Pertanika J. Trop. Agric. Sc. 43 (1): 19 - 34 (2020)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Review article

House Finch-Associated *Mycoplasma gallisepticum* Responsible for Epizootic Conjunctivitis in Passerines

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ABSTRACT

In 1994, *Mycoplasma gallisepticum* (MG) was reported to be responsible for conjunctivitis outbreak in the North American house finch population. This new course of MG infection in passerine was the result of spillover infections from the poultry strains. In severe cases of the disease, the conjunctival lesions might cause blindness and death, but in the mild form, there is a chance of recovery. The immune system of the recovered birds develops a resistance to the previous strains. However, the incomplete immune responses and the ability of MG to rapidly alter its surface antigens allow the pathogen to evolve new strains that can infect the birds that have already developed immune resistance. Although the rate of mortality decreases as a result of developing resistance, the persistence of the disease continues due to the increase in both virulence and the replication rate of the new strains. Therefore, the morbidity rate has remained steady, and new species of birds become infected as a result of evolutionary adaptation of the new strains. In this regard, the objective of this study is to provide a review of the mycoplasma conjunctivitis in passerine species, notably by looking at it from the host-pathogen interaction point of view.

Keywords: Conjunctivitis outbreak, evolutionary adaptation, house finch, host-pathogen interaction, *Mycoplasma gallisepticum*

ARTICLE INFO

Article history:
Received: 01 October 2019
Accepted: 02 January 2020
Published: 26 February 2020

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INTRODUCTION

Mycoplasma gallisepticum (MG) is primarily recognized as the causative agent of acute and chronic respiratory disease in birds (Raviv & Ley, 2013). In 1994, MG

ISSN: 1511-3701
e-ISSN: 2231-8542

was isolated in the samples collected from the house finch conjunctivitis outbreak and determined as the causative pathogen. This identification gave a new perspective on the pathogenicity of the MG that was not considered a primary pathogen in the wild population of passerine species previously (Ley et al., 1996). Additional analysis of the isolated pathogen revealed that a discrete MG strain (HFMG) was responsible for this outbreak (Dhondt et al., 1998, 2005; Tulman et al., 2012). Further studies showed the possibility of MG infection in a more diverse range of passerine species. Although most of these new cases were detected only through the conventional polymerase chain reaction (PCR) assay, some studies typed the strain of the detected MG in the birds (Allen et al., 2018). This strain typing led to the detection of different MG strains among the susceptible species (Cherry et al., 2006; Hochachka et al., 2013). This discovery has influenced researchers to investigate the fingerprints of these strains in wild birds to address the circulation of the MG in the wild.

The ability to rapidly change its surface antigens leads to fast host adaptation of MG; thus, making it a proper model to study and comprehend enzootic bacterial pathogens. In addition, the probability of spillover infections between the various species of birds is still open to interpretation. The study of the evolutionary developments of the host-pathogen interactions can provide a proper tool to answer such questions. For this concern, this paper describes a review of the *M. gallisepticum* infection associated with house finch and their interaction from various aspects.

Mycoplasma gallisepticum (MG)

Mycoplasmas belongs to the class of Mollicutes. Lack of cell wall, extraordinary reduction of the genome, and diminutive size distinguished this class from other bacteria. Adaptation to distinct hosts along with various tissue tropism plays a crucial role in the metabolism of mycoplasmas and regulates its austerity. Mucosal surfaces of the respiratory tracts, urogenital tracts, and joints are target regions for the colonization of the organism. Remarkable antigenic variation, despite its small genome size, revealed the capability of mycoplasma to survive in immunocompetent hosts. Limited capacity to synthesize the required nutrients and reduced genome have increased the dependency of mycoplasmas of their host cells. Therefore, cell membrane proteins (lipoproteins) play a vital role in the adherence of organism to host cells (Bencina, 2002). So far, 24 avian mycoplasma species have been identified, of which four of them are considered pathogenic to commercial poultry. These include *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae*. These 24 species are host-specific pathogens. They can also be commensals in non-host species, but in some birds like psittacine species, mycoplasmas may only be pathogenic (Lierz & Hafez, 2009). *Mycoplasma gallisepticum* is recognized as a multi-host microorganism. Its presence, however, is not always accompanied

by clinical signs. The cytadhesin genes that encode most of the surface proteins (lipoproteins) are involved in attachment to the host cells. The genes are comprised of putative variable protein gene (*pvpA*) and an operon encoding three genes: *mgc1*(*gapA*), *mgc2*, and *mgc3* (Boguslavsky et al., 2000; Yoshida et al., 2000).

MG Outbreak in House Finches

In February 1994, several cases of conjunctivitis in house finches (*Haemorhous mexicanus*) were reported that involved hundreds of infected birds at feeders and rescue centers (Doster, 1994). Conjunctival lesions were either unilateral or bilateral and ranged from mild to severe. The lesions were accompanied by serous to mucopurulent drainage and nasal discharge. Ley et al. (1996) attempted to identify the infectious agent. In this regard, a total of 25 conjunctival and infraorbital sinus swabs were collected from songbirds, especially house finches, and directly submerged in Frey's broth with 15% swine serum (Frey, 1968). Direct immunofluorescence (IF) was applied to identify the species. Of all these 25 samples, eleven isolates were detected positive using both direct IF and PCR. One of the two samples collected from blue jays was also detected positive to MG. Ultimately, researchers concluded that owing to the pathogenicity of mycoplasmosis in poultry; there may be subclinical long-term carriers within the wild population of songbirds (Ley et al., 1996). These findings introduced a new facet of the disease epidemiology in poultry and susceptible wild bird species like songbirds. Since then, conjunctivitis has been recognized as one of the typical clinical signs of MG infection in songbirds. This conjunctivitis outbreak was then spread readily to the eastern population of house finches inhabiting in Minnesota, Iowa, Missouri, Tennessee, and Mississippi states (Dhondt et al., 1998). The expansion of the disease was observed by experienced observers who participated in the ongoing Project Feeder Watch (Laboratory of Ornithology, Cornell University, Ithaca, New York, USA) from November 1994 to March 1997. An average of 24864 observations was recorded monthly. Based on this information, researchers were able to notice the spread of the disease and estimate the monthly prevalence of the infection. Data showed the rapid expansion of MG to eastern populations of passerine species. Irregular patterns of rising and fall were observed in the prevalence of the disease with the highest prevalence in the autumn due to dispersing juveniles. The high prevalence of MG infection among the breeding population had a devastating impact on the winter population of house finches in the eastern region of the US. For this concern, projects such as the feeder watch were established to monitor the status of conjunctivitis in finches. Data from the feeder watch project also indicated the occurrence of conjunctivitis in other species of songbirds. Hence, Hartup et al. (2000) tried to confirm the MG infection in selected songbird species that displayed conjunctivitis using culture,

PCR, and serology tests. Bird feeders were equipped with traps and nets year-round. Trapped birds were marked by applying a numbered aluminum leg band. Any signs of conjunctivitis such as swelling, erythema, exudation, or epiphora in eyelid were considered as conjunctivitis. Eye swabs were collected from the affected eye(s). Body condition score, wind chord, gender, and seasonal variation were considered as the confounding parameters and analyzed using logistic regression. In total, 1243 observations were made. Three species of songbirds, including house finch, goldfinch, and purple finch showed conjunctivitis and were found to be positive in the MG culture, PCR, or serology tests. In addition, two brown-headed cowbirds and four tufted titmice were positive in the plate agglutination test. None of the samples taken from these two birds was subjected to culture or PCR assay. Researchers concluded that the prevalence of mycoplasma conjunctivitis in this study area was lower than the northeast wintering house finch populations. Before 2003, the geographical distribution of the mycoplasmal conjunctivitis was primarily confined to the eastern populations of house finches. From then on, the disease was spread to the western populations (Figure 1).

House Finch-Associated MG in Other Wild Birds

Mycoplasma gallisepticum can be readily spread through horizontal transmission. Therefore, there is always a chance of disease transmission between different species of birds. In the case of mycoplasmal conjunctivitis, house finch was suspected to be responsible for the spread of MG infection in other passerine species in

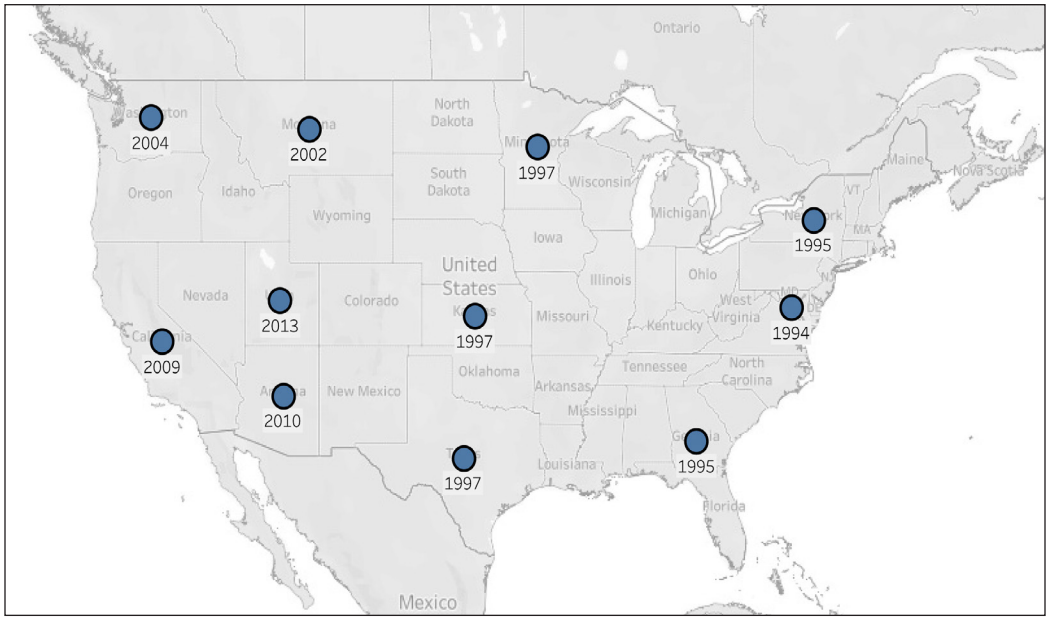

Figure 1. Geographical distribution of the HFMG in North America

North America. Therefore, further studies were conducted to reveal the role of house finches in the sustainability of the disease. To illustrate, in the study carried out by Luttrell et al. (2001), the prevalence of MG infection among passerine species that were living in poultry and non-poultry sites were measured and compared. The birds were captured using a mist net and assessed initially based on clinical signs and serum plate agglutination (SPA) test. Birds that were positive to the SPA test and had MG clinical signs were euthanatized and assessed further by culture, serology, PCR, and histopathology tests.

MG was only isolated from the house finches, and only the samples of house finches and tufted titmouse were positive for MG by PCR. However, histopathological lesions were observed in six house finches, five tufted titmice, three northern cardinals, one white-throated sparrow, and one yellow-rumped warbler. Ultimately, the higher prevalence of MG infection among the birds at poultry sites was statistically significant. These findings pointed towards the potential role of a house finch and tufted titmouse in the transmission of the disease. The MG infection in other songbirds was hypothesized to be the product of spillover infections that originated from the infected populations of house finches. In other words, the sporadic accidental infections in songbirds, excluding house finch, cannot break the transmission barriers between different species (Hartup et al., 2001). Therefore, after the exclusion of the regions with the historical presence of conjunctivitis (n=546), a total of 29266 observational data that were collected within four years were analyzed to test the hypothesis. The presence of the disease was confirmed when two wildlife veterinarians reported the signs of conjunctivitis. Bird species were divided into the target and non-target species based on the previous knowledge of susceptible species that was acquired from previous studies. A total of 297 cases of conjunctivitis from 27 non-target species recorded. A total of 187 of these cases occurred in American goldfinch, and 23 cases were reported in the Northern cardinal population. Logistic regression models were developed to determine the likelihood of conjunctivitis in birds by intervening in the occurrence of conjunctivitis in house finches on that particular temporospatial point. Results showed that the odds of observing conjunctivitis in both target and non-target species increased by the presence of house finches, especially during winters. Finally, the authors concluded that there is an epidemiological association between conjunctivitis in the population of American goldfinch, purple finch, and house sparrows with epizootics in house finch population, but the confirmation of the disease in other bird species required further investigations (Hartup et al., 2001). Therefore, Dhondt et al. (2014) aimed to investigate the risk factors involved in MG infection in wild songbirds. These factors included feeder usage, migration, and seasonal variation. For this concern, researchers set traps to catch birds at feeder and non-feeder sites between January 2007 and June 2010. Using culture,

PCR, and SPA tests, researchers were able to detect a broader range of hosts compared to the previous studies.

Eleven species of passerines were positive based on the findings from both SPA and PCR assays. Like the earlier study by Hartup et al. (2001), the occurrence of MG infection in wild bird species was shown to be directly associated with the incidence of MG infection in house finches. Moreover, it was found that seasonal change and migration, as the risk factors, influenced the occurrence of the disease as using feeder did not (Dhondt et al., 2014). Elaborate projects and facilities like the Feeder Watch Project and exclusive rehabilitation centers provided researchers with the proper tools to monitor the ecology and evolution of infectious diseases. This led to the identification of new MG susceptible passerine species (Ley et al., 2016). Rogers et al. (2019) were able to isolate and detect MG from conjunctival lesions of California scrub-jays (*Aphelocoma californica*) that were housed together with house finches at a rehabilitation center. The isolation of MG in the samples that were collected from these house finches and scrub jays were concurrent with the isolation of *M. synoviae*.

Impacts of *Mycoplasma gallisepticum* on House Finch Population

One of the early studies that assessed the impacts of mycoplasmal conjunctivitis on house finch populations was the study conducted by Hartup and Kollias (1999). Briefly, a total of 39 eggs and 110 nestling samples were assessed for the presence of MG using both culture and PCR. There was no MG isolated, and only two pooled choanal and conjunctival samples that were collected from two different broods of house finches tested positive for MG by PCR. These findings indicated that there is no evidence of vertical transmission in house finches. Therefore, direct contact with infected parents through brooding behavior, preening, or feeding was deemed as the possible route of infection in nestlings. To further examine the vertical transmission of MG in house finches, the reproductive success rate and the prevalence of MG among nestling were investigated by Nolan et al. (2004). A total of 280 nest boxes were used for sample collection and to check the presence of any clinical signs. Like the previous study, no evidence of vertical transmission was observed, and there was no significant impact on the hatchability rate. However, infected chicks had a smaller body size and relatively smaller tarsi that made them more accessible to predators. The contradiction between the new and initial course of the disease in terms of mortality and morbidity was the result of decreased virulence of MG strains, natural selection of the resistance house finches or the combination of both.

As a result of infection, the production of glucocorticoid, a stress hormone, can be incited and cause suppression of the host immune system (Dhabhar, 2009; Weidenfeld et al., 1995). Corticosterone, the main glucocorticoid in birds, was reported to be increased in house finches with conjunctivitis (Lindström et al., 2005).

It was also discovered that the pre-infection concentration of corticosterone has a negative correlation with the severity of clinical signs and sickness behaviors in house finches infected with MG (Adelman et al., 2015). Love et al. (2016) found a prolonged and late increase in the corticosterone level of the blood samples collected from house finches that were experimentally inoculated with low virulence MG strain. A higher concentration of corticosterone was also found to have a direct relationship with the severity of conjunctiva. This finding can be explained by the stimulation of cytokine activity due to the increase of corticosterone levels. These studies revealed the degree at which stress hormones can affect and alter the response of a house finch population to the MG infection.

Molecular Characterization of House Finch MG Isolates

Since the first report of mycoplasmal conjunctivitis (house finch eye disease), house finch disease survey (HFDS) has started to monitor the populations of house finches. The collected data were used for different reasons, such as measuring the mortality rate among the house finch populations. For instance, in the study conducted by Hochachka and Dhondt (2000), a high mortality rate (>50%) was reported from 1994 to 1999. The reports of high mortality rate and reduced reproductive success in house finches by Nolan et al. (2004) emphasized the need for an evolutionary study of the house finch-associated MG. Initially, the isolates were assessed by random amplification of polymorphic DNA (RAPD) test in which the banding patterns among the house finch MG strains (HFMG) were similar. However, different patterns of bands were observed between HFMG and three vaccine strains, including F, 6/85, and ts-11 (Hartup et al., 2000). Hong et al. (2005) used different techniques to discriminate different MG strains, including HFMG strains: K4997 and K4409. These techniques included sequencing the direct repeat (DR) region of *mgc2* gene amplified by PCR, RAPD, and amplified fragment length polymorphism (AFLP). The results showed that the gene target sequencing using *mgc2* DR region could differentiate these strains into seven groups. RAPD analysis of MG strains showed different 11 groups of strains highly similar to AFLP. However, AFLP is considered as a DNA typing method that can be linked to the database of the AFLP patterns, which allows researchers to use previous studies results. In all these techniques, three house finch isolates were classified as a unique group (Hong et al., 2005). In subsequent years, new technologies were employed to fingerprint the HFMG strains. For instance, Allen et al. (2018) developed a qPCR technique specific to HFMG strains. They compared the genome sequence of HFMG reference strains with those of low-passage R, F, S6, TS-11, 6/85, and A5969 strains to develop a primer specific to HFMG. Eight MG isolates from two American goldfinches, one purple finch, one house finch, two lesser goldfinches, one western scrub-jay,

and one American crow were assayed by the validated qPCR protocol and subjected to subsequent RAPD fingerprinting. The results have shown that all the isolates except that of American crow were favorable to the qPCR and demonstrated similar patterns.

The studies above indicated the circulation of one predominant MG strain in wild passerine populations. Some studies reported the presence of various HFMG strains. To illustrate, Liu et al. (2001) evaluated the sensibility of a molecular technique in which the *pvpA* gene was separated and amplified to distinguish between different MG strains, including house finch strains. PCR restriction fragment length polymorphism (PCR-RFLP) assay was conducted using PCR along with the restriction enzymes, including *Pvu*II, *Acc*I and *Scr*FI. PCR using selective primers for the gene of interest produced amplicons ranging from 266 to 497 bp. Four HFMG isolates displayed a 437 bp PCR product size and were placed in the F group of RFLP. One HFMG strain, however, showed a 266 bp product size and categorized as group H among RFLP groupings (Liu et al., 2001).

Further studies utilized more advanced techniques for phylogenetic analysis of HFMG. Cherry et al. (2006) designed a study to evaluate genomic variability between house finch isolates using both RAPD and AFLP. Samples were inclusive of 10 HFMG cultures isolated from different songbirds (one blue jay, one American goldfinch, and eight house finches) as well as six vaccine and reference strains isolated from poultry. The RAPD procedure was adapted from Ley et al. (1997), and primer sets were based on previous studies (Fan et al., 1995; Geary et al., 1994). AFLP fingerprinting technique was conducted based on the procedure described in a previous study (Kokotovic et al., 1999) in which the combination of *Bgl-II* and *Mfe-I* restriction enzymes were employed. The method that employed two sets of primers yielded at least two unique banding patterns of RAPD. The results of AFLP showed similar patterns among eight house finch isolates with the linkage level of 87%, indicating that these AFLP fragment patterns possibly belonged to one strain.

On the other hand, the AFLP pattern of one sample was unambiguously distinct from other songbird isolates with less than 78% linkage level. From the results of these two studies, it can be inferred that MG infection in songbirds might initially have a single point origin and the emergence of different strains of HFMG is the result of a molecular evolution after the first introduction and the following expansion of the infection. However, the clades or progenies from which this single point HFMG was evolved or originated were unclear until Hochachka et al. (2013) aimed to answer such questions by analyzing 107 isolates from poultry, house finches and other songbirds. Briefly, all the isolates were identified by direct immunofluorescence and then were subjected to molecular characterization based on partial genome sequencing. To make a comparison between these isolates, 13 sets of primers were employed to amplify the 8399 nucleotides of the whole genome in total. The result of

sequencing indicated two haplotypes among the isolates. One of these two haplotypes were from domesticated poultry, while the other one from house finch. This finding and the identical sequencing pattern between house finch and other songbirds indicated the primary interaction between poultry MG strains and the first HFMG strain. In other words, field strains of MG have been exchanged continuously between poultry and songbirds, especially house finches. Two aspects of host-pathogen interaction are involved here: 1) introduction into a new host, and 2) subsequent adaptation to new hosts that leads to minimal diversion among haplotypes. In an earlier study by Tulman et al. (2012), phylogenetic analysis of various MG isolates by using whole-genome sequencing indicated that HFMG strains were progenies of the poultry strains, notably between index isolate of MG in house finch (VA94) and F strain based on *vlhA* family group genes. In addition, significant divergence among HFMG strains implies the multi-points origin of the infection in the house finch. This indicates the existence of different primary lineages or earlier circulation of source strain in the wild before 1994. The identification of new hosts in subsequent years supported these results (Ley et al., 2016).

By considering all the findings reported from phylogenetic studies, it can be concluded that various MG strains isolated from house finches were progenies of poultry and/or ancestral wild strains (Tulman et al., 2012; Hochachka et al., 2013). Contradictory perspectives on the origin of HFMG might arise from the lack of sufficient index isolates. These findings emphasized the need for recognizing the potential of MG host among bird species through more elaborate trials. To illustrate, the potency of American goldfinches as a competent host for HFMG has been reported (Dhondt et al., 2013). However, such studies in different species of the Corvidae family are lacking. Conjunctivitis and identification of MG in blue jays had been reported since the first isolation of MG from house finches (Ley et al., 1996). In a recent study by Allen et al. (2018), the western scrub-jay was also reported positive of MG. These imply the potential role of the Corvidae family in sustainability and spillover infections of MG. A similar study revealed the susceptibility of tufted titmice to HFMG by displaying the significantly higher number of infected birds at poultry sites (Luttrell et al., 2001). However, there is a paucity of phylogenetic studies to comprehend what is the role of these birds in HFMG enzootics.

Experimentally Induced House Finch-Associated MG Infection

Inoculations of the HFMG were made for multiple reasons. For instance, Farmer et al. (2005) assessed the susceptibility of new hosts reported in previous studies (American goldfinches, eastern tufted titmice, house sparrows, pine siskins, chipping sparrows, purple finch, zebra finches, and budgerigars) by inoculation of an infective dose of a specific HFMG strain. Except for chipping sparrow, MG was detected in all species

using PCR. However, the clinical signs were not developed in house sparrows, zebra finches, and budgerigars. Another reason for conducting the experimental studies was to investigate the ways that MG can infect house finches. For this reason, Dhondt et al. (2007) designed a study to investigate whether fomites could transfer HFMG. They found that although HFMG strain was viable for 24 hours on the feeder's port tube and retained its infectious capability, the severity of the clinical signs was reduced when HFMG was inoculated to healthy house finches. Testing the theory of spillover infection and host jump events as the main reason behind the circulation of HFMG within wild passerine species was another reason to design the experimental studies. For instance, it was reported that the goldfinches are more susceptible than house sparrows to certain house finch MG strains; therefore, they are more infectious to house finches (Dhondt et al., 2008). This can be due to the different course of MG infection in goldfinches in which less severe clinical signs, faster improvement of conjunctivitis, and persistence of the pathogen in the conjunctiva were found. The consideration of American goldfinch as a competent host was assessed further and ultimately American goldfinch deemed as a competent host with more capability to spread HFMG, mostly because of its long-distance migration (Dhondt et al., 2013). As the infection spreads through enzootics, the MG will get the chance to endure and become enzootic in new areas by the introduction to new hosts. The virulence of MG will increase gradually, especially when birds infected with lower virulent strains are exposed to strains of higher virulence. High virulent strains will cause more severe signs; thus, they will shed copiously (Hawley et al., 2010, 2013; Williams et al., 2014). For further assessment, an experimental study was designed to find out whether the increase in virulence as a dynamic of enzootic disease is independent of the previous infection caused by a heterologous strain (Dhondt et al., 2017). The results of the study showed that the response to the re-infection was highly dependent on the pathogenicity of the former and new strain, regardless of whether the causative strains were heterologous or not. In other words, a high virulent strain can cause a more severe infection in house finches without any previous exposure compared to those with previous exposure. These findings were consistent with the primary expansion of the infection in new areas, especially during the fall and winter (Altizer et al., 2004; Dhondt et al., 2006; Hartup et al., 2001), and the subsequent decline in the number of infected house finches in enzootic regions due to the fact that juveniles recovered from previous infections became more resistance, regardless of the pathogenicity of the first strain (Hosseini et al., 2004). Drawing a comparison between exposed and non-exposed populations of house finches in terms of genetic variation and immune response can be very useful to understand how host resistance naturally influences bacterial virulence and replication rates. For instance, it was shown that the eastern

(the first exposed population) and western population of house finches had similar gene expression to MG inoculation until 2000. After this period, those house finches of the eastern population that were exposed to MG and survived, evolved genetic resistance by gaining the ability to mount a protective cell-mediated immune response (Bonneaud et al., 2011, 2012). In a recent study, the course of experimentally induced MG infection was compared between house finches with and without previous exposure to the pathogen. The results indicated that MG had become more virulent since recent strains caused more severe infections. In addition, as a result of immune adaptation, the possibility of developing lethal clinical signs in a previously exposed population would be reduced (Bonneaud et al., 2018). However, owing to the imperfect nature of the host immune memory, most of the responses to the infection were incomplete. These incomplete responses can provide a favorable condition for the evolution of more virulent strains (Fleming-Davies et al., 2018). These findings were consistent with a more elaborate study indicating that the initial spread of resistance to HFMG infection among house finches was the primary driver of the increasing virulence rather than the replication rate (Tardy et al., 2019). It was also observed that there is a direct association between the virulence of the HFMG and the expression of pro-inflammatory cytokines (Vinkler et al., 2018). In a recent immunological pathway study of both virulent and attenuated MG strains, a significant increase in the expression of the genes encoding proteins associated with pro-inflammatory responses was observed in the virulent strains. This indicates that the increase in the virulence of the pathogen leads to maladaptive and dysregulated immune responses of the host (Beaudet et al., 2019).

From these results and reports, it can be inferred that the recurrent induction of MG from the old host into the new host will ultimately end up with the adaptation to the new host. The host immune system plays a crucial role in this process, and MG infection in house finches is the result of the inefficiency of the immune system, especially the non-specific immune system, in the detection and elimination of the pathogen. By developing the immunity against the enzootic MG strains, through the prevention of the induced immune suppression instead of preventing the establishment of the pathogen, the proportion of infected house finches will drop, and the transmission of disease will be harder. On the other hand, the ability to alter the surface components, along with the predisposing environmental factors that affect the host immune system, can provide a suitable situation for MG to invade to new hosts and evade the immune system. This highlights the significance of monitoring programs in large scales such as the Feeder Watch Project for the fast identification of new competent hosts. From the studies investigating the effects of MG on house finch populations, it can be inferred that the higher rate of reproduction after high mortality and morbidity of the primary outbreaks was a result of the

adaptation of MG to its hosts whereby it can spread more readily by infecting the nestlings and even other species such as cowbirds. This interpretation is consistent with the general increase in virulence and evolving more virulent strains. To achieve this conclusion, however, direct examination of genital organs of adult house finches will be required to eliminate the possibility of vertical transmission of MG in house finches.

CONCLUSION

The mycoplasmal conjunctivitis outbreak caused by *Mycoplasma gallisepticum* revealed a new feature of the mycoplasmosis in birds. In this new course of MG infection, the increase in virulence as a result of spillover infections was observed. The spillover infections might lead to the evolution of new strains that might be capable of infecting the population of birds that evolved resistance to the ancestral strains. This emphasizes the role of free-living birds in the circulation of the pathogen. While mycoplasmal conjunctivitis mostly occurs in the house finches, almost every species of passerine birds can be the carrier of the pathogen, which makes the eradication of the disease from the wild more difficult. Therefore, the application of strict biosecurity in poultry farms to reduce the pathogen transmission between the commercial poultry and wild population of birds is highly advisable.

ACKNOWLEDGMENT

This study was granted by the Faculty of Veterinary Medicine, Universiti Putra Malaysia, which is highly appreciated.

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