Some Studies on the Diagnosis of *Mycoplasma Gallisepticum* in Chicken

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Abstract: Avian mycoplasmosis are considered one of the most important economic problems for chicken industry. The current research aims to obtain complete picture of (*M. gallisepticum)*. One hundred and eighty specimens were collected from sixty naturally infected chicken of different age, sex and breed from different localities in El-Sharkia Governorate. Bacteriological examination of the samples revealed that, the total incidence of mycoplasma were14.4% .The results of serelogical identification by SPA test for detecting *M. gallisepticum* antibodies showed that 31 isolates (51.6%) were positive and 29 isolates (48.3%) were negative,while the ELISA test revealed that 29 isolates (48.3%) were positive and 22 isolates (36.3%) were negative.The antibiotic sensitivity test of *M. gallisepticum* showed that all the isolates were sensitive to lincospectin, spectinomycin and tylosin, but all examined isolates were resistant to enrofloxacin, erythromycin, ampicillin, oxytetracycline and chloramphenicol.
[Lamyaa M. Reda and L.K. Abd El-Samie. **Some Studies on the Diagnosis of *Mycoplasma Gallisepticum* in Chicken.** *Nat Sci* 2012;10(12):247-251]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 37

Keywords: Mycoplasma Gallisepticum-chicken-SPA-ELIZA

1. Introduction

Avian Mycoplasmosis is considered as one of the major economic problems facing poultry industry all over the world. Avian mycoplasmosis can be cause by several species of Mycoplasma (class Mollicutes, order Mycoplasmatales, family Mycoplamataceae) including *Mycoplasma gallisepticum, M. synoviae, M. meleagridis* and *M. iowae*(Yoder *et al*., 1984) *M. gallisepticum* is a very small prokaryotic organism lacking a cell wall and bounded by plasma membrane.

The shape of mycoplasma cell is sphere, 0.3-0.8 µm in diameter. Mycoplasma is characterized by lacking of the cell wall that explains many of unique properties such as sensitivity to osmotic shock and detergents, resistance to penicillin and formation of the fried egg shaped colonies. The clinical signs seen in avian mycoplasmosis are coughing, sneezing ,ocular and nasal discharges, decreased feed intake and egg production, increased mortality, poor hatchability. *Mycoplasma species*are transmitted horizontally from bird to bird and vertically from dam to offspring through the eggs (Pitcher and Nicholas, 2005 and Razin and Hayflick, 2010). The aim of the present work was to compare the traditional and the recent techniques for the diagnosis of *Mycoplasma gallisepticum*,antibiotic sensitivity testing of isolated *Mycoplasma gallisepticum* for different antimicrobial agents.

2. Material and Methods

Samples:

A total of 180 lung,trachea and air sac specimens of 60 naturally infected chickens with respiratory manifestationsand nasal discharge were used in this study. Also, 60 blood samples were collected via wing vein puncture of each examined bird. All chicken were collected from El-Sharkia Governorate during September 2010 till August 2011 from different breeds with different ages. All the samples were submitted to the laboratory for bacteriological and serological examination.

Isolation and identification of *Mycoplasma species* (Sabry and Ahmed, 1975):

Tissue samples (trachea, lung and air sac) were minced under aseptic condition, diluted in mycoplasma broth (approximately 5gm tissue sample in 25 ml broth). Each swab sample from broth was inoculated into 5 ml. PPLO broth, then incubated at 37ºC for 3days, 0.02ml of broth culture was inoculated and streaked on PPLO agar. The agar plate was incubated at 37ºC in a moist candle jar under reduced oxygen tension. The plates were observed daily from the 3rd to the 10th day post incubation by dissecting microscope for the presence of fried-egg colonies.

Purification and maintenance of the isolates (Sabry, 1968)

A single with fried egg colony shape was picked up with an agar- block and transplanted into a broth medium for obtaining a pure culture of the isolates.The purified isolates were maintained at 20ºC in aform of agar blocks.

Genus determination using digitonin test: for differentiation between *Mycoplasma* and *Acholeplasma* species as described by **Freundt *et al*., (1973)**

Biochemical characterization tests: were carried out using glucose fermentation and arginine deamination

as previously mentioned by **Sabry,(1968).**

Serological identification:

A-Growth inhibition test as described by **Clyde (1983)**

B-Serum plate agglutination test by using stained *Mycoplasma gallisepticum* standard antigen that obtained from (Intervet International, B.V., Boxmeer, Holland, was applied as described by **Stipkovits and El-Ebeedy (1977)**

C- Enzyme linked immunosorbantassay:this test was performed according to **Higgings and Whithear (1986)** using *Mycoplasma gallispticum* antibody Kits (Kierkegaard and Perry Laboratories (KPL) Gaithersburg Maryland, U.S.A.

Antibiotic sensitivity test as described by **Clyde (1964):**

Mycoplasma gallisepticum isolates were culture on brain heart infusion agar using running drop technique, commercial antibiotic discs (Oxoid Lab.) were placed on the inoculated plates and pressed gently into agar. Plates were incubated at 37ºC in moist candle jar for 3-4 days, the plates were examined daily macroscopically and microscopically for inhibition of the growth of the colonies (inhibition zone) which were measured in millimeters.

3. Results and Discussion

Mycoplasma gallisepticum is a major poultry pathogens and causes severe economic losses for poultry industry. Therefore, methods for their control should be applied to protect against infection.

Incidence of Mycoplasma isolates from chicken:

Table (1) showed that the total number of Mycoplasma isolates was 26 with an incidence of (14.4%) out of 180 samples collected from 60 examined chicken, also **Metwalli (1980), El-Shater (1986) and Zeinab (1997)** isolated mycoplasma at different localities from Egypt with an incidence of (11.8%), (16.7%) and (13.3%) respectively. The highest rate of Mycoplasma was from the air sac with an incidence of (23.3%), also **Zeinab (1997)** who stated that the highest rate of mycoplasma isolated from air sac were (21.5%).

Table (1): incidence of Mycoplasma isolates from different organs of naturally infected chicken

Types of samples	No. of isolates/ No. of examined samples(%)
Air sac	14/60(23.3%)
Trachea	7/60(11.6%)
Lung	5/60(8.3%)
Total	26/180(14.4%)

Results of isolation and differentiation between *Mycoplasma* and *Acholeplasma* species:

Regarding the isolation of *Acholeplasma*, it could not be isolated from chicken samples and all the isolates were digitonin positive.

These results were also supported by that found by **Abd El-Latif (1999)** who reported that there was no isolation of *Acholeplasma* species from chicken samples.

Also, our data in were nearly similar with those obtained by **Erno and Stipkovits (1973) and Edward and Razin (1974)** but **Sokkar *et al*., (1986)** who isolated *Acholeplasmalaidlawii* and *Acholeplasmaamxanthum* by percentages of 28% and 32% respectively from trachea, sinus and air sac.

Table (2): Biochemical characterization of mycoplasma isolates from naturally infected chickens

Mycoplasma isolates from naturally infected chicken		
Type of organs	**Glucose +ve Arginine -ve**	**Glucose -ve Arginine +ve**
Air sac	13/14(92.8%)	1/14(7.14%)
Trachea	6/7(85.7%)	1/7(14.2%)
Lung	5/5(100%)	0/5(0 %)
total	24/26(92.3%)	2/26(7.6%)

Result of Biochemical characterization of mycoplasma isolates:

Biochemical characterization was carried out to simplify identification as shown in table (2).Two biochemical groups were be detected(group one was 92.3% which is glucose +ve, and arginine –ve and group two which is glucose –ve, and arginine +ve with an incidence of (7.6%) result is agreed with that mentioned by **Fatma (2004) , Rania (2005)** and **Sally (2010)** who classified mycoplasma isolates into the same two biochemical groups.

Serological identification of *Mycoplasma* isolates by GIT:

The results of serological identification of Mycoplasma isolates using known specific reference antisera showed that 24isolates were *M. gallisepticum* belonging to biogroup I and 2 isolates were antigenically related to *M. gallinarum* belonging to biogroupII. These results were agreed with the results obtained by **(Sabry1968)** who could isolate *M. gallisepticum* and *M. gallinarum*from respiratory tract of CRD infected chickens,but **Fawkia (1986) and Fatma (2004)** who could isolate different serotypes of avian mycoplasma related to ***M.gallisepticum, M.gallinarum, M.pullorum***and ***M.gallinaceum.***

Comparison between SPA and ELISA tests used for serelogical detection of *M.gallisepticum* antibodies in the sera of naturally infected chicken:

M.gallisepticum antibodies were detected by SPA, it was found that 31 serum samples were positive (51.6%) and 29 serum samples were negative (48.3%). These results nearly similar to that recorded by **El-Shabiny *etal.*, (1990), El-Shater *et al*.,(1990), El-Shabiny *et al*., (1997) ,Zeinab(1997) and Mohamed (2003)** who reported that antibodies of *M.gallisepticum* were detected by SPA in a rate of 48%, 53.3 %, 45%,45% and 56% respectively, but **Singab (1987)** who recorded *M. gallisepticum* in a rate of 40% .With regard to the result of ELISA for detection of antibodies of *M. gallisepticum* in 60 serum samples collected from naturally infected chickens, 29 serum samples were positive with an incidence of 48.3% and 22 serum samples were negative with an incidence of 36.6% and 9 serum samples were suspected as shown in Table (3). These findings nearly similar to that obtained by **Kempf *et al.*, (1994)** who examined serum samples for detection of *M.gallisepticum* antibodies by ELISA test and their results were 33% positive, 28% suspected and 29% negative, **(Talkington *et al*., 1985)** reported their ELISA to be less sensitive, but more specific than SPA test and more sensitive than haemagglutination inhibition test for mycoplasma. In our investigation we noticed that the overall recovery rate of mycoplasma isolates was low (14.4%) when compared with high rate of specific antibodies against mycoplasma in the sera of examined chicken, this finding is in agreement with those of **El-Shabiny *et al.*, (1990).** This may be due to mycoplasma is difficult to be grown in the artificial media. In addition of SPA is rapid and sensitive for detection of *M. gallisepticum,* but often give false positive reactions connected with antigen preparation techniques **(Optiz and Cyr, 1986)**, bad quality of sera tobe tested **(Bradbury and Jordan, 1972)** or use oil emulsion vaccines **(Yoder, 1989)**. Also the presence of *M. gallinarum* infection in flocks to be tested will give problem with cross reacting antibodies in serological tests **(Oslan, 1984)**.The results of serological tests proved that ELISA test gave better results and higher sensitivity than SPA test for detection of specific antibodies for *M. gallisepticum*, These results agreed with that recorded by **Abdel-Gawad (2005) and Sally (2010)**.

Table (3): Comparative results of SPA and ELISA testsused for serelogical detection of *M.gallisepticum* antibodies in the sera of naturally infected chicken

SPA results			ELISA results		
No. of +ve serum sample / total no. of examined serum sample (%)	No. of suspeceous sample/ total no. of examined serum sample (%)	No. of –ve serum sample / total no. of examined serum sample (%)	No. of +ve serum sample / total no. of examined serum sample (%)	No. of suspeceous sample/ total no. of examined serum sample (%)	No. of –ve serum sample / total no. of examined serum sample (%)
31/60 (51.6%)	Non	29/60 (48.3%)	29/60 (48.3%)	9/60 (15%)	22/60 (36.6%)

Antibiotic sensitivity tests for *M.gallisepticum* isolates:

From results recorded in table (4),it was clear that all isolates were highly sensitive to lincospectin, spectinomycin and tylosin, these results similar to those reported by **Soliman (1984) and Reece *etal*., (1986),** but these isolates were moderate sensitive to gentamycin and neomycin. On the other hand, the isolates were resistant to enrofloxacin, erythromycin, ampicillin, chloramphenicol and oxytetracycline.This resistance may be attributed to miss use of antibiotics in the field which resulted to development of acquired resistance of field isolates to these antibiotics as shown in table (4).

It could be concluded that mycoplasma infection in chicken should be considered as an important disease which act as a source of transmission of the disease to different species of chicken. Our data revealed that SPA that test can be used as an easy and simple screening test, while ELISA test can be used as a confirmatory test.

Table (4): Results of antibioticsensitivity tests for 20 isolates of *M.gallisepticum*

Antibiotic	Disc potency µg	No.of sensitivity isolates/total no(%)
Spectinomycin	100	18/20(90%)
Erythromycin	10	5/20(25%)
Lincospectin	100	19/20(95%)
Enrofloxacin	5	6/20(30%)
Ampicillin	10	7/20(35%)
Tylosin	100	17/20(85%)
Gentamycin	30	15/20(75%)
Neomycin	30	14/20(70%)
Oxytetracycline	30	9/20(45%)
Chloramphenicol	30	4/20(20%)

Moreover ELISA test had a higher degree of specifity. The antibiotic sensitivity test of *M. gallisepticum* showed that all the isolates were sensitive to lincospectin, spectinomycin and tylosin, but all examined isolates were resistant to enrofloxacin, erythromycin, ampicillin, oxytetracycline and chloramphenicol.

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10/21/2012