A survey of pathogenic avian mycoplasma involvement in multicausal respiratory disease in broiler flocks

Rahmani, E.[1], Hosseini, H.[2*]

[1]*Graduated from the Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*

[2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*

Key words:
broiler, molecular detection, multicausal respiratory disease, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*

Correspondence
Hosseini, H.
Department of Clinical Sciences, Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran
Tel: +98(21) 66434643
Fax: +98(21) 66121546
Email: hosseini.ho@gmail.com

Received: 10 January 2017
Accepted: 10 April 2017

Abstract:

BACKGROUND: *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the most important and pathogenic mycoplasma in chicken production. The tendency of avian mycoplasma for interaction with other pathogens is well-known. Interaction within several disease-producing factors in respiratory tract exacerbate the disease and is known as multicausal respiratory disease. **OBJECTIVES:** In recent years, high prevalence of multicausal respiratory disease in broiler flocks cause economic loss in Iran. The aim of the current study was to find the role of avian mycoplasma in recent outbreaks of respiratory diseases in broiler flocks. **METHODS:** Four hundred fifty tracheal or choanal cleft swabs were collected from 30 broiler farms with sever respiratory disease. The samples subjected to polymerase chain reaction (PCR) using specific primers for MG and MS. **RESULTS:** One flock (3.3%) and three flocks (10%) of broiler were found to be positive for MG and MS, respectively. **CONCLUSIONS:** The results show that mycoplasma (MG and MS) are not the major part of recent respiratory diseases and anti-mycoplasma drugs administration needs precise test to evaluated mycoplasma statues.

Introduction

Avian mycoplasma consist of more than 23 Mycoplasma species. *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the major avian pathogenic mycoplasmas and economically important pathogen in chicken flocks. *M. gallisepticum* known as chronic respiratory disease (CRD) and *M. synoviae* may cause respiratory disease or synovitis (Ferguson-Noel & Noormohammadi, 2013; Raviv & Ley, 2013). The tendency of avian mycoplasma for interaction with other pathogens is well-known. Interactions within several disease-producing factors in respiratory tract exacerbate the disease and are known as multicausal respiratory disease. Multicausal respiratory disease is a condition that multiple etiologies include a combination of infectious agents plus environmental factors may be involved. In this situation, harmless microbes which cause no disease in healthy bird imperil the bird's life (Glisson, 2013).

Recently, the high prevalence of multicausal respiratory disease in broiler flocks causes huge economic losses in Iran. While viral pathogens include Newcastle disease

virus (NDV), infectious bronchitis virus (IBV) and avian influenza virus (AIV) are considered as the main cause of the mortality in broiler, it is believed that different agents may be involved in exacerbation of disease. The role of mycoplasma in recent outbreaks of respiratory disease in Iranian broiler flocks is ambiguous. Considering avian mycoplasma as the main part multicausal respiratory disease, it is important to find the possible role of MG and MS in the respiratory disease. The finding can be helpful in designing logical control, prevention and treatment.

Materials and Methods

Mycoplasma strains: Reference MG strains included S6 strain (University of Liverpool), MG SS strain (GD Animal Health Service Ltd., The Netherlands) and commercial vaccine ts-11 strain (Bioproperties, Australia) and MS-H (Bioproperties, Australia) were used as positive control.

Specimen: Samples were taken from thirty broiler flocks located in Qazvin province during the period from summer to autumn of 2015. Live or dead birds from these broiler flocks were submitted to a poultry clinic in Qazvin with acute respiratory signs and exponential increase in mortality rate. Before postmortem examination fifteen swabs from upper respiratory tract (tracheal or choanal cleft swabs) were carefully taken from each flocks with sterile swabs. Postmortem findings predominantly consist of conjunctivitis with watering eye, nasal discharge, congestion, catarrhal or caseous exudate in trachea, airsacculitis and in some advanced cases fibrinous pericarditis, perihepatitis and peritonitis. Samples which were collected from multicausal respiratory disease-suspected flocks make up a total 450 swabs. Then, the tracheal or choanal cleft swabs submitted to PCR Veterinary Diagnostic Laboratory (Tehran, Iran).

DNA extraction: DNA was extracted from pooled swabs samples (three swabs) using CinnaPure-DNA (CinaClon, Iran) according to the manufacturer's procedure. Extracted DNA was stored at 4 °C for immediate or at -20 for later use.

Molecular detection: All samples were subjected to PCR using specific primers for MG and MS as described previously (Hosseini et al., 2006; Kleven & Bradbury 2008). Two pairs of oligonucleotide primers, (MG-14F: 5' GAG CTA ATC TGT AAA GTT GGT C 3' and MG-13R: 5' GCT TCC TTG CGG TTA GCA AC-3') and (MS-F: 5'-GAG AAG CAA AAT AGT GAT ATC A 3' and MS-R: 5' CAG TCG TCT CCG AAG TTA ACA A 3') were used for detection of MG and MS, respectively. The amplification was carried out in a total 20 µl reaction volume consisting of 2 µl 10 x PCR buffer, 1 µl of mixed primer (25 µM), 0.2 µl 10 mM dNTP, 1 µl 50 mM MgCl2, 0.25 µl Taq DNA polymerase (5U/µl) and 2 µl of template DNA. All amplification reactions were performed in a T100 Thermal Cycler (Bio-Rad, United States) as follows: 94 °C for 3 min, followed by 40 cycles of 94 °C for 10 sec, 55 °C for 10 sec, 72 °C for 10 sec, and a final extension at 72 °C for 5 min. The amplification products were electrophoresed on an agarose gel (2%) in TBE buffer. Gels were run for 1 hr. at 90 Volte, stained with ethidium bromide and visualized under UV light.

Results

A total of 30 broiler flocks with acute re-

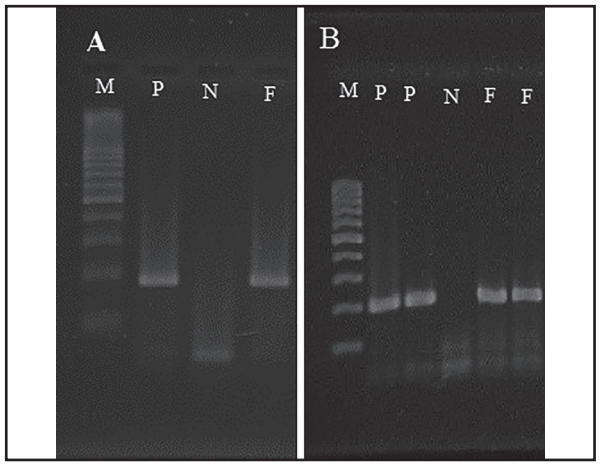

Figure 1. A: Amplification of 16S rRNA of *M. gallisepticum* B: Amplification of 16S rRNA of *M. synoviae*. M: GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific), P: positive N: negative F: field sample.

spiratory disease were investigated using specific PCR for MG and MS. In each flock 15 birds were swabbed. Swabs of each flock were pooled (three swabs) for examination. As a result, five pooled samples were tested for every affected flock.

The specific primers amplified a 185 bp and 207 bp of standard strains for MG and MS respectively (Fig. 1). Flock was considered positive if at least one sample gave positive result. Of all the flocks tested one flock (3/3%) only was positive in MG PCR and three flocks (10%) were positive in MS PCR. The majority of broiler flocks (26; 86.7%) were negative in MG and MS test.

Discussion

Poultry husbandry has changed since the 1950s. Keeping large numbers of birds in a small area with high density provides close contact between birds, in which infectious agent is easily transmitted. The respiratory tract of chicken harbors a variation of viruses and bacteria. In intensive poultry production infection with a single agent is an exception (Bradbury, 1984). In fact, in commercial condition multiple etiologies that are involved in respiratory disease are known as multicausal respiratory disease. The outcome of multicausal disease depends on many factors including virulence of pathogens, age. Numerous studies showed that MG and MS are the best example of agent involved in multicausal respiratory disease (Glisson, 2013). Traditionally mycoplasma infection is considered as an important part of multicausal respiratory disease. Considering vertical transmission and the economic importance of mycoplasma, a great deal of effort has been targeted at producing mycoplasma free parent flocks. It seems reasonable that mycoplasma status of parent stocks reflects directly in their progenies.

A great upsurge in respiratory diseases along with high rate of morbidity and mortality in recent years in Iranian broiler flocks suggest more than an etiological contagious agent may be involved. Conventionally, MG and MS are considered as the main exacerbating factors in this situation and usually anti-mycoplasma medication is administered. While there is no pathognomonic gross lesion for mycoplasmosis in postmortem examination, airsacculitis is recognized as CRD in a routine investigation. However, there are few reports in terms of mycoplasma status in broiler flocks and in a study from 2004 to 2007 more than 30% of boiler breeder flocks were shown to be infected with MS (Bayatzadeh et al, 2011). In addition, an earlier survey revealed the high prevalence of MS in boiler flock and about 60% of flocks were found to be positive (Pourbakhsh et al, 2010).

However, status of mycoplasma in boiler breeder flocks has changed significantly in recent years. This may lead to change in mycoplasma status in broiler flocks. MG

and MS transmit vertically and horizontally among poultry. As the mycoplasmas are susceptible outside of their host, transmission from hen to progeny through egg, i.e. vertical transmission is a very important route of spreading infection. So it is very important to acquire chicks from MG-free and MS-free parent stocks (Ferguson-Noel & Noormohammadi, 2013; Raviv & Ley, 2013; Kleven & Bradbury 2008). Strict biosecurity and sanitation along with depopulation of MG-positive flocks and repopulation with MG-free resulted in significant decrease in the MG-positive breeder broiler in Iran. While this approache was very effective for MG prevention and control, *M. synoviae* continued to circulate in broiler breeder. Vaccination of broiler breeder with MS-H was an alternative approach which was chosen in Iran from 2005. It is believed that vaccination can prevent the vertical transmission.

According to the results of this survey, the majority of broiler flocks were MG and MS-free and small number flocks were found to be positive. Low rate of mycoplasma infection in broiler can attribute to effectiveness of mycoplasma control applied in broiler breeder in recent years.

Anti-mycoplasma drugs including tylosin, tiamulin and tilmicosin are usually administered as part of the prevention or treatment program in broiler flocks. Medication is a short-term control method and has been of value in treating and controlling individual infected flocks (Ferguson-Noel & Noormohammadi, 2013; Raviv & Ley, 2013). However, the therapeutic and economic benefit of these expensive drugs in MG and MS-free flocks is a questionable decision and practice. It is not advisable to use anti-mycoplasma treatment based on clinical signs and postmortem finding.

In conclusion, the finding of this survey reveals that the percentage of MG and MS infected flocks is less than expected and mycoplasma are not a dominate part of multicausal respiratory diseases like they used to be. As a result, any treatment of mycoplasma in broiler flocks should be based on laboratory diagnosis and confirmation.

Acknowledgments

The authors would like to thank the PCR Veterinary Diagnostic Laboratory experts for their technical support.

References

Bayatzadeh, M.A., Pourbakhsh, S.A., Homayounimehr, A.R., Ashtari, A. Abtin, A.R. (2011) Application of culture and polymerase chain reaction (PCR) methods for isolation and identification of *Mycoplasma synoviae* on broiler chicken farms. Arch Razi Inst. 66: 87-94.

Bradbury J. M. (1984) Avian mycoplasma infections: prototype of mixed infections with mycoplasmas, bacteria and viruses. Annales de l'Institut Pasteur / Microbiologie. 135: 83-89.

Ferguson-Noel, N., Noormohammadi, A. H. (2013) *Mycoplasma synoviae* Infection. In: Diseases of Poultry. David, E., Swayne, J. R., Glisson, L. R., McDougald, L. K., Nolan, D. L., Suarez, V. L. Nair (eds.). (13th ed.) Wiley-Blackwell Publishing, Ames, Iowa, USA. p. 900-906.

Glisson, J.R. (2103) Multicausal Respiratory Diseases In: Diseases of Poultry. David, E., Swayne, J. R., Glisson, L. R., McDougald, L. K., Nolan, D. L., Suarez, V. L. Nair (eds.). (13th ed.) Wiley-Blackwell Publishing, Ames,

Iowa, USA. p. 1320-1322.

Hosseini, H., Bozorgmehrifard, M.H., Peighambari, S.M., Pourbakhsh, S.A., Razzazian, M. (2006) Random amplified polymorphic DNA (RAPD) fingerprinting of *Mycoplasma gallisepticum* isolates from chickens. Arch Razi Inst. 61: 67-71.

Pourbakhsh, S.A., Shokri, G.R., Banani, M., Elhamnia, F., Ashtari, A. (2010) Detection of *Mycoplasma synoviae* infection in broiler breeder farms of Tehran province using PCR and culture methods. Arch Razi Inst. 65: 75-81.

Kleven, S.H., Bradbury, J.M. (2008) Avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. synoviae*). In: OIE Terrestrial Manual chapter 2. 3. 5: 482-496.

Raviv, Z., Ley, D. H. (2013) *Mycoplasma gallisepticum* Infection. In: Diseases of Poultry. David, E., Swayne, J. R., Glisson, L. R., McDougald, L. K., Nolan, D. L., Suarez, V. L. Nair (eds.). (13th ed.) Wiley-Blackwell Publishing, Ames, Iowa, USA. p. 877-893.

مجله طب دامی ایران، ۱۳۹۶، دوره ۱۱، شماره ۳، ۲۵۳-۲۴۹

ارزیابی نقش مایکوپلاسماهای بیماریزای پرندگان در بیماری تنفسی چندعاملی گله‌های گوشتی

احسان رحمانی[۱] حسین حسینی[۲*]

۱) دانش آموخته دانشکده دامپزشکی، واحد کرج، دانشگاه آزاد اسلامی، کرج، ایران
۲) گروه علوم درمانگاهی، دانشکده دامپزشکی، واحد کرج، دانشگاه آزاد اسلامی، کرج، ایران

(دریافت مقاله: ۲۰ دی ماه ۱۳۹۵، پذیرش نهایی: ۲۱ فروردین ماه ۱۳۹۶)

چکیده

زمینه مطالعه: مایکوپلاسما گالی سپتیکوم و مایکوپلاسما سینوویه مهمترین و بیماریزاترین مایکوپلاسماها در پرورش ماکیان می‌باشند. تأثیر متقابل بین مایکوپلاسماهای پرندگان و سایر عوامل بیماریزا بخوبی شناخته شده است. این تأثیر متقابل با عوامل بیماریزا در دستگاه تنفسی سبب تشدید بیماری تنفسی می‌شود و بعنوان بیماری تنفسی چند عاملی شناخته می شود. **هدف:** در سال‌های اخیر شیوع بالای بیماری‌های تنفسی چند عاملی در گله‌های گوشتی سبب خسارات اقتصادی زیادی شده است. هدف از این مطالعه شناسایی نقش مایکوپلاسماهای پرندگان در شیوع‌های اخیر بود. **روش کار:** چهارصد و چهل سوآپ نای و شکاف کامی از گله‌های گوشتی مبتلا به بیماری حاد تنفسی جمع آوری گردید. با استفاده از پرایمرهای اختصاصی حضور مایکوپلاسما گالی سپتیکوم و مایکوپلاسما سینوویه مورد بررسی قرار گرفت. **نتایج:** از بین سی گله تنها یک گله (۳/۳٪) از نظر مایکوپلاسما گالی سپتیکوم و سه گله (۱۰٪) از نظر مایکوپلاسما سینوویه مثبت بودند. **نتیجه گیری نهایی:** نتایج نشان می‌دهد که مایکوپلاسماهای بیماریزا، نقش مهمی در شیوع‌های اخیر بیماری تنفسی ندارند و تجویز داروهای ضد مایکوپلاسمایی نیازمند ارزیابی دقیقی از وضعیت مایکوپلاسمای گله است.

واژه های کلیدی: گله گوشتی، شناسایی مولکولی، بیماری تنفسی چند عاملی، مایکوپلاسما گالی سپتیکوم، مایکوپلاسما سینوویه

---

*) نویسنده مسؤول: تلفن: ۶۶۴۳۴۶۴۳ (۲۱)۹۸+ نمابر: ۶۶۱۲۱۵۴۶ (۲۱)۹۸+ Email: hosseini.ho@gmail.com