CHAPTER V

DISCUSSION AND CONCLUSIONS

5.1 Discussion

The current study conducted comparative intradermal test in dairy cattle. Bovine PPD was inoculated at caudal region in 140 heads of cattle. Avian PPD was injected at cervical region in 138 heads of cattle. Comparative intradermal tuberculin test is currently used as screening test in both developed and developing countries and in most of bTB studies as well (Ameni et al., 2008, Proano-Perez et al., 2009, Barry et al., 2011, Kazwala et al., 2001, Munyeme et al., 2008).

This study compared skin thickness measurements before and after injections using paired T-test. And then differences in responses of PPDs were compared with Welch's T-test. All results of these comparisons were significant differences ($p < 0.01$). One study described that comparing the responses for bovine PPDs in cervical and caudal tuberculin tests, the results were significant different ($p < 0.01$). They also compared two kinds of bovine PPDs responses in caudal fold tests, the results were not significant different ($0.1 > p > 0.05$) (Errico et al., 1989).

As regard in avian PPD, skin swelling differences were larger than bovine PPD in this study. It can be assumed that environmental mycobacterium might have induced sensitization to avian PPD. One of the studies conducted in Uruguay described that mycobacteria were isolated from cattle with larger responses of avian PPD (Errico et al., 1989).

However, we cannot interpret the positive reactor using the interpretation criteria of comparative intradermal test. Positive reactors identification was according to OIE manual in caudal fold test. So, negative reactor, inconclusive and positive reactor were identified based on skin swelling differences ≤ 2mm, 3mm and ≥ 4mm receptively(OIE, 2009). Surveillance study of bTB in Indian cattle farms, declared the

positive animal with equal to or > 4mm skin swelling difference (Veerasami et al., 2012).

The current study found that five positive animals from two dairy farms amongst nine tested farms. Positive reactor 1 and 2 were found in one of the two farms and positive reactor 3, 4and 5 came from the remainder one. So, these two farms can be assumed that bTB positive farms in study area. Positive reactor 5 was purchased from Lop Buri Province, 6 yrs 2mths of age and showed the largest skin difference. The left four animals were born in the farm and aged around 1-2 years. In this study, the positive reactor cow purchased from Lop Buri Province was the eldest age among positive reactors. It was assumed that the positive response of young animal might be contributed from the old one. Moreover, the age of the animal was identified as the main individual risk factor in numerous studies (Kazwala et al., 2001, Humblet et al., 2009, Boukary et al., 2011). Introduction of new animals or purchasing of animals was the potential risk factor for bTB in some studies (Okafor et al., 2011, Humblet et al., 2009).

The findings of this study revealed that 4% of the tested animals were positive reactors. 86% showed negative responses for tuberculin test. However, it can be considered that bTB positive farm if at least one positive reactor found in that farm. There were two positive farms among nine tested farms in our study. Many studies in Africa identified positive farm or herd if at least one positive reactor was found (Tschopp et al., 2009, Munyeme et al., 2008).

Regarding the percentage of positive, inconclusive and negative reactors based on skin thickness differences of bovine PPD are 4% (≥ 4mm), 10% (3mm) and 86% (≤ 2mm) receptively. There was small amount of positive reactor and estimated prevalence was about 4-5% in Mae Wang District (DLD official data). Moreover the overall prevalence of bTB in cattle and buffaloes was 0.5% in 2011 and Thailand performed routine tuberculin test with bovine PPD for controlling bTB (DLD (PVS), 2012).

For avian PPD responses at cervical region, percentages of responses based on skin swelling differences were 81% (< 4mm), 17% (4-7mm) and 2% (> 7mm) receptively. There was no scientific interpretation for only avian PPD response.

However, the differences in skin thickness for avian PPD were larger than that of bovine PPD.

In this study, 46 dairy farm owners answered questionnaires. Amongst 85% of dairy farms were owned by men and the remaining 15% were owned by women. Nearly all of the farms are semi-intensive farming system and similar husbandry practice.

This study couldn't assess risk factors for bTB status in study area based on questionnaires answers but investigated the management system and biosecurity level of dairy farming.

Some of the studies in Africa were considered that risk factors of bTB status including direct contact of infected animals and drinking of unpasteurized milk. The review article illustrated that three levels of bTB risk factors contain animal level, herd level and region or country level (Humblet et al., 2009).

Risk factors identification was required to analyze association between positive reactor and questionnaires survey. But this study found that only 4% of positive response animals and couldn't received complete questionnaires answers. So, we can describe the condition of the farming system, feeding and management system of dairy farms in our study area.

Regarding in the management system, housing types were good ventilated. Good ventilation system can diminish aerogenic disease transmission. Overall, 40% of dairy farms had good drainage system, some farms used pressure pump for cleaning manure. There was a few farms stored manure in the tanks (6%), 33% of the farms directly used manure for vegetation. Manure management plays an important role for disease contamination. The study in South Africa report that *M.bovis* was isolated from feces samples up to 4 weeks (Tanner and Michel, 1999).

Feed storage condition was poor in some dairy farms due to lack of rodents and insects protections. Nearly all of the farms mixed the feed with separate barns, feed troughs were clean everyday and used feed bags before expire date. All dairy farms had manual feeding system, machine milking and most of the farms used mixed feed with concentrated and roughage. The review article of worldwide bTB risk factors in cattle summarized that many studies reported feed and manure storage were herd level risk factors (Humblet et al., 2009). In this study, underground water or

natural water was used in 50% of the farms, treat water was used in a few farms. bTB prevalence survey in Torodi described that 51% of households used only natural water to clean equipments, milking and milk processing (Boukary et al., 2011).

Dairy cows were annually administered with FMD vaccine and variations of deworming program were 1 or 2 or 4 times per year. It could be effective for low bTB transmission because high parasitic loads might decrease the animal resistance and make it more susceptible to bTB (Tschopp et al., 2009).

Eighteen farms owned 90-100% Holstein Friesian (HF) crossed-breed and sixteen farmers had 70-90% HF crossed-breed since AI was particularly used for breeding program. Breed of the cattle was identified as individual risk factor in some studies among imported and indigenous breeds. But it was depend on relation between different susceptibility and different managements including under intensive farming (Humblet et al., 2009, Kazwala et al., 2001).

About 60% of farmers informed the veterinarian if the animals were sick. Some farmers and volunteers also cured the sick animals. Respiratory diseases, reproductive diseases and gastrointestinal diseases were currently occurred. Clinical symptoms such as emaciation, coughing and anoxia were found in some farms but not frequently. Amongst them reproductive diseases were common.

In terms of biosecurity status, only a few farms had records for visitors. But washing facility, shower practice, disinfection, pet animals control and isolating the sick animals were found in more than 50% of the farms. As a result of this finding described that fifty percent of dairy farms in Mae Wang district had good farming practice.

Regarding the quarantine practice, 15 of 46 farmers implemented quarantine practice for new animals. But duration of quarantine period was about 7-10 days. It was not enough to prevent disease transmission. The current study presumed that although the farmers have knowledge of quarantine practice, they don’t apply in practical. Quarantine practice was not practically implemented in some developing countries particularly in rural area. Duration of quarantine period should be 3 to 5 weeks (20 to 40 days). This interval permits the farmers to detect general condition of the animals and clinical symptoms concerned with bTB (Boukary et al., 2011).

Only 14% of the farmers said they have been known bTB disease in cattle. Eleven percent of farmers said sometime they consumed raw milk. It would be at risk of bTB transmission since raw milk consumption is one of the transmissible routes. A recent bTB outbreak in Ireland was as a result of raw milk consumption (Torgerson and Torgerson, 2010). In terms of human TB, no one had been experience with human TB. The farmers in study area don't know *M.bovis* infection contributed to human TB. So, educating the farmers concerned with zoonotic TB is necessary.

Baseline information of Veterinary Service in Thailand reported to OIE described that bTB control program was annually performed and the overall prevalence of bTB was 0.5% (DLD (PVS), 2012). There was no potential risk factor identification in this study. However, this study emphasized farm management in dairy cattle husbandry. In order to success bTB eradication program, other susceptible animals such as beef cattle, buffaloes and pig, etc, are required to investigate. Moreover, bTB susceptible wildlife animals including elephants, tigers and cervids are also considered for disease control program.

Some poultry farms, dairy farms and pig farms were situated around tested dairy farms within 1-2 kilometers. The current study didn't investigate animal movement restriction in study area. Some poultry farms were located very closely in study area. It might be at risk for infectious disease transmission. We expected that further study should be investigated more detail than our study.

Besides, the findings of this study cannot imagine restriction of cattle movement during confirmation of tuberculin testing for inclusive reactors. Inclusive reactors may enhance the re-emerging of pathogens. It plays an important role of control and eradication program. So, further study need to investigate based on this fact. Results from this study highlight cell-mediated immune responses of bovine and avian PPDs.

In Thailand, Dairy Farming Organization distributes dairy farming knowledge to farmers in order to improve farming system. It was about 76% of the farmers were educated from Dairy Farming Promotion Organization of Thailand (DFPOT). In terms of education level, 40% of farmers had primary school level, 19% had secondary school level, 9% had high school level education and 33% were graduated

and professional. Although nearly half of the farmers had primary school level, they were educated for dairy farming.

5.2 Conclusions

The current study found that cell-mediated immune responses occurred due to cross reactivity of environmental Mycobacterium. Despite in bovine PPD positive responses were very low, avian PPD responses were high in study area. But this study cannot reach the detail interpretation for avian PPD. The results of the study postulated that the age of the animal, purchasing of animal from outside and inadequate quarantine period might be at risk for bTB status in Mae Wang. Besides, we assumed that biosecurity status was medium level in dairy farms based on questionnaires. Even a small percent of the farmers consumed raw or unpasteurized milk; most of the farmers never drink raw milk. In addition farm workers need to know relation between human TB and bTB so that they can prevent zoonotic infection contribute to human. Therefore, education of farmers concerned about bTB disease may facilitate improvement of preventive measures.

Recommendations based on our findings are:

a. Adequate duration of quarantine practice should be practically implemented if purchasing or introduction of new animal.
b. Improve biosecurity status for every farm in order to prevent transmission of diseases.
c. Feed storage condition would be increased in terms of good quality food for cattle.
d. Good drainage system and manure management should be improved in some dairy farms.
e. To educate the farmers, raw milk consumption is at risk of bTB transmission and important for public health concerned.
f. Relatedness of human TB and zoonotic TB is necessary to explain the farmers by co-operation with veterinarians and medical doctors.

g. Based on our findings, we strongly recommend that educate the farmers what importance of bTB and how to prevent zoonotic disease transmission in livestock husbandry are.

Overall recommendation for dairy farming system, good farming practice including good biosecurity status and quarantine practice should be implemented practically. Hence, zoonotic disease transmission from animal to human would be reduced. In order to success bTB control and eradication program, establishing the animal movement restrictions, culling the infected animals and sanitary slaughtering are required. Remuneration for culling animals is also essential for test and slaughter control program.

In addition, further studies need to conduct receiving of scientific data for bTB in developing countries. If properly implemented above suggestions, zoonotic disease transmission can be substantially eliminated and economic impact and public health risk from bTB would be decreased. To achieve these goals, integration of veterinary and human medicines, policy makers and farmers is necessary to solve the problems of animal origin.