Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2004

A Report of the Australian Mycobacterium Reference Laboratory Network

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Abstract

The Australian Mycobacterium Reference Laboratory Network collected and analysed laboratory data on new cases of disease caused by *Mycobacterium tuberculosis* complex in the year 2004. A total of 787 cases were identified by bacteriology, representing an annual reporting rate of 3.9 cases per 100,000 population. Almost all isolates were identified as *M. tuberculosis* (n=785), the remaining isolates being one each of *Mycobacterium africanum* and *Mycobacterium canettii.* Seven children under 10 years of age (female n=5, male n=2) had bacteriologically confirmed tuberculosis (gastric aspirate n=4, lymph node n=1, pleural n=1, thigh wound n=1). Results of *in vitro* drug susceptibility testing were available for all 787 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z). A total of 71 (9.0%) isolates of *M. tuberculosis* were resistant to at least one of these anti-tuberculosis agents. Resistance to at least both H and R (defined as multidrug resistance) was detected in 12 (1.5%) isolates; 10 were from the respiratory tract (sputum n= 7, bronchoscopy n= 3). The country of birth was known for 68/71 (95.8%) cases with a drug resistant strain; eight were Australian, 60 were overseas born, and three were unknown. Of the 60 migrants with drug resistant disease, 37 (61.7%) were from three countries; Viet Nam (n=20), China (n=9) and India (n=8). *Commun Dis Intell* 2006;30:102–108.

Keywords: Mycobacterium tuberculosis, Mycobacterium bovis, *laboratory diagnosis, tuberculosis, drug resistance, nucleic acid amplification test*

Introduction

Australia continues to have one of the lowest incidence rates of tuberculosis (TB) in the world. These rates have remained stable since the mid-1980s.[1] Tuberculosis remains a major health problem globally, and particularly in the World Health Organization regions of South East Asia and the Western Pacific.[2] In 2003, there were an estimated 9.7 million prevalent cases of TB, equivalent to 291 cases per 100,000 population, of which some 5 million were new cases. China and India accounted for 63 per cent of the incident cases for the two regions. Overall, the Western Pacific Region reported a notification rate of 57 cases per 100,000 population in 2003.

There are two sources of TB-related data for Australia. Since 1991, the National Notifiable Diseases Surveillance System (NNDSS) has provided statistics on cases of tuberculosis reported to public health authorities in Australia's states and territories. The second source, the Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986. Statistics compiled by the AMRLN relate to cases of bacteriologically confirmed tuberculosis whereas NNDSS data will have a proportion of cases that are identified on the basis of clinical and epidemiological information, or on non-bacteriological laboratory investigations. This report describes the bacteriologically confirmed TB diagnoses for the year 2004.

Methods

The data are based on clinical specimens that were culture-positive for *Mycobacterium tuberculosis* complex (MTBC). Although the Bacille Calmette-Guérin strain of *Mycobacterium bovis* is a member of the MTBC, no information on this organism is included in the present report. Almost all isolates of MTBC were referred to one of the five laboratories

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comprising the AMRLN for specific identification and drug susceptibility testing. Comparable methodologies are used in the reference laboratories. Relapse cases, as defined by the *National Strategic Plan for TB Control in Australia Beyond 2000* prepared by the National TB Advisory Committee, were included in the laboratory data as laboratories are generally unable to differentiate relapse cases from new cases.[3] Temporary visitors to Australia were included as were illegal aliens within correctional services facilities and asylum seekers located in detention centres or on temporary visas within Australia.

For each new bacteriologically confirmed case, the following information was collected (where available):

- demography: patient identifier, age, sex, HIV status and state of residence;
- specimen: type, site of collection, date of collection and microscopy result;
- isolate: species of mycobacterium and results of drug susceptibility testing;
- nucleic acid amplification testing: results of testing; and
- if the isolate was drug resistant: patient country of origin, and history of previous TB treatment to determine whether resistance was initial or acquired.

Data from contributing laboratories were submitted in standard format to the scheme coordinator for collation and analysis. Duplicate entries (indicated by identical patient identifier and date of birth) were deleted prior to analysis. Rates were calculated using mid-year estimates of the population for 2004 supplied by the Australian Bureau of Statistics.[4]

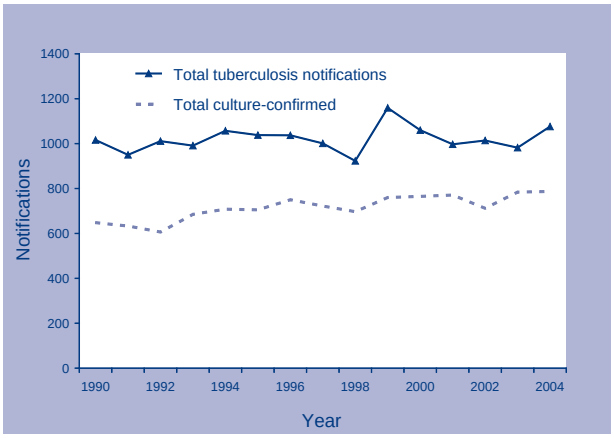
For each case, the nature of the first clinical specimen that yielded an isolate of MTBC was used to record the nominal site of disease. Culture-positive specimens collected at bronchoscopy or by gastric lavage were considered to indicate pulmonary disease. Cases with multi-site isolations, provided a sputum or bronchoscopy specimen was culture-positive, were listed as having pulmonary disease, the most important category for public health purposes. Cases for which there were multiple-site isolations were not categorised as having miliary or disseminated disease as differentiation is based on clinical findings that are generally not available to the reporting laboratories.

Drug resistance among new cases (as a proxy for primary resistance) was defined as the presence of resistant isolates of *M. tuberculosis* in patients, who in response to direct questioning, deny having had any prior anti-TB treatment (for as much as one month) and, in countries where adequate documentation is available, for whom there is no evidence of treatment. Drug resistance among previously treated cases (as a proxy for acquired resistance) was defined as the presence of resistant isolates of *M. tuberculosis* who, in response to direct questioning, admit having been treated for one month or more or, in countries where adequate documentation is available, for whom there is no evidence of such a treatment.[5]

Results

There were 787 bacteriologically confirmed cases of tuberculosis in 2004 (Figure 1), representing an annual rate of 3.9 cases per 100,000 population. State-specific reporting rates ranged from 1.7 to 10.5 cases per 100,000 population in Tasmania and the Northern Territory respectively (Table 1).

Figure 1. Comparison between tuberculosis notifications and laboratory data, Australia, 1990 to 2004



Causative organism

Almost all isolates were identified as *M. tuberculosis* (n=785), the remaining isolates being one each of *Mycobacterium africanum* and *Mycobacterium canettii.* No isolates of *Mycobacterium bovis* were reported for 2004.

Distribution by gender, age and site of disease

Complete information for gender and age were submitted for 785/787 (99.8%) of all patients, due to additional information provided by state and territory tuberculosis centres. Of the 787 MTBC isolates, 369 (46.9%) were from females, 417 (53.0%) were from males, and gender was unknown for a single case. Seven children aged under 10 years (female n=5, male n=2) had bacteriologically confirmed tuberculosis (gastric aspirate n=4, lymph node n=1, pleural n=1, thigh wound n=1). The rela-

Table 1. Bacteriologically confirmed cases of tuberculosis in Australia, 1994 and 2002–2004, cases and rate per 100,000 population, by state or territory

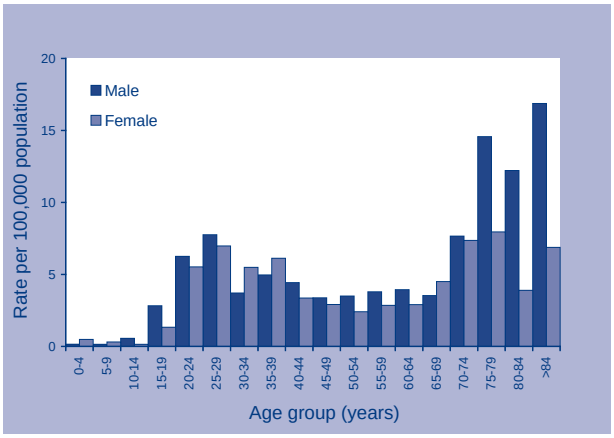
State or territory	2004		2003*		2002*		1994*	
	n	Rate	n	Rate	n	Rate	n	Rate
New South Wales†	308	4.4	325	4.6	301	4.3	278	4.4
Northern Territory	21	10.5	20	10.1	26	13.0	21	12.3
Queensland	88	2.3	91	2.4	97	2.6	88	2.8
South Australia	43	2.8	36	2.4	26	1.7	41	2.8
Tasmania	8	1.7	4	0.8	8	1.7	10	2.1
Victoria	262	5.3	254	5.2	208	4.3	217	4.8
Western Australia	57	2.9	54	2.8	46	2.4	53	3.1
Total	787	3.9	784	3.9	712	3.6	708	4.0

* Data from previous reports of the Australian Mycobacterium Reference Laboratory Network.

† Data from the Australian Capital Territory are included with those from New South Wales.

tionship of tuberculosis to age and gender is shown in Figure 2. The site of disease was dependent upon age and gender. The overall male:female ratio was 1.1:1. For males, there were two distinct age groups: a rise to 7.8 cases of TB per 100,000 population at 25–29 years and in the elderly male greater than 75 years (>10 cases of TB per 100,000 population). The age groupings for females was similar with 7.0 and >8 TB cases per 100,000 population for the 25–29 and >70–74 year age ranges respectively. For respiratory isolates, the male:female ratio was 1.4:1. The median age group for patients with bacteriologically confirmed disease was 20–24 years for males and 25–29 years for females. For lymph tissue, the male:female ratio was 1:1.6. For males the median age was 25–29 years with 2.0 TB cases per 100,000 population; the remaining age groups were all less than 1.0 per 100,000 population. In contrast, the median age range for females was 35–39 years, peaking at 2.7 TB cases per 100,000 population and 2.1 TB cases per 100,000 population in the 25–29 year age group. The predominant culture-positive specimen type was sputum (n=366, 46.5%); a further 100 (12.7%) were obtained via bronchoscopy, and five were from tissue/biopsies. Thirty-seven pleural specimens (28 fluid, 9 biopsy/tissue) were culture-positive; only two fluids were smear positive.

Figure 2. Laboratory confirmation of *Mycobacterium tuberculosis* complex disease, Australia, 2004, by age and sex



The most commonly encountered extrapulmonary culture-positive specimen was lymph tissue (n=172, 21.9%) followed by peritoneal (n=25, 3.2%), bone/joint (n=22, 2.8%), and genitorurinary tract (n=17, 2.2%).

Association with HIV

The AMRLN database recorded the HIV status of only 62 (7.9%) patients. No patient was identified as HIV seropositive.

Microscopy

Results of microscopy were available for 773 of 787 (98.2%) specimens; microscopy was not performed on 12 specimens and no results were provided for the remaining two specimens. Smears were positive for 202 of 366 (55.2%) sputum and for 43 of 100 (43%) bronchoscopy specimens respectively (Table 2). A total of 37 pleural specimens (9 biopsy and 28 fluids) were culture positive for *M. tuberculosis* with two fluid specimens smear positive (5.4%) only. Lymph node specimens were smear positive for only 33 of 172 (19.2%) cases.

Drug susceptibility testing

Results of *in vitro* drug susceptibility testing were available for all 787 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z). A total of 71 (9.0%) isolates of *M. tuberculosis* were resistant to at least one of the above anti-tuberculosis agents.

Table 2. Site of specimens smear- and culture-positive for *Mycobacterium tuberculosis* complex, 2004

	n	Smear positive (%)*
Sputum	366	202 (55.2)
Bronchoscopy	100	46 (46.0)
Lymph node	172	33 (19.2)
Pleural	37	2 (5.4)
Genito-urinary	17	ND
Bone/joint	22	ND
Peritoneal	25	ND
Skin	8	ND
Cerebrospinal fluid	5	ND

* Based on specimens that reported a microscopy result and excludes (i) microscopy not performed or (ii) result unknown.

ND The percentage of specimens smear positive was not calculated due to small numbers.

Results of testing for streptomycin (S) were available for 221 (28.1%) of 787 isolates with nine demonstrating S mono-resistance and another 10 resistant to S + H. Resistance to at least both H and R (defined as multi-drug resistance) was detected in 12 (1.5%) isolates. All of the MDR isolates were *M. tuberculosis* (Table 3). Of the 12 MDRTB isolates, 10 were from the respiratory tract (sputum n=7, bronchoscopy n=3); the remaining isolates were from a neck abscess and a peritoneal biopsy. Four of the MDRTB-positive sputum specimens were smear positive, as was the neck abscess. None of the bronchoscopy specimens were smear positive.

Mono-resistance to isoniazid, rifampicin, ethambutol, and pyrazinamide was detected in 31, 2, 4, and 5 isolates, respectively. There were 53 isolates that demonstrated resistance to H at a concentration of 0.1 mg/L. Of these, 38 (71.7%) demonstrated resistance to H at the higher level of 0.4 mg/L. For MDRTB strains, 11/12 (91.6%) demonstrated resistance at the higher concentration. Thirty-seven of 73 (50.7%) specimens culture-positive for drug resistant *M. tuberculosis*, including 28 of 48 (58.3%) sputum or bronchoscopy specimens, were smear-positive for acid-fast bacteria.

New case or previously treated, and country of birth

There were 71 *M. tuberculosis* isolates resistant to at least one of the standard drugs (H, R, E, Z). Of these, 45/52 (86.5%) were classified as being new cases, seven were considered as previously treated, and no information was available for 19 cases. The country of birth was known for 68/71 (95.8%) cases; eight were Australian, 60 were overseas born, and three were unknown. Of the 60 migrants with drug resistant disease, 37 (61.7%) were from three countries; Viet Nam (n=20), China (n=9) and India (n=8). The 12 patients with MDR-TB were from China (n=3), India (n=2), Viet Nam (n=2) with a single case each from Australia, Eritrea, Pakistan, South Africa and the Philippines.

Use of nucleic acid amplification tests

Nucleic acid amplification testing (NAAT) was performed on 173 of 787 (22.0%) specimens, all of which subsequently grew MTBC on culture. Of these, 103 specimens were of respiratory origin (sputum n=77; bronchoscopy n=22; tissue n=3; swab n=1), and 100 (97.1%) were NAAT positive. For smear positive respiratory specimens, 81 of 82 (98.8%) were NAAT positive whilst 19 of 21 (90.5%) smear negative respiratory specimens were NAAT positive.

Table 3. Drug resistance patterns in multi-drug resistant strains of *Mycobacterium tuberculosis* complex strains, Australia, 1994 to 2004

Resistance pattern (standard drugs)*	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995	1994
H+R only	7	4	8	8	3	2	2	6	10	3	2
H+R+E	2	2	1	1	1	1	1	1	1	1	0
H+R+Z	1	1	1	3	3	1	2	5	4	1	0
H+R+E+Z	2	0	2	0	1	0	1	2	0	0	0
Total (%)	12 (1.5)	7 (0.9)	12 (1.7)	12 (1.6)	8 (1.0)	4 (0.5)	6 (0.9)	14 (1.9)	15 (2.0)	5 (0.7)	2 (0.3)

* The streptomycin result was not considered for this table.

H = Isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide

There were 70 specimens of non-respiratory origin (tissue n=44; fluids n=14; aspirate n=9; pus n=3) and 44 (62.9%) were NAAT positive. For smear positive non-respiratory specimens, 16 of 18 (88.9%) were NAAT positive and 28 of 50 (56.0%) of smear negative non-respiratory specimens were NAAT positive. One specimen did not record a smear result, and one pleural biopsy yielded a non-interpretable result due to inhibitors.

Discussion

The finding of 787 cases of bacteriologically confirmed tuberculosis representing 3.9 cases per 100,000 population in 2004 was the same as for 2003 and is consistent with the results of previous AMRLN reports dating back to 1986. Similarly, distribution by gender, age and site of disease was consistent with previous reports.[6–17]

Once again, almost all isolates were *M. tuberculosis*, with only a single *M. africanum* and one case of the recently described *M. canettii*. Traditionally, the MTBC has contained four species: *M. tuberculosis*, *M. africanum*, *M. bovis* including the vaccination strain *M. bovis* (Bacille Calmette-Guérin) and *Mycobacterium microti*. More recent additions to the complex include *Mycobacterium canettii*, a rarely encountered human pathogen,[18] *Mycobacterium caprae* isolated from lymph node and lung of tuberculous goats,[19] and *Mycobacterium pinnipedii*, the causative agent of disease in seals from Australia, New Zealand, South America and Great Britain, and occasionally in human beings.[20,21] The possible evolutionary scenario for the emergence of the members of the MTBC has been discussed by Brosch, *et al*.[22]

M. canettii is an exotic member of the MTBC. Other than the initial Canetti strain, a further example of the species was cultured in 1993 from the lymph node of a 2-year-old Somali child. The peculiarity of this species is its abundant growth on solid media producing white, glossy colonies within six days of incubation. The Somali isolate was susceptible to H, R, and E but resistant to streptomycin. No result was reported for pryazinamide.[18] The *M. canettii* reported here was a lymph node isolate from a 36-year-old female.

The 'seal bacillus' associated with tuberculosis was isolated initially from three dead seals washed ashore in southern Western Australia. Comparison with other isolates cultured previously from seals and a trainer who worked with the infected seals found similar genetic profiles suggesting that the organism occupied a unique position within the MTBC.[20] It has been isolated from seals on four continents and is now recognised as *M. pinnepedii*, the causative agent of tuberculosis in seals, and occasionally humans. Transmission to humans is believed to be incidental, presumably by droplet aerosols.[21]

A longstanding member of the MTBC, *M. microti* is a causative agent of disease in wild voles or field mice but not in human beings. However, that view changed when two DNA fingerprinting methods unexpectedly found that *M. microti* had unique fingerprinting and spoligotyping profiles.[23] Similar profiles were subsequently identified in four human cases resident in The Netherlands; three of the four cases were immunocompromised (renal transplantation n=2; HIV n=1), the other case occurred in a 34-year-old immunocompetent male. From the clinical perspective, three patients were treated successfully with standard antituberculous therapy; the fourth died from overwhelming infection shortly after diagnosis. However, one patient with heavy smear positive lung disease was highly likely to have transmitted the organism to friends and a close relative. Another case of *M. microti* was confirmed from a resident of the United Kingdom although no other details were recorded.[24] The frequency of human disease caused by *M. microti* is unknown as the organism is difficult to cultivate, taking months rather than weeks to grow, and traditional phenotypic tests are unreliable.

For bacteriologically confirmed cases of tuberculosis in Australia, the most likely causative agent remains *M. tuberculosis* with the occasional *M. bovis* or *M. africanum.*[17] The most recent members of the MTBC are encountered infrequently by Australian laboratories, and indeed globally.

The level of acquired resistance in Australia remains low. Most cases with drug resistant strains occurred in the overseas born and reflects previous data.[14–17] These findings reflect more upon the performance of the TB program from their country of origin rather than the clinical management of these patients in Australia. Therefore, as a measure of performance of Australia's TB control program, the national drug resistance data has limited usefulness. For 2004, the proportion of isolates that were MDRTB was 1.5 per cent, and the number of isolates resistant to at least one of the first line anti-TB drugs was 71 (9.0%); these findings are consistent with previous reports.[6–17] In Australia, rifampicin resistance is relatively rare and is considered a useful surrogate marker for MDRTB. In the years 2000–2004, there were 3,819 bacteriologically confirmed cases of TB.[14–17] Only 58 (1.5%) demonstrated resistance to rifampicin and 51/58 (87.9%) of these isolates were MDRTB. Interestingly, 35/51 (68.6%) of the MDRTB isolates were from patients who had given a history of no previous TB treatment. For 2000–2004, the MDRTB cases were from 17 countries and of these, 11 countries were from the South East Asia and Western Pacific Regions.

In conclusion, the 2004 laboratory data for culture confirmed cases of TB demonstrates a steady-state situation for the number of cases reported, level of smear positivity for respiratory specimens, and drug resistance.

Acknowledgements

The Australian Mycobacterium Reference Laboratory Network comprises the Mycobacterium Reference Laboratories at the following facilities:

Institute of Medical and Veterinary Science, Adelaide, South Australia

Queensland Health Pathology Services, The Prince Charles Hospital, Chermside, Queensland

Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria

PathWest, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia

Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales

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References

1. Li J, Roche P, Spencer J, Bastian I, Christensen A, Hurwitz M, *et al.* Tuberculosis notifications in Australia, 2003. *Commun Dis Intell* 2004;28:464–473.

2. World Health Organization. Tuberculosis control in South East Asia and Western Pacific regions 2005. A bi-regional report.

3. Communicable Diseases Network Australia. *National Strategic Plan for TB Control in Australia Beyond 2000.* Commonwealth Department of Health and Ageing, Canberra, July 2002.

4. Australian Bureau of Statistics. Australian Demographic Statistics, June Quarter 2004.

5. World Health Organization. Anti-tuberculosis drug resistance in the world. Third global report. WHO/HTM/TB/2004.343.

6. Dawson D, Anargyros P, Blacklock Z, Chew W, Dagnia H, Gow B, *et al*. Tuberculosis in Australia: an analysis of cases identified in reference laboratories in 1986–88. *Pathology* 1991;23:130–134.

7. Dawson DJ, Cheah DF, Chew WF, Haverkort FC, Lumb R, Sievers AS. Tuberculosis in Australia, 1989–1992. Bacteriologically confirmed cases and drug resistance. *Med J Aust* 1995;162:287–290.

8. Curran M, Dawson D, Cheah D. Laboratory surveillance of *Mycobacterium tuberculosis* isolates in Australia, 1992. *Commun Dis Intell* 1994;18:337–339.

9. Curran M, Dawson D. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1993. *Commun Dis Intell* 1995;19:343–345.

10. Dawson D. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1994 and 1995. *Commun Dis Intell* 1997;21:245–249.

11. Dawson D. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1996. *Commun Dis Intell* 1998;22:183–188.

12. Dawson D. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1997. *Commun Dis Intell* 1999;23:349–353.

13. Dawson D. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1998 and 1999. *Commun Dis Intell* 2001;25:261–265.

14. Lumb R, Bastian I, Dawson D, Gilpin C, Haverkort F, Howard P, *et al*. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2000. *Commun Dis Intell* 2002;26:226–233.

15. Lumb R, Bastian I, Dawson D, Gilpin C, Haverkort F, James G, *et al*. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2001. *Commun Dis Intell* 2003;27:173–180.

16. Lumb R, Bastian I, Chew W, Gilpin C, Haverkort F, James G, *et al*. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2002. *Commun Dis Intell* 2003;27:459–465.

17. Lumb R, Bastian I, Crighton T, Gilpin C, Haverkort F, Sievers A. Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2003. *Commun Dis Intell* 2004;28:474–480.

18. van Soolingen D, Hoogenboezem T, de Haas PE, Hermans PW, Koedam MA, Teppema KS, *et al.* A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. *Int J Syst Bacteriol* 1997;47:1236–1245.

19. Aranaz A, Liebana E, Gomez-Mampaso E, Galan JC, Cousins D, Ortega A, *et al*. *Mycobacterium tuberculosis* subsp. caprae subsp. nov: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. *Int J Sys Bacteriol* 1999;49:1263–1273.

20. Cousins DV, Williams SN, Reuter R, Forshaw D, Chadwick B, Coughran D, *et al.* Tuberculosis in wild seals and characterisation of the seal bacillus. *Aust Vet J* 1993;70:92–97.

21. Cousins DV, Bastida R, Cataldi A, Quse V, Redrobe S, Dow S, *et al.* Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedi* sp. nov. *Int J Sys Evol Microbiol* 2003;53:1305–1314.

22. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc Natl Acad Sci USA* 2002;99:3684–3689.

23. van Soolingen D, van der Zanden AG, de Haas PE, Noordhoek GT, Kiers A, Foudraine NA, *et al.* Diagnosis of *Mycobacterium microti* infections among humans by using novel genetic markers. *J Clin Microbiol* 1998;36:1840–1845.

24. Kremer K, van Soolingen D, van Embden J, Hughes S, Inwald, J, Hewinson G. *Mycobacterium microti*: more widespread than previously thought. *J Clin Microbiol* 1998;36:2793–2794.