

Table. Ten-day LD₅₀* values (given in CFU) after intravenous, intraperitoneal, subcutaneous, intranasal, or oral introduction of NCTC 13178 or NCTC 13179 strains of *Burkholderia pseudomallei* into BALB/c or C57BL/6 mice

Route of infection	NCTC 13178		NCTC 13179	
	BALB/c	C57BL/6	BALB/c	C57BL/6
Intravenous	<10	5 × 10 ³	9 × 10 ³	6 × 10 ⁶
Intraperitoneal	1.2 × 10 ¹	9.7 × 10 ³	4.7 × 10 ⁵	2.1 × 10 ⁷
Subcutaneous	1 × 10 ³	9 × 10 ⁵	9 × 10 ²	>10 ⁸
Intranasal†	1.4 × 10 ²	1.8 × 10 ³	1.9 × 10 ⁶	>10 ⁸
Oral‡	7.2 × 10 ³	1.8 × 10 ⁶	4.8 × 10 ⁶	>10 ⁸

*50% lethal dose.

†20 µL of challenge dose was introduced intranasally onto the nostrils of the mice by using a pipette tip.

‡20 µL of challenge dose was introduced orally to the back of the throat of the mice by using a pipette tip.

each of the 5 routes. *B. pseudomallei* could be detected in the tissues of IV- and IP-infected mice earlier and in higher numbers than in those of intranasally and orally-infected mice, despite the fact that all mice received equal numbers of bacteria. This finding reflects differences in the innate immune response, depending on the route of infection. Bacterial numbers in mice infected by the IV or IP route reached >10⁶ CFU by day 2 postinfection, which indicates a failure of the innate immune response to control infection, leading to overwhelming sepsis and death.

Bacterial loads in tissues after challenge with a lethal dose of highly virulent NCTC 13178 did not indicate any tropism for the lung after intranasal infection. As early as day 1, bacterial loads were greatest in the liver and spleen, not lungs, of C57BL/6 and BALB/c mice following intranasal challenge. This finding suggests a very early systemic spread of *B. pseudomallei* from the lungs to other organs.

Bacteria were detected in the brains of all mice after infection by either the IV, IP, intranasal, or oral route. Colonies recovered from the brains of C57BL/6 mice infected by the intranasal or oral routes were mucoid in appearance. In comparison, bacteria recovered from brains of C57BL/6 mice that were challenged by the IV or IP route demonstrated the characteristic wrinkled shape on Ashdown agar and may have been a

consequence of the overwhelming septicemia that spilled over to all organs. Variation in colonial morphology of *B. pseudomallei* has been documented previously (8), and biofilm formation may be an adaptation of *B. pseudomallei* that enables it to evade host immune responses or to survive within unfavorable environments (9,10). The variation in colonial morphology on Ashdown agar observed in bacteria isolated from brains of C57BL/6 mice infected by the intranasal or oral route may reflect a change to biofilm formation of *B. pseudomallei* in this tissue.

In summary, the results of this study reiterate the validity of the mouse model for differential susceptibility to *B. pseudomallei*, regardless of the route of infection. The data also emphasize that virulence depends on the portal of entry of *B. pseudomallei*. Researchers should, therefore, be particularly cautious when comparing and extrapolating data from studies that use different methods of infection.

Jodie L. Barnes*
and Natkunam Ketheesan*

*James Cook University, Townsville, Queensland, Australia

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Address for correspondence: Jodie L. Barnes, School of Biomedical Sciences, James Cook University, Townsville, Queensland, Australia 4811; fax: 61-7-4779-1526; email: jodie.barnes@jcu.edu.au

Bordetella pertussis in Adult Pneumonia Patients¹

To the Editor: Although *B. pertussis* infection is well-characterized in children, the epidemiology and clinical spectrum of pertussis in ado-

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lescents and adults are less well defined. Instances of pneumonia complicating adult pertussis have been reported (1,2), yet the role of *B. pertussis* in adult pneumonia has not been rigorously evaluated.

This study searched specifically for evidence of *B. pertussis* infection in 304 adults (≥ 18 years) admitted to Christchurch Hospital (Christchurch, New Zealand) with community-acquired pneumonia from August 1999 to July 2000 (3). Nasopharyngeal samples and paired serum samples from these patients were stored and later tested for *B. pertussis* DNA and *B. pertussis* antibodies. Culture for *B. pertussis* was not performed because *B. pertussis* was not part of the original pneumonia study protocol.

Nasopharyngeal samples were centrifuged and tested for *B. pertussis* DNA by using the IS481 hemi-nested polymerase chain reaction (PCR) assay described previously (4). Serum samples taken from the patients during acute and convalescent phases of disease were tested for immunoglobulin (Ig)A and IgG antibodies against *B. pertussis* whole cell antigens by using enzyme-linked immunosorbent assay (ELISA) (Pan Bio, Queensland, Australia). All positive serum samples were tested for pertussis toxin (PT) IgG antibodies, the most specific serologic marker for recent *B. pertussis* infection (5). This assay, which uses highly purified PT as antigen, has been described in detail elsewhere (6).

Of the 304 adults, both acute and convalescent phase serum samples were available from 257 patients, only acute phase samples were available from 46 patients, and no samples were available from 1 patient; nasopharyngeal swabs samples were available for testing for 275 patients. Overall, 8 (3%) patients had definite recent *B. pertussis* infection based on *B. pertussis* DNA in nasopharyngeal samples (8 patients) or elevated levels of anti-PT IgG antibodies (single sample with an anti-PT IgG level ≥ 100 EU/mL, or

Table. Characteristics of adults with pneumonia and evidence of recent *Bordetella pertussis* infection

Characteristic	Definite evidence of recent <i>B. pertussis</i> infection (n = 8)	Possible evidence of recent <i>B. pertussis</i> infection (n = 18)
Median age (range)	68 (37–86) y	71 (34–95) y
Male:female	4:4	13:5
Current or ex-smokers	6 (75%)	16 (89%)
Median (range) duration of symptoms at admission	8.5 (1–21) d	3.5 (1–30) d
Presence of cough	8 (100%)	15 (83%)
Sputum production	6 (75%)	7 (39%)
Other respiratory tract pathogens identified	6 (75%)	11 (61%)

demonstration of a ≥ 4 -fold rise in anti-PT IgG level) (5 patients). Eighteen (6%) additional patients had evidence of possible recent *B. pertussis* infection based on elevated levels of IgA antibody or demonstrated IgG antibody seroconversion to whole cell lysate *B. pertussis* antigens, but had low levels of anti-PT IgG antibodies. A moderate degree of pertussis existed in the community during the study period, with 4–73 notifications per month in the Christchurch region (population 421,000).

Characteristics of the patients with evidence of recent *B. pertussis* infection are shown in the Table. Other respiratory pathogens identified from the patients with definite recent *B. pertussis* infection were *Streptococcus pneumoniae* (2 patients), *Haemophilus influenzae* (2 patients), respiratory syncytial virus (1 patient), and influenza A virus (1 patient). Respiratory pathogens identified in the group with possible recent *B. pertussis* infection were *S. pneumoniae* (6 patients), *H. influenzae* (2 patients), respiratory syncytial virus (2 patients), influenza A virus (2 patients), *Legionella pneumophila* (1 patient), adenovirus (1 patient), and *Pseudomonas aeruginosa* (1 patient). No patients died, but 2 were admitted to the intensive care unit. No clinical or laboratory variables distinguished patients with recent evidence of pertussis from other patients in the study, although the former group had higher proportion of current or ex-smokers

(85% vs. 65%; 95% confidence interval for the difference 5%–35%).

This study is the first to systematically search for evidence of *B. pertussis* infection in adults with community-acquired pneumonia. We found evidence of recent *B. pertussis* infection in 3% of adults admitted to the hospital with well-defined pneumonia during a period of increased pertussis activity, and weaker evidence in an additional 6%. In comparison, a community-based study of 122 adults with respiratory tract infections found serologic evidence of *B. pertussis* infection in 7% of the patients (7). Other studies have reported that pneumonia complicates $\approx 4\%$ of *B. pertussis* infections in adults (1,2), with the disease increasing with age (1).

B. pertussis infection can be difficult to diagnose, especially if symptoms have been present for many days, and we may have underestimated the number of patients with recent pertussis. However, the combination of PCR and serologic testing is one of the most sensitive approaches for diagnosing pertussis in adolescents and adults (5). The nasopharyngeal samples may not have been optimal for PCR testing because they were placed in viral transport media and had already been processed for viral studies. Although the viral transport media was not inhibitory to the PCR, the amount of cellular material may have decreased after this processing.

Our findings indicate that a small proportion of adults admitted to the

hospital with pneumonia had evidence of recent *B. pertussis* infection. In these persons, whether *B. pertussis* is a primary or secondary pathogen or an innocent bystander is not clear. Further work is needed to clarify the precise role of *B. pertussis* in developing adult pneumonia, the risk factors for *B. pertussis*-associated pneumonia, and the value of specific *B. pertussis* therapy in this setting. These data will also help inform about the role of pertussis vaccination in adults.

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Kirsten A. Beynon,*
Sheryl A. Young,*
Richard T.R. Laing,†
Timothy G. Harrison,‡
Trevor P. Anderson,* and
David R. Murdoch*†

*Canterbury Health Laboratories, Christchurch, New Zealand; †Christchurch School of Medicine and Health Sciences, Christchurch, New Zealand; and ‡Health Protection Agency, London, United Kingdom

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Address for correspondence: David R. Murdoch, Microbiology Unit, Canterbury Health Laboratories, P.O. Box 151, Christchurch, New Zealand; fax: 64-3-364-0238; email: david.murdoch@cdhb.govt.nz

SARS Risk Perception and Preventive Measures, Singapore and Japan

To the Editor: Healthcare workers accounted for 21% of all cases of severe acute respiratory syndrome (SARS) during the 2002–2003 outbreak (1). We studied perceptions of risk for SARS infection and preventive measures among healthcare workers in Singapore, who handled cases of SARS and where >41% of the cases occurred among healthcare workers, and in Japan, a SARS-free country.

A self-administered questionnaire was distributed to healthcare workers in various healthcare settings in Singapore (n = 15,025) and Japan (n = 9,978) from May to September 2003. Healthcare workers in Singapore were from 9 primary healthcare hospitals and 9 major institutional healthcare

settings, including 3 tertiary hospitals where cases of SARS occurred among healthcare workers, 1 specialized women and children's hospital, 2 community hospitals, and 2 tertiary dental centers. In Japan, study participants were healthcare workers at 7 tertiary-level hospitals distributed throughout Japan. Four of these are university-attached, 2 are municipal hospitals, and 1 is a private hospital.

A total of 10,511 (70% response) and 7,282 (73% response) valid questionnaires were returned in Singapore and Japan, respectively. A total of 43% and 45% of the healthcare workers in Singapore and Japan were nurses; others were doctors, physiotherapists, pharmacists, attendants, cleaning staff, and administrative or clerical staff. In terms of sociodemographic characteristics, the mean ages of the healthcare workers were 36.6 years in Singapore and 35.6 years in Japan, while the gender distribution was 82% female in Singapore and 70% female in Japan, respectively. Approximately half (57% and 45%, respectively) of healthcare workers in Singapore and Japan were married.

A similar proportion (about two thirds) of healthcare workers in both countries felt at great risk of exposure to SARS. However, a higher proportion (76%) was afraid of contracting SARS in Singapore as compared to Japan (55%). Nearly all healthcare workers (96%) in Singapore felt that implementation of protective measures at work was generally effective, and 95% were satisfied with the explanation of their necessity and importance. Slightly fewer (93%) agreed that clear policies and protocols for everyone to follow were in place. In contrast, among Japanese healthcare workers, only 65% agreed that clear policies and protocols were in place, and many fewer (31%) felt that protective measures at work were generally effective (Table).

As to the national experiences with the SARS outbreak, healthcare work-