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U.S. Poultry & Egg Association
Harold E. Ford Foundation
1530 Cooledge Road
Tucker, GA 30084-7303

(770) 493-9401
(770) 493-9257 (Fax)
research@poultryegg.org

Contact the researcher or USPOULTRY for more information.

March 2009

Project #F018

The Development of Advanced Diagnostic real-time TaqMan PCRs for the Four Pathogenic Avian Mycoplasmas

Ziv Raviv and Stanley H. Kleven
Department of Population Health
Poultry Diagnostic and Research Center
The University of Georgia
Athens, GA 30602-4875

"Development of Highly Specific Diagnostic Tests for Mycoplasma"

Mycoplasmas are very small prokaryotic organisms lacking a cell wall and bounded by a plasma membrane. Four avian mycoplasmas are commonly recognized as poultry pathogens: *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), *Mycoplasma meleagridis* (MM) and *Mycoplasma iowae* (MI). The four pathogenic avian mycoplasmas are important poultry pathogens that have significant implications on production and trading of poultry products.

There are three main approaches to the diagnosis of avian mycoplasmosis: isolation and identification, detection of antibodies, and molecular detection of the organism's nucleic acid by polymerase chain reaction (PCR). Culture is the "gold standard" for direct detection of the organism, but pathogenic avian mycoplasmas are slow-growing, fastidious organisms, and their isolation might be impaired by overgrowth of saprophytic, non-pathogenic mycoplasmas and contaminant bacteria and fungi. Serologic tests have long been the basis for avian mycoplasma surveillance, but are of lesser value for the detection of early infections. PCR has gained a pivotal role in the diagnosis of avian mycoplasma infection. The main advantages of PCR in avian mycoplasma diagnostics are the high specificity and sensitivity of the method along with its rapid turnover, ease and relatively inexpensive application, and the detection of the organism without the necessity to culture it. Several PCR assays for the 4 pathogenic avian mycoplasmas have been proposed since the early 90s. The earlier PCR assays targeted the 16S rRNA gene, a highly conserved genomic region among bacteria that might cross react with other mollicutes and prokaryotes; an example for such interspecies cross reaction with avian mycoplasmas was observed between MG and *Mycoplasma imitans*. Recent PCR assays successfully targeted more species-specific genes, but these targets appear to contain high levels of intraspecific genetic polymorphism that might result in reduced sensitivity of these assays to some of the members of a given mycoplasma species.

In recent years, real-time PCR technology has revolutionized the way laboratories diagnose infectious diseases. The combination of excellent sensitivity and specificity, ease of performance, speed, high throughput capabilities, inherent quantitative nature, and cost efficiency has made real-time PCR technology an appealing alternative to conventional PCR. So far, only a limited number of diagnostic real-time PCR assays were proposed for avian mycoplasma diagnosis. Our objectives were to develop a complete set of diagnostic real-time PCR assays for the four pathogenic avian mycoplasmas and to propose the utilization of real-time and high throughput technologies in avian mycoplasma diagnostics. We also aimed to optimize the species specificity and the intraspecific monomorphism of the genomic regions that were targeted by our assays.

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Four real-time TaqMan PCR assays for the detection of the four pathogenic avian mycoplasmas were developed. The selected genomic targets of the developed assays were species-specific and intraspecifically conserved and included the 16S-23S intergenic spacer region of MS and MM, the upstream region to the 16S rDNA of MI, and highly conserved foci of the *mgc2* gene of MG. The four assays were demonstrated highly specific and sensitive to their target avian mycoplasma, with detection limits of one copy per reaction mix for the MG assay and ten copies per reaction mix for the MS, MM and MI assays. The procedures are very rapid, have high throughput capability, and are more sensitive than conventional PCR.

We propose the addition of the real-time TaqMan PCR assays in the routine diagnosis of avian mycoplasmosis because the inclusion of these assays in avian mycoplasma diagnostics will contribute to the accuracy, efficiency, and feasibility of the diagnosis of these pathogens.

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