PREVALENCE OF TUBERCULLOSIS IN GOAT AT DINAJPUR DISTRICT IN BANGLADESH

A THESIS

BY

HODAN IBRAHIM NAGEYE
SEMESTER: JANUARY-JUNE, 2017
REGISTR ATION NO.: 1605138
SESSION: 2016-2017

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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

JUNE, 2017

PREVALENCE OF TUBERCULLOSIS IN GOAT AT DINAJPUR DISTRICT IN BANGLADESH

A THESIS
BY

HODAN IBRAHIM NAGEYE
SEMESTER: JANUARY-JUNE, 2017
REGISTR ATION NO.: 1605138
SESSION: 2016-2017

Submitted to the
Department of Pathology and Parasitology
Hajee Mohammad Danesh Science and Technology University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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

JUNE, 2017

PREVALENCE OF TUBERCULLOSIS IN GOAT AT DINAJPUR DISTRICT IN BANGLADESH

A THESIS

BY

HODAN IBRAHIM NAGEYE
SEMESTER: JANUARY-JUNE, 2017
REGISTR ATION NO.: 1605138
SESSION: 2016-2017

Approved as to style and contents by

(Prof. Dr. S.M. Harun-ur-Rashid)	**(Dr. Md. GolamAzam)**
Supervisor	**Co-Supervisor**

(Dr. Md. Haydar Ali)
Chairman
Examination Committee
&
Chairman

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

JUNE, 2017

DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGMENTS

First and foremost, my sincere gratitude goes to the Almighty Allah whose loving kindness had and continues to sustain my life.And for imbibing confidence on the author to complet this study education in veterinary science and to completeresearch work and the thesis for the degree of master science (M.S) degree in pathology successfully without any natural and unnatural obstruction.

The author express her deep sense of gratitude, sincere appreciation and Profound regards to her honorable teacher and research supervisor Dr. S. M. Harun-ur-Rashid,Professor,Department of Pathology and Parasitology, faculty of Veterinary and Animal Science, Hajee Mohammad Danish Science and Technology University. Not only did he offer enduring guidance, constructive criticisms, abundant help during every phase of this research work and preparation, but, they were a source of inspiration and good judgment and finally preparing the manuscript.

The author deems it a proud privilege pleasure and honour to express his heartfelt gratitude, ever, sincerity and profound regards to his beloved relevantfriendly,teacher and chairman, Dr. Md. Haydar Ali, Assistant Professor, Department of Pathology and Parasitology, faculty of veterinary and animal science , Hajee Mohammad Danesh Science and Technology University, Dinajpur for their encouragement valuable suggestion and kindly.

I would also like to express my sincere gratefulness and appreciation to my honorable teacher and Co-Supervisor Dr. Md. GolamAzam, AssistantProfessor, Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danish Science and Technology Universityfor his valuable suggestions, positive criticism and contribution during the research work and the preparation of this study.

I would wish to acknowledge with thanks and appreciation, the total contribution of all the staffs of Department of Pathology and Parasitology, faculty of veterinary and animal science, Hajee Mohammad Danish Science and Technology University, Dinajpur in providing support and generous assistance for the execution of this research work.

My deep appreciation goes to all my friends especially, Abdullahi Omar Mohamud, Abubakar Ali Jama, Hassan Nur Osman,Osman Hassan Afrah, and Yoonis Ahmed Nurfor their support and assistance towards the success of this research work.

Finally, my deep appreciation goes to all family members especially my father Ibrahim Nageye and my beloved mother Aamina Hussein sisters and brothers for their everlasting love, patience, unlimited sacrifice, blessing, endless inspiration and co-operation to complete my MS Degree.

The author

June 2017

ABSTRACT

Tuberculosis of goat is a zoonotic, highly infectious bacterial diseases caused by *Mycobacterium tuberculosis* complex subsp. *caprae*. The duration of the study was from January to June, 2017. According to this study there was no significant different of the disease between male and female (p=0.746). The highest number of positive TB was found in female goats that are equivalent to 7.14%, while Inclusive was 11.43% and the negative was 81.43%. In terms of males the positive rate was 4.44%, the inclusive test was 8.89% whereas the negative case was 86.67%. In this study showed there was non-significant difference of the disease among goat breeds (P=0.215) as demonstrated in (table 4.2). The highest prevalence rate11.42% was recorded in the breed of Jamunapari followed by Cross breed 6.90% in spite of the lowest prevalence 2.85% was found in indigenous breed. On the other result of the present that the highest number of positive TB in goat 10.00% was found in Mirjapur while the lowest positive rate (3.08%) was noted in Nayanpur. According to the previous history of tuberculosis and PPD test performed, most of the farmers 102 88.7% reported that there was no previous history of tuberculosis and 91(79.1%) of the farmers stated that there was no PPD test performed although13 (11.3%) of the farmers reported that there was previous history of tuberculosis 24 (20.9%) of them replied that there was PPD test performed. According to the positive case, females were higher and more susceptible than males. So the current investigation should be concluded that female goats were higher prevalence of tuberculosis than the males, body conditions of the studied goats were in poor condition, poor management practice such as housing system, nutrition and ventilation.

CONTENTS

CONTENTS (Cont'd.)

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LISTOF ABBREVIATIONS AND SYMBOLS

AFB	-	Acid –fast bacilli
BTB	-	Bovine tuberculosis
CFT	-	Caudal fold test
DNA	-	deoxyribonucleic acid
GTB	-	Goat tuberculosis
G+C	-	guanine plus cytosine
HSTU	-	Hajee Mohammed Danesh science and technology
IFN-Y	-	Interferon gamma
ITT	-	Interadermaltuberculine test
M	-	Mycobacterium
MS	-	Master of science
MTC	-	Mycobacterial tuberculosis complex
MIRU	-	mycobacterial interspersed repetitive unit
MIRU-VNTR	-	mycobacterial interspersed repetitive unit—variable number of tandem repeat
PCR	-	Polymerase chain reaction
PPD	-	Purified protein derivative
PTB	-	paratuberculosis
TB	-	Tuberculosis
DTH	-	delayed hypersensitivity type
OTF	-	officially TB-free
SICCT	-	single intradermal cervical comparative tuberculin
SIT	-	single intradermal tuberculin -
ELISA	-	Ezyme Linked Immmunosorbent Assay
DIVA	-	differentiation of infected and vaccinated animals
MDCT	-	multidetector computed tomography

CHAPTER I

INTRODUCTION

CHAPTER I

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).

Tuberculosis (TB) in the domestic goat (*Capra hircus*), mainly caused by *Mycobacterium caprae* (Aranaz, 2003), is an endemic disease in the Arabian berian Peninsula. *M. caprae* is widespread in goat herds and is an emerging infectious agent in cattle (Duarte *et al.,* 2008; Rodríguez *et al.,* 2011).

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).

Mycobacterium bovis and *M. caprae* are primarily a cattle pathogen; however, they have been isolated from goats, camels, horses, pigs, dogs, and cats amongst other animals including human being (Lepper and Corner, 1983; Erler *et al.,* 2004; Prodinger *et al.,* 2005; Thoen *et al.,* 2006).

Houlihan *et al.* (2008), Gutierrez *et al.* (1995) carry out 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).

TB in goat is caused by members of *Mycobacterium tuberculosis* complex predominantly by *Mycobacterium bovis* and *Mycobacterium caprae,* Cordes *et al.* (1981); Hiko and Agga (2011) 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); Tafess *et al.* (2011).

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).

In the region of Dinajpur district, no such study was performed to investigate the prevalence of Goat tuberculosis under these circumstances, it is necessary to explore a study to detect the prevalence of TB infection in goat.

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

- To determine the prevalence of GTB in the villages of Sadar Upazilla of Dinajpur.
- To find out the tuberculin positive test in relation to sex, breed of goat at Dinajpur district.
- To evaluate the spread of the disease in endemic area.
- To observe the management practice of goat in that area.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 *M. caprae* tuberculosis

Mycobacterium tuberculosis complex isolates recovered from goats were originally classified as *Mycobacterium tuberculosis* subsp. *caprae*; however, this subspecies was recently reclassified as *Mycobacterium bovis* subsp. *caprae*. Besides biochemical (sensitivity to pyrazinamide) and epidemiological features, strains of this unusual member of the *M. tuberculosis* complex show a special combination of pncA, oxyR, katG and gyrA gene polymorphisms. Sequence analysis of the gyrB gene in these strains reveals special nucleotide substitutions not found in other members of the *M. tuberculosis* complex that can be used to differentiate *caprine* mycobacterial strains from *M. bovis* and other members of the *M. tuberculosis* complex. *M. tuberculosis* subsp. *caprae* now appears not to be restricted to Spanish goats, as strains of this organism have been isolated from cattle, wild boar and pigs. Its occurrence has also been reported in France, Austria and Germany. By (Aranaz *et al.,* 2003).

2.2 Epidemiology

2.2.1 Etiology

Mycobacterium caprae, a recently defined member of the *Mycobacterium tuberculosis* complex, causes tuberculosis among animals and, to a limited extent, in humans in several European countries. To characterize *M. caprae* in comparison with other *Mycobacterium tuberculosis* complex members and to evaluate genotyping methods for this species, they analyzed 232 *M. caprae* isolates by mycobacterial interspersed repetitive unit (MIRU) genotyping and by spoligotyping. The isolates originated from 128 distinct epidemiological settings in 10 countries, spanning a period of 25 years. We found 78 different MIRU patterns (53 unique types and 25 clusters with group sizes from 2 to 9) but only 17 spoligotypes, giving Hunter-Gaston discriminatory indices of 0.941 (MIRU typing) and 0.665 (spoligotyping). For a subset of 103 *M. caprae* isolates derived from outbreaks or endemic foci, MIRU genotyping and IS 6110 restriction fragment length polymorphism were compared and shown to provide similar results. MIRU loci 4, 26, and 31 were most discriminant in *M. caprae*, followed by loci 10 and 16, a combination which is different than those reported to discriminate *M. bovis* best. *M. caprae* MIRU patterns together with published data were used for phylogenetic inference analysis employing the neighbor-joining method. *M. caprae* isolates were grouped together, closely related to the branches of classical *M. bovis*, *M. pinnipedii*, *M. microti*, and ancestral M. tuberculosis, but apart from modern M. tuberculosis. The analysis did not reflect geographic patterns indicative of origin or spread of *M. caprae*. Altogether, our data confirm *M. caprae* as a distinct phylogenetic lineage within the *Mycobacterium tuberculosis* complex, reported by (Prodinger *et al.,* 2005).

Mycobacterium tuberculosis complex isolates recovered from goats were originally classified as *Mycobacterium tuberculosis* subsp. *caprae*; however, this subspecies was recently reclassified as *Mycobacterium bovis* subsp. *caprae*. Besides biochemical (sensitivity to pyrazinamide) and epidemiological features, strains of this unusual member of the *M. tuberculosis* complex show a special combination of pncA, oxyR, katG and gyrA gene polymorphisms. Sequence analysis of the gyrB gene in these strains reveals special nucleotide substitutions not found in other members of the *M. tuberculosis* complex that can be used to differentiate *caprine* mycobacterial strains from *M. bovis* and other members of the *M. tuberculosis* complex. *M. tuberculosis* subsp.

caprae now appears not to be restricted to Spanish goats, as strains of this organism have been isolated from cattle, wild boar and pigs. Its occurrence has also been reported in France, Austria and Germany. Two studies on the evolution of the *M. tuberculosis* complex based on the presence/absence of regions of difference have shown that the group of *caprine* isolates (or its ancestor) is older than *M. bovis* (or its ancestor). These findings reinforce the original suggestion that the *caprine* mycobacterial strains are a taxon of the *M. tuberculosis* complex, independent of *M. bovis* (Aranaz *et al.,* 2003).

2.2.2 Incidence and prevalence

A survey of 143 hunter-harvested red deer for tuberculosis was conducted in an Alpine area in Western Austria over two subsequent years. There, single tuberculosis cases caused by *Mycobacterium caprae* had been detected in cattle and red deer over the preceding decade. The area under investigation covered approximately 500 km^2, divided into five different hunting plots. Lymph nodes of red deer were examined grossly and microscopically for typical tuberculosis-like lesions and additionally by microbiological culturing. Executing a detailed hunting plan, nine *M. caprae* isolates were obtained. Six out of nine originated from one single hunting plot with the highest estimated prevalence of tuberculosis, that is, 23.1%. All isolates were genotyped by mycobacterial interspersed repetitive unit—variable number of tandem repeat (MIRU-VNTR) typing of 24 standard loci plus VNTR 1982. All nine isolates belonged to a single cluster termed “Lechtal” which had been found in cattle and red deer in the region, demonstrating a remarkable dominance and stability over ten years. Conducted by (Schoepf *et al.,* 2012)

The prevalence of the *Mycobacterium bovis* subsp. *caprae* and *M. bovis* subsp. *bovis* among German tuberculosis cases caused by the bovine tubercle bacillus from 1999 to 2001 was determined. Isolates from 166 patients living in Germany and 10 animals were analyzed by conventional laboratory procedures, spoligotyping, and partly by PCR-restriction fragment length polymorphism analysis of the gyrB gene. By spoligotyping, 55 of 176 isolates (31%) could be identified as *M. bovis* subsp. *caprae*, and 121 (69%) were confirmed as *M. bovis* subsp. *bovis*. In general, a low variability of spoligotypes with 59 distinct patterns and a cluster rate of 77% (136 isolates/19 clusters) was determined. About half of all isolates were grouped in the three main clusters with 29, 30, and 35 isolates, respectively. Differences in age and gender between the patient groups infected with *M. bovis* subsp. *bovis* and *M. bovis* subsp. *caprae* did not reach statistical significance. However, marked differences in the geographical prevalence of *M. bovis* subsp. *caprae* were observed, ranging from fewer than 10% of all *M. bovis* isolates in the north up to more than 80% of isolates in the south of Germany. In conclusion, *M. bovis* subsp. *caprae* accounts for a high ratio of human *M. bovis*-associated tuberculosis cases in Germany and was more frequently found in the southern part on this study was reported by (Kubica *et al.,* 2003).

Tuberculosis in goats (caused by *Mycobacterium caprae* and *M. bovis*) has become a significant concern in recent years because of its high prevalence in certain *caprine* herds in Spain and other European countries, and also due to the potential transmission to other animals and human beings. In the present study, a transthoracic model of tuberculosis infection was performed on goats. Animals were selected based on the serological response used to detect *paratuberculosis* in goats (negative and positive results). The kinetics of the immune response was evaluated using the interferon-γ (IFN-) assay, skin tests and serology of *paratuberculosis* during nine months post-challenge. At the end of the study the animals were necropsied, tuberculosis-lesions were scored and culture (*M. caprae* and *M. avium* subsp. *paratuberculosis*) was performed to determine the true infection status. Animals were positive to the IFN- assay 15 days post-challenge and the values were fluctuating throughout the study. A varied performance of the assay was observed between tuberculosis and tuberculosis-*paratuberculosis* mixed infection regarding both the number of positive results and the OD values obtained after stimulation with bovine and avian PPDs. Furthermore, the single intradermal comparative cervical tuberculin test did not detect all *M. caprae*-infected animals. At necropsy, a positive correlation between pathology score and bovine PPD specific IFN-response was founded by (Bezos *et al.,* 2010).

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 (A., O., J.A., C., & J.A., M. (2011).

2.2.2 Source of transmission

Infected cattle are important sources of infection for healthy cattle, but wildlife reservoirs of infection have also been reported in many regions (Wedlock *et al.,* 2002; Philips *et al.,* 2003; Kaneene and Pfeiffer, 2006; Thoen *et al.,* 2009); thereby complicating the epidemiological picture. Bovine TB is an FAO—OIE "List B" disease because of its important socio-economic and public health impact (Benkirane. 1998; CRIB, 2008).

2.2.3 Routes of transmission of the tubercle bacilli

There are several routes of entry and transmission of the tubercle bacilli but the respiratory and gastrointestinal tracts are the primary routes and less frequently incisions in the skin (Neill *et al.,* 1994; Goodchild and Clifton-Hadley, 2001; Philips *et al.,* 2003; Ayele *et al.,* 2004; Biet *et al.,* 2005; Kaneene and Pfeiffer, 2006; Wilsmore and Taylor, 2008).

Respiratory transmission through direct inhalation of contaminated aerosols is the most important route of infection in groups of susceptible hosts that remain in repeated close contact or in a confinement with infected individuals (Goodchild and Clifton-Hadley, 2001; Hussain *et al.,* 2003; Cassidy, 2006; Kaneene and Pfeiffer, 2006; Palmer and Waters, 2006).

The oral route is accomplished when feed or water contaminated with mucous, nasal secretion, saliva, discharging lesions, faeces and urine that contain the infective organism; and unpasteurized milk or raw meat from an infected animals are consumed by the healthy host (Moda *et al.,* 1996; Ayele *et al.,* 2004; Biet *et al.,* 2005; Kaneene and Pfeiffer, 2006; Palmer and Waters, 2006). Also, the ingestion of *M. bovis* directly from cows to nursing calf and indirectly from contaminated pasture or farm tools to other animals (O'Reilly and Dabom, 1995: Cosivi *et al.,* 1998; Ayele *et al.,* 2004; Good, 2006; Goodchild and Clifton-Hadley, 2006; Delahay *et al.,* 2007) could be common in some regions.

Other modes of transmission though less common, include the transcutaneous mode of transmission of *M. bovis* from animals to humans who handle infected carcasses, with the spread of infection through cuts and abrasions; for example butcherE s wart in humans (Grange and Yates, 1994).

Congenital infections, genital transmission and vertical transmission have also been noted to occur when the reproductive organs are affected (Neill *et al.,* 1994; Cosivi *et al.,* 1998; Philips *et al.,* 2003; Figueiredo *et al.,* 2008), but these modes of transmission are rarely reported in regions with strict eradication programmes (Ayele *et al.,* 2004).

2.5.4. Incidence of infection The incidence of the disease is higher in the developing nations because of the absence of any national control and eradication program, is also accelerating worldwide particularly in the Asian, African and Latin American countries (Bonsu *et al.,* 2001).

2.3 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).

2.4 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.

2.5 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).

2.6 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).

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).

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)

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).

2.7 PPD skin test

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).

Tuberculosis in goats (caused by *Mycobacterium caprae* and *M. bovis*) has become a significant concern in recent years because of its high prevalence in certain *caprine* herds in Spain and other European countries, and also due to the potential transmission to other animals and human beings. In the present study, a transthoracic model of tuberculosis infection was performed on goats. Animals were selected based on the serological response used to detect *paratuberculosis* in goats (negative and positive results). The kinetics of the immune response was evaluated using the interferon (IFN) assay, skin tests and serology of *paratuberculosis* during nine months post-challenge. At the end of the study the animals were necropsied, tuberculosis-lesions were scored and culture (*M. caprae* and *M. avium* subsp. *paratuberculosis*) was performed to determine the true infection status. Animals were positive to the IFN assay 15 days post-challenge and the values were fluctuating throughout the study. A varied performance of the assay was observed between tuberculosis and tuberculosis-*paratuberculosis* mixed infection regarding both the number of positive results and the OD values obtained after stimulation with bovine and avian PPDs. Furthermore, the single intradermal comparative cervical tuberculin test did not detect all *M. caprae*-infected animals. At necropsy, a positive correlation between pathology score and bovine PPD specific IFN observed by Bezos *et al.* (2010)

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).

This paper describes an outbreak of tuberculosis (TB) caused by *Mycobacterium bovis* in a dairy goat herd on a farm in Ireland, where 66.3 per cent of the herd tested positive to the single intradermal comparative tuberculin test (SICTT) at initial detection. An epidemiological investigation was conducted to determine the origin of the outbreak, considering issues such as animal movements and herd management practices. Infection was introduced with a consignment of goats, as determined by the variable number tandem repeat profile. Infection was eradicated using a test and cull programme involving the SICTT, the interferon-γ assay and a multiplex immunoassay (Enferplex TB) mentioned by Shanahan *et al.* (2011).

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).

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).

2.8 Zoonotic finding

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).

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).

Tuberculosis is an important disease among many zoonoses, because both *Mycobacterium tuberculosis* and *Mycobacterium bovis*, which are the major causes of tuberculosis, are highly pathogenic, infect many animal species and thus are likely to be the source of infection in humans. In particular, monkeys are highly susceptible to these bacteria and are important spreaders. Recently, two outbreaks of *M. tuberculosis* occurred in four different kinds of monkeys and humans were also infected with the disease in Japan. In zoos, tuberculosis was reported not only in monkeys, but also in several different kinds of animals, including elephants. Pets such as dogs and cats are believed to be generally less susceptible to M. tuberculosis, but in this article we introduce a case of infection from man to dog by close contact. Japan is one of the few countries that have been able to control *M. bovis* infection. In other countries, however, cases of bovine tuberculosis and human *M. bovis* infection have been reported, and thus further attention is still required in the future (Reported by Une and Mori, 2007).

Tuberculosis in goats (caused by *Mycobacterium caprae* and *M. bovis*) has become a significant concern in recent years because of its high prevalence in certain *caprine* herds in Spain and other European countries, and also due to the potential transmission to other animals and human beings. In the present study, a transthoracic model of tuberculosis infection was performed on goats. Animals were selected based on the serological response used to detect *paratuberculosis* in goats (negative and positive results). The kinetics of the immune response was evaluated using the interferon-γ (IFN-γ) assay, skin tests and serology of *paratuberculosis* during nine months post-challenge. At the end of the study the animals were necropsied, tuberculosis-lesions were scored and culture (*M. caprae* and *M. avium* subsp. *paratuberculosis*) was performed to determine the true infection status. Animals were positive to the IFN-γ assay 15 days post-challenge and the values were fluctuating throughout the study. A varied performance of the assay was observed between tuberculosis and tuberculosis-*paratuberculosis* mixed infection regarding both the number of positive results and the OD values obtained after stimulation with bovine and avian PPDs. Furthermore, the single intradermal comparative cervical tuberculin test did not detect all *M. caprae*-infected animals. At necropsy, a positive correlation between pathology score and bovine PPD specific IFN-γ response was found γ 2009 Elsevier B.V. All rights reserved by Bezos *et al.* (2010).

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).

2.9 Treatment and Control

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. 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)

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).

Caprine tuberculosis (TB) has increased in recent years, highlighting the need to address the problem the infection poses in goats. Moreover, goats may represent a cheaper alternative for testing of prototype vaccines in large ruminants and humans. With this aim, a *Mycobacterium caprae* infection model has been developed in goats. Eleven 6-month-old goats were infected by the endobronchial route with 1.5 × 10(3) CFU, and two other goats were kept as noninfected controls. The animals were monitored for clinical and immunological parameters throughout the experiment. After 14 weeks, the goats were euthanized, and detailed postmortem analysis of lung lesions was performed by multidetector computed tomography (MDCT) and direct observation. The respiratory lymph nodes were also evaluated and cultured for bacteriological analysis. All infected animals were positive in a single intradermal comparative cervical tuberculin (SICCT) test at 12 weeks post-infection (p.i.). Gamma interferon (IFN-γ) antigen-specific responses were detected from 4 weeks p.i. until the end of the experiment. The humoral response to MPB83 was especially strong at 14 weeks p.i. (13 days after SICCT boost). All infected animals presented severe TB lesions in the lungs and associated lymph nodes. *M. caprae* was recovered from pulmonary lymph nodes in all inoculated goats. MDCT allowed a precise quantitative measure of TB lesions. Lesions in goats induced by *M. caprae* appeared to be more severe than those induced in cattle by *M. bovis* over a similar period of time. The present work proposes a reliable new experimental animal model for a better understanding of *caprine* tuberculosis and future development of vaccine trials in this and other species. Find out by (Pérez De Val *et al.,* 2011).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental animals, areas duration and experimental protocol

This study was design to focus the prevalence of *Caprine* tuberculosis in Sadar Upazilla farm and villages in Dinajpur distract (Nayanpur and Mirjapur). The experiment 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 January to June, 2017

3.2 The major work of the present study

- Tuberculin test in selected household farm.
- To compare the prevalence of goat tuberculosis of different age and sex.

3.3 Test material

- Mammalian tuberculin PPD (purified protein derivative)
- Tuberculin gum
- Mask
- Ice carrier with ice pack
- Cloves
- Cotton
- Providone iodine
- Scissors/blade
- Water
- Mark pen
- Gumboot and apron

Figure 1: Tuberculin gum

Figure 2: Mammalian tuberculin PPD (Purified)

Figure 3: PPD Tuberculin

3.4 Selection and grouping of animal

To determine the prevalence of goat tuberculosis infection in 115 goats from different housekeeping goat in Dinajpur district was selected using CFT test. Of the 115 goat 45 male and 70 female, according to area sex, and breed other managemental information was record by questionnaire.

3.5 Experimental layout

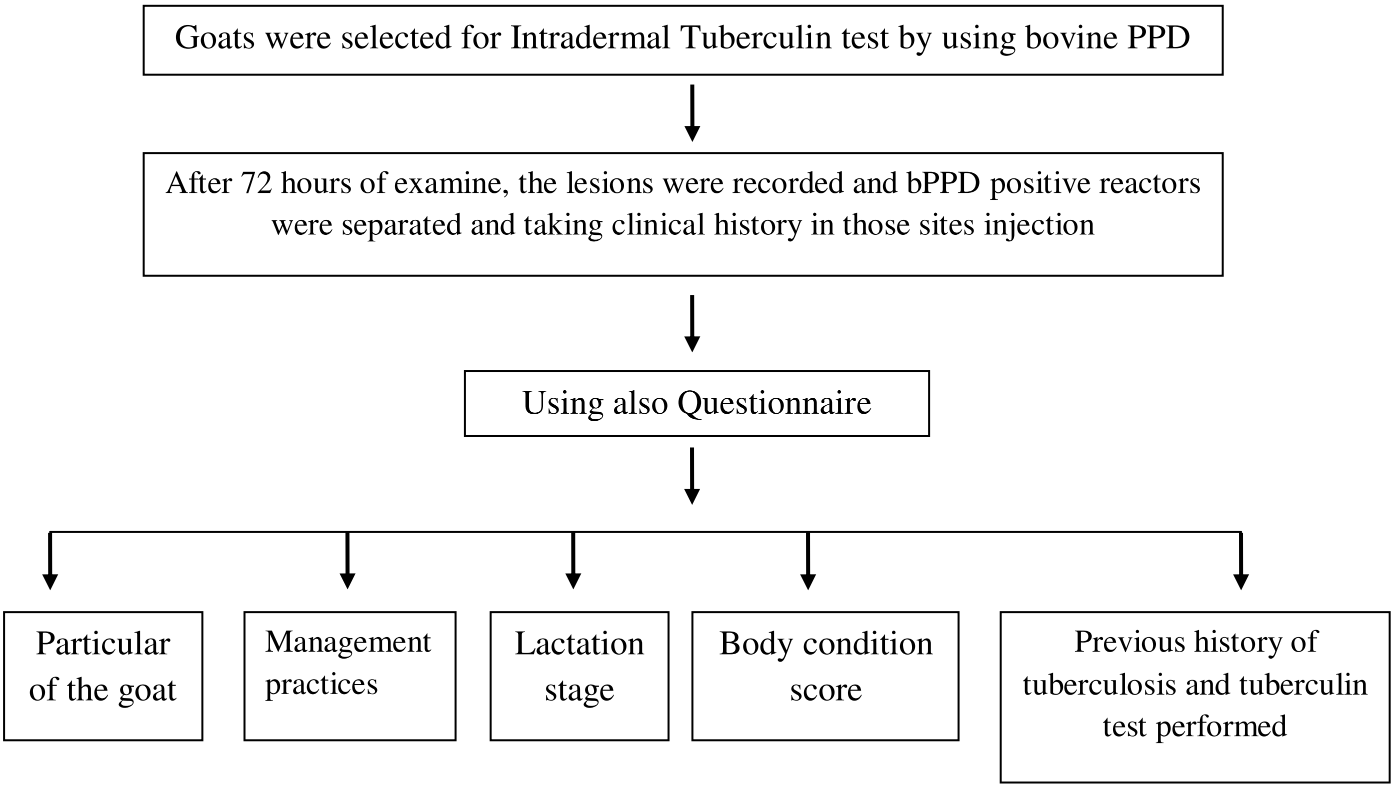


Figure 6: Experimental layout

3.6 Test procedure

The test used is the single intradermal injection of bovine tuberculin purified protein derivative (PPD) (SID test), commonly known as the tuberculin skin test, 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* and ‘avian’ tuberculin derived from *M. avium* (the bacterium that causes tuberculosis in birds) into the deep layer of the skin.

The skin is first clipped and the thickness of the skin fold measured in millimetres using special callipers before the tuberculin’s are injected in the middle of the neck 0.1 ml. After 72 + 4 hours, the Veterinary surgeon returns and re-measures the skin at the site of the injections to determine if the animal is classified as a reactor, inconclusive or pass.

Table 1: Schedule and procedure of inoculation:

Test	**Reagent**	**Site**	**Rout**	**Dose**	**Time of observation post incubation**
Single intradermal injection test	bPPD	Skin fold	i/d	0.1 ml	72

3.7 Reading of the results of inoculations

In CFT test the reading was take 72 hours after the inoculation. The positive tuberculin reaction was evident from an inflammation of sensitive nature at the point of inoculation.

The swelling was either soft and edematous or somewhat hard in nature.

The results were interpreted according to OIE standards (OIE, 2004):

a. If the reaction is ≥ 4.0 mm greater than the test is considered reactor.

b. If the reaction is between 3.0 and < 4.0 mm than the test is considered suspect.

c. If the reaction is < 3.0 mm than the test is considered negative.

The swelling was felt and estimate by palpation at the site of inoculation, while animal expressed the sign of pain.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 A Purified Protein Derivative (PPD) Skin Test

According to this study there was no significant different of the disease between male and female (p=0.746) (Table 1). The highest number of positive TB was found in female goats that are equivalent to 7.14%, while Inclusive was 11.43% and the negative was 81.43%. In terms of males the positive rate was 4.44%, the inclusive test was 8.89% whereas the negative case was 86.67%.

Table 2: Purified Protein Derivative (PPD) Skin Test based on sex

Sex	No. of Tested	Positive cases		Inclusive cases		Negative cases		Chi-Square
		No. of Positive	Percentage (%)	No. of goat Inclusive	Percentage (%)	No. of goat Negative	Percentage (%)	
Male	45	2	4.44	4	8.89	39	86.67	0.587(0.746)
Female	70	5	7.14	8	11.43	57	81.43	
Total	115	7	11.58	12	20.32	96		

4.2 Purified Protein Derivative (PPD) Skin Test based on breed

According to this study there was non-significant difference of the disease among goat breeds (P= 0.215) as demonstrated in (table 4.2). The highest prevalence rate (11.42%) was recorded in the breed of Jamunapari followed by Black Bengal breed (6.90%) in spite of the lowest prevalence (2.85%) was found in indigenous breed.

Table 3: Purified Protein Derivative (PPD) Skin Test based on breed

Breed	No. of observed	**Positive cases**		**Inclusive cases**		**Negative cases**		**Chi-Square**
		No.	(%)	No.	(%)	No.	(%)	
Black bengal	51	1	2.85	3	0.06%	47	97.09	5.797(0.215
Jamunapari	35	4	11.42	5	14.29	26	74.29	
Cross breed	29	2	6.90	4	13.79	23	79.31	

4.3 Purified Protein Derivative (PPD) Skin Test based on Area

According to this study there was no significant different of the disease between Nayanpur and Mirjapur (p=0.126) (Table 2). The highest number of positive TB in goats (10.00%) was found in Mirjapur in contrast, the lowest positive rate (3.08%) was noted in Nayanpur.

Table 4: Effect of T.B in goats in the study Area

Area	**No. of observed**	**No. of positive**		**No. of negative**		**Chi-square**
		No.	**%**	**No.**	**%**	
Nayanpur	65	2	3.08	63	96.92	2.370(0.126)
Mirjapur	50	5	10.00	45	90.00	

4.4 Previous history of tuberculosis and PPD test performed

According to the previous history of tuberculosis and PPD test performed, most of the farmers 102 (88.7%) reported that there was no previous history of tuberculosis and 91(79.1%) of the farmers stated that there was no PPD test performed although13 (11.3%) of the farmers reported that there was previous history of tuberculosis and 24 (20.9%) of them replied that there was PPD test performed. This may be that there was no prevalence of tuberculosis in this area or may be no accurate examination done on this area.

Table 5: Pervious history of tuberculosis and PPD test performed

Characteristics	No. TB in Previous History	Percentage (%)	PPD Test Performed Previously	Percentage (%)
Yes	13	11.3	24	20.9
No	102	88.7	91	79.1
Total	115	100.0	115	100.0

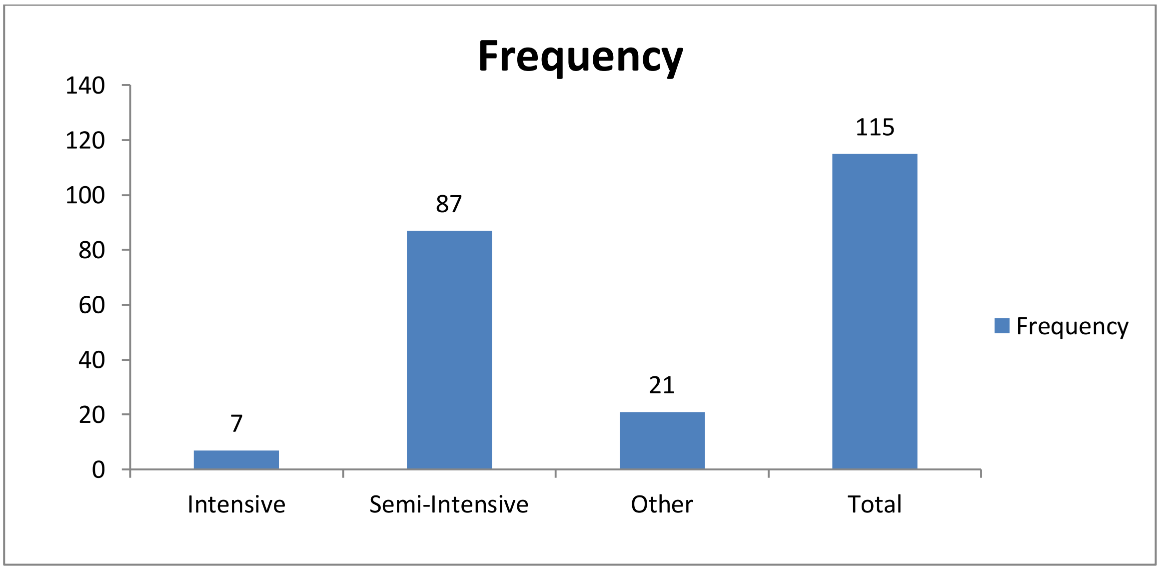
4.5 Management system

4.5.1 Housing system

Housing is one of the most important for the animal, which protect against predators, temperature, and other factors. According to this study most of the animal owners practice Semi-intensive system which was 87 out 115 that is equivalent to 75.7 %, while 21 farmers representing 18.3% of the study used other system for goat housing, and finally 7 farmers that is 6.1% used for intensive system. This study showed that most of farmers used semi-intensive system because it cheaper than the other systems and needs less labor.

Table 6: Housing System

Characteristics	Frequency	Percent	Cumulative Percent
Intensive	7	6.1	6.1
Semi-Intensive	87	75.7	81.7
Other	21	18.3	12.2
Total	115	100.0	100.0



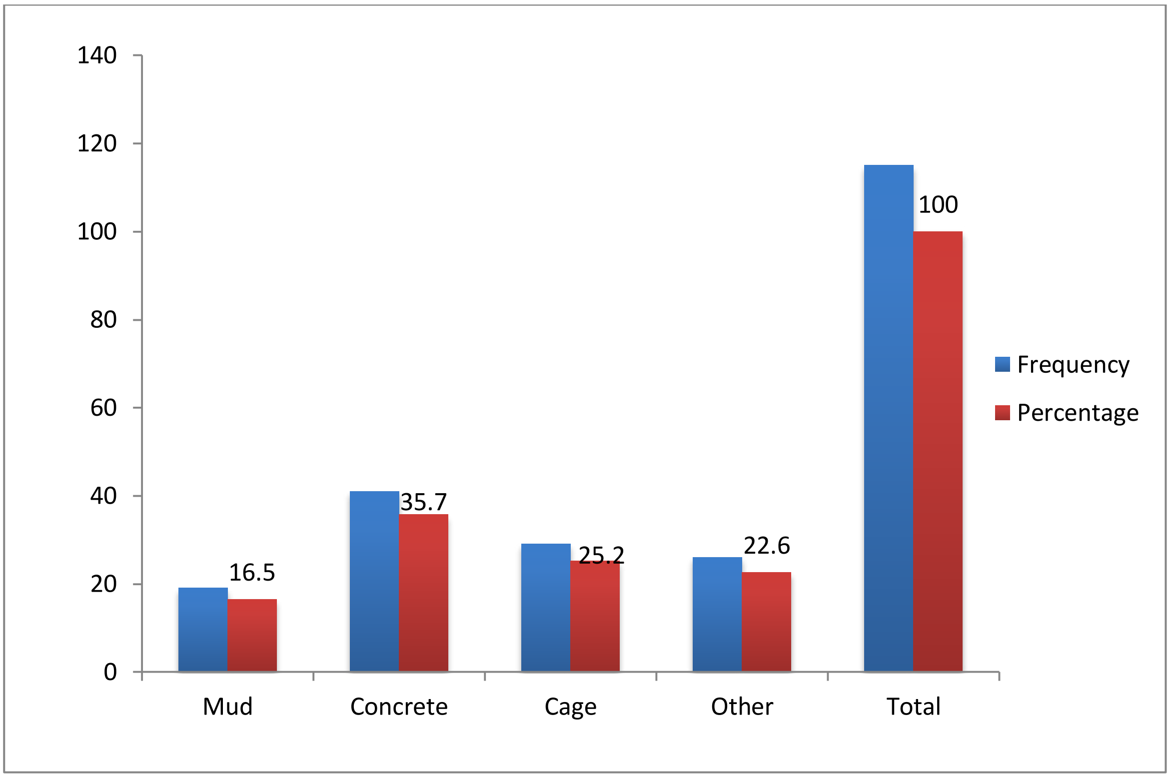
Graph-1: graphical representations of Housing system

4.5.2 Housing floor condition

Housing floor is very important for goat farming business, for staying at night, security, preventing them from adverse climate, cold, sunlight. According to the housing floor system, the majority of the farmers 41 (35.7%) used for concrete in floor condition but 29 (25.2%) of the farmers under study used cage and 26 (22.6%) of them used other condition. However 19 farmers which was (16.5%) of the study used mud (over the ground). The data showed that most of the farmers under this study used concrete for flooring system and it is easy to clean although it is expensive compare to other systems followed by cage system.

Table 7: Housing Floor Condition

Characteristics	Frequency	Percentage (%)	Cumulative Percent
Mud	41	35.7	81.7
Concrete	19	16.5	6.1
Cage	29	25.2	100.0
Other	26	22.6	
Total	115	100.0	



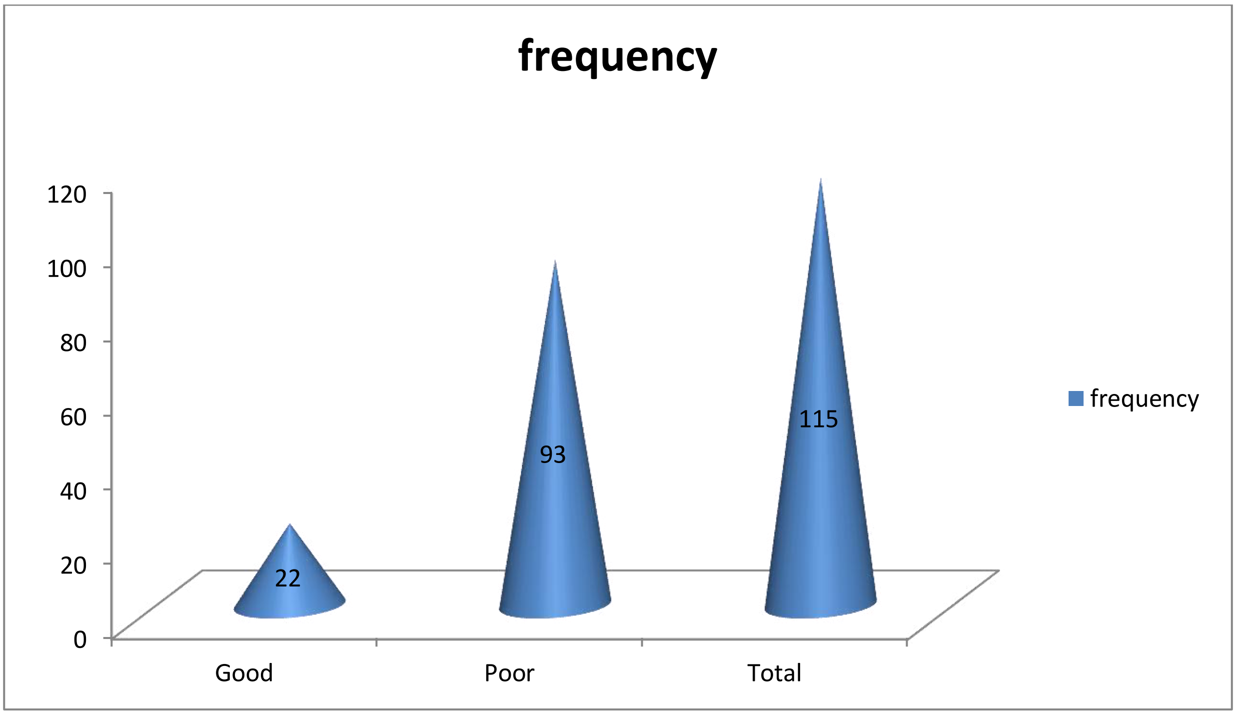
Graph-2 Housing floor condition

4.5.3 Ventilation Facilities

Good ventilation is an important part of any livestock housing system, the collected data from the farm owners exhibited that most of them 93 (80.9%)practiced poor ventilation facilities for animal herd while 22 (19.1%) adopted good ventilation. This may be poor economic condition of the farmers who are not able to build houses with good ventilation.

Table 8: Ventilation Facility

Characteristics	Frequency	Percent	Cumulative Percent
Good	22	19.1	19.1
Poor	93	80.9	100.0
Total	115	100.0	



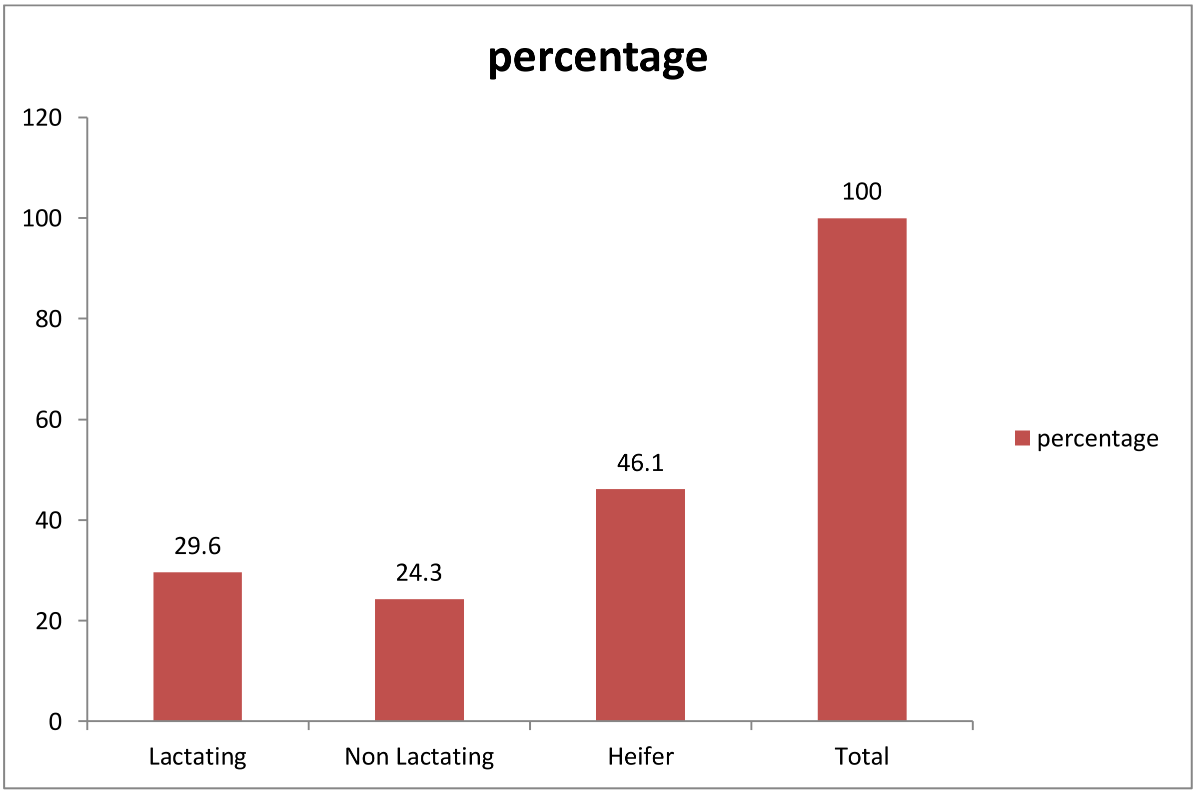
Graph-3 Ventilation Facilities for the house

4.6 Lactation Stage for goats

The examined animal included different lactation period, 34 (29.6%) Out of 115 are lactating stage while28 (24.3%) non lactating period and remaining others are Heifers 53 (46.1%).

Table 9: Lactation Stage for goats

Characteristics	Frequency	Percent	Cumulative Percent
Lactating	34	29.6	29.6
Non Lactating	28	24.3	53.9
Heifer	53	46.1	100.0
Total	115	100.0	



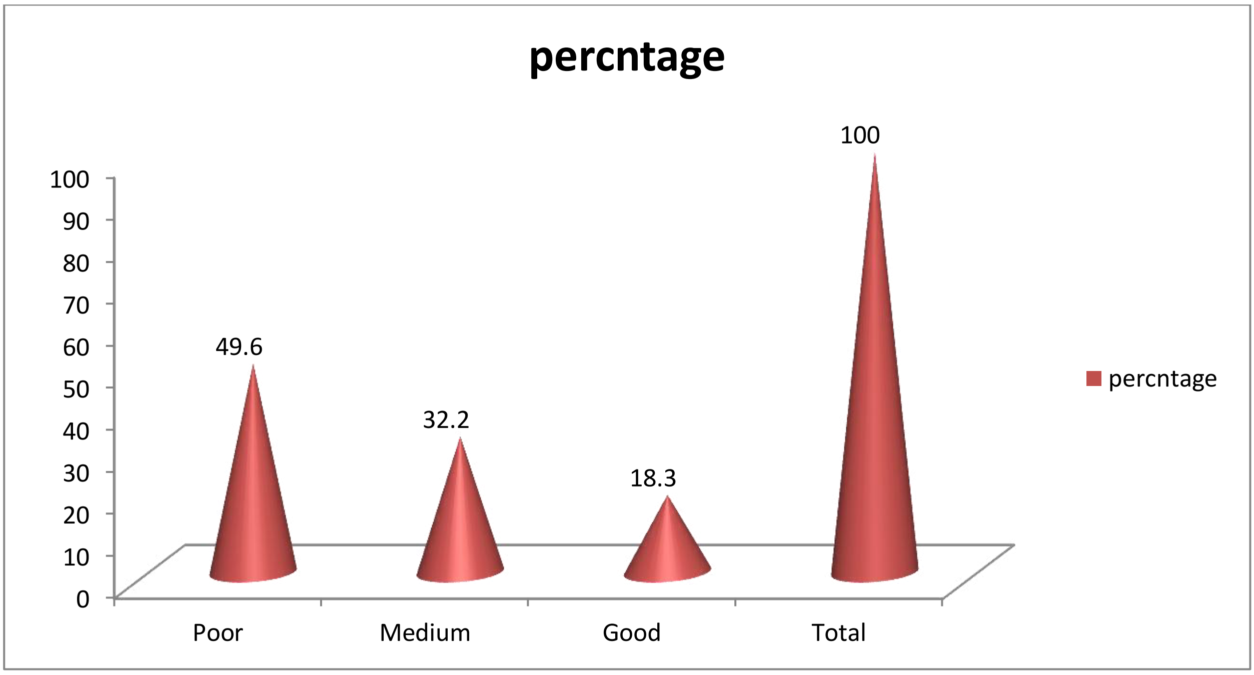
Graph-4: Lactation Stage for goats

4.7 Body Condition Score

Body condition score has shown the animal condition weather normal condition or abnormal condition, so the studied animal showed that the highest number of the examined animal which is 57 (49.6%) are poor body condition whereas 37 (32.2%) medium condition and 21(18.3%) are good body condition. Nearly half of the studied animals were poor condition and this may be poor healthy condition of the goats.

Table 10: Body Condition Score

Level	Frequency	Percent	Cumulative Percent
Poor	57	49.6	49.6
Medium	37	32.2	81.7
Good	21	18.3	100.0
Total	115	100.0	



Graph-5: Graph presented of Body Condition Score

Figure 4: Inoculation for caprine tuberculosis

Figure 7: Picture of goats shown positive reaction swelling after 72 hours by intradermal injection of mammalian tuberculin PPD

Figure 5: Measurement scale for skin swelling

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

Goat tuberculosis is an infectious bacterial disease, zoonotic important which affects both human and animal cased by genus *Mycobacterium tuberculosis* subsp. *caprae*. However the current study was performed in different areas at Dinajpur district. The current study presented that the overall prevalence rate of *M. caprae* tuberculosis was (11.58%). My result is higher than the result of Pignata *et al.,* (2009) who reported the prevalence of tuberculosis in goats was 0.47% in Brazil. One study reported a prevalence of tuberculosis was 3.5% in domestic goats (Sanson, 1998), while another reported 0.5% in goats (Arellano *et al.,* 1999). 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 this study there was no significant different of the disease between male and female (p=0.746). The highest number of positive TB was found in female goats that are equivalent to 7.14%, while Inclusive was 11.43% and the negative was 81.43%. In terms of males the positive rate was 4.44%, the inclusive test was 8.89% whereas the negative case was 86.67%. Offiong *et al.* (2014) conducted a study, they found that female goats had the highest level of infection with TB with number of 68 (53.1%) whiles the male 60 (46.9%). 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. (Eckert and Deplazes, 2003).

According to this study there was non-significant difference of the disease among goat breeds (P= 0.215) as demonstrated in (table 4.2). The highest prevalence rate (11.42%) was recorded in the breed of Jamunapari followed by cross breed (6.90%) in spite of the lowest prevalence (2.85%) was found in inigenous breed. Jamunapari (2.85%) breed of goat had 3.5 times higher percentage of reactors as compared to indigenous breed (0.83%) studied by (Rahman *et al.,* 2013)

According to the previous history of tuberculosis and PPD test performed, it was found in this study that most of the farmers 102 (88.7%) reported that there was no previous history of tuberculosis and 91(79.1%) of them stated that there was no PPD test performed although 13(11.3%) of the farmers reported that there was previous history of tuberculosis and 24 (20.9%) of them replied that there was PPD test performed. This may be that there was no prevalence of tuberculosis in this area or may be no accurate examination done on this area. Tafess *et al.* (2011) conducted a study and found 9.6% of prevalence of tuberculosis in Ethiopia. The presently recorded prevalence was almost similar to previous studies (Hiko, 2005; Nigussie, 2005).

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

This study was designed to focus the prevalence of *M. caprae* tuberculosis in Sadar Upazilla farm and villages in Dinajpur distract (Nayanpur and Mirjapur). The experiment 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, 2017. According to this study there was no significant different of the disease between male and female (p=0.746) (Table 1). The highest number of positive TB was found in female goats that are equivalent to 7.14%, while Inclusive was 11.43% and the negative was 81.43%. In terms of males the positive rate was 4.44%, the inclusive test was 8.89% whereas the negative case was 86.67%. In this study showed there was non-significant difference of the disease among goat breeds (P=0.215) as demonstrated in (table 4.2). The highest prevalence rate 11.42% was recorded in the breed of Jamunapari followed by cross breed 6.90% in spite of the lowest prevalence 2.85% was found in indigenous breed. On the other result of the present that the highest number of positive TB in goat 10.00% was found in Mirjapur while the lowest positive rate 3.08% was noted in Nayanpur. According to the previous history of tuberculosis and PPD test performed, most of the farmers 102 (88.7%) reported that there was no previous history of tuberculosis and 91 (79.1%) of the farmers stated that there was no PPD test performed although 13 (11.3%) of the farmers reported that there was previous history of tuberculosis 24 (20.9%) of them replied that there was PPD test performed.

Whereas body condition score of the goat examined were in poor condition and the examined animal included different lactation period, 29.6% of them were lactating stage while 24.3% of them were non lactating period and remaining goats were heifers 46.1%. Moreover the study showed that most of farmers used semi-intensive system because it is cheaper than the other systems and needs less labor. Furthermore, most of the farmers under this study used concrete for flooring system because of it is easy to clean although it is expensive when compared to other systems.

Tuberculosis is zoonotic high contagious diseases caused by *Mycobacterium tuberculosis* complex (MTC or MTBC) occurs both human animal, so the current investigation should be concluded that female goats were higher prevalence of tuberculosis than the males, body conditions of the studied goats were in poor condition, poor management practice such as housing system, nutrition and ventilation and it is also recommended the following:

- To identify and diagnosis detail about the specific strain of goat tuberculosis.
- To make proper management practices such as housing, ventilation, and nutrition
- To separate infected and non-infected animal.
- To give relevant treatment to the goats affected by tuberculosis.
- To determine further investigation on Tuberculosis strain to get the most suitable treatment.
- To make awareness to the farmers to contact to the nearest veterinary hospitals if seen any cases of this disease.

REFERENCES

REFERENCES

A., O., J.A., C. and J.A., M. (2011). Present epidemiology of tuberculosis. Prevention and control programs. *Enfermedades Infecciosas Y Microbiologia Clinica*. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=2011163603.

Aimé B., Lequen L., Balageas A., Haddad N. and Maugein J. (2012). *M. bovis* and *M. caprae* infections in Aquitaine: A clinico-epidemiologic study of 15 patients. *Pathologie-Biologie*, 60(3): 156–159.

Alvarez J., de Juan L., Bezos J., Romero B., Sáez J.L., Gordejo F.J.R. and Aranaz A. (2008). Interference of *paratuberculosis* with the diagnosis of tuberculosis in a goat flock with a natural mixed infection. *Veterinary Microbiology*, 128(1–2): 72–80.

Aranaz A., Cousins D., Mateos A. and Dominguez L. (2003). Elevation of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz *et al.* 1999 to species rank as *Mycobacterium caprae* comb. nov., sp. nov. *Int. J. Syst. Evol. Microbiol.,* 53: 1785–1789.

Aranaz A., Cousins D., Mateos A. and Domínguez L. (2003). Elevation of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz *et al.,* 1999 to species rank as *Mycobacterium caprae* comb. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *53*(6): 1785–1789.

Arellano R.B., Ramirez C.I.C., Diaz A.E., Valero E.G. and Santillan F.M.A. 1999. Diagnosis of tuberculosis in goat flocks using the double intradermal test and bacteriology. *Tecnica Pecuaria en Mexico,* 37: 55-58.

Arellano R.B., Ramirez C.I.C., Diaz A.E., Valero E.G. and Santillan F.M.A. (1999). Diagnosis of tuberculosis in goat flocks using the double intradermal test and bacteriology. *Tecnica Pecuaria en Mexico,* 37: 55-58.

Bangladesh Economic Review (2009). Ministry of Finance, Government of Bangladesh.

BARC Bangladesh (2010). Agricultural Research Priority: Vision-2030 and Beyond (Sub-sector: Livestock). Bangladesh Agriculture Research Council, Farmgate, Dhaka.

Benkirane A. (1998) bovine tuberculosis in Africa, livestock and human needs, conflict or compatibility? World Animal Review, Food and Agriculture Organization of the United Nation, *Animal Production and Health Division*. Vol. 1.

Bezos J., de Juan L., Romero B., Álvarez J., Mazzucchelli F., Mateos A. and Aranaz A. (2010). Experimental infection with *Mycobacterium caprae* in goats and evaluation of immunological status in tuberculosis and *paratuberculosis* co-infected animals. *Veterinary Immunology and Immunopathology*, 133(2–4): 269–275.

Biet F., Boschiroli M.L, Thorel M.F. and Guilloteau L.A. (2005). Zoonotic aspects of *Mycobacterium bovis* and *Mycobacterium avium*- interacellular complex (MAC). *Veterinary Research,* 36: 411-436.

Cadmus S.I., Adesokan H.K., Jenkins A.O. and Van Soolingen D. (2009). *Mycobacterium bovis* and *M. tuberculosis* in goats, Nigeria," Emerging Infectious Diseases, 15(12): 2066–2067.

Cassidy J.P. (2006). The pathogenesis and pathology of bovine tuberculosis with insights from studies of tuberculosis in humans and laboratory animal models. Veterinary microbiology 112: 151-161.

Cfsph (2009). Bovine Tuberculosis. Institute for international cooperation in Animal Biologics an OIE Collaborating Center, Iowa State University, College of Veterinary Medicine, USA.

Chartier C., Mercier P., Pellet M. P. and Vialard J. (2012). Effect of an inactivated *paratuberculosis* vaccine on the intradermal testing of goats for tuberculosis. *Veterinary Journal,* 191(3): 360–363.

Cordes D.O., Bullians J.A., Lake D.E. and Carter M.E. (1981). Observations on tuberculosis caused by *Mycobacterium bovis* in sheep. *New Zealand Veterinary Journal,* 29(4): 60–62.

Cosivi O., Grange J.M., Dabon C.J., Raviglione M.C., Fujikura T., Cousins D., Robinson R.A., Huchzermeryex H.F.A.K., de Kantor I. and Meslin F.-X. (1998). Zoonotic Tuberculosis due to *Mycobacterium bovis* I Developing Countries. Emerging Infectious Diseases 4: 59-70.

Danneberg A.M. (2001). Pathologenesis of pulmonary *Mycobacterium bovis* infection, basic principles established by the rabbit model. *Tuberculosis*, 81: 87-96.

De la Fuente J., Díez-Delgado I., Contreras M., Vicente J., Cabezas-Cruz A., Tobes R. and Gortazar C. (2015). Comparative Genomics of Field Isolates of *Mycobacterium bovis* and *M. caprae* Provides Evidence for Possible Correlates with Bacterial Viability and Virulence. *PLoS Neglected Tropical Diseases*, 9(11): 1–22.

Delahay R.J., Smith G.C., Barlow A.M., Walker N., Harrix A., Clifton-Hadley R.S. and Cheeseman C.L. (2007). Bovine tuberculosis infection in wild mammalsin the South- West region of England , A survey of prevalence and a semi- quantitative assessment of the relative risks to cattle. *The Veterinary Journal*, 173: 287-301.

Department of Livestock Services (2012). Ministry of Fisheries and Livestock Services, Government of Bangladesh.

Deresa B., Conraths F.J. and Ameni G. (2013). Abattoir-based study on the epidemiology of *caprine* tuberculosis in Ethiopia using conventional and molecular tools. *Acta Veterinaria Scandinavica,* 55: 15.

Duarte E.L., Domingos M., Amado A. and Botelho A. (2008). Spoligotype diversity of *Mycobacterium bovis* and *Mycobacterium caprae* animal isolates. *Vet. Microbiol.* 130: 415–421.

Eckert J.P. and Daplez. (2003). Echinococcosis in Animals, Clinical aspect, diagnosis and treatment, pp. 72-99.

Erler W., Martin G., Sachse K., Naumann L., Kahlau D., Beer J., Bartos M., Nagy G., Cvetnic Z., Zolnir-Dovc M. and Pavlik I. (2004). Molecular fingerprinting of *Mycobacterium bovis* subsp. *caprae* isolates from Central Europe.

Food Safety Authority of Ireland (FSAI) (2008). Zoonotic tuberculosis and food safety, 2nd. Ed. L. Lower Abbey Street Dublin I, Ireland. pp. 7-28.

Good M. (2006). Bovine tuberculosis eradication in Ireland. *Irish Veterinary Journal,* 59: 154-162.

Goodchild A.V. and Clifton-Hadly R.S. (2001). Cattle –to Cattle transmission of *Mycobacterium bovis*. *Tuberculosis*, 81: 23-41.

Grange J.M. and Yates M.D. (1994). Zoonotic aspects of *Mycobacterium bovis* infection. *Veterinary microbiology*, 40: 137-151.

Griffin J.F.T. and Buchan G.S. (1994). Aetiology, pathogenesis and diagnosis of *Mycobacterium bovis* in deer. *Veterinary Microbiology,* 40: 193-205.

Gupta U.D. and Katoch V.M. (2005). Animal models of tuberculosis. *Tuberculosis,* 85: 277-293.

Gutierrez M., Samper S., Gavigan J.-A., Garcia, Marin J.F. and Martin C. (1995). Differentiation by molecular typing of *Mycobacterium bovis* strains causing tuberculosis in cattle and goats. *J. Clin. Microbiol.,* 33: 2953-2956.

Hiko A. (2005). Preliminary study on *caprine* tuberculosis in Modjo Export Abattoir. DVM Thesis, Faculty of Veterinary Medicine, Debre- Zeit, Ethiopia.

Hiko A. and Agga G.E. (2011). First-time detection of *Mycobacterium* species from goats in Ethiopia, *Tropical Animal Health and Production*, 43(1): 133–139.

Houlihan M.G., Williams S.J. and Poff J.D. (2008). *Mycobacterium bovis* isolated from a sheep during routine surveillance. *Vet. Rec.,* 163: 94-95.

Jones T.C., Hunt R.D. and King N.W. (1997). *Veterinary pathology,* 6th edition, pp. 489-497.

Kaneen J.B. and Pfeir D. (2006). Epidemiology of *Mycobacterium bovis*, In, C.O.

Kassa G.M., Abebe F., Worku Y., Legesse M., Medhin G., Bjune G., and Ameni G. (2012). Tuberculosis in goats and sheep in Afar Pastoral Region of Ethiopia and isolation of *Mycobacterium tuberculosis* from goat. *Veterinary Medicine International*, 2012.

Kubica T., Rüsch-Gerdes S. and Niemann S. (2003). Mycobactetium *bovis* subsp. *caprae* caused one-third of human *M. bovis*-associated tuberculosis cases reorted in Germany between 1999 and 2001. *Journal of Clinical Microbiology*, 41(7): 3070–3077.

Lepper A.W.D. and Corner L.A. (1983). Naturally occurring mycobacteriosis of animals. In: Ratladge C., Stanford J. (eds.): The Biology of the Mycobacteria. 1st ed. Vol. 2. Academic Press, London, pp. 417–524.

Lieban E., Johnson L., Gough J., Durr P., Jahans K., Clifton-Hadley R., Spencer Y., Hewinson R.G. and Downs S.H. (2008). Pathology of naturally occurring bovine tuberculosis in England and Wales. *The Veterinary journal,* 176: 354-360.

Liébana E., Aranaz A., Urquía J.J., Mateos A. and Domínguez L. (1998). Evaluation of the gamma-interferon assay for eradication of tuberculosis in a goat herd. *Australian Veterinary Journal*, 76(1): 50–3.

McAdams H.P., Erasmus J. and Winter A. (1995). Radiologic manifestation of pulmonary tuberculosis. *Radiologic Clinics of North America,* 33: 655-678.

Moda G., Daborn C.J., Grange J.M. and Cosivi O. (1996). The Zoonotic importance of *Mycobacterium bovis*. *Tubercle and lung Disease,* 77: 103-108.

Neil S.D., Pollock J.M., Bryson D.B. and Hanna J. (1994). Pathogenesis of *Mycobacterium bovis* infection in cattle. Veterinary Microbiology 40: 41-52.

Nigussie, T. (2005). Preliminary study on Tuberculosis in small ruminants slaughtered at HELIMEX export abattoir. DVM, Thesis Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.

Nigussie T. (2005). Preliminary study on Tuberculosis in small ruminants slaughtered at HELIMEX export abattoir. DVM, Thesis Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.

O'Reilly L.M. and Daborn C.J. (1995). The epidemiology of *Mycobacterium bovis* infection in animals and man, A review. *Tubercle and Lung Disease*, 76: 1-46.

Offiong E.E.A., Obioku E.O., Etim U.M., Habib M. and Nkeme K.K. (2014). Incidence of tuberculosis in goats in Abak local Government Area - A case study of Abak Abattoir, *International Journal of Development and Sustainability*, 3(4): 692-695.

OIE (2004). Manual of Standards for Diagnostic Tests and Vaccines of World Organization for Animal Health, 4th edition.

OIE (2009). Manual of Diagnosis Tests and Vaccines for Terrestrial Animals. World Organization for Animal Health, Paris, France.

Palmer, M.V. and Waters, W.R. (2006). Advances in bovine tuberculosis diagnosis and pathogenesis, what policy makers need to know. *Veterinary Microbiology*, 112: 181-190.

Palomino J., Le'ao S. and Ritacco V. (2007). Tuberculosis in 2007 from basic science to patients care. 1st ed. www. Tuberculosis Textbook.com. pp. 687.

Pavlik I. (2006). The experience of new European Union Member States concerning the control of bovine tuberculosis. *In Veterinary Microbiology*, 112: 221–230.

Pavlik I., Bures F., Janovsky P., Pecinka P., Bartos M., Dvorska L., Matlova L., Kremer K. and Van-Soolingen D. (2002). The last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995 was caused by *Mycobacterium bovis* subspecies *caprae*. *Vet. Med. Czech,* 47: 251-263.

Pérez De Val B., López-Soria S., Nofrarías M., Martín M., Vordermeier H.M., Villarreal-Ramos B. and Domingo M. (2011). Experimental model of tuberculosis in the domestic goat after endobronchial infection with *Mycobacterium caprae*. *Clinical and Vaccine Immunology,* 18(11): 1872–1881.

Philips C.J.C., Foster C.R.W., Morris P.A. and Teverson R. (2003). The transmission of *Mycobacterium bovis* infection to cattle. *Research in Veterinary Science*, 74: 1-15.

Pignata W.A., Alves C.J., Azevedo S.S., Dantas A.F.M., Gomes A.A.B., Remígio F.R., Lima F.S. and Mota P.M.P. (2009). Prevalence for *caprine* tuberculosis in semi-arid of Paraíba. *Pesq. Vet. Bras.* 29: 7.

Prodinger W.M., Brandstatter A., Naumann L., Pacciarini M., Kubica T., Boschiroli M.L., Aranz A., Nagy G., Cvetnic Z., Ocepek M., Skrypnyk A., Erler W., Niemann S., Pavlik I. and Moser I. (2005). Characterization of *Mycobacterium caprae* isolates from Europe by mycobacterial interspersed repetitive unit genotyping. *Journal of Clinical Microbiology*, 43: 4984–4992.

Prodinger W.M., Indra A., Koksalan O.K., Kilicaslan Z. and Richter E. (2014). *Mycobacterium caprae* infection in humans. Expert Review of Anti-Infective Therapy, 12(12): 1501–1513.

Quinn P., cartel M., Malkey B. and cartel G. (2004). *Mycobacterium* species In Clinical Veterinary Microbiology. 5th ed. Mosby International Limited, London, Great Britain, pp. 156-169.

Tschopp R., Bobosha K., Aseffa A. *et al.* (2011). Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia *BMC Infectious Diseases,* 11: 318.

Radostis O.M., Gay C.C., Blood D.C and Hinchdiff K.W. (2000). Veterinary Medicine, A text book of the diseases of cattle, sheep, pigs, goats and horses. 9th edition, 909-917.

Rahman M.M., Ferdousur M., Khan R., Nazmul K.H.M., Nazir H. and Rahman M.B. (2013). Prevalence of bovine and avian tuberculosis in sheep and goat population of Bangladesh. *Scientific Journal of Microbiology*, 2(21): 1–8.

Rahman M.M., Ferdousur M., Khan R., Nazmul K.H.M., Nazir H. and Rahman M.B. (2013). Prevalence of bovine and avian tuberculosis in sheep and goat population of Bangladesh. *Scientific Journal of Microbiology*, 2(21): 1–8.

Rainy F., Klattle S., Krappenstedf R. and Stackebrandet E. (1995). Ditizia, a new genus including Ditiziamaliscomb. nov. Formerly *Rhod. Intern. J. of system. Bact.*, 45: 32-36.

Ramirez I.C., Santillan M.A. and Dante V. (2003). The goat as an experimental ruminant model for tuberculosis infection. *Small Ruminant Research*, 47(2): 113–116.

Raval S.K., Trivedi S., Belsare V.P., Kanani A.N. and Patel P.R. (2006). Diagnosis of tuberculosis in animal and man. *Intas- Polivet*, 7(2): 247-250.

Rodríguez E., Sánchez L.P., Pérez S., Herrera L., Jiménez M.S., Samper S. and Iglesias M.J. (2009). Human tuberculosis due to *Mycobacterium bovis* and *M. caprae* in Spain, 2004-2007. *International Journal of Tuberculosis and Lung Disease*, 13(12): 1536–1541.

Schoepf K., Prodinger W.M., Glawischnig W., Hofer E., Revilla-Fernandez S., Hofrichter J. and Schmoll F. (2012). A Two-Years' Survey on the Prevalence of Tuberculosis Caused by *Mycobacterium caprae* in Red Deer (*Cervus elaphus*) in the Tyrol, Austria. ISRN Veterinary Science, 1–7.

Seifert H. (1996). Tropical Animal Health. 2nded. Kluwer Aczadamic Publishers, Dordrecht, the Netherlands. pp. 343-352.

Shanahan A., Good M., Duignan A., Curtin T. and More S.J. (2011). Tuberculosis in goats on a farm in Ireland: epidemiological investigation and control. *The Veterinary Record*, 168(18): 485.

Tafess K., Dawo F., Sori Te. and Ameni G. (2011). Prevalence of *caprine* tuberculosis in Mid- Rift valley area of Oromia, Ethiopia. *African Journal of Microbiology Research*, 5(12): 1473-1478.

Thoen C.O., Steele J.H. and Gilsdorf M.J. (2006). *Mycobacterium bovis* Infection in Animals and Humans. 2nd ed. Blackwell Publishing, Boston, Mass., USA, 329.

Thoen C.O., LoBue P.A., Enarson D.A., Kaneene J.B. and de Kantor I.N. (2009). Tuberculosis, a re-emerging disease of animals and humans. *Veterinaria Italiana*, 45: 135-181.

Thoen C.O. and Barletta R.G. (2006). Pathogenesis of mycobacterium bovis in: C.O. Thoen' Stecle, J.H.; Gilsdorf, M.J. (eds.) mucobacterium bovis infection in animals and humans. Black well publishing Ltd. Lowa;

Une Y. and Mori T. (2007). Tuberculosis as a zoonosis from a veterinary perspective. Comparative Immunology, Microbiology and Infectious Diseases, 30(5–6): 415–425.

United Nations (2011). Department of Economic and Social Affairs, Population Division: World Population Prospects: The 2010 Revision. New York.

Val B.P., de Vidal E., Nofrarías M., López-Soria S., Cardona P.-J. and Domingo M. (2014). Assessment of Goat Tuberculosis Model for Use in Vaccine Trials. *Procedia in Vaccinology*, 8: 43–49.

Wedlock D.N., Skinner M.A., de Lisle G.W. and Buddle B.M. (2002). Control of *Mycobacterium bovis* infections and the risk to human populations. Microbes and infection, 4: 471-480.

Wilsmore T., Taylor N. (2008). Bovine Tuberculosis, an update. Veterinary Epidemiology and Economics Reseach Unit, The University of Reading, UK 132.

Zanardi G., Boniotti M.B., Gaffuri A., Casto B., Zanoni M. and Pacciarini M.L. (2013). Tuberculosis transmission by *Mycobacterium bovis* in a mixed cattle and goat herd. Research in Veterinary Science, 95(2): 430-433.

APPENDIX

APPENDIX

Questionnaire sheet

1. Particulars of the owner

i. Name…………………………………………………………………

ii. Address……………………………………………………………

2. Particular of goat

i. Breed : Indigenous ☐ Jamunapari ☐ Cross breed ☐ Others ☐

ii. Sex : Male ☐ Female ☐

3. Management practice

i. Housing system : Intensive ☐ semi- intensive ☐ Others ☐

ii. Housing floor condition : Mud ☐ Concrete ☐ Cage ☐ Other ☐

iii. Ventilation facilities : Good ☐ Poor ☐

4. Lactation stage: Lactating ☐ Non-lactating ☐ Heifer ☐

5. Body condition score : Poor Medium Good

6. Previous history of tuberculosis: Yes ☐ No ☐

7. Tuberculin test performed previously: Yes ☐ No ☐

8. Clinical signs (if any)

9. CITT: POSATIVE ☐ Negative ☐

Supervisor Signature

………………………