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Genotypic Characterization of *Mycobacterium avium* Strains Recovered from Animals and Their Comparison to Human Strains

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Mycobacterium avium **was recovered from 21 birds and 10 pigs. Bird isolates carried IS*901* and a few copies of IS*1245* and appeared highly related by pulsed-field gel electrophoresis. Pig isolates showed features previously described in human isolates: a lack of IS*901*, a high copy number of IS*1245*, and marked polymorphism by pulsed-field gel electrophoresis.**

Mycobacterium avium represents an important pathogen in both humans (5, 7, 12, 19, 25) and animals (8, 16). For humans, water has been identified as a definitive source of infection (24). However, the observed genetic diversity of *M. avium* strains recovered from AIDS patients (1) and the occurrence of *M. avium* in animals and various environmental samples suggest the existence of additional reservoirs (17). The purpose of this study was to characterize *M. avium* strains recovered from animal sources within a defined geographical area—the German-speaking part of Switzerland—by using a number of genotype markers, namely, the presence of insertion elements IS*900* (9), IS*901* (15), and the novel element IS*1245* (10), and by evaluating strain relatedness by pulsed-field gel electrophoresis (PFGE).

Materials investigated (n = 398) included mandibular and mesenteric lymph nodes from 120 slaughtered pigs collected from two different abattoirs; 103 samples from 25 chickens and 15 eggs from eight different flocks; organs (n = 21) from seven additional birds, including five exotic birds, in which acid-fast bacilli had been detected (Institute for Veterinary Bacteriology, University of Berne); and environmental samples, such as those from soil (n = 8), pig and chicken litter (n = 9), pondwater (n = 1), and chicken feed (n = 1).

Samples were homogenized, decontaminated with sodium dodecyl sulfate-sodium hydroxide, and neutralized, and the sediments were resuspended in phosphate-buffered saline (20). One BACTEC 12B vial supplemented with Panta Plus (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.), one pyruvate-containing Löwenstein-Jensen slant, and one slant of Herrold's medium were inoculated. Slants were incubated at 37°C for 12 weeks and inspected for growth weekly. BACTEC 12B vials were processed for 12 weeks with the BACTEC-460 TB apparatus (Johnston Laboratories Inc., Sparks, Md.) according to the manufacturer's recommendations. For identification, mycobacterial isolates underwent PCR-restriction enzyme analysis of the gene coding for the 65-kDa heat shock protein of mycobacteria (21) and conventional biochemical tests (13).

Insertion sequence analysis was performed by PCR with the following primers: IS900_U (5′-TTGATCTGGACAATGACG GT) and IS900_D (5′-GGGAATATAAAGCAGCCGCT), positions 203 to 491 (9); IS901_U (5′-AAGCCGAGGTGGTGTA TGT) and IS901_D (5′-AGGGAAGATGGCGGTGAGCAT), positions 456 to 712 (15); and IS1245_U (5′-GCCGCCGAAAC GATCTAC) and IS1245_D (5′-AGGTGGCGTCGAGGAAG AC), positions 135 to 561 (10).

DNA fingerprinting was performed with a specific IS*1245* DNA digoxigenin-labeled, PCR-generated probe (6).

PFGE was performed as previously described (4). Briefly, isolates were grown at 37°C to late exponential phase (3 to 5 days) in Middlebrook 7H9 medium supplemented with 10% OADC enrichment and 0.1% Tween 80. Cycloserine (1 mg ml^{-1}) and ampicillin (0.1 mg ml^{-1}) were added, and the cultures were incubated an additional day prior to harvesting. The pellet was washed in cold 0.1% Tween 80 in TE buffer (10 mM Tris–1 mM EDTA), snap-frozen in liquid nitrogen, and stored at −60°C. After resuspension in 1 ml of TE buffer, the pellets were mixed with 2% low-melt agarose (Bio-Rad Laboratories, Richmond, Calif.) and poured into plug molds. Plugs were digested in TE buffer containing 2 mg of lysozyme ml^{-1} and 1 μl of 2-mercaptoethanol ml^{-1} followed by TE buffer containing 1% sodium dodecyl sulfate and 0.5 mg of proteinase K

TABLE 1. Mycobacteria recovered from diverse animal and environmental sources

Source	Mycobacterium (a) recovered	No. of strains recovered
Chickens	*M. avium* subsp. *avium*	19
Duck	*M. avium* subsp. *avium*	1
Wood pigeon	*M. avium* subsp. *silvaticum*	1
Pet birds	*M. genavense*	5
Pigs[a]	*M. avium* subsp. *avium*	10
	M. fortuitum	13
	M. intracellulare	4
	M. nonchromogenicum	1
	M. terrae	1
	M. xenopi	2
	Mycobacterium spp.	1
Environment	*M. fortuitum*	1
	M. nonchromogenicum	4
	M. terrae	1

[a] In two pigs, more than one mycobacterium species was recovered.

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TABLE 2. Insertion sequence analyses and DNA fingerprinting of the recovered *M. avium* strains

Source (no.)	IS*900*	IS*901*	IS*1245*
Birds (52)	−	+	+ (3 copies)
Pigs (10)	−	−	+ (≥7 copies)
Humans (30)[a]	−	−	+ (≥7 copies)

[a] Data for human isolates are added for reference only (4, 10).

ml^{-1}. They were washed in TE buffer containing 1 mM phenylmethylsulfonyl fluoride and then in TE buffer. For DNA restriction, plugs were incubated with restriction enzyme *Ase*I (New England Bio Labs) and loaded in a 1% pulsed-field certified agarose gel (Bio-Rad). Electrophoresis was performed with a CHEF-DR III system (Bio-Rad).

Sources yielding mycobacteria are listed in Table 1. *Mycobacterium genavense* (2) appeared as a significant pathogen among exotic birds. As previously suggested (11), these might represent a source of this mycobacterial infection for AIDS patients (3, 18).

In total, 64 strains of *M. avium* were isolated from 10 pigs, 19 chickens, one duck, and one wood pigeon. According to phenotype characteristics (23), the wood pigeon strain was considered *M. avium* subsp. *silvaticum*. The remaining isolates were classified as *M. avium* subsp. *avium* (23). In the following discussion, *M. avium* refers to the latter subspecies.

Results of the genotype analysis were compared with data available for human *M. avium* isolates (4, 10) (Table 2). Of 63 *M. avium* isolates, 62 could be successfully evaluated by PFGE. Chicken isolates from various organs of the same bird as well as isolates from different chickens within a flock showed identical PFGE patterns. Chicken isolates from geographically distant farms as well as the duck isolate were shown by PFGE (Fig. 1) and IS*1245* to represent a population of highly related strains. In contrast, porcine isolates exhibited marked restriction fragment polymorphism by PFGE.

IS*900* was absent in all the strains. IS*901* was identified in all avian isolates, but in none of those of porcine origin. DNA fingerprinting with IS*1245* (10) showed elevated copy numbers of this novel element among porcine isolates as compared with avian isolates. Our data support the findings of Kunze et al. (14, 15), who reported that *M. avium* strains containing IS*901* differed in their host ranges from those lacking IS*901*. However, the authors' conclusion that the source of *M. avium* infection in humans is unlikely to be animals (14) needs careful reconsideration, because porcine and human strains in our area shared various genetic characteristics (a lack of IS*901*, a high copy number of IS*1245*, and marked polymorphism by PFGE) rendering them inseparable (4, 10).

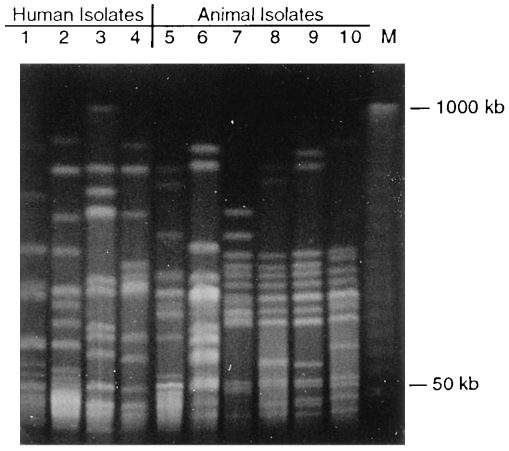

FIG. 1. Restriction patterns from *Ase*I digests of *M. avium* DNA resolved by PFGE. Lanes 1 to 4, different human isolates; lanes 5 and 6, different porcine isolates; lanes 7 to 9, different chicken isolates; lane 10, duck isolate; lane M, lambda phage concatemers (multiples of 48.5 kb).

For our area, two main conclusions can be drawn: (i) poultry are infected with a limited number of *M. avium* clones exhibiting genotypic characteristics that differ greatly from those of human and porcine isolates, and (ii) pigs and humans are infected with singular strains sharing several genotypic characteristics. Whether the latter finding reflects exposure of pigs and humans to a common environmental reservoir or whether this points to pigs or pig products as vehicles for *M. avium* infection of humans needs to be determined. If the latter was demonstrated, consumption of inadequately cooked meat or meat products from *M. avium*-positive pigs would represent a potential hazard for immunocompromised patients.

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