ESTIMATION OF THE PREVALENCE OF PATHOGENIC MYCOBACTERIA IN ORGANIC BROILER FARMS IN GREECE BY THE POLYMERASE CHAIN REACTION

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Introduction

Avian mycobacteriosis is usually caused by *Mycobacterium avium* complex (MAC) serovars 1 to 3 (*M. avium* subsp. *avium)* and *M. genavense* (8, 9). In most cases, incidences are sporadic and they rarely refer to intensive farms since modern breeding practices have decreased exposure of birds to the parameters that were traditionally linked to the spread of mycobacterial infections, such as contact with soil, feces, wild animals and birds (8, 9).

Organic farming reintroduces the parameters mentioned above to the management practice followed by many producers around Europe. Effectively concern is raised with regard to the probability of increased exposure-risk of organically reared fowl to mycobacterial infections. Although several groups have already studied the prevalence of different pathogens in organic farms of birds, there is no evidence to this day regarding mycobacteria (4, 5).

For this reason we incorporated the Polymerase Chain Reaction (PCR) to the investigation of the prevalence of the major mycobacterial pathogens (*M. tuberculosis complex, M. avium complex, M. genavense*) in organically reared broilers in Greece.

Materials and methods

Sampling plan: In order to allow a 95% level of confidence for a minimum of 5% prevalence of mycobacteria (STATGRAPHICS Plus) (9), liver, spleen, and gonads were aseptically collected from 500 of total 35370, 81-150 days old, organic broilers reared in total 25 registered organic farms in Greece, in 2004. The tissue samples of each bird were pooled in container, were kept at 4-6^{o}C and transported to laboratory within 6 hours, were homogenised and proceeded to DNA isolation.

DNA isolation: 0.5 ml of tissue homogenates were used for DNA isolation with the NucleoSpin® Tissue kit (Macherey- Nagel). The product was evaluated with regard to purity and integrity using spectrophotometry, gel-electrophoresis and PCR targeted to an in-house gene (cytochrome b) (10).

PCR: For detection and differential identification of mycobacteria we relied on a PCR-based diagnostic algorithm (1), modified to include *M. genavense* (Figure 1). Within this context we applied 5 PCR assays targeted to 16S-rRNA gene (for *Mycobacterium spp*.), IS6110 (for *M. tuberculosis complex),* IS1245 (for *M. avium complex),* IS901 (for *M. avium subsp. avium)* and hsp65 (for *M. genavense* by PCR-RFLP) (Table 1).

Results

Fifty of the 500 samples (10%) were found positive for pathogenic mycobacteria. Eight (1.6%) were identified as MAC, 18 (3.6%) as Mycobacterium tuberculosis complex, and 24 (4.8%) as pathogenic mycobacteria not belonging to any of the mycobacterial species mentioned above (16S rRNA-positive).

After exclusion of a farm that presented 90% positivity and was therefore considered an outlier, the prevalence of pathogenic mycobacteria was finally estimated to 6.6% (32/480) and 5-35% with regard respectively to broiler chickens and farms. Hypothesis test proved that mycobacterial prevalence in organic broilers' population was similar to the 5% estimated prevalence in conventional broilers population (p-value=0.12 >0.05).

The mean prevalence of pathogenic mycobacteria using PCR, in organic broilers of Greece, with a 95% confidence interval, was estimated to 4.4-8.8%. The relevant percentage with regard to the mycobacterial species that were investigated was 0.17-2.03% for MAC, 2.11-3.39% for MTB, and 0.66-3.08% for other pathogenic mycobacteria not belonging to any of the above species**.**

Discussion

Our results indicate that DNA belonging to pathogenic mycobacteria can be found in visceral samples (excluding intestine or other tissues that could carry environmental mycobacteria) collected from organic broilers in Greece. Although the absence of any disease symptoms can be attributed to the young age of birds and to the fact that none of the positive samples referred to the mycobacterial species pathogenic to fowl, presence of mycobacterial DNA in the visceral organs of birds indicates active infection.

The absence of PCR-positive results for any of the mycobacterial pathogens that are considered typically pathogenic to birds (*M. avium* subsp. *avium* and *M. genavense*) was expected, since avian tuberculosis has been eradicated from the conventional farms of Greece. Considering that the latter consist in most cases the source of organically reared livestock, the absence of avian mycobacterial pathogens in these farms may very well reflect the situation of conventional farms.

The chance of our results representing the wider spread of pathogenic mycobacteria in nature cannot be underestimated. However the fact that positive results were recorded from only 48% of the farms that were tested may indicate that infection was introduced only to the certain farms from an unknown source. In this event, our results can be considered indicative of the existence of some epidemiological link between human/animal mycobacterial pathogens and atypical, and therefore silent, mycobacterial infections of broilers.

Conclusion

DNA belonging to human and animal, but not to avian mycobacterial pathogens, was detected using PCR, in the viscera of a considerable percentage of organically reared broilers in Greece. Considering that organic farming has been only recently introduced to the EU, our results cannot be safely attributed to the specific conditions of organic farming system, which is supported by the fact that the mycobacterial prevalence that we recorded was similar to that of conventional poultry farming.

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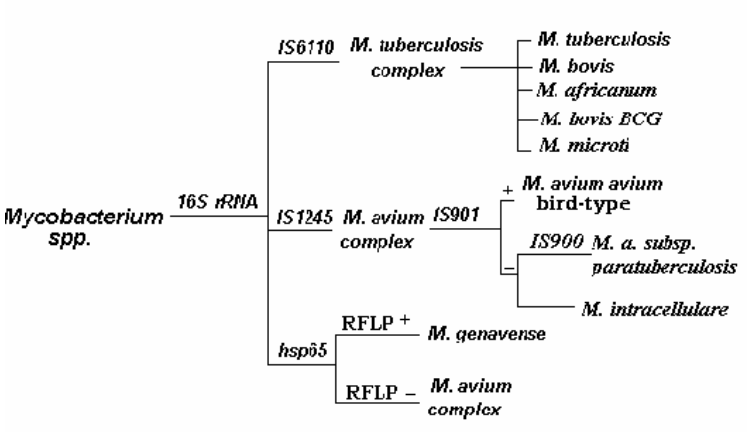

Figure 1: PCR-based diagnostic algorithm for the identification of major pathogenic mycobacteria

Mycobacterium spp.	***Target***	***Primer sequence (5'→3')***	***Amplicon size (bp)***	***Reference***	***Nucleotide region***
Mycobacterium spp.	*16S rRNA*	*F:-ACGGTGGGTACTAGGTGTGGGTTTC- R:- TCTGCGATTACTAGCGACTCCGACTTCA-*	*543*	*3*	*752 -1295*
M. tuberculosis complex	*IS6110*	*F:- CGTGAGGGCATCGAGGTGGC - R:-GCGTAGGCGTCGGTGACAAA-*	*245*	*3*	*3158-3402*
M. avium complex	*IS1245*	*F:- AGGTGGCGTCGAGGAAGAC- R:-GCCGCCGAAACGATCTAC-*	*427*	*2*	*16732-17159*
M. avium avium	*IS901 (nested)*	*NP1:-TTAACACGATGAGTCATGCG- NP2:-GCTTATCGATGTCCTTGATC-*	*509*	*7*	*489-998*
		NP3:-GTACCCGGCGAAGACCTGG- NP4:-AAGTCCAGCAGCCGTGCTG-	*376*		*593-969*
M. genavense	*hsp65*	*MKMTB 13A : - AGGCGATGGACAAGGT- MPTB II : - CCTCGATGCGGTGCTTGC-*	*693*	*6*	*609-1310*

Table 1. Characteristics of the PCR assays that were incorporated in the study.