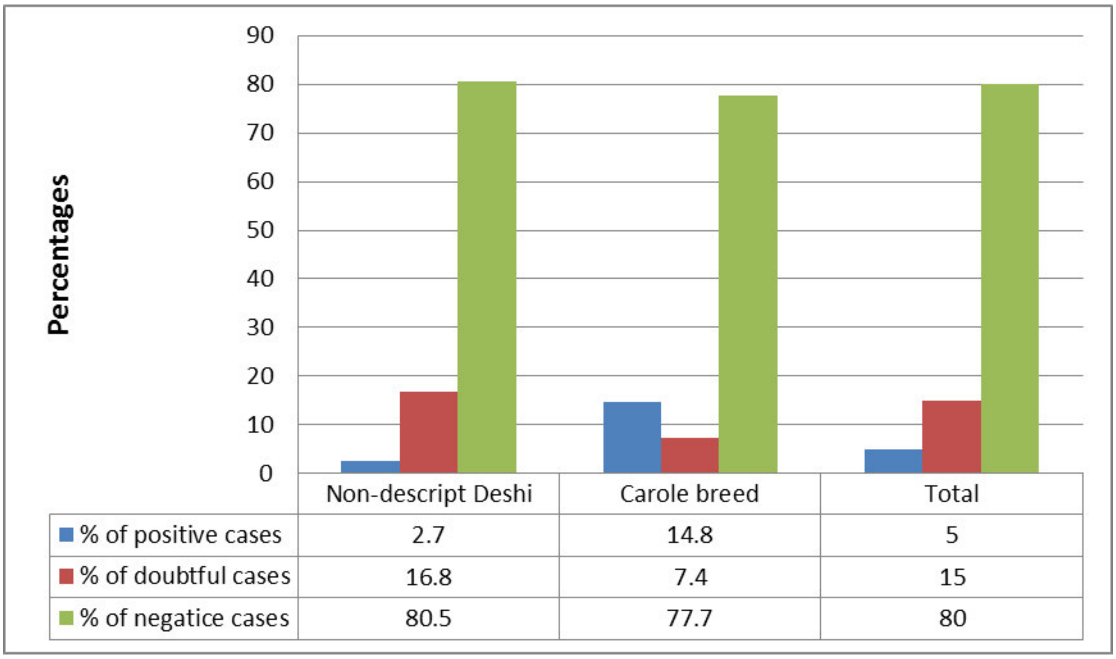
Graph 2: Results of tuberculin skin test based on age

4.6 Results of tuberculin skin test based on breed

According to this study the highest positive rate 14.8% was recorded in the breed of carole breeds followed by non-descript Deshi 2.7%, while doubtful and negative cases were 21%, 16.8%, 80.5%, and 77.7%. In non-descript Deshi breeds and Exotic breeds respectively.

Table 6: Results of tuberculin skin test based on breed

Type of Breed	**No of observed**	**Positive Cases**		**Doubtful Cases**		**Negative Cases**	
		No	**%**	**No**	**%**	No	%
Non-descript Deshi	113	3	2.7	19	16.8	91	80.5
Carole Sheep	27	4	14.8	2	7.4	21	77.7
Total	140	7	5	21	15	112	80



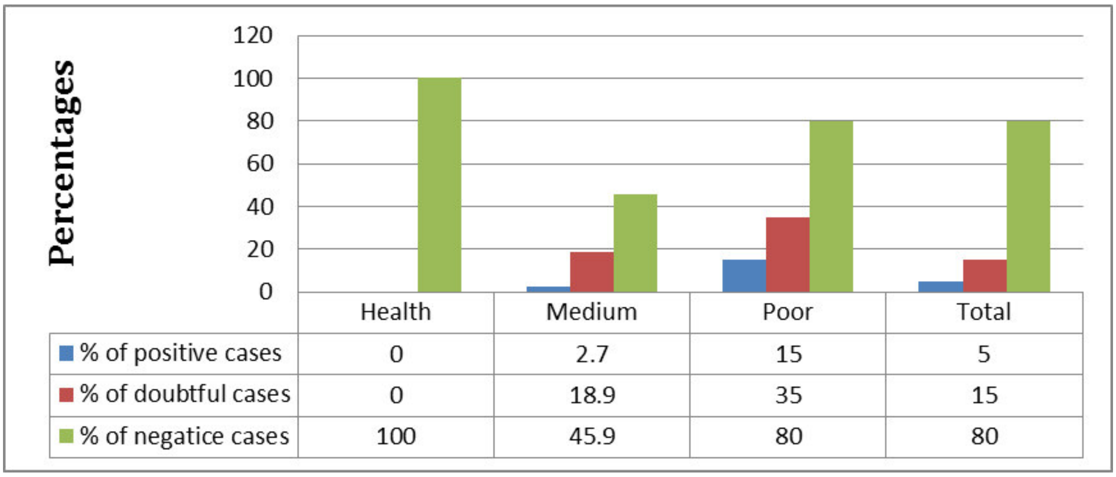
Graph 3: Results of tuberculin skin test based on breed

4.7 Results of tuberculin skin test based on body condition

All the samples were categorized in three groups i.e. good, medium and poor according to their body condition. Sheep of poor body condition showed high prevalence of sheep TB which was found to be 15% as compared to sheep of medium condition in which the disease was prevalent in 2.7 % of sheep. No positive reactor was recorded in sheep having good body condition status, while doubtful and negative cases were recorded 32%, 18.9%, 0, 80%, 45.9% and 100% respectively in poor, medium and good body condition.

Table 7: Results of tuberculin skin test based on body condition

Body Condition	No of observed	Positive Cases		Doubtful Cases		Negative Cases	
		No	%	No	%	No	%
Good	63	0	0	0	0	63	100
Medium	37	1	2.7	7	18.9	17	45.9
Poor	40	6	15	14	35	32	80
Total	140	7	5	21	15	112	80



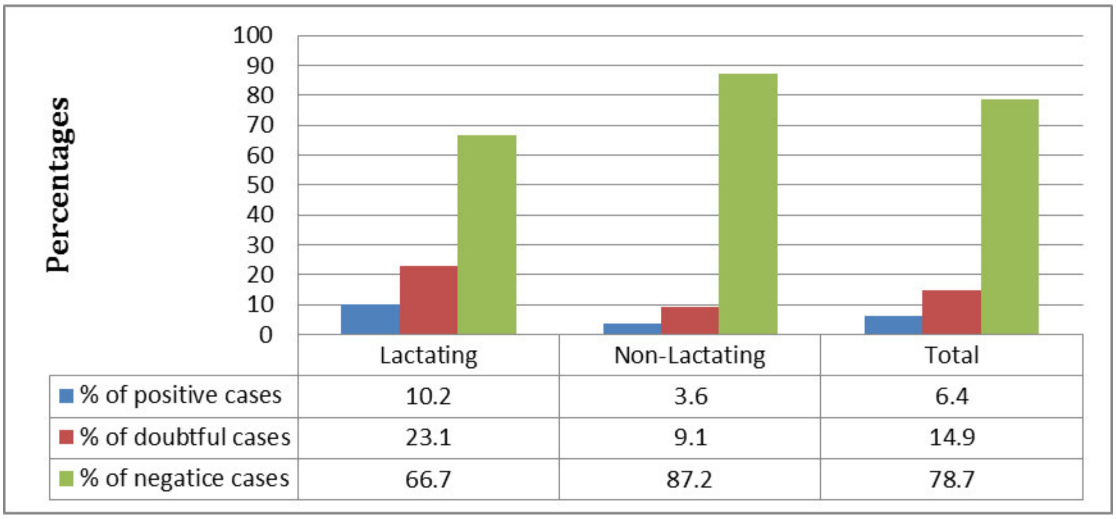
Graph 4: Results of tuberculin skin test based on body condition

4.8 Results of tuberculin skin test based on lactating status

Among 140 sheep, 94 were females in which 39 were lactating while 55 were dry (non-lactating). Positive cases of sheep TB was found in 10.2% in lactating females which was higher than in non-lactating females in which the prevalence was recorded in 3.6% females, while highest doubtful cases were 23.1% in lactating females, was higher than in non-lactating females 9.1%, the negative cases were 87.2% in non-lactating females, was higher than in lactating females 66.7%.

Table 8: Results of tuberculin skin test based on lactating status

Lactation Status	No of observed	Positive Cases		Doubtful Cases		Negative Cases	
		No	%	No	%	No	%
Lactating	39	4	10.2	9	23.1	26	66.7
Non Lactating	55	2	3.6	5	9.1	48	87.2
Total	94	6	6.4	14	14.9	74	78.7



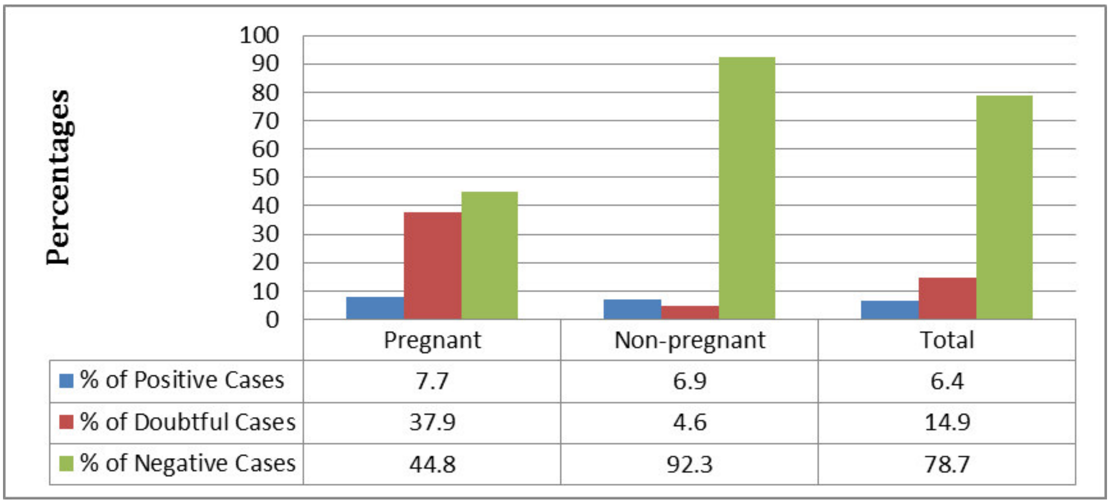
Graph 5: Results of tuberculin skin test based on lactating status

4.9 Results of tuberculin skin test based on production status

Among 94 samples of ewes in which 29 were pregnant, while 65 were Non- pregnant. positive cases of sheep TB was found in 7.7% in Pregnant ewes which was higher than in Non- pregnant females in which the positive was recorded in 6.9% females, while highest doubtful cases were 37.9% in pregnant ewes, was higher than non-pregnant females 4.6%, the negative cases were 92.3% in non-pregnant ewes was higher than in pregnant ewes 44.8%.

Table 9: Results of tuberculin skin test based on production status

Production status	No of observed	Positive Cases		Doubtful Cases		Negative Cases	
		No.	%	No.	%	No	%
Pregnant	29	5	7.7	11	37.9	13	44.8
Non-pregnant	65	2	6.9	3	4.6	60	92.3
Total	94	6	6.4	14	14.9	74	78.7



Graph 6: Results of tuberculin skin test based on production status

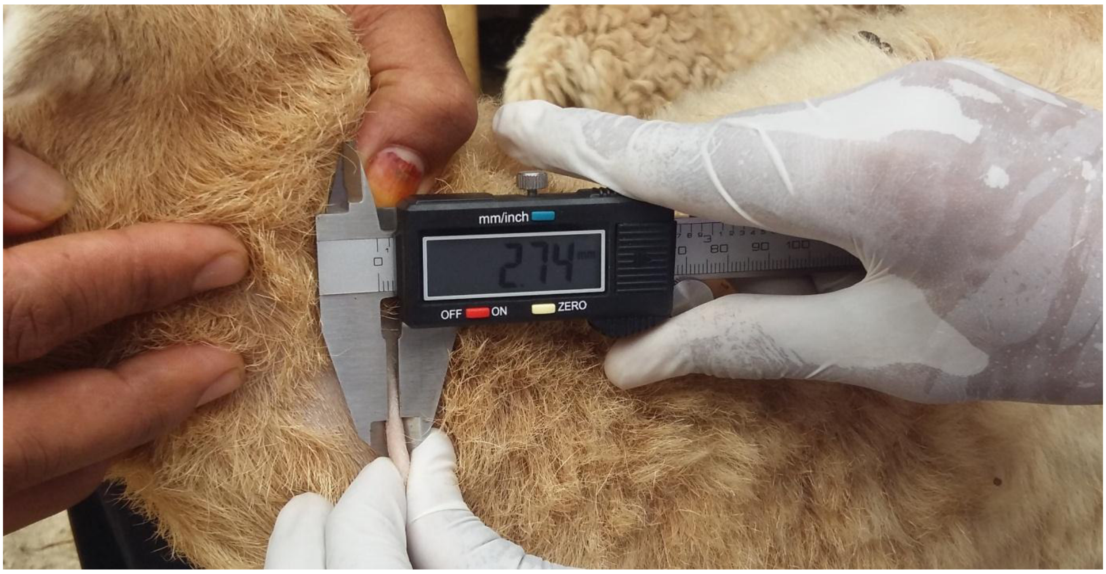


Figure 8: Measuring of skin thickness before injection the tuberculin skin test

Figure 9: Injection for bPPD skin test on the sheep neck region

Figure 10: Picture of sheep shown positive reaction swelling after 72 hours

Figure 11: Measuring of skin thickness response

CHAPTER V

DISCUSSIONS

CHAPTER V

DISCUSSIONS

Tuberculosis (TB) in sheep, caused by members of the mycobacterium tuberculosis complex (MTC), has been reported in New Zealand, Sudan, Italy, Ireland and the United Kingdom (Cordes *et al.,* 1981). However the current study was performed in Sadar and Parbatipur Upazila under Dinajpur District. In this study, total 140 sheep were tested with bovine purified protein derivative using tuberculin gun, the test were performed in the mid-neck region (OIE, 2009), out of 140, seven positive, 21 doubtful and 112 were negative. the current study presented that the overall prevalence rate of Mycobacterium tuberculosis complex (MTC) *Sub-species bovis* was (5%), compared with previous studies Rahman *et al.* (2013) also undertook a study to investigate the occurrence of bovine and avian tuberculosis in sheep and goat population in Bangladesh. They found that the percentage of responders to CFT test in sheep and goat were 9.15% and 1.29% respectively. And the overall percentages of reactors to CCT test in sheep and goats were 1.46% and 1.29% respectively, In CCT testing within 10 days of the initial inoculation, 1.46% cases were proved as reactor in sheep and 1.29% reactor in goats so in case of sheep the CFT test gives 7.95% false reaction. These results were found in accordance with the findings of Grooms and Moles worth, 2000 who stated that the response to CFT test may be caused by infection with *Mycobacterium bovis*, exposure to or infection with other closely related bacteria, such as *Mycobacterium avium* (avian tuberculosis) and *Mycobacterium paratuberculosis* (Johne's disease). This is referred to as a false-positive test. It is expected that false-positive results on the CFT test will occur in a normal population approximately 5 percent of the time. In herds that have increased exposure to *Mycobacterium avium* (avian tuberculosis) or *Mycobacterium paratuberculosis*, the response rate may be greater than 5 percent (Grooms and Molesworth, 2000). Ashraf *et al.,* 1986 reported that the prevalence of tuberculosis in sheep was 2% in Pakistan. The overall percentage of reactor animals to SCCIT at farms in sheep and goats were 0.9% and 2.4% respectively (Javed *et al.,* 2010).

In the present study, to know the history of sheep tuberculosis in the area we were asking the owners of the sheep if they were seen any cases of TB in the sheep before based on this information the sheep samples were divided in two groups i.e. Numbers of Sheep had Previous History of TB and second group had PPD Skin Test Performed by Using TST (tuberculin skin test) 100% as demonstrated in table (3), were not any cases, because this is first time performed in Sheep population of Sadar and Parbatipur Upazila under Dinajpur District, so there was no prevalence of tuberculosis in sheep in the area except M. Mashiur R. *et al.* (2013) conducted bovine and avian tuberculosis which may cause dangerous effects on human health as well as livestock of Bangladesh, also Gezahegne M. K**,** *et al.* (2012) made a cross sectional study was conducted on 2231 small ruminants in four districts of the Afar Pastoral Region of Ethiopia to investigate the epidemiology of tuberculosis in goats and sheep using comparative intradermal tuberculin skin test, postmortem examination, mycobacteriological culture and molecular typing methods.

In this study the highest number of positive sheep TB was found in female sheep as demonstrated in (table 4), that are equivalent to 6.4%, while Doubtful cases were 14.9% and the negative was 78.7%. In terms of males the positive rate was 2.2%, the doubtful cases were 15.2% whereas the negative case was 82.6%. Such type of study in sheep has not undertaken as yet in this area so we correlates with Prevalence was also recorded higher (4.51%) in female animals than in male animals (2.94%). Salgado *et al.* (2009) also found higher prevalence in females (5.9%) than males (3.1%). Similar results were recorded by Phaniraja *et al.* (2010), Nwanta *et al.* (2011), Arshad *et al.* (2012) and Trangadia *et al.* (2013) also (Eckert and Deplazes, 2003) reported that female animals had higher infection were found to be mostly infected which may be due to some physiologic conditions such as pregnancy, lactation etc. that rendered the animal vulnerable to disease conditions.

According to Age the high prevalence was recorded to be 23.5% in animals of more than 4 years and 4.1% in between 2 and 4 years of age while no positive animal was detected in animals of less than 2 years of age as demonstrated in (table 5), Salgado *et al.* (2009) also found TB infection most commonly among adult and female animals, Our results are also in agreement with the findings of Khan *et al.* (2007), Phaniraja *et al.* (2010), Sharma *et al.* (2011) and Trangadia *et al.* (2013) who also recorded higher prevalence of the TB in old animals as compared to young animals. Gezahegne M K *et al.* (2012) reported older goat and sheep have higher proportion of positivity in tuberculin test results which might be related to the fact that older animals have longer duration and repeated chance of exposure to mycobacterial infection with their age. Similar results have been reported by other researchers in cattle.

According to this study the high positive rate 14.8% was recorded in the breed of carole breeds followed by non-descript Deshi 2.7% as demonstrated in table (6), this study we correlate with Rahman *et al.,* 2013 make same pattern in Jamunapari (Exotic breeds) of goat had 3.5 times higher percentage of reactors as compared to Black Bengal breed (Indigenous breeds) (0.83%) because of lack of previous study in sheep breeds.

All the samples were categorized in three groups i.e. Good, medium and poor according to their body condition score. Sheep of poor body condition showed high prevalence of sheep TB which was found to be 15% as compared to sheep of medium condition score in which the disease was 2.7 % of the sheep. No positive reactor was recorded in sheep having good body condition status as demonstrated in table (7), Arshad *et al.* (2012) also found 2.8 times higher prevalence in animals with poor physical condition than the animals with good health conditions.

Among 140 Sheep, 94 were females in which 39 were lactating while 55 were dry (Non-lactating). Positive cases of sheep TB was found in 10.2% in lactating females which was higher than in non-lactating females in which the prevalence was recorded in 3.6% females as demonstrated in table (8), Rahman and Samad. (2008) also found the prevalence of TB in cattle and found a higher prevalence of 35.29% in lactating cows as compared to 20.0% in non-lactating cows. Arshad *et al.* (2012) had also reported similar results.

In my study Among 94 samples of ewes in which 29 were pregnant, while 65 were Non-pregnant. Positive cases of sheep TB was found in 7.7% in pregnant ewes which was not more high than in non-pregnant females in which the positive was recorded in 6.9% females, previous studies reported such as (center of food security and public health 2009 in bovine tuberculosis) In cattle disseminated cases, multiple small granulomas may be found in numerous organs. Lesions are sometimes found on the female genitalia.

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

This study was designed to focus the investigation of sheep tuberculosis in Sadar and Parbatipur Upazila under Dinajpur District. In the present study, there was not previous history of TB and PPD skin test performed by using TST (tuberculin skin test) performed in that area, The experiment design was performed in the Department of pathology and Parasitology faculty of veterinary and animal sciences, Hajee Mohammed Danesh science and Technology University (HSTU), Dinajpur, Bangladesh, The duration of the study was from January to June, 2018. According to this study total of 140 samples were investigated through TST (tuberculin skin test). Out of 140 sheep 7 were found to be positive and overall prevalence was 5% were positive, 15% were doubtful and 80% were negative. The highest number of positive sheep TB was found in female sheep that are equivalent to 6.4%, while doubtful cases were 14.9% and the negative cases were 78.7%. In terms of males the positive cases were 2.2%, the doubtful Cases were 15.2% whereas the negative cases were 82.6%.

In this study the high prevalence was recorded to be 23.5% in animals of more than 4 years and 4.1% in animals between 2 and 4 years of age while no positive animal was detected in animals of less than 2 years of age, while high doubtful cases were 29.5% in sheep of more than 4 years, secondly 16.4% in sheep between 2 and 4 years of age least in sheep less than 2 years of age was 8%, the negative cases were 92%, 79.5% and 47.1% in sheep less than 2 years, between 2 and 4 years of age and more than 4 years respectively, more than 4 years showed higher proportion of positivity in tuberculin test results which might be related to the fact that older animals have longer duration and repeated chance of exposure to mycobacterial infection with their age as demonstrated table (5). According this study the highest positive rate 14.8% was recorded in the crossbreed followed by undescribed Deshi breeds 2.7%, while doubtful and negative cases were 21%, 16.8%, 80.5%, and 77.7% in undescribed Deshi breeds and Carole breeds respectively. According to body condition scores, sheep with poor body condition showed high prevalence of sheep TB which was found to be 15% followed by 2.7% in medium and no positive reactor was recorded in sheep having good body condition status, while doubtful and negative cases were recorded 0, 32%, 18.9%, 100%, 80%, and 45.9% respectively in good body condition Score, medium and poor. Among 140 Sheep, 94 were females in which 39 were lactating while 55 were dry (Non-lactating), Positive cases of were found in 10.2% in lactating females was higher than in non-lactating females was 3.6%, while Highest Doubtful and Negative cases were higher in non-lactating females as demonstrated in table (8). This present study the no high positive cases were recorded 7.7% in pregnant ewes as compared with Non- pregnant ewes in which the positive was recorded in 6.9%, while high doubtful cases were 37.9% in pregnant ewes, which higher than Non- pregnant ewes 4.6%, the negative cases were 92.3% in Non- pregnant ewes which higher than in pregnant ewes 44.8%.

Tuberculosis is zoonotic high contagious diseases caused by *Mycobacterium tuberculosis* complex (MTC or MTBC) occurs both human animal, Sheep are considered susceptible to *M. bovis* infection, however, published reports tend to describe individual cases rather than outbreaks. Some authors argue that the low incidence of TB in sheep is a consequence of management and behavioral factors, which tend to reduce their exposure to this pathogen. Sheep are extensively managed and grazed predominantly during daylight hours. They also tend to flock together, both when grazing and when resting and ruminating. The main trait of *M. bovis* is its broad host range, the largest of any member of the *M. tuberculosis* complex. *Mycobacterium bovis* causes disease in a wide range of domestic, farmed, free-range, and wildlife animals, as well as humans. Only a small proportion of the species that become infected can act as maintenance hosts of these organisms. In maintenance, or reservoir, hosts such as wildlife species, cattle, buffalo, and goats, infection can persist through horizontal transfer in the absence of any other source of *M. bovis* and may be transmitted to other susceptible hosts as well. Spillover hosts, such as sheep, on the other hand, can become infected with *M. bovis* when the challenge level is relatively high, although sheep do not seem to maintain infection within the species in the absence of continuing acquisition of infection from maintenance hosts, as reported Marianelli *et al.* (2010) so the current investigation should be concluded that ewes were higher prevalence of tuberculosis than the rams.

Recommending the following:

- To identify and Isolate *Mycobacterium tuberculosis* complex (MTC or MTBC) in sheep which have been reacted with tuberculin skin test.
- To make comparative study between avium PPD and bovine PPD by injecting caudal folds and neck region skin in this area
- To make proper management practices.
- Generally it should use the strategies of prevention such as: In developed countries, regular testing and removal of infected animals.
- To enhance the awareness of farmers to contact the nearest veterinary hospitals.

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