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Title: High genetic relatedness among *Mycobacterium avium* strains isolated from pigs and humans revealed by comparative IS *1245* RFLP analysis

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1 **High genetic relatedness among *Mycobacterium avium* strains isolated from pigs**  
2 **and humans revealed by comparative IS1245 RFLP analysis**

3

4

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21 Key words: molecular epidemiology, zoonosis, food safety.

22

23 **Abstract**

24 Members of the *Mycobacterium avium* complex cause pig mycobacteriosis and opportunistic  
25 human infections. Infections due to environmental mycobacteria are increasing in both industrial  
26 and developing countries. *Mycobacterium*-infected pig carcasses can pass for human consumption  
27 due to the poor specificity of meat control by visual detection at the slaughter houses.

28 The genetic relatedness of porcine and human MAC isolates in Finland has been unknown. *M.*  
29 *avium* isolates isolated from pig organs (n=16) and clinical samples (n=13) were compared by  
30 IS1245 RFLP analysis to evaluate the similarity of the isolates obtained from human and porcine  
31 samples.

32 Nearly identical multicopy *Mycobacterium avium* subsp. *hominissuis* IS1245 RFLP fingerprints  
33 were obtained for isolates of porcine and human origin. IS1245 RFLP patterns of 38 % of the  
34 porcine and human *M. a. hominissuis* isolates were >90% similar. The RFLP patterns of 2 porcine  
35 and 2 human isolates showed >95 % similarity. The high similarity of the IS1245 RFLP patterns of  
36 the human and porcine *M. a. hominissuis* isolates indicates close genetic relatedness, suggesting that  
37 *M. a. hominissuis* is transmitted between pigs and humans, or that pigs and humans share common  
38 environmental sources of infection. Porcine and human isolates with RFLP patterns differing by  
39 only 1 or 2 bands were found, which shows that the same *M. a. hominissuis* strains may infect both  
40 humans and pigs.

41

42 **INTRODUCTION**

43 Animal mycobacteriosis causes major economical losses world wide and a potential health risk to  
44 humans (Biet et al., 2005; Thorel et al., 2001). Pig mycobacteriosis is a significant problem in  
45 several European countries, with a prevalence of 0.34% in Finland, 0.5% in the Netherlands (Ali-  
46 Vehmas et al., 2004; Komijn et al., 1999) and 10% in Germany in an area where pigs were fed peat  
47 (von Durring et al., 1998). Economic losses worth approximately 0.5 million euros per the annually  
48 processed 2.1 million carcasses (Information Centre of the Ministry of Agriculture and Forestry of  
49 Finland, 2005) are caused in Finland due to condemnation of pork infected by mycobacteria as only  
50 conditionally edible after processing.

51 Martín-Casabona et al. (2004) reported 36,099 human infections by non-tuberculous mycobacteria  
52 (NTM) and 22,884 NTM isolates identified to the species level in 14 countries world wide between  
53 1991 and 1996. A total of 3,961 NTM isolates were obtained from human specimens in Finland  
54 between 1995 and 2004 (Iivonen et al., 2005). Human NTM infection occurs mainly in  
55 immunosuppressed individuals and is most frequently caused by members of the *Mycobacterium*  
56 *avium* complex (MAC), which occurred in 1,360 (34%) of the 3,961 human cases in Finland and  
57 6,633 (29%, including *M. intracellulare*) of the 22,884 human cases in the multi-country survey by  
58 Martín-Casabona et al. (2004).

59 Komijn et al. (1999) compared human and porcine *Mycobacterium avium* subsp. *hominissuis* (*M. a.*  
60 *hominissuis*) isolates in the Netherlands by the *IS1245* restriction fragment length polymorphism  
61 (RFLP) method standardized by van Soolingen et al. (1998) and discovered that the RFLP patterns  
62 of 61% of the human and 59% of the porcine isolates were > 75% similar, showing close genetic  
63 relatedness, and that pigs may be a vehicle for human *M. a. hominissuis* infections, or that pigs and  
64 humans share common sources of infection. Direct transmission of NTM between animals and  
65 humans has not been proven. Möbius et al. (2006) found close genetic relatedness between porcine  
66 and human *M. a. hominissuis* isolates in Germany using the *IS1245* RFLP method and pulse field

67 gel electrophoresis. The IS1245 probe used in the standard method may cross hybridize with the  
68 IS1311 element, whereby the RFLP method is not strictly specific to IS1245 (Johansen et al., 2005).  
69 In the present study we used the IS1245 RFLP analysis to study the genetic relatedness of *M. a.*  
70 hominissuis isolates isolated from slaughter pigs and humans in Finland with regard to public health  
71 aspects.

## 72 MATERIAL AND METHODS

### 73 Bacterial strains

74 We randomly selected 13 clinical *M. avium* isolates collected 2001 to 2004 from patients living in  
75 Finland, and stored in the culture collection of the Mycobacterial Reference Laboratory, National  
76 Public Health Institute, Turku, Finland, for comparison. The clinical isolates were originally  
77 obtained from sputum, bronchial washings and lung biopsies. *M. avium* subsp. *avium* reference  
78 strains were supplied by culture collections as indicated by strain code (Fig. 1) and the reference  
79 strain IWGMT 49 kindly provided by Dick van Soolingen.

### 80 Isolation of mycobacteria from porcine tissue

81 Specimens of liver (n=27), lung (n=10), spleen (n=3), lymph node (n=2) and heart (n=1) tissue with  
82 visible tuberculous lesions were collected from 9 pigs raised in Southern Finland and Western  
83 Central Finland and slaughtered at the slaughterhouse in Nurmo Finland in 2002. Mycobacteria  
84 were isolated on Mycobacterium 1 and Mycobacterium 2 modified Löwenstein–Jensen culture  
85 medium (Orion Diagnostica, Finland) and Middlebrook 7H10 agar medium (Difco Laboratories,  
86 USA) and incubated for 14 to 28 days at 28 °C. Samples were incubated at 28°C to enable the  
87 isolation of mycobacterial species that grow slowly at temperature above 28°C. The colonies  
88 obtained were stained by the Ziel-Nielsen Carbol Fuchsin method. Representatives of each acid-fast  
89 colony type were subcultured on Middlebrook 7H10 agar plates, incubated at 28°C and stored in  
90 glycerol at –70 °C.

### 91 Identification of isolates

92 59 bacterial cultures were identified to species level by partial sequencing of the 16S rDNA gene as  
93 described by (Kirschner et al, 1993). The sequences were compared with the RIDOM (Harmsen et  
94 al., 2002) and GenBank (Benson et al. 2006) databases and a 100% match was required for species  
95 identification.

#### 96 **RFLP-typing and data analysis**

97 For the genetic typing of 16 porcine and 13 human *M. avium* isolates IS1245 Restriction Fragment  
98 Length Polymorphism (RFLP) was used as described by van Soolingen et al. (1998). 5 porcine  
99 isolates and one human isolate were run in duplicate to confirm the reproducibility of the RFLP  
100 patterns.

101 The RFLP patterns were analyzed with the Bionumerics program (version 4.0, Applied Maths,  
102 Kortrijk, Belgium). Autoradiograms of the IS1245 patterns were superimposed on the  
103 autoradiograms of internal markers for normalization. The dice coefficient of similarity for all pair  
104 wise comparisons of patterns was calculated. A dendrogram of relatedness among the patterns was  
105 constructed by the unweighted pair group method with arithmetic averages clustering method.

## 106 **RESULTS**

### 107 **Isolation and identification of mycobacteria from porcine tissue**

108 43 tissue specimens from 9 pigs with tuberculous lesions were investigated for the presence of  
109 mycobacteria (Table 1). 18 tissues (42%) were culture positive. Culture positive specimens were  
110 obtained from all 9 pigs. A total of 59 pure mycobacterial cultures were obtained. All porcine  
111 mycobacterial isolates (n=59) had 16S rRNA gene sequences 100% identical with the 16S rRNA  
112 gene sequence of *M. avium* (GeneBank accession number CP000479), which supports the view of  
113 *M. avium* as the principal pig pathogen. The partial 16S rRNA gene sequence of the strain AM26  
114 was deposited in the EMBL nucleotide database with the accession number AM698088.

### 115 **RFLP-typing of *M. avium* isolates**

116 Genetic fingerprints were generated for 16 porcine and 13 human *M. avium* isolates by RFLP  
117 analysis of the IS1245 element. Fig. 1 shows the IS1245 RFLP patterns of 16 porcine and 13 human  
118 *M. avium* isolates and 5 *M. avium* reference strains. 29 different RFLP patterns were obtained for  
119 the porcine and human isolates. The replicate DNA preparations produced identical RFLP patterns  
120 for each strain. In 6 pigs 2 or more isolates with different RFLP patterns were isolated. In one case  
121 (pig 3247), 4 strains with different RFLP patterns were obtained from 4 specimens from the same  
122 pig (2 lung, 1 liver, 1 lymph node specimen).

### 123 **Cluster analysis**

124 The genetic relatedness of the porcine and human *M. avium* isolates was studied by cluster analysis  
125 of the IS1245 RFLP patterns. All porcine and human isolates showed high-copy IS1245 RFLP  
126 patterns (>5 bands) typical for *M. avium* subsp. hominissuis (Mijs et al., 2002) (Table 1) and  
127 differed from the low-copy number patterns of the *M. a. avium* isolates (Fig. 1). The similarity of  
128 RFLP patterns between the *M. a. avium* reference strains was 100%. Similarity of the IS1245  
129 RFLP patterns among the porcine isolates was >50% and among the human isolates >40%. Ten  
130 porcine and seven human isolates grouped together with >80% similarity. Six porcine (38%) and  
131 five human (38%) isolates had RFLP patterns with >90% similarity. In two cases porcine (AM23B  
132 and AM26) and human (H03965/1 and H0147/2) isolates grouped together with >95% similarity.  
133 The human isolate H03965/1 was analyzed as a duplicate to ensure the reproducibility of the close  
134 clustering. The high similarity between the RFLP patterns of porcine and human *M. avium* isolates  
135 shows that they are closely genetically related. Four isolates with IS1245 RFLP patterns 50% to  
136 80% similar were obtained from the pig 3247, which suggests simultaneous infection by four  
137 different strains.

### 138 **DISCUSSION**

139 The prevalence of tuberculous lesions in slaughter pigs has increased ten-fold in Finland during the  
140 last decade (Ali-Vehmas et al., 2004). Approximately one in 10,000 individuals in Finland is yearly



141 infected by environmental mycobacteria, and the infections are caused by members of MAC more  
142 often than any other NTM species (Iivonen et al., 2005).

143 In this study porcine *M. avium* isolates were identified by sequencing of the 16S rRNA gene and  
144 compared to human isolates using the IS1245 RFLP standard method. No earlier reports on  
145 comparative RFLP typing of human and porcine *M. avium* isolates from Finland exist. RFLP  
146 analysis showed that both pigs and humans were infected with strains carrying a large number of  
147 the IS1245 element. These isolates produced more than 15 identical bands, which is the typical  
148 IS1245 RFLP pattern for *M. avium* subsp. *hominissuis* (Mijs et al., 2002). Almost identical IS1245  
149 RFLP fingerprints were found in strains of porcine and human origin. The high similarity (>90%)  
150 of the IS1245 RFLP patterns of ~40% of the human and porcine *M. a. hominissuis* isolates  
151 evidences close genetic relatedness between human and porcine *M. a. hominissuis* isolates in  
152 Finland. The results of this study support the earlier studies reporting close genetic relatedness  
153 between human and porcine *M. a. hominissuis* isolates in the Netherlands (Komijn et al., 1999),  
154 Sweden (Ramasoota et al., 2001), and Germany (Möbius et al., 2006) and the hypothesis about a  
155 common source of *M. a. hominissuis* infection for pigs and humans or pigs as a vehicle for human  
156 infections.

157 Isolation of several IS1245 genotypes of *M. avium* from one pig suggests simultaneous infection by  
158 several *M. avium* strains. This finding supports the study of Matlova et al. (2005) where one isolate  
159 from a single pig contained several mycobacterial clones with different RFLP types. Simultaneous  
160 infection by several strains can be the result of a heavy load of *M. a. hominissuis* in the piggery  
161 environment, or susceptibility of some pigs to *M. a. hominissuis* infection. *M. avium* and other  
162 NTM have been shown to occur in piggery bedding materials such as peat, sawdust, and straw and  
163 also in drinking water (Matlova et al., 2003, 2004, 2005; Pakarinen et al., 2006; Windsor et al.,  
164 1984). The high prevalence of pig mycobacteriosis and the common occurrence of mycobacteria in

165 the piggery environment indicate that piggeries may be reservoirs of infective environmental  
166 mycobacteria.

167 Pig mycobacteriosis is diagnosed at slaughterhouses by a visual detection of tuberculous lesions in  
168 pig organs. Komijn et al. (1999) reported isolation of *M. a. hominissuis* from the mesenteric lymph  
169 nodes of 48 out of 345 (13.9 %) healthy slaughter pigs without tuberculous lesions in the lymph  
170 nodes, which shows that visual inspection is a poor method for detection of pig mycobacteriosis.

171 There is long-term evidence that ingestion can be a route of human *M. avium* infection (Argueta et  
172 al., 2000; Yoder et al., 1999). In some cases temperature of up to 70 °C is required for inhibition of  
173 *M. avium* (Nichols et al., 2004), whereby mycobacteria may survive in poorly heated pork.

174 Effective methods are needed for detection, quantification, identification and genetic profiling of  
175 environmental mycobacteria in order to trace the environmental reservoirs of human and animal  
176 mycobacteriosis. Using IS1245 RFLP we identified nearly identical *M. a. hominissuis* from pig  
177 organ samples and clinical cases and showed that pigs may be a vehicle for *M. a. hominissuis*  
178 infections in humans, or that pigs and humans share common sources of infection in Finland.

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### 182 **References**

- 183 1. Ali-Vehmas, T., Moisander, A.-M., Soini, H., 2004. , Mycobacteriosis – A review and  
184 survey in Finland. Finn. Vet. J. 110, 79-84 (in finnish).
- 185 2. Argueta, C., Yoder, S., Holtzman, A.E., Aronson, T.W., Glover, N., Berlin, O.G., Stelma,  
186 G.N. Jr, Froman, S., Tomasek, P., 200. Isolation and identification of nontuberculous  
187 mycobacteria from foods as possible exposure sources. J. Food Prot. 63, 930-933.
- 188 3. Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L., 2006. GenBank.  
189 Nucleic Acids Res. 34, D16-20.

- 190 4. Biet, F., Boschioli, M.L., Thorel, M.F., Guilloteau, L.A., 2005. Zoonotic aspects of  
191 *Mycobacterium Bovis* and *Mycobacterium avium-intracellulare* complex (MAC). Vet. Res.  
192 36, 411-436.
- 193 5. Harmsen, D., Rothgänger, J., Frosch, M., & Albert, J., 2002. RIDOM: ribosomal  
194 differentiation of medical microorganisms database. Nucleic Acids Res. 30, 416-417
- 195 6. Iivonen, J., Kela, E., Kuusi, M., Lyytikäinen, O., Ruutu, P., 2005. Infectious diseases in  
196 Finland 1995 - 2004, National Public Health Institute, Helsinki, p. 44.
- 197 7. Johansen, T.B., Djonne, B., Jensen, M.R., Olsen, I., 2005. Distribution of IS1311 and  
198 IS1245 in *Mycobacterium avium* subspecies revisited. J. Clin. Microbiol. 43, 2500-2502.
- 199 8. Information Centre of the Ministry of Agriculture and Forestry of Finland, 2005.  
200 Teurastukset 2002 - Slaughtering statistics in 2002 (in Finnish), [Online.]  
201 [http://tike.mmm.fi/Tilasto/teurastukset\\_2002.pdf](http://tike.mmm.fi/Tilasto/teurastukset_2002.pdf).
- 202 9. Kulikova, T., Akhtar, R., Aldeber, P., Althorpe, N., Andersson, M., Baldwin, A., Bates, K.,  
203 Bhattacharyya, S., Bower, L., Browne, P., Castro, M., Cochrane, G., Duggan, K., Eberhardt,  
204 R., Faruque, N., Hoad, G., Kanz, C., Lee, C., Leinonen, R., Lin, Q., Lombard, V., Lopez, R.,  
205 Lorenc, D., McWilliam, H., Mukherjee, G., Nardone, F., Garcia-Pastor, M.P., Plaister, S.,  
206 Sobhany, S., Stoehr, P., Vaughan, R., Wu, D., Zhu, W., Apweiler, R. 2007. EMBL  
207 Nucleotide Sequence Database in 2006. Nucleic Acids Research 35, D16-D20.
- 208 10. Kirschner, P., Springer, B., Vogel, U., Meier, A., Wrede, A., Kiekenbeck, M., Bange, F.-C.,  
209 Böttger, E.C., 1993, Genotypic identification of mycobacteria by nucleic acid sequence  
210 determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol.  
211 31:2882-2889.
- 212 11. Komijn, R.E., De Haas, P.E., Schneider, M.M.E., Eger, T., Nieuwenhuijs, J.H.M., Van Den  
213 Hoek, J., Bakker, D., Van Zijderveld, F.G., Van Soolingen, D., 1999. Prevalence of  
214 *Mycobacterium avium* in slaughter pigs in the Netherlands and comparison of IS1245

- 215 restriction fragment length polymorphism patterns of porcine and human isolates. J. Clin.  
216 Microbiol. 37, 1254-1259.
- 217 12. Martín-Casabona, N., Bahrmand, A.R., Bennedsen, J., Thomsen, V.O., Curcio, M., Fauville-  
218 Dufaux, M., Feldman, K., Havelkova, M., Katila, M.L., Koksalan, K., Pereira, M.F.,  
219 Rodrigues, F., Pfyffer, G.E., Portaels, F., Urgell, J.R., Rusch-Gerdes, S., Tortoli, E.,  
220 Vincent, V., Watt, B., Spanish Group for Non-Tuberculosis Mycobacteria, 2004. Non-  
221 tuberculous mycobacteria: patterns of isolation. A multi-country retrospective survey. Int. J.  
222 Tuberc. Lung Dis. 10, 1186-1193.
- 223 13. Matlova, L., Dvorska, L., Bartl, J., Bartos, M., Ayele, W.Y., Alexa, M., Pavlik, I., 2003.  
224 Mycobacteria isolated from the environment of pig farms in the Czech Republic during the  
225 years 1996 to 2002. Vet. Med. Czech. 48, 343-357.
- 226 14. Matlova, L., Dvorska, L., Palecek, K., Maurenc, L., Bartos, M., Pavlik, I., 2004. Impact of  
227 sawdust and wood shavings on pig bedding on pig tuberculous lesions in lymph nodes, and  
228 IS1245 RFLP analysis of *Mycobacterium avium* subsp. *Hominissuis* of serotypes 6 and 8  
229 isolated from pigs and environment. Vet. Microbiol. 102, 227-236.
- 230 15. Matlova, L., Dvorska, L., Ayele, W.Y., Bartos, M., Amemori, T., Pavlik, I., 2005.  
231 Distribution of *Mycobacterium avium* complex isolates in tissue samples of pigs fed peat  
232 naturally contaminated with mycobacteria as a supplement. J. Clin. Microbiol. 43, 1261-  
233 1268.
- 234 16. Mijs, W., de Haas, P., Rossau, R., van der, L.T., Rigouts, L., Portaels, F., van Soolingen, D.,  
235 2002. Molecular evidence to support a proposal to reserve the designation *Mycobacterium*  
236 *avium* subsp. *avium* for bird-type isolates and '*M. avium* subsp. *hominissuis*' for the  
237 human/porcine type of *M. avium*. Int. J. Syst. Evol. Microbiol. 52, 1505-1518.
- 238 17. Möbius, P., Lentzsch, P., Moser, I., Naumann, L., Martin, G., Köhler, H., 2006.  
239 Comparative macrorestriction and RFLP analysis of *Mycobacterium avium* subsp. *avium*

- 240 and *Mycobacterium avium* subsp. *hominissuis* isolates from man, pig, and cattle. Vet.  
241 Microbiol. 117, 284-291.
- 242 18. Nichols, G., Ford, T., Bartram, J., Dufour, A., Portaels F., 2004. The epidemiology of  
243 environmental mycobacteria. In: Pedley, S., Bartram, J., Rees, G., Dufour, A., Cotruvo, J.A.  
244 (Eds), Pathogenic Mycobacteria in Water: A Guide to Public Health Consequences,  
245 Monitoring and Management. WHO Emerging Issues in Water & Infectious Disease Series,  
246 IWA Publishing, London, UK, pp. 5-6.
- 247 19. Pakarinen, J., Nieminen, T., Tirkkonen, T., Tsitko, I., Ali-Vehmas, T., Neubauer, P.,  
248 Salkinoja-Salonen, M., 2006. Proliferation of mycobacteria in a piggery environment  
249 revealed by *Mycobacterium*-specific real-time quantitative PCR and 16S rRNA sandwich  
250 hybridization. Vet. Microbiol. 120, 105-112.
- 251 20. Primm, T.P., Lucero, C.A., Falkinham III J.O., 2004. Health impacts of Environmental  
252 Mycobacteria. Appl. Environ. Microbiol. 17, 98-106.
- 253 21. Ramasoota, P., Chansiripornchai, N., Källenius, G., Hoffner, S., Svenson, S.B., 2001.  
254 Comparison of *Mycobacterium avium* complex (MAC) strains from pigs and humans in  
255 Sweden by random amplified polymorphic DNA (RAPD) using standardized reagents. Vet.  
256 Microbiol. 12, 251-259.
- 257 22. Thorel, M.F., Huchzermeyer, H.F., Michel, A.L., 2001. *Mycobacterium avium* and  
258 *Mycobacterium intracellulare* infection in mammals. Rev. Sci. Tech. Off. Int. Epiz. 20, 204-  
259 218.
- 260 23. van Soolingen, D., Bauer, J., Ritacco, V., Leao, S.C., Pavlik, I., Vincent, C., Rastogi, N.,  
261 Gori, A., Bodmer, T., Garzelli, D., Garcia, M.J., 1998. IS *1245* restriction fragment length  
262 polymorphism typing of *Mycobacterium avium* isolates: proposal for standardization. J.  
263 Clin. Microbiol. 36, 3051-3054.

- 264 24. von Dürrling, H., Ludewig, H., Uhlemann, J., Gericke, R., 1998. Torf als Quelle einer  
265 Infektion mit aviären Mykobakterien bei Schweinen. Tierärztl. Umschau 53, 259-261.
- 266 25. Windsor, R.S., Durrant, D.S., Burn, K.J., 1984. Avian tuberculosis in pigs: *Mycobacterium*  
267 *intracellulare* infection in a breeding herd. Vet. Rec. 114, 497-500.
- 268 26. Yoder, S., Argueta, C., Holtzman, A., Aronson, T., Berlin, O.G., Tomasek, P., Glover, N.,  
269 Froman, S., Stelma, G. Jr., 1999. PCR comparison of *Mycobacterium avium* isolates  
270 obtained from patients and foods. Appl. Environ. Microbiol. 65, 2650-2653.

271

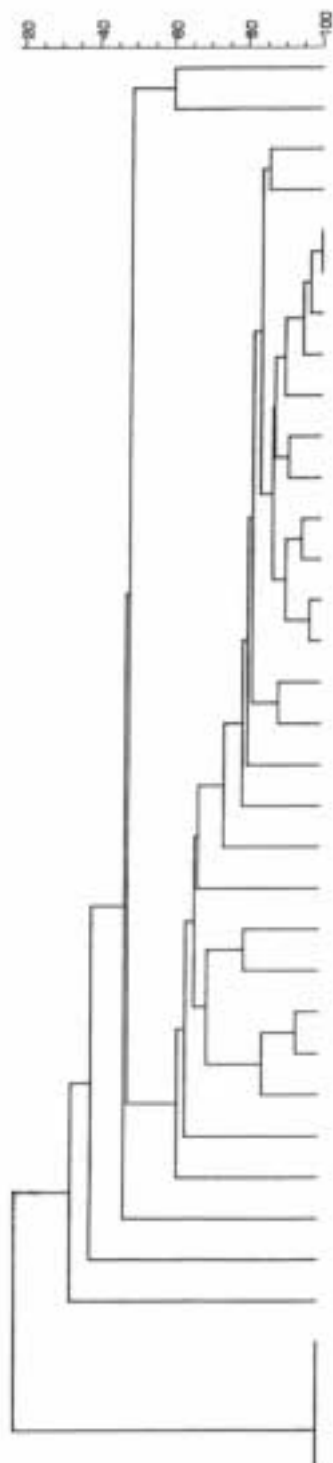
272 Table 1. Isolation and characterization of mycobacteria in porcine tissues.

	Mycobacterial culture of porcine tissues			Characterization of mycobacterial isolates					
				Sequencing of the 16S rRNA gene			IS/245 RFLP analysis		
	Examined	Positive		Examined	MA <sup>a</sup>		Examined	MAH <sup>b</sup>	
	No.	No.	%	No.	No.	%	No.	No	%
Liver	27	11	41	40	40	100	9	9	100
Lung	10	3	30	7	7	100	4	4	100
Spleen	3	2	67	9	9	100	2	2	100
Lymph node	2	1	50	1	1	100	1	1	100
Heart	1	1	100	2	2	100	0	0	0
Total	43	18	42	59	59	100	16	16	100

273 <sup>a</sup>Identified as *M. avium*274 <sup>b</sup>Identified as *M. avium* subsp. *hominissuis*

275 **Fig. 1.** Dendogram of the IS1245 RFLP patterns of porcine, human and avian *M. avium*  
276 isolates. Source: OUH: Oulu University Hospital, HUH: Helsinki University Hospital, PHCH:  
277 Päijät-Häme Central Hospital, EP: Etelä-Pohjanmaa Hospital District, TAUH: Tampere  
278 University Hospital, TUH: Turku University Hospital, LCH: Lappi Central Hospital, SCH:  
279 Seinäjoki Central Hospital. Isolates >80% similar marked with "\*". Isolates >90% similar  
280 marked with "†". Four isolates obtained from a single pig marked by emboldened text.





<u>Strain Code</u>	<u>Source</u>	<u>Specimen</u>
H0923/01	Human OUH	N.D.
<b>AM87B</b>	<b>Pig 3247</b>	<b>lung 1</b>
H0854/04*	Human HUH	lung biopsy
H0187/02*	Human HUH	sputum
H0935/01*†	Human HUH	sputum
H0935/01*†	Human HUH	sputum
AM23B*†	Pig O906	liver
AM53A*†	Pig 2107	liver
AM38*	Pig 7277	spleen
H0341/02*†	Human PHCH	sputum
AM9B2*†	Pig 4393	liver
H1081/01*†	Human HUH	sputum
<b>AM81*†</b>	<b>Pig 3247</b>	<b>liver</b>
H0147/02*†	Human EP	sputum
AM26*†	Pig 5509	liver
<b>AM85*</b>	<b>Pig 3247</b>	<b>lung 2</b>
AM45B*	Pig 5483	liver
AM49B	Pig 5483	lung
AM67	Pig 6761	liver
AM30B	Pig 5509	lung
H1045/04	Human TAUH	bronchial washing
<b>AM84B</b>	<b>Pig 3247</b>	<b>lymph node</b>
H1078/03	Human TUH	sputum
AM58B*†	Pig 2107	spleen
H0277/03*†	Human TUH	bronchial washing
AM69B*	Pig O950	liver
AM34B	Pig 7277	liver
H1149/04	Human LCH	sputum
H0914/04	Human SCH	sputum
H1303/04	Human HUH	bronchial washing
IWGMT49		
ATCC 35712	Chicken	
ATCC 25291	Hen	
ATCC 15769	Hen	
ATCC 35712	Chicken	