CHAPTER V
DISCUSSION

Melioidosis is an important public health problem in Southeast Asia and Northern Australia (Ashdown & Guard, 1984; Dance, 2000; Leelarasamee & Bovornkitti, 1989). The *B. pseudomallei* infection is most common in rice-farming communities, where the likelihood of infection depends on the degree of exposure to this environmental organism (Suputtamongkol et al., 1994). As a result, the risk is very high during the ploughing and planting of rice paddies. The infection exhibits broad clinical manifestation in human and animals, varies form acute sepsis to a chronic localized infection. Furthermore, recurrent infection can occur despite adequate and prolong therapy (Chaowagul et al., 1993; White, 2003). The mechanisms underlying the development of different forms of disease remain poorly understood. The different of the disease outcomes were depended on the infective dose, the bacterial strain used, host factors and route of infection (Hoppe et al., 1999; Leakey et al., 1998; Liu et al., 2002; Santanirand et al., 1999). The septicemia form occurred in 60% of patients with melioidosis (Wuthiekanun et al., 1990). The mortality rate of the septicemia form is very high, most patients are die within 2 to 3 days if they are not properly diagnosed and treated.

The kinetics of antigen and antibody responses in various form of melioidosis patients at different times are not clearly investigated. In the present study, we generated BALB/c mice model for acute and chronic infections. These animal models were used because the disease outcomes is similar to of human melioidosis. This animal was used to investigate the kinetic response of virulent *B. pseudomallei* (A2) and antibodies responses. In acute infection model, which received very high dose of virulent *B. pseudomallei*, the bacteria in blood was detected at 12 h then increased and all infected mice have bacteremia after 24 h post infection. All mice died within 3 days after infection. This indicated acute fatal infection in these animals. The bacteria could be detected in blood, liver and spleen by culture and PCR starting from 12 h after infections. However the percent positive was found to be higher in spleen and liver (60%), where as only 20% was detected in the blood. At this time, the sensitivity

of PCR and culture were similar. The bacteria in blood at 24 h post infection could be detected in all infected mice. The course of bacteremia was different to that described by Leakey et al., (Leakey et al., 1998) in the same mice model, they demonstrated that the bacteria in blood of BABL/c mice following intravenous inoculation with 37 CFU of *B. pseudomallei* could be detected starting from 24 h post infection then rapidly increasing, peaked at 72 h and all infected animals died at 96 h post infection. The result of the present study demonstrated that *B. pseudomallei* can be detected in blood of acute infection starting 12 h after exposure. However, all acute infected mice had the bacteria in their blood at 24 h which peaked at 60 h post infection. The use of culture gave 100% positive after 24 h of infection. This result is similar to previous reports (Gauthier et al., 2001; Liu et al., 2002). The bacteria number of in the spleen is higher and persisted longer than any other organs. This correlated with the bacteria in blood determined at the different time points of infection. The bacterial load in liver was peaked at 48h post infection then rapidly decreased. This might be related to the clearance mechanism in early stage of the infection. Hoppe et al. reported the lower bacterial load in livers than in spleens during intravenous infection and found evidence for a phagosome-lysosome fusion process resulting in the degradation of *B. pseudomallei* in hepatocytes (Hoppe et al., 1999).

By using PCR method for detection of *B. pseudomallei* DNA. Previous studies demonstrated a highly sensitive, specific and simple method to detected *B. pseudomallei* DNA in blood sample (Rattanathongkom et al., 1997). They used LPS1 and LPS2 primer to amplify 178-base pair sequence in *B. pseudomallei* isolates. To our surprise, no amplification of this sequence was seen in *B. pseudomallei* (A2) tested under the same conditions used. This result suggested that it might have sequences variation in amplicon of LPS1/LPS2 in our A2 *B. pseudomallei* template that made it failed to detected *B. pseudomallei* (A2) DNA in our current study. However when the amplicon sequence (putative transposase) was blast against *B. pseudomallei* genome databases, this sequence was found in all available *B. pseudomallei* genome databases (K96243, 1106a, 1710b and 668).

The PCR method described here can be used for the detection of *B. pseudomallei* (A2). Primers wcbGfor/wcbGrev were able to amplify a 323-base pair fragment of *B. pseudomallei* capsule gene. The sensitivity of this method is 1 pg and at least 5 CFU

was needed for detection using the described PCR technique. However the capsule gene cluster of *B. pseudomallei* was used to design primers for PCR because it was demonstrated to be related the virulence of the bacteria in infected hamsters (Reckseidler-Zenteno et al., 2005). This therefore made them lower sensitivity of detection since the gene is normally expressed in low levels and could be up regulated in the presence of 30% normal human serum (Reckseidler-Zenteno et al., 2005). Moreover the wcbG is found only in *B. pseudomallei* and *B. mallei* but not in *B. thailandensis* (Kim et al., 2005).

The specific antibody responses to a CF were analyzed by using ELISA method, this antigen was found to be highly sensitive and specific for the detection (92.6% and 96.8%, respectively) (Wongratanacheewin et al., 1995). The mean levels of Ig against *B. pseudomallei* in plasma of acute infected mice was low and was not significantly different from PBS control group ($P > 0.05$). This data demonstrated that *B. pseudomallei* antibody could not be detected in early time of *B. pseudomallei* infection. These findings are similar to those of Appassakij and coworkers (Appassakij et al., 1990), who demonstrated that patients with acute sepsis do not exhibit significant IHA antibody levels, thus resulting in false negative reports. It is reasonable to mention here that this is a limited role for antibody determining in acute melioidosis patients. In acute phase, we therefore suggested using either culture or PCR for diagnosis.

In chronic model which 6 CFU of *B. pseudomallei* (A2) was given intraperitoneally, most of infected animals gave negative blood culture. The few number of bacteria (mean±SE = 1.82±1.82 – (3.23±3.21)x10^2 CFU/ml) was detected in blood between 1st to 5th day after infection. Four of five mice died in 1 day later they have bacteremia and another one (1/5) died in 2 day later. This might not be chronic infection because they have symptom and die within 5 days. These results associated with previous studies, which showed high mortality rates of septicaemic melioidosis (Chaowagul et al., 1993; Chaowagul et al., 1989; Smith et al., 1995). Our results indicated that when lower numbers of bacteria were used to infect the mice, most animals did not give positive bacteremia as detected by PCR and culture. This model might be extrapolated to human melioidosis in case of chronic and subclinical

infection. We therefore recommended to use antibody detection in stead of PCR or culture.

For the specific *B. pseudomallei* antibody detection, the mean of specific Ig levels was first detected at 5th days, peaked at 14th days post-infection and enhanced relative to control mice (P = 0.001) whereas in acute mice the antibodies was still low until day 3rd that the time of all infected mice were died. Their specific antibody persisted until 21st day post-infection and then decreased. By day 28 post infection (as the end time point of study) antibody levels were still higher than those observed in PBS injected mice (P = 0.05). This information indicated indirectly that the mice were infected and they might be chronic or subclinical infection. Our result revealed that in contrast to acute infection, in the low dose infection, the antibodies will go up earliest as 5 days after infection and it could be used as a marker for diagnosis.

In summary, this study demonstrated the kinetics of antibody and bacterial loads in blood and different organs in BALB/c mice infected with low and high doses of *B. pseudomallei*. The results demonstrated that blood culture and PCR are very good tools for early detection of the bacteria in acute infection whereas antibody levels were useful for infection in mice with infection more than 5 days. The present study might be used as guideline to develop sensitive and specific diagnosis in human melioidosis. Further study will be extended to the relapse mouse model in order to investigate the antigen and antibody response for diagnosis.