行政院衛生署疾病管制局 95 年度自行研究計畫

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計畫名稱：二仁溪流域之類鼻疽流行病學調查

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目錄

中文摘要

南台灣二仁溪流域從 2001 年 11 月至 2006 年 8 月觀察類鼻疽年發生率(36-125/100,000)，發現呈明顯增加趨勢。另外，比較南台灣地區類鼻疽菌土壤分離率與土壤類鼻疽菌 PCR 陽性檢測率(NSC95-DC-1008 研究案)，發現類鼻疽菌的最高分離與 PCR 檢測陽性位址，亦座落於二仁溪流域，並且分離率與血清抗體升高的驅勢有相互關聯性。故本計畫以此區域為範圍，抽取當地居民(n=624)手臂血清，調查類鼻疽之鞭毛抗體血清盛行率高達 10.9-36.6％ 。此外，本計畫於血清抗體調查之同時，亦對取樣居民進行問卷訪視(n=624)，顯示血清抗體陽性民眾，都具有居家淹水與赤足於田間工作經驗。交差比對後，居家淹水因素，更明顯較赤足工作重要，這可能是台灣地區罹患類鼻疽病的重要流行病學因子。本計畫的研究結果顯示，台灣地區二仁溪流域確實是高度類鼻疽盛行的地區，並且是目前國際間第一個位於大於北緯 20°的地方流行區域。

中文關鍵詞：類鼻疽症、二仁溪流域、血清盛行率

英文摘要

From Nov, 2001 to Aug, 2006, a raised annual incidence of melioidosis (36-125/100,000) was observed in the Er-Ren River Basin, southwestern Taiwan. In this area, sero-surveillance of melioidosis (n=624) indicated the 10.9-36.6 % of sero-positive rate for inhabitants. Using bacterial isolation combined with PCR-based detection of *B. pseudomallei* from soil specimens demonstrated the geographical distribution of this bacterium was targeted at a focus site within the Er-Ren River Basin. The presence of this bacterium in environment tended to be correlated with a higher rate of melioidosis sero-positive individuals. In addition, a questionnaire survey of local individuals (n=624) indicated that sero-positivity was linked to experiencing flooding and walking barefoot on soil; these would seem to be potential risks factors associated with acquiring melioidosis. Results indicated that the Er-Ren River Basin, southwestern Taiwan, is a hyper-prevalent area for melioidosis. This is the first reported hyper-prevalent area located north of >20°N latitude.

Keyword: melioidosis, the Er-Ren River Basin, sero-positive rate

前言(Introduction)

As a potentially fatally infectious disease, melioidosis typically occurs as a result of infection with *B. pseudomallei* and is endemic to Southeast Asia and northern Australia *(1).* Human infection with *B. pseudomallei* is usually via inhalation or subcutaneous inoculation and only rarely through ingestion *(2).* Clinical manifestation includes a variety of symptoms ranging from an unapparent localized chronic infection to a fully blown systemic infection. After the onset of acute septicemia, the mortality is around 40% *(3).* Worldwide, fatal pulmonary melioidosis has been increasingly recognised among returning travellers from endemic areas *(30).*

B. pseudomallei is a saprophyte and widely distributed in tropical soil and water but with an uneven distribution *(2).* The presence of *B. pseudomallei* in soil is associated to some degree with areas having a highly incidence of melioidosis *(6-8).* Most patients with melioidosis in Thailand were farmers suffering from heavy exposure to *B. pseudomallei* during agricultural activity *(31).* Human exposure to *B. pseudomallei* may occur early because detectable specific antibodies against melioidosis in human sera have been found at birth when the child dwells in melioidosis-endemic areas such as northeast Thailand *(11).* In non-endemic areas, the seropositive rate is relatively low because individuals have few chances to contact with the pathogenic bacterium *(10, 16).* However, the rate of seroprevalence is raised when a country develops towards endemicity, for example, East Timor *(9).* The distribution of sero-positive titres tends to correlate with the annual incidence rate of melioidosis, and is also correlated with the geographical distribution of *B. pseudomallei* in the environment *(5, 32).*

Melioidosis in Taiwan was first described in 1984 and was diagnosed as a

pulmonary infection after a drowning incident near Manila, Philippines *(12)*. Since 1994, the numbers of reported cases of melioidosis in Taiwan have been steadily increasing *(13)*. In clinical experiences, this infection appears to have become indigenous rather than arising from travel to endemic areas *(13, 21-23)*. The clinical manifestations of melioidosis are quite protean and therefore clinical diagnosis is often difficult. Thus, the true incidence of melioidosis may actually be higher than is currently believed *(4, 14)*. Previously, we have demonstrated that *B. pseudomallei* can be isolated from cropped soil in southern Taiwan *(15)* and that city dwelling individuals have a sero-positive rate for melioidosis of about 5% *(16)*. Whether Taiwan will develop overt and widespread melioidosis is not clear. From Nov, 2001 to Aug, 2006, the case distribution of melioidosis was investigated and this indicated that the Er-Ren River Basin had the highest rate of annual incidence of melioidosis in Taiwan. In this study, a range of epidemiological evidence including sero-prevalence, geographical distribution of *B. pseudomallei* and a questionnaire set to local inhabitants demonstrated that there is a higher risk of acquiring melioidosis in the Er-Ren River Basin.

材料與方法(Materials and Methods)

Patients, serum sampling and questionnaire

From November 1, 2001, to August 31, 2006, a total of 133 melioidosis cases were officially documented by the Centers for Disease Control (CDC), Taiwan. The etiology of *B. pseudomallei* infection in documented patients allows the identification of the disease by biochemical tests and morphological characterization of clinical isolates from blood or localized infected sites. Over the period of February 2005 to September 2005, 21 serum samples (group 1) were collected from individual documented patients with melioidosis. From April to May, 2006, 22 volunteers who had acquired melioidosis with definitive hospital identification in the last 7-9 months ago were requested to donor their blood for this study (group 2). Between these two sampling, 6 out of 22 of the serum samples overlapped. All specimens were collected by a staff nurse following an IRB procedure established by CDC.

Approximately, 66,103 persons have addresses in the 32 villages surrounding the Er-Ren Basin. Between February 2006 and April 2006, 624 serum samples were collected from individuals living in this area by random sampling from each village based on the allocation of area's demography. All sera were kept at -80^{o}C. At the same time as the blood sampling, the same 624 inhabitants were asked to fill in a lifestyle questionnaire.

Serodiagnosis

The serum samples were tested for melioidosis using indirect ELISA *(17).* Briefly, 96-well polystyrene microtiter plates were coated with *B. pseudomallei* flagellin (0.5 μg/ml) in coating buffer (50 mM carbonate/bicarbonate buffer [pH 9.6])

at 4°C overnight. The plates were blocked for 2 h using 100 µl of bovine serum albumin (1 mg/ml; GIBCO, Grand Island, N.Y.). After being washed with saline-Tween solution (0.9% [wt/vol] NaCl and 0.05% [vol/vol] Tween 20 in phosphate-buffered saline [PBS]) three times, the wells were incubated at 37°C for 1 h with twofold serial dilutions of the sera in PBS. The wells were then washed with saline-Tween solution and incubated with diluted (1:1,000) anti-human IgG conjugated with peroxidase (Zymed, South San Francisco, Calif.) at 37°C for 1 h. The wells were washed again with PBS three times, and 100-µl volumes of 1-Step Turbo TMB-ELISA substrate (Pierce) were added. The OD_{450} of each well was determined using a microplate reader (Anthos 2010). When the average of the OD readings of the test sample was greater than that of the negative controls plus 2 standard deviations, the test sample was considered to be positive for the specific antibody. The highest dilution of the tested sample that still gave a positive result was considered the endpoint titer and listed on the data sheet.

Soil sampling

Soil samples were collected from various cropped fields that were located on both sides of the main Er-Ren River and its branches. The sampling sites were separated by between 0.5 km and 1 km and stretched from Kuan-Yin village to Wan-Fu village. In total, there were 311 sampling sites where a hole with a depth of 30-60 cm was dug. Approximately 100 g of soil sample were obtained from the bottom of the hole and placed into a sterile tube. Each site was independently sampled three times during the survey.

Polymerase chain reaction (PCR) detection

The genomic DNA of soil bacteria was isolated using a purification kit (IsoQuick; ORCA Research Inc. USA). Two primer sets (forward: 5'-CGG CAG CGC GGG CTT CGG-3', reverse: 5'-TGT GGC TGG TCG TCC TCT C-3' and 5'-CAC TCC GGG TAT TAG CCA GA-3', for 16S RNA gene; forward: 5'-CTG TCG TCG ACG GCC GTG-3', reverse: 5'-ATT GTT GAC CGT CGC GAG-3', for flagella gene) were used to amplifying species specific amplicons (243 and 405 bp for 16S RNA gene; 267 bp for flagella gene) *(15, 18)*. The PCR reaction mixture consisted of 1 pg genomic DNA, 0.5 µmol of each primer, 250 µmol/L deoxynucleotide (dNTP), 1X PCR buffer and 1 U Taq polymerase with a final volume of 50 µl. The PCR profile consisted of 40 cycles of 1 min at 94^{o}C, 30 s at 60^{o}C, and 1 min at 72^{o}C, with a final extension step of 10 min at 72^{o}C. The products were visualized by 1.5% of agarose electrophoresis. When amplicons of both the 16S RNA gene and the flagella gene were observed, the sample was considered to be positively for *B. pseudomallei (15).*

Enrichment, culture and identification of *B. pseudomallei*

A 15-g soil sample was placed into 50 ml of Ashdown's broth *(19)* in a 250-ml flask. The flask was shaken vigorously for 5 min and then incubated at 150 rpm/min and 42^{o}C for 2 d. The cultures were repeatedly streaked onto Ashdown's medium. The plates were incubated at 37^{o}C for 2-6 d to allow the dry, wrinkled, violet-to-purple colonies typical of *B. pseudomallei* to grow. These typical colonies were stored in Luria-Bertani (LB) broth containing 15% glycerol at -80^{o}C for further identification. The environmental isolates of *B. pseudomallei* were confirmed by biochemical tests and their molecular characteristics. The biochemical tests were performed using a ID32 GN profile (API system; bioMerieux, France). The molecular characteristics

were accorded to the presence of the specific amplicons for the 16S RNA and flagella genes (see above).

Statistical evaluation

Statistical analyses were carried out using the chi-square test (Epi Info, version 5.01b, 1991) and the chi-square exact test (StatXact, version 2.05, 1991). The significance of differences between two groups was defined as $p<0.005$.

結果(Results)

In a previous study, we demonstrated that 93.8% of melioidosis with acute septicemia in Taiwan can be detected by the presence of specific antibodies against the flagella protein (flagellin) of *B. pseudomallei* in the patient's sera with a cutoff value of 1:512 *(17)*. In this study, 19 out of 21 (90.5%) of the serum samples that were afresh collected from individual patients with systemic or localized melioidosis were still sero-positive (Table 1). A group of serum samples collecting from melioidosis patients up to six months before and this gave a detection rate of 72.7% (16/22) for seropositivity. Amongst the duplicate samples from within this group, 50 % (3/6) were demonstrated to be seropositivity seven months after the illness (Table 1). The results indicate that detectable anti-flagellin antibodies are generated at 10.8±6.7 days after commencement of identifiable symptoms in a patient and remain detectable for at least 7 months.

From November 2001 to August 2006, 133 cases of melioidosis were documented in Taiwan. Out of these cases, 72 (54.1%) lived in the area surrounding the Er-Ren River Basin, southwestern Taiwan (Fig. 1). It would be useful to determine if persons dwelling in this area have a higher chance of exposure to *B. pseudomallei*. Therefore, 624 local inhabitants from the 32 villages surrounding in the Er-Ren River Basin were randomly sampling for serum based on the allocation of district demography (Table 2). According to significantly raised seropositivity ($p<0.005$) in the inhabitants, Er-Ren River Basin could divided into three regions for the ranking of seropositive rate (A>B>C). Each region could subdivided into several sites (A1 and A2; B1, B2 and B3; C1, C2 and C3) in accordance to geographical barriers such as

river segmenting or discontinuous location (Fig. 1). No significant difference in seropositive rate was found in each sub divisible site. Inhabitants in region A shows a significantly higher sero-positive rate (36.6%) and this region had a reported rate of 125/100,000 for the annual incidence of melioidosis (Table 2). In neighboring region B (B1-B3), there was a sero-positive rate of 21.6% and an annual incidence of 63/100,000. An earlier study showed that 5% of city dwelling adults in southern Taiwan were sero-positive for anti-flagellin antibodies *(16).* Furthermore, the sero-positive rate for region C of 10.9% was little higher than the rate for southern cities in Taiwan (p=0.056), which is linked to a lower annual incidence rate for melioidosis of 36/100,000 (Table 2). These results indicate that the sero-positive rate was positively correlated with incidence of melioidosis in the Er-Ren River Basin region of Taiwan.

The geographical distribution of *B. pseudomallei* was surveyed to try and link exposure to this bacterium in the Er-Ren River Basin with infection. Only at an eastern site within region A, site B3, had a positively bacterial isolation (26.4%) for *B. pseudomallei* from the cropped fields. Nevertheless, an extensive region was detected to presence of *B. pseudomallei* with PCR-based techniques. There was 6.7% and 3.1% in region A1 and A2; was 11.9%, 2.6% and 33.0% in region B1, B2 and B3; was 0%, 5.6% and 0% in region C1, C2 and C3, respectively (Table 2). It seemed that small number of bacteria or unculturable bacteria were present in most regions. However, if *B. pseudomallei* was absent in cropped fields, the seroprevalence and annual incidence of melioidosis in Er-Ren River Basin was predisposed to low, likely in region C (Table 2).

To address the possible methods of exposure to *B. pseudomallei*, the inhabitants

of regions A, B or C were evaluated using a lifestyle questionnaire and this was analyzed with respect to their sero-positive or sero-negative status. No individual different events of this lifestyle questionnaire were surprisingly found amongst subdivisions of sero-positive regions in Er-Ren River Basin. The significant events detected for the sero-positive individual were as follows. Firstly, the sero-positive inhabitants in regions A and C said that they frequently worked in nearby paddy fields. Secondly, however, only people living in region A said that they often walked barefoot on the soil. Thirdly, inhabitants of regions A and B complained that their houses had been flooded within the last 6 months. Finally, however, the inhabitants of region B said that they were seldom exposed to soil either at home or work (Table 3). In this study, the sero-surveillance reveals that inhabitants of regions A and B had raised seropositivity for melioidosis (Table 2) and it seemed that contact with water was a major contributing factor and exposure to soil was a minor contributing factor to infection with *B. pseudomallei* for inhabitants of regions A and B of Er-Ren River Basin.

討論(Discussion)

Melioidosis in Taiwan has been recognized as an emerging disease *(13).* However, the prevalence of melioidosis in Taiwan has not yet been fully evaluated although sporadic cases of this disease have substantially increased over recent years *(13, 21-23).* According to the officially available information on melioidosis for 2001-2006, the case-distribution of melioidosis was not even and was mostly localized to the Er-Ren River Basin, southwestern Taiwan. Especially, the annual incidence in 2005 was exhibited to 36-125/100,000. In this study, we have confirmed that inhabitants in the certain areas of the Er-Ren River Basin exhibited either 21.6% or 36.6% sero-positivity for melioidosis, which is significantly higher than the 2.5% to 5% that has been reported for the city adults in Taiwan *(16).* Moreover, a survey using a PCR-based technique and bacterial cultures demonstrated the presence of detectable amounts of *B. pseudomallei* in these areas. In particular, the sero-prevalence of melioidosis was correlated with PCR detection and bacterial isolation of *B. pseudomallei* at site B3. Taken together, the Er-Ren River Basin in Taiwan should be described as a hyper-prevalent area. This is the first area located above latitudes of 20°N, where hyper-prevalence has been demonstrated by case-distribution, sero-prevalence and geographical distribution of *B. pseudomalle*i within an epidemiological analysis.

B. pseudomallei inhabits soil or water in tropical areas and in particular is found between latitudes 20°N and 20°S *(1).* Earlier, environmental isolates of *B. pseudomallei* have been divided into pathogenic (arabinose non-assimilation) and non-pathogenic (arabinose assimilation) isolates; however, non-pathogenic strains are now classified as *B. thailandensis* strains *(33)* and are easily distinguished from

pathogenic strains based on specific 16S RNA gene and flagella gene amplicons *(18, 34)*. Using the presence of these specific amplicons detected by a PCR-based technique, it was demonstrated that *B. pseudomallei* existed in various soil samples; however, these bacteria might unculturable or present in the soil in very low numbers in some areas of the Er-Ren River Basin. A PCR-positive test detects the presence of 10 gene copies per gram of soil or 1 cell per test *(15, 20)*. Since we have previously demonstrated *B. pseudomallei* is capable of survival and growth in soil media mimicking the Taiwan environment for 6 months *(24)*, a very low level of bacteria or the presence of unculturable bacteria might be the origin of the risk of infection when conditions that restrict the growth of *B. pseudomallei* are removed.

The geographical distribution of *B. pseudomallei* was uneven in Er-Ren River Basin, which is similar to the distribution in endemic areas *(5)*. For example, the isolation rate was 37% in Pattalung, but only 3% in Trang, both being neighboring provinces in southern Thailand *(5)*. In this study, the isolation rate was as highest, 26.4%, at site B3, where the presence of the bacteria was significantly focused relative to the whole area surveyed. This is the first report of a *B. pseudomallei* distribution being focused in an area above latitudes of 20°N. In the region B3, inhabitants showed a significantly raised sero-positive rate for melioidosis, indicating that they indeed had a higher chance of exposure to *B. pseudomallei* in this environment. Beyond region B3, the distribution of *B. pseudomallei* is low although the presence of the bacteria can occasionally be detected by PCR. Physical factors (soil types or water conditions) or biological antagonists (protozoa or phages) in environment may restrict the multiplication of *B. pseudomallei* in the out of region B3 *(25-27)*.

In one particular instance, during an outbreak of melioidosis in Australia, it was found that the disease was spread by a water conduit *(29).* Since the geographical distribution of *B. pseudomallei* is usually uneven, the transmission of the infectious organism causing melioidosis from one site to another has been proposed to occur through the vector of floodwater or wind *(28)*. In this study, most inhabitants who were sero-positive for melioidosis in region A shared common experiences of flooding and walking barefoot on soil. However, similar inhabitants in region B had only experienced floods. Although inhabitants of regions A and B had a significantly higher sero-positive rate than other areas, only region B3 was highly positive by PCR detection and bacterial isolation for *B. pseudomallei*. It would seem that there are two different transmission modes occurring in these areas. It is possible that inhabitants with melioidosis in region A were originally infected by the bacterium from region B3 due to spreading through floodwater. Alternatively, there could have been direct contact with propagating previously unculturable bacteria that had rapidly proliferated in suitable conditions.

Before 1994, the clinical cases of melioidosis in Taiwan were rare and sporadic *(3)*. After 2001, the incidence of melioidosis substantially increased and there was a rapid accumulation of 163 cases from 2001 to 2006. Epidemiological data, including case-prevalence data, sero-prevalence data, and geographical distribution of *B. pseudomallei*, indicate that the Er-Ren River Basin, southwestern Taiwan, has become a hyper-prevalent area for melioidosis. Physicians engaged in medical treatment in this area should be aware when they examine a patient for an unknown fever or community-acquired pneumonia, that the infectious causative organism may be *B. pseudomallei*. With subclinical or recovery patients with specific antibodies in their

sera, it is still necessary to aware, because patients can re-activate or relapse easily, especially if immunocompromised and this can lead to death *(1, 35)*.

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結論與建議

經過二仁溪流域之 32 村落的隨機抽樣，類鼻疽的血清抗體陽性率明顯分怖於二仁溪下游(台南縣與高雄線交界處)，與類鼻疽通報病例數的分佈相關，顯示該地區確實係類鼻疽的盛行區域。並且藉由當地居民問卷調查顯示，血清抗體陽性者多具有淹水與赤足田間工作經驗。特別是淹水經驗，顯然與血清抗體陽性檢測率相關，顯示台灣地區曝露 *B. pseudomallei* 的方式，可能藉由淹水，致使污染源的病菌傳播給易感宿主。因此，我們建議：

(1) 依據二仁溪流域類鼻疽血清抗體調查經驗，對特定地區或族群實施血清抗體盛行率調查。

(2) 今年(2006)於墾丁牧場發生類鼻疽羊隻感染事件，應可對該地工作人員實施抗體追蹤調查，則可瞭解其曝露情形。可即時實施衛教，提出適時的醫療警訊。

(3) 發展類鼻疽血清檢測試劑，使血清抗體陽性率更為準確。

(4) 建立台灣地區類鼻疽血清抗體檢測標準方法。

(5) 繼續對特定報告地區，如：墾丁牧場、高屏沿海居民，監測其血清抗體效價，以瞭解類鼻疽潛在的危險性，並建立台灣地區類鼻疽血清抗體盛行率基礎圖譜。

(6) 有系統的建置臨床分離株與環境分離株的基因圖譜比對。

(7) 釐清環境分離株的毒性與導至致死性類鼻疽的可能性。

(8) 台灣本土分離 *B. pseudomallei* 的基因表徵可能與國際間報告者不同，暗示著其潛在毒性與致病力亦有不同。雖然目前可證實台灣部份地區的耕植土，蘊藏著 *B. pseudomallei*。但這些菌株的毒性差異，需要進一步的釐清，以瞭解民眾接觸後之潛在危險性。

本計畫重要研究成果

本計畫之重要研究成果：

1. 清楚的界定類鼻疽於台灣地區二仁溪流域的發生率，並釐清台灣高度盛行類鼻疽之二仁溪區域之血清抗體效價與環境土壤分離 *B. pseudomallei* 的相關性。
2. 依據當地居民之經歷調查，指出居家淹水或赤足工作可能是台灣地區傳播類鼻疽的主要因素
3. 利用本計畫研究以及疾病管制局之相關計畫(DOH95-DC-1008; 計畫主持人:陳亞雷; DOH95-DC-2047 計畫主持人:楊效偉)，釐清南台灣地區類鼻疽盛行區域，以及可能存在的感染模式。本研究成果，已投稿於 Emerging Infectious Disease 雜誌。

參考文獻

1. Cheng AC, Currie BJ. Melioidosis: epidemiology, pathophysiology, and management. Clin Microbiol Rev. 2005;18:383-416.
2. Dance DA. Ecology of *Burkholderia pseudomallei* and the interactions between environmental *Burkholderia spp*. and human-animal hosts. Acta Trop. 2000;74:159-68.
3. Chaowagul W, White NJ, Dance DA, Wattanagoon Y, Naigowit P, Davis TM, Looareesuwan S, Pitakwatchara N. Melioidosis: a major cause of community-acquired septicemia in northeastern Thailand. Infect Dis. 1989;159:890-9.
4. Dance DA. Melioidosis as an emerging global problem. Acta Trop. 2000;74:115-9.
5. Finkelstein RA, Atthasampunna P, Chulasamaya M. *Pseudomonas (Burkholderia) pseudomallei* in Thailand, 1964-1967: geographic distribution of the organism, attempts to identify cases of active infection, and presence of antibody in representative sera**.** Am J Trop Med Hyg. 2000;62:232-9.
6. Trakulsomboon S, Vuddhakul V, Tharavichitkul P, Na-Gnam N, Suputtamongkol Y, Thamlikitkul V. Epidemiology of arabinose assimilation in *Burkholderia pseudomallei* isolated from patients and soil in Thailand. Southeast Asian J Trop Med Public Health. 1999;30:756-9.
7. Vuddhakul V, Tharavichitkul P, Na-Ngam N, Jitsurong S, Kunthawa B, Noimay P, Noimay P, Binla A, Thamlikitkul V. Epidemiology of *Burkholderia pseudomallei* in Thailand. Am J Trop Med Hyg. 1999;60:458-61.
8. Wuthiekanun V, Mayxay M, Chierakul W, Phetsouvanh R, Cheng AC, White NJ,

Day NP, Peacock SJ. Detection of *Burkholderia pseudomallei* in soil within the Lao People's Democratic Republic. J Clin Microbiol. 2005;43:923-4.

9. Armstrong PK, Anstey NM, Kelly PM, Currie BJ, Martins N, Dasari P, Krause V. Seroprevalence of *Burkholderia pseudomallei* in East Timorese refugees: implications for healthcare in East Timor. Southeast Asian J Trop Med Public Health. 2005;36:1496-502.
10. Chen WT, Chen YS, Chye SM, Wu TR, Hong WG, Lin YN, Bair HT, Yang SC, Chen YL. Seroprevalence of melioidosis in diabetic patients in Taiwan. J Microbiol Immunol Infect. 2005;38:267-70.
11. Wuthiekanun V, Chierakul W, Langa S, Chaowagul W, Panpitpat C, Saipan P, Thoujaikong T, Day NP, Peacock SJ. Development of antibodies to *Burkholderia pseudomallei* during childhood in melioidosis-endemic northeast Thailand. Am J Trop Med Hyg. 2006;74:1074-5.
12. Lee N, Wu JL, Lee CH, Tsai WC. *Pseudomonas pseudomallei* infection from drowning: the first reported case in Taiwan. J Clin Microbiol. 1985;22:352-4.
13. Hsueh PR, Teng LJ, Lee LN, Yu CJ, Yang PC, Ho SW, Luh KT. Melioidosis: an emerging infection in Taiwan? Emerg Infect Dis. 2001;7:428-33.
14. Dance DA. Melioidosis: the tip of the iceberg? Clin Microbiol Rev. 1991;4:52-60.
15. Kao CM, Chen SC, Chen YS, Lin HM, Chen YL. Detection of *Burkholderia pseudomallei* in rice fields with PCR-based technique. Folia Microbiol (Praha). 2003;48:521-4.
16. Chen YS, Chen SC, Wu TR, Kao CM, Chen YL. Seroprevalence of anti-flagellin antibody against *Burkholderia pseudomallei* in Taiwan. Jpn J Infect Dis. 2004;57:224-5.

17. Chen YS, Shiuan D, Chen SC, Chye SM, Chen YL. Recombinant truncated flagellin of *Burkholderia pseudomallei* as a molecular probe for diagnosis of melioidosis. Clin Diagn Lab Immunol. 2003;10:423-5.
18. Dharakul T, Tassaneetrithep B, Trakulsomboon S, Songsivilai S. Phylogenetic analysis of Ara+ and Ara- *Burkholderia pseudomallei* isolates and development of a multiplex PCR procedure for rapid discrimination between the two biotypes. J Clin Microbiol. 1999;37:1906-12.
19. Wuthiekanun V, Dance DA, Wattanagoon Y, Supputtamongkol Y, Chaowagul W, White NJ. The use of selective media for the isolation of *Pseudomonas pseudomallei* in clinical practice. J Med Microbiol. 1990;33:121-6.
20. Brook MD, Currie B, Desmarchelier PM. Isolation and identification of *Burkholderia pseudomallei* from soil using selective culture techniques and the polymerase chain reaction. J Appl Microbiol. 1997;82:589-96.
21. Chen YH, Peng CF, Hwang KP, Tsai JJ, Lu PL, Chen TP. An indigenous melioidosis: a case report. Kaohsiung J Med Sci. 1999;15:292-6.
22. Lee SS, Liu YC, Chen YS, Wann SR, Wang JH, Yen MY, Wang JH, Lin HH, Huang WK, Cheng DL. Melioidosis: two indigenous cases in Taiwan. J Formos Med Assoc. 1996l;95:562-6.
23. Luo CY, Ko WC, Lee HC, Yang YJ. Relapsing melioidosis as cause of iliac mycotic aneurysm: an indigenous case in Taiwan. J Vasc Surg. 2003;37:882-5.
24. Chen YS, Chen SC, Kao CM, Chen YL. Effects of soil pH, temperature and water content on the growth of *Burkholderia pseudomallei*. Folia Microbiol (Praha). 2003;48:253-6.
25. Wuthiekanun V, Smith MD, Dance DA, White NJ. Isolation of *Pseudomonas*

pseudomallei from soil in north-eastern Thailand. Trans R Soc Trop Med Hyg. 1995;89:41-3.

26. Inglis TJ, Foster NF, Gal D, Powell K, Mayo M, Norton R, Currie BJ. Preliminary report on the northern Australian melioidosis environmental surveillance project. Epidemiol Infect. 2004;132:813-20.
27. DeShazer D. Genomic diversity of *Burkholderia pseudomallei* clinical isolates: subtractive hybridization reveals a *Burkholderia mallei*-specific prophage in *B. pseudomallei* 1026b. J Bacteriol. 2004;186:3938-50.
28. Currie BJ, Jacups SP. Intensity of rainfall and severity of melioidosis, Australia. Emerg Infect Dis. 2003;9:1538-42.
29. Currie BJ, Mayo M, Anstey NM, Donohoe P, Haase A, Kemp DJ. A cluster of melioidosis cases from an endemic region is clonal and is linked to the water supply using molecular typing of *Burkholderia pseudomallei* isolates. Am J Trop Med Hyg. 2001;65:177-9.
30. Currie BJ. Melioidosis: an important cause of pneumonia in residents of and travellers returned from endemic regions. Eur Respir J. 2003;22:542-50.
31. Suputtamongkol Y, Hall AJ, Dance DA, Chaowagul W, Rajchanuvong A, Smith MD, White NJ. The epidemiology of melioidosis in Ubon Ratchatani, northeast Thailand. Int J Epidemiol. 1994;23:1082-90.
32. Heng BH, Goh KT, Yap EH, Loh H, Yeo M. Epidemiological surveillance of melioidosis in Singapore. Ann Acad Med Singapore. 1998;27:478-84.
33. Brett PJ, DeShazer D, Woods DE. *Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species. Int J Syst Bacteriol. 1998;48 Pt 1:317-20.
34. Sonthayanon P, Krasao P, Wuthiekanun V, Panyim S, Tungpradabkul S. A simple

method to detect and differentiate *Burkholderia pseudomallei* and *Burkholderia thailandensis* using specific flagellin gene primers. Mol Cell Probes. 2002;16:217-22.

35. Currie BJ, Fisher DA, Anstey NM, Jacups SP. Melioidosis: acute and chronic disease, relapse and re-activation. Trans R Soc Trop Med Hyg. 2000;94:301-4.

Legends

Fig. 1. The map of case-distribution and seroprevalence of melioidosis. The melioidosis cases in Taiwan (2001-2006) were indicated as right site. One black dot means one melioidosis patient. Using numbering indicated the raised cases in certain areas. The surrounding areas of Er-Ren River Basin were amplified in the map (left). The English letters indicated the regions with distinct seropositive rate to melioidosis or different isolation rate for *B. pseudomallei* (see text).

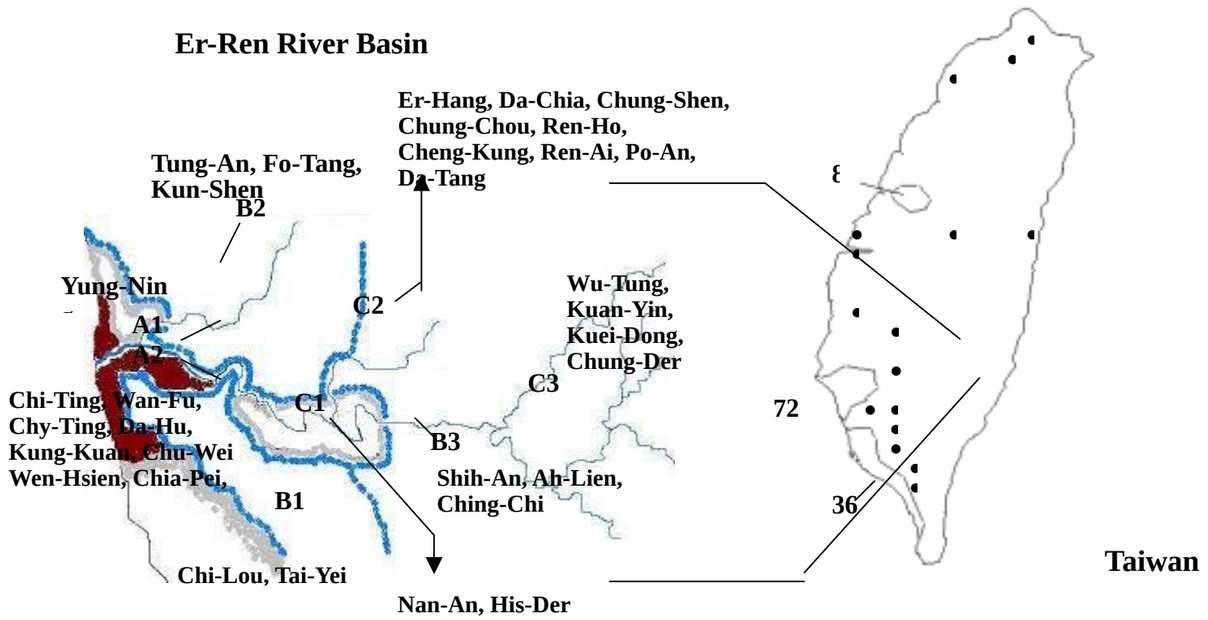
Er-Ren River Basin
Er-Hang, Da-Chia, Chung-Shen,
Chung-Chou, Ren-Ho,
Cheng-Kung, Ren-Ai, Po-An,
Da-Tang
Tung-An, Fo-Tang,
Kun-Shen
B2
Yung-Nin
A1
A2
C2
Wu-Tung,
Kuan-Yin,
Kuei-Dong,
Chung-Der
C3
Chi-Ting, Wan-Fu,
Chy-Ting, Da-Hu,
Kung-Kuan, Chu-Wei
Wen-Hsien, Chia-Pei,
C1
B3
Shih-An, Ah-Lien,
Ching-Chi
B1
Chi-Lou, Tai-Yei
Nan-An, His-Der
72
36
Taiwan

Fig. 1

Table 1. The characteristics of patient information and serodiagnosis

	Patient information			Serodiagnosis	
	Clinical manifestations	Origins#	Onset†	Collection‡	Results§
Group 1					
1	Pneumonia, bil; SS; arthritis	Blood	05/07/20	05/08/08	P
2	Pneumonia, RML; sepsis	Blood, Sputum	05/07/24	05/08/05	P
3	Pneumonia, RLL; sepsis	Blood	05/07/23	05/08/05	P
4	Septic pulmonary emboli; SS	Blood, Urine	05/07/24	05/08/19	P
5	Septic pulmonary emboli; SS	Blood	05/08/15	05/08/22	P
6	Pneumonia, LLL; cellulittis	Blood	05/08/01	05/08/07	P
7	Pneumonia, LUL	Blood, Sputum	05/08/01	05/08/07	P
8	Pulmonary nodular lesion, LLL	Sputum	05/07/21	05/08/06	P
9	Pneumonia, LLL; cellulittis	Blood	05/08/01	05/08/07	P
10	Pneumonia, LUL	Blood, Sputum	05/08/01	05/08/07	P
11	Pneumonia	Sputum	05/07/21	05/08/06	N
12	Pneumonia, LUL	Sputum	05/07/25	05/07/29	P
13	Splenic abscess	Splenic abscess	05/02/09	05/04/06	P
14	Liver abscess; sepsis	Blood	05/07/18	05/08/02	P
15	Peritonitis	Ascites	05/07/25	05/08/05	P
16	Primary septicemia	Blood	05/08/05	05/08/09	P
17	Pneumonia, LUL	Sputum	05/07/25	05/07/29	P
18	Pneumonia, bil; SS	Blood	05/08/07	05/08/13	P
19	Pneumonia; SBP	Ascites	05/07/20	05/08/13	P
20	Pneumonia; PE	Blood	05/07/23	05/07/29	P
21	Pneumonia, bil; SS	Blood	05/07/22	05/08/05	N
Group 2					
1	Primary septicemia	Blood	05/09/05	06/04/19	P
2	Primary septicemia	Blood	05/07/28	06/04/15	P
3	Pneumonia, RUL	Sputum	05/07/30	06/04/15	P
4	Pneumonia, RLL	Sputum	05/07/25	06/04/15	P
5	Pneumonia, RUL	Blood	05/07/25	06/04/15	P
6	Pneumonia, LML	Unknown	05/07/25	06/04/15	P
7	Pneumonia, bil; SS	Blood	05/08/07	06/04/15	P (18)
8	Pneumonia, RUL; PE	Sputum	05/08/13	06/04/15	N
9	Pneumonia, LUL	Blood, Sputum	05/08/01	06/04/15	N (10)
10	Septic pulmonary emboli; SS	Blood, Urine	05/07/24	06/04/10	P (4)
11	Primary septicemia	Blood	05/07/20	06/04/10	P
12	Pneumonia, LLL; cellulittis	Blood	05/08/01	06/04/10	P (6)
13	Pneumonia, bil	Blood	05/05/25	06/04/11	P
14	Knee infectious bursitis	Pus	05/09/10	06/04/12	P
15	Primary septicemia; SA	Blood	05/08/01	06/04/19	N
16	Primary septicemia	Blood	05/07/31	06/04/28	N
17	Necrotizing fascitis	Pus	05/07/20	06/04/28	P
18	Pneumonia; PE	Blood	05/07/23	06/05/01	N (20)
19	Primary septicemia	Blood	05/07/18	06/04/28	P
20	Primary septicemia	Blood	05/07/28	06/05/01	P
21	Unknown	Unknown	Unknown	06/05/01	P
22	Pneumonia, LUL	Sputum	05/07/25	06/05/01	N (12)

#: The “origins” means the isolation site for *B. pseudomallei* from patients. †: The “onset” was defined as the date (year/month/day) with commence of identifiable symptom by hospital. ‡: The “Collection” was defined as the date (year/month/day) for collecting serum specimens. §: The “Results” means the outcome of ELISA. “P” means positive and “N” means negative. The parentheses indicated that the patient in group 2 same as (patient no) in group 1. Abbreviation, PE: pleural effusion; SS: septic shock; SA: septic arthritis; LLL: left lower lobe; LUL: left upper lobe; RUL: right upper lobe; RLL: right low lobe; RML: right middle lobe; LML: left middle lobe; bil: bilateral; SBP: spontaneous bacterial peritonitis.

Table 2. Summary in case-prevalence, sero-prevalence and geographical distribution of *B. pseudomallei* in Er-Ren River Basin

Region	A		B			C		
Demographics								
Villages (n)	9		8			15		
Population (n)	19135		18944			28024		
Case number (2001-2006)	30		22			21		
Annual incidence (2005)	125/100,000		63/100,000			36/100,000		
Sero-prevalence								
Sampling size (n)	183		176			265		
Sero-positive rate (%)	36.6%		21.6 %			10.9 %		
Significance (p<0.05)						A>B>C		
Subdivisions	A1	A2	B1	B2	B3	C1	C2	C3
Geographical distribution								
Detection sites (n)	30	32	42	38	91	24	18	36
Isolation rate (%)	0.0 %	0.0 %	0.0 %	0.0 %	26.4 %	0.0 %	0.0 %	0.0 %
PCR-positive rate (%)	6.7 %	3.1 %	11.9 %	2.6 %	33.0 %	0.0 %	5.6 %	0.0 %

Table 3. Summary in lifestyle questionnaire

Variables	Characteristics of survey responders: Questionnaire at Area (responders; n) A (n=67)	B (n=38)	C (n=29)	Significance (p<0.05)
Sex (n)				
Male	32	13	14	
Female	35	25	15	
Age (n)				
21-40	2	3	1	
41-60	23	11	10	
>60	42	24	18	
Lifestyle (%)				
Had been endemic areas	29.9 %	26.3 %	27.6 %	No
Farmers	20.9 %	21.1 %	20.7 %	No
Working or living nearby paddy	49.2 %	21.1 %	48.3 %	A≅C>B
Frequently worked in nearby paddy	34.3 %	23.7 %	37.9 %	No
Often barefoot on soil	29.9 %	15.8 %	17.2 %	A>B≅C
Had been flooded within 6 month	32.8 %	34.2 %	13.4 %	A≅B>C

附件一

申明書

本人同意接受『二仁溪流域類鼻疽血清盛行率調查』計劃所需的問卷調查，與接受 3 mL 的血液採集，做為檢測類鼻疽抗體之用。

樣張

同意人：(簽名)

身份證字號(後三碼)：XXXXXX□□□

出生：　　年　　月　　日

性別：□女、□男

教育程度：□國小或國小以下、□高中、□大專、□研究所或以上

(本聯屬保密資料，由計劃主持單位收執)

----------(裁剪線)----------

問卷單 (共兩頁)

(本聯由訪視者當面提問、受訪者依序回答。劃線部份可視情況免答)

1. 出生地點：　　縣(市)、＿＿＿＿鄉(鎮)。
2. 居住地點：□ 本地、□ 非本地 [　　縣(市)、　　鄉(鎮)]。已於居住地居住　　年＿＿＿月。(非本地居民免答)
3. 從居住環境前往最接近的耕植地(農田、果園、菜園、花埔等，但自用、自賞的小塊耕植地除外)、約：□走路3分鐘、□走路10分鐘、□騎(乘)機車5分鐘、□騎(乘)機車約10分鐘以上。
4. 從工作地前往最接近的耕植地約：□走路 3 分鐘、□走路 10 分鐘、□騎(乘)機車 5 分鐘、□騎(乘)機車約 10 分鐘以上。
5. 職業：

工人：□清潔工、□建築工、□一般非技術工。

農人：□稻農、□果農、□菜農、□園藝、□其它農作。

漁人：□海漁、□池漁。

□軍、警人員、□公務、教育、行政人員。□經商。□服務、商店銷售人員。□機台及機械操作員及裝配員。□學生(全日制)、□家庭主婦。□退休。⑤待業。□醫、護人員。

6. 生活及工作直接接觸泥巴(泥土)的機會：□幾乎沒有、□很少、□經常、□非常頻繁
7. 曾經出差或旅遊前往東南亞或澳洲：□沒有、□有
8. 是否會在室外赤足工作、休閒或活動：□幾乎沒有、□很少、□經常、□非常頻繁
9. 對於一般性傷口(三-五分鐘內會止血)的處理態度是：□讓它自然痊癒、□擦些消毒水、紅藥水、小護士等、□貼上黏貼膠布或紗布、□讓醫生處理
10. 是否曾經聽過類鼻疽病：□ 是、□ 否。(答否者、以下免答)
11. 是否知道去年(七月)海棠颱風來襲後，在南部地區爆發類鼻疽疫情：□ 知道 □ 不知道
12. 類鼻疽傳染途徑為 (複選)：□空氣傳染□傷口進入□食入受污染泥土或水

□吸入受污染泥土或水□人傳人。

13. 預防類鼻疽要注意甚麼重點（複選）：

□ 曾在地方性流行地區接觸到水或土壤且本身是有慢性潛在性疾病疾病的人，是感染的高危險群

□ 身體有耗弱性疾病的人（如糖尿病、酗酒、肝硬化、慢性腎病及外傷者），應盡量避免暴露受污染泥土或水中

□ 在地方性流行地區中，皮膚有傷口，若接觸到受污染水或土壤，應盡快以水沖洗乾淨並就醫

□ 類鼻疽臨床症狀（複選）：□發燒 □畏寒 □肺炎 □肌肉痛

訪視員：（簽名）

日　期：