

# Current status of vaccine research, development, and challenges of vaccines for *Mycoplasma gallisepticum*

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**ABSTRACT** *Mycoplasma gallisepticum* (MG) is an important avian pathogen that causes significant economic losses in the poultry industry. Surprisingly, the limited protection and adverse reactions caused by the vaccines, including live vaccines, bacterin-based (killed) vaccines, and recombinant viral vaccines is still a major concern. *Mycoplasma gallisepticum* strains vary in infectivity and virulence and infection may sometimes unapparent and goes undetected. Although extensive research has been carried out on the biology of this pathogen, information is lacking about the type of immune response that confers protection and selection of

appropriate protective antigens and adjuvants. Regardless of numerous efforts focused on the development of safe and effective vaccine for the control of MG, the use of modern DNA vaccine technology selected in silico approaches for the use of conserved recombinant proteins may be a better choice for the preparation of novel effective vaccines. More research is needed to characterize and elucidate MG products modulating MG-host interactions. These products could be used as a reference for the preparation and development of vaccines to control MG infections in poultry flocks.

**Key words:** vaccine, *Mycoplasma gallisepticum*, live vaccines, attenuated vaccines, new approaches

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## INTRODUCTION

*Mycoplasma gallisepticum* (MG) is an avian pathogen, belongs to the class *mollicutes* and characterized by a special feature, the lack of cell wall (Razin and Herrmann, 1998; Lu et al., 2017). The pathogen primarily infects chickens and turkeys (Ley, 2003), but also some other birds, such as house and wild finches (Ley et al., 1996; Dhondt et al., 2014) and geese (Benoina et al., 1988). It is well documented that MG is the causative agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys (Chin et al., 1991; Cecchini et al., 2007; Wijesurendra et al., 2015; Beaudet et al., 2017; Kanci et al., 2018). The disease is often slowly

developed and accompanied with severe inflammation in the respiratory tract of birds (Harry and Yoder, 1990). The pathogen often remains undetected in flocks and caused latent infections (Evan et al., 2005). Previous reports showed that factors such as high feeding density, heat and/cold stresses, excessive ammonia, accumulation of feces, fouling of the chicken house, excessive temperature differences, and sudden changes in climate can contribute to the spread and outbreak of disease (Hochachka and Dhondt, 2000; Ley, 2003). *Mycoplasma gallisepticum* infection caused major economic losses in terms of reduced weight gain, egg production and hatchability, downgrading carcass quality, and the infected birds become susceptible to other diseases (Beaudet et al., 2017; Ishfaq et al., 2019a). *Mycoplasma gallisepticum* is a continuing problem in poultry and efforts to prevent the losses in commercial flocks particularly in layer and breeders including bacterin-based vaccines, killed vaccines, and live vaccines. These efforts have been successful up to some extent in reducing the severity of respiratory diseases, maintaining constant egg production, controlling excess vaccine reactions,

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and reducing horizontal and vertical transmission (Butcher, 2002). Among these vaccines, some vaccines are semi-virulent, often have adverse effect, and provide only partial or transient immunity (Jacob et al., 2014, 2015; Peebles et al., 2014, 2015). Moreover, in view of the increasing antimicrobial resistance and decreasing effectiveness of antibiotics in controlling MG infection, and alarmingly, exacerbation of disease is often associated with vaccine improvement, the need for effective and novel vaccines has become even more necessary (Kleven et al., 1984; Whithear, 1996; Kleven, 2008). Table 1 shows the currently available vaccines against MG infection. Numerous studies so far, focused on live-attenuated vaccine, killed vaccines, bacterin-based, or recombinant proteins (Whithear, 1996; Hussein et al., 2007; Rabie and Amin Girh, 2020). This review assesses and summarizes the description of the problem, challenges, and vaccines under development or in use and highlighted developments that may be better for the preparation of effective vaccines in the future.

## CHALLENGES

Despite extensive research on the pathology of MG, there are several challenges that hinder the triumph of vaccines to prevent MG infection. Among these challenges are immune status of host animals, colonization of MG in host respiratory tract, presence of other respiratory pathogens, phase or antigenic variation that lead to immune escape from the host immune system and transmission of infection in the flocks (Levisohn et al., 1995; Staley and Bonneaud, 2015; Beaudet et al., 2017; Beaudet et al., 2019). Adhesion plays a pivotal role in the colonization and pathogenesis of *Mycoplasmas* infection (Henderson and Jensen, 2006). *Mycoplasma gallisepticum* adhere to the host cells with the help of a terminal bleb, which in turn damage cell architecture and the surface layer of epithelial cells lose its cilia, leading to increased chances of other secondary infections (Uppal and Chu, 1977; Bredt, 1979). Up till now, several adhesion proteins have been reported to involve in adhesion and colonization of MG. For instance, Glycolytic enzymes such as glyceraldehyde-3-phosphate

dehydrogenase (Alvarez et al., 2003; Dumke et al., 2011), PvpA (Yogev et al., 1994), GapA (Goh et al., 1998), and Crm A or *mgc3* (Yoshida et al., 2000; Indiková et al., 2013), MGC2 (Hnatow et al., 1998), pyruvate dehydrogenase- $\alpha$  and pyruvate dehydrogenase- $\beta$  (Qi et al., 2018), and OsmC-like protein MG1142 (Jenkins et al., 2007). The infection caused the release of catarrhal exudate from the goblet cells and lead to mucosal thickness of the respiratory epithelium and has the ability to invade various host tissues and cells such are chicken erythrocytes, fibroblasts, and HeLa-229 cells. This invasive capability contributes to the dissemination of MG to various distant sites in the host body and the successful establishment of persistent and recurrent infection (Baseman et al., 1995; Mohammed et al., 2007; Majumder et al., 2014; Rosales et al., 2017; Ishfaq et al., 2019b, 2019c). However, factors such as strain of MG, infected dose, passage, and host immunity also greatly affects the establishment of MG infection. Moreover, studies demonstrated that the host often failed to recognize MG because of its antigenic/phase variation of membrane surface proteins, which are associated with evasion from the host immune system leading to infection (Gorton and Geary, 1997; Markham et al., 1998; Glew et al., 2000; Mazin et al., 2014). *Mycoplasma gallisepticum* modulate host immune system through activation of toll-like receptors (TLR) including TLR-2/TLR-4/TLR-6, nod-like receptors, and NF- $\kappa$ B pathway (Takeda et al., 2002; Gaunson et al., 2006; Majumder et al., 2014; Li et al., 2019; Chen et al., 2020). Increased number of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes were found in chicken tracheal mucosa after 1 wk of infection (Gaunson et al., 2000, 2006). Another study reported that MG infection increased the expression of CXCL13, CXCL14, lymphotactin, RANTES, IL-1 $\beta$ , MIP-1 $\beta$ , and IFN- $\gamma$  gene in chicken trachea (Mohammed et al., 2007; Majumder and Silbart, 2016). Similarly, Beaudet et al., reported immune dysregulation in immune responses over a course of 7 D MG infection in chickens. Various inflammation and immune-related signaling pathways including TLR, mitogen-activated protein kinase, Jak-STAT, and the nucleotide oligomerization domain-like

**Table 1.** List of commercial vaccines available in the market.

S. no.	Name	Vaccine type and strain	Manufacturer
1	AviPro104 MG BACTERIN	Bacterin, Strain R	Lohmann Animal Health International, United States.
2	<i>Mycoplasma gallisepticum</i> vaccines	Live-attenuated vaccine, Strain R, Strain F-36	Shandong Lvdu Biosciences and Technology Co., Ltd. Some other companies in different cities of China.
3	VAXSAFE MG VACCINE	Live-attenuated vaccine, Strain ts-11	Bioproperties Pty LTD Australia
4	Mycoplasma Gallisepticum Vaccine	Live-attenuated vaccine, Strain ts-11	Boehringer Ingelheim Animal Health Business Unit
5	Nobilis MG 6/85	Live vaccine Strain 6/85	MSD Animal Health PHILIPPINES, INC.
6	CEVAC MG F	Live-attenuated vaccine, Strain F	Ceva Santé Animale, United Kingdom.
7	Mycoplasma Gallisepticum Vaccine (TS-11)	Live vaccine Strain ts-11	MERIAL, INC. (POULTRY BIOLOGICS), GAINESVILLE, GA, 30,503, United States.
8	MG-Bac Vaccine	Bacterin	United States.
9	Vaxsafe MG (Strain TS-11)	Live-attenuated vaccine, Strain ts-11	Rhone Ma Malaysia Sdn Bhd.

receptor pathways were differentially expressed, depicting the complex inflammatory response during MG infection (Beaudet et al., 2017). Some other in vivo and in vitro studies examined inflammatory response, induction of apoptosis, and triggering of autophagy following MG infection (Majumder and Silbart, 2016; Tian et al., 2016; Wu et al., 2019; Bao et al., 2020; Zhang et al., 2020). The interaction and modulation of host immune responses is of major importance in the preparation of effective vaccine against MG infection. More importantly, infection by other respiratory pathogens must be taken into account in preparation of vaccine against MG. For instance, several studies reported co-infection of MG with influenza virus, *Escherichia coli* and infectious bronchitis virus (Stipkovits et al., 2012; Sid et al., 2016; Bwala et al., 2018; Awad et al., 2019; Canter et al., 2019; Wu et al., 2019). A previous report assessed the efficacy of MG vaccine in a co-infection model and suggested that ts-11 and 6/85 provided protection up to some extent against virulent MG strain. The 2 vaccines provided nonspecific protection. Whereas, the ts-11 proved to be more effective than 6/85 vaccine in trachea, bursa, and air sacs but not in lungs (Bwala et al., 2018). In addition, a recombinant adenovirus-based vaccine candidate was developed and evaluated for co-infection against infectious bronchitis virus and MG in chickens. A recombinant adenovirus was constructed containing the TM-1 protein of MG (pBH-S1-TM-1-EGFP) and S1 spike glycoprotein of infectious bronchitis virus. The recombinant bivalent pBH-S1-TM-1-EGFP adenovirus construct vaccine provides potential protective effects against bronchitis and MG infection (Zhang et al., 2018). Therefore, preventive measures against MG infection and other respiratory pathogens must take into account by vaccination and/or antimicrobial agents. The use of a challenge model in vaccination trial that reproduces the disease also plays a crucial role. There are several other factors and challenges that affect the potency of vaccine such as strain of MG, dose, route, and age of animals that are necessary to be considered during developing a vaccine against MG infection (Levisohn and Kleven, 2000; Branton et al., 2002; Evan et al., 2005; Evans et al., 2009). These challenges are often ignored in the preparation of vaccine, and therefore, care must be taken to overcome these challenges to successfully prevent and eradicate this devastating organism.

## MG VACCINE CANDIDATES

Till now, the vaccines available for MG infection consist of live-attenuated vaccines, inactivated vaccines such as oil-emulsion bacterins. Some of the commonly used vaccines available in the world are listed in Table 1. Adler for the first-time suggested vaccination against *mycoplasmas* infections in poultry in 1960 (Adler et al., 1960). Later on, Luginbuhl et al. and Fabricant used it in field trials (Luginbuhl et al., 1967; Fabricant, 1975). A large number of studies focused on vaccination against MG infection, which is mostly used

in commercial flocks, particularly in broiler breeder and layers (Whithear, 1996). A study proved that immunization with vaccine partially depended on bursal-dependent lymphoid cells but not on thymus (Lam and Lin, 1984). Recently, a study reported the possible differences in host response between F-strain-based vaccines. The genomes of the F99 parent strains and AviPro vaccine were sequenced for comparison with the already sequenced F-strain vaccine (Leigh et al., 2019). Control of MG infection needs investigation of MG-infection free flocks. For this reason, rapid and cost-effective PCR-based assays were developed for the simultaneous detection of ts-11, 6/85, and F vaccine strains from field isolates (Sulyok et al., 2019). There are 2 main candidates of MG vaccine known as live-attenuated vaccine and killed vaccine/bacterins.

## Live-Attenuated Vaccines

Three live MG vaccines were commercially approved including 6/85 strain, ts-11, and F strain. The 3 strains have effectively reduced losses related with MG infection in the field (Carpenter et al., 1981; Whithear et al., 1990a, 1990b; Evans and Hafez, 1992) and different in protection afforded, pathogenicity, and transmissibility (Branton et al., 2002). The F-strain vaccine was found to be efficacious and virtually nonpathogenic under field conditions (Levisohn and Kleven, 1981). Another study reported that adjusting for strain differences, the average egg production of uninfected flocks, MG (-), was 8.7 eggs hen housed greater than that of MG (vacc) flocks; production was 222.9 and 214.2, respectively (Carpenter et al., 1981). In addition, F strain vaccination reduced antibiotic requirements and mortality (Luginbuhl et al., 1967; Branton and Deaton, 1985; Self, 2003). F strain is preferable on sites where wild-type MG is very virulent and has the potential to displace a virulent MG strain in a commercial flock (Kleven et al., 1990). However, the disadvantages associated with the F strain is pathogenic and transmissible to broilers and turkeys (Vance et al., 2008). The other MG vaccines, ts-11 and 6/85, were found safer because of less pathogenicity and transmissibility toward young broilers and turkeys. While, these strains were found less effective in field challenge than F strain (Abd-El-Motelib and Kleven, 1993; US Animal Health Association, 2002). The effects of 6/85 or ts-11 strain vaccine on production have not been extensively investigated. It has been demonstrated that a single vaccination is often enough for a layer flock. Both 6/85 and ts-11 are preferable to F strain vaccine because of their low potential spread and risk to nearby unvaccinated flocks and safety characteristics. However, secondary vaccinations have been done in previously vaccinated flocks. For instance, a layer flock vaccinated with ts-11 or 6/85 have been revaccinated because of MG breaks with F strain (Gingerich, 2002). The F strain vaccine can be administered as early as 2 wk before infection by intranasal and intraocular and by coarse spray (Levisohn and Kleven, 2000). 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 et al., 2003). Further research can investigate this strain as an effective and safe vaccine for both chickens and turkeys. However, a limiting factor is the ability of these strains used in vaccines to revert to their pathogenic counterparts. Recently, vectors or carriers have gained immense importance in protecting against pathogens that are localized in the mucosal respiratory tract of the host (Mingozzi and High, 2013; Athanasopoulos et al., 2017; Humphreys and Sebastian, 2018). Bacteria such as both gram-negative and gram-positive have been used as carriers of recombinant antigens. Nonpathogenic and attenuated bacteria may be used as vectors, but nonpathogenic species are preferred (Liljeqvist and Stahl, 1999). The development of recombinant vaccines by cloning and identification of important MG surface antigens and colonization factor, development of expression and transformation strategies bewitch the interest of scientist (Zhang et al., 2010, 2018; Shil et al., 2011). Gene transfer is accomplished in mycoplasma species by electroporation (Liu et al., 2000; Pour-El et al., 2002; Mudah-Orenstein et al., 2003) and *Enterococcus faecalis*-mediated conjugation (Ruffin et al., 2002). The transposons Tn4001 and Tn916 were found functional in mycoplasma (Knudtson and Minion, 1993; Cao et al., 1994; Dybvig et al., 2000; Pour-El et al., 2002; Mudah-Orenstein et al., 2003) and can be used in mutant construction, protein functional analysis, cellular tagging, and gene expression (Dybvig and Voelker, 1996). For example, wild-type GapA, multiple genes encoding surface antigens and putative colonization factors have been characterized and cloned, and LacZ fusion protein studies were completed to date (Hnatow et al., 1998; Boguslavsky et al., 2000; Papazisi et al., 2002a; Liu et al., 2002; Jenkins et al., 2007; Yavlovich et al., 2007; Qi et al., 2018). The use of these proteins and technologies in recombinant vaccine can provide an effective and safe MG vaccine, but it would be complicated because of variable expression of these proteins. Recently, an attenuated and genetically modified MG strain known as GT5 was developed in laboratory (Mohammed et al., 2007; Gates et al., 2008). An in vivo study investigated that GT5 vaccinated chickens were from MG-induced disease. Specifically, the infection-associated lesions in trachea and colonization abilities of virulent MG strain were reduced (Papazisi et al., 2002b). Different vehicles serve for expression of MG antigens and recognized by host immune system. To this end, live-attenuated viruses also used as vehicles. For instance, USDA-approved recombinant fowl pox virus encoding genes for MG and used in turkeys and chickens. Studies demonstrated that chickens vaccinated with the vaccine containing vehicle fowl pox virus produced desirable effects and protected chickens from MG challenge (Evan et al., 2005). However, further investigation is necessary to determine the level of protection by vaccine in which virus is used as a vehicle. Another recombinant vaccine is the expression

of MG antigens in nonpathogenic bacteria, with the benefits of vector effectivity, stability and safety, and selection of a proper host. The limitation is that the nonpathogenic strains have poorly characterized genetic systems that are residing in the avian respiratory tract. For example, MG was considered as nonpathogenic, isolated from the respiratory and reproductive tract of chickens (Wang et al., 1990), and evaluated as a possible vector for MG antigen expression (Evan et al., 2005). Recently, the efficacy of K-strain as a live-attenuated vaccine was reported to be safe in chickens (Ferguson-Noel and Williams, 2015). Moreover, another study compared the protection elicited by K-strain and ts-11 in layers and efficacy of K-strain and F-strain in broilers. Their results showed that K-strain vaccine has equivalent efficacy and potential to protect vaccinated birds from field challenge (Ferguson-Noel and Williams, 2015). F strain vaccine was administered in ovo to layers to reduce cost of labor and evaluate it as an effective vaccination to control MG infection in commercial layer flocks. However, the results showed higher embryo mortality rate and further research is needed to investigate and optimize the dose and immune response offered by this method (Elliott et al., 2017). The short-term and long-term efficacies of MG vaccines, strains ts-11 and 6/85, were demonstrated in chickens through aerosol and eye drop inoculations. The results showed the level of protection offered by the 2 vaccines were found similar after 36 wk postvaccination (Noormohammadi and Whithear, 2019). A live-attenuated vaccine named Vax-safe MG (strain ts-11) has been reported to provide protection in chickens. The study proved that the vaccine can also be used in turkeys and found safe and efficacious in 3-wk-old turkeys (Kanci et al., 2018). While, limited information is available about the use of *Mycoplasma gallinarum* as a vector and host, and therefore, further studies are encouraged to scrutinize the organism's genetic system in detail.

### **Killed/Inactivated Vaccines/Bacterins**

More complex vaccines consist of 1 or more purified antigens, killed pathogens, or bacterins with an oil adjuvant that stimulate the immune response. These vaccines are protective, but their use is limited because of cost (Ley, 2003). Bacterins are inactivated so these vaccines are safer than live vaccines. Studies demonstrated that bacterins were efficacious in prevention of respiratory lesions in chickens and proved beneficial in reducing transmission and production losses (Rimler et al., 1978; Yoder, 1979, 1983; Hildebrand, 1985). However, some researchers have demonstrated that bacterins were not so effective as live vaccines, as bacterins can temporarily control MG infection and have negligible effect in protecting host respiratory system from MG (Kleven, 1985). Therefore, bacterins are of minimal value in commercial flocks where long-term control of MG infection is needed. In addition, bacterins are costly and expensive and difficult to vaccinate as each bird need individual

vaccination, and often 2 doses are needed as repeated vaccination found more effective than single dose.

## CONCLUSIONS AND FUTURE DIRECTIONS

The use of vaccines is an alternative means for the prevention and control of MG, particularly in commercial layer and breeder flocks with the benefits of avoiding considerable economic losses. Live-attenuated vaccines such as F strain, ts-11 strain, and 6/85 strain and inactivated vaccines such as bacterins are commonly used in commercial flocks, but the sudden onset of MG infection could occur in some circumstances. For instance, latent infections and transmission both by horizontal and vertical means are possible among different and same flocks. Therefore, biosurveillance and biosecurity practices are recommended to carry out for the complete eradication of infected flocks. Live vaccines often showed pathogenicity and adverse side-effects, whereas bacterins have high cost, and often repeated doses are required to boost avian immune system. Hence, new novel recombinant vaccines are needed to be developed which are more efficacious and less expensive. Limited information is available about the type of immune response, key protective antigens, and optimal adjuvant formulations. Research on all possible antigens for their potential through reverse vaccinology (Rappuoli, 2000) may be needed as this is more advanced method to identify recombinant proteins that provide protection against experimental challenge (Perez-Casal et al., 2015; Nkando et al., 2016). Research demonstrated that MG vaccination in turkeys and chickens produced a high antibody titer including increased CD4<sup>+</sup> and CD8<sup>+</sup> expressing lymphocytes in trachea. This suggested that these cells contributing toward the pathogenesis of the disease (Wijesurendra et al., 2017). Therefore, agents which enhance the production of humoral immune response including lymphocytes production could be considered for the possible protection trial of MG infection in future studies. In addition, research on in silico approaches had not yet been reported for MG. While, some researchers used in silico approaches for the identification of virulence candidates for other *Mycoplasma* species such as *Mycoplasma pneumoniae* type 2a strain 309 and *Mycoplasma agalactiae* (Shahbaaz et al., 2015; Gaurivaud et al., 2016). Thus, future studies are recommended on in silico approaches for the development of effective vaccines. Besides, several studies focused on adjuvants such as chitosan and other adjuvants to boost host immune responses infected with MG (Barbour and Newman, 1990; Limsatanun et al., 2018). The role of adjuvants in enhancing immune responses cannot be neglected. Adjuvants increased the immunogenic responses of antigens and enhanced the efficacy and potency of vaccines (Bastola et al., 2017). Recently, studies reviewed the fundamental knowledge for the application of adjuvants in vaccines (Kwissa et al., 2007; Riese et al., 2013; Jin et al., 2019). However, limited information is available about adjuvants used for MG vaccine preparation (Barbour and Newman, 1989, 1990; Hussein et al.,

2007; Limsatanun et al., 2018). Hence, it is of prime importance to screen new and more effective adjuvants for potential benefits and effective vaccine preparation to control MG infection. Moreover, a strong support is needed from funding organizations to invest more in research associated with vaccine preparation against MG infections, as these researches are necessary for the elucidation of molecular mechanisms required for the development of effective vaccines or new treatments to protect from MG infection.

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## REFERENCES

- Abd-El-Motelib, T. Y., and S. H. Kleven. 1993. A comparative study of *Mycoplasma gallisepticum* vaccines in young chickens. *Avian Dis.* 37:981–987.
- Adler, H. E., D. McMartin, and M. Shifrine. 1960. Immunization against *Mycoplasma* infections of poultry. *Am. J. Vet. Res.* 21:482–485.
- Alvarez, R. A., M. W. Blaylock, and J. B. Baseman. 2003. Surface localized glyceraldehyde-3-phosphate dehydrogenase of *Mycoplasma genitalium* binds mucin. *Mol. Microbiol.* 48:1417–1425.
- Awad, N., M. I. Abd El-Hamid, Y. M. Hashem, A. M. Erfan, B. A. Abdelrahman, and H. I. Mahmoud. 2019. Impact of single and mixed infections with *Escherichia coli* and *Mycoplasma gallisepticum* on Newcastle disease virus vaccine performance in broiler chickens: an in vivo perspective. *J. Appl. Microbiol.* 127:396–405.
- Athanasopoulos, T., M. M. Munye, and R. J. Yáñez-Muñoz. 2017. Nonintegrating gene therapy vectors. *Hematol. Oncol. Clin. North Am.* 31:753–770.
- Bao, J., Z. Wu, M. Ishfaq, Y. Miao, R. Li, A. C. Clifton, L. Ding, and J. Li. 2020. Comparison of experimental infection of Normal and Immunosuppressed chickens with *Mycoplasma gallisepticum*. *J. Comp. Pathol.* 175:5–12.
- Barbour, E. K., and J. A. Newman. 1989. Comparison of *Mycoplasma gallisepticum* subunit and whole organism vaccines containing different adjuvants by western immunoblotting. *Vet. Immunol. Immunopathol.* 22:135–144.
- Barbour, E. K., and J. A. Newman. 1990. Preliminary data on efficacy of *Mycoplasma gallisepticum* vaccines containing different adjuvants in laying hens. *Vet. Immunol. Immunopathol.* 26:115–123.
- Baseman, J. B., M. Lange, N. L. Criscimagna, J. A. Giron, and C. A. Thomas. 1995. Interplay between mycoplasmas and host target cells. *Microb. Pathog.* 19:105–116.
- Bastola, R., G. Noh, T. Keum, S. Bashyal, J. E. Seo, J. Choi, Y. Oh, Y. Cho, and S. Lee. 2017. Vaccine adjuvants: smart components to boost the immune system. *Arch. Pharm. Res.* 40:1238–1248.
- Beaudet, J., E. R. Tulman, K. Pflaum, J. A. Canter, L. K. Silbart, and S. J. Geary. 2019. Immunologic pathways in protective versus Maladaptive host responses to attenuated and pathogenic strains of *Mycoplasma gallisepticum*. *Infect. Immun.* 87:e00613–e00618.
- Beaudet, J., E. R. Tulman, K. Pflaum, X. Liao, G. F. Kutish, S. M. Szczepanek, L. K. Silbart, and S. J. Geary. 2017. Transcriptional profiling of the chicken tracheal response to virulent *Mycoplasma gallisepticum* strain Rlow. *Infect. Immun.* 85:e00343–17.
- Benoina, D., T. Tadina, and D. Dorrer. 1988. Natural infection of geese with *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and egg transmission of the mycoplasmas. *Avian Pathol.* 17:925–928.
- Boguslavsky, S., D. Menaker, I. Lysnyansky, T. Liu, S. Levisohn, R. Rosengarten, M. García, and D. Yogev. 2000. Molecular

- characterization of the *Mycoplasma gallisepticum* pvpA gene which encodes a putative variable cytoadhesin protein. *Infect. Immun.* 68:3956–3964.
- Branton, S. L., S. M. Bearson, B. Bearson, B. D. Lott, W. R. Maslin, S. D. Collier, G. T. Pharr, and D. L. Boykin. 2002. The effects of 6/85 live *Mycoplasma gallisepticum* vaccine in commercial layer hens over a 43-week laying cycle on egg production, selected egg quality parameters, and egg size distribution when challenged before beginning of lay. *Avian Dis.* 46:423–428.
- Branton, S. L., and J. W. Deaton. 1985. Egg production, egg weight, eggshell strength, and mortality in three strains of commercial layers vaccinated with F strain *Mycoplasma gallisepticum*. *Avian Dis.* 29:832–837.
- Bredt, W. 1979. Motility. Pages in 141–155 in M. F. Barile M. F. and S. Razin eds, Vol. 1. Academic Press, New York.
- Butcher, G. D. 2002. *Mycoplasma Gallisepticum-A* continuing problem in commercial poultry. Accessed July 2020. <https://edis.ifa.ufl.edu/ps034>.
- Bwala, D. G., P. Solomon, N. Duncan, D. B. R. Wandrag, and C. Abolnik. 2018. Assessment of *Mycoplasma-gallisepticum* vaccine efficacy in a co-infection challenge model with QX-like infectious bronchitis virus. *Avian Pathol.* 47:261–270.
- Cao, J., P. A. Kapke, and F. C. Minion. 1994. Transformation of *Mycoplasma gallisepticum* with Tn916, Tn4001, and integrative plasmid vectors. *J. Bacteriol.* 176:4459–4462.
- Canter, J. A., E. R. Tulman, J. Beaudet, D. H. Lee, M. May, S. M. Szczepanek, and S. J. Geary. 2019. Transcriptional and Pathological host responses to Coinfection with virulent or attenuated *Mycoplasma gallisepticum* and low-pathogenic avian influenza A virus in chickens. *Infect. Immun.* 88:e00607–e00619.
- Carpenter, T. E., E. T. Mallinson, K. F. Miller, R. F. Gentry, and L. D. Schwartz. 1981. Vaccination with F-strain *Mycoplasma gallisepticum* to reduce production losses in layer chickens. *Avian Dis.* 25:404–409.
- Cecchini, K. R., T. S. Gorton, and S. J. Geary. 2007. Transcriptional responses of *Mycoplasma gallisepticum* strain R in association with eukaryotic cells. *J. Bacteriol.* 189:5803–5807.
- Chen, C., J. Li, W. Zhang, S. Shah, and M. Ishfaq. 2020. *Mycoplasma gallisepticum* triggers immune damage in the chicken thymus by activating the TLR-2/MyD88/NF- $\kappa$ B signaling pathway and NLRP3 inflammasome. *Vet. Res.* 51:52.
- Chin, R. P., B. M. Daft, C. U. Meteyer, and R. Yamamoto. 1991. Meningoencephalitis in commercial meat turkeys associated with *Mycoplasma gallisepticum*. *Avian Dis.* 35:986–993.
- Dhondt, A. A., J. C. DeCoste, D. H. Ley, and W. M. Hochachk. 2014. Diverse wild bird host range of *Mycoplasma gallisepticum* in eastern North America. *PLoS One* 9:e103553.
- Dumke, R., M. Hausner, and E. Jacobs. 2011. Role of *Mycoplasma pneumoniae* glyceraldehyde-3 phosphate dehydrogenase (GAPDH) in mediating interactions with the human extracellular matrix. *Microbiology* 157:2328–2338.
- Dybvig, K., C. T. French, and L. L. Voelker. 2000. Construction and use of derivatives of transposon Tn4001 that function in *Mycoplasma pulmonis* and *Mycoplasma arthritis*. *J. Bacteriol.* 182:4343–4347.
- Dybvig, K., and L. L. Voelker. 1996. Molecular biology of mycoplasmas. *Annu Rev Microbiol* 50:25–57.
- Elliott, K., S. Branton, J. Evans, P. Gerard, and E. Peebles. 2017. Layer chicken embryo survival to hatch when administered an in ovo vaccination of strain F *Mycoplasma gallisepticum* and locations of bacteria prevalence in the newly hatched chick. *Poult. Sci.* 96:3879–3884.
- Evans, J. D., S. L. Branton, and S. A. Leigh. 2009. Effect of dosage and vaccination route on transmission of a live attenuated *Mycoplasma gallisepticum* vaccine: a broiler model. *Avian Dis.* 53:416–420.
- Evan, J. D., S. A. Leigh, S. L. Branton, S. D. Collier, G. T. Pharr, and S. M. D. Bearson. 2005. *Mycoplasma gallisepticum*: current and developing means to control the avian pathogen. *J. Appl. Poult. Res.* 14:757–763.
- Evans, R. D., and Y. S. Hafez. 1992. Evaluation of a *Mycoplasma gallisepticum* strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. *Avian Dis.* 36:197–201.
- Fabricant, J. 1975. Immunization of chickens against *Mycoplasma gallisepticum* infection. *Am. J. Vet. Res.* 36:566–567.
- Ferguson, N. M., D. Hermes, V. A. Leiting, and S. H. Kleven. 2003. Characterization of a naturally occurring infection of a *Mycoplasma gallisepticum* house finch-like strain in Turkey breeders. *Avian Dis.* 47:523–530.
- Ferguson-Noel, N. M., and S. M. Williams. 2015. The efficacy of *Mycoplasma gallisepticum* K-strain live vaccine in broiler and layer chickens. *Avian Pathol.* 44:75–80.
- Gates, A. E., S. Frasca, A. Nyaoke, T. S. Gorton, L. K. Silbart, and S. J. Geary. 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.
- Gaunson, J. E., C. J. Philip, K. G. Whithear, and G. F. Browning. 2000. Lymphocytic infiltration in the chicken trachea in response to *Mycoplasma gallisepticum* infection. *Microbiology* 146:223–229.
- Gaunson, J. E., C. J. Philip, K. G. Whithear, and G. F. Browning. 2006. The cellular immune response in the tracheal mucosa to *Mycoplasma gallisepticum* in vaccinated and unvaccinated chickens in the acute and chronic stages of disease. *Vaccine* 24:2627–2633.
- Gaurivaud, P., E. Baranowski, C. Pau-Roblot, E. Sagné, C. Citti, and F. Tardy. 2016. *Mycoplasma agalactiae* Secretion of  $\beta$ -(1 $\rightarrow$ 6)-Glucan, a Rare Polysaccharide in Prokaryotes, is Governed by high-frequency phase variation. *Appl. Environ. Microbiol.* 82:370–3383.
- Gingerich, E. 2002. Vaccine and vaccination for *Mycoplasma gallisepticum* (MG), Proc. Pages 2–5 in Cornell Poult. Conf., Ithaca, NY.
- Glew, M. D., G. F. Browning, P. F. Markham, and I. D. Walker. 2000. pMGA phenotypic variation in *Mycoplasma gallisepticum* occurs in vivo and is mediated by trinucleotide repeat length variation. *Infect. Immun.* 68:6027–6033.
- Goh, M. S., T. S. Gorton, M. H. Forsyth, K. E. Troy, and S. J. Geary. 1998. Molecular and biochemical analysis of a 105 kDa *Mycoplasma gallisepticum* cytoadhesin (GapA). *Microbiology* 144:2971–2978.
- Gorton, T. S., and S. J. Geary. 1997. Antibody-mediated selection of a *Mycoplasma gallisepticum* phenotype expressing variable proteins. *FEMS Microbiol. Lett.* 155:31–38.
- Harry, W., and Yoder, Jr. 1990. Chapter- avian mycoplasmas. Pages 333–341 in Diagnostic Procedure in Veterinary Bacteriology and Mycology. G. R. Carter and John R. Cole, Jr, eds. 5th ed. Academic Press, San Diego, CA.
- Henderson, G. P., and G. J. Jensen. 2006. Three-dimensional structure of *Mycoplasma pneumoniae*'s attachment organelle and a model for its role in gliding motility. *Mol. Microbiol.* 60:376–385.
- Hildebrand, D. 1985. Immunology and prophylaxis associated with the use of a *Mycoplasma gallisepticum* bacterin in chickens. *Clin. Vet. (Milano)* 108:89–94.
- Hnatow, L. L., C.L. Keeler, Jr, L. L. Tessmer, K. Czymmek, and J. E. Dohms. 1998. Characterization of MGC2, a *Mycoplasma gallisepticum* cytoadhesin with homology to the *Mycoplasma pneumoniae* 30-kilodalton protein P30 and *Mycoplasma genitalium* P32. *Infect. Immun.* 66:3436–3442.
- Hochachka, W. M., and A. A. Dhondt. 2000. Density-dependent decline of host abundance resulting from a new infectious disease. *Proc. Natl. Acad. Sci. USA.* 97:5303–5306.
- Humphreys, I. R., and S. Sebastian. 2018. Novel viral vectors in infectious diseases. *Immunology* 153:1–9.
- Hussein, A., T. M. El-Shaib, S. M. Saoud, N. A. Shalaby, H. Sultan, and A. M. Ragab. 2007. Protective immune response of *Mycoplasma gallisepticum* vaccines in poultry. *Egypt. J. Immunol.* 14:93–99.
- Indiková, I., P. Much, L. Stipkovits, K. Siebert-Gulle, M. P. Szostak, R. Rosengarten, and C. Citti. 2013. Role of the GapA and CrmA cytoadhesins of *Mycoplasma gallisepticum* in promoting virulence and host colonization. *Infect. Immun.* 81:1618–1624.
- Ishfaq, M., W. Zhang, W. Hu, S. Waqas Ali Shah, Y. Liu, J. Wang, Z. Wu, I. Ahmad, and J. Li. 2019a. Antagonistic effects of Baicalin on *Mycoplasma gallisepticum*-induced inflammation and apoptosis by restoring energy metabolism in the chicken lungs. *Infect. Drug Resist.* 12:3075–3089.
- Ishfaq, M., W. Zhang, S. W. Ali Shah, Z. Wu, J. Wang, L. Ding, and J. Li. 2019b. The effect of *Mycoplasma gallisepticum* infection on energy metabolism in chicken lungs: through oxidative stress and inflammation. *Microb. Pathog.* 138:103848.
- Ishfaq, M., C. Chen, J. Bao, W. Zhang, Z. Wu, J. Wang, Y. Liu, E. Tian, S. Hamid, R. Li, L. Ding, and J. Li. 2019c. Baicalin

- ameliorates oxidative stress and apoptosis by restoring mitochondrial dynamics in the spleen of chickens via the opposite modulation of NF- $\kappa$ B and Nrf2/HO-1 signaling pathway during *Mycoplasma gallisepticum* infection. *Poult. Sci.* 98:6296–6310.
- Jacob, R., S. L. Branton, J. D. Evans, S. A. Leigh, and E. D. Peebles. 2014. Effects of live and killed vaccines against *Mycoplasma gallisepticum* on the performance characteristics of commercial layer chickens. *Poult. Sci.* 93:1403–1409.
- Jacob, R., S. L. Branton, J. D. Evans, S. A. Leigh, and E. D. Peebles. 2015. Effects of different vaccine combinations against *Mycoplasma gallisepticum* on the internal egg and eggshell characteristics of commercial layer chickens 1,2,3. *Poult. Sci.* 94:912–917.
- Jenkins, C., S. J. Geary, M. Gladd, and S. P. Djordjevic. 2007. The *Mycoplasma gallisepticum* OsmC-like protein MG1142 resides on the cell surface and binds heparin. *Microbiology* 153:1455–1463.
- Jin, Z., S. Gao, X. Cui, D. Sun, and K. Zhao. 2019. Adjuvants and delivery systems based on polymeric nanoparticles for mucosal vaccines. *Int. J. Pharm.* 572:118731.
- Kanci, A., D. S. Wijesurendra, N. K. Wawegama, G. J. Underwood, A. H. Noormohammadi, P. F. Markham, and G. F. Browning. 2018. Evaluation of *Mycoplasma gallisepticum* (MG) ts-304 vaccine as a live attenuated vaccine in turkeys. *Vaccine* 36:2487–2493.
- Kwissa, M., S. P. Kasturi, and B. Pulendran. 2007. The science of adjuvants. *Expert Rev. Vaccin.* 6:673–684.
- Kleven, S. H., J. R. Glisson, M. Y. Lin, and F. D. Talkington. 1984. Bacterins and vaccines for the control of *Mycoplasma gallisepticum*. *Isr. J. Med. Sci.* 20:989–991.
- Kleven, S. H. 1985. Tracheal populations of *Mycoplasma gallisepticum* after challenge of bacterin-vaccinated chickens. *Avian Dis.* 29:1012–1017.
- Kleven, S. H., M. I. Khan, and R. Yamamoto. 1990. Fingerprinting of *Mycoplasma gallisepticum* strains isolated from multiple-age layers vaccinated with live F strains. *Avian Dis.* 34:984–990.
- Kleven, S. H. 2008. Control of avian mycoplasma infections in commercial poultry. *Avian Dis.* 52:367–374.
- Knudtson, K. L., and F. C. Minion. 1993. Construction of Tn4001lac derivatives to be used as promoter probe vectors in mycoplasmas. *Gene* 137:217–222.
- Lam, K., and W. Lin. 1984. Resistance of chickens immunized against *Mycoplasma gallisepticum* is mediated by bursal dependent lymphoid cells. *Vet. Microbiol.* 9:509–514.
- Leigh, S. A., J. D. Evans, and S. L. Branton. 2019. Complete genome sequences of two vaccine strains and one field isolate of *Mycoplasma gallisepticum*. *Microbiol. Resour. Announc.* 8:e01237-19.
- Levisohn, S., and S. H. Kleven. 1981. Vaccination of chickens with nonpathogenic *Mycoplasma gallisepticum* as a means for displacement of pathogenic strains. *Isr. J. Med. Sci.* 17:669–673.
- Levisohn, S., R. Rosengarten, and D. Yogeve. 1995. In vivo variation of *Mycoplasma gallisepticum* antigen expression in experimentally infected chickens. *Vet. Microbiol.* 45:219–231.
- Levisohn, S., and S. H. Kleven. 2000. Avian mycoplasmosis (*Mycoplasma gallisepticum*). *Rev. Sci. Tech.* 19:425–442.
- Ley, D. H., J. E. Berkhoff, and J. M. McLaren. 1996. *Mycoplasma gallisepticum* isolated from house finches (*Carpodacus mexicanus*) with conjunctivitis. *Avian Dis.* 40:480–483.
- Ley, D. H. 2003. *Mycoplasma gallisepticum* infection. Pages 722–743 in *Diseases of Poultry*. Y. M. Saif, ed. 11th ed. Iowa State Press, Ames, IA.
- Li, J., Z. Qiao, W. Hu, W. Zhang, S. W. A. Shah, and M. Ishfaq. 2019. Baicalin mitigated *Mycoplasma gallisepticum*-induced structural damage and attenuated oxidative stress and apoptosis in chicken thymus through the Nrf2/HO-1 defence pathway. *Vet. Res.* 50:83.
- Liljeqvist, S., and S. Stahl. 1999. Review article: production of recombinant subunit vaccines: protein immunogens, live delivery systems and nucleic acid vaccines. *J. Biotechnol.* 73:1–33.
- Limsatanun, A., J. Sasipreeyajan, and S. Pakpinyo. 2018. Chitosan-adjuvanted *Mycoplasma gallisepticum* bacterin via intraocular administration enhances *Mycoplasma gallisepticum* protection in commercial layers. *Poult. Sci.* 97:1934–1940.
- Liu, L., K. Dybvig, V. S. Panangala, V. L. Van Santen, and C. T. French. 2000. GAA trinucleotide repeat region regulates M9/pMGA gene expression in *Mycoplasma gallisepticum*. *Infect. Immun.* 68:871–876.
- Liu, L., V. S. Panangala, and K. Dybvig. 2002. Trinucleotide GAA repeats dictate pMGA gene expression in *Mycoplasma gallisepticum* by affecting spacing between flanking regions. *J. Bacteriol.* 184:1335–1339.
- Lu, Z., D. Xie, Y. Chen, E. Tian, I. Muhammad, X. Chen, Y. Miao, W. Hu, Z. Wu, H. Ni, J. Xin, Y. Li, and J. Li. 2017. TLR2 mediates autophagy through ERK signaling pathway in *Mycoplasma gallisepticum*-infected RAW264.7 cells. *Mol. Immunol.* 87:161–170.
- Luginbuhl, R. E., M. E. Tourtellotte, and M. N. Frazier. 1967. *Mycoplasma gallisepticum* - control by immunization. *Ann. NY. Acad. Sci.* 143:234–238.
- Majumder, S., and L. K. Silbart. 2016. Interaction of *Mycoplasma gallisepticum* with chicken tracheal epithelial cells contributes to macrophage chemotaxis and activation. *Infect. Immun.* 84:266–274.
- Majumder, S., F. Zappulla, and L. K. Silbart. 2014. *Mycoplasma gallisepticum* Lipid associated membrane proteins up-regulate inflammatory genes in chicken tracheal epithelial cells via TLR-2 Ligation through an NF- $\kappa$ B dependent pathway. *PLoS One.* 9:e112796.
- Markham, P. F., M. D. Glew, G. F. Browning, K. G. Whithear, and I. D. Walker. 1998. Expression of two members of the pMGA gene family of *Mycoplasma gallisepticum* oscillates and is influenced by pMGA-specific antibodies. *Infect. Immun.* 66:2845–2853.
- Mazin, P. V., G. Y. Fisunov, A. Y. Gorbachev, K. Y. Kapitskaya, I. A. Altukhov, T. A. Semashko, D. G. lexeev, and V. M. Govorun. 2014. Transcriptome analysis reveals novel regulatory mechanisms in a genome-reduced bacterium. *Nucleic Acids Res.* 42:13254–13268.
- Mingozzi, F., and K. A. High. 2013. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood* 122:23–36.
- Mohammed, J., S. Frasca, Jr, K. Cecchini, D. Rood, A. C. Nyaoke, S. J. Geary, and L. K. Silbart. 2007. Chemokine and cytokine gene expression profiles in chickens inoculated with *Mycoplasma gallisepticum* strains Rlow or GT5. *Vaccine* 25:8611–8621.
- Mudahi-Orenstein, S., S. Levisohn, S. J. Geary, and D. Yogeve. 2003. Cytadherence deficient mutants of *Mycoplasma gallisepticum* generated by transposon mutagenesis. *Infect. Immun.* 71:3812–3820.
- Nkando, I., J. Perez-Casal, M. Mwirigi, T. Prysliak, T. Maina, H. Townsend, J. Kuria, J. Mugambi, R. Soi, A. Liljander, J. Jores, V. Gerds, A. Potter, J. Naessens, and H. Wesonga. 2016. Recombinant *Mycoplasma mycoides* proteins elicit protective immune responses against contagious bovine pleuropneumonia. *Vet. Immunol. Immunopathol.* 171:103–114.
- Noormohammadi, A. H., and K. G. Whithear. 2019. Comparison of the short-term and long-term efficacies of the *Mycoplasma gallisepticum* vaccines ts-11 and 6/85. *Avian Pathol.* 48:238–244.
- Papazisi, L., S. Frasca, Jr, M. Gladd, X. Liao, D. Yogeve, and S. Geary. 2002a. GapA and CrmA coexpression is essential for *Mycoplasma gallisepticum* cytodherence and virulence. *Infect. Immun.* 70:6839–6845.
- Papazisi, L., L. K. Silbart, S. Frasca, D. Rood, X. Liao, M. Gladd, M. A. Javed, and S. J. Geary. 2002b. A modified live *Mycoplasma gallisepticum* vaccine to protect chickens from respiratory disease. *Vaccine* 20:3709–3719.
- Peebles, E. D., R. Jacob, S. L. Branton, and P. D. Gerard. 2014. Effects of *Mycoplasma gallisepticum* vaccination on serum  $\alpha$ -1-acid glycoprotein concentrations in commercial layer chickens. *Poult. Sci.* 93:1396–1402.
- Peebles, E. D., R. Jacob, S. L. Branton, J. D. Evans, S. A. Leigh, and P. D. Gerard. 2015. Effects of different vaccine combinations against *Mycoplasma gallisepticum* on the digestive and reproductive organ characteristics of commercial egg-laying hens. *Poult. Sci.* 94:2898–2904.
- Perez-Casal, J., T. Prysliak, T. Maina, Y. Wang, H. Townsend, E. Berverov, I. Nkando, H. Wesonga, A. Liljander, J. Jores, J. Naessens, V. Gerds, and A. Potter. 2015. Analysis of immune responses to recombinant proteins from strains of *Mycoplasma mycoides* subsp. *mycoides*, the causative agent of contagious bovine pleuropneumonia. *Vet. Immunol. Immunopathol.* 168:103–110.

- Pour-El, I., C. Adams, and F. C. Minion. 2002. Construction of mini-Tn4001tet and its use in *Mycoplasma gallisepticum*. *Plasmid* 47:129–137.
- Qi, J., F. Zhang, Y. Wang, T. Liu, L. Tan, S. Wan, M. Tian, T. Li, X. Wang, C. Ding, and S. Yu. 2018. Characterization of *Mycoplasma gallisepticum* pyruvate dehydrogenase alpha and beta subunits and their roles in cytoadherence. *PLoS One*. 13:e0208745.
- Rabie, N. S., and Z. Amin Girh. 2020. Bacterial vaccines in poultry. *Bull. Natl. Res. Cent.* 44:15.
- Rappuoli, R. 2000. Reverse vaccinology. *Curr. Opin. Microbiol.* 3:445–450.
- Razin, S., and R. Herrmann. 1998. Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.* 62:1094–1156.
- Riese, P., K. Schulze, T. Ebensen, B. Prochnow, and C. A. Guzmán. 2013. Vaccine adjuvants: key tools for innovative vaccine design. *Curr. Topics Medicinal Chemistry* 13(20):2562–2580.
- Rimler, R. B., R. B. Davis, R. K. Page, and S. H. Kleven. 1978. Infectious coryza: preventing complicated coryza with *Haemophilus gallinarum* and *Mycoplasma gallisepticum* bacterins. *Avian Dis.* 22:140–150.
- Rosales, R. S., R. Puleio, G. R. Loria, S. Catania, and R. A. J. Nicholas. 2017. Mycoplasmas: Brain invaders? *Res. Vet. Sci.* 113:56–61.
- Ruffin, D. C., V. L. van Santen, Y. Zhang, L. L. Voelker, V. S. Panangala, and K. Dybvig. 2002. Transposon mutagenesis of *Mycoplasma gallisepticum* by conjugation with *Enterococcus faecalis* and determination of insertion site by direct genomic sequencing. *Plasmid* 44:191–195.
- Self, J. 2003. Cal-Maine Foods, Inc. Personal communication, Jackson, MS.
- Shahbaaz, M., K. Bisetty, F. Ahmad, and M. I. Hassan. 2015. In silico approaches for the identification of virulence candidates amongst hypothetical proteins of *Mycoplasma pneumoniae* 309. *Comput. Biol. Chem.* 59(Pt A):67–80.
- Shil, P. K., A. Kanci, G. F. Browning, and P. F. Markham. 2011. Development and immunogenicity of recombinant GapA(+) *Mycoplasma gallisepticum* vaccine strain ts-11 expressing infectious bronchitis virus-S1 glycoprotein and chicken interleukin-6. *Vaccine* 29:3197–3205.
- Sid, H., S. Hartmann, H. Petersen, M. Ryll, and S. Rautenschlein. 2016. *Mycoplasma gallisepticum* modifies the pathogenesis of influenza A virus in the avian tracheal epithelium. *Int. J. Med. Microbiol.* 306:174–186.
- Staley, M., and C. Bonneaud. 2015. Immune responses of wild birds to emerging infectious diseases. *Parasite. Immunol.* 37:242–254.
- Stipkovits, L., L. Egyed, V. Palfi, A. Beres, E. Pitlik, M. Somogyi, S. Szathmary, and B. Denes. 2012. Effect of low-pathogenicity influenza virus H3N8 infection on *Mycoplasma gallisepticum* infection of chickens. *Avian Pathol.* 41:51–57.
- Sulyok, K. M., Z. Kreizinger, K. Bekó, B. Forró, S. Marton, K. Banyai, S. Catania, C. Ellis, J. Bradbury, O. M. Olaogun, Á. B. Kovács, T. Cserép, and M. Gyuranecz. 2019. Development of molecular methods for rapid differentiation of *Mycoplasma gallisepticum* vaccine strains from field isolates. *J. Clin. Microbiol.* 57:e01084-18.
- Takeda, K., O. Takeuchi, and S. Akira. 2002. Recognition of lipopeptides by Toll-like receptors. *J. Endotoxin Res.* 8:459–463.
- Tian, W., C. Zhao, Q. Hu, J. Sun, and X. Peng. 2016. Roles of Toll-like receptors 2 and 6 in the inflammatory response to *Mycoplasma gallisepticum* infection in DF-1 cells and in chicken embryos. *Dev. Comp. Immunol.* 59:39–47.
- Uppal, P. K., and H. P. Chu. 1977. Attachment of *Mycoplasma gallisepticum* to the tracheal epithelium of fowls. *Res. Vet. Sci.* 22:259–260.
- US Animal Health Association. 2002. Report of the Committee on Transmissible Diseases of Poultry and Other Avian Species. USAHA, Richmond, VA.
- Vance, A. M., S. L. Branton, S. D. Collier, P. D. Gerard, and E. D. Peeble. 2008. Effects of time-specific F-strain *Mycoplasma gallisepticum* inoculation overlays on prelay ts11-strain *Mycoplasma gallisepticum* inoculation on performance characteristics of commercial laying hens. *Poult. Sci.* 87:655–660.
- Wang, Y., K. G. Whithear, and E. Ghiocas. 1990. Isolation of *Mycoplasma gallinarum* and *M. gallinaceum* from the reproductive tract of hens. *Aust. Vet. J.* 67:31–32.
- Whithear, K. G., K. E. Soeripto, Haringan, and E. Ghiocas. 1990a. Safety of temperature sensitive mutant *Mycoplasma gallisepticum* vaccine. *Aust. Vet. J.* 67:159–165.
- Whithear, K. G., K. E. Soeripto, Haringan, and E. Ghiocas. 1990b. Immunogenicity of a temperature sensitive mutant *Mycoplasma gallisepticum* vaccine. *Aust. Vet. J.* 67:168–174.
- Whithear, K. G. 1996. Control of avian mycoplasmoses by vaccination. *Rev. Sci. Tech.* 15:1527–1553.
- Wijesurendra, D. S., A. Kanci, K. A. Tivendale, B. Bacci, A. H. Noormohammadi, G. F. Browning, and P. F. Markham. 2015. Development of a *Mycoplasma gallisepticum* infection model in turkeys. *Avian Pathol.* 44:35–42.
- Wijesurendra, D. S., A. Kanci, K. A. Tivendale, J. M. Devlin, N. K. Wawegama, B. Bacci, A. H. Noormohammadi, P. F. Markham, and G. F. Browning. 2017. Immune responses to vaccination and infection with *Mycoplasma gallisepticum* in turkeys. *Avian Pathol.* 46:464–473.
- Wu, Z., C. Chen, Y. Miao, Y. Liu, Q. Zhang, R. Li, L. Ding, M. Ishfaq, and J. Li. 2019. Baicalin Attenuates mycoplasma gallisepticum-induced inflammation via Inhibition of the TLR2-NF-κB pathway in chicken and DF-1 cells. *Infect. Drug Resist.* 12:3911–3923.
- Yavlovich, A., H. Rechnitzer, and S. Rottem. 2007. Alpha-enolase resides on the cell surface of *Mycoplasma fermentans* and binds plasminogen. *Infect Immun* 75:5716–5719.
- Yoder, H. W. 1983. Laboratory studies with inactivated oil-emulsion *Mycoplasma gallisepticum* vaccines. *Avian Dis.* 27:339–340.
- Yoder, H. W. 1979. Serologic response of chickens vaccinated with inactivated preparations of *Mycoplasma gallisepticum*. *Avian Dis.* 23:493–506.
- Yogev, D., D. Menaker, K. Strutzberg, S. Levisohn, H. Kirchhoff, K. H. Hinz, and R. Rosengarten. 1994. A surface epitope undergoing high-frequency phase variation is shared by *Mycoplasma gallisepticum* and *Mycoplasma bovis*. *Infect. Immun.* 62:962–968.
- Yoshida, S., A. Fujisawa, Y. Tsuzaki, and S. Saitoh. 2000. Identification and expression of a *Mycoplasma gallisepticum* surface antigen recognized by a monoclonal antibody capable of inhibiting both growth and metabolism. *Infect. Immun.* 68:3186–3192.
- Zhang, W., Y. Liu, Q. Zhang, S. Waqas Ali Shah, Z. Wu, J. Wang, M. Ishfaq, and J. Li. 2020. *Mycoplasma gallisepticum* infection Impaired the structural Integrity and immune function of bursa of Fabricius in chicken: Implication of oxidative stress and apoptosis. *Front. Vet. Sci.* 7:225.
- Zhang, D., Y. Long, M. Li, J. Gong, X. Li, J. Lin, J. Meng, K. Gao, R. Zhao, and T. Jin. 2018. Development and evaluation of novel recombinant adenovirus-based vaccine candidates for infectious bronchitis virus and *Mycoplasma gallisepticum* in chickens. *Avian Pathol.* 47:213–222.
- Zhang, G. Z., R. Zhang, H. L. Zhao, X. T. Wang, S. P. Zhang, X. J. Li, C. Z. Qin, C. M. Lv, J. X. Zhao, and J. F. Zhou. 2010. A safety assessment of a fowlpox-vectored *Mycoplasma gallisepticum* vaccine in chickens. *Poult. Sci.* 89:1301–1306.