Nested-PCR Assays for the Detection of Avian Pathogenic Mycoplasmas in Poultry*

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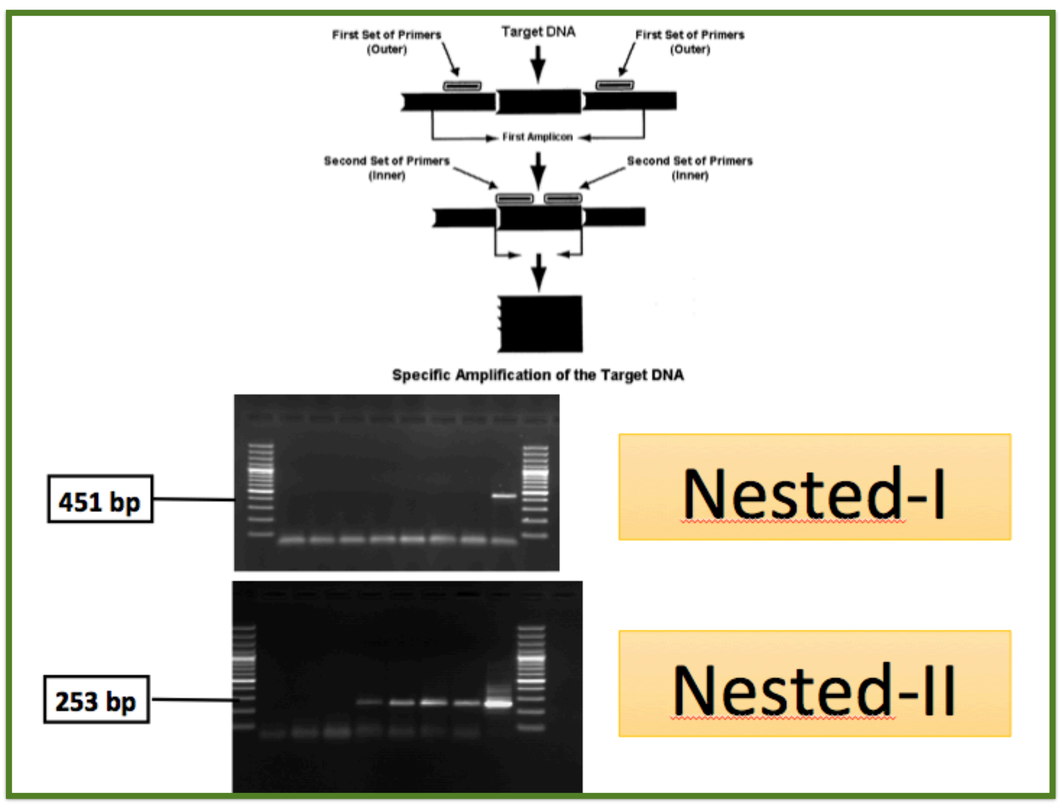
INTRODUCTION

- Avian pathogenic mycoplasmas are known to cause economic losses in commercial poultry production.
- *Mycoplasma gallisepticum* causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys.
- *M. synoviae* most frequently occurs as subclinical upper respiratory infection and synovitis in chickens and turkeys.
- *M. iowae* causes a decrease in hatchability and high embryo mortality in turkeys.
- *M. meleagridis* causes primary lesions in airsacs in the progeny, which leads to lower hatchability and skeletal abnormalities in young turkeys.

MATERIALS AND METHODS

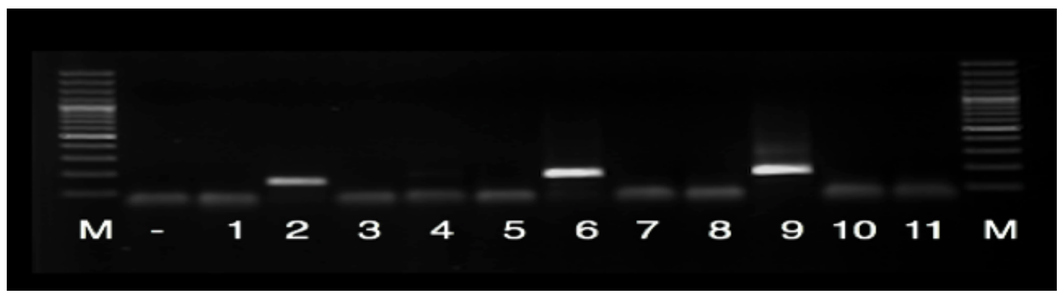
Nested-PCR Primers						
Primer	Oligonucleotide sequence	Length	Ta	Product	Agent	Target
MgcF1	CAGGTGCTGGGTTGATTGTTG	21	60	495 bp	MG	*mgc2*
MgcR1	GGAAGTTTGGTCTTGGTGGC	20				
MgcF2	CAAAATAAAACAGAAGCGATTGAG	24	59	183 bp	MG	*mgc2*
MgcR2	AATTCTTTGTTGGGGATTAGGA	22				
MsHsapF1	TGTTGGTGCAATGCCTGGAAAA	22	61	634 bp	MS	*Hsap*
MsHsapR1	TCCACCGTGTGTTGAATATGCT	22				
MsHsapF3	AATTGTAAAAGGTGTTGCTAAAG	23	54	407 bp	MS	*Hsap*
MsHsapR3	CAGCTTCTCTAGTGTAACGATTA	23				
Ms23SF1	AGAGTGTGTAGTGGATGCCTTG	22	57	290 bp	MS	*23SrRNA*
Ms23SR1	GGCTGTCCTACAACCCCAATAA	22				
Ms23SF2	GTAGTGGATGCCTTGGGTCTTA	22	60	250 bp	MS	*23SrRNA*
Ms23SR2	CTCTTCCAGTTTCGCTCGCC	20				
P34-75F1	ACAAGCCCCTGATGTAGCTAGA	22	60	300 bp	MM	*P34-75 geni*
P34-75R1	GAATTTGAAGAGTCGATTGTTTCACT	26				
P34-75F2	ATAGAGTAAGTGAAGTTCAAGT	22	46	150 bp	MM	*P34-75 geni*
P34-75R2	TTTTGTCATTTGCTGCTTTA	20				
MiSpi1F1	TGTAAATCGGGTTTCACTTGGAGT	24	63	451 bp	MI	*Spi1*
MiSpi1R1	TGCACCAATTCCAATAGCTTTTCAA	25				
MiSpi1F2	CTAAACAGAAAGCACACAAAC	21	52	253 bp	MI	*Spi1*
MiSpi1R2	ATTGGTCCTCTTCTGTTTCTAT	22				



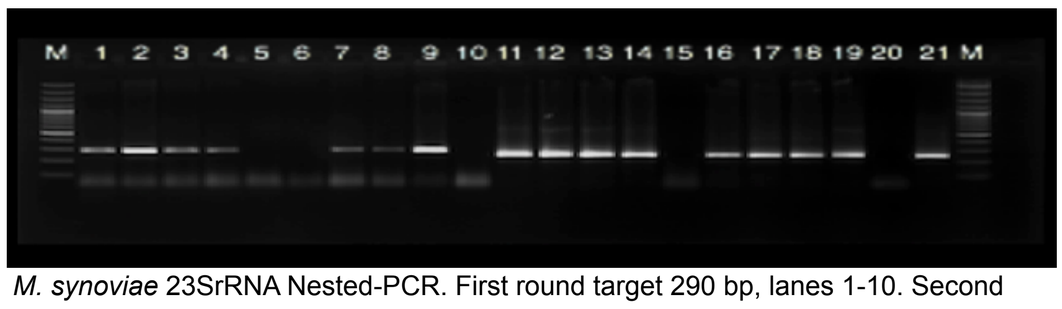
M. iowae spi1 Nested-PCR. First round target 451 bp. Second round target 253 bp. Positive detections from direct tissue samples (lungs and tracheas) after second Nested-II PCR. Last lane positive control.

RESULTS

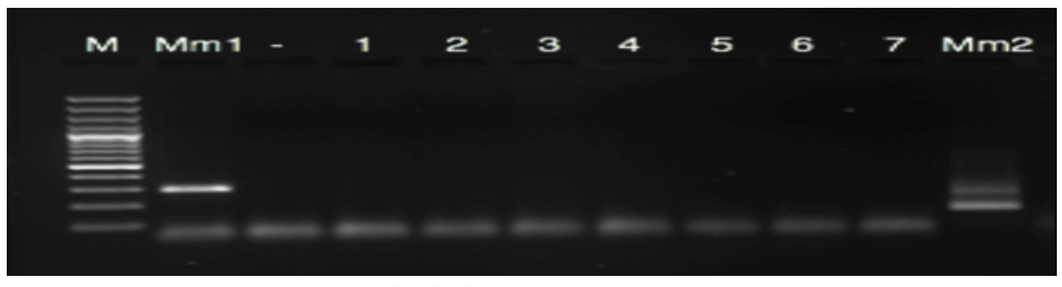
- Novel two-step nested-polymerase chain reaction (nested-PCR) assays were developed and optimized to detect four pathogenic species of avian mycoplasmas.
- Following in-silico analyses with relevant sequences of the pathogens in nucleotide databases, target genes were determined for primer design.
- Nested primers were designed targeting *Mgc2* gene encoding cytadhesin protein of *M. gallisepticum*, *Hsap* gene encoding heat shock ATP dependent protease gene of *M. synoviae*, P34-75 gene of *M. meleagridis*, and *spi1* gene encoding species protein 1 of *M. iowae*.



M. gallisepticum mgc2 Nested-PCR. First round target 495 bp. Second round target 183 bp.



M. synoviae 23SrRNA Nested-PCR. First round target 290 bp, lanes 1-10. Second round target 250 bp, lanes 11-20; 21 positive control.



M. meleagridis P34-75 Nested-PCR. First round target 300 bp. Second round target 150 bp. Mm1: *M. meleagridis* positive control PCR1. Mm2: *M. meleagridis* positive control PCR2. -: Negative control. 1,2: *M. gallisepticum*. 3,4: *M. synoviae*. 5: *M. imitans*. 6,7: *M. iowae*.

DISCUSSION&CONCLUSION

- These assays are thought to provide fast, reliable, specific and sensitive alternatives to other diagnostic methods regarding the confirmation of *Mycoplasma* isolates and direct detection of the pathogens in the clinical and necropsy samples, as well.
- We believe after the completion of thorough validation studies, these assays could be used for the identification and control of avian pathogenic mycoplasma infections.