



Review

Avian *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: Advances in diagnosis and control

Abdelrahman A. Abdelrahman¹, Salama A. S. Shany¹, Mansy A. A. Dardeer², Kareem E. Hassan¹, Ahmed Ali¹ and Magdy F. El-Kady^{1*}

¹ Poultry Diseases Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 65211, Egypt

² Mycoplasma Research Department, Animal Health Research Institute, Agricultural Research Center, P.O. Box, 264, Dokki, Giza 12618, Egypt



Article History:

Received: 06-Jun-2021

Accepted: 02-Jul-2021

*Corresponding author:

Magdy F. El-Kady

E-mail: mfelkady@yahoo.com

Abstract

Both *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) infections are the most common mycoplasma infection in domestic poultry. The disease is associated with economic losses in poultry. MG and MS are commonly spread within chicken (*Gallus gallus domesticus*) and turkey (*Meleagris gallopavo domesticus*) flocks; however, they are frequently isolated from quails (*Coturnix coturnix*) and several avian species. Diagnosis of MG or MS infections is confirmed by isolating the organism in a cell-free medium or directly detecting its DNA in infected tissues or swab samples. Serological tests are also widely used for diagnosis. However, advances in molecular biology represented a rapid and sensitive alternative to the traditional culture methods requiring specialized techniques and sophisticated reagents. Several mycoplasma molecular diagnostic tests are implemented, including polymerase chain reaction (PCR), Random Amplified Polymorphic DNA (RAPD), arbitrary primed polymerase chain reactions (AP-PCR), and Multiplex real-time polymerase chain reaction. Current control practices against mycoplasma infection include intense biosecurity, medication, and vaccination. However, the egg-borne nature of avian mycoplasma infection complicates controlling the infection. This review focuses on the advances in diagnosis and control of avian mycoplasma infection, especially MG and MS infections.

Keywords: *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, Chickens, Turkeys, Diagnosis, Vaccination

Citation: Abdelrahman A. A., Shany, S. A. S., Dardeer, M. A. A., Hassan, K. E., Ali, A. and El-Kady, M. F. 2021. Avian *Mycoplasma gallisepticum* and *Mycoplasma synoviae*: Advances in diagnosis and control. Ger. J. Vet. Res. 1 (2): 46-55. <https://doi.org/10.51585/gjvr.2021.2.0019>

Introduction

Avian mycoplasma belongs to *Mycoplasma* genus, family *Mycoplasmataceae*, class *Mollicutes* based on molecular data, differentiation in morphology, nutritional requirements, and ecological habitat. Mycoplasma is the smallest microorganism capable of self-replication with a size diameter as small as 150:300 nm and can pass through 0.45 μ m pore size filters (Cordova et al., 2016). The mycoplasmas lack a cell wall, making them resistant to β -lactam antibiotics and unstainable by Gram stain; mycoplasma cells consist of plasma membrane enclosing cytoplasm containing ribosomes and circular double-stranded DNA (B'eb'ear and Pereyre, 2005).

The matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) can identify 27 species of avian mycoplasma, of which about 13 are associated with diseases in domestic poultry. *M. gallisepticum* (MG), *M. synoviae* (MS), *M. meleagridis*, *M. iowa*, *M. gallinarum*, *M. iners*, *M. gallopavonis*, *M. gallinaceum*, *M. pullorum*, *M. lipo*

faciens, *M. glycyphilum*, *M. cloacale*, and *M. imitans* have been isolated from chickens and turkey (Baudler et al., 2019). Mycoplasmas may be transmitted horizontally through infectious aerosols of infected birds and contaminated feed, water, contact, and communicant birds. Transmission occurs vertically from parents to their offspring, through contamination of laid eggs, "transovarial transmission" (Levisohn and Kleven, 2000; Nascimento et al., 2005). The egg-borne route is the most common means of spreading the disease. The egg-borne nature of avian mycoplasma infection adds complexity and difficulty in controlling the infection. Therefore, the logical approach to preventing mycoplasma spread is to break the cycle by establishing primary breeding and multiplier flocks free of the infection to eradicate the disease.

With rare exceptions, mycoplasma is usually spread within the same species (because of their host selectivity) and between closely related species. MG and MS infections occur primarily in chickens and

turkeys. However, they have been frequently isolated from quails (*Coturnix coturnix*) and several avian species (Nascimento et al., 2005). In this review, the advances in diagnosis and control of MG and MS, representing the most important avian mycoplasma infection in poultry, are discussed.

Avian mycoplasma host range

Avian mycoplasmas are frequently isolated from domestic poultry and other avian species. Their distribution seems to be worldwide. Most mycoplasma isolates belong to the genus *Mycoplasma*, although *Acholeplasma* species and *Ureaplasmas* have also been isolated. Mycoplasmas were isolated from chickens, chicken embryos, turkeys, ducks, geese, pigeons, Japanese quail, and their embryos. Surveillance of mycoplasma in 792 birds and embryos has revealed a 52% prevalence of mycoplasma. Where MS and MG were the most frequently isolated species. *M. anatis* was found only in ducks and geese; *M. columbinasale*, *M. columbinum*, and *M. columborale* only in pigeons, while *M. meleagridis* and *M. gallopavonis* isolates are confined to turkeys. *M. gallinarum*, *M. gallinaceum*, *M. pullorum*, *M. glycyphilum*, and *M. lipofaciens* were not uncommon but were mainly confined to chicken (Sawicka et al., 2020). Currently, the species names have replaced the earlier serotype designations for avian Mycoplasmas. (Ferguson-Noel et al., 2020) (Figure 1).

Avian mycoplasma virulence and pathobiology

Pathogenic mycoplasma poultry species can hemagglutinate and/or hemadsorb erythrocytes. Hemagglutination (HA) and hemadsorption (HAD) are different properties of mycoplasmas (García-Morales et al., 2014). The pathogenic avian mycoplasmas, MG, MS, *M. meleagridis*, *M. iowae*, and *M. imitans* synthesize hemagglutinins that are immunogenic, variably expressed surface proteins. The hemagglutinins of MG (pMGA), MS (VlhA), and *M. imitans* are lipoproteins encoded by related multigene families that appear to have arisen by horizontal gene transfer.

MG also has genes encoding cytoadhesins in its genome, but these are present as single copies, while the pMGA gene family contains 30 to 70 genes. The switch in the expression of distinct pMGA genes (e.g., pMGA1.1 to pMGA1.9) generates antigenic variation, which is thought to be important in immune evasion but also has significance in the preparation of MG antigens for serological diagnosis (May et al., 2014; Orlov et al., 2018). The molecular mechanisms of MG attachment and subsequent virulence have identified a specialized terminal organelle, or bleb-like structure, that serves as an attachment tip. Other potential adhesion structures include surface proteins containing highly reiterated domains. These proteins are members of large gene families, and individual members often undergo high-frequency phase variation, which is thought to promote evasion of the host immune system (Papazisi et al., 2002).

Virulence factors associated with MG include motility, cytoadhesion, phenotypic variation, nutrient

acquisition, the ability to invade host cells, and modulate the host's immune response to infection. MG moves by gliding, allowing the organism to access target tissues and break host physical barriers, such as respiratory mucus and ciliary activity (Mizutani and Miyata, 2019). The attachment of MG to host cells (cytoadhesion) is a prerequisite for successful colonization and subsequent pathogenesis. MG possesses a gene encoding a putative cysteine protease that is capable of digesting chicken IgG, suggesting a novel prolonged MG survival mechanism against active host antibody response (Cizelj et al., 2011; Chen et al., 2020).

Clinical disease in poultry

The MG incubation period varies from 6 to 21 days in experimentally infected chickens or turkeys with uniform and high dosages. Sinusitis often develops in experimentally inoculated turkeys within 6–10 days. However, the onset and extent of clinical signs following a known exposure can vary depending on MG strain virulence, complicating infections, bird age, and environmental and other stressors (Gaunson et al., 2006).

Chickens and turkeys often develop clinical infections near the onset of egg production, suggesting a subclinical infection that becomes clinical in response to stressors. Seropositivity may be the first indicator of MG infection with less virulent strains in older birds. Infectious synovitis has been seen in 6-day-old chicks, suggesting that the incubation period can be relatively short in birds infected by egg transmission. The incubation period following contact exposure is generally 1–21 days. Antibodies may be detected before the clinical disease becomes evident. In birds experimentally infected at 3–6 weeks of age, the incubation period varies from 2 to 20 days, depending on the administration route (Sawicka et al., 2020). Intra-tracheal inoculation results in infection of the trachea and sinuses as early as four days and readily spreads to contact birds. Air sac lesions appear 17–21 days after the aerosol challenge. The incubation period also varies depending on the inoculum titer and pathogenicity (Ferguson-Noel et al., 2020).

The most characteristic clinical signs of naturally occurring MG infection in adult flocks are tracheal rales, nasal discharge, coughing, reduced feed consumption, and weight loss. In laying flocks, egg production declines but is usually maintained at a lowered level. However, poultry flocks may have serologic evidence of infection with no apparent clinical signs, especially if they are recovered carriers. Male birds may have the most evident symptoms, which are often more severe during winter (Stipkovits et al., 2012). Severe outbreaks with high morbidity and mortality observed in broilers are frequently observed due to concurrent infections and environmental factors.

Cases of keratoconjunctivitis caused by MG infection in commercial layer pullets are associated with facial edema, eyelid swelling, increased lacrimation, and conjunctivitis. MG infection colonizes the respiratory tract, causing tracheitis, airsacculitis, and pneumonia (Figure 2).
















Species	Usual Host	Glucose Fermentation	Arginine Hydrolysis
<i>M. gallisepticum</i>	Chicken  Turkeys  House finch 	●	●
<i>M. synoviae</i>	Chicken  Turkeys 	●	●
<i>M. gallinarum</i>		●	●
<i>M. gallinaceum</i>		●	●
<i>M. glycophilum</i>	Chicken 	●	●
<i>M. iners</i>		●	●
<i>M. lipofaciens</i>		●	●
<i>M. pullorum</i>		●	●
<i>M. anatis</i>	Duck 	●	●
<i>M. anseris</i>	Geese 	●	●
<i>M. gallopavonis</i>		●	
<i>M. iowae</i>	Turkeys 	●	●
<i>M. meleagridis</i>		●	●
<i>M. cloacale</i>	Turkeys  Geese 	●	●
<i>M. imitans</i>	Ducks  Geese  Partridge 	●	●
<i>M. columbinasale</i>		●	●
<i>M. columbinum</i>	Pigeon 	●	●
<i>M. columborale</i>		●	●

Figure 1: Host range and biochemical criteria of some avian *Mycoplasma* species (Ferguson-Noel et al., 2020). Green and red dots denote positive and negative reactions, respectively.

Occasionally, MG infections are associated with conjunctivitis, salpingitis, arthritis, and fatal encephalopathy (Stipkovits et al., 2012).

MS infection causes infectious synovitis and sometimes the upper respiratory disease of chicken and turkey, especially when co-infected with Newcastle Disease (ND), Low Pathogenic Avian Influenza (LPAI), and Infectious Bronchitis (IB) viruses or their vaccines (de Cássia Figueira Silva et al., 2008). MS infection may cause a reduction of 5-10% in egg production and 5-7% in hatchability, with more than 5% mortality in the offspring without obvious clinical symptoms in breeder flocks (Kursa et al., 2019; Cisneros-Tamayo et al., 2020).

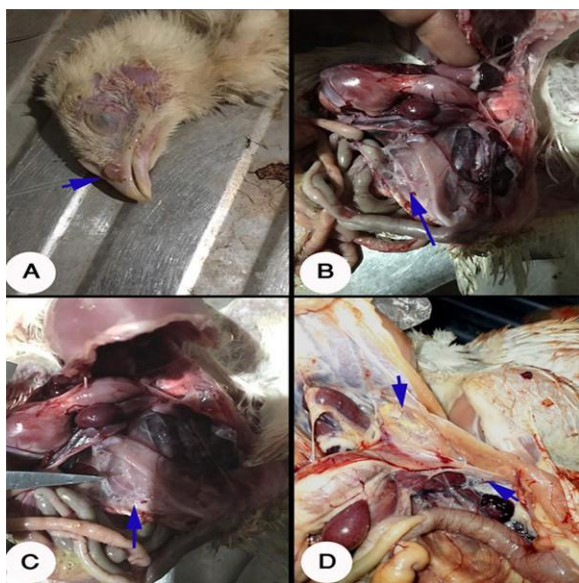


Figure 2: Postmortem findings in mycoplasma infection. (A) nasal discharge and swollen infraorbital sinuses, (B&C) airsacculitis, (D) complicated case with pericarditis and airsacculitis (photos were obtained from field cases submitted to the Poultry Diseases Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt).

Lesions may be found throughout the upper and lower respiratory tract. However, the severity of these lesions varies depending on the virulence and pathogenicity of the infecting strain, concurrent respiratory pathogens, and stress factors. Catarrhal exudates may be present in the nasal passages, infraorbital sinuses, trachea, and bronchi. In chickens, mild sinusitis, tracheitis, and airsacculitis are observed in uncomplicated cases of mycoplasmosis. Interstitial pneumonia and salpingitis are seen in chickens and turkeys. Other findings may include conjunctivitis, corneal opacities, and periocular edema (Pattison et al., 2008). Thickening and turbidity of the air sacs, exudative accumulations, fibrinopurulent pericarditis, and perihepatitis are seen in *E. coli* concurrently infected chickens. Lesions in complicated cases sometimes include swelling and edema of periarticular tissue, excess joint fluid, erosion of the

surface (arthritis), inflammation of tendovaginal sheaths, bursae, the synovial membrane (synovitis), and pale areas in the cerebrum may be observed (Stipkovits et al., 2012).

MS infection in chickens causes infectious synovitis with visible yellowish purulent exudate or a yellow cheese-like substance accumulated in the joint and wing cavities (Sun et al., 2017; Xu et al., 2020). Systemic MS infections in broiler chickens cause septicaemic lesions and increased carcass condemnation rate. Condemnations at the processing plant were due to airsacculitis and keel bursitis. However, the involvement of several organs, including the keel bursa, liver, spleen, brain, choroid of the eye, nerves, and skeletal muscle associated with vasculitis, were reported (Tebyanian et al., 2014).

Advances in the diagnosis of avian mycoplasmosis

MG or MS infections can be confirmed by isolating the organism in a cell-free medium or directly detecting its DNA in infected tissues or swab samples. Serological tests are also widely used for diagnosis. The diagnosis of MG and MS infection is traditionally made by serology (Pattison et al., 2008). Several serological tests have been used to detect MG and MS antibodies, but specificity and sensitivity have been somewhat lacking in all of them (Ali et al., 2015). The rapid plate agglutination test (Figure 3) is the only test that detects both IgM and IgG antibodies; this allows earlier detection of the infection from a few days up to one week in advance compared to the ELISA test (Levisohn and Kleven, 2000).

For MG culture, swabs from the trachea or choanal cleft, oropharynx, esophagus, trachea, cloaca, and phallus are sampled.

Airsacculitis lesions may be sampled; however, MG organisms tend to disappear from lesions after a few weeks but persist in the upper respiratory tract (Ferguson-Noel et al., 2020). Samples from dead birds may be taken from the nasal cavity, infra-orbital sinuses, trachea, and air sacs. Exudates can be aspirated from the infra-orbital sinuses and joints in MS (Asgharzade et al., 2013). Samples for MS isolation include hock joints with synovitis. However, MS can be isolated from the trachea and air sacs of chickens with air sacculitis without hock joint lesions. Generally, choanal cleft swabs yield higher isolation rates and are more easily obtained than tracheal swabs. Suspension of tracheal or air sacs exudates, lungs and infra-orbital sinuses exudate can be inoculated directly to Pleuropneumonia-like Organism (PPO) broth or agar medium. Recently, direct MG and MS detection by PCR and qPCR in the collected swabs have been adequate for molecular detection (Ball et al., 2020). The isolation of mycoplasma from field samples can be easily and rapidly attained in the early infection before the organism is eliminated or overwhelmed by the invasion of the tissue by other microorganisms, especially *E. coli*. Also, the anti-mycoplasma substances such as antibiotics in tissue, antisera, or inhibitors released from tissues after death may influence the recovery of mycoplasma.

Mycoplasma media generally contain protein digest and meat infusion bases supplemented with serum or serum fraction, yeast extract, glucose, and bacterial inhibitors. MG can be isolated on Frey's medium consisting of PPLO broth base (2.1%), PPLO agar base (1%), horse or swine serum (12-15%), glucose (1%), yeast extract (10%), thallium acetate (1%), and penicillin G sodium (0.5%) is an ideal media for isolation of MG (Heleili et al., 2011). In contrast, MS can grow in Frey's media after adding 0.02% if reduced nicotinamide adenine dinucleotide (NAD+) and L-cysteine (Hennigan et al., 2012). Colonies appear as circular and smooth with dense raised centers (fried egg appearance) (Figure 4). The survival periods of MG inoculated into liquid media ranged from 7-185 days, depending on the media components and temperature. MG strains can survive for at most 28 days at 4°C, while at 30°C, they can't survive for more than 14 days (Nagatomo et al., 2001).

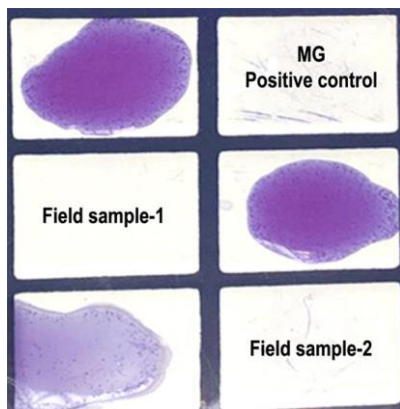


Figure 3: Seroagglutination test showing positive reaction as definite clumps to *Mycoplasma* isolates from field cases (Poultry Diseases Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt).

Culturing of mycoplasma is laborious, can take 3–4 weeks, and may fail in mycoplasma detection or be hampered by mixed infections. Therefore, rapid and sensitive detection methods, like the polymerase chain reaction (PCR) method, can be advantageous. The pooling of samples can also reduce PCR costs, especially for epidemiological studies (Asgharzade et al., 2013; Fraga et al., 2013).

Polymerase chain reaction (PCR)

The PCR represents a rapid and sensitive alternative to the traditional culture methods requiring specialized techniques and sophisticated reagents. A major advantage of implementing MG-PCR technology is diagnosing a wide range of poultry diseases, for which PCR is now one of the tests of choice. PCR techniques are interesting alternatives for the detection and analysis of DNA with high specificity, sensitivity, and capability for a large scale of data and economic viability. These tests have been used for the rapid detection and identification of MG and MS from cultures or directly from clinical samples without the need for isolation (Hess et al., 2007).

The development of molecular typing presents new opportunities for epidemiological studies and the identification of reservoirs of infection. A multi-species PCR and restriction fragment length polymorphism targeting a 780-base-pair DNA fragment within the 16S rRNA gene of MG, MS, and MI does not amplify other avian mycoplasmas or other bacteria (Garcia et al., 1995). PCR primers specific to MG contain a unique 660-nucleotide intergenic spacer region (IGSR) between the 16S and the 23S rRNA genes. The MG IGSR PCR was tested on 18 avian mollicute species and confirmed as MG-specific (Raviv et al., 2007). Reverse transcription-polymerase chain reaction (RT-PCR) of mycoplasma's 16S ribosomal nucleic acid (rRNA) has been developed to detect viable MG. The 16S rRNA of MG was detected up to approximately 20-25 h at 37°C, 22-25 h at 16°C, and 23-27 h at 4°C (Tan et al., 2014). The RT-PCR method was applied during the *in vivo* study of drug efficacy using embryonated commercial broiler-breeder eggs treated with Macrolide after MG inoculation via the yolk. The PCR and RT-PCR combination has been proven to be capable of detecting and differentiating viable from non-viable MG (Tan et al., 2014).

The increased use of MG live vaccines requires a rapid test to differentiate the MG strains from the live vaccine strains ts-11 and 6/85 (Sulyok et al., 2019). PCR is diagnostic enough to differentiate vaccine strain ts-11 and MG field isolates based on identifying restriction enzyme sites in the 300-base pair (bp) *mgc2*- PCR amplicon present in ts-11 and missing in MG isolates from field outbreaks (Lysnyansky et al., 2005).

Random Amplified Polymorphic DNA (RAPD) arbitrary primed polymerase chain reactions (AP-PCR)

Random amplified polymorphic DNA (RAPD) or arbitrary primed polymerase chain reaction (AP-PCR) is a DNA fingerprinting method that has proven very useful for strain differentiation. RAPD banding patterns are prone to variability and are difficult to reproduce and interpret. Amplified fragment length polymorphism (AFLP) has provided accurate and reproducible strain differentiation but is somewhat complex. The isolation of MG in pure culture is a preliminary requirement for these DNA fingerprinting techniques (Ferguson-Noel et al., 2020). The RAPD technique is used to differentiate among different strains of MG. The similarity percent of DNA profiles of different MG species is higher between homologous and heterologous species.

RAPD analysis produced reproducible banding patterns based on which various distinct amplification patterns can be detected. MG isolates demonstrated specific RAPD profiles compared to reference strains (S6 and Mg SS) and vaccine strains (ts-11). The AP-PCR fingerprinting was used to successfully amplify a characteristic fragment of 369 bp of the 16s rRNA genes of avian *Mycoplasma* spp. MG and MS field and reference strains. However, *M. pullorum*, *M. gallinaceum*, *M. iowae*, and *M. gallinarum* are not amplified (El-Shater et al., 2002).

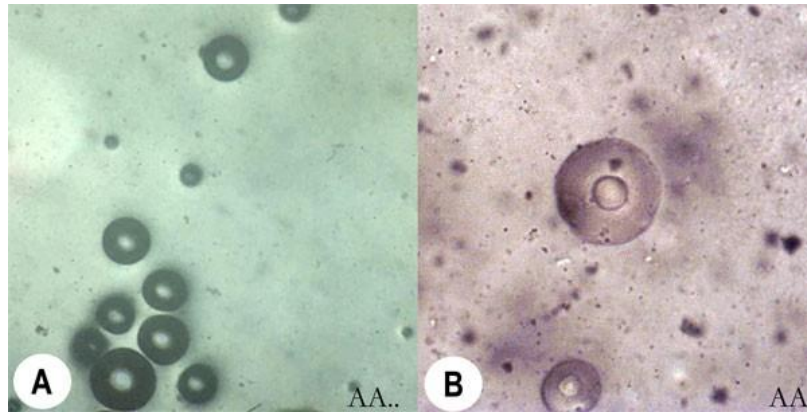


Figure 4: Characteristic colony morphology of *Mycoplasma* spp. isolated from broiler chickens showing fried egg appearance of colonies. (A) 4× magnification (B) 10× magnification (Isolates from field cases submitted to the Poultry Diseases Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt).

Therefore, identifying the genotypic diversity and heterogeneity among MG isolated from the field can be used for epidemiological studies (Peighambari et al., 2006).

Multiplex real-time polymerase chain reaction (Multiplex MGMS)

Multiplex MGMS is designed with primers and probes specific for different *Mycoplasma* spp. The test showed 100% specificity and sensitivity in the MG analysis and 94.7% sensitivity and 100% specificity in the MS analysis from several studies. Another developed TaqMan® real-time polymerase chain reaction (Taq- Man RT-PCR) targeting the surface protein (*mgc2*) exhibited higher sensitivity and applicable accuracy than other molecular and serological techniques (Fraga et al., 2013).

Recently, a field-deployable POKKIT™ device consisting of an insulated isothermal polymerase chain reaction (iiPCR) assay to detect MS in the farm was developed. The detection limit has shown 95% of nine genome equivalents by testing serial dilutions of standard DNA. The detection endpoint for MS genomic DNA was comparable to a real-time reference PCR. No cross-reaction and the test was MS-specific, as confirmed by negative reactions with avian reovirus, MG, *Staphylococcus aureus*, *E. coli*, *Pasteurella multocida*, and *Salmonella Pullorum* (Kuo et al., 2017).

Agarose gel-based mismatch amplification mutation assays (MAMAs)

The MAMAs is a new technique used for rapid differentiation of MG vaccine strains from field isolates based on mutations in the *crmA*, *gapA*, *lpd*, *plpA*, *pot C*, *glpK*, and *hlp2* genes. Therefore, it supports routine diagnostics by determining the successful vaccination or confirming the MG-free status of poultry flocks. The technique is highly specific; thus, it is applied directly to clinical samples, avoiding technical problems associated with isolation, which is particularly important in the case of mycoplasmas. Additionally, the MAMAs technique is a helpful tool for detecting mixed infections with a sensitivity similar to those of the wild-type and vaccine-type strains.

Another advantage is that the technique is designed with the same thermal profile; thus, it can be simultaneously applied and can be performed on basic real-time PCR platforms and conventional PCR equipment coupled with agarose gel electrophoresis (Sulyok et al., 2019).

Real-time Loop-mediated Isothermal Amplification assay (LAMP)

the LAMP assay has emerged as an affordable and rapid molecular diagnostic technique based on amplifying the mycoplasma *mgc2* gene (Kursa et al., 2015; Ehtisham-ul Haque et al., 2017). With the advantage of not requiring specialized instrumentation, the technique can obviate the limitations associated with PCR (Aryan et al., 2010; Mansour et al., 2015). A LAMP reaction takes place under isothermal conditions of 60–65°C with high strand displacement activity performed by *Bst* or *Bsm* DNA polymerase (Zhang et al., 2015) with 2 inner, 2 outer, or 2 loop primers that recognize 6-8 specific sequences on the target DNA (Woźniakowski et al., 2011; Kursa et al., 2015).

Mycoplasma prevention and control

Current control practices against mycoplasma infection include intense biosecurity and surveillance via serological monitoring of flocks, mycoplasma isolation techniques, and DNA-based detection methods. Biosecurity and serological surveillance measures have mainly been successful at minimizing mycoplasma outbreaks among the turkey and chicken breeder flocks, in which outbreaks occur only in a sporadic nature. Furthermore, the "all-in-all out" nature of the meat-type turkey and broiler industries allows for the complete eradication of infected flocks (Evans et al., 2005).

Since all pathogenic avian mycoplasmas are vertically transmitted, obtaining replacement stock from mycoplasma-free sources is essential (Armour and Ferguson-Noel, 2015). The major poultry breeders have eradicated MG, MS, and *M. meleagridis* from their genetic lines, and significant progress is being made to eliminate *M. iowae* from genetic lines.

In the event of infection of genetic lines or grandparent flocks with MG, MS, or *M. meleagridis*, the primary breeding companies will eliminate infected poultry flocks (Kleven, 2008; Buim et al., 2009).

The most significant risk factor for avian mycoplasma is the presence of infected flocks nearby. MG and MS can survive in the environment for longer periods than previously assumed, thus increasing the risk of contaminating flocks by indirect exposure (Kleven, 2008). A consistently applied monitoring system is essential for the prevention of mycoplasma infections. Besides, an early detection system is extremely important to prevent contamination of other flocks. Adding young males to improve fertility in older breeder flocks is especially hazardous (Fiorentin et al., 2003). In general, monitoring fewer samples more frequently is preferred to testing large numbers of samples less regularly.

Though time-consuming and laborious, the isolation and identification of mycoplasma remain the "gold standard" for diagnosing mycoplasma infections (Razin and Hayflick, 2010). The PCR test application is a rapid, sensitive, and specific method instead of culture to detect specific mycoplasma species DNA (Fraga et al., 2013).

Medication

The lack of cell walls makes mycoplasmas resistant to β -lactam antibiotics such as penicillin or cephalosporins (Chernova et al., 2016). However, they tend to be sensitive to macrolides, tetracyclines, fluoroquinolones, and others (Abd El-Hamid et al., 2019). Antibiotic medication has been used to reduce egg transmission and improve egg production in MG-infected commercial layers (Kleven, 2008). Dipping of hatching eggs in antibiotic solution and/or injection of individual eggs has been used to reduce or eliminate MG and *M. meleagridis* egg transmission. Currently, tylosin or tetracyclines are the most commonly used products in the U.S. to minimize egg transmission or prophylactic treatment to prevent respiratory disease in broilers or commercial turkeys (Puva'ca et al., 2020). Effective antibiotic medication can be practical and useful in preventing economic losses associated with avian mycoplasma infections, but it should not be considered a long-term solution.

Highly effective products such as enrofloxacin or tilmicosin are not approved for use in poultry in the US (Hong et al., 2015).

A typical treatment program in infected breeding stock may consist of continuous medication in the feed or treatment for 5–7 days each month. Treatment may reduce MG populations in the respiratory tract, potentially reducing the risk of spreading to neighboring flocks. Medication of naturally infected birds with enrofloxacin was highly effective in reducing or eliminating upper respiratory infection with MG but had little effect on MS populations (Garmyn et al., 2019). Nevertheless, even though antibiotic medication can be an effective tool for reducing egg transmission, clinical signs, and lesions. However, medication cannot be depended on to eliminate the infection from a flock, and the misuse of antibiotics may result in resistance development.

The antimicrobial use after mycoplasma vaccination may interfere with vaccine strain persistence and efficacy (field strains were more commonly observed in flocks that had treatments after immunization). Increased minimum inhibitory concentrations (MIC) to various antimicrobials were reported in different mycoplasma isolates. A recent study demonstrated low MICs of tetracyclines, tiamulin, and tylosin; however, increased tilmicosin and high tylosin MICs were observed in both MS and MG isolates (Morrow et al., 2020). The MIC₅₀ of spiramycin and tilmicosin was higher in turkeys than in chicken isolates of MG, while the enrofloxacin and tylosin MIC₅₀ values were higher for chickens compared to turkey isolates. In MS, high MIC₉₀ values of macrolides "spiramycin, tylosin, and tilmicosin" in chicken isolates compared to turkey isolates (de Jong et al., 2021).

Vaccination

Vaccination is an option for controlling MG or MS when biosecurity measures fail to prevent poultry flock infection. Both live vaccines and killed vaccines (bacterins) are currently available.

Inactivated vaccines/Bacterins

The inactivated mycoplasma vaccines are protective, but their use is not cost-effective (Ishfaq et al., 2020). Studies demonstrated that bacterins were efficacious in preventing respiratory lesions in chickens and proved beneficial in reducing transmission and production losses. However, these bacterins' efficacy was less effective than live vaccines due to their temporary capability to control MG infection with negligible effect in protecting the chicken respiratory system from MG. Therefore, bacterins are of minimal value in commercial flocks where long-term control of MG infection is needed (Kleven, 2008; Ishfaq et al., 2020). Recently, aerosolized liposomal nanoparticles vaccine, encapsulating antigens of H9N2 and MG and *Echinacea* extract induced local immunity in broilers against infection and improved production performance in challenged birds (Kumosani et al., 2020).

Live attenuated vaccines

Live MG vaccines include the F-strain and attenuated strains ts-11 and 6/85. The F-strain vaccine is efficacious and virtually nonpathogenic under field conditions; it also reduces antibiotic requirements and mortality. The F-strain vaccine is preferable on sites where wild-type MG is very virulent and can displace a virulent MG strain in a commercial flock. The F-strain vaccine can be administered as early as 2 weeks before infection by intranasal, intraocular, and coarse spray (Leigh et al., 2018). However, the F-strain's disadvantages are mainly due to its pathogenicity and transmissibility to broilers and turkeys (Liu et al., 2013; Leigh et al., 2018).

The strain ts-11 is less virulent and less infectious than the F-strain and provides somewhat weaker but usually effective, long-term vaccine-dose-dependent protective immunity (Raviv et al., 2008; Vance et al., 2008).

This strain is administered by eye drops, persists in the chicken for long periods, and stimulates a detectable, although variable, systemic antibody

response (Bíró et al., 2005; Gates et al., 2008). Strain ts-11 can be used safely in combination with respiratory virus vaccines (Bíró et al., 2005; Armour and Ferguson-Noel, 2015). Strain 6/85 also stimulates a weaker protective immune response than F-strain and of low virulence and infectivity (Viscione et al., 2009). This strain is administered by aerosol, does not persist in vaccinated birds, and may fail to stimulate a detectable systemic antibody response (Peebles et al., 2008; Viscione et al., 2009). Another novel live-attenuated MG vaccine (K5054) was isolated from turkeys and proved to be effective in turkeys and chickens against virulent strains of MG (Ferguson-Noel et al., 2012; Ferguson-Noel and Williams, 2015). The K-strain is a vaccine strain with, at minimum, equivalent efficacy to two commercially available live MG vaccines and has the potential to protect vaccinated birds from respiratory and reproductive lesions, as well as colonization with field strains (Ferguson-Noel and Williams, 2015).

Conclusions

Avian mycoplasma infections are widely spreading among poultry flocks, causing a great economic loss in the poultry industry. Although MG and MS are the most important pathogens in poultry involved in chronic respiratory disease (CRD), other types of mycoplasma are isolated from chicken flocks suffering from CRD. Mycoplasma in chickens is not only causing CRD but also opens the way for other co-infecting pathogens such as *E. coli*, LPAI (H9N1), and IB by evading the immune system. Isolation of mycoplasma by traditional culture method is still the gold standard in the diagnosis of avian mycoplasmosis, but it is a laborious, difficult, and time-consuming method; therefore, PCR is still an alternative to the traditional culture method as it is sensitive, rapid, and easy to apply.

Prevention and control of mycoplasma infection in poultry farms are mainly achieved by obtaining chicks from breeder flocks free from mycoplasma, and this can be achieved by the frequent vaccination of these flocks by both live and inactivated vaccines, so the current field state requires further studies for development of other types of vaccines have the ability to prevent the infection or at least prevent the shedding and vertical transmission of mycoplasma.

Article Information

Funding. This research received no external funding.

Conflict of Interest. The author declares no conflict of interest.

References

- Abd El-Hamid, M.I., Awad, N.F.S., Hashem, Y.M., Abdel-Rahman, M.A., Abdelaziz, A.M., Mohammed, I.A.A., Abo-Shama, U.H., 2019. *In vitro* evaluation of various antimicrobials against field *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates in Egypt. *Poultry Science* 98, 6281–6288. [10.3382/ps/pez576](https://doi.org/10.3382/ps/pez576).
- Ali, M.Z., Rahman, M.M., Sultana, S., 2015. Seroprevalence of *Mycoplasma gallisepticum* antibody by ELISA and serum plate agglutination test of laying chicken. *Veterinary World* 8, 9–14. [10.14202/vetworld.2015.9-14](https://doi.org/10.14202/vetworld.2015.9-14).
- Armour, N.K., Ferguson-Noel, N., 2015. Evaluation of the egg transmission and pathogenicity of *Mycoplasma gallisepticum* isolates genotyped as ts-11. *Avian Pathology* 44, 296–304. [10.1080/03079457.2015.1044890](https://doi.org/10.1080/03079457.2015.1044890).
- Aryan, E., Makvandi, M., Farajzadeh, A., Huygen, K., Bifani, P., Mousavi, S.L., Fateh, A., Jelodar, A., Gouya, M.M., Romano, M., 2010. A novel and more sensitive loop-mediated isothermal amplification assay targeting IS6110 for detection of *Mycobacterium tuberculosis* complex. *Microbiological Research* 165, 211–220. [10.1016/j.micres.2009.05.001](https://doi.org/10.1016/j.micres.2009.05.001).
- Asgharzade, S., Zaeri, S., Hasanzade, M., Ahmadi, M., Talebi, A.R., 2013. Detection of *Mycoplasma gallisepticum* in experimentally infected broiler chickens using culture, SPA, ELISA, and PCR methods. *Comparative Clinical Pathology* 22, 1051–1055.
- Ball, C., Felice, V., Ding, Y., Forrester, A., Catelli, E., Ganapathy, K., 2020. Influences of swab types and storage temperatures on isolation and molecular detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Avian Pathology* 49, 106–110. [10.1080/03079457.2019.1675865](https://doi.org/10.1080/03079457.2019.1675865).
- Baudler, L., Scheufen, S., Ziegler, L., Moller Palau-Ribes, F., Ewers, C., Lierz, M., 2019. Identification and differentiation of avian Mycoplasma species using MALDI-TOF MS. *Journal of Veterinary Diagnostic Investigation* 31, 620–624. [10.1177/1040638719856932](https://doi.org/10.1177/1040638719856932).
- Buim, M.R., Mettifofo, E., Timenetsky, J., Kleven, S., Ferreira, A.J.P., 2009. Epidemiological survey on *Mycoplasma gallisepticum* and *M. synoviae* by multiplex PCR in commercial poultry. *Pesquisa Veterinária Brasileira* 29, 552–556.
- B'eb'ear, C.M., Pereyre, S., 2005. Mechanisms of drug resistance in *Mycoplasma pneumoniae*. *Current Drug Targets. Infectious Disorders* 5, 263–271. [10.2174/1568005054880109](https://doi.org/10.2174/1568005054880109).
- B'ir'o, J., Povaz's'an, J., Kor'osi, L., Gl'avits, R., Hufnagel, L., Stipkovits, L., 2005. Safety and efficacy of *Mycoplasma gallisepticum* TS-11 vaccine for the protection of layer pullets against challenge with virulent *M. gallisepticum* R-strain. *Avian Pathology* 34, 341–347. [10.1080/03079450500179913](https://doi.org/10.1080/03079450500179913).
- Chen, C., Li, J., Zhang, W., Shah, S.W.A., Ishfaq, M., 2020. *Mycoplasma gallisepticum* triggers immune damage in the chicken thymus by activating the TLR-2/MyD88/NF- κ B signaling pathway and NLRP3 inflammasome. *Veterinary Research* 51, 52. [10.1186/s13567-020-00777-x](https://doi.org/10.1186/s13567-020-00777-x).
- Chernova, O., Medvedeva, E., Mouzykantov, A., Baranova, N., Chernov, V., 2016. Mycoplasmas and their antibiotic resistance: The problems and prospects in controlling infections. *Acta Naturae* 8.
- Cisneros-Tamayo, M., Kempf, I., Coton, J., Michel, V., Bougeard, S., de Boiss'eson, C., Lucas, P., B'ayon-Auboyer, M.H., Chiron, G., Mindus, C., 2020. Investigation on eggshell apex abnormality (EAA) syndrome in France: isolation of *Mycoplasma synoviae* is frequently associated with mycoplasma pullorum. *BMC Veterinary Research* 16, 1–13. [10.1186/s12917-020-02487-0](https://doi.org/10.1186/s12917-020-02487-0).
- Cizelj, I., Ber'ci'c, R.L., Du'sani'c, D., Narat, M., Kos, J., Dov'c, P., Ben'cina, D., 2011. *Mycoplasma gallisepticum* and *Mycoplasma synoviae* express a cysteine protease CysP, which can cleave chicken IgG into fab and fc. *Microbiology* 157, 362–372. [10.1099/mic.0.045641-0](https://doi.org/10.1099/mic.0.045641-0).
- Cordova, C.M.M., Hoeltgebaum, D.L., Machado, L.D.P.N., Santos, L.D., 2016. Molecular biology of Mycoplasmas: from the minimum cell concept to the artificial cell. *Anais da Academia Brasileira de Ciências* 88 Suppl 1, 599–607. [10.1590/0001-3765201620150164](https://doi.org/10.1590/0001-3765201620150164).
- de C'assia Figueira Silva, R., do Nascimento, E.R., de Almeida Pereira, V.L., Barreto, M.L., do Nascimento, M.d.G.F., 2008. *Mycoplasma synoviae* infection on Newcastle disease vaccination of chickens. *Brazilian Journal of Microbiology* 39, 384–389. [10.1590/S1517-83822008002000033](https://doi.org/10.1590/S1517-83822008002000033).
- El-Shater, S., Eissa, S., Hassan, A., 2002. Detection of pathogenic Mycoplasmas (*M. gallisepticum* and *M. synoviae*) in samples from chickens and turkeys by polymerase chain reaction (PCR) and identification by arbitrary primed PCR (AP-PCR). *Egyptian Journal of Agricultural Research* 78, 57–67.
- Evans, J., Leigh, S., Branton, S., Collier, S., Pharr, G., Bearson, S., 2005. *Mycoplasma gallisepticum*: Current and developing means to control the avian pathogen. *Journal of*

- Applied Poultry Research 14, 757–763. [10.1093/japr/14.4.757](https://doi.org/10.1093/japr/14.4.757).
- Ferguson-Noel, N.M., Laibinis, V.A., Kleven, S.H., 2012. Evaluation of *Mycoplasma gallisepticum* K-strain as a live vaccine in chickens. *Avian Diseases* 56, 44–50.
- Ferguson-Noel, N.M., Williams, S.M., 2015. The efficacy of *Mycoplasma gallisepticum* K-strain live vaccine in broiler and layer chickens. *Avian Pathology* 44, 75–80. [10.1080/03079457.2015.1005054](https://doi.org/10.1080/03079457.2015.1005054).
- Ferguson-Noel, N., Armour, N.K., Noormohammadi, A.H., El Gazzar, M., Bradbury, J.M., 2020. Mycoplasmosis, in: Swayne, D.E., Boulianne, M., Logue, C.M., McDougald, L.R., Nair, V., Suarez, D.L., Wit, S., Grimes, T., Johnson, D., Kromm, M., Prajitno, T.Y., Rubinoff, I., Zavala, G. (Eds.), *Diseases of Poultry*. Wiley, pp. 907–965. [10.1002/9781119371199.ch21](https://doi.org/10.1002/9781119371199.ch21).
- Fiorentin, L., Mores, M.A.Z., Trevisol, I.M., Antunes, S.C., Costa, J.L.A., Soncini, R.A., Vieira, N.D., 2003. Test profiles of broiler breeder flocks housed in farms with endemic *Mycoplasma synoviae* infection. *Brazilian Journal of Poultry Science* 5, 37–43. <https://www.scielo.br/j/rbca/a/4zHvgDyShMvJXTvcm8BDqxR/?lang=en>.
- Fraga, A.P., de Vargas, T., Ikuta, N., Fonseca, A.S.K., Celmer, J., Marques, E.K., Lunge, V.R., 2013. A multiplex real-time PCR for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in clinical samples from Brazilian commercial poultry flocks. *Brazilian Journal of Microbiology* 44, 505–510. [10.1590/S1517-83822013000200028](https://doi.org/10.1590/S1517-83822013000200028).
- Garcia, M., Jackwood, M.W., Levisohn, S., Kleven, S.H., 1995. Detection of *Mycoplasma gallisepticum*, *M. synoviae*, and *M. iowae* by multi-species polymerase chain reaction and restriction fragment length polymorphism. *Avian Diseases* 39, 606–616. <https://www.ncbi.nlm.nih.gov/pubmed/8561747>.
- García-Morales, L., González-González, L., Costa, M., Querol, E., Pinol, J., 2014. Quantitative assessment of *Mycoplasma* hemadsorption activity by flow cytometry. *Plos One* 9, e87500. [10.1371/journal.pone.0087500](https://doi.org/10.1371/journal.pone.0087500).
- Garmyn, A., Vereecken, M., De Gussem, K., Depondt, W., Haesebrouck, F., 2019. Efficacy of tylosin and tilmicosin against experimental *Mycoplasma gallisepticum* infection in chickens. *Avian Diseases* 63, 359. [10.1637/11991-110818-Reg.1](https://doi.org/10.1637/11991-110818-Reg.1).
- Gates, A.E., Frasca, S., Nyaoke, A., Gorton, T.S., Silbart, L.K., Geary, S.J., 2008. Comparative assessment of a metabolically attenuated *Mycoplasma gallisepticum* mutant as a live vaccine for the prevention of avian respiratory mycoplasmosis. *Vaccine* 26, 2010–2019. [10.1016/j.vaccine.2008.02.010](https://doi.org/10.1016/j.vaccine.2008.02.010).
- Gaunson, J.E., Phillip, C.J., Whithear, K.G., Browning, G.F., 2006. Age-related differences in the immune response to vaccination and infection with *Mycoplasma gallisepticum*. *Vaccine* 24, 1687–1692. [10.1016/j.vaccine.2005.09.045](https://doi.org/10.1016/j.vaccine.2005.09.045).
- Ehtisham-ul Haque, S., Kiran, M., Waheed, U., Younus, M., 2017. Real-time loop-mediated isothermal amplification (LAMP) of *mgc2* gene of *Mycoplasma gallisepticum*. *Journal of Veterinary Research* 61, 439–444. [10.1515/jvetres-2017-0058](https://doi.org/10.1515/jvetres-2017-0058).
- Heleili, H., N, N., Mamache, M., Chelili, B., A, A., 2011. Incidence of avian Mycoplasmosis in the region of Batna, Eastern Algeria. *Veterinary World*, 101. [10.5455/vetworld.2011.101-105](https://doi.org/10.5455/vetworld.2011.101-105).
- Hennigan, S.L., Driskell, J.D., Ferguson-Noel, N., Dluhy, R.A., Zhao, Y., Tripp, R.A., Krause, D.C., 2012. Detection and differentiation of avian mycoplasmas by surface-enhanced Raman spectroscopy based on a silver nanorod array. *Applied and Environmental Microbiology* 78, 1930–1935. [10.1128/AEM.07419-11](https://doi.org/10.1128/AEM.07419-11).
- Hess, M., Neubauer, C., Hackl, R., 2007. Interlaboratory comparison of ability to detect nucleic acid of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by polymerase chain reaction. *Avian Pathology* 36, 127–133. [10.1080/03079450701203082](https://doi.org/10.1080/03079450701203082).
- Hong, Y.H., Kwon, J.S., Lee, H.J., Song, C.S., Lee, S.W., 2015. Eradication of *Mycoplasma synoviae* from a multi-age broiler breeder farm using antibiotics therapy. *Poultry Science* 94, 2364–2368. [10.3382/ps/pev232](https://doi.org/10.3382/ps/pev232).
- Ishfaq, M., Zhang, W., Ali Shah, S.W., Wu, Z., Wang, J., Ding, L., Li, J., 2020. The effect of *Mycoplasma gallisepticum* infection on energy metabolism in chicken lungs through oxidative stress and inflammation. *Microbial Pathogenesis* 138, 103848. [10.1016/j.micpath.2019.103848](https://doi.org/10.1016/j.micpath.2019.103848).
- de Jong, A., Youala, M., Klein, U., El Garch, F., Simjee, S., Moyaert, H., Rose, M., Gautier-Bouchardon, A.V., Catania, S., Ganapathy, K., Gyuranecz, M., Moller Palau-Ribes, F., Pridmore, A., Ayling, R.D., 2021. Minimal inhibitory concentration of seven antimicrobials to *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolates from six European countries. *Avian Pathology* 50, 161–173. [10.1080/03079457.2020.1861216](https://doi.org/10.1080/03079457.2020.1861216).
- Kleven, S.H., 2008. Control of avian mycoplasma infections in commercial poultry. *Avian Diseases* 52, 367–374. [10.1637/8323-041808-Review.1](https://doi.org/10.1637/8323-041808-Review.1).
- Kumosani, T., Yaghmoor, S., Abdulaal, W.H., Barbour, E., 2020. Evaluation in broilers of aerosolized nanoparticles vaccine encapsulating immuno-stimulant and antigens of avian influenza virus/*Mycoplasma gallisepticum*. *BMC Veterinary Research* 16, 319. [10.1186/s12917-020-02539-5](https://doi.org/10.1186/s12917-020-02539-5).
- Kuo, H.C., Lo, D.Y., Chen, C.L., Tsai, Y.L., Ping, J.F., Lee, C.H., Lee, P.Y.A., Chang, H.F.G., 2017. Rapid and sensitive detection of *Mycoplasma synoviae* by an insulated isothermal polymerase chain reaction-based assay on a field-deployable device. *Poultry Science* 96, 35–41. [10.3382/ps/pew228](https://doi.org/10.3382/ps/pew228).
- Kursa, O., Pakula, A., Tomczyk, G., Paśko, S., Sawicka, A., 2019. Eggshell apex abnormalities caused by two different *Mycoplasma synoviae* genotypes and evaluation of eggshell anomalies by full-field optical coherence tomography. *BMC Veterinary Research* 15, 1. [10.1186/s12917-018-1758-8](https://doi.org/10.1186/s12917-018-1758-8).
- Kursa, O., Woźniakowski, G., Tomczyk, G., Sawicka, A., Minta, Z., 2015. Rapid detection of *Mycoplasma synoviae* by loop-mediated isothermal amplification. *Archives of Microbiology* 197, 319–325. [10.1007/s00203-014-1063-2](https://doi.org/10.1007/s00203-014-1063-2).
- Leigh, S.A., Evans, J.D., Collier, S.D., Branton, S.L., 2018. The impact of vaccination route on *Mycoplasma gallisepticum* vaccine efficacy. *Poultry Science* 97, 3072–3075. [10.3382/ps/pey188](https://doi.org/10.3382/ps/pey188).
- Levisohn, S., Kleven, S.H., 2000. Avian mycoplasmosis (*Mycoplasma gallisepticum*). *Revue Scientifique et Technique (OIE)* 19, 425–442.
- Liu, J.J., Ding, L., Wei, J.Z., Li, Y., 2013. Influences of F-strain *Mycoplasma gallisepticum* vaccine on productive and reproductive performance of commercial parent broiler chicken breeders on a multi-age farm. *Poultry Science* 92, 1535–1542. [10.3382/ps.2012-02999](https://doi.org/10.3382/ps.2012-02999).
- Lysnyansky, I., García, M., Levisohn, S., 2005. Use of *mgc2*-polymerase chain reaction-restriction fragment length polymorphism for rapid differentiation between field isolates and vaccine strains of *Mycoplasma gallisepticum* in Israel. *Avian Diseases* 49, 238–245. [10.1637/7285-10020R](https://doi.org/10.1637/7285-10020R).
- Mansour, S.M.G., Ali, H., Chase, C.C.L., Cepica, A., 2015. Loop-mediated isothermal amplification for diagnosis of 18 World Organization for Animal Health (OIE) notifiable viral diseases of ruminants, swine, and poultry. *Animal Health Research Reviews* 16, 89–106. [10.1017/S1466252315000018](https://doi.org/10.1017/S1466252315000018).
- May, M., Dunne, D.W., Brown, D.R., 2014. A sialoreceptor binding motif in the *Mycoplasma synoviae* adhesin VlhA. *Plos One* 9, e110360. [10.1371/journal.pone.0110360](https://doi.org/10.1371/journal.pone.0110360).
- Mizutani, M., Miyata, M., 2019. Behaviors and energy source of *Mycoplasma gallisepticum* gliding. *Journal of Bacteriology* 201. [10.1128/JB.00397-19](https://doi.org/10.1128/JB.00397-19).
- Morrow, C.J., Kreizinger, Z., Achari, R.R., Bek'oo, K., Yvon, C., Gyuranecz, M., 2020. Antimicrobial susceptibility of pathogenic mycoplasmas in chickens in Asia. *Veterinary Microbiology* 250, 108840. [10.1016/j.vetmic.2020.108840](https://doi.org/10.1016/j.vetmic.2020.108840).

- Nagatomo, H., Takegahara, Y., Sonoda, T., Yamaguchi, A., Uemura, R., Hagiwara, S., Sueyoshi, M., 2001. Comparative studies of the persistence of animal mycoplasma under different environmental conditions. *Veterinary Microbiology* 82, 223–232. [10.1016/S0378-1135\(01\)00385-6](https://doi.org/10.1016/S0378-1135(01)00385-6).
- Nascimento, E.R., Pereira, V.L.A., Nascimento, M.G.F., Barreto, M.L., 2005. Avian mycoplasmosis update. *Brazilian Journal of Poultry Science* 7, 1–9. URL: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2005000100001&nrm=iso.
- Orlov, M., Garanina, I., Fisunov, G.Y., Sorokin, A., 2018. Comparative analysis of *Mycoplasma gallisepticum* vH4 promoters. *Frontiers in Genetics* 9. [10.3389/fgene.2018.00569](https://doi.org/10.3389/fgene.2018.00569).
- Papazisi, L., Frasca, S., Gladd, M., Liao, X., Yogev, D., Geary, S.J., 2002. GapA and CrmA coexpression is essential for *Mycoplasma gallisepticum* cytoadherence and virulence. *Infection and Immunity* 70, 6839–6845. [10.1128/IAI.70.12.6839-6845.2002](https://doi.org/10.1128/IAI.70.12.6839-6845.2002).
- Pattison, M., McMullin, P., Bradbury, J., Alexander, D.J., 2008. *Poultry Diseases*. volume 6th. Saunders Elsevier, London, UK. [10.1016/B978-0-7020-2862-5.X5001-6](https://doi.org/10.1016/B978-0-7020-2862-5.X5001-6).
- Peebles, E.D., Viscione, K.A., Branton, S.L., Vance, A.M., Gerard, P.D., Whitmarsh, S.K., 2008. Effects of pre-lay 6/85-strain *Mycoplasma gallisepticum* inoculation alone or in conjunction with the inoculation of F-strain *Mycoplasma gallisepticum* during lay on the blood characteristics of commercial egg-laying hens. *Poultry Science* 87, 2000–2004. [10.3382/ps.2008-00178](https://doi.org/10.3382/ps.2008-00178).
- Peighambari, S.M., Bozorgmehrifard, M.H., Hosseini, H., Pourbakhsh, S.A., Razzazian, M., 2006. Random amplified polymorphic DNA (RAPD) fingerprinting of *Mycoplasma gallisepticum* isolates from chickens. *Archives of Razi Institute* 61, 67.
- Puva'ca, N., Lika, E., Tufarelli, V., Bursi'c, V., Ljubojevi'c Peli'c, D., Nikolova, N., Petrovi'c, A., Prodanovi'c, R., Vukovi'c, G., Levi'c, J., Giannenas, I., 2020. Influence of different tetracycline antimicrobial therapy of mycoplasma (*Mycoplasma synoviae*) in laying hens compared to tea tree essential oil on table egg quality and antibiotic residues. *Foods* 9. [10.3390/foods9050612](https://doi.org/10.3390/foods9050612).
- Raviv, Z., Callison, S., Ferguson-Noel, N., Laibinis, V., Wooten, R., Kleven, S.H., 2007. The *Mycoplasma gallisepticum* 16S-23S rRNA intergenic spacer region sequence as a novel tool for epizootiological studies. *Avian Diseases* 51, 555–60. [10.1637/0005-2086\(2007\)51\[555:tmgsrj\]2.0.co;2](https://doi.org/10.1637/0005-2086(2007)51[555:tmgsrj]2.0.co;2).
- Raviv, Z., Callison, S.A., Ferguson-Noel, N., Kleven, S.H., 2008. Strain differentiating real-time PCR for *Mycoplasma gallisepticum* live vaccine evaluation studies. *Veterinary Microbiology* 129, 179–187. [10.1016/j.vetmic.2007.11.017](https://doi.org/10.1016/j.vetmic.2007.11.017).
- Razin, S., Hayflick, L., 2010. Highlights of mycoplasma research: Historical perspective. *Biologicals* 38, 183–190. [10.1016/j.biologicals.2009.11.008](https://doi.org/10.1016/j.biologicals.2009.11.008).
- Sawicka, A., Durkalec, M., Tomczyk, G., Kurska, O., 2020. Occurrence of *Mycoplasma gallisepticum* in wild birds: A systematic review and meta-analysis. *Plos One* 15, e0231545. [10.1371/journal.pone.0231545](https://doi.org/10.1371/journal.pone.0231545).
- Stipkovits, L., Glavits, R., Palfi, V., Beres, A., Egyed, L., Denes, B., Somogyi, M., Szathmary, S., 2012. Pathologic lesions caused by coinfection of *Mycoplasma gallisepticum* and H3N8 low pathogenic avian influenza virus in chickens. *Veterinary Pathology* 49, 273–283. [10.1177/0300985811415702](https://doi.org/10.1177/0300985811415702).
- Sulyok, K.M., Kreizinger, Z., Bek'ó, K., Forró, B., Marton, S., Bányai, K., Catania, S., Ellis, C., Bradbury, J., Olaogun, O.M., Kovács, B., Cser'ep, T., Gyuranecz, M., 2019. Development of molecular methods for rapid differentiation of *Mycoplasma gallisepticum* vaccine strains from field isolates. *Journal of Clinical Microbiology* 57. [10.1128/JCM.01084-18](https://doi.org/10.1128/JCM.01084-18).
- Sun, S.K., Lin, X., Chen, F., Wang, D.A., Lu, J.P., Qin, J.P., Luo, T.R., 2017. Epidemiological investigation of *Mycoplasma synoviae* in native chicken breeds in China. *BMC Veterinary Research* 13, 115. [10.1186/s12917-017-1029-0](https://doi.org/10.1186/s12917-017-1029-0).
- Tan, C.G., Ideris, A., Omar, A.R., Yii, C.P., Kleven, S.H., 2014. Polymerase chain reaction-based discrimination of viable from non-viable *Mycoplasma gallisepticum*. *The Onderstepoort Journal of Veterinary Research* 81. [10.4102/ojvr.v81i1.708](https://doi.org/10.4102/ojvr.v81i1.708).
- Tebyanian, H., Mirhosseini, S.H., Kheirikhah, B., Hassanshahian, M., Farhadian, H., 2014. Isolation and identification of *Mycoplasma synoviae* from suspected ostriches by polymerase chain reaction, in Kerman Province, Iran. *Jundishapur Journal of Microbiology* 7, e19262. [10.5812/jjm.19262](https://doi.org/10.5812/jjm.19262).
- Vance, A.M., Branton, S.L., Collier, S.D., Gerard, P.D., Peebles, E.D., 2008. Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on pre-lay ts11-strain *Mycoplasma gallisepticum* inoculation on performance characteristics of commercial laying hens. *Poultry Science* 87, 655–660. [10.3382/ps.2007-00492](https://doi.org/10.3382/ps.2007-00492).
- Viscione, K.A., Branton, S.L., Vance, A.M., Gerard, P.D., Womack, S.K., Peebles, E.D., 2009. Effects of 6/85-strain *Mycoplasma gallisepticum* vaccination alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the reproductive and digestive organs of commercial egg-laying hens. *Poultry Science* 88, 567–570. [10.3382/ps.2008-00394](https://doi.org/10.3382/ps.2008-00394).
- Woźniakowski, G., Samorek-Salamonowicz, E., Kozdrun', W., 2011. Rapid detection of Marek's disease virus in feather follicles by loop-mediated amplification. *Avian Diseases* 55, 462–467. [10.1637/9668-012711-ResNote.1](https://doi.org/10.1637/9668-012711-ResNote.1).
- Xu, B., Liu, R., Ding, M., Zhang, J., Sun, H., Liu, C., Lu, F., Zhao, S., Pan, Q., Zhang, X., 2020. Interaction of *Mycoplasma synoviae* with chicken synovial sheath cells contributes to macrophage recruitment and inflammation. *Poultry Science* 99, 5366–5377. [10.1016/j.psj.2020.08.003](https://doi.org/10.1016/j.psj.2020.08.003).
- Zhang, F., Bao, S., Yu, S., Cheng, J., Tan, L., Qiu, X., Song, C., Dai, Y., Fei, R., Ding, C., 2015. Development of a loop-mediated isothermal amplification targeting a gene within the pyruvate dehydrogenase complex, the pdhA gene, for rapid detection of *Mycoplasma gallisepticum*. *Journal of Veterinary Diagnostic Investigation* 27, 260–267. [10.1177/1040638715584155](https://doi.org/10.1177/1040638715584155).