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Relationship between IS*901* in the *Mycobacterium avium* Complex Strains Isolated from Birds, Animals, Humans, and the Environment and Virulence for Poultry

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A total of 738 strains of *Mycobacterium avium* complex (MAC) were examined in biological experiments on poultry by use of PCR methods with primers for detection of the insertion sequence IS*901*. Serotype strains of MAC from all known 28 serotypes were examined. Further strains were isolated from human immunodeficiency virus (HIV)-negative and HIV-positive patients, 6 animal species, 17 bird species, and the environment. Of 165 strains virulent for poultry, characterized by generalized tuberculosis, 164 strains contained IS*901*, a result which is statistically highly significant (*P*, 0.01). The remaining 573 strains were nonvirulent; however, IS*901* was present in 24 strains. From among 20 strains of serotypes 1, 2, and 3, IS*901* was found in 15 strains, only 5 of which were virulent for poultry. The remaining 111 strains, of serotypes 4 to 28, were nonvirulent and did not incorporate IS*901*. None of the 152 strains isolated from humans was virulent for poultry, including 12 strains which were IS*901* positive.

The importance of mycobacterial infections caused by strains of *Mycobacterium avium* complex (MAC) in animals and humans is continuously increasing (11, 18). In the human population, the condition is aggravated by the spread of human immunodeficiency virus (HIV) infection. In AIDS patients, the incidence of disseminated mycobacterial infection caused by MAC strains can reach up to 55% (34, 47). In poultry, swine, and cattle farms and in farmed red deer, avian tuberculosis imposes the highest financial losses (5, 9, 30, 39, 40).

In veterinary epidemiology, virulent strains inducing avian generalized tuberculosis are the most important. One of the oldest and most frequently used methods for virulence assessment of MAC strains in birds is the challenge assay with poultry; nevertheless, mice, guinea pigs, hamsters, and rabbits also have been used (2, 8, 10, 31, 42, 43, 51, 52). Serotyping has partly replaced time-consuming experimentation on laboratory animals. Originally, serotypes 1 and 2, virulent for birds, were classified as *M. avium*, and 13 nonvirulent serotypes were classified as *M. intracellulare* (39, 41). Later, a third virulent serotype of *M. avium*, serotype 3, was described (21). Piening et al. denominated those three MAC serotypes as the *M. avium* group, in accordance with previous studies. Strains of serotypes 12 to 20 were absolutely nonvirulent for poultry and were denominated as the *M. intracellulare* group. Strains of serotypes 4 to 6 and 8 to 11, causing changes only at the site of inoculation after intramuscular injection, were denominated as an intermediate MAC group (33).

As further serotypes were continuously investigated, in 1979 the number of serotypes belonging to *M. intracellulare* increased to 25 (serotypes 4 to 28) (49, 50). However, research results obtained in the early 1980s led to a new approach to the taxonomic classification of each MAC serotype. DNA-DNA hybridization was used to show the relationship of strains of serotypes 1 to 6 and 8 to 11 (3). These and other results led in 1990 to the suggestion for a new taxonomy for MAC strains, "wood pigeon" (mycobactin-dependent strains isolated from wood pigeons) and *M. paratuberculosis* strains. MAC strains of serotypes 1 to 6 and 8 to 11, and 21 were denominated as *M. avium* subsp. *avium*, wood pigeon strains were denominated as *M. avium* subsp. *silvaticum*, and *M. paratuberculosis* strains were denominated as *M. avium* subsp. *paratuberculosis* (44).

These results, especially for strains belonging to *M. avium* subsp. *avium* (serotypes 1 to 6, 8 to 11, and later 21), were supported by the sequencing of 16S rRNA and internal transcribed spacer 16S-23S ribosomal DNA from strains of all 28 serotypes (6, 12, 46). Restriction fragment length polymorphisms (RFLP) have been used to divide the MAC strains into the group *M. avium* subsp. *avium*, with serotypes 1 to 6 and 8 to 10, and the group *M. intracellulare*, with serotypes 7 and 11 to 21, according to McFadden et al. (24). Serotypes 1 to 6, 8 to 11, and 21 were further classified with 16S rRNA probes from Gen-Probe Inc., San Diego, Calif., as *M. avium* subsp. *avium*, and serotypes 7, 12 to 17, 19, 20, and 25 were classified as *M. intracellulare* (37, 38). The same results were later obtained in seven laboratories within a comparative study of the International Working Group on Mycobacterial Taxonomy (48).

A new approach to the differentiation of MAC strains was obtained with the description of repetitive insertion sequence IS*900* in *M. avium* subsp. *paratuberculosis* strains and IS*901* or IS*902* in *M. avium* subsp. *silvaticum* strains (15, 19, 27). It was also found that MAC strains containing IS*901* are more virulent for mice after intravenous infection (20). IS*901* was found in 97.8% of strains from birds, in 74.1% of strains from animals, and in 12.5% of strains from the environment. Examination of serotyped strains revealed IS*901* only in strains of serotypes 1, 2, and 3 (28).

The presence of IS*901* only in strains of serotypes 1, 2, and 3 and in strains virulent for mice suggests that the presence of IS*901* may be connected to the virulence of MAC for birds. In this study, we therefore examined all available serotyped MAC strains and a range of strains isolated from birds, animals, the environment, and humans by using parallel challenge experimentation with poultry and an IS*901* PCR detection method.

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TABLE 1. MAC strains

Origin of the strains	Total no. of strains	Poultry strains					
		Virulent			Nonvirulent		
		No.	IS*901*$^{+b}$	%	No.	IS*901*$^{+}$	%
Serotypes 1–3[a]	20	5	5	100	15	10	66.7
Serotypes 4–28[a]	111	0	0	0	111	0	0
Birds	56	49	49	100	7	0	0
Animals	261	106	105	99.1	155[6]	2	1.3
Environment	138	5	5	100	133	0	0
HIV-negative patients	98	0	0	0	98	8	8.2
HIV-positive patients	54	0	0	0	54	4	7.4
Total no. (%)	738 (100)	165 (22.4)	164	99.4	573 (77.6)	24	4.2

[a] Strains were obtained from the following sources: Mykobakteriologie, National Referenzzentrum für Mykobakterien, Forschungszentrum Borstel (S. Rüsch-Gerdes; originally submitted from W. B. Schaefer to K. H. Schröder) Borstel, Germany; National Institute for Leprosy Research, Shimane Medical University (H. Saito and H. Tomioka), Tokyo, Japan; Veterinary Research Institute (originally submitted from W. B. Schaefer to M. Pavlas), Brno, Czech Republic; National Institute of Public Health, Unit and Reference Laboratory for Mycobacterial Infections, The Czechoslovak National Collection of Type Cultures (CNCTC) (M. Slosarek; originally submitted from W. B. Schaefer to M. Kubin), Prague, Czech Republic; and National Institute of Public Health and Environment (D. van Soolingen), Bilthoven, The Netherlands. Five strains inoculated intramuscularly caused a maximum of four isolated nodules (poppy-seed size) within 8 to 10 weeks of infection.
[b] IS*901*$^{+}$, IS901 positive.

MATERIALS AND METHODS

Origin of MAC strains. A total of 738 MAC strains were examined (Tables 1, 2, and 3). Each field strain was isolated from one host or from one environmental sample. Serotyped strains were obtained from the culture collections of five laboratories. Field strains from animals, birds, and the environment were isolated in our laboratory or were obtained from eight other laboratories. Human strains were obtained from 29 other laboratories.

Serotype MAC strains. MAC strains (n = 131) (Table 1) of all 28 serotypes were examined. Each serotype strain from each laboratory was considered a separate strain due to long-term storage in various collections. There were 20 strains of serotypes 1, 2, and 3 and 111 strains of serotypes 4 to 28.

Field veterinary MAC strains. Fifty-six strains (Table 2) from 17 bird species and 38 locations were examined; 49 of these were isolated from parenchymatous organs with generalized tuberculosis, and 7 were isolated from unchanged parenchymatous organs or gut contents. In addition, 261 strains (Table 2) from six animal species and 89 locations were examined: 237 strains were isolated from swine, 12 were isolated from cattle, 10 were isolated from sheep, 1 was isolated from a goat, and one was isolated from a horse. Environmental strains (n = 138) (Table 2) were obtained from 51 infected swine and cattle herds and included samples of sawdust, drinking water, litter, stable environment, and feed.

Human MAC strains. A total of 152 strains (Table 3) isolated from HIV-negative and HIV-positive patients in 11 countries were examined. The strains were isolated from blood (19.6%), bone marrow (3.0%), feces (3.0%), lymph nodes (4.5%), sputum (67.6%), and gastric lavage (2.3%). The strains isolated from patients in the Czech Republic and Slovakia were divided, based on isolation period, into two groups: group A contained 29 strains isolated from 1968 to 1978 and stored at the Czechoslovak National Collection of Type Cultures in Prague, Czech Republic, and group B contained 20 strains isolated from 1996 to 1997 and provided in the first subculture by various laboratories.

Subculturing of MAC strains. Mycobacterial strains were subcultivated on Stonebrink, Löwenstein-Jensen, Herrold, and Middlebrook (7H11) solid media and in a liquid serum medium for mycobacterial cultivation (SEVAC, Prague, Czech Republic).

Virulence assessment of MAC strains. Strain virulence was assessed by biological experiments on 6- to 8-week-old pullets (two pullets for each strain) of the egg type breed ISA Brown after intramuscular injection with 1 to 5 mg of bacterial wet substance per kg of live weight (31). Gross lesions in parenchymatous organs (liver, spleen, kidney) were assessed 8 to 10 weeks after infection. The finding of organized, small tuberculous nodules which were poppy-seed to lentil size in both pullets was designated a positive finding.

PCR method (IS*901*). In this study, the PCR procedure of Kunze et al. was used (20). The primers (5′-GCAACGGTTGTTGCTTGAAA-3′ and 5′-TGATACGGCCGGAATCGCGT-3′) used for the detection of IS*901* were derived from positions 76 and 1184 of fragment IS*901*, thus amplifying a PCR product of 1,108 bp.

Statistical evaluation. The χ^2 test from the STAT Plus package (23) was used for statistical evaluation.

RESULTS

A total of 165 of 738 MAC strains tested were highly virulent for poultry in challenge experimentation (generalized tuberculosis); IS*901* was detected in 164 strains (Table 1). No gross lesions were detected in 573 strains after infection, except for 5 strains isolated in swine that caused sporadic small nodules (up to four tubercles) of poppy-seed size to occur in the liver of infected poultry. Among 573 nonvirulent strains, IS*901* was present in 24 strains. A highly significant (P, 0.01) relationship was found between the presence of IS*901* and virulence for poultry after intramuscular infection (generalized tuberculosis).

Serotype MAC strains. Only 15 of 131 serotype MAC strains were found to contain IS*901* (Table 1). These were all serotype 1, 2, or 3. Another five isolates of serotype 1, 2, or 3, however, were IS*901* negative. Only 5 of 15 IS*901*-positive strains of serotypes 1, 2, and 3 induced generalized tuberculosis in poultry. The remaining 111 strains of other serotypes (4 to 28) were nonvirulent for poultry.

MAC strains from birds. Of 56 MAC strains isolated from birds, 49 were IS*901*-positive strains which were all fully virulent for poultry in challenge experimentation (Table 2). The other seven, IS*901*-negative strains, isolated from unchanged tissues of parenchymatous organs or intestinal contents of the examined birds, were nonvirulent.

MAC strains from animals. A total of 261 MAC strains were isolated from six animal species (Table 2). A total of 107 were IS*901*-positive strains, 105 of which (98.1%) were virulent for poultry. No IS*901* was detected in the DNA of 154 animal strains, and of these strains, only 1 was virulent for poultry. Five strains induced sporadic changes in the liver of infected poultry in the form of small nodules. Of 236 animal strains originating from swine, 90 were IS*901*-positive strains, 88 of which (97.8%) were virulent for poultry. Of 146 IS*901*-negative strains, only 5 induced sporadic nodules in the liver, the remaining strains being nonvirulent. A relationship between IS*901* and virulence for poultry was found in all other strains isolated from wild pigs, cattle, sheep, goats, and a horse, except for one of the strains (IS*901* negative) isolated from a goat, which did induce generalized tuberculosis in poultry.

Environmental MAC strains. Of 138 environmental strains, 5 contained IS*901* and were virulent for poultry (Table 2); 3 of these strains were isolated from drinking water, 1 was from stable scrapings, and 1 was from the sawdust of swine bedding. All five IS*901*-positive strains from the environment were isolated from three herds of swine in which infection by these

TABLE 2. MAC strains isolated from animals and the environment

Type of strain	Origin of the strain	Total no. of strains	No. of:					
			Localities	IS*901*$^{+aa}$ strains	Virulent strains	Localities	IS*901*$^{-aa}$ strains	Virulent strains
Bird	Geese[a,g]	2	2	2	2	0	0	0
	Sparrow[a,i]	3	2	2	2	1	1	0
	Poultry[a,b,d]	23	16	22	22	1	1	0
	Pheasant[a,b,d,j]	5	4	4	4	1	1	0
	Partridge[a,k]	4	1	4	4	0	0	0
	Quail[a,d,l]	7	4	6	6	1	1	0
	Tengmalm's owl[e,m]	1	1	1	1	0	0	0
	Tawny owl[e,n]	1	1	1	1	0	0	0
	Pelican[b,o]	1	1	1	1	0	0	0
	Heron[a,p]	1	1	1	1	0	0	0
	Wagtail[a,q]	1	0	0	0	1	1	0
	Starling[a,r]	1	0	0	0	1	1	0
	Pigeon[a,s]	1	1	1	1	0	0	0
	Vulture[g]	2	2	2	2	0	0	0
	Little owl[g,t]	1	1	1	1	0	0	0
	Fieldfare[g,u]	1	1	1[w]	1	0	0	0
	Ostrich[g,v]	1	0	0	0	1	1	0
	Total no. (%)	56	38	49[w] (100)	49 (100)	7	7 (100)	0 (0)
Animal	Swine[a,b,c,d,g,h]	236	70	90	88	71	146[z]	0
	Wild swine[a]	1	1	1	1	0	0	0
	Cattle[a,b,e]	12	8	8	8	3	4	0
	Sheep[b]	10	6	7	7	1	3	0
	Goat[a]	1	0	0	0	1	1	1
	Horse[a]	1	1	1	1	0	0	0
	Total no. (%)	261	86	107 (100)	105 (98.1)	76	154 (100)	1 (0.7)
Environment	Drinking water for swine[a,d]	29	1	3	3	9	26	0
	Dust (swine stable)[a]	1	0	0	0	1	1	0
	Concentrate (pigs)[a]	6	0	0	0	5	6	0
	Soil (cattle pasture)[a]	2	0	0	0	1	2	0
	Scrapings (floor)[a,x]	6	1	1	1	4	5	0
	Sawdust[a]	23	0	0	0	6	23	0
	Sawdust litter (pigs)[a]	71	1	1	1	35	70	0
	Total no. (%)	138	3[y]	5 (100)	5 (100)	51[y]	133 (100)	0 (0)
	Grand total no. (%)	455	120[y]	161 (100)	159 (98.8)	122[y]	294 (100)	1 (0.3)

[a] Veterinary Research Institute, Brno, Czech Republic.
[b] State Veterinary Diagnostic Institute (V. Stika and I. Parmova [70 strains]), Prague, Czech Republic.
[c] State Veterinary Diagnostic Institute (K. Kovarik [18 strains]), Brno, Czech Republic.
[d] State Veterinary Diagnostic Institute (I. Melicharek [85 strains]), Nitra, Slovakia.
[e] State Veterinary Diagnostic Institute (Z. Vasilova [2 strains]), Michalovce, Slovakia.
[f] Central Veterinary Laboratory (F. Saxegaard [2 strains]), Oslo, Norway.
[g] National Institute of Public Health and Environment (D. van Soolingen) Bilthoven, The Netherlands.
[h] Statens Serum Institut (J. Bauer and V. Thomsen) Copenhagen, Denmark.
[i] *Passer domesticus*.
[j] *Phasianus colchicus*.
[k] *Perdix perdix*.
[l] *Coturnix coturnix*.
[m] *Aegolius funereus*—wood pigeon strain (*M. avium* subsp. *silvaticum*).
[n] *Strix aluco*—wood pigeon strain (*M. avium* subsp. *silvaticum*).
[o] *Pelecanus onocrotalus*.
[p] Common heron (*Ardea cinerea*).
[q] Pied wagtail (*Motacilla alba*).
[r] *Sturnus vulgaris*.
[s] *Columba domestica*.
[t] *Athene noctua*.
[u] *Turdus pilaris*.
[v] *Struthio camelus*
[w] All strains were isolated from avian liver with generalized tuberculosis.
[x] Scrapings from the floor of stables for swine and cattle.
[y] The total number of localities is lower because some strains were isolated from different environmental material in the same swine herd and from the lymph nodes.
[z] Five strains inoculated intramuscularly caused a maximum of four isolated nodules (poppy-seed size) within 8 to 10 weeks of infection.
[aa] IS*901*$^{+}$, IS901 positive; IS901^{-}, IS*901* negative.

TABLE 3. MAC strains isolated from humans

HIV status	Origin of strain	Total no. of strains	IS*901*$^+$ strains	No. of strains from:					
				Blood culture	Bone marrow	Feces	Lymph nodes	Sputum[m]	Gastric lavage
Negative	Czech Republic[a]	32	1	0	0	1	2	29/1	0
	Denmark[b]	7	0	0	1	0	1	5	0
	Germany[c]	21	1	0	0	0	1	17/1	3
	Russia[d]	6	0	0	0	0	0	6	0
	Slovakia[e]	11	5	0	0	0	0	11/5	0
	Slovenia[f]	3	0	0	0	0	0	3	0
	Spain[g]	6	0	0	1	0	3	2	0
	The Netherlands[h]	1	1	?	?	?	?	?	?
	United States[i,j]	11	0	0	0	0	0	11	0
	Total no. (%)	98 (100)	8 (8.2)	0 (0)	2 (2.1)	1 (1.0)	7 (7.2)	84/7 (86.6)	3 (3.1)
Positive	Czech Republic[k]	6	1	0	0	0	0	6/1	0
	Denmark[b]	6	0	1	0	2	0	3	0
	Germany[c]	18	0	11	1	3	0	3	0
	Spain[g]	15	1	11/1	2	0	0	2	0
	Sweden[l]	3	0	1	1	1	0	0	0
	The Netherlands[h]	1	1	?	?	?	?	?	?
	Argentina[h]	1	1	?	?	?	?	?	?
	United States[j]	4	0	4	0	0	0	0	0
	Total no. (%)	54 (100)	4 (7.4)	28/1 (53.9)	4 (7.7)	6 (11.5)	0 (0)	14/1 (26.9)	0 (0)
	Grand total no. (%)	152 (100)	12 (7.9)	28/1 (18.8)	6 (4.0)	7 (4.7)	7 (4.7)	98/8 (65.8)	3 (2.0)

[a] Research Institute of Tuberculosis (CNCTC strains TBCa6/68 and My85/72), Prague, Czech Republic; Bulovka Hospital (K. Dvorsky; CNCTC strain TBCa8/68), Prague, Czech Republic; University Hospital Veleslavin (CNCTC strains TBCa11/68 and My71/72), Prague, Czech Republic; Hygienic Station (CNCTC strain TBCa13/68), Plzen, Czech Republic; Sanatorium for Tuberculosis (CNCTC strain TBCa14/68), Dobris, Czech Republic; Sanatorium for Tuberculosis (CNCTC strain My73/72), Kostelec nad Cernymi Lesy, Czech Republic; Sanatorium for Tuberculosis (CNCTC strain My74/72), Jablunkov, Czech Republic; Hygienic Station (CNCTC strain My75/72), Ceske Budejovice, Czech Republic; Hygienic Station (CNCTC strain My76/72), Prague, Czech Republic; Hygienic Station (CNCTC strains TBCa9/68 and My78/72), Pardubice, Czech Republic; Hygienic Station (J. Sytarova; CNCTC strain My150/73), Brno, Czech Republic; Hygienic Station (Z. Horak; CNCTC strain My151/73), Usti nad Labem, Czech Republic; Sanatorium for Tuberculosis (M. Slosarek; CNCTC strain My155/73), Paseka, Czech Republic; Hygienic Station, Reference Laboratory for *Mycobacterium kansassi* (J. Kaustova; CNCTC strains My224/78, 350/93, 427/93, 435/93, 9684/96, 5654/96, 5052/96, 1341/96, 2534/96, 1192/96, 3899/96, 13030/96, and 757/96), Ostrava, Czech Republic; Hygienic Station (CNCTC strain TBCa10/68), Hradec Kralove, Czech Republic; and Hygienic Station (L. Mezensky; CNCTC strains LP 63/96 and LP 135/96), Brno, Czech Republic.

[b] Statens Serum Institute (J. Bauer and V. Thomsen), Copenhagen, Denmark.

[c] National Referenzzentrum für Mykobakterien, Forschungszentrum Borstel (S. Rüsch-Gerdes), Borstel, Germany.

[d] Research Institute for Tuberculosis, Academy of Medical Sciences (N. Makarevic; CNCTC strains My163/73, My165/73, My166/73, My167/73, My168/73, and My169/73), Moscow, Russia.

[e] Research Institute for Tuberculosis (R. Grigelova; CNCTC strains TBCa7/68, My72/72, My170/73, My171/73, My172/73, My173/73, My174/73, and My176/73), Bratislava, Slovakia; and Sanatorium for Tuberculosis (CNCTC strains My178/73, My181/73, and My182/73), Vysne Hagy, Slovakia.

[f] Sanatorium for Tuberculosis (A. Gasi; CNCTC strains My148/73, My153/73, and My154/73), Golnik, Slovenia.

[g] University of Madrid, (M. J. Garcia; strains 1, 3, 14, 15, 16, 18, 28, 35, 37, 38, 42, 44, 50, 51, 54, 1B, 7B, 15B, 23B, 26B, and 27B), Madrid, Spain.

[h] National Institute of Public Health and Environment (D. van Soolingen), Bilthoven, The Netherlands.

[i] Department of Public Health (T. S. Hosty; CNCTC strains My156/73, My159/73, My160/73, My161/73, and My162/73), Montgomery, Ala.

[j] University of Wisconsin—Milwaukee (R. S. Lambrecht; strains NA2, NA3, NA6, NA8, NA11, NA12, A14, A15, A16, and 101), Milwaukee.

[k] National Institute of Public Health, Unit and Reference Laboratory for Mycobacterial Infections, CNCTC (M. Havelkova and M. Slosarek; strains D10/93, D11/93, D24/93, D70/93, D99/93, and D123/94), Prague, Czech Republic.

[l] Strains originally sent to St. George's Hospital, Medical School, University of London (J. Hermon-Taylor and T. Bull), London, United Kingdom, from Swedish Institute for Infectious Disease Control (S. Hoffner), Stockholm, Sweden.

[m] Respiratory tract, pleural fluid, or bronchoalveolar lavage. Numbers after slashes indicate numbers of IS*901*-positive (IS*901*$^+$) strains.

strains was diagnosed. None of the other 133 strains contained IS*901*, nor were they virulent for poultry.

MAC strains from humans. All 152 MAC strains from humans were nonvirulent for poultry, including 12 IS*901*-positive strains (Table 3). Of 98 strains isolated from HIV-negative patients, IS*901* was detected in 8 strains, which were all isolated from sputum. From 54 HIV-positive patients, four IS*901*-positive strains were detected (three strains from sputum and one strain from blood). MAC strains from the Czech Republic and Slovakia were divided into two groups based on the date of their isolation. Group A comprised 29 strains isolated from 1968 to 1978; IS*901* was detected in 6 strains (5 strains originated from Slovakia). Group B contained 20 strains isolated from 1996 to 1997; no IS*901* was detected.

Statistical evaluation of the results. Monitoring of strain virulence for poultry together with the presence of IS*901* in the strains revealed that the presence of IS*901* in strains virulent for poultry was of statistically higher significance than that in nonvirulent strains (P, 0.01). In addition, the frequency of strains with IS*901* in individual hosts was analyzed. Statistically highly significant differences (P, 0.01) were found for the presence of IS*901* in birds and animals, animals and humans, birds and environment, animals and environment, and birds and humans. No statistical differences were found for the presence

of IS*901* in strains isolated from the environment and from humans. Study of the relationship between IS*901*-positive isolation and HIV infections did not reveal any significant difference for the occurrences of IS*901*-positive strains in HIV-positive and HIV-negative patients.

DISCUSSION

The virulence of MAC strains for poultry in this study was tested by intramuscular administration (31). The use of intraperitoneal infection in parallel with intramuscular infection is advisable for efficient assessment of virulence (C. Thoen [U.S. Department of Agriculture, Ames, Iowa], personal communication). Intravenous infection is a further alternative; however, inoculation with strains of serotypes 8 and 9 resulted in mycobacteremia and the death of the experimental poultry without prior formation of tubercles in parenchymatous organs (infection type Yersin). Other studies with the oral or intramuscular route of infection showed the strains of these serotypes to be nonvirulent for poultry (31). In addition, oral infection is impractical, as it prolongs the experiment for several months (16).

Statistically high significance (P, 0.01) was found between virulence and the presence of IS*901* in all freshly isolated strains from birds, animals, and the environment. However, no such association was seen in reference serotype and human MAC strains stored for a long time. Strains of serotypes 1, 2, and 3 incorporated IS*901*-negative nonvirulent strains, IS*901*-positive nonvirulent strains, and IS*901*-positive virulent strains (Table 1). Some of these strains were isolated several decades ago, and the loss of virulence has occurred gradually due to mechanisms so far unknown. Anz and Meissner assigned the loss of virulence for poultry of some MAC strains of serotypes 1, 2, and 3 to their adaptation to laboratory conditions (1). Loss of virulence in mycobacterial strains during laboratory passage is well known. The first BCG vaccine was obtained in this way (14).

The virulence of MAC strains for poultry has been associated with various factors, including their growth type (rough and smooth colony types) and the presence of plasmids (13, 22, 26). Our results indicate that although the virulence of MAC strains is multifactorial, IS*901* plays a significant role. All of these virulence factors (including IS*901*) could be lost during a long-term laboratory passage and storage.

All MAC strains isolated from humans were avirulent for poultry. However, 12 of these were IS*901* positive. Humans as well as animals are not typical hosts for IS*901*-positive strains virulent for poultry. This fact is in agreement with previous serotyping studies suggesting that the passage of MAC in humans leads to a loss of virulence for poultry (2). A reduction of virulence was also observed during storage of these strains and subculturing at 37°C, a temperature lower than a bird's body temperature (42°C). This difference may be another factor in virulence attenuation (40). Consistent virulence in MAC strains isolated from animals may be due to the short duration of the infection associated with a relatively short life expectancy of infected animals. In contrast, MAC infection in humans may be subclinical and therefore involve a long-term process (up to several years), thereby exposing the strains for a longer time to "unfavorable conditions." When field strains of MAC are examined, it is necessary to take into account a relatively broad spectrum of hosts. Our results are consistent with previous studies (7, 20, 36) which have indicated that IS*901*-positive strains are most frequently isolated from birds (Tables 1 and 2), possibly because birds are the natural hosts of avian tuberculosis.

Our study revealed that IS*901* was present in only 3.6% of the 138 environmental strains examined (Table 2). Pigs are in contact with IS*901*-negative MAC strains through drinking water, sawdust, and other substances (17). Our findings agree with those of other authors (4, 7, 31, 35), that is, IS*901*-negative MAC strains not virulent for poultry prevail in the environment. Nevertheless, certain environmental conditions can serve as a reservoir for IS*901*-positive MAC strains.

The frequency of IS*901*-positive isolation in human and environmental strains was relatively low, suggesting similar infection sources. Immunocompetent humans, although in contact with MAC strains in the environment, are not usually infected under normal conditions (25). It is interesting that 6 of 12 strains originated from the former Czechoslovakia from 1968 to 1978 (Table 3), when avian tuberculosis was frequent in birds, pigs, and other farm animals. Results published during this period showed a large proportion (up to 40%) of MAC strains of serotypes 1 and 2 isolated from humans and subsequently shown to be virulent for poultry (2).

Gene probes (Accu-Probe) have been used for the rapid identification of *M. avium* subsp. *avium* and *M. intracellulare*. However, the results do not correspond to the virulence of the strains for poultry, as *M. avium* subsp. *avium* includes the IS*901*-positive serotypes 1 to 3 but also serotypes 4 to 6, 8 to 11, and 21, which do not contain IS*901* and are not virulent for poultry (37, 38, 45, 48). In the Czech Republic and Slovenia, serotypes 4, 8, and 9 (nonvirulent for poultry) are the most commonly isolated from swine and the environment (29, 30). In veterinary laboratories, strain virulence for poultry can be readily and rapidly assessed by the PCR method for the detection of IS*901*, thereby circumventing the need for biological experiments on poultry (20). Biological experiments could be used for virulence assessment of strains which are stored in collections for long periods. Therefore, a rapid diagnostic method using PCR would be useful for reducing the spread of virulent MAC strains among other animals in infected herds and for minimizing financial losses.

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