Mycoplasma iowae Isolation from Broiler Breeder Chickens in Turkey*

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Development of PCR-Based Tests for the Detection of Bacterial Respiratory System Pathogens in Chickens and Turkeys

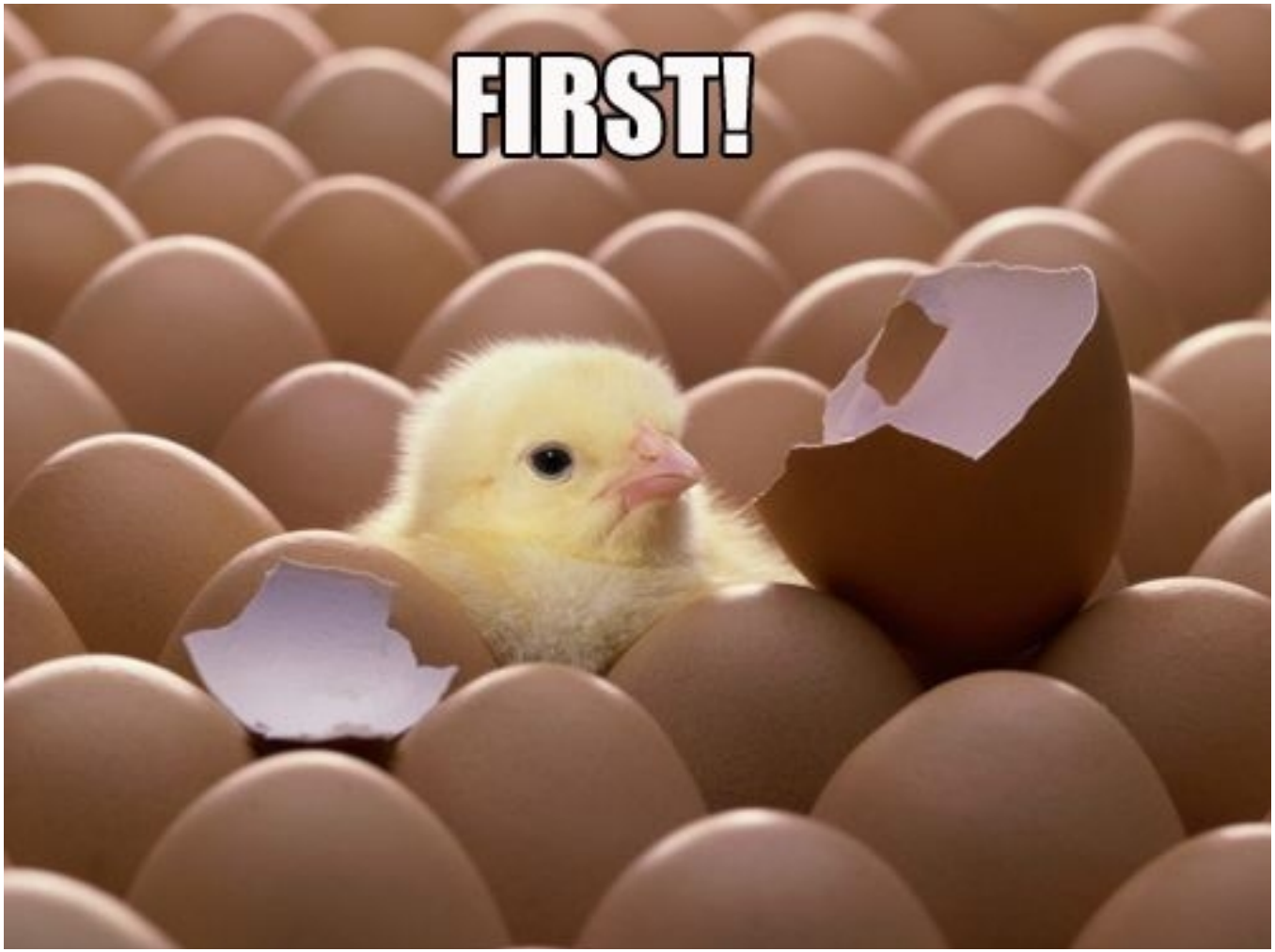
- *Mycoplasma gallisepticum*,
- *M. synoviae*,
- *M. meleagridis*,
- *M. iowae* (turkeys),
- *Pasteurella multocida*,
- *Ornithobacterium rhinotracheale*,
- *Avibacterium paragallinarum*, *Avibacterium gallinarum*,
- *Bordetella avium*,
- *Riemerella anatipestifer*

The Scientific and Technological Research Council of Turkey (TUBITAK-TOVAG) Project No. 112O334.

Presentation Outline

- Introduction
- Materials and Methods
- Results
- Discussion
- Comments and Conclusion

INTRODUCTION



Mycoplasma iowae

- Reduced hatchability (2-5%) and embryo mortality in turkeys
- Experimentally induce
  - mortality in turkey and chicken embryos
  - mild to moderate airsacculitis and leg abnormalities in chickens and turkeys
- 6 serotypes (I, J, K, N, Q, R)
- Family *Mycoplasmataceae*, class Mollicutes
- Characteristic colony morphology (fried-egg appearance), no cell wall, growth requirement for sterol
- Worldwide in distribution

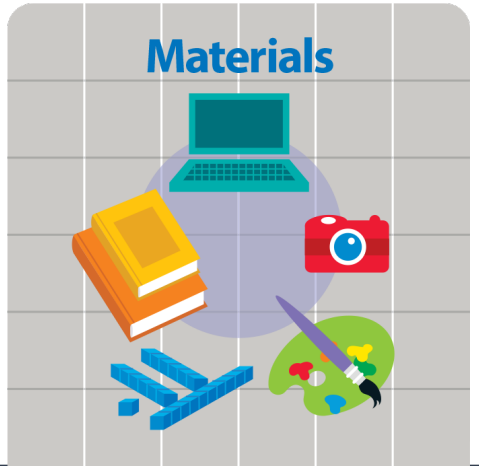
Mycoplasma iowae

- Predilection for the gastrointestinal tract
- Less is known about the natural infection in chickens
- Virulence factors have not been studied for *M. iowae*.
- Very little is known about the pathogenesis of *M. iowae*
- Humoral response in turkeys and chickens is poor, there is no reliable serologic test available for use in the field
- Diagnosis is currently by culture and PCR detection methods

MATERIALS AND METHODS

Materials

- More than 2600 field materials (tracheal swabs, palatinal cleft swabs, trachea, lungs, infraorbital sinus contents, etc.) from 121 flocks
- Broilers, layer and broiler breeder chickens, turkeys
- Showing respiratory system symptoms
- ATCC strains of different *Mycoplasma* species and strains as controls
- *M. iowae* specific primers (previous studies/recently designed)



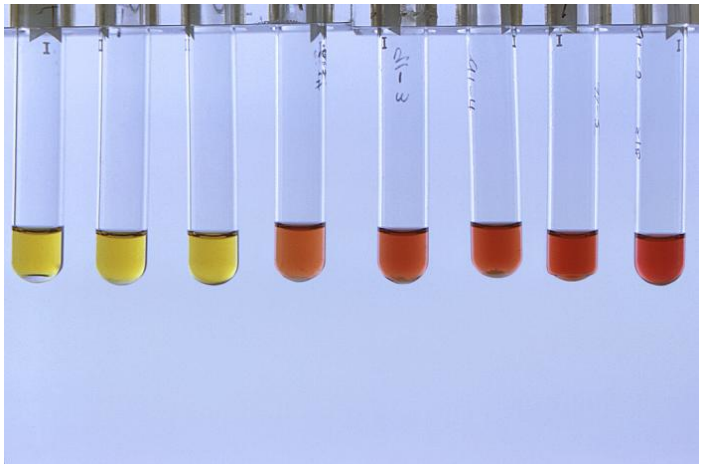
- *Mycoplasma* isolation (regarding all four pathogenic species)
  - Frey's medium
- Bacteriological investigation+PCR (other respiratory pathogens)
- PCR confirmation (with primers from published literature and primers designed in the project)
  - *M. iowae specific* 16S rRNA gene
  - *M. iowae* species protein 1 (*Spi1*) gene (This study)
  - *M. iowae* ribonuclease P RNA *(rnpB)* gene (This study)
- 16SrRNA gene sequencing for bacterial identification
- Nested-PCR
  - *M. iowae* species protein 1 (*Spi1*) gene (This study)

Flock History

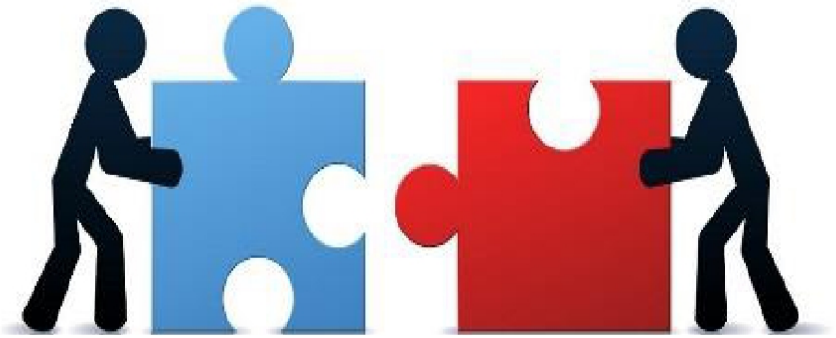
- Broiler breeder flocks
- Respiratory symptoms
- One flock was 6 weeks old
- Other flock was 31 weeks old


RESULTS

- *M. iowae* was isolated from two broiler breeder chicken flocks
- MI was isolated from lungs but no other samples



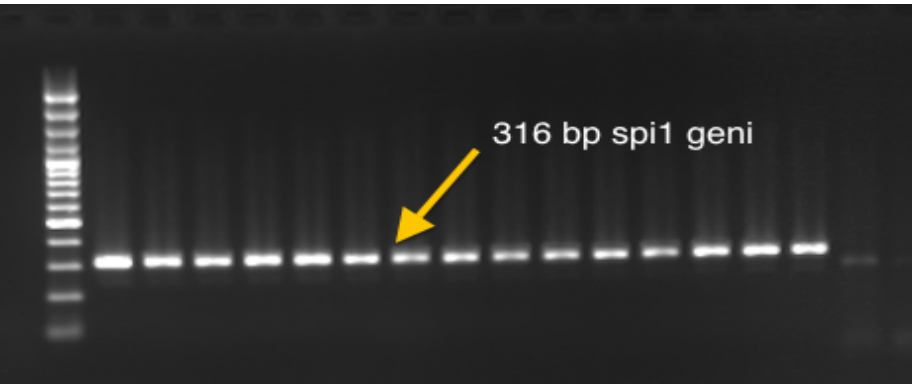
- Isolates were confirmed by three different PCRs
- Isolates were also confirmed by 16SrRNA identification with DNA sequencing
- *M. iowae* DNA could not be detected in the direct PCR investigation of the infected tissues
- Nested-PCR were able to detect *M. iowae* DNA in the infected tissues



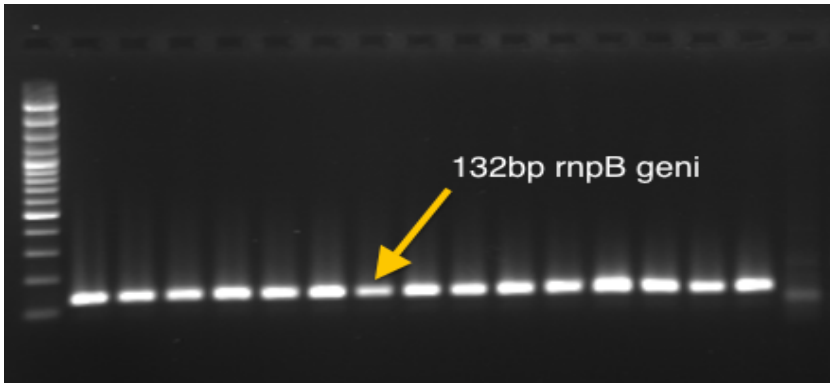
***Mycoplasma iowae* PCR I**
MI-F: ATGAGTCCATTATTTATGCTTCC
MI-R: TCCATTTCTTTTGAACGTGCATT

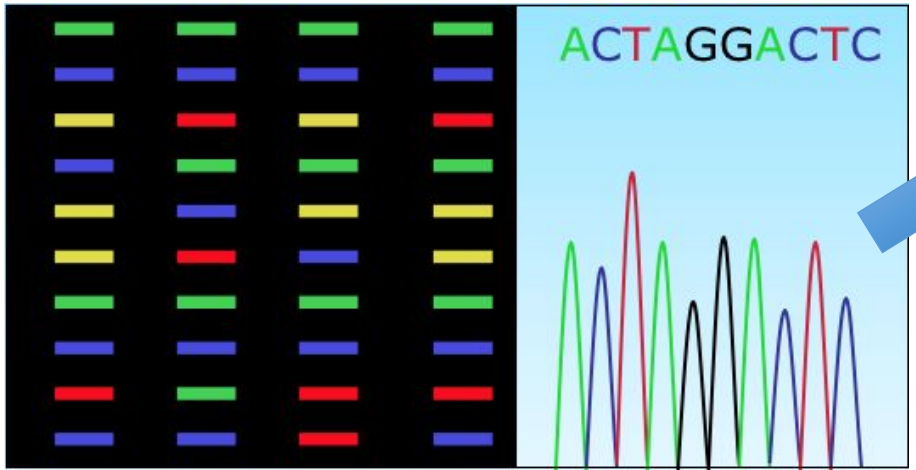
126 bp specific Raviv and Kleven, 2009

***M. iowae* PCR II**



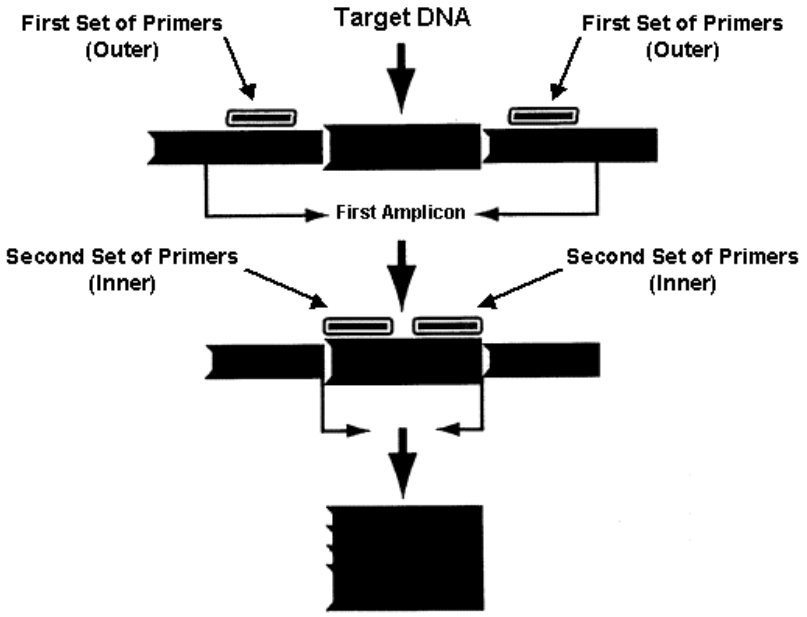
***M. iowae* PCR III**



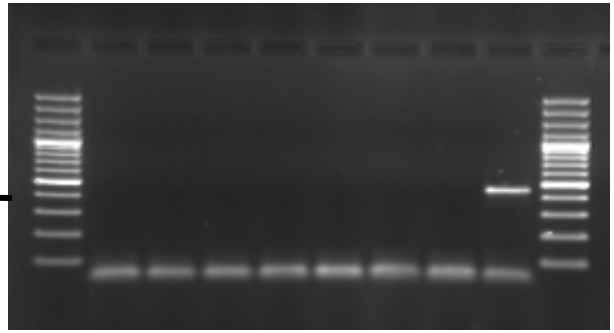


BLAST

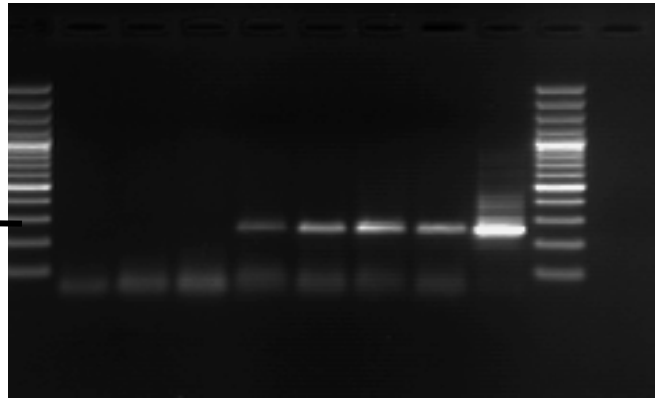
M. iowae



Specific Amplification of the Target DNA



Nested-I



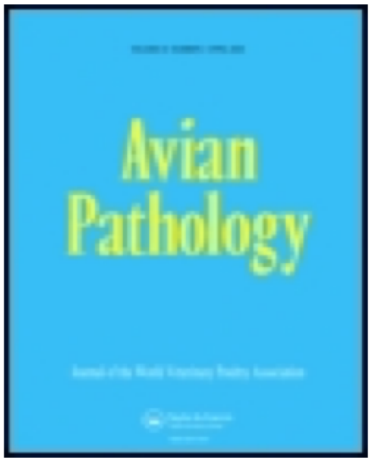
Nested-II

DISCUSSION

QUESTIONS RAISED AFTER THE STUDY

- Can we rely on PCR-based assays alone especially for the direct investigation of tissue samples?
- Do mycoplasmas have certain and specific clinical outcomes due to animal species basis?---Host specificity?!!!
- *M. iowae* ----- lungs, chicken, breeders!!!

- Can we rely on PCR-based assays alone especially for the direct investigation of tissue samples?
  - Isolation is the gold standard method
  - There are PCR inhibitors in tissue samples. Target DNA/inhibitor ratio could effect the result of PCR assays
  - A well designed nested-PCR could be a good strategy especially working with tissues
- Host specificity?!!!
  - Vignault et al., (1982) isolated the agent from an apple seed



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Isolation of Mycoplasma bovis from broiler chickens in Turkey

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- *M. iowae* ----- lungs, chicken, breeders!!!
  - Artificial inoculation of I day-old broiler breeder chickens resulted in stunting and poor feathering in addition to leg lesions
  - Few clinical reports, however, describe airsacculitis or leg problems in chickens or turkeys, or embryo mortality in chickens under field circumstances

- What is left to be done?
  - Investigate the relationship of the two isolates?
  - Investigate comprehensively whether *M. iowae* has a role in the clinical case?
  - Reconsider the efficacy of the molecular identification methods---even the validated ones!...
  - Retest all the samples for the existence of *M. iowae*

We will be after *M. iowae*!!!

Thank you
for your patience and attention!

Questions, Contributions

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