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Subspecies Identification and Significance of 257 Clinical Strains of *Mycobacterium avium*

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Mycobacterium avium **is abundant in the environment. It has four subspecies of three types: the human or porcine type, *M. avium* subsp. *hominissuis*; the bird type, including *M. avium* subsp. *avium* serotype 1 and serotype 2, 3 (also *M. avium* subsp. *silvaticum*); and the ruminant type, *M. avium* subsp. *paratuberculosis*. We determined the subspecies of 257 *M. avium* strains isolated from patients at the M.D. Anderson Cancer Center from 2001 to 2010 and assessed their clinical significance. An assay of multiplex PCR was used for the typing. Results showed *M. avium* subsp. *hominissuis* to be most common ($n = 238$, 92.6%), followed by *M. avium* subsp. *avium* serotype 1 ($n = 12$, 4.7%) and serotype 2, 3 ($n = 7$, 2.7%). No strains of *M. avium* subsp. *paratuberculosis* were found. Of the 238 patients with *M. avium* subsp. *hominissuis*, 65 (27.3%) showed evidence of definite or probable infections, mostly in the respiratory tract, whereas the rest had weak evidence of infection. The bird-type subspecies, despite being infrequently isolated, caused relatively more definite and probable infections (10 of 19 strains, 52.6%). Overall, women of 50 years of age or older were more prone to *M. avium* infection than younger women or men of all ages were. We therefore conclude that *M. avium* subsp. *hominissuis* is the dominant *M. avium* subspecies clinically, that the two bird-type subspecies do cause human infections, and that *M. avium* infects mainly postmenopausal women. The lack of human clinical isolation of the ruminant type subspecies may need further investigation.**

Nontuberculous mycobacteria are ubiquitous in the environment and can cause significant disease in animals and humans (1–5). *Mycobacterium avium* and *Mycobacterium intracellulare* were the two original main species belonging to the *M. avium-M. intracellulare* complex (MAIC). *M. avium* is an important opportunistic pathogen that causes respiratory tract, lymph node, and, occasionally, soft tissue infections in healthy immunocompetent individuals (6, 7). Moreover, secondary infections with *M. avium* have gained increased attention over the decades due to the longevity of the immunocompromised population, i.e., individuals living with HIV and AIDS, and the administration of immunosuppressive chemotherapeutics in cancer patients (8).

With the development of new molecular identification methods, *M. avium* has been further subdivided into four major subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *silvaticum*, and *M. avium* subsp. *hominissuis* (9, 10), each with distinct pathogenic characteristics and host preference and all considered possible zoonoses. These *M. avium* subspecies are considered to be a broad group, particularly *M. avium* subsp. *avium* and *M. avium* subsp. *paratuberculosis* as independently evolved pathogenic clones (9, 10).

M. avium subsp. *avium* represents bird-type subspecies and is well recognized as the causative agent of avian tuberculosis (11). This subspecies has three recognized serotypes, 1, 2, and 3; they are considered virulent strains for wild and domestic birds and are capable of infecting other animal species (12, 13), including humans (14–16). *M. avium* subsp. *silvaticum*, another bird-type subspecies, known as the wood pigeon mycobacterium, also causes bird tuberculosis and paratuberculosis in other species (9, 17). This subspecies is generally undifferentiable from *M. avium* subsp. *avium* serotypes 2 and 3.

The human/porcine type of *M. avium*, designated *M. avium* subsp. *hominissuis*, has been isolated from patients with HIV, respiratory disease, and lymphadentitis (3, 18–20) as well as from asymptomatic swine with granulomatous lesions of the head and mesenteric lymph nodes (21). These findings indicate that both hosts are largely susceptible to this particular organism. Recently, there have been reports of domestic animals infected with *M. avium* subsp. *hominissuis*, which may suggest the emergence of this opportunistic pathogen in veterinary medicine (22–24) and put forth the idea that animals may serve as a source of infection or that there may be exposure to common environmental reservoirs (25).

M. avium subsp. *paratuberculosis*, also referred to as Johne's disease agent, is a significant pathogen in veterinary medicine that causes chronic granulomatous enteritis in ruminants, leading to substantial economic losses in the agricultural industry (2, 26). Infection with this subspecies has been diagnosed in domestic and wildlife animals, and it is thought to be transmissible across different species (26, 27). This organism has also been implicated as a possible etiological agent of Crohn's disease in humans and so far has been isolated from samples of intestinal tissue, breast milk, and blood (28–30). However, there is a lack of definitive evidence to prove *M. paratuberculosis* infection in Crohn's patients (31–33). Thus, the relationship between *M. paratuberculosis* and Crohn's disease remains unsettled.

In view of common isolation of MAIC from patients with cancer in our institution, we performed a study earlier to differentiate *M. avium* and *M. intracellulare* and to compare the clinical significances and epidemiologic features of those infections (34). The

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present study was an expansion of the previous study to focus on *M. avium* and its subspecies. We typed the *M. avium* strains by a multiplex PCR assay and assessed the clinical significance of these strains by review of medical records.

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MATERIALS AND METHODS

Study setting, data acquisition, and assessment. This study was conducted from January 2001 to December 2010 at the University of Texas M.D. Anderson Cancer Center, a 550-bed tertiary care hospital located in Houston, TX. Approximately two-thirds of the patients at the institution were from the greater Houston area and other parts of Texas. In view of the environmental origin of *M. avium*, the climate in Houston included abundant rainfall (around 140 cm each year) and warm temperature (monthly means of 12.4°C in the coldest January to 29.1°C in the warmest July and August).

The 257 patients all had a cancer diagnosis or were suspected as such. The electronic medical records of these patients were reviewed for the clinical significance of the isolated *M. avium* strains. The isolation sources of the *M. avium* strains were categorized as tissue, respiratory tract, abdominal source, and others. The clinical significances were categorized as definitive, probable, or possible infection or contaminant based on the American Thoracic Society criteria (35) and our previous experience (34). Objective criteria, such as sterile specimens, radiographic abnormalities, and multiple isolations, were emphasized. All patients had chest X-ray examinations, and around 80% of them also had at least one chest computed tomography for better resolution of lung lesions. Briefly, definite infection required isolation of *M. avium* from a sterile source (such as a lung biopsy specimen) with compatible histopathology (such as granulomas) and/or radiographic evidence (such as a tree and bud appearance of the lung image). A nonsterile source of isolation (such as a bronchial wash and/or bronchoalveolar lavage), radiographic evidence, and clinical signs and symptoms constituted probable infection. Lack of these features constituted weak evidence of infection (contamination or possible infection).

For the purpose of this study, underlying disease states were categorized as hematologic cancer, solid tumors, and no cancer. Due to the severity of underlying cancer and/or out-of-state referral, data on treatment and outcome of the patients might be lacking or inadequate, which precluded assessment of these aspects of patient management.

Culture and identification of *M. avium*. Isolates of mycobacteria were recovered from patient specimens using standard culture practices with Lowenstein-Jensen slant and liquid 7H9-based medium with supplemental nutrients in an automated system (BacT/Alert MB; bioMérieux, Durham, NC). Positive cultures were initially identified via acid-fast stains (Kinyoun method). Further identification of various mycobacterial species was achieved through the sequencing analysis of the 16S rRNA gene upon PCR amplification as previously described (36). This method resolved 600 bp of the gene, which identified *M. avium* confidently by separating it from all other mycobacteria, particularly *M. intracellulare*, in which the two species differed by seven nucleotides in the amplicon (36). All *M. avium* subspecies had identical 16S gene sequences.

DNA extraction. Bacteria from 2 to 3 colonies or from the pellet of 2 ml of liquid medium from the original positive culture were resuspended in 200 μl of extraction solution (PrepMan Ultra, Applied Biosystems). The suspension was boiled for 10 min at 105°C and centrifuged at 12,000 × *g* for 10 min to pellet the cellular debris while retaining DNA in the supernatant. The extracted DNA sample was stored frozen at −80° until further testing in this study. One microliter of the supernatant was used as the template DNA in subsequent multiplex PCRs.

Multiplex PCR analysis. Multiplex PCRs were performed to detect four target genes, *DT1*, *IS900*, *IS311*, and *IS901*, in order to differentiate the *M. avium* subspecies, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *avium* (serotype 2, 3)/*M. avium* subsp. *silvaticum*, *M. avium* subsp. *avium* (serotype 1), and *M. avium* subsp. *hominissuis*, using previously published primers and amplification conditions (22, 37). However, while establishing the described method, amplification of *IS901* was unsuccessful somehow with control strain ATCC 35716 in the multiplex format and in single-*IS901* PCR. Therefore, we redesigned the *IS901* primers by locating them approximately 40 nucleotides downstream of the reported primers (37); all primers used in this study are listed in Table 1.

TABLE 1 PCR primers used in the multiplex reaction

Target gene	Sequence (5′ to 3′) of paired primers	Product size (bp)	Reference
DT1	CGTTGGCTGGCCATTCACGAAGGAGT GCTAGTTGGATCGCGCCGAACACCGG	296	Shin et al. (37)
IS900	TGGACAATGACGGTTACGGAGGTGG CGCAGAGGCTGCAAGTCGTGG	398	Shin et al. (37)
IS311	GCGTGAGGCTCTGTGGTGAA ATGACGACCGCTTGGGAGAC	608	Shin et al. (37)
IS901	CGACGACAGGAGTAGCGGTATGGC CCGTGCTGCGAGTTGCTTGATGAG	754	This study

In brief, the multiplex PCR assays were performed in a final volume of 25 μl, containing 10 mM Tris-HCl (pH 8.8), 2.5 mM $MgCl_2$, 50 mM KCl, 1 M betaine, 0.2 mM (each) deoxynucleotide triphosphate (Promega, Madision, WI), 10 pmol of *IS311* primers, 20 pmol of *DT1* primers, 20 pmol of *IS901* primers, 10 pmol of *IS900* primers, 2.5 U of GoTaq DNA polymerase (Promega), and 1 μl of DNA as the template. DNA amplification was performed in an Eppendorf Mastercycler (Eppendorf North America, Hauppauge, NY) under the following conditions: initial denaturation at 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 40 s, and extension at 72°C for 35 s and a final extension at 72°C for 10 min. PCR products were electrophoresed through a 2% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized on a UV transilluminator. DNA samples from *M. avium* subsp. *avium* Chester serotype 2 (ATCC 35716) and *M. avium* subsp. *paratuberculosis* (ATCC 19698) were used as PCR-positive controls.

The multiplex identification criteria used in this study were adapted from Shin et al. (37). The PCR product sizes of 296 bp, 398 bp, 608 bp, and 754 bp corresponded to amplification of the *DT1*, *IS900*, *IS311*, and *IS901* targets, respectively. The amplification of *IS311* only but not other genes was interpreted as representing *M. avium* subsp. *hominissuis*; amplifications of *IS311* and *IS900* were interpreted as *M. avium* subsp. *paratuberculosis*; amplifications of *IS311* and *IS901* indicated *M. avium* subsp. *avium* (serotype 1); and amplifications of *IS311*, *IS901*, and *DT1* indicated *M. avium* subsp. *avium* (serotype 2, 3) or *M. avium* subsp. *silvaticum*. Figure 1 shows a representative electrophoresis gel with these amplicons and corresponding subspecies. All 257 strains were typed successfully.

Statistical analysis. Where appropriate, statistical analyses with a chi-square test or Student *t* test were used. A *P* value of 0.05 or less was considered significant.

RESULTS

During the 10-year study period, a total of 257 patients had isolation of *M. avium* at least once. The demographic features of these patients are shown in Table 2. There were 141 (54.9%) men and 116 women, and they had a mean age of 59.7 years (range, 9 to 95). There were 123 patients with hematologic cancers, 122 with solid tumors, and 12 with no tumors. Among all 257 patients, definite and probable infections by the *M. avium* were found in 75 (29.2%) whereas the rest, 182 patients (70.8%), showed weak evidence of infection. The infected patients included 49 women, 42.2% of all 116 women, and 26 men, 18.4% of all 141 men, suggesting that

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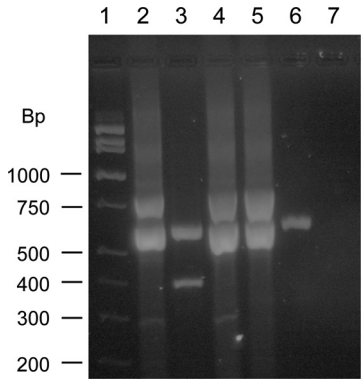

FIG 1 A representative gel of a multiplex PCR. Lane 1, molecular weight marker; lane 2, *M. avium* subsp. *avium* serotype 2 Chester strain (ATCC 35716) with amplicons of *IS311*, *IS901*, and *DT1*; lane 3, *M. avium* subsp. *paratuberculosis* (ATCC 19698) with amplicons of *IS311* and *IS900*; lane 4, a strain of *M. avium* subsp. *avium* serotype 2, 3 or *M. avium* subsp. *silvaticum* with amplicons of *IS311*, *IS901*, and *DT1*; lane 5, a strain of *M. avium* subsp. *avium* serotype 1 with amplicons of *IS311*and *IS901*; lane 6, a strain of *M. avium* subsp. *hominissuis* with only an *IS311* amplicon; lane 7, blank control without target DNA.

women are more vulnerable to *M. avium* infection than men (odds ratio = 3.2, $P < 0.0001$). In addition, the mean age of the 49 infected women was 65.5 years (range, 42 to 95), significantly higher than that of women with weak evidence of infection (mean, 56.3; range, 20 to 85) ($P = 0.0005$). Alternatively, for the 90 women of 50 years of age or more, 45 (50.0%) were infected, in comparison to the infection of 4 of the 26 women aged 49 or less (15.4%) (odds ratio = 5.5, $P = 0.002$). Therefore, women of postmenopausal age are prone to *M. avium* infection. In contrast, such age differences were not seen among men with strong versus weak evidence of infection.

Subspecies typing of the 257 *M. avium* strains, consisting of 280 isolates, owing to multiple isolates from 19 patients, revealed the vast majority (238 strains, 92.6%) to be *M. avium* subsp. *hominissuis*, the human/porcine type. The rest (19 strains, 7.4%) were identified as *M. avium* subsp. *avium*, the bird-type subspecies, including 12 strains of serotype 1, and 7 strains of serotype 2, 3 or *M. avium* subsp. *silvaticum*. There were no *M. avium* subsp. *paratuberculosis* strains, the ruminant type subspecies. The 19 patients with multiple isolates all had the same subspecies.

TABLE 2 Clinical features of patients with isolation of various *M. avium* subspecies

Feature	No. (%) or age (mean ± SD)		
	Men	Women	Total
By underlying cancer			
Hematologic	77 (54.6)	46 (39.7)	123 (47.9)
Solid	61 (43.3)	61 (52.6)	122 (47.5)
No cancer	3 (2.1)	9 (7.8)	12 (4.7)
By evidence of infection[a]			
Strong (definite and probable)	26 (18.4)	49 (42.2)	75 (29.2)
Weak (possible and contaminant)	115 (81.6)	67 (57.8)	182 (70.8)
By yrs of age[b]			
All	59.3 ± 13.7	60.2 ± 14.5	59.7 ± 14.0
With strong evidence of infection	60.7 ± 13.2	65.5 ± 11.9	63.9 ± 12.5
With weak evidence of infection	59.0 ± 13.8	56.2 ± 15.1	58.0 ± 14.3
Total	141 (100)	116 (100)	257 (100)

[a] By comparisons of women to men, $\chi^2 = 17.4$, $P < 0.0001$, odds ratio = 3.2.
[b] By comparisons (Student *t* test) of patients with strong evidence of infection to those with weak evidence of infection, for men, the results were not statistically significant; for women, $t = 3.59$, $P = 0.0005$; for men and women combined, $t = 3.11$, $P = 0.002$.

TABLE 3 Clinical source and significance for 238 patients with isolation of *M. avium* subsp. *hominissuis*

Feature	Total (%)	No. of patients with evidence of infection			
		Definite	Probable	Possible or part of mix	Contaminant
By isolation source					
Respiratory tract					
BAL fluid and/or bronchial wash[a]	139 (58.4)	6	25	33	75
Sputum	62 (26.0)	3	13	16	30
Lung tissue	16 (6.7)	14		1	1
Pleural fluid	2 (0.8)	2			
Abdominal or pelvic abscess	8 (3.4)			8	
Polymicrobic wound	4 (1.7)		1	1	2
Other tissue	2 (0.8)	1 (neck pus)			1 (abdomen)
Urine	5 (2.1)			3	2
By no. of isolates					
Single isolate	220 (92.4)	19	34	56	111
Two isolates (separate days)	13 (5.5)	3	4	6	
Three or more isolates	5 (2.1)	4	1		
Total (%)	238 (100)	26 (10.9)	39 (16.4)	62 (26.1)	111 (46.6)

[a] BAL, bronchoalveolar lavage.

The clinical sources and significance of the 238 patients with isolation of *M. avium* subsp. *hominissuis* are shown in Table 3. Sixty-five patients (27.3%) showed evidence of definite or probable infection whereas the rest had weak evidence of infection, i.e., either possible infection (26.1%) or contamination (46.6%). The vast majority of the bacterial strains were isolated from the respiratory tract specimens (92.0%, 219 of 238). Single isolation also accounted for most strains (92.4%, 220 of 238). Among the 18 patients with two or more isolates from specimens spanning 3 days to 4 years, 12, or two-thirds of them, showed definite or probable infection. It is noteworthy that there were 8 strains isolated from abdominal or pelvic abscess or drainage, along with concurrent isolation of other typical enteric bacteria from routine cultures, such as enterococci and/or coliforms, suggesting colonization of this mycobacterium in the lower gastrointestinal tract.

Table 4 shows the clinical sources and significance of the 19 patients with isolation of *M. avium* subsp. *avium* serotype 1 or serotype 2, 3 or *M. avium* subsp. *silvaticum*. Ten of them (52.6%) showed evidence of definite or probable infection, with the radiographic appearance and tissue reactions being similar to those caused by *M. avium* subsp. *hominissuis*. Thus, despite being infrequently isolated, these bird-type subspecies did cause human infections, in a significantly higher percentage than the human/porcine subspecies did (27.3%, 65 of 238) ($\chi^2 = 5.46$, $P = 0.019$). Seventeen of the bacterial strains were from respiratory tract specimens. One strain was notably from a pelvic abscess, consistent with colonization of *M. avium* subsp. *avium* serotype 2, 3 or *M. avium* subsp. *silvaticum* in the lower gastrointestinal tract of the patient.

The 12 patients without tumor diagnosis were examined sep-

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TABLE 4 Clinical source and significance for 19 patients with isolation of *M. avium* subsp. *avium* (serotype 1) or *M. avium* subsp. *avium* (serotype 2, 3) or *silvaticum*

M. avium subsp. *avium* serotype + source	Total	Evidence of infection			
		Definite	Probable	Possible or part of mix	Contaminant
Serotype 1					
BAL and/or bronchial wash	7		2	3	2
Sputum	2			1	1
Lung tissue	3	2	1		
Subtotal	12	2	3	4	3
Serotype 2, 3 or *M. avium* subsp. *silvaticum*					
BAL fluid and/or bronchial wash[a]	4		3[b]	1	
Sputum	1		1		
Other	2	1[c]		1[d]	
Subtotal	7	1	4	2	
Total	19	3	7	6	3

[a] BAL, bronchoalveolar lavage.
[b] Data include one with two isolates spanning 2 weeks.
[c] From abdominal tissue.
[d] From pelvic abscess.

arately. They all presented with nodules in the lungs (11 patients) or mediastinum (1 patient) for diagnostic work-up; 9 of them were found to have evidence of definite or probable infection by the *M. avium*. These patients included three men (2.1% of all 141 men) and 9 women (7.8% of all 116 women) with a mean age of 65.9 years, a finding consistent with female susceptibility to *M. avium* ($\chi^2 = 4.53$, $P = 0.033$). The cultured bacterial subspecies included 8 *M. avium* subsp. *hominissuis* and 4 *M. avium* subsp. *avium* serotype 1. This proportion of bird-type subspecies in these non-cancer patients (33.3%, 4 of 12) was 5-fold higher than that recovered from patients with underlying cancer (6.1%, 15 of 245) ($\chi^2 = 12.4$, $P = 0.0004$). This finding, along with that of a higher proportion of infection of all 19 patients with *M. avium* subsp. *avium* as shown above, suggests that these bird-type subspecies have higher pathogenicity than that of *M. avium* subsp. *hominissuis*, although the human/porcine subspecies is far more abundant. In general, patients with cancer are more vulnerable to weak opportunistic pathogens, and frequent diagnostic procedures also recover more contaminants. The treatment and follow-up data of the 12 patients were inadequate for assessment due to out-of-state referral.

DISCUSSION

Clinical isolates of *M. avium* are likely on the rise due to advancements in identification methods, more awareness of this organism and its infections, and the increase of the immunocompromised population. In our earlier study (34), we noted that the frequent isolation of *M. avium* from patients with cancer was mainly due to the use of more diagnostic bronchoscopy procedures instead of being due to true infections. In fact, only 16.2% of all 62 patients with *M. avium* isolation showed evidence of definite or probable infection in that study whereas 63.1% of 65 patients with *M. intracellulare* exhibited such evidence of infection.

The present study extended our previous *M. avium* study (34) with longer durations of study years (and thus longer follow-up) and far greater numbers of patients and bacterial strains. We successfully typed 257 *M. avium* strains and assessed their clinical significance. We found that the most commonly encountered subspecies was the human/porcine-type *M. avium* subsp. *hominissuis*, followed by the bird-type *M. avium* subsp. *avium* serotypes, but no *M. avium* subsp. *paratuberculosis*. Seventy-five (29.2%) of the patients showed evidence of definite or probable infections, which is higher than our previous data showing 16.2% infection (34). The percentage of increase can be explained by the longer follow-up and larger cohort of the present study. The 75 cases of infection included 65 caused by *M. avium* subsp. *hominissuis* and 10 by *M. avium* subsp. *avium* serotypes. These results support and extend the view that human infections by the bird-type subspecies do occur and account for a considerable proportion of the infections (13.3%, 10 of 75). The two bird-type subspecies, despite being far less frequently isolated, caused relatively more infections (52.6%, 10 of 19) than the human-porcine subspecies did (27.3%, 65 of 238). The bird-type subspecies were also recovered more often from a subset of 12 patients who did not have underlying cancer (4 of 12) than from patients with underlying cancer (15 of 245), suggesting higher pathogenicity of the bird-type subspecies. This finding extends earlier studies (14–16) of *M. avium* subspecies from human sources that reported isolation of the bird-type subspecies only without clinical assessment. However, the patient population precluded our assessment of treatment and follow-up in this study, which may require further investigation of the infections caused by *M. avium* subsp. *avium*.

The dominance of *M. avium* subsp. *hominissuis* in this study agrees with other previous reports (15, 18, 25, 37). Pavlik and colleagues tested a collection of 152 human clinical *M. avium* strains from the United States and 10 European countries for *IS901* and noted 140 strains (92.1%) being *IS901*-negative *M. avium* subsp. *hominissuis* and the rest (7.9%) being positive for this *M. avium* subsp. *avium* marker (15). Shin et al. typed 77 human clinical strains (excluding those from patients with Crohn's disease) collected from several institutions in different countries and found only *M. avium* subsp. *hominissuis* (37). Mobius et al. in Germany typed 45 strains from humans and 42 strains from swine and cattle and noted only *M. avium* subsp. *hominissuis* from humans and a nearly even split of *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis* from animals (25). And Alvarez et al. in Spain also noted 35 clinical strains of *M. avium* subsp. *hominissuis* without *M. avium* subsp. *avium* (18).

It is well known that women of postmenopausal age are prone to chronic MAIC infection with associated bronchiectasis and lung nodularity (7, 34, 38–40). Studies have further attributed ~70% of these MAIC infections to *M. intracellulare* and the rest to *M. avium* (34, 40). In the present study, we further noted that half of postmenopausal women with isolation of *M. avium* were infected, more than the proportion of premenopausal women (15.4%) and that of men of all ages (18.4%). The mean age of the *M. avium*-infected women was 65.5 years, close to the 69.4 years of age of the women with *M. intracellulare* infection as noted previously (34). Preliminary research suggests that the gamma interferon pathways may be deficient in elderly women, which may cause susceptibility to MAIC lung disease (41).

In the present study, we did not find the ruminant type of subspecies *M. avium* subsp. *paratuberculosis* among the 257 *M.*

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avium strains that were recovered using standard mycobacteriologic culture techniques for human-derived specimens. This result could represent a true lack of this subspecies in association with human infection or colonization or could have been due to an inadequate culture method used to recover this animal pathogen or both in a case of very rare occurrence. In veterinary medicine, recovering this subspecies requires addition of mycobactin J to the culture media. In the reports of successful cultivation of *M. avium* subsp. *paratuberculosis* from the breast milk or blood of patients with Crohn's disease, the standard culture media were also supplemented with mycobactin J (28–30). However, two similar subsequent studies failed to replicate such culture results (32, 33). To add to the perplexity, *M. avium* subsp. *paratuberculosis* has also been detected in healthy individuals with no history or signs of ulcerative colitis or Crohn's disease (28, 33).

Among our 257 patients, we found that 9 of them (3.5%) were colonized in the lower gastrointestinal tract with *M. avium* subsp. *hominissuis* or *M. avium* subsp. *avium*. If *M. avium* subsp. *paratuberculosis* were one of the causes of Crohn's disease, one would reasonably expect to find colonization of the organism in the lower gastrointestinal tract of some individuals well before the start of disease manifestation. In order to see this, it might be necessary to incorporate mycobactin J into the standard mycobacteriologic culture media. The logistics and feasibility of this potential practice need to be considered.

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