Epidemiol. Infect. (1996), **116**, 41–49

Genetic characterization of *Mycobacterium avium* isolates recovered from humans and animals in Australia

M. M. FEIZABADI[1], I. D. ROBERTSON[1], D. V. COUSINS[2], D. DAWSON[3], W. CHEW[4], G. L. GILBERT[4] AND D. J. HAMPSON*[1]

[1] *School of Veterinary Studies, Murdoch University, Murdoch, Western Australia 6150*
[2] *Australian Reference Laboratory for Bovine Tuberculosis, Department of Agriculture, South Perth, Western Australia, 6151*
[3] *State Health Laboratory, Brisbane, Queensland 4000*
[4] *Mycobacterium Reference Laboratory, Westmead Hospital, Sydney, New South Wales 2145*

(*Accepted 8 September 1995*)

SUMMARY

Genetic relationships amongst 115 mainly Australian isolates of *Mycobacterium avium* were assessed using multilocus enzyme electrophoresis (MEE). The isolates were divided into 58 electrophoretic types (ETs), with a mean genetic diversity of 0·29. Isolates from humans were closely related to but distinct from those cultured from birds, whilst some porcine isolates belonged to the same ETs as certain human isolates. Pulsed field gel electrophoresis (PFGE) was used to differentiate related isolates, and those from birds and some from other animals, including pigs, were distinguished from the human isolates. The results of MEE and PFGE suggested that certain strains of *M. avium* may be transmitted between birds and pigs, but there was no clear evidence of transmission to humans. The serovar of the *M. avium* isolates was not obviously related to their ET assignment or their PFGE type.

INTRODUCTION

Mycobacterium avium has long been recognized as a primary pathogen of birds [1], and in 1943 it was shown to infect humans [2]. The organism is present in the environment, may be found in the faeces of healthy people [3], and can be isolated from granulomas in animals [4, 5] and in human patients without any predisposing conditions [6, 7]. It is considered to be an opportunistic pathogen in humans, and has been reported to cause disseminated infection in up to 50% of patients with AIDS in the USA [8, 9], and in 17% of such patients in Australia [10, 11]. The resistance of the organisms to antimycobacterial drugs makes them important clinically [12, 13].

Several techniques have been developed to type the organisms for epidemiological studies. Among these, a seroagglutination test was introduced by Shaefer [14], and modified by Reznikov and Leggo [15], and has been used by several researchers [10, 16]. Others have used thin layer chromatography for subtyping [11, 17], and this has the advantage that it can differentiate isolates which autoagglutinate or are untypable in the seroagglutination test. Correlations have been reported between the distribution of isolates in different geographical areas and their serovar [10, 16, 18] but molecular genetic techniques, such as restriction fragment length polymorphism of DNA (RFLP), have shown such isolates to be closely related [19, 20]. Conversely isolates of a given serovar may be genetically diverse [21–23].

Multilocus enzyme electrophoresis (MEE), which has been used for genetic analysis, and for determining the population structure of various groups of bacteria, including mycobacteria [24, 25], has the advantage of

* Corresponding author.

measuring the proportional difference between the genomes of different isolates [26]. Pulsed field gel electrophoresis (PFGE) also has been shown to be useful for identification of specific strains [27–31].

The current investigation examined the epidemiology of *M. avium* infections in Australia by determining their genetic relationships using MEE. Individual strains were identified using PFGE and possible inter-species transmission was deduced. A number of non-Australian strains also were examined for comparison.

MATERIALS AND METHODS

Bacterial isolates

Seventy-two Australian isolates of *M. avium* from humans and 25 from other mammals and birds were examined, and compared with 18 non-Australian strains [32] (Tables 1 and 2).

The human isolates were provided by the State Health Laboratories in Queensland ($n = 27$) and Western Australia ($n = 4$), and Westmead hospital, New South Wales ($n = 41$). Animal isolates were obtained from the Laboratories of the Department of Primary Industries and Fisheries in Tasmania ($n = 18$), the Department of Agriculture in Western Australia ($n = 5$) and the State Health Laboratories in Queensland ($n = 2$).

Table 1. *Serovars and electrophoretic type (ET) of non-Australian strains of* M. avium* *included for comparison*

ET	Reference number	Serovar
1	SJB#2 (USA)	8
2	2993	21
4	11907-300	1
5	1602-1965	10
6	TMC 1461 (USA)	10
12	17584-286	9
13	25546-759	5
14	6450-204	9
22	13528-1079	4
22	14816-124 (USA)	11
22	TMC 1462 (USA)	11
23	16741 Cardiff	2
28	4443-1237	5
42	14141-1395 (USA)	2
47	128 Germany	3
48	6195	3
49	B-92	1
57	TMC715 (USA)	2

* Used in the study by Wayne and colleagues [32], except ET 57.

Bacterial culture and cell preparation

All isolates were grown and identified using standard bacteriological procedures [33]. Isolates were cultured for 2 weeks at 37 °C in 200 ml of Middlebrook 7H9 medium (Difco) supplemented with 10% OAD (0·06% saponified oleic acid, 0·5% bovine serum albumin and 2% glucose). Cells were harvested by centrifugation at 10000 g at 4 °C for 10 min, washed twice in phosphate buffered saline (PBS) and then stored at −20 °C overnight. Approximately 70 μl of packed cells were removed and kept in a microfuge tube for DNA extraction for PFGE, and the remainder (approximately 500 μl) were washed twice with PBS and transferred to a glass bijou bottle containing 1·5 ml of sonication buffer (10 mM-Tris-1 mM-EDTA-0·5 mM-NADP, pH 6·8) and 500 mg of glass beads (Sigma, Catalogue number G-4649). These cells were kept at 4 °C and disrupted by four cycles of sonication, each of 1 min duration, using a 50 W sonic probe (Lab Sonic 1510). Disrupted cells were microfuged at 13000 g for 20 min at 4 °C, and the supernatant dispensed into 100 μl amounts and stored at −70 °C.

Enzyme electrophoresis

The supernatants were subjected to electrophoresis in horizontal 11·4% starch gels, and the electrophoretic mobilities of 17 enzymes determined by staining for specific enzyme activity [34], except for peroxidase [35]. These enzymes included arginine phosphokinase (APK), esterase (EST), isocitrate dehydrogenase (IDH), fructose 1-6 diphosphate dehydrogenase (FDP), fumarase (FUM), glucose-6-phosphate dehydrogenase (GPD), leucyl-glycine peptidase (LGG), leucyl-proline peptidase (LP), leucyl-tyrosine peptidase (LTT) 1 and 2, peroxidase (PER), maleate dehydrogenase (MDH), nucleoside phosphorylase (NP), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (PGD), glucose-6-phosphate dehydrogenase (GPD), and super oxidase dismutase (SOD). Four different buffer systems [34] were used for electrophoresis as follows: buffer A for PGI, PGM, NP, IDH, MDH, FUM, PER and SOD; buffer B for PGD

https://doi.org/10.1017/S0950268800058945 Published online by Cambridge University Press

Table 2. *Electrophoretic type (ET), number of pulsed field gel electrophoresis (PFGE) patterns, serovar and source of Australian isolates used in the study*

ET	No of isolates	Number of PFGE patterns*	Serovar†	Origin‡	Australian State§
1	4	2	8	S, F, BL	NSW
2	1	2	2	BM	NSW
3	1	NE	4	LIV	NSW
6	8	6	4, 5, 8, 21	BW, BM, Mm, F, S	NSW, VIC
7	1	NE	2	BM	NSW
8	10	6	4, 5, 8, 9, 1/8/21, Auto	INF, BL, S, F, BM, LN	NSW, QLD, SA, WA
9	1	1	5	LT	QLD
10	3	2	8, Auto, NT	BW, BM, S	NSW, QLD, WA
11	4	3	1, 4,	S, BL, INF, Mm	NSW, QLD
12	1	1	4	LIV	QLD
13	1	1	Auto	BW	NSW
15	1	1	4	F	NSW
16	1	NE	9	BL	NSW
17	1	NE	2	F	NSW
18	1	NE	10	POR	WA
19	2	NE	ND	POR	WA
20	1	NE	5	F	NSW
21	2	1	8/1/21, 9	BM, BL	NSW
24	1	1	8	BM	NSW
25	1	NE	1/2/3	AV	TAS
26	1	NE	1	POR	WA
27	1	NE	ND	POR	WA
29	1	NE	1	F	NSW
30	2	1	Auto, UN	BM, F	NSW
31	1	NE	1	BL	NSW
32	1	NE	1	LIV	NSW
33	1	1	Auto	F	NSW
34	15	8	1, 8, 21, Auto	BM, BL, MB, F	NSW
35	1	1	8	BW	QLD
36	1	NE	Auto	BM	NSW
37	1	1	8	BL	NSW
38	1	1	1	BM	NSW
39	1	NE	10	S	WA
40	1	1	4	NR	VIC
41	3	2	2	AV, POR	TAS
42	1	1	1/2/3	POR	TAS
43	1	1	1/2/3	POR	TAS
44	1	NE	2	AV	TAS
45	2	NE	2	AV	TAS
46	1	NE	2	AV	TAS
47	1	NE	1/2/3	AV	TAS
49	1	NE	1/2/3	POR	TAS
50	3	2	2	AV, POR	TAS
51	1	NE	ND	AV	TAS
52	1	NE	1/18/21	INF	NSW
53	1	NE	Auto	LIV	NSW
54	1	NE	9	S	NSW
55	1	NE	Auto	LIV	QLD
56	1	NE	2/1/03	AV	TAS
58	1	NE	UN	BL	NSW

* The number of different PFGE patterns obtained. NE, isolates not examined.

† Strains were serotyped by using a microtube agglutination test [15]: Auto, autoagglutination; ND, serotyping not determined; UN, untypable.

‡ All isolates from humans unless indicated: Mm, mammalian isolate; Av, avian isolate; BL, blood; BM, bone marrow; BW, bronchial wash; F, faeces; INF, infants; LN, lymph node; LIV, liver; LT, lung tissue; NR, not recorded; POR, porcine isolate; S, sputum. Specimens are underlined where they originate from humans who are not suffering from AIDS.

§ NSW, New South Wales; VIC, Victoria; QLD, Queensland; SA, South Australia; WA, Western Australia; TAS, Tasmania.

https://doi.org/10.1017/S0950268800058945 Published online by Cambridge University Press

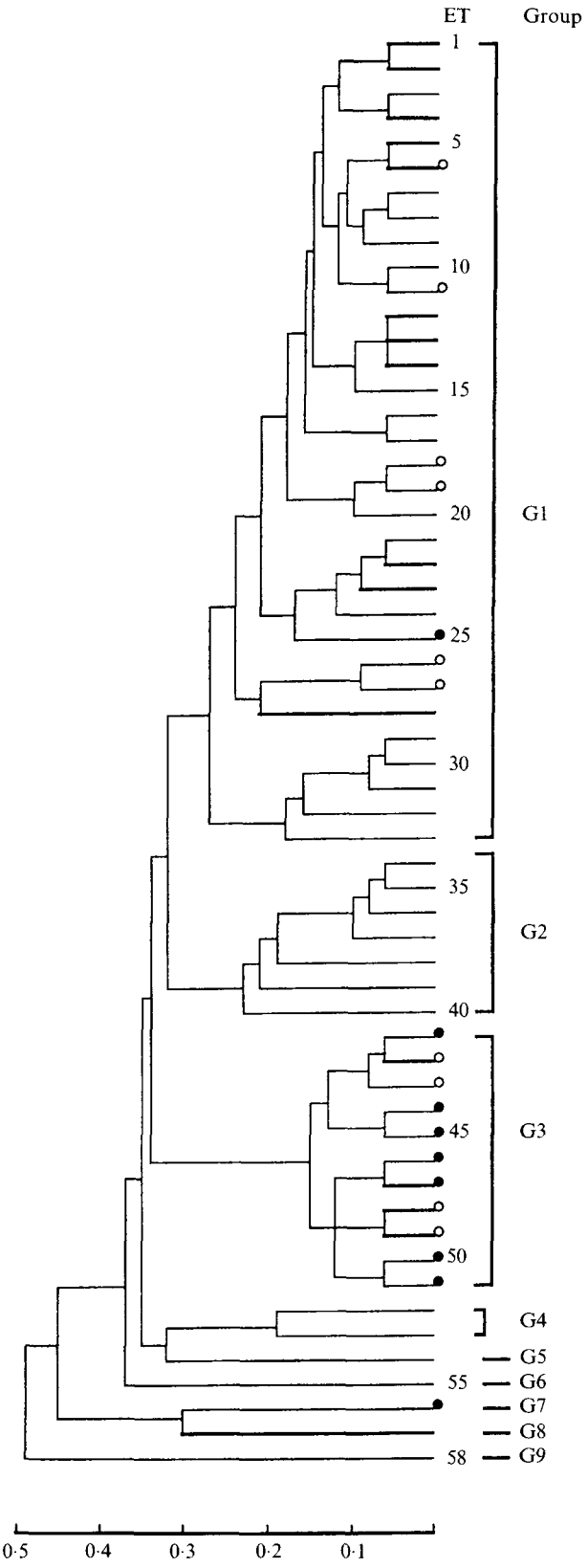

Fig. 1. Genetic variation among strains of *M. avium*. The scale shows the genetic distance (expressed as percent fixed allelic differences) among 58 ETs clustered by the unweighted pair group method with average strategy. G; group. Circles show the ETs containing Australian porcine and bovine isolates (unfilled) or avian isolates from Tasmania (filled). The other ETs contain Australian human or non-Australian strains. The position of the ETs containing the non-Australian strains are outlined in bold.

and GPD; buffer D for APK, FDP, and EST; buffer G for LGG, LTT and LP.

DNA extraction for PFGE

Packed cells (70 μl) from each isolate were washed twice in 50 mM-EDTA and the DNA extracted by the methods outlined by Lévy-Frébault and colleagues [36], with minor modifications. Briefly, cells were suspended in 400 μl of prelysing solution (6 ml of 50 mM-EDTA, 6 ml of 10 mM-Tris, 0·1 M sodium citrate, 150 μl β-mercaptoethanol, and 2 mg of lyticase (Sigma)) and the cell suspension was mixed with an equal volume of 1% low melting point agarose (Bio-Rad Laboratories) prepared in 125 mM-EDTA (pH 8), cooled to 45 °C. This mixture was poured into plug moulds. Agarose plugs were kept at 4 °C for 30 min and then transferred to 4 ml tubes containing 0·5 M-EDTA plus 7·5% β-mercaptoethanol and incubated for 24 h at 37 °C in a water bath. Agarose plugs were washed four times with TE buffer (10 mM-Tris, 1 mM-EDTA, pH 8) for 10 min each, and then incubated for 5 h in 3 ml of TE buffer containing 3 mg of lysozyme. The solution was changed to 0·5 M-EDTA containing 2 mg/ml proteinase K (Boehringer Gmbh), and 1% sodium lauroyl sarcosine (Sigma), and incubated at 55 °C for 48 h. The plugs were washed at room temperature three times for 30 min with TE, and then incubated at 55 °C in TE plus 0·04 mg/ml of phenylmethylsulfonyl fluoride (Sigma), to inactivate the proteinase K. Agarose plugs were washed three times with TE and stored in 0·5 M-EDTA at 4 °C. They were washed with TE three times for 30 min each, before being subjected to restriction endonuclease digestion.

Restriction endonuclease digestion

Agarose plugs were cut with a scalpel to fit the wells of cast gels (4 × 3 mm), washed in restriction buffer at 4 °C for 30 min, and digested for 24 h with 25 U of *Xba* I (Boehringer Gmbh) or *Vsp* I (Promega) in the buffer recommended by the supplier, supplemented with 2·5 μl of bovine serum albumin (10·27 mg/ml, Pharmacia).

https://doi.org/10.1017/S0950268800058945 Published online by Cambridge University Press

Pulsed-field gel electrophoresis (PFGE)

Sixty-one isolates were selected and examined by PFGE (Table 2). Plugs containing digested DNA were loaded into a 1% agarose gel, prepared and subjected to electrophoresis in 0·5 M-TBE buffer (1 M-TBE containing 0·025 M-Tris, 0·5 mM-EDTA, and 0·025 boric acid). PFGE was carried out with a contour-clamped homogenous electric field-DR II system (Bio-Rad Laboratories) at 14 °C for 24 h at 180 V and 12 A. Pulse time was ramped from 1–30 sec for *Xba* I (Boehringer) or 1–40 sec for *Vsp* I (Promega) digestion. Gels were stained with 0·5 μg/ml of ethidium bromide for 30 min and photographed under UV light with polaroid film. Bacteriophage lambda or *Saccharomyces cervisiae* chromosomal DNA (Bio-Rad) were used as molecular mass markers.

Analysis

Genetic diversity (h) for each enzyme locus examined in MEE was calculated from the formula $h = (1-\Sigma P_i^2)[n/(n-1)]$, where P_i is the frequency of the ith allele and n is the number of ETs or isolates in the sample [37]. Total genetic diversity (H) was calculated as the mean of h over all loci. Genetic diversities among isolates of serovars 1, 2, 4, 5, 8, 9, 21 and autoagglutinating isolates, which all were represented by five or more isolates, also were calculated separately. The genetic diversity for Australian isolates from humans and other animals respectively also were calculated. Genetic distances between ETs were calculated as the proportion of fixed loci at which dissimilar alleles occurred, and the unweighted pair-group method of arithmetic averages clustering fusion strategy was used to create a phenogram to show the relationships between isolates [38].

Statistical analysis

The matrix of coefficients which was used for allelic mismatches between each pair of isolates also was used for calculating the index of association (I_A). This index, which describes multilocus linkage disequilibrium in bacterial populations [39], is significantly different from zero for a clonal population.

RESULTS

Multilocus enzyme electrophoresis

The 115 isolates of *M. avium* were divided into 58 ETs (Table 2). These ETs were clustered into nine groups at a genetic distance (percent of allele mismatches) of 0·3 (Fig. 1). Group 1 was the largest and consisted of ETs 1–33 mainly containing isolates from humans, but including 9 isolates from Australian animals and 13 non-Australian strains, mostly recovered in the USA from bovine lymph nodes. All porcine isolates recovered from granulomas at Western Australian abattoirs belonged to this group. Group 2 (ETs 34–40) contained human isolates from New South Wales, Queensland, Victoria, and Western Australia. ET 34, containing 15 isolates from New South Wales, was located in this group. Group 3 (ETs 41–51) contained 15 isolates cultured from mammals or birds in Tasmania, and 4 non-Australian strains. Group 4 consisted of two ETs containing isolates from New South Wales, which were separated from group 3 by a genetic distance of 3·5. Groups 5–9 included ETs 54–58, with a single isolate in each, recovered from human infections or a bird (ET 56).

The enzymes, except for PGM and SOD, were polymorphic with between two (IDH) and seven (6 PGD) alleles, with a mean of 3·8 alleles. The mean genetic diversity was 0·29 for 58 ETs and 0·23 for all the isolates. Values of 0·27 were obtained for the 35 ETs containing human isolates, and 0·24 for the 18 ETs containing isolates from other animals. Isolates of serovar 2 showed the greatest genetic diversity (0·309), followed by serovar 1, autoagglutinated isolates, serovar 4, serovar 8 and serovar 5 (genetic diversity of 0·064). The genetic diversity at each enzyme locus varied for isolates of different serovars. For example, the genetic diversity for PGI varied from zero for serovar 5 to 0·733 for serovar 2. Serovar 2 also showed the greatest diversity for LT2 and EST. Each group contained several ETs that subgrouped according to their enzyme profiles. Some ETs such as 1, 6, 8, 11, 22 and 34 contained isolates belonging to different serovars. There was no apparent consistent relationship between the ET and either serovar, or the geographical origin of the isolate.

Non-Australian strains were distributed throughout the phenogram, and in several cases such as in ETs 1, 6, 12, 42, 47 and 49, were clustered with Australian isolates. Some of the porcine isolates (ETs 17 and 18) were closely related to the strains recovered from humans, while others (such as in ETs 42, 43 and 53) were identical or closely related to isolates from birds and other mammals. Two isolates from pigs (ETs 42 and 49) also were identical to certain non-Australian strains. ETs 41–50 contained isolates from birds and pigs.

https://doi.org/10.1017/S0950268800058945 Published online by Cambridge University Press

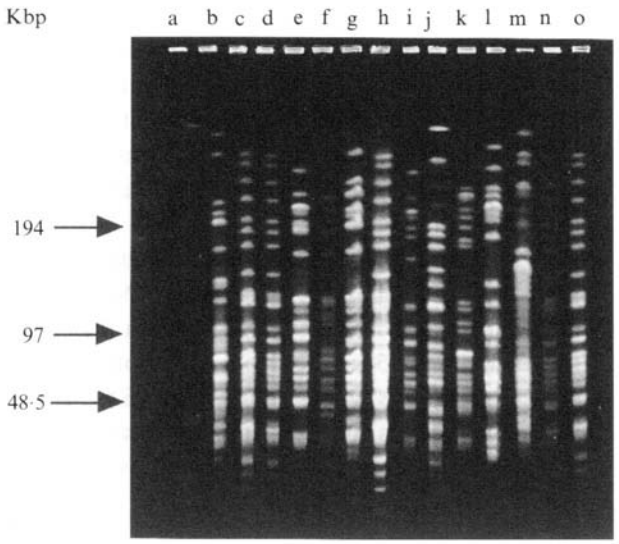

Fig. 2. DNA polymorphisms among Australian clinical isolates of *M. avium* belonging to ETs 34, 8, 6, 35, 11 and 24: Lane a, Lambda marker; Lanes b–e, i, n and o, isolates from ET 34; lane f, an isolate from ET 8; lane h and j, isolates from ET 6, lane k, an isolate from ET 35; lane l, an isolate from ET 11; lane m, an isolate from ET 24.

The index of association (I_A) was calculated as $0{\cdot}46 \pm 0{\cdot}169$ ($P < 0{\cdot}05$) for all ETs of *M. avium*. This was significantly different from zero, suggesting that this collection of organisms represented a clonal population.

Pulsed field gel electrophoresis

Sixty-one isolates of different ETs, including the major groups such as ETs 1, 6, 8, 34, 41 and 50, were further analysed using PFGE (Table 2). The two restriction enzymes *Xba* I and *Vsp* I differentiated the isolates into the same groups. This technique was more discriminating than MEE and detected up to eight different subgroups for each major MEE group. For example, isolates in ET 34 were of serovars 1, 8 or 21, or autoagglutinated. When these were analysed using PFGE, eight different subgroups were found, with each group showing differences in one to several DNA bands (Fig. 2, lanes b–e, i, n, and o). Isolates from patients with AIDS were closely related (lanes b and c), while isolates of that ET from other animals were distinct (Fig. 2; lane d). Eleven isolates in ET 8 were clustered into six different groups according to the geographical area of origin. Isolates in ET 6 also were clustered in seven subgroups. Animal isolates in the two major ETs (6 and 11) were differentiated from other isolates of these ETs by PFGE. Similarly, the non-Australian strain SJB#2 was distinct from other isolates in ET 1. Of three isolates in ET 50, one from a pig and one from a chicken were identical by both techniques, while the third differed in a single DNA band. Similar findings were observed in ETs 41 and 43. Overall there was no apparent relationship between the DNA patterns and the serovar.

Table 3. *Single and mixed infections with* Mycobacterium avium *in patients from New South Wales for which more than one isolate was examined*

Patient identity	ET	PFGE type	Serovar	Tissue/specimen
B	34	34/2	1	Blood
B	34	34/2	Auto*	Blood
C	34	34/1	21	Bone marrow
C	34	34/1	21	Blood
C	34	34/1	21	Faeces
F	6	6/7	5	Bone marrow
F	6	6/7	5	Bone marrow
H	1	1/1	8	Sputum
H	1	1/1	8	Faeces
H	1	1/1	8	Blood
H	1	1/1	8	Faeces
O	34	34/1	8	Faeces
O	38	38	1	Bone marrow
Q	15	15	4	Faeces
Q	8	8/4	4	Blood

* Auto; autoagglutinated.

Most of the isolates of *M. avium* recovered from different organs of the same patients proved to be identical (Table 3). In patient O, isolates from faeces and bone marrow belonged to distinct ETs (34 and 38), and also had distinct PFGE groups and serovars, while two isolates from patient Q were distinct by both techniques, but they were of the same serovar. In patient B (ET 34) two isolates cultured from blood belonged to the same ET and PFGE group, but were of different serovars.

DISCUSSION

This study demonstrated considerable genetic diversity amongst *M. avium* isolates from humans and other animals in Australia. Although the diversity of the isolates from humans was slightly greater than that for the other animal isolates, this may have been due to the larger number of human isolates being examined (72 versus 25). No comparison was made of

https://doi.org/10.1017/S0950268800058945 Published online by Cambridge University Press

diversity amongst human isolates from AIDS or non-AIDS patients, since there were too few from the second group for analysis. The mean genetic diversity of 2·8 for the 58 ETs was less than 3·85 previously reported for a collection of *M. avium* and *M. intracellulare* isolates [25], but greater than that reported for isolates of serovars 4 and 8 (2·30) [24].

MEE proved valuable for the genetic analysis of *M. avium*. In some cases it was more discriminatory than DNA sequencing: for example the two bovine strains TMC 1461 (ET 6) and TMC 1462 (ET 22), which have been reported to be different in one nucleotide in the complete 16–23S rDNA internal transcribed spacer sequence (ITS) [40], were differentiated by the enzymes FUM, PGI and EST. This discrimination also was supported by the results of PFGE. PFGE could be used to subtype isolates of the major groups obtained by MEE, and this demonstrated additional variation amongst isolates (Table 2). Some ETs, such as 6, 11 and 34, contained isolates originating from both humans and other animals. When these were analysed by PFGE, the isolates were differentiated, with animal isolates being distinct from the human isolates in one or more DNA bands. PFGE also differentiated isolates from different states in Australia, and non-Australian strains (ETs 6, 8, 10 and 11). These results demonstrate the ability of PFGE to differentiate between closely related isolates. Both of the restriction enzymes *Xba* I and *Vsp* I could be used to differentiate between isolates of *M. avium*, although *Xba* I was cheaper and hence more practical for large-scale studies.

Calculation of the index of association (I_A) for the 58 ETs of *M. avium* demonstrated that the population was clonal. The same was true for the subset of isolates in ETs 1–40, and of these, all except a single isolate in ET 25 have been found to be of the same RFLP type (RFLP type A) [D. V. Cousins, unpublished data]. Using RFLP, McFadden and others [41] examined several hundred strains of *M. avium* from different sources, and again these fell into a very limited number of highly conserved RFLP types. They suggested that the limited amount of genetic variation in these strains might have been due to transposition [41].

Isolates of different serovars were located in the same genetic groups (ETs 6, 8, 10, 11, 21, 30, 34 and 52), and similar findings have been reported by other researchers using different techniques [20, 22–25, 40]. On the other hand, isolates of the same serovar were not necessarily closely related. This was demonstrated even when they were isolated from different sites in the same patient (Patient Q, Table 3). Similarly, although isolates of serovar 2 are commonly recovered from birds [42], none of the isolates of this serovar from patients with AIDS were related to avian isolates of this serovar. Thus, the serovar is not a particularly useful marker for epidemiological studies since it does not reflect the genetic identity of an *M. avium* isolate.

Isolates from infected humans were distributed throughout the different groups. Most ETs containing human isolates were separated from each other by a genetic distance of 0·059, and similar findings were reported by Yakrus and colleagues [24] for the major groups of serovars 4 and 8. However, three of the isolates from Australian AIDS patients were particularly genetically distinct (ETs 53, 55 and 58).

Where several isolates were available from an individual, such as from patient O, isolates from normally sterile organs, such as the bone marrow, differed from the isolate cultured from faeces. In other patients, such as patient C, isolates from the blood and bone marrow were identical by PFGE to an isolate from faeces. These findings suggest that in some cases systemic infection may have arisen via the gastrointestinal tract. Mixed infections with *M. avium* have been reported in 14–20% of patients when samples were taken at the same time, or in 33% of patients when samples were collected at intervals of between 8–192 days [33].

The results of MEE demonstrated that animals can be infected with different strains of *M. avium*, and there may be host specificity for different strains. While most of the bovine strains (including five non-Australian strains) were closely related to human isolates (except ET 42), pigs were found to be infected by different genetic groups of *M. avium* (ETs 18, 19, 26, 27, 41, 49 and 50). In contrast, a specific group of *M. avium* isolates was found to be responsible for avian tuberculosis in Australia.

Some ETs contained isolates from pigs and chickens (ETs 41 and 50). Isolates in these ETs were of the same serovar and also were identical by PFGE. Interestingly, they were isolated from poultry and pigs originating from the same area, suggesting the occurrence of transmission of infection between these animal species. As tuberculous lesions in pigs due to infection with *M. avium* have been found in several countries [5], contact between birds and pigs may cause these cases of porcine tuberculosis. The current finding is different from a previous report from

Switzerland [43], where porcine and avian isolates were found to be distinct.

Recently Guerrero [44] detected a new Insertion Element numbered IS*1245*, which was reported to be specific for *M. avium*. They used this IS as a probe for RFLP typing of *M. avium*, and reported that the genome of *M. avium* strains isolated from pigs and humans have the same copy number of this insertion element. This implied that isolates infecting humans and pigs are related, and suggests the possibility of cross-species transmission. This finding is similar to our results for porcine isolates from Western Australia, however all six porcine isolates from Tasmania were distinct from human isolates by all the techniques used. More evidence is needed to demonstrate the possibility of transmission of such strains from pigs to humans. Since porcine isolates were distributed into different groups (Groups 1 and 3), there are likely to be other sources of infections for pigs. Future studies should examine isolates from the environment or water, and compare these with those from human beings and other animals.

ACKNOWLEDGMENTS

This work was supported by a grant from Murdoch University. M. M. Feizabadi was sponsored by the Iranian Ministry of Culture and Higher Education. We thank Darren Trott and Suzette Williams for their excellent technical assistance.

REFERENCES

1. Thoen CO, Karlson AG. Avian tuberculosis. In: Calnek BW, Barnes HJ, Beard CW, Reid WM, Yoder HW eds. Diseases of poultry. Iowa: Iowa State University Press, 1991: 172–85.
2. Feldman WH, Davis R, Moses HE, Andberg W. An unusual mycobacterium isolated from sputum of a man suffering from pulmonary disease of long duration. Am Rev Tub 1943; **48**: 82–93.
3. Portaels F, Larsson L, Smeets P. Isolation of mycobacteria from healthy persons, stools. Int J Lep 1988; **56**: 468–71.
4. Corner LA, Barrett RH, Lepper AW, Lewis V, Pearson CW. A survey of mycobacteriosis of feral pigs in the Northern Territory. Aust Vet J 1981; **57**: 537–42.
5. Morita Y. Maruyama S, Katsube Y. Prevalence of atypical mycobacteriosis in slaughtered swine in Gunma prefecture and the serovars of the isolates. J Vet Med Sci 1994; **56**; 475–9.
6. Prince DS, Peterson DD, Steiner RM, et al. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. New Eng J Med 1989; **321**: 863.
7. Debrunner M. Salfinger M, Brändli O, Von Graevenitz A. Epidemiology and clinical significance of non-tuberculous Mycobacteria in patients negative for human immunodeficiency virus in Switzerland. Clin Infect Dis 1992; **15**: 330–45.
8. Young LS, Inderlied CB, Berlin OG, Gottlieb MS. Mycobacterial infection in AIDS patient, with an emphasis on the *Mycobacterium avium* complex. Rev Infect Dis 1986; **8**: 1024–33.
9. Yakrus MA, Good RC. Geographic distribution, frequency, specimen source of *Mycobacterium avium* complex serotypes isolated from patients with Acquired Immunodeficiency Syndrome. J Clin Microbiol 1990; **28**: 926–9.
10. Dawson DJ. Infection with *Mycobacterium avium* complex in Australian patients with AIDS. Med J Aust 1990; **153**: 466–8.
11. Chew W, Sorrell TC, Gilbert GL. Subtyping of *Mycobacterium avium* complex (MAC) isolates by thin-layer chromatography-distribution of subtypes from patients with AIDS compared with clinically non-significant isolates. Epidemiol Infect 1994; **112**: 543–9.
12. Mitchison DA, Ellard GA, Grosset J. New antibacterial drugs for the treatment of mycobacterial diseases in man. Brit Med Bull 1988; **44**: 757–74.
13. Hopewell PM, Cynamon M, Starke J, Iseman M, O'Brien R. General guidelines for the evaluation of new antimycobacterial drugs for the treatment of systematic mycobacterial infection. Clin Infect Dis 1992; **15**: 282–95. (Suppl 1).
14. Schaefer WB. Serological identification classification of the atypical mycobacteria by their agglutination. Am Rev Res Dis 1965; **92** (Suppl.): 37–42.
15. Reznikov M, Leggo JH. Modification of Shaefer's procedure for serotyping of organisms of the *Mycobacterium avium-M. intracellulare-M. scrofulaceum* complex. Appl Microbiol 1981; **14**: 448–51.
16. Falkinham JO, Parker BC, Gruft H. Epidemiology of infection by nontuberculous mycobacteria. Am Rev Res Dis 1980; **121**: 931–7.
17. Denner JC, Tsang AY, Chatterjee D, Brennan PJ. Comprehensive approach to identification of serovars of *Mycobacterium avium* complex. J Clin Microbiol 1992; **30**: 473–8.
18. Ruf B, Peters M, Schröder HJ, Pohle HD. *Mycobacteria avium-intracellulare* serovars in German AIDS patient. Lancet 1989; **ii**: 1101.
19. Hampson SJ, Portales F, Thompson J, et al. DNA probes demonstrate a single highly conserved strain of *Mycobacterium avium* infecting AIDS patients. Lancet 1989; **i**: 65–8.
20. Wards BJ, Collins DM, de Lisle GW. Restriction endonuclease analysis of members of the *Mycobacterium avium-M. intracellulare-M. scrofulaceum* serocomplex. J Clin Microbiol 1987; **25**: 2309–13.
21. McFadden JJ, Butcher PD, Thompson J, Chiodini R, Hermon-Taylor J. The use of DNA probes identifying restriction-fragment length polymorphisms to examine the *Mycobacterium avium* complex. Mol Microbiol 1987; **1**: 283–91.

22. Kunze ZM, Portaels F, McFadden JJ. Biologically distinct subtypes of *Mycobacterium avium* differ in possession of insertion sequence IS 901. J Clin Microbiol 1992; **30**: 2366–72.
23. Peillon R, Drouet EB, Bruneau S, Panteix G, Denoyel GA DeMontclos HP. Discrimination of *Mycobacterium avium-Mycobacterium intracellulare* strains by genomic DNA fingerprinting with a 16S rRNA gene probe. FEMS Microbiol Lett 1994; **24**: 75–80.
24. Yakrus MA, Reeves MW, Hunter S. Characterization of *Mycobacterium avium* serotypes 4 and 8 from patients with AIDS by multilocus enzyme electrophoresis. J Clin Microbiol 1992; **30**: 1474–8.
25. Wasem CF, McCarthy CM, Murray LW. Multilocus enzyme electrophoresis analysis of the *Mycobacterium avium* complex and other mycobacteria. J Clin Microbiol 1991; **29**: 264–71.
26. Maslow JN, Mulligan ME Arbeit RD. Molecular epidemiology: Application of contemporary techniques to the typing of microorganisms. Clin Infect Dis 1993; **17**: 153–64.
27. Arbeit RD, Slutsky A, Barber TW, et al. Genetic diversity among strains of *Mycobacterium avium* causing monoclonal and polyclonal bacteremia in patients with AIDS. J Infect Dis 1993; **167**: 1384–90.
28. Burns N, Wallace RJ, Schultz ME, et al. Nosocomial outbreak of respiratory tract colonization with *Mycobacterium fortuitum*: demonstration of the usefulness of pulsed-field gel electrophoresis in an epidemiological investigation. Am Rev Res Dis 1991; **144**: 1153–9.
29. Mazurek GH, Hartman S, Zhang Y, et al. Large DNA restriction fragment polymorphism in the *Mycobacterium avium-M. intracellulare* complex: a potential epidemiological tool. J Clin Microbiol 1993; **31**: 390–4.
30. Von Reyn CF, Maslow JN, Barber TW, Falkinham JO, Arbeit RD. Persistent colonisation of potable water as a source of *Mycobacterium avium* infection in AIDS. Lancet 1994; **343**: 1137–1141.
31. Slutsky AM, Arbeit RD, Barber TW, et al. Polyclonal infections due to *Mycobacterium avium* complex in patients with AIDS detected by pulsed-field gel electrophoresis of sequential clinical isolates. J Clin Microbiol 1994; **32**: 1773–1778.
32. Wayne LG, Good RC, Tsang A, et al. Serovar determination and molecular taxonomic correlation in *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*; a cooperative study of the international working group on mycobacterial taxonomy. Int J Syst Bacteriol 1993; **43**: 482–489.
33. Vestal, A. Procedures for the isolation and identification of mycobacteria. Training and Consultative Division Centre for Disease Control, Atlanta 1975.
34. Selander RK, Caugant DA, Ochman H, Musser JO, Gilmour MN, Whittam TS. Methods of Multilocus Enzyme Electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; **51**: 873–884.
35. Lygren, ST, Closs O, Bercouvier H, Wayne LG. Catalase, peroxidase, and superoxide dismutase in *Mycobacterium leprae* and other mycobacteria studied by crossed immunoelectrophoresis and polyacrylamide gel electrophoresis. Infect Immun 1986; **54**: 662–72.
36. Lévy-Frébault V, Thorei MF, Varnerot A, Gricquel B. DNA polymorphism in *Mycobacterium paratuberculosis*, wood pigeon mycobacteria, and related mycobacteria analysed by field inversion gel electrophoresis. J Clin Microbiol 1989; **27**: 2823–6.
37. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 1987; **89**: 583–90.
38. Burr EJ. Division sorting with mixed character types II. Fusion strategies. Aust Comp J 1970; **2**: 98–103.
39. Maynard Smith J, Smith NH, O'Rourke M, Spratt BG, How clonal are bacteria? Proc Natl Acad Sci USA 1993; **90**: 4384–8.
40. Frothingham R, Wilson KH. Sequence-based differentiation of strains in the *Mycobacterium avium* complex. J Bacteriol 1993; **175**: 2818–25.
41. McFadden JJ, Kunze Z, Seechurn P. DNA probes for detection and identification. In; McFadden JJ, ed. Molecular biology of mycobacteria. London: Surrey University Press in association with Academic Press, 1990: 139–72.
42. Grange JM, Yates MD, Boughton E. The avian tubercle bacillus and its relatives. J Appl Bacteriol 1990; **68**: 411–31.
43. Bono M, Jemmi T, Bernasconi C, Burki D, Telenti A, Bodmer T. Genotypic characterization of *Mycobacterium avium* strains recovered from animals and their comparison to human strains. Appl Environ Microbiol 1995; **61**: 371–3.
44. Guerrero C, Bernasconi C, Burki D, Bodmer T, Telenti A. A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. J Clin Microbiol 1995; **33**: 304–7.