BACTERIOLOGICAL STUDIES ON *MYCOPLASMA GALLISEPTICUM* INFECTION IN QUAILS IN CORRELATION TO THEIR AGES

BY

***Mosaad, A.A.;**Abdel-Tawab, A.A. and *Rasha Zahran, N.**
*Minufiya Univ. Fac. Vet. Med. (Sadat City) Dept. of Bacteriology, Immunology and Mycology. **Zagazig Univ. Fac. Vet. Med, (Moshtohor) Dept. of Bacteriology, Immunology and Mycology

SUMMARY

One hundred quails of different ages were collected from private and governmental farms. Out of these, eighty (80) quails were from flocks suffering from respiratory manifestations (Coughing, sneezing, ralling, wet eyes, nasal discharge and sometimes swollen of infraorbital sinuses either bilateral or unilateral) and the remaining twenty (20) quails were apparently healthy ones. Bacteriological examination revealed that 16 isolates M. gallisepticum were recovered with an over all incidence of 16.0%. Regarding the distribution and frequency of occurrence of Mycoplasma species isolated from quails in relation to the age of examined birds it can be concluded that quails aged more than 8 weeks showed the highest incidence of M. gallisepticum (9.0%), then quails aged 7-8 weeks (2.0%) followed by those aged one to six weeks (1 2 %). M. gallisepticum strains were isolated with higher incidence from trachea (10.0%) followed by lungs (6.0%). Biochemical identification of Mycoplasma species recovered from examined quails revealed that, the isolated Mycoplasma gallisepticum (16 isolates) were positive for glucose fermentation and tetrazolium reduction tests; in contrast, they were negative for arginine hydrolysis test. Serological typing of mycoplasma isolates by using growth inhibition test (G.I.T.) and growth precipitation test (G.P.T) revealed that 15 isolates obtained from diseased quails showing respiratory affection were identified as M. gallisepticum (18.8 %). Meanwhile, one isolate obtained from apparently healthy quails also was identified serologically as M. gallisepticum with an incidence of (5.0%). From the obtained results, it can be concluded that, all ages of quails are susceptible for infection with Mycoplasma gallisepticum

INTRODUCTION

Owing to rapidly increasing of human population in Egypt and consequently the increasing demand for cheapest protein source, special attention is being paid to the newly types of birds such as quails as trails to fulfill excessive demands of the animal proteins *(Hamouda, 1992)*. Economically quails are better source of protein than chickens as their condornicies put egg each 22 hours, and for a dozen of eggs of condorniz is necessary only 300 grams of food, and the egg of condorniz contain only 0.7% of cholesterol, with constant position of condorniz and constant couple during all the year; and it is found mature to begin to put eggs to the 42 days with high resistance to infectious agents.

Nowadays, quails become widely distributed in Egypt as a source of meat and egg production. So, the zoonotic importance of quails play a considerable role in dissemination of pathogens and their role for the transmission of Mycoplasma, Escherichia coli and Salmonella microorganisms are still the point of researches *(Abu-El-Makarem and Ali, 1997)*.

Respiratory diseases are one of the most important poultry diseases due to highly contagious affect nearly all classes of wild and domestic birds. It is usually appear as septicemic diseases with highly mortality and morbidity rates but it may persist as chronic one. Respiratory diseases cause serious economic losses due to high morbidity and mortality rate, decrease in growth rate, poor in feed conversion, poor uniformity and consuming medications during the course of disease condition, *El-Shater et al (1990) and Yoder (1990 and1991)* isolated *Mycoplasma gallisepticum* from the respiratory tract of chickens exhibiting chronic respiratory disease *(CRD). Saif Edin (1997)* stated that *Mycoplasma gallisepticum* (MG) infection was responsible for chronic respiratory disease after complication with respiratory viruses such as (ND) (Newcastle disease), IB (Infectious Bronchitis) and *E. coli* infection. .The most prevalent bacterial isolates in the respiratory affected quails was *M. gallisepticum (El-Sheshtawy and Shaker, 1999)*. So, the presented study was planned for:

1- Studying the prevalence rate of *Mycoplasma species* infection in quails in relation to their ages

2-Isolation and Biochemical identification of mycoplasma microorganisms which affect on the respiratory tract of apparently healthy and naturally infected quails.
3-Serological Identification of mycoplasma microorganisms isolated from both apparently healthy and naturally infected quails

MATERIALS and METHODS

I- Materials

1.1. Samples:

One hundred quails of different ages were collected from private and governmental farms. Out of these, eighty (80 quails) were from flocks suffering from respiratory manifestations (Coughing, sneezing, ralling, wet eyes, nasal discharge and sometimes swollen of infraorbital sinuses either bilateral or unilateral) and conjunctivitis and postmortem examination revealed that there were congestion of lung and trachea. Meanwhile the remaining twenty (20) quails were apparently healthy ones. The quails were also subjected to bacteriological examination.

1.2. The used culture media:

1.2.1. PPLO agar and broth media: (Difco) :They were used for isolation and maintenance of *Mycoplasma Spp.*
1.2.2. Media used for biochemical reaction: The following biochemical media were used according to *Cruickshank et al. (1975) and Quinn et al. (1994).*
1.2.2. Arginine deamination test medium: (Difco)
Used for differentiation of *Mycoplasma* and *Acholeplasma* species.

2. Methods:

2.1. Collection of samples:

Trachea, lung and nasal swabs were collected aseptically from both apparently healthy as well as diseased quails. All samples were sent immediately to the laboratory with minimum of delay for bacteriological, biochemical and serological identifications of mycoplasma microorganisms. All collected quails were examined for any clinical manifestations and postmortem lesions (Coughing, sneezing, ralling, wet eyes, nasal discharge and sometimes swollen of infraorbital sinuses either bilateral or unilateral) and conjunctivitis and postmortem examination revealed that there were congestion of lung and trachea).

2.2. Bacteriological examination:

Isolation and identification of mycoplasma:

1-Preparation of media: (Shaker, 1991).

2-Preparation and inoculation of the samples: (Razin and Tully, 1983):- The collected samples were aseptically removed into a sterile mortar, cut by sterile scissors and ground with aid of sterile sand and 5 ml of broth which was then added. From the mixture, about 0.2 – 0.3 ml was transferred into the PPLO broth and incubated at 37 °C for 3 days, then sub cultured onto PPLO agar plates, which were further incubated at 37 °C under reduced oxygen tension in humidified candle jars. The plates were examined for suspected colonies after 45 hours under a stereomicroscope using oblique light on every other day up to 7 – 10 days.

3- Purification of mycoplasma isolates: (Sabry, 1968)

4- Maintenance of mycoplasma isolates: Colonies were identified by their characteristic morphology (Fried-egg appearance) then agar blocks from positive plates were cut and transferred into corresponding broth isolation medium and aerobically incubated for 48 – 72 hours. After insuring the viability, sub culturing onto the isolation agar medium and purification were performed for characterization and identification of mycoplasma and then kept in a deep freezer at – 30 °C.

5-Differentiation of Mycoplasma and Acholeplasma: "Digitonin sensitivity test": (Erno and Stipkovits, 1973).

6-Biochemical identification: Suspected typical mycoplasma colonies were identified biochemically by using the following tests:

a-Glucose fermentation test and Arginine deamination test (*Sabry, 1968*)

b- Tetrazolium reduction test: (*Erno and Stipkovits, 1973*)

7-Serological identification:

a-Growth inhibition test: (*Clyde, 1964*), Filter paper discs soaked in 20 µl of antiserum prepared in rabbits were placed on the surface of the inoculated plates by the running drop technique. The plates were incubated at 37 °C in a CO_2 incubator for 3 – 7 days. Observing the zone of inhibition around the antisera discs and interpretation were made.

b-Slide agglutination test (SAT), One drop of isolate suspension was mixed with one drop of antiserum on a slide and the reaction was read within 2-minutes.

RESULTS

I. *Mycoplasma species*:

I.1.Incidence of Mycoplasma species in relation to age and site of isolation from examined quails:

As shown in Table (1) bacteriological examination of 100 quails including apparently healthy (20) and diseased quails (80) suffering from respiratory manifestations revealed the isolation of 16 isolates of *M. gallisepticum* with an over all incidence of 16.0%. Regarding the distribution and frequency of occurrence of members of mycoplasma isolated from quails in relation to the age of examined quails it can be concluded that quails aged more than 8 weeks showed the highest incidence of *M. gallisepticum* (9.0%), then quails aged 7-8 weeks (2.0%) followed by those aged 1 6 weeks (1- 2%). As shown in Table (2), *M. gallisepticum* strains were isolated with higher incidence from trachea (10.0%) and then lungs (6.0%) and negative for isolation through nasal cavity.

4.4.4.2. Results of biochemical identification of *Mycoplasma species* recovered from examined quails: The obtained isolates of mycoplasma (16 isolates) were positive for glucose fermentation and tetrazolium reduction tests; in contrast, they were negative for arginine hydrolysis test.

4.4.4.3. Serological typing of mycoplasmas:

Serological typing of mycoplasma isolates by using growth inhibition test (G.I.T.) and slide agglutination test revealed that 15 isolates obtained from diseased quails with respiratory affection were all identified serologically as *M. gallisepticum* (18.8 %). Meanwhile, one isolate obtained from apparently normal quails also was identified serologically as *M. gallisepticum* with an incidence of 5.0%.

DISCUSSION

Recently, great attention was directed to the non classical types of birds like quails in Egypt to meet the increasing demand of animal protein. Quails are regarded without any doubt, the most appropriate source of protein supply of high nutritive value and very low cholesterol contents making it a suitable source of protein of high biological value, quails are affected by common poultry diseases but are more disease resistant when compared with other avian species. The quails are migratory birds and may play an important role in transmitting the infection. One of the major problems in quail's farms is the control of bacterial diseases such

as mycoplasma microorganisms which affecting the respiratory tracts. However, until now such infections have not yet been thoroughly studied especially in Egypt as being a real problem, which may affect poultry rising in general.

Accordingly, great attention and efforts are required be paid or to eliminate these microorganisms causing respiratory infection in quails. Diagnosis must be confirmed by the isolation and complete identification of these microorganisms to determine the species of mycoplasma causing the disease.

Mycoplasma organisms are the predominant bacteria that cause significant economic loss in all phases of the avian industry. *El-Shater et al. (1990)* had found that *M. gallisepticum* to be a common cause of respiratory disease. Also, *Nascimento et al., (1986)* isolated *M gallisepticum* from sinuses of Japanese quails Moreover, *Abo- El-Makarem and Ali (1997)* collected lung tissues from living and slaughtered quails suffering from respiratory disease affection for isolation of *M. gallisepticum, M. pullorum and M. gallinaceum.*The isolation of *M.gallisepticum* from diseased quails was periodically recorded recorded by *Saif Edin (1997)* and *Murakami et al., (2002),* they stated that *Mycoplasma gallisepticum (MG)* infection was responsible for chronic respiratory disease These organisms were identified in an incidence of 38.9%, 5.6% and 18.8%, respectively. It is worth to record that in the present work *M. gallisepticum* was isolated in an incidence of 5% from apparently normal quails and (18.8%) from diseased quails with an over all incidence 16% as shown in Table (1 and 2).

The previously mentioned results agree with the findings of *Levisohn (1984); Sandoval et al. (1994), Abo- El-Makarem and Ali (1997) and El-Shater and Oraby (2001).*

Also, from the results obtained in Table (1) it can be concluded that, the isolation of *M. gallisepticum* from both apparently healthy and diseased quails. In the same time the results revealed that the diseased quails can be infected at any age with such microorganism. The isolation of the mycoplasma microorganisms in quails aged more than 8 weeks indicated that the mycoplasma microorganisms normally inhabit the respiratory system of quails and under stress factors either environmental or infection with other species of bacteria enhance the mycoplasma infection as shown in Table (1) also similar results were recorded

by *Molokowa etal., (1987)* and *Abo- El-Makarem and Ali, (1997)* serotyped mycoplasma from diseased chicken into *Mycoplasma gallisepticum*. Fifteen mycoplasma isolates were recovered from diseased quails and serotyped as *M. gallisepticum* with an incidence of 18.8%. Meanwhile, one isolate obtained from apparently normal quails was identified serologically as *M. gallisepticum. Similar results were obtained by Nascimento et al., (1986);Muirhead (1994); Janet et al., (2001); Jordan et al., (2001) and Murakami et al., (2002).*

REFERENCES

Abo- El-Makarem, M. and Ali, A. R. (1997): Bacteriological studies on Mycoplasma and *E. coli* in quails. J. Egypt. Vet. Med. Ass. 57, No. 4: 1319 1329.

Clyde, W. A. (1964): Mycoplasma species identification based upon growth inhibition by specific antisera.Immunol. 92: 958 965.

Cruickshank, R.; Dugid, J. P.; Marino, B. P. and Swain, R. H. A. (1975): Medical Microbiology. 12th Ed., and S. Livingstone Limited, Edinburgh and New York.

El-Shater, S. A. A.; Khair E-Din, A. A. W. and Oraby, F. A. (1990): Incidence of ***mycoplasma*** in gallinaceous birds. Fourth Sic. Cong; fac. Vet. Med., Assuit Univ., PP. 1003 1010.

El-Shater, S. A. A. and Oraby, F. A. I. (2001): Incidence of mycoplasma infection in chicken farms in Matrouh Governorate by using recent techniques. Egypt. Vet. J. Medicine Assc. 61(2): 293 301.

El-Sheshtawy, E. A. and Shaker, M. M. (1999): Some studies on mycoplasma and bacteria infections in quails. J. Egypt. Vet. Med. Ass. 59 (6): 1573 1584.

Erno, H. and Stipkovits, L. (1973): Bovine mycoplasmosis. Cultural and biochemical studies. Act Vet. Scand., 14: 450 463.

Hamouda, A.M. (1992): Mycoplasma prevalent in quails and some wild bird's .PhD. Thesis, Faculty of Vet. Med. ,Zagazig Univ.

Janet, M. B.; Christine, A. Y. and Cynthia, M. D. (2001): Mycoplasma and respiratory disease in pheasants and partridges. Avian Path., 30 (3): 391 396.

Jordan, F.; Pattison, M.; Alexander, D. and faragher, T. (2001): Avian mycoplasmosis. Poultry Dis., 5th Eds. PP. 178 - 186. Sounders, W. B. London, Edinburg. New York. Toronto.

Levisohn, S. (1984): Early stages in the interaction between *Mycoplasma gallisepticum* and the chick trachea, as related to pathogenicity and immunogenicity. Israel. J. Med. Sci., 20 (10): 982 984.

Hamouda, A. M. (1992): Mycoplasmas prevalent in quails and some wild birds. Ph. D. Thesis, Faculty of Vet. Med., Zagazig Univ., PP. 90.

Molokwa, J. U. A.; Degbaye, D. S. and Emejuaiwe, S. (1987): Studies on chronic respiratory disease of poultry in Nigeria. Bull. Anim. Hlth. Prod., Africa, 35 (4), 240 343.

Murakami, S.; Miyama, M.; Ogawa, A.; Shimada, J. and Nakane, T. (2002): Occurrence of conjunctivitis, sinusitis and upper region tracheitis in Japanese quail (Coturnix Japonica), possibly caused *Mycoplasma gallisepticum* accompanied cryptosporidium Sp. infection . Avian Pathol., 31 (4): 363 – 70.

Nascimento-M-da-GF-do; Nascimento-ER-do; Do- Nascimento-M-da-GF; Do- Nascimento-ER (1987): Infectious sinusitis in cornix quails in Brazil. Avian Dis. 30 (1): 228-230.

Quinn, P. J.; Carter, M. E.; Markey, B. K. and Carter, G. R. (1994): Clinical veterinary microbiology. Wolfe Publishing Livestock, London.

Razin, S. and Tully, J. (1983): Methods in Mycoplasmology. Vol. I, mycoplasma characterization, 3rd Ed. Acad. Press, N. Y.

Sabry, M. Z. (1968): Characterization and classification of avian *mycoplasma*. Ph. D. Thesis, Cornell Univ., USA.

Saif Edin, M. (1997): Situation of mycoplasma infection among chickens in Upper Egypt with evaluation of different diagnostic techniques. Assuit Vet. Med. J. 37 (73) : 220-230

Sandoval, V. E.; Terzoto, H. R. and Blackall, P. J. (1994): Complicated infection coryza outbreaks in Argentina. Avian Dis., 38 (3): 672 – 678.

Shaker, M. (1991): Affection mycoplasmosis in some poultry farms with special reference to Ureaplasma. M. V. Sc. Thesis, Microbiol., Fac. Vet. Med. Alex. Univ.

Yoder, H. W. Jr. (1990): Mycoplasma In "Diagnostic Procedure in Veterinary Bacteriology and Mycology". PP. 333. Ed. Carter, G. R. and Cole, J. R. Academic, Press Inc.

Yoder, H. W. Jr. (1991): *Mycoplasma gallisepticum infection.* Poultry Diseases. Ninth Eds PP. 198 – 212.

Table (1): Incidence of *Mycoplasma gallisepticum* in relation to the age of examined quails.

Age per week	Apparent healthy (20)		Diseased quails (80)			Total (100)	
	No of examined cases	No.	%	No of examined cases	No.	%	No of examined cases	No.	%
1 2	4	0	0.0	19	2	10.2	23	2	2.0
3 4	9	0	0.0	18	1	5.5	27	1	1.0
5 6	3	0	0.0	7	2	28.6	10	2	2.0
7 8	2	0	0.0	8	2	25.0	10	2	2.0
More than 8-weeks	2	1	50.0	28	8	28.6	30	9	9.0
Total	20	1	05.0	80	15	18.8	100	16	16.0

No: Number of positive cases, %: Was calculated according to the number of examined cases.

Table (2): Distribution of *Mycoplasma gallisepticum* isolates in organs of apparently healthy and diseased quails

Site of isolation	General health condition					
	Apparently healthy quails (20)		Diseased quails (80)		Total (100)	
	No	%	No	%	No	%
Lungs	0	0.0	6	7.5	6	6.0
Trachea	1	5.0	9	11.3	10	10.0
Nasal swabs	0	0.0	0	0.0	0	0.0
Overall	1	5.0	15	18.8	16	16.0

No: Number of positive cases
%: Was calculated according to the number of examined cases

الملخص العربي

دراسات بكتريولوجية عن العدوى بميكروب الميكوبلازما جاليسيبتكم في السمان وصلته بالأعمار المختلفة

عبد العزيز عبد الخالق مسعد* أشرف عواد عبد التواب** , ورشا نبيل زهران*

*قسم البكتريولوجى والمناعة والفطريات- كلية الطب البيطرى السادات جامعة المنوفية

** قسم البكتريولوجى - كلية الطب البيطرى بمشتهر جامعة الزقازيق

أجريت هذه الدراسة على عدد 100 من السمان الذى تم جمعه من مزارع خاصه وحكوميه من أماكن متعددة منهم عدد 20 سمان سليم ظاهريا وعدد 80 سمان مريض يعانى من أعراض تنفسية متنوعة كحه وعطس وضيق فى التنفس وارتشاح من الجيوب الأنفية وكذلك انتفاخهاز وبالفحص البكتريولوج تم عزل ميكروب الميكوبلازما (بنسبه اجمالية 16 %) كما تم تصنيف الميكوبلازما المعزولة من السمان المريض سيرولوجيا بنسبة 18.8% للنوع ميكوبلازما جاليسيتكم.كما تم تصنيف الميكوبلازما المعزولة من السمان السليم ظاهريا سيرولوجيا بنسبة 5% لنوع ميكوبلازما جاليسيتكم . وبمقارنة الدراسة ومعدل العزل من الأعمار المختلفة تبين ان أعلى معدل للعزل من السمان عند عمر اكثر من ثمانية اسابيع بنسبة 9 % وبنسبة 2% عند عمر 7-8 اسبوع بينما عند اعمر 1-6 أسبوع كانت نسبة العزل تتراوح من 1-2% فقط . كما أنه وجد ان الميكوبلازما جاليسيتكم تم عزلها غالبا من القصبة الهوائية بنمسبة عزل 10% يليها الرئتين بنسبة 6%. ومن هذه الدراسة يتضح ان الأعمار المختلفة من السمان عرضة للآصابة بميكروب الميكوبلازما جاليسيبتكم وحيث أنه من الطيور المهاجرة فمن الممكن ان يكون له دور فى انتشار هذا النوع من الميكوبلازما والذى يسبب اصابة فى النواع الأخرى من الطيور.

****