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1 **Estimation of the spread of pathogenic mycobacteria in organic broiler**
2 **farms by the polymerase chain reaction**

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4

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13

14 **Abstract**

15 Organic poultry breeding allows for increased exposure of birds to soil, faeces,
16 and wildlife, which have been associated with the transmission of mycobacterial
17 infections. Therefore the aim of this study was to investigate the spread of the major
18 pathogenic mycobacteria in organically reared broilers in Greece using a diagnostic
19 algorithm that relied on a combination of the Polymerase Chain Reaction (PCR) and the
20 Restriction Fragment Length Polymorphism analysis (RFLP).

21 Liver, spleen and gonads from 81-150 days old broilers were aseptically
22 collected post-mortem. 500 broilers from a population of 35370, reared in the 25
23 registered as organic farms in Greece for the 2005 were used. DNA was isolated and
24 incorporated to PCR targeted to 16s-rRNA gene (for *Mycobacterium spp.*), IS6110 (for
25 *M. tuberculosis complex*-MTBc), IS1245 (for *M. avium complex*-MAC), IS901 (for *M.*
26 *avium* subsp. *avium* - MAA) and hsp65 (for *M. genavense*, by PCR-RFLP). The mean
27 prevalence of mycobacteria detected by PCR with a 95% confidence interval was
28 estimated to 4.4 - 8.8%. The relevant percentage with regard to the mycobacterial
29 species that were included in this study was 0.17-2.03% for MAC, 2.11-3.39% for
30 MTBc and 0.66-3.08% for mycobacteria not belonging to any of the above groups.
31 None of the mycobacteria detected were identified as MAA or *M. genavense*.

32 Considering that avian tuberculosis has been eradicated from conventional farms,
33 the level and the pattern of positivity recorded here, indicates that our results may be
34 associated with the specific conditions that apply to organic breeding.

35

36 **Keywords:** mycobacteriosis, epidemiology, poultry, organic farming

37

38

39

40 Introduction

41

42 Avian tuberculosis is usually caused by *Mycobacterium avium* complex (MAC)
43 serovars 1 to 3 (*M. avium* subsp. *avium* - MAA) and *M. genavense* (Böttger et al., 1993;
44 Tell et al., 2001; Saif, 2003; Shitaye et al., 2008). The incidence of the disease is
45 sporadic and it is rarely reported in intensively reared flocks (Tell et al., 2001). Organic
46 farming is gradually becoming very popular among farmers and consumers in Europe,
47 but it increases exposure of fowl to soil and faeces, and has been associated with the
48 spread of mycobacterial infections (Hoop et al., 1996; Martin and Schimmel, 2000;
49 Gonzalez et al., 2002; Saif, 2003). Despite the assessment of the prevalence of various
50 pathogens in organic farms of birds, there seems to be, to the best of our knowledge, no
51 evidence with respect to mycobacteria (Permin and Nansen, 1996; Lund and Algers,
52 2003; Maag et al., 2003).

53 The aim of this study was therefore to investigate the spread of the major
54 pathogenic mycobacteria (*M. avium* complex, *M. genavense*, *M. tuberculosis* complex)
55 in organically reared broilers in Greece using a diagnostic algorithm that relied on a
56 combination of the Polymerase Chain Reaction (PCR) and the Restriction Fragment
57 Length Polymorphism analysis (RFLP).

58

59

60 Materials and Methods

61 *Samples*

62 The sampling plan covered 25 broiler farms that represented the total of those
63 registered as organic in Greece in the year 2005, based on the records of the National
64 Authorized Certification Institutes. Liver, spleen, and gonads were collected aseptically
65 post-mortem, from 500 of the 35370, 81-150 days old, organically reared broilers. The
66 required sample size was calculated (STATGRAPHICS Plus 5.1) to estimate a 95%
67 confidence interval for the prevalence of mycobacteria no wider than 4%, when the
68 expected mycobacteria prevalence is reported to be 5% (Tell et al., 2001; Saif, 2003).
69 The sample size that was thus estimated (n=457) was finally defined to 500 for practical
70 reasons, and was distributed equally among the farms, the broiler population of which
71 was considered homogenous. Fowl within each flock was randomly selected among

72 clinically healthy broilers, based on the records of the farm and the evaluation of the
73 birds by Veterinary Inspectors. Collection of samples was performed from June to
74 August 2005.

75 The tissue samples of each bird were pooled in 20 ml, screw-cup containers and
76 were stored at 4-6°C for up to 6 hours, until transportation to the laboratory. The
77 material was then homogenised in a stomacher blender at 260 rpm for 5 min and it was
78 processed for DNA isolation.

79

80 **DNA isolation**

81 A portion of 0.5 ml of the tissue homogenate of each sample was used for DNA
82 isolation with the NucleoSpin® Tissue kit (Macherey-Nagel, Germany) according to the
83 instructions of the manufacturer. The DNA solution was evaluated for purity and
84 integrity by spectrophotometry and gel-electrophoresis (Sambrook et al., 1989). The
85 presence of PCR inhibitors in the DNA preparations was assessed by a PCR assay
86 targeted to an in-house gene (cytochrome b), based on a method originally described by
87 Zehner et al., 1998 (data not shown). DNA samples of low quality, and those failing to
88 produce the expected amplification product from the PCR assay mentioned above, were
89 discarded and DNA isolation was repeated from the original material.

90

91 **PCR**

92 The detection and the differential identification of mycobacteria relied on a
93 PCR-based diagnostic algorithm (Dvorska et al., 2001), modified to include *M.*
94 *genavense* (Mendenhall et al., 2000), (Figure 1). Within this context a combination of 5
95 PCR assays was applied targeted to 16s-rRNA gene (for *Mycobacterium spp.*), IS6110
96 (for *M. tuberculosis complex*), IS1245 (for *M. avium complex*), IS901 (for *M. avium*
97 subsp. *avium*), and hsp65 followed by RFLP (for *M. genavense*) (Table 1).

98 PCR products were analysed by submerged electrophoresis on a 2% agarose gel.
99 Samples were evaluated and processed together with positive and negative controls that
100 consisted of confirmed, PCR-positive and negative, DNA preparations, respectively.
101 Test tubes, containing only the reaction mixture and water of the same source used for
102 the dilution of DNA, were also included in each PCR assay, as negative controls.

103 Results

104

105 Fifty of the 500 (10%) samples examined were found positive for mycobacteria. Of
106 these, 8 (1.6%) were identified as *Mycobacterium avium complex* (2 positive to 16s-
107 rRNA and IS1245, 6 positive to 16S-rRNA and hsp65), 18 (3.6%) as *Mycobacterium*
108 *tuberculosis complex* (MTBc) (16S-rRNA and IS6110-positive, with the exception of
109 one sample that reacted positively only to IS6110), and 24 (4.8%) as mycobacteria not
110 belonging to any of the groups (OM-other mycobacteria) previously mentioned (16s-
111 rRNA-positive), (Table 2). After exclusion of a farm in which mycobacteria-specific
112 DNA was detected by PCR in 90% of the tested samples and was therefore considered
113 an outlier, the above percentages were formulated respectively to the following: 1%
114 MAC (5/480), 3,75% MTBc (18/480), and 1,87% OM (9/480).

115 Twelve of the 25 (48%) flocks tested where found PCR-positive with prevalence
116 ranging (excluding the outlier flock) from 5 to 35%. Within this group, the majority of
117 the farms (6/11, 54,5%) were positioned at 10% (4/11) and 20% (2/11). Of the reactor
118 flocks, 3 were found positive only to OM, 3 to MAC, 5 to MTBc, and 1 to a
119 combination of OM and MAC.

120 Hypothesis tests proved that mycobacterial prevalence in organic broilers'
121 population was similar to the 5% estimated prevalence in conventional broilers
122 populations (p-value=0.12 >0.05).

123 The mean prevalence of mycobacteria detected by PCR in organic broilers in
124 Greece, with a 95% confidence interval, was estimated to 4.4 - 8.8%. The relevant
125 percentage with regard to the mycobacterial species that were investigated was 0.17-
126 2.03% for *Mycobacterium avium complex*, 2.11-3.39% for *Mycobacterium tuberculosis*
127 *complex* and 0.66-3.08% for other mycobacteria not belonging to any of the above
128 groups.

129

130

131 Discussion

132

133 The results of this study show that DNA that belongs to pathogenic mycobacteria
134 can be detected in viscera (not inclusive of the intestine which might contain

135 environmental mycobacteria) of clinically healthy organic broilers in Greece. The
136 young age of the birds examined and possibly the fact that none of the positive reactions
137 referred to mycobacteria known to specifically infect fowl (*M. avium* subsp. *avium* and
138 *M. genavense*) may account for the absence of any disease symptoms in the tested
139 population, even though the presence of mycobacterial DNA in the visceral organs
140 indicates invading bacteria (Ashour, 1972; Ashford et al., 2001).

141 In studies such as the one described here, reservations on the reliability of the
142 results can be attributed mainly to false positive reactions usually due to the carry over
143 effect or the use of low specificity PCR assays. To avoid this, the evaluation of the
144 samples was performed under vigorous precautions such as the use of dedicated space,
145 equipment, and consumables, as well as negative controls that were processed together
146 with the tested material during both, DNA isolation and PCR. The low specificity of the
147 methodology followed in this study was eliminated by the use of PCR assays
148 thoroughly evaluated and repeatedly used in the past by the same and other groups
149 (Hance et al., 1989; Huard et al., 2003; Ikonopoulou et al., 2004; Ikonopoulou,
150 2006; Ikonopoulou et al., 2007). Furthermore, the use of the specific diagnostic
151 algorithm was selected because in addition to the identification of the targeted
152 mycobacteria, it allowed confirmation of the results by the amplification of more than
153 one gene for each of the pathogens detected.

154 This approach was however responsible, for some discrepancies results because
155 of the expected inconsistency among the positive reactions of PCR assays designed for
156 gene-targets of the same mycobacterial groups (Hance et al., 1989; Gazouli et al., 2002
157 Ikonopoulou, 2006; Ikonopoulou et al., 2007). Indeed this was observed for 2
158 IS1245 and 16rRNA-positive samples, which came from the same farm that reacted
159 negatively to the PCR assay targeted to hsp65. Furthermore, one IS6110-positive
160 sample did not produce the expected 16S rRNA-positive result, despite the fact that
161 evaluation of these samples was repeated more than once from the same DNA
162 preparation and the original material. These observations could be attributed to atypical
163 strains, were not considered unreliable and therefore they were included in the final
164 evaluation of the tested population.

165 A high level of awareness was also imposed by the effort to eliminate false
166 negative reactions, especially since these could easily be the cause of considerable
167 under-evaluation, given that the expected positivity of the tested material was small.
168 Therefore all our DNA preparations were assessed before PCR by several methods
169 (electrophoresis, measurement of optical density, and PCR targeted to an in-house gene)
170 for DNA fragmentation and presence of PCR inhibitors. Apparently, detection and
171 identification of mycobacteria was always performed in correlation with positive
172 controls that represented no less than 10% of the tested samples of each batch, and
173 consisted of DNA preparations of visceral organs inoculated with pure growth of each
174 of the mycobacteria included in our investigation.

175 Considering that avian tuberculosis has been eradicated from conventional farms
176 and that nowadays the disease is reported mostly in wild (Tell et al., 2001; Milan et al.,
177 2004), or captive fowl (Portaels et al., 1996), and pet birds (Bottger et al 1993; Hoop et
178 al., 1996; Hoop, 2002), the level of positivity recorded here may be associated with the
179 specific conditions that apply to organic farming. According to the relevant regulations,
180 birds have to be housed in specifically designed facilities but never in cages, and they
181 have to be able to spend at least one third of their lives in open fields covered with
182 vegetation (Council of European Communities-EEL198, 22/7/1991). Evidently this
183 increases exposure of fowl to worms, rodents, arthropods, and wild birds that have all
184 been found to carry mycobacteria and play an important role in their spread in nature
185 and the livestock (Fischer et al., 2001; Fischer et al., 2003a; Fischer et al., 2003b;
186 Tadesse et al., 2003). This may justify the high percentage of OM detected in the
187 samples, which probably represent non-pathogenic mycobacteria broadly found in the
188 environment. The characteristics of the organic farming system mentioned above may
189 also account for the level of PCR-positivity of the tested samples recorded in a specific
190 flock that was finally considered an outlier, since detection of mycobacterial DNA in all
191 the other farms was comparatively much lower (90% compared to 14.5% mean
192 average). Admittedly this finding may consist an indication of a mycobacterial outbreak
193 that could be associated with organic farming or just malpractice, something that could
194 not be investigated by a study such as this. On the other hand failure to detect any *M.*
195 *genavense* or MAA-positive samples may result from the fact that as opposed to animal
196 tuberculosis, the disease in fowl has been eradicated and effectively exposure of birds to

197 these pathogens is small. The negativity of the samples to the mycobacteria that
198 specifically infect fowl may be reflecting, at least in Greece, the situation in
199 conventional farms for yet another reason: based on the regulations mentioned above,
200 the fowl of organic farms often derive from conventional flocks with the restriction to
201 be of no more than 3 days of age, since this is the suggested alternative in the event that
202 availability of organic breeding stock is small.

203 **In conclusion**

204

205 DNA belonging to MAC, MTBc, and MO, but not to *M. genevense* or MAA, was
206 detected by PCR in the viscera of organically reared broilers in Greece. The level and
207 the pattern of sample positivity recorded, indicate that our results may be associated
208 with the specific conditions that apply to organic farming, probably with connection to
209 increased exposure of birds to the environment.

210

211

212 **References**

- 213 Ashford, D.A., Whitney, E., Raghunathan, P., Cosini, O., 2001. Epidemiology of
214 selected mycobacteria that infect humans and other animals. Rev. Sci. Tech. Off.
215 Int. Epiz. 20, 325-337.
- 216 Ashour, N., 1972. Contribution to the experimental infection of young chickens with
217 *Mycobacterium avium*. Acta. Vet. Brno. 41, 421-427.
- 218 Böttger, E.C., Hirschell, B., Coyle, M.B., 1993. *Mycobacterium genevense* sp. nov.
219 Int. J. Syst. Bacteriol. 43, 841-843.
- 220 Dvorska, L., Bartos, M., Martin, G., Erler, W., Pavlik, I., 2001. Strategies for
221 differentiation, identification and typing of medically important species of
222 mycobacteria by molecular methods. Vet. Med. Czech. 46, 309-328.
- 223 Fischer, O.A., Matlova, L., Dvorska, L., Svastova, P., Bartl, J., Melicharek, I.,
224 Weston, R.T., Pavlik, I., 2001. Diptera as vectors of mycobacterial infections in
225 cattle and pigs. Med. Vet. Entomol. 15, 208-211.
- 226 Fischer, O.A., Matlova, L., Dvorska, L., Svastova, P., Pavlik, I., 2003a. Nymphs of
227 the Oriental cockroach (*Blatta orientalis*) as passive vectors of causal agents of
228 avian tuberculosis and paratuberculosis. Med. Vet. Entomol. 17, 145-150.

- 229 Fischer, O.A., Matlova, L., Bartl, J., Dvorska, L., Svastova, P., du Maine, R.,
230 Melicharek, I., Bartos, M., Pavlik, I., 2003b. Earthworms (*Oligochaeta*,
231 *Lumbricidae*) and mycobacteria. *Vet. Microbiol.* 91, 325-338.
- 232 Gazouli, M., Ikonopoulou, J., Trigidou, R., Foteinou, M., Kittas, C., Georgoulis,
233 V., 2002. Assessment of mycobacterial, propionibacterial, and human herpesvirus 8
234 DNA in tissues of Greek patients with sarcoidosis. *J. Clin. Microbiol.* 8, 3060-3063.
- 235 Gonzalez, M., Rodriguez-Bettos, A., Gimeno, I., Flores, J.M., Pizarro, M., 2002.
236 Outbreak of avian tuberculosis in 48-week-old commercial layer hen flock. *Av. Dis.*
237 46, 1055-1061.
- 238 Guerrero, C., Bernasconi, C., Burki, D., Bodmer, T., Telenti, A., 1995. A novel
239 insertion element from *Mycobacterium avium*, IS1245, is a specific target for
240 analysis of strain relatedness. *J. Clin. Microbiol.* 33, 304-307.
- 241 Hance, A.J., Grandchamp, B., Levy-Frebault, V., Lecossier, D., Raugier, J., Bocart,
242 D., Giquel, B., 1989. Detection and identification of mycobacteria by amplification
243 of mycobacterial DNA. *Mol. Microbiol.* 3, 843-849.
- 244 Hoop, R.K., Bottger, E.C., Pfyffer, G.E., 1996. Etiological agents of mycobacterioses
245 in pet birds between 1986 and 1995. *J. Clin. Microbiol.* 34, 991-992.
- 246 Hoop, R.K. 2002. *Mycobacterium tuberculosis* infection in a canary (*Serinus canaria*
247 *L.*) and a blue-fronted Amazon Parrot (*Amazona amazona aestiva*). *Avian Dis.* 46,
248 502-504.
- 249 Huard, R.C., Lazzarini, L.C.O., Butler, W.R., van Soolingen, D., Ho, J.L., 2003. PCR-
250 based method to differentiate the subspecies of *Mycobacterium tuberculosis*
251 *complex* on the basis of genomic deletions. *J. Clin. Microbiol.* 41, 1637-1650.
- 252 Ikonopoulou, J., 2006. Evidence that sheep and goats are a reservoir for
253 mycobacteria in Greece. *Online J. Vet. Res.* 10, 162-176.
- 254 Ikonopoulou, J., Gazouli, M., Zacharatos, P., Xylouri, E., Papalambros E.,
255 Georgoulis, V., 2004. Comparative evaluation of PCR assays for the robust
256 molecular detection of *Mycobacterium avium* subsp. *paratuberculosis*. *J. Microbiol.*
257 *Methods.* 56, 315-321.
- 258 Ikonopoulou, J., Balaskas, C., Kantzoura, B., Fragiadaki, E., Pavlik, I., Bartos, M.,
259 Lukas, J., Gazouli, M., 2007. Comparative evaluation of positive tests to
260 *Mycobacterium avium* subsp. *paratuberculosis* in clinically healthy sheep and goats

- 261 of South -West Greece using molecular techniques, serology, and culture. *Vet. J.*
262 174, 337-343.
- 263 Lund, V., Algers, B., 2003. Research on animal health and welfare in organic farming
264 - a literature review. *Livestock Prod. Sci.* 80, 55-68.
- 265 Maag, E.N., Permin, A., Christensen, J.P., Bisgaard, M., 2003. Mortality in organic
266 free-range chickens and molecular characterization of the involved pathogens.
267 Poster presented at XIII Congress of the World Veterinary Poultry Association,
268 Denver, Colorado, July 19-23.
- 269 Martin, G., Schimmel, D., 2000. *Mycobacterium avium* infections in poultry-a risk for
270 human health or not? *Dtsch. Tierarztl. Wochenschr.* 107, 53-58. In German
- 271 Mendenhall, M.K, Ford, S.L., Emreson, C.L., Wells, R.A., Gines, L.G., Eriks, I.S.,
272 2000. Detection and differentiation of *Mycobacterium avium* and *Mycobacterium*
273 *genavense* by polymerase chain reaction and restriction enzyme digestion analysis.
274 *J. Vet. Diagn. Invest.* 12, 57-60.
- 275 Milan, L., Gortazar, C., Villafuerte, R., 2004. *Mycobacterium avium* disease in wild
276 red-legged partridges (*Alectoris rufa*). *Eur. J. Wildl. Res.* 50, 97-99.
- 277 Pavlik, I., Svatova, P., Bartl, J., Dvorska, L., Rychlik, I., 2000. Relationship between
278 IS901 in the *Mycobacterium avium complex* stains isolated from birds, animals,
279 humans and the environment and virulence for poultry. *Clin. Diagn. Lab. Immunol.*
280 7, 212-217.
- 281 Permin, A. Nansen, P., 1996. Parasitological problems in organic poultry production.
282 Beretning Fra Statens Husdyrbrugsforsog. Danish Institute of Animal Science,
283 Tjele, Denmark, 729 91-96.
- 284 Portaels, F., Realini, L., Bauwens, L., Hirschell, B., Meyers, W.M., De Meurichy, W.,
285 1996. Mycobacteriosis caused by *Mycobacterium genavense* in birds kept in a zoo:
286 11-year survey. *J. Clin. Microbiol.* 34, 319-323.
- 287 Saif, Y.M., 2003. Diseases of poultry- 11th edition. Iowa State Press, 836-844.
- 288 Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular Cloning: A Laboratory*
289 *Manual*, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring, N.Y.
- 290 Shitaye, J.E., Matlova, L., Horvathova, A., Moravkova, M., Dvorska-Bartosova, L.,
291 Treml, F., Lamka, J., Pavlik, I. 2008. *Mycobacterium avium* subsp. *avium*

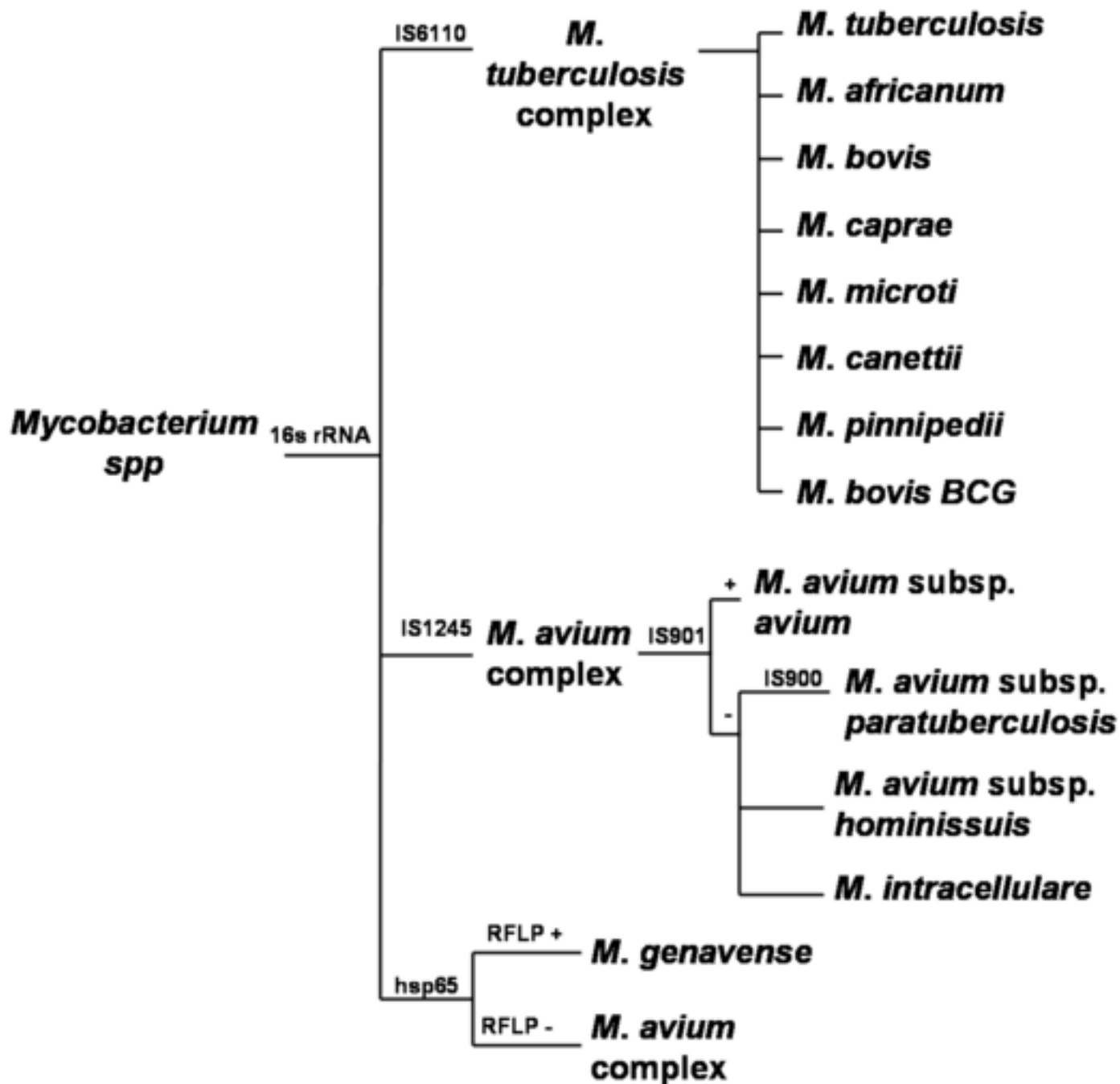
292 distribution studied in a naturally infected hen flock and in the environment by
293 culture, serotyping and IS901 RFLP methods. *Vet. Microbiol.*, 127, 155-164.

294 Tadesse, S., Woldemeskel, M., Molla, B., Tibbo, M., Kidane, D., Medhim, G.,
295 Britton, S., 2003. Avian Mycobacteriosis in domestic chickens from selected agro-
296 climatic regions in Ethiopia. *J. App. Res. Vet. Med.* 62, 254-260.

297 Tell, L.A., Woods, L., Cromie, R.L., 2001. Mycobacteriosis in birds. *Rev. Sci. Tech.*
298 *Off. Int. Epiz.* 20, 180-203.

299 Zehner, R., Zimmermann, S., Mebs, D., 1998. RFLP and sequence analysis of the
300 cytochrome b gene of selected animals and man: methodology and forensic
301 application. *Int. J. Legal. Med.* 111, 323-327.

302



Species	Target	Primer sequence* (5'→3')	Amplicon size (bp)	Reference
<i>Mycobacterium 16S spp.</i>	<i>rRNA</i>	F:-ACGGTGGGTACTAGGTGTGGGTTTC- R:- TCTGCGATTACTAGCGACTCCGACTTCA-	543	Huard <i>et al.</i> , 2003
<i>M. tuberculosis complex</i>	<i>IS6110</i>	F:- CGTGAGGGCATCGAGGTGGC - R:-GCGTAGGCGTCCGGTGACAAA-	245	Huard <i>et al.</i> , 2003
<i>M. avium complex</i>	<i>IS1245</i>	F:- AGGTGGCGTTCGAGGAAGAC- R:-GCCGCCGAAACGATCTAC-	427	Guerrero <i>et al.</i> , 1995
<i>M. avium avium</i>	<i>IS901 (nested)</i>	NP1:-TTAACACGATGAGTCATGCG- NP2:-GCTTATCGATGTCCTTGATC-	509	Pavlik <i>et al.</i> , 2000
		NP3:-GTACCCGGCGAAGACCTGG- NP4:-AAGTCCAGCAGCCGTGCTG-	376	
<i>M. genavense</i>	<i>hsp65</i>	MKMTB 13A : - AGGCGATGGACAAGGT- MPTB II : - CCTCGATGCGGTGCTTGC-	693	Mendenhall <i>et al.</i> , 2000

*F: forward, R: reverse

Table 1. Characteristics of the PCR assays used for the detection and identification of mycobacterial pathogens from our tissue samples.

Reactor Farm Code No	Number of samples positive to					Total*
	<i>16S rRNA</i>	<i>IS1245</i>	<i>IS6110</i>	<i>hsp65</i>	<i>IS901</i>	
E1	18	0	0	3	0	18
E2	5	0	0	0	0	5
E3	2	0	2	0	0	2
E4	2	0	0	2	0	2
E5	7	0	7	0	0	7
E6	4	0	4	0	0	4
E7	1	0	0	1	0	1
E8	3	0	0	0	0	3
E9	1	0	0	0	0	1
E10	3	0	3	0	0	3
E11	3	2	0	0	0	2
E12	1	0	2	0	0	2
						50

* Total number of positive samples

Table 2. The results recorded by PCR for each of the targeted genes for all the reactor farms