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Review

# *Mycoplasma gallisepticum* infection: An avian challenge

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## ABSTRACT

*Mycoplasma gallisepticum* is an avian respiratory and reproductive tract pathogen, which has a significant economic impact on all sectors of the poultry industry worldwide. It is a persistent, highly transmissible chicken and turkey pathogen. *M. gallisepticum* belongs to the class of Mollicutes evolved from AT-rich, gram-positive bacteria. The class Mollicutes is different from other bacteria in its very small size and total absence of cell wall which accounts for its characteristic "fried egg" type of colonial morphology, complete resistance to antibiotics that affect cell wall synthesis, and its complex nutritional requirements due to its small genome size. *Mycoplasma gallisepticum* infection is of continuing economic concern in commercial broiler breeder chicken and meat turkey flocks, despite the great efforts of poultry industries made towards eradication of pathogenic mycoplasmas from poultry flocks. *M. gallisepticum* infection is a contagious, notifiable disease to the World Organization for Animal Health (OIE). Major clinical signs observed in avian *M. gallisepticum* infection include coughing, rales, sneezing, nasal discharges, airsacculitis and poor growth. Turkeys typically experience more severe disease, often accompanied by swelling of the paranasal (infraorbital) sinus. Conjunctivitis with frothy ocular exudates is common in turkeys and occurs occasionally in chickens. The symptoms of avian mycoplasmosis are typically slow to develop, and the course of the disease can be prolonged. However, acute respiratory disease sometimes occurs in young birds. More frequently the severity of the disease is characterized by the degree of secondary infection with viruses and/or bacteria such as Newcastle disease, infectious bronchitis, and *Escherichia coli* in chickens. In this paper, *M. gallisepticum* and its effects on the poultry industry are discussed.

**KEYWORDS:** *Mycoplasma gallisepticum*, infection, poultry

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## INTRODUCTION

Mycoplasmas belong to a class of Mollicutes evolved from AT-rich, gram-positive bacteria that become the smallest self replicating organisms known to date with a genome size ranging from 580 kbp of *M. genitalium* to 2220 kbp of *Spiroplasma ixodetis* [1, 2]. Mycoplasmas are believed to undergo degenerative evolution, leading to reduced genome size and loss of many genes common to most bacteria [3, 4, 5]. Primarily, mollicutes (*mollis* = soft, *cutis* = skin) lost the genes involved in the synthesis of a cell wall, which differentiate and make them unique from other bacteria. The loss of cell wall implies an intrinsic resistance to antimicrobial agents that inhibit cell wall synthesis, sensitivity to osmotic shock and an ability to pass filters typically used to sterilize solutions [6]. Moreover, because of their small genomes, these bacteria have limited biosynthetic capabilities and occur as obligate parasites in a wide diversity of plant and animal hosts [7, 8]. Because of this character, they are hard to grow in the laboratory and are often

missed as pathogenic causes of disease. The microorganisms of the class Mollicutes (Wall-less bacteria) were first identified in 1898 as the etiologic agent of the contagious bovine pleuropneumonia (CBPP) named pleuropneumonia-like (PPLO-like) organisms [7] and currently over 190 species, widely distributed among humans, animals, insects and plants are known [9].

Among *Mycoplasma* species affecting poultry industry, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae* are the most significant pathogens known worldwide [10, 11]. Of which *Mycoplasma gallisepticum*, a causative agent for chronic respiratory disease (CRD), is the most important affecting gallinaceous and few of the non-gallinaceous avian species and responsible for economic losses in commercial broiler breeder chicken flock industry causing substantial losses in both performance and production [12, 13, 14]. *M. gallisepticum* before it received this name [15] was described for the first time associated to the etiologic agent of CRD, the pathogen responsible for the infectious sinusitis of turkeys as a member of the Pleuropneumonia-Like Organisms (PPLO) group [16, 17].

The consequences of widespread *M. gallisepticum* infection in poultry industries (layer or breeder operation) are devastating from direct and indirect losses throughout the production cycle [18, 19]. The losses that occur can be attributed to:

- Decreased egg production and quality
- Decreased hatchability
- Reduced egg selection pressure because of the reduced hatching egg availability
- Reduced day old chick quality
- Increased chick mortality because of the exacerbated consequences of concurrent infection
- Increased mortality and carcass condemnations
- Increased medication cost
- Reduced growth rate and feed conversion
- Costly control measures involving biosecurity and vaccination
- Costly eradication measures involving depopulation and site cleaning
- Costly monitoring programs involving serology and PCR

Therefore, this review will focus on *M. gallisepticum* infection in poultry.

### *M. gallisepticum*

*M. gallisepticum* is a bacterial pathogen lacking cell wall, with a circular DNA genome of 996,422 bp length with 742 coding DNA sequence, causing chronic respiratory diseases in poultry.

### *M. gallisepticum* transmission and distribution

*M. gallisepticum* can spread by both lateral/ horizontal and vertical routes. The lateral transmission occurs through direct contact and indirect mechanical means such as fomites and mechanical vectors [18]. Vertical transmission can occur [20], despite vaccination [21, 22] or treatment [23]. Due to its contagious and international biosecurity threat, *M. gallisepticum* infection is among the list of the notifiable diseases to the World Organization for Animal Health (OIE) [24], obligating member countries to report any incidence of the infection within the country. Its incidence in a country may have a grave consequence in its international trade due to suspensions and/or ban of any poultry and poultry products exportation. In this regard besides OIE, regional and country wise legislation that govern reporting of occurrence, control measures and trade managements have been developed. The best example for such a case is the European Community legislation (Directive 90/539/EEC) [25] which governs the control of *M. gallisepticum* and *M. meleagridis* in intra-Community trade in poultry and hatching eggs, with the United Kingdom (UK), and also reflects similar directive to that of members of European Union (EU) countries in laying down control measures for *M. gallisepticum* and *M. meleagridis* but not *M. synoviae*. The legislation also applies to imports from other countries. In contrast, the United States Department of Agriculture National Poultry Improvement Plan legislation encompasses all the four *Mycoplasma* species involved in CRD [26].

In Asia, avian mycoplasmosis has been considered a significant problem in chicken flocks in Japan since 1954, and has been recognized serologically or with isolation of the bacteria in other countries including Korea, Malaysia,

The Philippines, Taipei, China, Israel, Thailand, Vietnam, Bangladesh, India, and Indonesia [14, 27].

Vertical transmission was found to peak at 25-50% approximately 4 weeks after infection and then declined to 3-5% [22, 28]. Although under field conditions egg transmission rates are generally very low, these infected birds invariably cause entire flock to become *M. gallisepticum* positive through the lateral spread [14].

Spread within a flock initially occurs via contaminated droplets projected from one bird to another during coughing and sneezing [29]. The infection rate within the flock increases the level of environmental contamination and the possibility for indirect spread via contaminated fomites [18].

As described by McMartin *et al.* [30], spread within a flock occurs in 4 phases according to antibody response which are: first latent phase when the source-causes incubate the disease and develop an immune response, second early phase when the lateral spread is initiated, third late phase when lateral spread is rapid and fourth terminal phase where the remainder of the flock become antibody positive. Resistance to infection varies depending on host immune system which is the most critical host factor determining the consequences of *M. gallisepticum* infection. *M. gallisepticum* is fragile and unable to survive for more than a few days outside the host making horizontal transmission with direct contact between susceptible and clinically or sub-clinically infected birds most important [18]. However, other reports have also indicated that at optimum room temperature, *M. gallisepticum* can survive as long as 2 weeks and at 4ºC can stay as long as 8 weeks [31]. This persistence of the organism in the environment creates a good opportunity for the transmission of the infection in and between flocks. Besides, carrier birds including previously infected convalescent birds weather treated or not are substantial sources of infections and contact should be avoided with such birds [20, 22, 23]. Furthermore, complications in *M. gallisepticum* control include the organism's ability to transmit both vertically and horizontally and survive outside the host [32].

### *M. gallisepticum* infection and host

*M. gallisepticum* infection can cause primary disease in young chickens characterized by respiratory symptoms including coughing, rales, sneezing, nasal discharges, airsacculitis and poor growth. Turkeys typically experience more severe disease, often accompanied by swelling of the paranasal (infraorbital) sinus. Conjunctivitis with frothy ocular exudates is common in turkeys and occurs occasionally in chickens [14, 33, 34]. The symptoms of avian mycoplasmosis are typically slow to develop, and the course of the disease can be prolonged. However, acute respiratory disease sometimes occurs in young birds, particularly turkeys. More often, the severity of the disease is characterized by the degree of secondary infection with viruses and/or bacteria such as Newcastle disease, infectious bronchitis, and *Escherichia coli* in chickens. In turkeys there is synergism with avian pneumovirus infection with a more chronic form of the disease causing reduced egg production in breeders and layers [33, 34].

In addition to chickens and turkeys, *M. gallisepticum* causes disease in game birds including pheasants, chukar partridges, bobwhite quail, Japanese quail and peafowl. The organism has also been isolated from ducks and geese, as well as yellow-naped Amazon parrots, pigeons and greater flamingos. It has been found in wild peregrine falcons in Spain [35, 36]. Since 1994, *M. gallisepticum* epidemics have been reported in house finches (*Carpodacus mexicanus*) in the U.S. This organism has also been confirmed by culture or polymerase chain reaction (PCR) in American goldfinches (*Carduelis tristis*), purple finches (*Carpodacus purpureus*), eastern tufted titmice (*Baeolophus bicolor*), pine grosbeaks (*Pinicola enucleator*), evening grosbeaks (*Coccothraustes vespertinus*) and a captive blue jay (*Cyanocitta cristata*) [35, 37]. PCR-positive mourning doves (order Columbiformes) have also been reported, but these birds remained seronegative and culture negative, and may have been infected by a related species of Mycoplasma. Other passerine species have tested positive by serology. House sparrows (*Passer domesticus*) and budgerigars (*Melopsittacus undualtus*) have been infected experimentally with some strains [36, 38].

Although *M. gallisepticum* affects poultry industries worldwide, its pathogenesis toward avian species is not well understood [39, 40, 41]. Research has indicated that attachment of *M. gallisepticum* to specific target cells via sialic acid residues along the respiratory epithelium is required prior to initiation of the disease processes and that a complex multifactorial process mediates cytodherence [39, 40]. This adherence could then lead to mediation of apoptosis, innocent bystander damage to host cell due to intimate membrane contact, molecular (antigen) mimicry that may lead to tolerance, and mitotic effect for B and/or T lymphocytes, which could lead to suppressed T-cell function and/or production of cytotoxic T cell, besides mycoplasma byproducts, such as hydrogen peroxide and superoxide radicals [7, 38].

The pathogenesis of *M. gallisepticum* is complicated by the organism's ability to alter its antigenic profile and thereby evade the host's immune system with multiple membrane proteins, and lipoproteins have been characterized with size- or phase-variant forms that occur at high frequency and confer phenotypic or antigenic variation to *M. gallisepticum* that include PvpA and pMGA (also termed vlhA) proteins [12, 42, 43, 44]. Additionally, at least 3 other membrane proteins have been identified as phase variant [42]. The pathogenicity of *M. gallisepticum* may be further complicated by its ability to trigger Calcium ion release from epithelio-tracheal cells [45]. All these factors contribute to chronic form of the infection.

### *M. gallisepticum* detection

Definitive identification of *M. gallisepticum* infections can be made using serologic seroagglutination reaction (SAR) and hemagglutination inhibition (HI) tests. Additionally, immunofluorescence, immunoperoxidase staining, a growth inhibition test, metabolism inhibition or PCR are also commonly used tests for the diagnosis of *M. gallisepticum*. These tests can be followed by isolation and identification through culturing of the organism. Biochemical tests can be useful in preliminary identification, but indirect Polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) may be necessary to distinguish *M. gallisepticum* from *M. imitans*. These two species can also be differentiated by immunofluorescence using serial dilutions of antisera to *M. gallisepticum* and *M. imitans* in parallel. Moreover, *M. gallisepticum* is frequently confused with other respiratory disease(s), including Newcastle disease, infectious bronchitis and *Escherichia coli* infection [33, 34, 46] and it must be differentiated from these common respiratory diseases in chickens. In poultry industry, for monitoring of flocks for the presence of *M. gallisepticum* antibodies, rapid serum plate agglutination test (RSPA) is frequently used due to its high sensitivity and low cost, however it does not distinguish vaccine stimulated humoral antibody complicating the evaluation of antibodies to monitor flocks and also express false positive titers. The false positive RSPA tests can be eliminated by serum dilution. The wide use of enzyme linked immunosorbent assay (ELISA) procedure has been aroused by the need for a reproducible test with enhanced specificity, sensitivity, and the potential for automation. Nevertheless, at present, isolation and molecular identification provide the only effective and accurate method to distinguish a vaccine reaction to *M. gallisepticum* infection. For this purpose PCR detection was developed for *M. gallisepticum* infection [47, 48, 49], which is also accepted worldwide for detection of all avian mycoplasmas, either in specific DNA amplification for diagnosis [50, 51] or in nonspecific DNA banding pattern (RAPD) for strain identification [52].

*M. gallisepticum* can be detected in tissue fragments of affected organs like trachea, air sacs and lungs. Swabs from trachea and choanal cleft constitute excellent specimens, mainly for isolation or PCR, which are used as confirmation tools for monitoring *M. gallisepticum* infections in live birds [38, 53]. Other sources for isolation of avian mycoplasma and/or PCR detection are synovial, ocular and infraorbital sinus exudates, and swabs from trachea and air sacs, and pipped embryos [15, 54, 55]. The colonies of *M. gallisepticum* are tiny, circular, smooth and translucent, and sometimes have a "fried egg" appearance with a central dense mass.

Symptomatic diagnoses with gross and microscopic examinations have been used to help the diagnosis

of avian mycoplasmosis in naturally infected birds. The lesions found with natural infection were similar to experimentally infected birds [15, 55]. *M. gallisepticum* with *E. coli* showed gross lesions which include edematous airsacculitis with fibrin deposition extending to pericarditis and perihepatitis as well as infiltrative microscopic lesions of the lung in experimental CRD in broilers [38, 54]. The serological tests which are by far cheap, simple and quick and which were used for monitoring of flock exposure to *M. gallisepticum* had a diagnosis problem with the advent of live vaccines and the appearance of low virulent strains of *M. gallisepticum,* although these tests have been published to differentiate between poorly vaccinated, well vaccinated and field strain challenged flocks based on sera dilutions from sampled birds of the flocks [56, 57, 58]. PCR can be employed to distinguish between field and vaccine strain of *M. gallisepticum.* Although the PCR test is a lot more expensive than serological assays, the specificity and sensitivity of this technique makes it an attractive alternative especially when samples are polled together where the cost could be reduced significantly [59].

### *M. gallisepticum* treatment and control

*M. gallisepticum* can be introduced into a flock by live birds or hatching eggs, as well as the movement of people and fomites. Attempts to control *M. gallisepticum* must be balanced against the need for greater efficacy in production cost. Test and slaughter eradication has been reported the most effective control [15]. This approach is very expensive and emergence of multiage complexes in the commercial layer poultry industries makes it difficult (impractical) to implement [12, 14, 60]. However, successful Mycoplasma control begins with a Mycoplasma free breeding flock due to the difficulty to prevent vertical transmission [26], for this reason strict biosecurity and the implementation of all in-all out production system are invariably adequate to avoid lateral infection and spread. The all in-all out manageability in the meat-type turkey and broiler industries allows for complete eradication of infected flocks in USA with biosecurity and biosurveillance measures that have been largely successful at minimizing *M. gallisepticum* infection outbreaks among the breeding stock of the turkey and chicken industries, in which outbreaks occur only in a sporadic nature [12, 10]. Despite the greater need for biosecurity under the high risk conditions generated by industry growth, control programs are frequently compromised by cost saving initiatives, furthermore, for short term return, investing in biosecurity is difficult to justify. Control of *M. gallisepticum* infection by chemotherapy is also reported as the most practical way to minimize economic losses [13], although it does not eliminate *M. gallisepticum* from the flock [17]. The most significant antimicrobial agent used for treatment and control of *M. gallisepticum* infection is Timicosin, a broad spectrum bacteriostatic tylosin synthesized for veterinary use only [13]. Other antibiotics for which *M. gallisepticum* is sensitive includes tetracyclines (oxytetracycline, chlortetracycline and doxycycline), macrolides (erythromycin, tylosin, spiramycin, lincomycin, and kitasamycin), quinolones (imequil, norfloxacin, enrofloxacin and danofloxacin) or tiamulin. Drugs that accumulate in high concentrations in the mucosal membranes of the respiratory and genitourinary tracts are often highly preferred [17], such as tiamulin and enrofloxacin [13].

Infected flock with virulent strains of *M. gallisepticum* early in life have been reported to have a lower incidence of vertical transmission compared to flocks during lay; although degree of resistance develops subsequent to infection, many flocks remain subclinical carrier after recovery [61]. Although control of avian mycoplasmosis infection by vaccination is limited due to few vaccination available [13], it is an alternative approach chosen for the control of *M. gallisepticum* infection especially in the layer industry due to the unique structures [12]. Effective control of *M. gallisepticum* infections is hindered by the organism's inherent ability to evade the host's immune system. Like other Mycoplasma species and pathogens of other genera, *M. gallisepticum* strains have the ability to change the expression of surface antigens and thereby to alter the ''antigenic profile'' presented to the host's immune system [62]. In addition, immune responses to *M. gallisepticum* are not well understood and complications toward

*M. gallisepticum* control include the organism's ability to transmit both vertically and horizontally and survive outside the host [32], and the lack of rapid and specific means of detection that differentiates field and vaccine strains.

Currently, there are 3 live *M. gallisepticum* vaccines approved and commercially available including F strain (FVAX-MG, Schering-Plough Animal Health), 6/85 (Mycovac-L, Intervet Inc.), and ts-11 (MG vaccine, Merial Select) and an inactivated bacterins vaccine used both in broiler and layer flocks. Although each is distinct, varying in pathogenicity, protection afforded, and transmissibility [63], each has been shown to effectively reduce losses associated with *M. gallisepticum* field strain challenge [57, 58, 60, 64] and reduction of egg production loss. The F strain was the first available attenuated live *M. gallisepticum* vaccine and has been described as the most economic in terms of initial cost and application-associated labor [65, 66]. However, due to the limitations of available live vaccines, alternative vaccines are being sought [12]. In conclusion, current means of controlling *M. gallisepticum* infections among avian species are limited. Beside the biosecurity and biosurveillance practices, which are practiced by different countries, only 3 vaccines (live-attenuated *M. gallisepticum*) have been approved for use in most countries.

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