Avian Mycoplasmosis

Nagwa S, Ata[(1)]; Riham H, Hedia[(1)]; Azza. S. M. Abu Elnaga[(1)] and Mona S, Zaki [(2)]

[1]Dept. Microbiology and Immunology, National Research Center, Giza, Egypt.
[2] Dept. Hydrobiology, National Research Center, Giza, Egypt.
dr_mona_zaki@yahoo.co.uk

Abstract: Avian Mycoplamosis is one of the major problems among avian disease and was caused by several pathogenic mycoplasmas, belongs to class *Mollicutes*, found on mucosal surfaces and can be transmitted vertically and horizontally and cause drop of egg production and considerable economical losses .The present literature view the classification, symptoms, transmission, treatment and vaccination. The present study concluded that it is important to control Mycoplasmosis in Egypt and does more effort for production of new vaccines to achieve the elimination of *Mycoplasma* from Egypt.

[Nagwa S, Ata; Riham H, Hedia ; Azza. S. M. Abu Elnaga and Mona S, Zaki. **Avian Mycoplasmosis.** *Life Sci J* 2013;10(2):1014-1017] (ISSN:1097-8135). http://www.lifesciencesite.com. 142

Key word: *Mycoplasma gallisepticum*, PCR, *Mycoplasma synoviae*, digitonin sensitivity test.

1. Introduction

Poultry provide humans with companionship, food and fiber in the form of eggs, meat and feathers. Many people love to raise and show chickens and other poultry species at fairs and other poultry shows. Others just love to raise them for backyard pets and for fresh eggs every day. Also there is a large commercial chicken industry that provides us with eggs and meat.

Avian Mycoplamosis is a collective term for infectious diseases caused by the micro-organisms called mycoplasmas and causes considerable economical losses to the poultry industry, especially in chickens all over the world. with the main ones being: *Mycoplasma gallisepticum* (MG), which affects a number of bird species including chickens, turkeys, gamebirds and pigeons; *M. synoviae* (MS), which affects chickens and turkeys; and, *M. meleagridis* (MM), which only affects turkeys.

Other avian mycoplasmas may exhibit pathogenicity under certain circumctances. For example, *M. gallinarum* was shown to be involved in an outbreak of respiratory disease in commercial broilers **(Kleven *et al*., 1978)**, and *M. pullorum* has been associated with turkey embryo mortality in France **(Moalic *et al*., 1997).**

Mycoplasmas cause decrease egg production and reduce feed conversion efficiency **(Carpenter *et al*., 1981; Ley and Yoder, 1997)** and production losses between 10 and 20% have been reported in layers **(Bradbury, 2001)**. High economical losses are caused by *Mycoplasma* infection in poultry and turkey flocks, solely or in conjunction with other pathogenic organisms. Infection with *Mycoplasma* is associated with high condemnation rate, decreased final weight and drop of egg production, higher conversion ratio **(Kapetanov *et al*., 2010)**.

M. gallisepticum is a cause of respiratory disease and the most economically important of the avian *Mycoplasma*. *M. gallisepticum* is responsible for chronic respiratory disease in chickens. In broiler, it causes reduction in weight gain, decrease in feed conversion efficiency, increase in mortality rate and increased condemnations in slaughter houses **(Gharaibeh and Al Roussan, 2008)**.

Mycoplasmas are very small prokaryotes devoid of cell walls, bounded by a plasma membrane only **(Razin *et al*., 1998)**. This accounts for the "fried egg" type of colony morphology, resistance to antibiotics that affect cell wall synthesis, and complex nutritional requirements, several fast growing *Mycoplasma* species in particular *Mycoplasma glycophilum, Mycoplasma gallinaceum* and *Mycoplasma pullorum* were isolated frequently and were thought to be impeding the isolation of *Mycoplasma gallisepticum* by outgrowing it **(Bradbury *et al*., 2001)**.

Classification:

According to classification scheme, *Mycoplasma* spp. belongs to the family of *Mycoplasmataceae*, order *mycoplasmatales* and class Mollicutes. More than a hundred *Mycoplasma* species are able to infect humans and animals and twenty species infect birds.

Transmission:

This disease may be transmitted both horizontally and vertically and remain in the flock constantly as subclinical form **(Bencina *et al*., 1988)**. *Mycoplasma* species were found on mucosal surfaces of the conjunctiva, nasal cavity and oropharenx. So

they are host specific and survive for short periods in the environment **(Quinn *et al.*, 2002)**.All of these mycoplasmas can be transmitted vertically and so can be introduced into the flock through infected eggs, including venereal transmission by males for MM. Vertical transmission has been greatly reduced through the establishment and maintenance of MG, MS and MM-free breeder flocks. They can spread through bird-to-bird contact and contact with exhaled respiratory droplets either as aerosols or on equipment, people and surroundings. Birds recovered from MG and MM remain shedders of the organisms.

Diagnosis:

Clinical signs:

It causes respiratory disease and can weaken the bird's immune system sufficiently for them to pick up any disease that they come into contact with. Small bubbles in the corners of eyes and swollen sinuses are usually the first sign of Mycoplasma, In laying flocks, egg production declines but usually is maintained at a lowered level **(Mohamed *et al.*, 1987)**. Once birds have been infected, they become carriers and remain infectious for life. Some birds seem to have a good resistance to MG. and out of an infected flock, a few may die, others may become ill and recover and some may not show any symptoms at all. The first time they are ill seems to be the worst and subsequent outbreaks seem to be milder.

Laboratory diagnosis:

Serological identification:

Serum plate agglutination (SPA) antigen used for the detection of antibodies to MG is commercially available. Because the SPA test is quick, relatively inexpensive, and sensitive, it has been widely used as an initial screening test for flock monitoring and serodiagnosis **(Kleven and Levisohn. 1996)**. However, nonspecific reactors occur in some flocks infected with *M. synoviae* due to cross-reactive antigens **(Ben Abdelmoumen and Roy 1996)**, or in flocks recently vaccinated with oil-emulsion vaccines and/or vaccines of tissue-culture origin against various agents **(Yoder 1989)**. The hemagglutination-inhibition (HI) test has been commonly used to confirm reactors detected by SPA or, more recently, enzyme- linked immunosorb. Commercial ELISA test kits are now commonly used for flock monitoring and serodiagnosis.

Isolation and identification:

Samples for isolation may be swabs, organs, tissues and exudates, diluted tissue homogenates**,** Broth and agar are used for isolation, but it is normally necessary to obtain *Mycoplasma* colonies on agar before attempting identification. Nutritional requirements require a protein-rich medium containing 10–15% added animal serum. Further supplementation with some yeast-derived component is often beneficial. Growth of *M. synoviae* requires the addition of nicotinamide adenine dinucleotide (NAD). A medium described by **Frey *et al.*, 1968**. Or a medium described by **Bradbury 1977** is commonly used for the cultivation of avian mycoplasmas. *Mycoplasma* organisms tend to grow rather slowly usually prefer 37–38°C, and are rather resistant to thallium acetate and penicillin, which are frequently employed in media to retard growth of contaminant bacteria and fungi. Fried egg colonies form on agar media after 3–10 days at 37°C because *Mycoplasma* is devoid of cell walls and it is bounded by a plasma membrane only **(Razin *et al.*, 1998)**, digitonin sensitivity test is applied to differentiate between *Acholeplasma* and *Mycoplasma*. Basic biochemical tests as glucose fermentation test and arginine deamination test can be helpful in preliminary classification of isolates **(Enro and stipkovits 1973)**, but final identification is by immunological tests, the most satisfactory being fluorescent antibody and immunoperoxidase tests.

Molecular techniques:

In recent years PCR assays have become widely used as methods to confirm the presence of mycoplasmae in poultry flocks **(Kajhn *et al.*, 2009)**. General *Mycoplasma* polymerase reaction (PCR) to generate amplicon (DNA amplification product) from nine avian *Mycoplasma* species is applied by **Laureman *et al.* (1995)**, also **Fan *et al.* (1995)** distinguish the DNA heterogeneity among strains and isolates of *Mycoplasma gallisepticum* with arbitrary primed polymerase chain reaction (AP-PCR) method according to banding pattern and the differences between isolates of *Mycoplasma gallisepticum.* **Charlton *et al.* (1999)** used RAPD analysis to differentiate 7 strains of *M.gallisepticum*, real time polymerase chain reaction (Q-PCR) was developed by **Mekkes and Febrwee (2005)** for qualitative and quantitative detection of *M. gallisepticum* in clinical samples. Also GTS analysis of surface-protein genes was a sensitive and reproducible typing method by **Ferguson *et al.* (2005)**. Also **Mardassai *et al.* (2005)** developed a duplex PCR assay targeting the hemagglutinin multigene families, v1hA and pMGA, of *Mycoplasma synoviae* and *Mycoplasma gallisepticum*, respectively. Also **Carrion *et al.*, 2013** demonstrated that PCR-RFLP is an appropriate method of diagnosis of mycoplasmosis in our environment, to differentiate vaccine strains from field strains obtained from tracheal swab samples taken at commercial farms.

Treatment:

Although antibiotics are commonly used to reduce the effects of MG infections, they have proven ineffective at clearing MG infections **(Ley and Yoder 1997)**. *Mycoplasma* is sensitive to tetracyclines (oxytetracycline, chlortetracycline and doxycycline), macrolides (erythromycin, tylosin, spiramycin, lincomycin, and kitasamycin), quinolones (imequil, norfloxacin, enrofloxacin and danofloxacin) or tiamulin. Drugs that accumulate in high concentrations in the mucosal membranes of the respiratory and genitourinary tracts, such as tiamulin and enrofloxacin for CCRD. Biosecurity and biosurveillance measures have been largely successful at minimizing MG outbreaks among the breeding stock of the turkey and chicken industries, in which outbreaks occur only in a sporadic nature. Efforts should be made to reduce dust and secondary infections. Improve the ventiliation for having good results of medicine.

Prevention:

1. Establishment of Mycoplasma free breeding flocks.
2. Treating infected hatching eggs with the antibiotic Tylosin to kill the organism contained in the eggs.
3. Before purchasing chicks from a hatchery, it should be confirmed that they are free from CRD. Chicks should be raised at the place where there is no approach of infected birds and complete fencing of the breeding farms and sufficient isolation of prevent iarborne infections from infected flocks.
4. Disposing of dead birds by incineratin, deep burial or by means of special disposal pits.
5. Using vaccines that are free from contamination of Mycoplasma gallisepticum. Construction of the houses must be done in such a way that probibit the entrance of any type of wild birds and wandering animals. Vaccination against *Mycoplasma gallisepticum* (MG) or *M. synoviae* (MS) can be a useful long-term solution in situations where maintaining flocks free of infection is not feasible, especially on multi-age commercial egg production sites **Kleven**., **2008**.
6. Prohibition of visitors in the farm, and before coming in contact with flocks, workmen should take shower and put on special clothes. Strict biosecurity measures should be adopted.

Reference

1- Ben Abdelmoumen, B. and R. S. Roy. 1995. Antigenic relatedness between seven avian mycoplasma species as revealed by Western blot analysis. *Avian Dis* 39:250–262.

2- Bradbury, J. M. 1977. Rapid biochemical tests for characterization of the Mycoplasmatales. *J Clin Microbiol* 5: 531–534.

3- Bradbury, J.M. (2001): Avian mycoplasmosis, In: Jordan, F. et al. (eds) Poultry Diseases.5th edn. W. B. Saunders Company, Iowa. pp:178-193.

4- Bradbury, J.M.; Abdul Wahab, O.M.S.; Yavari, C.A.; Dupiellet, J.P. and Bove,J.M.(2001): *Mycoplasma imitates* Sp.nov. is related to *Mycoplasma gallisepticum* and found in birds. Int. J. Syst. Bacteriol., 43:721-728.

5- Carpenter, T.R.; Mallinson, E.T.; Miller,K.F.; Gentry,R.F. and Schwartz, L.D. (1981): Vaccination with Fstrain *Mycoplasma gallisepticum* to reduce production losses in layer chickens. *Avian Dis., 25: 404-409.*

6- Carrion, L. J; Ferrer, R.B.; Nieto, G.C.R and Alfonso, V.J.V. (2012): Differentiation of *Mycoplasma gallisepticum* Strains through RFLP. Rev. Med. Vet. 24: 113-121.

7- Charlton, B. R.; Bickford, A. A.; Walker, R.L. and Yamamoto, R. (1999): Complementary randomly amplified polymorphic DNA (RAPD) analysis patterns and primer sets to differentiate *Mycoplasma gallisepticum* strains. *J. Vet. Diag. Invest.* 11 (2): 158-161.

8- Enro, H. and Stipkovits, L. (1973): Bovine Mycoplasmas. Cultural and biochemical studies. *Acta Vet. Scand., 14: 450-463*.

9- Fan, H. H.; Kleven, S. H. and Jackwood, M.W. (1995): Application of polymerase chain reaction with arbitrary primers to strain identification of *Mycoplasma gallisepticum. Avian Dis.,* 39 (4):729-735.

10- Ferguson, N.M.; Hepp, D.; Sun, S.; Ikuta, N.; Levisohn, S.; Kleven, S. H.; and Garcia, M. (2005): Use of molecular diversity of *Mycoplasma gallisepticum* by gene targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. *Microbiology (2005), 151, 1883-1893.*

11- Frey, M. L., R. P. Hanson, and D. P. Anderson. 1968. A medium for the isolation of avian Mycoplasmas. *Am J Vet Res* 29: 2163–2171.

12- Gharaibeh, S. and Al Roussan,D. (2008): The use of molecular techniques in isolation and characterization of *Mycoplasma gallisepticum* from commercial chickens in Jordan. *Inter. J. Poult. Sci. 7(1):28-35*

13- KÃjhn, S.; Spergser, J.; Ahlers, C.; Voss, M.; Bartels, T.; Rosengarten, R. and Krautwald-Junghanns, M.E.(2009): Prevalence of Mycoplasmas in commercial layer flocks during

laying period.*Berl Munch Tierarztl Wochenschr; 122(5-6):186-92.*

14- Kapetanov, M.; Orlic,D.; Potkonjak,D.; Velhner, M.; Stojanov, I.; Milanov, D. and Stojanovic, D. (2010): *Mycoplasma* in poultry flocks in the year 2009 compared to the year 2000 and significance of the control measures. *Lucrari Stiintifice Medicina Veterinara (1):249-253.*

15- Kleven, S. H. and S. Levisohn. 1996. Mycoplasma infections of poultry. In J. G. Tully Molecular and Diagnostic Procedures in Mycoplasmology. Volume II—Diagnostic Procedures, (283–292) New York: Academic Press, Inc.

16- Kleven, S. H. (*2008*) Control of Avian Mycoplasma Infections in Commercial Poultry. Avian Diseases: September 2008, Vol. 52, No. 3, pp. 367-374.

17- Laureman, L. H.; Chilina, A.R.; Closser, J. A. and Johansen, D. (1995): Avian *Mycoplasma* identification using Polymerase reaction amplicon and restriction fragment length polymorphism analysis. *Avian Dis. 39:804-811.*

18- Ley, D. H. and Yoder, H. W. (1997): *Mycoplasma gallisepticum* infection In: Disease of poultry, 10th ed. Calnek, B.W.; Barnes, H.J.; Beard, C.W.; McDougald, L.R. and Saif, Y. M. eds. Iowa state, University Press, Ames. Iowa, pp: 194-207.

19- Mardassi, B. B.; Mohamed, R. B.; Gueriri, I.; Boughattas, S. and Mlik, B. (2005) : Duplex PCR to differentiate between *Mycoplasma synoviae* and *Mycoplasma gallisepticum* on the basis of conserved species-specific sequences of their hemagglutinin genes. *J Clin. Microbiol. 43(2):948-958.*

20- Mekkes, D.R. and Feberwee, A. (2005): Real-time polymerase chain reaction for the qualitative and quantitative detection of *Mycoplasma gallisepticum. Avian pathol., 34:348-354.*

21- Mohammed, H. O., T. E. Carpenter, and R. Yamamoto. 1987. Economic impact of Mycoplasma gallisepticum and M. synoviae in commercial layer flocks. *Avian Diseases* 31:477–482.

22- Razin, S., D. Yogev, and Y. Naot. 1998. Molecular biology and pathogenicityof mycoplasmas. *Microbiol Mol Biol Rev* 62: 1094–1156.

23- Stipkovits, L., E. Csiba, G. Laber, and D. G. Burch. 1992. Simultaneous treatment of chickens with salinomycin and tiamulin in feed. *Avian Dis* 36:11–16.

24- Winner, F., R. Rosengarten, and C. Citti. 2000. *In vitro* cell invasion of Mycoplasma gallisepticum. *Infect Immun* 68: 4238–4244.

25- Yoder, H. W., Jr. 1989. Nonspecific reactions to Mycoplasma serum plate antigens induced by inactivated poultry disease vaccines. *Avian Diseases* 33:60–68.

3/2/2013