CHAPTER II

LITERATURE REVIEW

2.1 Tubercle bacillus

The bacteria of genus *Mycobacteria* are gram positive, non-motile and non-sporulated rods, slow growing acid fast microorganisms. They have high lipid content in the wall, probably the highest amongst all of the bacteria. Lipids involve more than half of the dry weight of the mycobacteria. The composition of lipids may vary the duration of life cycle in culture, depending on the accessibility of nutrients. They are classified in the suprageneric rank of actinomycetes that, extraordinarily, have a high content (61-71 %) of guanine plus cytosine (G+C) in the genomic deoxyribonucleic acid (DNA). Idiosyncratic characteristics of the genus *Mycobacteria* are acid fastness, extreme hydrophobicity, resistance to injury, many antibiotics, and distinctive immunological properties (Palomino, 2007).

2.1.1 Lineage of *Mycobacterium Tuberculosis* Complex

Kingdom	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Subclass	Actinobacteridae
Order	Actinomycetales
Suborder	Corynebacterineae
Family	Mycobacteriaceae
Genus	*Mycobacterium*
	unique genus
Species	*M. tuberculosis*
	M. bovis
	M. africanum
	M. microti
	"M. canettii"
	M. caprae
	M. pinnipedii

Source: http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Tree&id=1760&lvl=3&lin=f &keep=1&srchmode=1&unlock (Palomino, 2007)

2.1.2 *Mycobacterium tuberculosis* complex (*M. tuberculosis* complex)

Genus Mycobacterium comprises more than 100 species. Mycobacterium species are found in the environment and are not normally correlated with disease in humans or animals. However, a small number of these are pathogenic. These pathogenic mycobacteria are similar morphology and staining characteristics with saprophytic mycobacteria. Mycobacteria are slow growing organisms and it takes 3 to 6 weeks to grow on culture media (Kaneene and Thoen, 2004). Mycobacteria are resistant to acid decolorizing agents. Although several staining procedures are available for acid-fast bacilli, Ziehl-Neelsen or Kinyoun techniques with carbol fuchsin are most widely used.

Generally *M. tuberculosis* complex bacteria (MTBC) are host adapted but with the ability to spill over into other species (Strain et al., 2011). *M. bovis* and *M. microti* are causative agents of TB in animals, and can be transmitted to human. Sometimes *M. pinnipedii*, and *M. caprae* are identified as *M. bovis* subspecies or variants; they have been isolated from goat and seals (Palomino, 2007).

Identification of *M. bovis* and *M. tuberculosis* is not easy due to their closely relatedness. Generally, *M. tuberculosis* strains produce luxuriant (eugenic) growth on egg-based media and produce large amount of niacin whereas *M. bovis* does not produce this vitamin and there is poor (dysgenic) growth on egg-based media. According to the problems encountered in the identification of *M .bovis*, and the need to strains isolation by culture, only a few laboratories can differentiate it from *M. tuberculosis*. In developing countries, this reason caused limited information on the zoonotic important worldwide (Moda et al., 1996).

Different host species susceptibilities vary for *M. tuberculosis* complex based on the route of exposure, dose of organisms and virulence of the strain. Theon et al., (2009) summarized that human, non-human primates and guinea-pigs are very susceptible to *M. tuberculosis*. Cattle, rabbit and cats are quite resistant to *M. tuberculosis*, but susceptible to *M. bovis*. Swine and dogs are susceptible to both *M. bovis* and *M. tuberculosis*. Generally wild hoofed animals are susceptible to *M. bovis*; however a few reports are available on the isolation of *M. tuberculosis*. In Thailand,

M. tuberculosis infection was confirmed in Asian elephants using conventional and molecular diagnostic assay (Angkawanish et al., 2010).

2.1.3 Maintenance hosts of *M. bovis* and susceptible species

Despite cattle are primary hosts for *M. bovis*, other domesticated and wild mammals can also be infected. It has been known that brush–tailed opossums (and possibly ferrets) in New Zealand, badgers in the United Kingdom and Ireland, bison and elk in Canada, and kudu and African buffalo in southern Africa were identified as maintenance hosts. White-tailed deer in the United States (Michigan) have been classified as maintenance hosts. Some authors supposed this species perhaps spillover host when population density is high. Species reported to be spillover hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas, many species of wild ruminants including deer and elk; elephants, rhinoceroses, foxes, coyotes, mink, primates, opossums, otters, seals, sea lions, hares, raccoons, bears, warthogs, large cats (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents. Most mammals may be susceptible to *M. bovis* (CFSPH, 2007a).

2.2 Importance of zoonotic tuberculosis

There were 1,415 pathogens known to infect human, 61% were zoonotic (https://en.wikipedia.org/wiki/Zoonosis). Zoonoses have been realized that major global threat to human health. Zoonotic disease, an infectious disease, transmitted from animals to human and other than human to human or other animals (Palmer and Whipple, 2006). Since bTB is one of the notifiable zoonotic diseases, public health concerned worldwide and economic impact in animal trade and animal product, need to address effective control and eradication scheme to be established. It also occur major socio-economic impact. The expense of diagnosis and treatment of cattle and human and the costs of correct disposal of infected animal carcasses have an additional impact (Proaño-Perez et al., 2009).

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2.2.1 bTB risk in human

Zoonotic TB or bTB is an OIE listed disease. Although most people are at low risk to infect with *M. bovis*, people who work with cattle, bison or cervids, or products from these animals including hides, milk, or meat are at high risk. (Eg. people working in a dairy farming or a slaughter house as a butcher, and hunting) Raw milk drinking and consumption of dairy products made from raw milk are also at high risk. It is hypothesized bTB is a food born disease transmitted by milk because of declining numbers of human cases despite massive increases in affected cattle is consistent (Torgerson and Torgerson, 2010).

2.2.2 Route of transmission and clinical presentations in human

In human, symptoms of *M. bovis* infection are similar to the symptoms of *M. tuberculosis*; including fever, night sweats, and weight loss. Other symptoms might occur due to different part of the body affected by the disease. For example, disease in the lungs produces cough and disease occur in gastro-intestinal can cause abdominal pain and diarrhea. If untreated, person can die of disease (http://www.cdc.gov/tb) (mbovis.pdf).

Clinical symptoms of human disease due to *M. bovis* are related to the route of transmission from animals. The respiratory route of infection most frequently found in workers exposed to infected animals (farmers, veterinarians, slaughterhouse workers) and rural people who live in close contact with their animals. It may cause pulmonary disease. Infection through break skins causes cutaneous, tendon and localized lymph nodes lesions in person handling of infected carcasses. It also caused the preliminary disease in children involved the cervical lymph nodes (scrofula), and the consequence of alimentary route of infection is extra-pulmonary forms of tuberculosis occurred in intestinal tract, kidneys, bones and central nervous system. Contaminated raw milk and related products are the main source of infection. Secondary contamination of milk from the environment and tuberculosis udder by a single cow can excrete a large number of bacteria sufficient to infect pooled milk. Post-mortem inspection of

affected organs or whole carcasses decreases hazards to consumers (Moda et al., 1996).

The three main explanations for the lack of accuracy and systematic estimation of the contributation of *M.bovis* to the global TB burden are; (i) there is no difference between TB caused by *M. tuberculosis* or that of *M. bovis* at the clinical and radiological level; (ii) most laboratories use Löwenstein-Jensen culture medium with glycerol, which does not encourage *M. bovis* growth. Moreover, cultivation is always an expensive option for many low-income countries compared to the cheaper and faster acid-fast staining. (iii) treatment of TB caused by *M. tuberculosis* or *M. bovis* was the same in most cases; therefore, there was no clinical interest in differentiating the causative agent (Palomino, 2007). Last three to four decades, bTB was eradicated in many developed countries by tuberculin skin testing and mandatory slaughtering of animals. In these countries, human TB due to *M. bovis* dramatically decreased and sporadic cases occur in elderly people by reactivation of ancient infections or in immigrants from countries where bTB has not been eradicated (Palomino, 2007) .

In England and New Zealand, bTB was not completely eliminate and re-emergence due to wildlife animals such as brush–tailed opossums and badgers (CFSPH, 2007a). The persistence of *M. bovis* in wildlife is frequently indicated as the main cause of this re-emergence. Alternatively, in many low-income countries, bTB continues to be an important animal health problem.

2.2.3 Symptoms and microscopic pathology of bTB in cattle

In cattle, clinical signs vary with the distribution of tubercles in the body. Formation of tuberculous lesions in lungs, retropharyngeal, bronchial and mediastinal lymph nodes are characteristic lesions of bTB infection. Lesions can also found in the other organs such as mesenteric lymph nodes, liver, spleen and on serous membrane. Clinical signs are not specifically distinctive in subclinical stage. Clinical evidence of disease may not become apparent in chronic cases until the terminal stage of disease. The animals may show signs of dyspnoea with an associated cough where progressive pulmonary disease exists. An enlarged spleen and/or liver may be palpated on

physical examination if visceral lesions are present. Regional lymph nodes may be enlarged in advanced cases and in some cases may rupture and drain to the surface. Emaciation is as a result of inappetence in chronic cases (Thoen et al., 2009). In advanced tuberculosis, clinical sings include weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and cough (OIE, 2009).

In microscopic examination, necrosis and mineralization are found in the central areas of the tubercles, with borders of epithelioid cells and well-defined capsules composed of fibrous connective tissue. Multinucleated giant cells are commonly observed. The expression of a tubercle is a granulomatous lesion, characteristically composed of a caseous, necrotic centre bordered by a zone of epithiloid cells, some of which may have formed multinucleated giant cells, an accumulation of lymphocytes, a few granulocytes and encapsulation of fibrous connective tissue of varying thickness (Thoen et al., 2009).

2.2.4 *M. bovis* cases in human TB

In UK, TB caused by *M. bovis* was a major public health issue before the introduction of milk pasteurization in the 1960s. Within this period, around 2,500 people died annually from bTB. Therefore control measures were introduced to eliminate bTB from UK. Although bTB was eliminated most part of Britain in 1970s, re-emerged in 2007 resulting new herd breakdowns in England and Wales (Torgerson and Torgerson, 2010).

Cattle are the main host of *M. bovis* but the most frequent cause of zoonotic TB occurs in man. Infection caused by *M. bovis* is difficult to distinguish with TB caused by *M. tuberculosis* with regard to pathogenesis, lesion and clinical findings. Before milk pasteurization, *M. bovis* was an important cause of human TB, especially intestinal TB in children (Palomino, 2007).

The proportion of human TB cases caused by *M.bovis* produces considerable regional variation depending on the presence and extent of disease in the cattle population. It also based on social and economic situation, food hygiene practice and implementation of preventive measure. The reported proportion is lower than actual

proportion in some region due to failure of laboratory facilities to differentiate with *M. bovis* and *M. tuberculosis* (Moda et al., 1996).

In the last 50 years, research on zoonotic TB was influenced by scientific trends, societal worries such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and contaminated food, as well as by the availability of tools for the identification of the bTB bacillus (Palomino, 2007).

All of human TB cases caused by *M. bovis*, 2.1% were pulmonary and 9.4% were extra- pulmonary (Cosivi et al., 1998). In global, around 9 million new TB cases and 2 million deaths are annually reported (CDC, 2007). Only 0.1% of TB caused by *M. bovis* occurred in USA where tuberculosis in cattle was virtually eradicated. But the proportion rises in 3% near the border with Mexico where bTB are still widespread. In Egypt, the proportion of human disease due to *M. bovis* varied 0.4% to 6% in different hospitals. Moreover, in some developing countries, HIV pandemic is favourable human to human transmission of *M. bovis*. It is one of the additional important factors leading rapidly to disease (Moda et al., 1996).

2.2.5 Economic important of zoonotic tuberculosis

In England and Wales, there were 4172 new herd breakdowns occurred in 2007 (Torgerson and Torgerson, 2010). The expenditure is due to program implementation and there was no report for animal health costs. Although bTB control program can provide some benefits to cattle industry, none of the cost-effective studies in terms of animal health, welfare and productivity. The economic effect of the blocking of live cattle exports is important consideration if bTB were terminated. However, the cost of the bTB control program is in excess of the live exports from the UK (Torgerson and Torgerson, 2010).

Additionally, different epidemiological scenarios can be observed. For example, in Argentina, Brazil, Mexico, and Venezuela, meat and bovine products are important resources and the number of cattle equals or exceeds that of the human population and it would be bTB high risk. Livestock industry is less developed and intensive in these countries. Cattle husbandry is a family business for milk consumption or retail commercialization. In Central American countries, African

countries and China, cows are preserved for milk and meat is consumed from other species; for example sheep and swine that are less susceptible to *M. bovis.* Although a high proportion of people do not eat cattle meat in India, do consume milk and are close contact with cattle. It may increase at risk of *M. bovis* infection. bTB has eradicated in some low income countries including Cuba, Mongolia, and Costa Rica, because the cattle population is relatively small in these countries (Palomino, 2007).

2.3 Global tuberculosis

Human tuberculosis (TB) caused by the bacillus *Mycobacterium tuberculosis* mainly affects the lungs (pulmonary TB) and can also affect the other sites (extra-pulmonary TB). In particular, TB can be transmitted by inhalation of air droplets from infected person or animals. It is an important example of re-emerging diseases that become a problem, again for significant part of the population, after decreasing the incidence rate (G. Quaglio et al., 2012). Nowadays, more than one third of the world's population is infected with tubercle bacilli. People dying with tuberculosis are more than other disease. It has been estimated that the disease results in the deaths of 2 to 3 million people each year (Kaneene and Thoen, 2004).

Over 95% of TB deaths occur in low and middle income countries (http://www.who.int/mediacentre/factsheets/fs104/en/index.html). According to WHO report, 8.8 million new TB cases in 2010 , 1.1 million deaths from TB among HIV-negative people and an additional 0.35 million deaths from HIV- associated TB. Additionally, 10 million children were orphans as a consequence of parental deaths caused by TB (2009). In 2010, there were 3.2 million TB cases 0.32 million of women deaths from TB. 13% of TB cases occur in HIV infected people. It has been recognized that TB is the second leading cause of deaths from an infectious disease worldwide (WHO, 2011).

TB Infection is more common in men than women, and mostly affected in economically productive age groups; 15-59 years of aged groups. It can be estimated that around two-thirds of cases occur within these aged groups (WHO, 2011) .

In the World Health Organization (WHO) European Region, 418,000 new TB cases were estimated in 2010. More than 60,000 deaths in the WHO Region were

estimated as being due to TB.TB notifications have been decreasing since 2005, indicating the lower incidence of TB. However, the prevalence of MDR-TB among new TB cases increased from 12% in 2009 to 13.7% in 2010. In total, the WHO Region reported more than 29,000 MDR-TB patients, of whom 13.2% are estimated to be already extensively drug resistant (XDR-TB). Indeed, despite a decrease in TB incidence, drug-resistant TB is becoming a major concern(G. Quaglio et al., 2012). TB is still on the priority list of the re-emerging diseases and it is threatening to both human and animal health issues around the world.

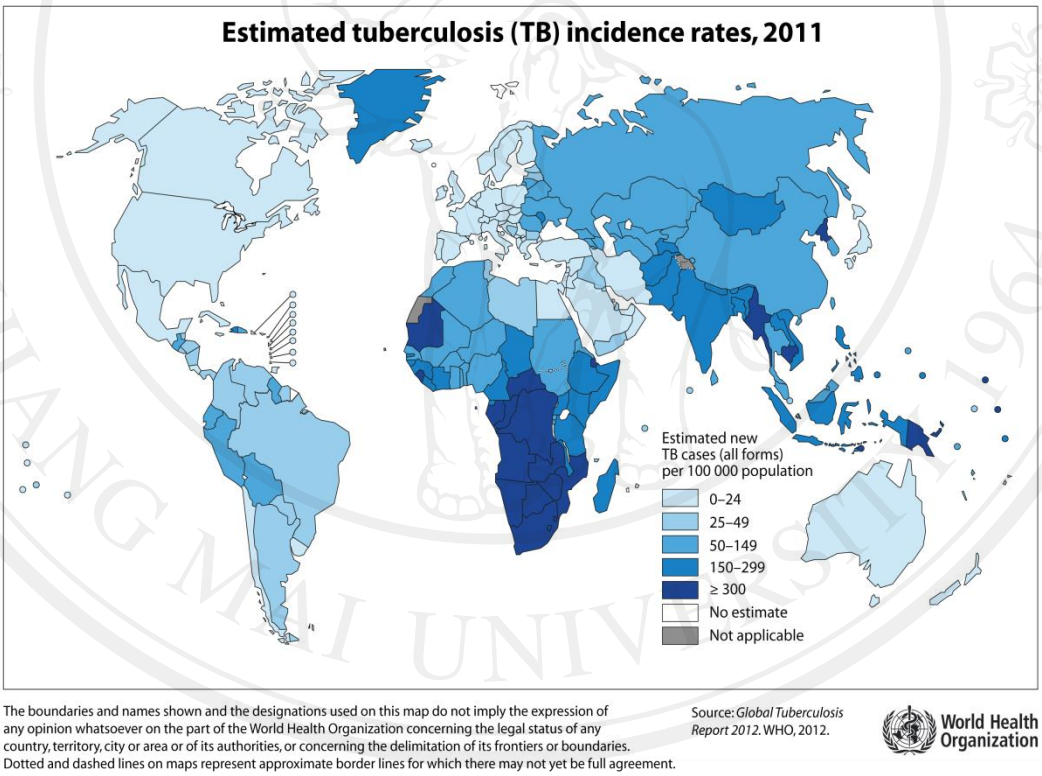

Figure 2: Estimated TB incident rates, 2011

Source: http://reliefweb.int/sites/reliefweb.int/files/resources/map_3148.pdf

2.4.1 Viability of *M. bovis*

Various durations of environmental survival of *M. bovis* are reported in the literature depending on the conditions under which the research has been conducted. Early work suggested that *M. bovis* is a highly resistant organism surviving in cow feces, for at least 5 months in winter, 4 months in autumn, 2 months in summer up to 2 years in soil; 4 months in liquid manure stored underground, and 1-2 months in soil during the summer months (Williams and Hoy, 1930).

The study of *M. bovis* viability under different environmental conditions reported that survival time was the longest in winter in infected specimens. Viable *M. bovis* could be recovered for up to 6 weeks from lung tissues and up to 4 weeks from feces. In tissue specimens, viability showed a five-fold decrease from winter to summer, whilst in fecal samples, survival times of *M. bovis* were vary only by 1 week throughout the year. Subterranean specimens were found to be negative for *M. bovis* at all times except for spring when a successful isolation was made from specimens buried for 5 days. Survival time in feces was slightly longer in winter at moist but not in sunny sites (Tanner and Michel, 1999).

The study conducted in Michigan described that survival time of *M. bovis* on various feed and effect of maintenance temperature. *M. bovis* survived on all feedstuffs at all temperatures tested for at least 7 days. At 23 °C, it could be isolated from samples of apple, corn and potatoes at 112 days (Palmer and Whipple, 2006).

Fine et al., (2011) reported that persistence of *M. bovis* in the environment was significantly shorter in the spring or summer season and *M. bovis* persisted up to 88 days in soil, 58 days in water and hay, and 43 days on corn.

2.4.2 Transmission of *M. bovis* and minimum infectious dose

Transmissible route of tubercle bacillus was through the air, contaminated feed and water. Mycobacteria can be isolated from the nasal secretions of cattle reacting to tuberculin. Pulmonary exudates of the cows are usually swallowed; consequently the organisms pass with the feces to contaminate the ground and feed. Congenital transmission of TB has been recorded. Genital lesions have been reported; therefore,

the importance of spread by this route should be considered in a herd where infection persists (Thoen et al., 2009).

The minimum infectious dose is highly dependent on the route of infection. However, 1 CFU of *M. bovis* developed pulmonary pathology of bovine tuberculosis in calves that were infected with intratracheal route of infection (Dean et al., 2005). In this study, 20 calves from bTB free farms were randomly selected and inoculated intratracheally with *M. bovis* field strain GB (AF 2122/97). This route of infection selected was similar to natural infection and lesions were more closely occurred in naturally infected cattle. Variation doses were 1 CFU, 10 CFU, 100CFU, 1000 CFU and then infected animals were tested with tuberculin test. All of the skin positive animals produced visible lesion in respiratory lymph node and lung lobes (Dean et al., 2005).

One review article described many studies reported that infection via oral route required greater doses than infection via aerosol. Indeed, doses as low as 1-5 bacilli resulted in infection via aerosol, while 10-20 million bacilli were required to infect via the oral route (Good and Duignan, 2011).

2.4.3 Detection of bovine tuberculosis

bTB is a complex multiple species endemic disease (Krebs, 2012). It has been realized that from 176 countries as one of the important bovine diseases causing great economic losses (Hines et al., 1995). *M. bovis* and *M. avium* are animal origins (Thoen, 1994) and *M. bovis* infection has been identified as a zoonotic disease with most cases of human infection attributable to animal sources (Rabozzi et al., 2012). Zoonotic tuberculosis is of great consequence in public health concern worldwide. In addition to the economic losses it causes, because of the zoonotic property of the disease and its chronic progressive nature, effective eradication program is needed. In the study reviewed by (Good and Duignan, 2011) described Finland was the first country to commence a successful bTB eradication program using tuberculin test in the late 1890s. The effectiveness of test and removal program commenced for bovines was rapidly declined bTB incidence in the population. Thus, economic losses due to

bTB declined simultaneously as the cattle population became healthier (Good and Duignan, 2011).

In some developed countries, due to the proper eradication efforts, infections caused by *M. bovis* have been reduced significantly (Moda et al., 1996). In developing countries, particularly in Africa and parts of Asia, TB caused by *M. bovis* still increases due to the lack of proper eradication program (Thoen et al., 2009). In Asia, bTB control policy was adopted in a few countries; there are very few publications on zoonotic TB. It is needed a more active search for *M. bovis* on the Asian continent, where high TB burden (Palomino, 2007).

Eradication programs are proceed in other European countries, New Zealand, the United States, Mexico, some countries of central and South America. Besides, nations currently classified as tuberculosis free involve Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel. Although control programs had been eliminated in domesticated animals, white-tailed deer in Michigan, badgers in U.K and Ireland and infected brush-tailed possums in New Zealand are causes of wildlife complicated eradication efforts (Thoen et al., 2009).

TB is difficult to diagnose only based on the clinical signs. Although bacteria culture is the gold diagnostic method of bTB, mycobacteria grow slowly and cultures are incubated for eight weeks. The standard method for the detection of bTB is the tuberculin test, called delayed hypersensitivity test, used in test and slaughter control program (OIE, 2009).

The comparative intradermal skin test with bovine PPD and avian PPD is used to identify the infected animal which is caused by *M. bovis* or other mycobacteria (OIE, 2009). In U.S, the single intradermal test (bovine PPD only) is used for the preliminary screening of the cattle and reactors are re-tested with comparative intradermal test. But in Europe, a comparative intradermal test is used for the initial screening of the cattle (CFSPH, 2007b).

In addition, diagnosis blood tests are now available, for example, the lymphocyte proliferation assay, the gamma-interferon assay and enzyme-linked immunosorbent assay (ELISA). The lymphocyte proliferation test may be useful in

wildlife and zoo animals and uncommon in cattle. All of the blood tests are relatively expensive (OIE, 2009).

Theon et al., (2009) described that slaughter surveillance is useful in some animals to identify typical gross lesion of tuberculosis. Tissue culture and PCR methods are the only way to confirm the diagnosis of tuberculosis. In addition many pathogenic strains of mycobacteria are slow growing and cultures are incubated at 37^{o} C for 8-10 weeks. Currently, PCR and RFLP are available in most reference diagnostic laboratories (Thoen et al., 2009).

The effectiveness of the diagnostic test is mainly dependent upon the prospects placed upon the test. The diagnostic test may probably need to detect all kinds of animals such as infected animals, infectious animals or all animals exposed to the pathogen. The tests are necessary to identify all infected animals in most cases. Nevertheless it can be argued that only those animals infectious or likely to become infectious need be identified by current diagnostic tests in terms of disease control at the national level over time. For example an animal infected with bTB but unlikely to become infectious, either because it will be slaughtered at a young age or it is able to control the infection, if not, it can contribute to the maintenance of the disease in long term period. This may be particularly important in the case of tuberculosis. In the case of human TB most infected individuals will not progress to active disease (Strain et al., 2011).

2.4.3.1 Tuberculin test (Delayed Hypersensitivity test)

The tuberculin skin test is the primary diagnosis test for tuberculosis in both human and cattle. It is called Mantoux test in human TB detection and performed by intracutaneous injection of 5TU(Tuberculin Units) of PPD (Thoen et al., 2009). But in animals particularly in cattle intradermally injected tuberculin PPD; doses based on disease situations. For caudal fold test, bovine PPD injection at least 2000 International Units (IU) is recommended in OIE manual. In the comparative tuberculin test, the doses should be no lower than 2000 IU each. The interpretation varies on the basis of the test method used (OIE, 2009). Moreover a higher dose of bovine tuberculin is needed in cattle with weaken allergic sensitivity and in national

eradication campaigns, doses up to 5000 IU are recommended. It should be recognized that the volume of each injection dose must not exceed 0.2 ml (OIE, 2009).

Variations of injected areas for tuberculin skin tests depend on animal species; in exotic cloven hoofed animals, tests are inoculated in the cervical region; in camelids, the tests are conducted in the axillary region just behind the front leg. In non-human primates, 0.1ml (5000 TU of USDA-PPD) is usually administered into an upper eyelids and the site is detected at 24, 48 and 72 hours after injection. Skin test is conducted in dorsal surface of the ear and vulva in swine using the dose of 5000 TU PPD. In horse, skin tests conducted in an eyelid or in the cervical region but some horses produce no response to tuberculin. Skin test can be applied in dogs and the injections sites are chosen as an eyelid, in the cervical region or in the medial aspect of a rear leg. 5000TU of *M. bovis* and *M. avium* PPD can be used and the injection should be examined at 48 hours (Thoen et al., 2009).

It is also called delayed hypersensitivity test as a result of inflammatory reactions initiated by mononuclear leukocytes. The term delayed is used to differentiate a secondary cellular response, which appears 48-72 hours after antigen exposure, from an immediate hypersensitivity response, which generally appears within 12 minutes of an antigen challenge. T cells and macrophages are primarily involved in delayed hypersensitivity reactions. First, local immune and inflammatory responses at the site of foreign antigen up-regulate endothelial cell adhesion molecule expression, enhancing the accumulation of leukocytes at the tissue site. The antigen is engulfed by macrophages and monocytes and is processed and presented to a T cell that has a specific receptor for that processed antigen. The characteristic histological appearance of the macrophage–T-cell infiltrate is a granuloma. This type of infiltrate in the tissue is called granulomatous inflammation. So, these reactions are mediated by T cells and monocytes/macrophages rather than by antibodies and they are also termed by type IV hypersensitivity reactions (Abramson, 2011).

Delayed hypersensitivity may not progress for a period of 3–6 weeks after infection. So, if an animal is suspected in contact very recently with infected animals, delaying testing may cause false-negatives. Likewise, in chronically infected animals with severe pathology, the tuberculin test may be unresponsive. As the sensitivity of

the test is less than 100%, it is unlikely that eradication of tuberculosis from a herd will be achieved with only a single tuberculin test (OIE, 2009).

The sites of injection can be chosen in the mid-neck region and caudal fold of the tail. There is no validation to use tuberculin test in non-bovid and non-cervid species.

Tuberculins are crude antigen preparations derived from heat-killed cultures of mycobacteria and contain mixture of proteins, polypeptides, nucleic acids, and substantial amounts of polysaccharides (Angus, 1978).

Tuberculin purified protein derivative, bovine (Tuberculin PPD, bovine) is a preparation obtained from the heat-treated products of growth and lysis of *Mycobacterium bovis* capable of revealing a delayed hypersensitivity in an animal sensitized to micro-organisms of the same species. The most commonly used strains are *M. bovis* AN5 or Vallee.

The organism is cultured in liquid synthetic medium; the active fraction of the filtrate containing protein is isolated by precipitation, washed and re-dissolved. An antimicrobial preservative that does not give rise to false positive reactions, such as phenol, may be added. The final sterile preparation, free from mycobacteria, is dispersed aseptically into sterile, neutral glass containers which are then closed so as to prevent contamination. The preparation may be freeze-dried (OIE, 2009).

2.4.3.2 Single Intradermal test and Comparative Intradermal test

The single intradermal test (SIDT) contains only bovine PPD. In SIDT test, the positive reactors may either be caused by *M. bovis* infection or other mycobacteria. In addition, inconclusive reactors animals tested with SIDT test should be subjected to another test after an interval of 42 days to allow desensitisation to wane(in some areas 60 days)(OIE, 2009) .

The comparative intradermal test (CIDT) can be used to distinguish between animals infected with *M. bovis* and other mycobacteria those are responding to bovine tuberculin. This sensitization can be attributed to the antigenic cross-reactivity among mycobacterial species and related genera. CIDT test involves both bovine and avian tuberculin PPDs and inject into different sites of the neck. The distance between the

two injections should be approximately 12–15 cm. The skin-fold thickness of each injection site is measured before injection and 72 hours after injection. The same person should measure skin thickness before and after injection.

Within the European Union, the intradermal skin test is one of two currently approved tests for bovine TB. It is based upon the measurement of a delayed type hypersensitivity response to intradermally injected tuberculins usually conducted either in the neck or caudal fold. In general the skin of the neck is regarded as more sensitive. In order to compare antigenic cross- reactivity, particularly other mycobacteria, many countries have adopted a comparative test approach. In these cases comparisons are made between the responses to bovine PPD and avian PPD, with reactions characterized as positive where there is a greater response to bovine PPD (Strain et al., 2011). In addition, animals with thinner skin thickness produced larger tuberculin response influenced on CIDT test (Muma et al., 2013b).

In cattle tuberculin tests are based on detection of the specific immunological response following exposure to *M. bovis* or indeed *M. caprae* at some period previously. Infection will have occurred following exposure and probably either progressive or become inactive based on the infective dose or inherent immune system of the animal. The caudal fold test is widely used in the USA and New Zealand and was also used in Australia during their bovine TB eradication campaign. There are also other regions of the world where this is the routine test of choice with or without use of the CIDT before animal removal (Good and Duignan, 2011).

2.4.3.3 Performance of the CIDT

Indeed, the performance of the tuberculin skin test can be affected by environmental factors, the prevalence of TB, host factors such as status of immunity, genetics, etc., and the nature of the tuberculin test used (Boukary et al., 2011). The CIDT test remains the best single test currently used (Strain et al., 2011) and primary bTB surveillance tool in several countries worldwide. It can be used as a confirmatory test of caudal-fold and mid-cervical test reactors. The application of this test is central to restoring the officially TB-free status of infected herds in Great Britain (Karolemeas et al., 2012).

A large number of evidence supports that CIDT test has high specificity with the typical estimation of greater than 99.9%. On the other hand, the sensitivity of the test remains moderate and typically estimates that 50-60% with the standard test interpretation. It mainly depends on the quality and potency of the reagents used i.e. bovine and avian PPDs. These reagents are principal reagents for both CIDT test and gamma interferon test. There is substantial evidence of considerable variation in the quality of PPD tuberculins produced around the world due to some producing very poor potency and leads to failure detection of disease with poor specificity in some cases. It is indicated that further study to improve PPD standard potency and performance in order to give additional assurance of their quality (Strain et al., 2011).

On the other hand, using more stringent skin test interpretations leads to increase test sensitivity but with reduced specificity. It has been shown that the application of severe interpretations will lead to improve disease control and clearly identify the situations where increased sensitivity is desirable and a somewhat reduced specificity acceptable (Strain et al., 2011). Although cutoff value of CIDT test is > 4mm used in worldwide, the study conducted in central Ethiopia reported that the sensitivity of CIDT test using cutoff value > 2mm was 69% without affecting specificity (97%) (Ameni et al., 2008).

Estimation of sensitivity and specificity for diagnostic tests can be dependent upon the animal population studied and the disease characteristics within the population. Moreover, there is now substantial evidence that other factors influence not only the course of the disease but also the sensitivity and perhaps the specificity of the most commonly used diagnostic tests. These important factors are co-infection with parasites particularly liver fluke and Johne's disease which may influence the diagnostic sensitivity of both the comparative skin test and the interferon-gamma test (Strain et al., 2011). In the study conducted in England and Wales, cattle with co-infection of *Fasciola hepatica* and bTB disease failure to response of CIDT test diagnosis. Nonetheless, there was negative association with bTB and *F. heptica* infection (Claridge et al., 2012).

2.5 bTB worldwide distribution

The global distribution of *M. bovis* infection in animals and humans varies widely. Several North American and western European countries have established bTB eradication program using test and slaughter method, consequently very low or sporadic occurrence of the disease Elimination schemes are progress in Australia and New Zealand, eastern Europe, Israel, Japan and some central and south American countries (Moda et al., 1996). However, bTB prevalence can be influenced by several factors. Overview of tuberculin test surveys in Ecuador demonstrated that prevalence of bTB varied by study areas. One of the bTB surveys in Ecuador used both single and comparative intradermal tests although in most studies PPD antigens used were not reported (Proaño-Pérez et al., 2011).

2.6 Prevalence and risk factors of bovine tuberculosis worldwide

There are numerous risk factors for bTB identified in cattle so far in different parts of the world. Risk factors of bTB are classified by animal level, herd level and region/country levels (Humblet et al., 2009). However, risk factors may vary across the region and operate at different scales (Skuce et al., 2012). For example, the age of the animals is one of the main individual risk factors identified by several studies in both developed and developing countries (Humblet et al., 2009).

Mostly bTB studies in Africa highlighted individual level risk factors associated with bTB transmission in which lack of effective bTB control program and large number of cattle population. However even bTB eradication program had been costly implemented, is still endemic in the United Kingdom and Ireland because of wildlife population (Skuce et al., 2012). In addition, purchasing of cattle, the occurrence of bTB in contiguous herds, and/or the surrounding area as well as in herd size were identified herd level risk factors of bTB in one epidemiological study (Johnston et al., 2005). Some studies have identified at risk on herds and farm management practice such as the use of housing types, the spreading of slurry, farms having multiple premises, and the use of silage clamps (Skuce et al., 2012).

A case-control study conducted in UK revealed that two housing variables, namely covered yard housing and other housing types, operating herds over multiple premises were associated with an increased risk of herd break down. This study described that movement of cattle onto the farm from market or farm sales was the strongest risk factor associated with an increased risks of bTB (Johnston et al., 2005).

Regarding the bTB studies in Africa, the overall prevalence of bTB in Zambia was 4.8% (4mm cutoff value) and 6.3% (3mm cutoff value) (Muma et al., 2013a). The study conducted in the Southern Highlands of Tanzania has been mentioned that overall prevalence of bTB was 13.2% and 51% in herds tested animals. Age, gender and cattle with exotic blood were at risk of bTB in this study. It also described that there was no influence of reactivity to tuberculin on the reproductive status and lactating cows (Kazwala et al., 2001).

In some countries, a number of factors have been examined the association between bTB and cattle herds. The other studies had also demonstrated that herd size has an influence on the prevalence of bTB (R.R.kazwala, 2001, Ameni et al., 2008, Kazwala et al., 2001). bTB prevalence survey in Torodi (Niger) demonstrated that overall apparent individual animal prevalence of tuberculin reactors was 3.6%, whereas the individual true prevalence was estimated at 0.8%. This study also described household level risk factor with the presence of coughing animals in the herds (Boukary et al., 2011).

In Zambia, true prevalence of herd level bTB was estimated at 49.8% (95% CI: 37.9, 61.7%) after weighing according to sampling fraction. This study identified geographical area and being transhumant were significant risk factors associated with bTB status (Munyeme et al., 2008). One of the studies carried out in Ethiopia estimated the apparent prevalence was 32% by performing three kinds of tests; CIDT test, Bovigam IFN-γ assay and lateral flow assay. The prevalence was varied from 23-25% when a pair of test was used and from 9% to 15% when a single test was used. Holstein, cross-breeds and zebus cattle were performed in this study. In addition, since Ethiopia has no routine bTB screening test, the use of tests which measure cellular and antibody responses may help for maximum detection of disease (Ameni et al., 2009).

In 2009, the study performed in four regions of Ethiopia reported the overall prevalence was 3.1% and amongst the highest prevalence was 7.9% and the lowest one was 1.2%. This study interested in rural livestock production system and ninety-nine percent of tested animals were traditional Zebu cattle. It also identified significant risks for bTB were purchase of cattle and presence of other livestock in the herd (Tschopp et al., 2009).

Moreover Ethiopia has the largest cattle population in Africa and overall herd prevalence of bTB in dairy cattle was over 50% in central Ethiopia. This study also described age, herd sizes and education level of owners were significant risk of bTB status (Firdessa et al., 2012).

Clearly, three studies in Ethiopia represented bTB prevalence varied from different regions in different breeds of cattle and variations of significant risk factors based on geographical situations (Tschopp et al., 2009, Firdessa et al., 2012).

The prevalence and distribution of bTB in Latin America is poorly understood. The disease is officially endemic to 7 of the 34 countries in Latin America. Prevalence is high only in the Dominican Republic, 12 countries have reported as sporadic/ low occurrence of bTB, and there are no data for the remainder (Cosivi et al., 1998). The cross-sectional study carried out in north of Ecuador revealed that true prevalence of bTB was 7.14% in 2007 and 7.13% in 2008. The number of skin positive animals also increased with age, contact with other animals and introduction of new cattle and these factors were identified at risk for bTB. According to the results of CIDT test, herd size was recognized as a significant risk factor because of large herds having a much higher prevalence. Based on the result of this study herd prevalence in Ecuador was 55% in 2007 and 65% in 2008 (Proano-Perez et al., 2009).

Humblet et al., (2009) summarised that animal level risk factors are age, gender, body condition, immune status, genetic resistance and susceptibility to bTB, vertical and pseudo-vertical transmission and auto-contamination .

The arrival of new animal should be performed tuberculin test if there is either unknown history of bTB or suspected closely contact with bTB infected animal. Skuce et al., (2012) summarized some previous studies, mostly in the UK and Ireland recognized a number of risk factors associated with bTB herd breakdowns. These studies described that the purchase of cattle, the occurrence of bTB in contiguous

herds and /or the surrounding area were risks for TB herd breakdown. Cattle movements and trading were most frequently identified risks for herd-herd transmission. In order to produce more effective ways of reduce transmission, important to understand risk factors which influence the presence or absence of bTB in cattle herds. However, risk factors operate at different scales and may vary across regions (Skuce et al., 2012).

The occurrence of *M. bovis* infection in domesticated animals and human indicates that the more research to be done to understand the risk factors for the future control of the disease in the world. The real picture and incidence of the *M. bovis* in developing countries especially in Asian countries is underestimated and it is crucial to explore the pattern and nature of the disease in there even though a significant progress for the control of the disease in advanced industrialized countries (Enarson, 1995).