Avian mycoplasmosis

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Abstract: Avian mycoplasmosis is an important disease of poultry of great economic importance. It is caused by four pathogenic mycoplasma species namely *Mycoplasma gallisepticum* (MG), Mycoplasma synoviae (MS), *Mycoplasma meleagridis* (MM) and *Mycoplasma iowae* (MI); although other Mycoplasma species have also been incriminated in the disease. The disease causes cough, rales, ocular and nasal discharges, decreased feed intake, decreased feed conversion, decreased egg production and hatchability. Avian mycoplasmosis can lead to a significant reduction in egg production of between 10-20% in infected layer and broiler breeder flocks. It also causes infectious sinusitis in turkeys. It can be prevented and controlled by the acquisition of birds free from mycoplasma, maintenance of replacements from mycoplasma free sources in a single-age, all in all out management system, proper hygiene and biosecurity measures. This review will focus mostly on MG and MS and focused on clinical signs, transmission, economic significance, methods of diagnosis, treatment, prevention and control.
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I. Introduction

Avian mycoplasmosis is a disease which is worldwide in occurrence and is extremely important to both the broiler grower and the table-egg producer **(Ley and yoder, 1997)**. It is caused by mycoplasma organisms of the Class Mollicutes. These organisms are different from other bacteria; they are of very small sizes and do not have a cell wall **(Khan *et al.*, 2010).** These characteristics account for the "fried egg" type of colonial morphology exhibited by mycoplasmas, their complete resistance to antibiotics that affect cell wall synthesis and their complex nutritional requirements**.** Avian mycoplasmas are also host specific (for instance, *Mycoplasmameleagridis* infects turkeys only) **(Kleven, 1998)**. Avian mycoplasmosis which is an important disease condition in birds is caused by four commonly recognized pathogens: *Mycoplasma gallisepticum* (MG), Mycoplasma synoviae (MS), Mycoplasma meleagridis (MM) and Mycoplasma iowae (MI) (Buim et al.,2009). Other mycoplasmas have also been incriminated in mycoplasma infections in birds **(Bradbury, 2005)** *Mycoplasma gallisepticum*, the pathogen responsible for chronic respiratory disease in chickens, is the most economically important species of *Mycoplasma* that affect poultry **(Ley, 2008)**. Symptoms of MG infection in chickens include respiratory rales, coughing, nasal discharge, and airsacculitis. The economicloss associated with this infection is realized by increased carcass condemnation and mortality in broilers and decreased feed efficiency and reduced egg production in layers. Reduced egg size has also been reported in layers in response to *MG* infections **(Branton *et al.*, 1999)**. Outbreaks of infectious disease are a constant risk for the agricultural industry and *Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of gallinaceous and certain non-gallinaceous avian species. It causes chronic respiratory disease in chickens and other fowl **(Evans *etal.*,2005).** *Mycoplasma gallisepticum* can be found worldwide **(Ley and Yoder, 1997)**, is a fragile (has no cell wall), host-adapted (to avian hosts only), fastidious (has specialised growth requirements) organism **(Kleven., 2003)**. Owing to the substantial losses caused in both performance and production, *MG has* been described as the most economically important of the four pathogenic *Mycoplasma* species affecting poultry **(Evans et al., 2005)**. Losses attributed to mycoplasmosis, mainly *MG* infection, a redue to:– a decrease in egg production and quality– poor hatchability (a high rate of embryonic mortality and culling of day-old birds)– poor feed efficiency– an increase in mortality and carcass condemnations– medication costs **(Nascimento *et al.*, 2005)**. Moreover, mycoplasmosis can spread quickly through an entire flock. The carcasses of birds sent to slaughter may also be downgraded **(Bradbury, 2001)**. Since *MG* is frequently complicated with other respiratory disease (s), including Newcastle disease, infectious bronchitis and *Escherichia coli* infection **(Soeripto *et al*, 1989)**, *MG* must be differentiated from these common respiratory diseases in chickens. *Mycoplasma* *gallisepticum* infections are notifiable to the World Organisation for Animal Health (OIE,2004).

II. Aetiologies/ Species Affected

Avian mycoplasmosis is caused by Mycoplasma which belongs to the Class Mollicutes, Order Mycoplasmatales and Family Mycoplasmataceae **(OIE, 2007)**. These are differentiated based on differences in morphology, genome size and some nutritional requirements **(Khan et al., 2010)**. The disease is caused by four commonly recognized pathogens: MG, MS, MM and MI **(Hossain *et al.*, 2007)**. Several strains of these mycoplasmas exist and they vary in their pathogenicity for different species of birds. Of all these, MG has been reported to be the most economically significant mycoplasma pathogen of gallinaceous and certain non gallinaceous avian species and causes chronic respiratory disease in chickens and infectious sinusitis in turkeys **(Osman *et al.*,2009)**. MG and MS are pathogenic for chickens and turkeys; MI is pathogenic primarily for turkeys while MM infects turkeys only **(Ley and Yoder, 1997)**. MG has been reported to have been isolated from infected falcons, parrots, pheasants, geese, quails, patridges, ducks and geese **(Garner *et al.*, 2006)**. Other species that have been incriminated in avian mycoplasmosis are *Mycoplasma (M) anseris* (affects geese), *M. columbianum* (affects pigeons); *M. gallinarum, M. gallinaceum, M. lipofaciens* and *M. pullorum* which affect chickens **(Cookson and Shivaprasad, 1994)**. Others are *M. gallopavonis, M. iners, M. columbinasale. M. glycophilum, M. cloacale*, Ureaplasmalaidlawii. These are not pathogenic; therefore they are not of major concern to the poultry industry **(Nascimento, 2000)**.

III. Pathogenicity

Mycoplasmas make use of some pathogenicity mechanisms to survive within the host organism, induce disease and evade the host immune system. Some of these mechanisms include adherence to host target cells mediation of apoptosis, damage to host cell due to intimate membrane contact **(Razin and Tully, 1995)**. Latency is also common to mycoplasmas. During this period, the mycoplasma may not be recognized by the host immune system due to its intracellular location **(Razin *et al*, 1998)**. Mycoplasma therefore induces disease after the host is affected by other disease causing agents or after an episode of host weakness.

All ages of chickens and turkeys are susceptible to avian mycoplasmosis although young birds are more prone to infection than the older ones **(Nunoya *et al*, 1995)**.

IV. Transmission

Mycoplasmas are transmitted laterally by contact **(Kleven, 1998)**; infectious aerosols coughed and sneezed by infected birds, through contaminated feed, water, contact personnel and communicant animals mainly birds **(Nascimento *et al*., 2005)** and vertically through the eggs **(OIE, 2007)**. Veneral transmission is particularly important in the case of MM **(Whithear, 1976)**. MS infection can also be through the conjunctiva and upper respiratory tract **(McMullin, 2004)**. It has been reported by **(Wang *et al*., 1990)** that *M. gallinarum* and *M. gallinaceum* have been isolated from the oviduct of chickens. This suggests that egg transmission of this species is possible. According to infected birds carry MG for life and can remain asymptomatic until they are stressed **(OIE, 2007).**

V. Economic Significance .

MG has been ascribed to be the most economically important of the pathogenic mycoplasma species affecting poultry due to the significant losses occurring from decrease in egg production, egg quality, poor hatchability (high rate of embryonic mortality and culling of day old birds), poor feed efficiency, an increase in mortality and carcass condemnations and medication costs **(Yoder, 1991b)**. Economic losses in the poultry industry caused by this infection have been noted to be significant **(Ahmad *et al*., 2008)**; the infection has been reported by **(Bradbury, 2001)**. to reduce egg production in layers and broiler breeder chickens by 10-20%. In 1984 in the USA, MG infected chickens were found to lay 15.7 eggs less than healthy ones; this contributed to a loss of 127 million eggs corresponding to an annual loss of 125 million dollars **(Mohammed *et al*., 1987)**. Also, losses over a 6 month period in 1999 in a North Carolina company were conservatively estimated to be between 500,000 and 750,000 dollars **(Rhorer, 2002)**.

VI. Clinical Signs

The incubation period of avian mycoplasmosis varies ranging between 6-21 days for experimentally infected poultry and is variable under natural infection. Infected birds may be asymptomatic for days or months until stressed **(OIE, 2007)**. Presence of concurrent infection with New castle disease virus, infectious bronchitis virus, *Esherichia coli* or other pathogens make avian mycoplasmosis more severe **(Bradbury, 2001)**.

The syndromes caused by avian mycoplasmosis are chronic respiratory disease an upper respiratory disease primarily seen in chickens and infectious sinusitis of turkeys caused by MG; infectious synovitis caused by MS and air sacculitis caused by MG, MS and MM **(Yoder, 1991b)**. Chickens with MG exhibit coughing, sneezing, rales, ocular and nasal discharges, decrease in feed consumption, decrease in egg production, increased mortality and poor hatchability. In turkeys, there is swelling of the infra orbital sinus (es), conjunctivitis accompanied by frothy exudates. This is common in turkeys but also occurs occasionally in chickens. However, respiratory disease often occurs in young birds particularly turkeys **(OIE, 2007)**. MS infection manifests as a milder form of respiratory involvement; lameness, pale comb and head, swollen hock and foot pad can be observed. Although most of the symptoms of MM are mild or in apparent, impaired hatchability and embryo pipping, increased embryo mortality and poor weight gain can be seen **(Charlton *et al*.,1996).**

VII. Post Mortem Lesions .

Gross post mortem lesions On post mortem examination, lesions may be found throughout the upper and lower respiratory tracts. Catarrhal exudates may be present in the nasal passages, infra orbital sinuses, trachea and bronchi **(McMullin, 2004)**. Mild sinusitis, tracheitis and air sacculitis are observed in uncomplicated cases of mycoplasmosis in chickens. Thickening and turbidity of the air sacs, Exudative accumulations, fibrinopurulent pericarditis and perihepatitis may be seen in cases where the chicken is concurrently infected with *E. coli* **(OIE,2007)**. In turkeys severe mucopurulent sinusitis may be found with variable severe tracheitis and air sacculitis **(Cookson and Shivaprasad, 1994)**. Interstitial pneumonia and Salpingitis are often seen in chickens and turkeys **(Charlton *et al*., 1996)**; other findings may include conjunctivitis, corneal opacities and peri ocular edema **(Pattison *et al*., 2008)**. The severity of these lesions is variable depending on the virulence and pathogenicity of the infecting strain, concurrent respiratory pathogens and stress factors **(Salami-Shinaba, 2009).**

VIII. Diagnosis

Mycoplasma species are difficult to grow from clinical specimens. This is due to their fastidious nature, intimate dependence on their host species and slow growth on artificial media **(Yoder., 1984)**. This infection can be diagnosed by clinical signs and isolation and identification of the organism by culturing on mycoplasma media **(Bencina, 2002)**; mycoplasma colonies are tiny, circular, smooth and translucent having a “fried egg” appearance with a central dense mass **(Adesiyun and Abdu, 1985)** Mycoplasmosis can also be diagnosed by post mortem lesions (gross and microscopic), serological tests such as sero agglutination reaction and hemagglutination inhibition test (HI); polymerase chain reaction (PCR) **(Yoder, 1991b)**, Enzyme linked immune sorbitant assay (ELISA), indirect immunofluorescence, immune peroxidase staining or growth inhibition test are also diagnostic for avian mycoplasmosis. In live poultry, swab samples for diagnosis are taken from the choanal cleft, cloaca and phallus **(OIE, 2007)**. At post mortem, samples for diagnosis can be obtained from affected organs such as trachea, air sacs and lungs. Others are synovial, ocular and infra orbital sinus exudates and pipped embryos **(Ley and Yoder, 1997)**. Swabs of the yolk sac endothelium are also used to isolate egg transmitted mycoplasmas form pipped embryos **(Kleven and Yoder, 1989)**. Swabs can be taken from the phallus, oviduct and semen for the isolation of MM from mature turkeys **(Whithear, 1976)**. Tissue or swab samples should be transported in mycoplasma broth and sent to the laboratory as soon as possible after collection **(OIE, 2007**). It was reported by **(Salami, (1994)**. that sinus and trachea (upper respiratory tract) are more reliable tissue sites for mycoplasma isolation rather than the lower respiratory tract in clinically mild infections butboth upper and lower respiratory tracts sites can be used in severe clinical poultry mycoplasmosis. Traditionally, the test of choice for confirmatory serology has been haemagglutination-inhibition (HI), which can be performed with fresh culture of a haemagglutinating teststrain of MG **(Kleven *et al*., 1996)** or with standardised preserved antigen **(Animal and Plant Health Inspection Service (APHIS), 1997)**. Diagnostically significant titres in the HI test may not be detected until three or more weeks after infection. However, the test is highly specific, even to the level of differentiation among strains **(Kleven *et al*., 1988)** Commercial enzyme-linked immunosorbent assay (ELISA) kits are widely available and are increasingly used for serological confirmation **(Kempf *et al*., 1994)**

IX. Treatment

The treatment of mycoplasma infected breeders with anti microbials decreases the rate of clinical manifestations and consequently also decreases the risk of transovarian transmission. It was stated by **(Stipkovits and Kempf, 1996)** that although this procedure is recommended for laying hens, it doesn’t eliminate MG, MS OR even MM from the flock. Many antimicrobial agents such as oxytetracycline, amino glycosides, lincosamides, fluoroquinolones, tylosin and tiamulin have been shown to possess different degrees of in vitro activity against various veterinary mycoplasmas **(Hannan *et al*., 1997a)**. An impressive effect of tylosin on Mycoplasma infected chickens has been recently reported by **(Kalu *et al*., 2015)**. However, increasing resistance of mycoplasma against tetracyclines **(Hannan, 1997a)**, macrolides **(Gautier-Bouchardon *et al*., 2002)** and quinolones **(Wu *et al*., 2000)**. has been reported in animal and human species. Mycoplasmas have higher mutation rates than conventional bacteria which mean that they can rapidly develop resistance to other drugs including the oxytetracyclines and tylosin as has been reported in Europe **(Thomas *et al*., 2003)**. The massive use of antimycoplasma drugs resulted in development of antimycoplasma drug resistant MS and MG strains **(Stipkovits, 2000)** However, the carrier status of infected flocks is not eliminated by treatment. It only suppresses the excretion of the micro organism in respiratory exudates and eggs **(Timms *et al*., 1989)**.

X. Control and Prevention Strategies

The prevention of mycoplasmosis in poultry includes the acquisition of birds free from MG, MS, MM or MI and constant monitoring of breeder flocks. These flocks free of MG should be sustained by maintaining replacements from mycoplasma-free sources in a single-age, all in all out management system. Control of avian mycoplasmosis consists of good biosecurity and proper hygiene. Although medication can be very useful in preventing clinical signs and lesions as well as economic losses, it cannot eliminate infection from a flock, it is not a satisfactory long term solution **(Kleven, 2008)**. Control by medication is necessary to compliment biosecurity measures to minimize economic losses, lateral and vertical transmissions **(Behbahan *et al*., 2008)**. It has been reported by **(Levisohn and Kleven., 2000)** that vaccination against MG and MS can be a useful long term solution in situations where maintaining flocks free of infection is not feasible especially in multi-age commercial egg production sites. Vaccines generally prevent egg production losses and reduce respiratory disease impact in commercial layers and can also help in the eradication or reduction of egg transmission in breeder flocks. Infections can be eliminated from a farm by depopulation of the flock, followed by thorough cleaning and disinfection of the premises. Most commonly used disinfectants are thought to be effective for MG. Recommended disinfectants for buildings and equipment include phenolic or cresylic acid disinfectants, hypochlorite, and 0.1% glutaraldehyde. Mycoplasmas are typically fragile and only survive in the environment for a few days therefore, birds can be re-introduced after two weeks **(OIE, 2007)**. Within the poultry industry, intense biosecurity and biosurveillance through serologic testing, *M. gallisepticum*-isolation, and DNA-based detection methods, are the most common control strategies used against avian mycoplasmosis **(Levisohn and Kleven, 2000)**. However, vaccination may be the most practical option in endemically infected multi-age commercial layer facilities as anintermediary step toward eradication **(Branton *et al.*, 1988; Kleven *et al*., 1984; Kleven, 2008)**. Various types of vaccines used against avian mycoplasmosis include inactivated oil-emulsion bacterins, live attenuated vaccines, and recombinant live pox virus vaccines, which express key protective MG antigens **(Kleven, 2008)**. As the result of extensive control programs under the National Poultry Improvement Plan, the incidence of *M. gallisepticum* in commercial poultry in the United States decreased considerably during the past 50 yr. However, MG **infections are still classified as sporadic by the USDA Animal Plant and Health Inspection Service (2013)**. The live F-strain MG vaccine, which is relatively mild when used in commercial layer flocks, was the first MGLAV approved foruse by the USDA **(Branton *et al*., 1999)**. Continuous use of this FMG LAV in commercial layer houses has been shown to protect birds from virulent field-strain.

MG infections **(Kleven, 1990, 1998)**. **Liu*et al.* (2013)** also indicated that when used as a prelayvaccine for commercial broiler breeders, FMG facilitated their reproductive performance, while protecting them against field-strain MG infections. However, **Burnham *et al*. (2002)** reported that the prelay inoculation of commercial layers with FMG could lead to a delay in the onset of lay by 1 wk and cause a decrease in total EP. Nevertheless, EP was reduced without having any effects on eggshell thickness, orpimpling and meat spot incidences when FMG was inoculatedvia eye drop at 45 wk of age **(Branton *et al*., 1988).**

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