THE 1990 - 1991 OUTBREAK OF MELIOIDOSIS IN THE NORTHERN TERRITORY OF AUSTRALIA : EPIDEMIOLOGY AND ENVIRONMENTAL STUDIES

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Abstract. From November 1990 to June 1991 33 acute cases of melioidosis occurred in the Northern Territory, Australia; 25 cases were reported in the capital city, Darwin. We carried out an epidemiological investigation to exclude a common source outbreak, describe the risk factors for disease, and develop and institute appropriate control measures. We compared population based attack rates among various risk groups using logistic regression, and the demographic, medical and behavioral risk factors for melioidosis by a matched case-control study. Environmental Health Officers collected soil, surface water and cooling tower water specimens for *Pseudomonas pseudomallei* culture. The crude attack rate of melioidosis during the outbreak was 52 per 100,000. Age, gender, race, diabetes and alcohol abuse were independent risk factors for disease. The relative risk of disease in diabetic patients was 12.9 (95% CI 5.1 - 32.7; $p < 0.001$) and 6.7 in alcoholic patients (95% CI 2.9 - 15.2; $p < 0.001$). We found no significant difference between cases and controls in matched pair analysis for any of several exposure factors studied. We isolated *Pseudomonas pseudomallei* from 4% of soil samples and 9% of surface water samples. Our study confirms the importance of host factors in the development of melioidosis, and attempts to quantify the risk of disease during the Darwin epidemic. *Pseudomonas pseudomallei* is widespread in the soil of urban Darwin.

INTRODUCTION

Melioidosis is a bacterial disease of humans and animals caused by the soil saprophyte *Pseudomonas pseudomallei,* and is endemic in northern Australia above latitude 20°S (Crotty *et al,* 1963; Woods *et al,* 1992; Rode and Webling, 1981; Thomas *et al,* 1979; Ashdown *et al,* 1980; Guard *et al,* 1984; Ketterer *et al,* 1986; Thomas, 1981; Loyd *et al,* 1988). Although the incidence of disease is low, the high case fatality rate and significant morbidity associated with the infection make it an important disease clinically. The first case of human melioidosis reported from the Northern Territory (NT) of Australia was in 1960 (Crotty *et al,* 1963). The patient was a 40 year old male diabetic who presented with pneumonia, and died three weeks later of *P. pseudomallei* septicemia. Woods *et al* (1992) reported 52 NT cases of culture-confirmed or serologically diagnosed cases of melioidosis from January 1984 to October 1990, a mean incidence of eight cases annually. Most presented during the northern Australian wet season, November through April. The majority of these cases lived in rural and semi-rural communities remote from Darwin, and many had outdoor occupations which brought them into frequent contact with soil. In contrast, from 1 November 1990 to 30 June 1991, 33 cases of melioidosis were confirmed in the region.

The incidence of melioidosis in humans and in veterinary practice has a seasonal periodicity in endemic areas (Woods *et al,* 1992; Rode and Webling 1981; Thomas *et al,* 1979; Ashdown *et al,* 1980; Guard *et al,* 1984; Ketterer *et al,* 1986; Strauss *et al,* 1969), peaking during the months of heaviest rainfall and after flooding (Ketterer *et al,* 1986). The near record rainfall experienced by the NT in January 1991 was associated with a considerable increase in the number of patients presenting with melioidosis in the same month, clustering in the capital city, Darwin. The temporal clustering of urban cases necessitated urgent exclusion of a common source outbreak. We were particularly concerned about the environmental contami-

nation and possible aerosolization in a public access facility. Previous studies identified inoculation (Rode and Webling 1981; Ashdown *et al,* 1980; Thin *et al,* 1970; De Buse *et al,* 1975), inhalation of dust (Ashdown *et al,* 1980; De Buse *et al,* 1975) or aerosols (Ketterer *et al,* 1986; Thin *et al,* 1970; Clayton *et al,* 1970), and ingestion (Thomas *et al,* 1979) as possible routes of transmission of *P. pseudomallei* in both humans and animals. Strauss *et al* (1969a,b) found a positive correlation between recovery rates of *P. pseudomallei* from soil and ground water samples and *P. pseudomallei* antibody prevalence in Malaysia. Serosurveys of military personnel who served in Vietnam suggested that helicopter crewmen were infected by inhaling aerosols produced by helicopter rotors (Clayton *et al,* 1970). These studies also suggested that *P. pseudomallei* was ubiquitous in soil in these countries.

We began epidemiological and environmental investigations in January 1991. This paper presents the outbreak investigation results of the 25 acute cases of melioidosis in the greater Darwin region from November 1990 to June 1991. The accompanying article also includes eight additional cases reported during the abservation period which did not fulfil the case definition used in this study.

MATERIALS AND METHODS

Interview of the first cases or their proxies excluded a common source outbreak within Darwin. Most gave a history of frequent exposure to wet soil or pooled surface water during the rains, and the early cases reported pre-morbid trauma to the feet or infected ingrown toenails.

We conducted a matched case-control study and environmental sampling to test three hypotheses suggested by the descriptive epidemiology and clinical features of the first 15 cases; first, that soil samples and standing water from the greater Darwin region harbored *P. pseudomallei;* second, that *P. pseudomallei* infected cases through exposure to contaminated soil or water; and third, that laceration or maceration of the skin of the extremities, particularly the feet, facilitated transmission. Demonstration of environmental contamination would be used to guide the nature and extent of a proposed prevention campaign. We also compared attack rates for various groups using population denominators enumerated from published sources.

Case definition

The eligible case subjects were 25 patients with acute melioidosis; 24 were admitted to the Royal Darwin Hospital (RDH), or attended the medical outpatient clinic at the Royal Darwin Hospital from November 1990 to June 1991, and one patient was diagnosed with melioidosis at autopsy. All lived in the Darwin Statistical Division during the 1990/91 wet season, an area of 303 square kilometres and a population of approximately 73,000 people. All but two were bacteriologically confirmed. The two culture negative patients presented with clinical signs of acute melioidosis, and had IFA-IgM (Ashdown, 1981; Khupulsup and Petchclai, 1986) or IgM-ELISA (Ashdown *et al,*1989) antibodies to *P. pseudomallei,* with no other causative pathogens identified.

We maintained active surveillance for cases during the "wet season" by reviewing acute admissions to the RDH, the principal hospital of two in the Darwin Statistical Division. We asked physicians, laboratory staff at the RDH and private laboratories, and rural clinic staff to notify all cases of melioidosis to the Communicable Diseases Center. General practitioners reported cases on a voluntary basis. We distributed a newsletter to all private practitioners informing them of the outbreak and defining the clinical features and patients at risk of melioidosis (Rode and Webling 1981; Ashdown *et al,* 1980; Guard, 1987; Leelarasamee and Bovornkitti, 1989). They were encouraged to review susceptible patients who had presented with pneumonia, pyrexia of unknown origin genitourinary symptoms or unusual skin infections in the preceding month, to request *P. pseudomallei* serology when clinically indicated during the wet season, and to maintain heightened surveillance. Although the study began in January 1991, we also carried out an audit of the case notes of patients diagnosed with melioidosis in November and December 1990, and interviewed the patient or a suitable proxy.

Selection of controls

Because 88% of our cases (22 patients) had a recognized medical risk factor for melioidosis, we selected controls from among diabetic, alcohol abusing, oncology or steroid dependent patients

in whom clinical melioidosis had been excluded. They were not screened for the presence of *P. pseudomallei* antibodies. We intended to select two control subjects matched to each case from consecutive admissions to the general medical or surgical wards of the RDH. We reduced this target to one control per case as the study progressed because of the paucity of suitable in-patient controls. We also extended selection of controls to patients attending the RDH medical out-patient clinics, clients of the RDH Detoxification Unit, and diabetic patients registered with Diabetes Australia Northern Territory.

Eligible controls included 7 female and 27 male Darwin residents, matched to the cases by age (to plus or minus 6 years for patients aged less than 30 years, and to plus or minus 10 years for older patients), gender, and race (Aboriginal or other). The number of controls per case ranged from one to three; 17 cases were matched to one control, 7 to two, and one case to three controls. The final number of controls selected was 34.

Interviews

All cases or their proxies, and all of the control subjects agreed to be interviewed. We interviewed proxies if the patient was deceased or too ill to talk to us. Informed verbal consent was obtained from all particpants or their proxies. One of the investigators (CMN) conducted the interviews unblinded to the case-control status of the study population, and aware of the risk factors for melioidosis under investigation. Questions were asked in a standard manner. The cases or their proxies were interviewed within two weeks of the diagnosis. We attempted to obtain exposure histories for the three month period before the onset of illness among the cases, and for the same period before the day of the interview for the controls. The exposure variables included occupation; frequency of occupational and recreational exposure to soil, mud or standing water, type of footwear most commonly worn at work and recreation (waterproof shoes or boots, open footwear or bare feet); history of injury to the extremities, including surgical procedures and cutaneous infections such as infected insect bites; severity of the skin trauma graded by a specialist physician (BC) as mild, moderate or severe; past medical history and intercurrent illness; alcohol use and smoking history. We considered exposure to soil to be frequent if the subject worked outdoors, or spent at least half of their leisure time outdoors each week. We specifically asked about activites such as gardening, digging for molluscs and crustaceans in the mud flats around Darwin, fishing, and camping.

Environmental studies

Environmental Health Officers from the Public Health Branch of the NT Department of Health and Community Services obtained a convenience sample of surface soil and water specimens from the gardens of patients, controls and popular recreational areas. They generally sampled wet shady areas, reported in the medical literature to yield the highest isolation rates of *P. pseudomallei* (Thomas 1981; Strauss *et al,* 1969a). Soil was collected from around the residences of the first 22 cases and eight control subjects. They collected topsoil with stainless steel spoons, which were wiped clean and sterilized with heat between collections. No attempt was made to sample deeper soil layers. They also collected water from the cooling towers of the main public access buildings in Darwin, and from the private air conditioning units of two cases. Surfaces of the domestic air conditioning units were swabbed.

Laboratory methods

We prepared culture plates for *P. pseudomallei* from 68 soil and 11 surface water samples, and 7 water samples and 3 surface swabs from the cooling towers and air-conditioning systems tested.

Soil: Each 1kg sample of soil was divided into 4 portions each weighing 250g, apportioned into 4 containers, and 300ml of Ashdown modified broth was added to each container (Ashdown, 1979a). These containers were to have been incubated at 35°C for a minimum of 48 hours, but the smell in the laboratory was prohibitive after 14 - 18 hours. The specimens were moved outside, where the ambient temperature of 27 - 34°C would have been adequate to sustain the growth of *P. pseudomallei.* 50ml of supernatent were removed from these specimens on day 2,3,4 or 5, centrifuged at 3,000 rpm for 15 minutes, the supernatent discarded, and the pellet plated onto Pp *(P. pseudomallei)* selective plates.

Water: Water samples were filtered though Whatman® number 1 filter paper (W and R Balston

Ltd, UK), and then through 0.45 mm millipore filters. The filter papers were incubated in 30ml of Ashdown modified broth for 48 hours at 35°C.

All inoculate plates were incubated for a minimum of 48 hours, and Pp colonies were identified by their characteristic macroscopic and microscopic morphology, and confirmed with API 20E (Ashdown, 1979b).

Statistical analysis

We obtained denominators for calculating population-based attack rates (AR) from the Australian Bureau of Statistics population census (ABS, 1989). We excluded residents of the Darwin Statistical Division less than 20 years of age from all denominators.

We used two methods to estimate the number of diabetics in Darwin, because the true prevalence of diabetes is unknown. We applied the age- and sex-specific diabetes rates reported in the prevalence study in Busselton, Western Australia (Glathaar *et al,* 1985) to the Darwin population to estimate the prevalence in non-Aboriginals, and the corresponding rates described in central Australia (Phillips *et al,* 1990) for the prevalence in Darwin Aborigines. We adjusted both estimates by the ratios of undiagnosed to diagnosed diabetics in each ethnic group reported in the two studies. This ratio varied with age for non-Aboriginal diabetics, but we used a fixed ratio of one undiagnosed case for each known case in all Aboriginal age categories. The denominator for each racial group was the sum of diagnosed and undiagnosed diabetics.

We used the 1990 ABS survey of alcohol and tobacco consumption in Darwin (ABS, 1991) to estimate the age- and sex-specific denominators of alcohol drinkers in the "moderate" and "high risk" consumption categories, at least 50ml of pure alcohol per day in men and at least 25ml per day in women. These rates also enabled us to estimate denominators for diabetics drinking alcohol at these levels, assuming that the proportions of moderate and high risk drinkers among diabetics and among Aborigines are the same as reported for the general Darwin population.

We estimated the population engaged in outdoor work using the following occupation classifications used by the ABS: farmers and farm managers; natural scientists; building professionals and engineers; electrical, building and vehicle tradespersons; police officers; road and rail transport drivers; mobile plant operators; amenity horticulturalists; and agricultural, construction and mining, and miscellaneous laborers.

We calculated descriptive statistics, relative risks (RR) and their 95% confidence intervals (CI) for the incidence data using the epidemiological program Epi Info Version 5 (Dean *et al,* 1990), and matched odds ratios (OR_M) and their 95% CI (Robins *et al,* 1986) for the case - control study. Because of small sample size, we collapsed all categorical data into a maximum of three categories for the matched-pair analysis. For example, we recoded the five levels of severity of pre-morbid injury or skin lesions (nil, minor, moderate or severe injury, and non-traumatic heavy soil exposure) into "nil", "minor injury or heavy soil contact" and "moderate to severe injury or existing lesion" categories.

We used logistic regression on our incidence data to estimate the predictors of melioidosis with GLIM statistical software (Baker, 1985). The odds ratios derived from the model approximate relative risk estimates in this study, since the outcome is rare. We selected a subset of predictors based on initial univariate analysis ($\alpha = 0.05$), and employed logistic regression to select the final set of significant predictors ($p < 0.05$). The predictors fitted to the final model were age, gender, race, and history of diabetes and alcohol abuse. The adjusted relative risks and 95% Cls were calculated from the logistic regression using the formula $\exp(\beta \pm 1.96*SE)$, where β and SE are the estimated regression coefficient and the standard error respectively (Kleinbaum *et al,* 1982).

RESULTS

Attack rates

The epidemic curve of the outbreak is presented in Fig 1. The mean age of the case subjects was 52.1 years (SD 13.5 years), with a range of 20 - 80 years. Six were female; 8 were Aboriginal. Table 1 presents the risk factors of the cases and controls. The clinical data of the cases will be presented elsewhere. Eighty percent of cases (20 patients) had a history of diabetes and/or current alcohol abuse. Seven patients had two, and two

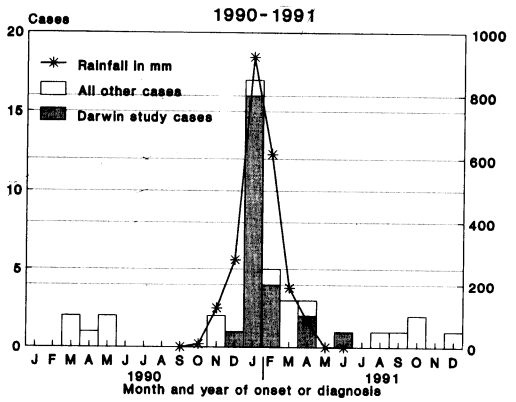

Fig 1—Epidemic curve of melioidosis in the Northern Territory.

patients had three or more risk factors for melioidosis. One patient had a 15 year history of topical steroid use for psoriasis as his only recognized risk factor, and another had a past history of mastectomy for carcinoma of the breast. Only three patients had no relevant medical histories; two cases in this latter group had frequent occupational exposure to soil. They were males aged 63 and 44 years, and both worked in the building trade. The former had sustained a welding spark burn over his right tibia; he required daily dressings for approximately five weeks and oral antibiotics to control secondary cellulitis. The second patient was a tiler who frequently injured his hands. Seven of the 10 cases with outdoor occupations, and both cases working indoors, described frequent recreational exposure to soil.

Table 2 presents the attack rates stratified by various demographic and risk factors. The crude attack rate of melioidosis in Darwin residents aged 20 years and over was 52 per 100,000. The highest attack rate (2,128 per 100,000) occurred in diabetic patients abusing alcohol. Age 50 years and over, ethnicity, male gender, diabetes and alcohol abuse are significant univariate exposure factors, and infrequent occupational exposure to soil was protective in this analysis.

The similarity in attack rates between cases frequently exposed to soil at work, and those outside the workforce, may be explained by the high proportion of Aboriginal patients in the latter group (7/8 cases compared to 6/17 non-Aboriginal cases; Fisher exact two-tailed test, $p = 0.03$). Six Aboriginal patients admitted to heavy soil exposure from activities such as fishing and collecting shellfish (75%), while 47% of non-Aboriginal patients (8/17) engaged in frequent outdoor recreation; this difference was not statistically significant ($X^2 1df = 0.81$). Age, gender, history of trauma to the extremities, and the presence of intercurrent medical risk factors were similar between ethnic groups. The difference between the ethnic groups in the rate of heavy occupational exposure to soil, 13% for Aboriginal and 53% non-Aboriginal patients, was not statistically significant.

The adjusted relative risks from the logistic regression are shown in Table 3. Age, gender, ethnicity, the presence of diabetes and alcohol abuse remained significant predictors of melioidosis after adjustment. The relative risk of melioidosis for Darwin residents are approximately 8, 13 and 7 in the presence of age 50 years and over, history of diabetes, and alcohol abuse respectively. There is no interaction (effect modification) between alcohol and diabetes and the relationship with melioidosis in this population ($X^2 1df = 0.011$; $p = 0.91$).

The case-control study

We found no significant difference between cases and controls for any of the predictor variables tested by univariate matched pair analysis (Table 1), so we did not proceed to multivariate analysis. The only significant finding was that more cases than controls were employed at the time of interview ($OR_M = 5.0$; 95% CI 1.0 – 24.2; $p < 0.05$).

The ecological study

One ground water specimen (9.1%) and three soil samples (4.4%) yielded *P. pseudomallei* on culture. The Environmental Health Officers collected the postive water sample on the property of one of the cases. Soil collected from the stockpile of a commercial topsoil distributor in Darwin, the garden of a volunteer outside the investigation, and an area of water seepage on a vacant lot, all yielded *P. pseudomallei.* We failed to isolate the organism in soil samples from the other commercial topsoil distribution sites, and from the cooling towers and air-conditioning systems examined.

DISCUSSION

This epidemic of melioidosis in urban Darwin

Table 1

Matched-pair analysis of 25 cases with melioidosis and 34 control subjects, Darwin, 1990 - 91.

Risk factor	OR_M	95% CI of OR_M	P value
Occupational exposure to soil			
Not in the workforce	1.0		
Frequent soil / water contact	undefined		
Infrequent soil / water contact	0.8	0.1 - 6.3	0.73
Recreational exposure to soil			
Infrequent soil / water contact	1.0		
Frequent soil / water contact	1.3	0.4 - 4.2	0.88
Type of recreational exposure			
Other *eg* digging for molluscs	1.0		
Gardening	0.5	0.2 - 1.8	0.49
History of pre-morbid injury or heavy soil water exposure			
No	1.0		
Yes	1.3	0.4 - 4.0	0.84
Severity of the pre-morbid injury			
Nil	1.0		
Minor injury or heavy soil contact	3.0	0.7 - 13.2	0.22
Moderate to severe injury or existing lesion	0.8	0.1 - 10.0	0.63
Footwear at work			
Closed shoes	1.0		
Bare feet or open shoes	2.0	0.2 - 22.1	1.00
Footwear during recreation			
Closed shoes	1.0		
Bare feet or open shoes	0.8	0.2 - 4.5	0.83
Medical risk factors			
Nil known or other risk factors[1]	1.0		
Diabetes or alcohol abuse, alone or in combination with other risk factors	0.4	0.1 - 2.3	0.49
Smoking history			
No	1.0		
Yes	1.0	0.3 - 3.3	0.80

1 Other medical risk factors includes a patient with a past history of carcinoma of the breast, and another on long term treatment with topical steroids for psoriasis as their only known medical risk factors for melioidosis.

provided a unique opportunity for an analytic study of the epidemiology of the disease. This unusual outbreak is probably associated with the heavy summer rainfall recorded in northern Australia from November 1990 to April 1991 (Woods *et al,* 1992). A similar increase in case numbers was observed in northern Queensland during the same season, when 28 cases were re-

Table 2

Population based attack rates of melioidosis by risk category, Darwin, 1990 - 91.

Risk factor	Cases	Population estimate	AR per 100,000	RR	95% CI of RR
Age					
20 - 49 years	8	39,020	21	1.0	
50 years and over	17	8,670	196	9.6	4.1 - 22.1[1]
Gender					
Female	6	22,720	26	1.0	
Male	19	24,970	76	2.9	1.2 - 7.2[2]
Ethnicity					
Non-Aboriginal	17	45,090	38	1.0	
Aboriginal	8	2,600	307	8.1	3.5 - 18.8[1]
Occupational soil exposure					
Not in workforce	13	16,130	81	1.0	
Infrequent	2	21,330	9	0.1	0.0 - 0.5[3]
Frequent	10	10,230	97	1.2	0.5 - 2.8[4]
Medical risk factors					
Nil and other[5]	5	33,760	15	1.0	
Alcohol	8	12,530	64	4.3	1.4 - 13.2[1]
Diabetes	6	1,120	535	36.0	11.0 - 118.0[1]
Alcohol and diabetes	6	280	2,128	142.0	43.5 - 462.0[1]
Total	25	47,690	52		

1 $p < 0.001$
2 $p < 0.05$
3 $p < 0.01$
4 Not significant
5 Other medical risk factors includes a patient with a past history of carcinoma of the breast, and another on long term treatment with topical steroids for psoriasis as their only known medical risk factors for melioidosis.

ported over a six month period (Allen, 1991).

Growth of *P. pseudomallei* in the environment requires temperatures between 18 - 42°C, humidity and consistent rainfall. During heavy rainfall, the rising water table may leach the organism out of the lower soil layers to the surface where favourable environmental conditions facilitate growth and replication (Thomas *et al,* 1979). The highest levels of environmental contamination with *P. pseudomallei* have been reported in Thailand and Malaysia. The organism is found widely in the soil and surface water of rice paddies, cleared fields, marshes and monsoon drains in endemic areas. Strauss *et al* (1969a) isolated *P. pseudomallei* in 28% of soil samples from cleared fields, and in 33% of ground water samples collected in wet rice fields in West Malaysia, although the crude rates of contamination across 10 states were 3.8% and 7.6% for soil and water samples respectively. Finkelstein *et al* (1966) found isolation rates ranging from 30 - 50% in southern Thailand. Piggott and Hochholzer (1970) observed that discrete areas of hyperendemicity occur within endemic areas. In Australia, Thomas *et al* (1979) sampled a field implicated in melioidosis in sheep over a two year period, and cultured *P. pseudomallei* from 10% of 30 water samples and from 1% of 700 soil samples.

Table 3

Population based adjusted relative risks for melioidosis risk factors from multiple logistic regression, Darwin, 1990 - 91.

Risk factor	Adjusted relative risk	95% CI of RR	P value
Age			
20 - 49 year	1.0		
50 years and over	8.1	3.3 - 19.9	< 0.001
Gender			
Female	1.0		
Male	2.8	1.1 - 6.9	0.03
Ethnicity			
Non-Aboriginal	1.0		
Aboriginal	3.2	1.2 - 8.8	0.02
Diabetes			
Absent	1.0		
Present	12.9	5.1 - 32.7	< 0.001
Alcohol abuse			
No	1.0		
Yes	6.7	3.0 - 15.2	< 0.001

The relative risks for the 5 risk factors were adjusted for each other.
There was no interaction (effect modification) between diabetes and alcohol abuse, and the relationship with melioidosis (X^2 1df = 0.011).

They suspected that the high isolation rate from the water samples was a result of sampling mostly muddy water from bore holes rather than surface water.

Our crude isolation rate of 5% from a small number of surface water and soil samples, the lack of geographical clustering of cases within urban Darwin during the epidemic, and their apparent lack of common source exposure suggest that *P. pseudomallei* is widely distributed in the soil of Darwin. Anecdotal evidence from long term residents suggests that following Cyclone Tracy which devastated Darwin in 1974, topsoil from a local source was used to landscape severely affected suburbs. Soil yielding cultures of *P. pseudomallei* collected in the garden of the volunteer who lived in one of the re-landscaped suburbs, and from a commercial distributor of topsoil, were linked to this source of topsoil. This supplier delivered soil to a building site where one of the melioidosis cases worked as a concreter. These anecdotes suggest widespread distribution of *P. pseudomallei* contaminated soil in Darwin. Failure to culture the organism from soil collected from the other commercial distributors of topsoil does not preclude its presence, so we did not recommend any specific action against the implicated outlet.

The case-control study did not identify any behavioral factors predisposing to melioidosis. The almost universal exposure of Darwin residents to wet soil and standing water during the 1990/91 wet season supports the importance of medical risk factors in the development of disease. The wide confidence intervals for the matched odds ratios do not exclude the possibility of either a positive or a negative (protective) effect. We attribute these results to small numbers, and possibly to lack of specificity of the questionnaire for the relevant soil contact behaviors, or to unequal

levels of soil contamination with *P. pseudomallei.*

We had to conduct this study rapidly, so we selected easily accessible controls, most of whom were unemployed; 66.2% of the Darwin population aged 20 years and older were part of the workforce during the 1986 census compared to only 23.5% of the control subjects in this study. The employment status of cases also differed significantly from that of their matched controls at the time of interview. Our assessment of the role of occupational exposure in the development of melioidosis is therefore invalid.

The risk of melioidosis in cases with heavy occupational exposure to soil was approxmately 10 times that of cases who worked indoors in the univariate population-based analysis, even though their recreational exposure was similar. Two of the three patients without predisposing medical risk factors for melioidosis were engaged in outdoor work. Fifty percent of the cases from 1960 - 1990 also worked outdoors; most of the remaining cases were rural Aborigines (Woods *et al,* 1992). These data suggest that occupational exposure was an important risk factor in this outbreak.

Elsewhere melioidosis is a rural disease, affecting mainly men aged 40 - 50 years, but cases occur in all age groups (Guard *et al,* 1984; Guard, 1987). Although urban Aborigines in Darwin live less traditional lifestyles, 75% of the Aboriginal cases in this study had engaged in activities with heavy soil exposure, such as gathering sea foods from mangrove swamp areas.

Our investigation supports existing evidence that skin inoculation with soil or water contaminated with *P. pseudomallei* is an important route of transmission, although inhalation of aerosols containing the bacteria has not been excluded. The adjusted relatives risks indicate that underlying medical risk factors are more important than demographic variables and environmental exposure in the development of disease. Clinical melioidosis is known to be associated with conditions causing immunosuppression such as diabetes mellitus and chronic alcohol abuse (Rode and Webling 1981; Ashdown *et al,*1980, Guard, 1987, Leelarasamee *et al,* 1989; Ashdown and Guard 1984). A serosurvey in north Queensland involving 9,047 residents found that seroprevalence rates in people with alcoholism and chronic infections (15%), liver disease (13%), and diabetes (9%), were higher than the crude seroprevalence rate of 5.7% (Ashdown and Guard 1984). The high attack rates of melioidosis in diabetics and alcoholics in our study support these observations. A prospective study of seronegative, "high risk" individuals would theoretically improve our understanding of the risk of infection and the natural history of the disease in the immunocompromised. The low incidence of melioidosis, the long latency between infection and disease, sometimes measured in decades, the small size and transient nature of the NT population, and the logistical problems inherent in cohort studies, hinder such an investigation (Rode and Webling 1981; Guard, 1987; Morrison *et al,* 1988).

Although most patients in our series were diagnosed after admission to hospital or at post mortem, close contact with the laboratories in Darwin, and referral of all patients to physicians for treatment, meant that our case ascertainment was very high. Only one additional case of mild cutaneous melioidosis was detected by one of the authors (BC) during an retrospective audit of the admission log books of the private hospital in Darwin. Our methods of estimating risk factor-specific denominators may be inexact, but the orders of magnitude of the attack rates and adjusted relative risks are large enough to remain robust against sizable variations in the denominator populations.

The degree of recall bias of both cases and control subjects in this study may have been considerable, especially after extensive media coverage of the epidemic. We anticipated bias towards recollection of a pre-morbid exposure event, such as minor trauma to the extremities, when interviewing relatives or friends of deceased patients. The data do not show a significant difference in the frequency of pre-morbid events between the deceased patients and surviving cases. The validity of the case-control study is compromised by our reliance on self-reported data of behavioral risk factors, the small number of cases, problems in finding suitable control subjects, and possible over-matching of cases and controls. A control group selected from patients with a malignancy, diabetes, or alcoholism may be inappropriate for the study of melioidosis risk factors in "low risk" cases. We considered a second, population-based, control group, but time and resource constraints

prevented such additional analytic study. However, our control group was appropriate for the investigation of a common source outbreak.

Our current melioidosis awareness campaigns advise the public with recognized risk factors, including outdoor occupations, to avoid exposure to soil and untreated water during the "wet season" by using protective foot and hand wear when indicated (Morrison *et al,* 1988; Johnson, 1967). We also emphasize the need for a high level of clinical suspicion of melioidosis among health care professionals, especially when dealing with patients at risk of the disease. Long term surveillance will be needed to evaluate the effectiveness of these prevention strategies.

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