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FURTHER STUDIES ON QUAIL MYCOPLASMOSIS IN ASSUIT GOVERNORATE

(With 5 Tables)

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دراسات مستفيضة عن الميكوبلازما في السمان بمحافظة أسيوط

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تمت هذه الدراسة على أعمار مختلفة من السمان من مزرعة كلية الزراعة جامعة أسيوط ومن المزارع المختلفة المنتشرة في محافظة أسيوط وكانت محاولة العزل الأولى للميكوبلازما من طيور سليمة ظاهريا وأخرى تعانى أعراضا تنفسية. وقد أخذت مسحات من القصبة الهوائية والرئة والأكياس الهوائية. وكان إجمالي عدد العترات المعزولة ٤٠ عترة من ١٢٥ حالة ميتة وسليمة ظاهريا. وتم تقسيم العترات المعزولة بيوكيميائيا وسيرولوجيا باستخدام المانع للنمو الى ١٢ عترة ميكوبلازما جالسيتيكم (٣٠%) و ١١عترة ميكوبلازما جالينيروم (٢٧,٥%)، ٨ عترات ميكوبلازما بالوروم (٢٠%) ولم يتم تصنيف باقى العترات. ولتحديد ضراوة العترات المعزولة تم حقن السمان عمر ٢ أسبوع في داخل الانف وكانت فترة الملاحظة ٣ أسابيع وقد تم ذبح السمان بعد هذه الفترة. وقد أوضحت التجربة عدم وجود وفيات بالأنواع المختلفة للميكوبلازما. وقد تم وصف الأعراض الاكلينيكية والصفات التشريحية والتي كانت عبارة عن زيادة في سمك الأكياس الهوائية وعكارتها وكذلك احتقان الرئات والكبد. وقد أجرى اختبار حساسية العترات المعزولة ضد ١٠ من المضادات الحيوية الشائعة معمليا وقد وجد أن جميع العترات حساسة لكل من اللنكوسبكتين وسبيكتينوميسين والنيلوزين والجنتاميسين والنيومايسين والكانميسن وغير حساسة للتتراسيكلين والاوكسى تتراسيكلين والامبيسلين والكلورومفينيكول.

SUMMARY

In this study mycoplasma was isolated from 125 Coturinx quails from faculty of Agriculture poultry farm and also from local breeds at Assiut Governorat, Swabs for culturing were obtained from trachea of 80 bird clinically healthy as well as from these showing signs of mild respiratory disease and from lungs and air sacs of 45 dead birds. A total of 40 (32%) isolates were recovered from 125 examined birds based on biochemical

as well as serological tests, 12 (30 %) isolates were M. gallisepticum, 11 (27.5 %) M gallinarum, 8 (20%) M pullorum and 9 (22.5%) untyped .For detection of virulence of isolated strains, thirty – 2 weeks old quails were inoculated intranasal with 10^7 cfu of the isolated M. gallisepticum, M gallinarum and M pullorum, 10 birds for each isolate. The experiment revealed no mortality with the different types of Mycoplasma isolates. PM lesions were recorded in birds including congestion of the lungs and livers as will as turbidity and thickening of the air sacs. The recovered strains were tested against the available antibiotics by the in-vitro sensitivity test, where all strains were highly sensitive to Lincospectin, Spectinomycin and Tylosin, and moderate senstive to Kanamycin Gentamycin and Neomycin but resistant to Tetracycline, Oxy-tetracycline, Ampicillin and Chloramphinichol.

Keywords: *Mycoplasmosis in Quail*

INTRODUCTION

During recent years there has been a noticeable increase in the number of quail farms in Egypt which are considered an important alternative source of high quality protein with low amount of cholesterol. Quails and other migratory birds play a considerable role in dissemination of many pathogens and act as a reservoir and carrier of microbial agents for domestic birds and human. This supports the importance of sanitation and sound management in poultry farms against such birds (El-Attar *et al*., 1996). Mycoplasma plays an important role in chronic respiratory disease of domestic fowl and infectious sinusitis of turkeys (Yoder *et al*, 1964). M. gallisepticum has been isolated from natural infections in several species of birds including commercial Japanese quail suffering from purulent sinusitis (Tiong, 1978) and from bob-white quail with chronic respiratory disease (Madden *et al*., 1967), but Cookon and Shiraprasad (1994) reported that Mycoplasma infection in quails should be considered not only as a potential source of Transmission to other birds but also as a potential selection pressures for the generation of new variant and pathogenic strains. Yoder and Hofstad (1964) reported that nine of 12 quails inoculated with the first yolk passage of M. gallisepticum developed evidence of infection in the form of detectable antibodies by the heamagglutination inhibition procedure.

Ahmed (1996) isolated M. gallisepticum, M. gallinarum, M. gallinaceum and M. pullorum from quails and used ELISA test for

detection of antibodies against Mycoplasma species. Murakami *et al.* (2002) isolated M. gallisepticum from a farm of Japanese quail used for egg production that showed clinical signs of nasal discharge, increase lacrimation and decreased egg production with large, gelatinous masses of caseous exudate in the sinuses and air sacs. They concluded that M. gallisepticum play important role in respiratory disease in quails.

This work was designed to investigate the local distribution of coturnix quails mycoplasmosis in the area of Assiut province, studying the pathogenicity of isolated strains in quails and testing of the in-vitro sensitivity of the isolated strains to antimycoplasmal agents available in the field.

MATERIALS and METHODS

Materials were collected to cover different ages of living Coturnix quails from the poultry farms of Agriculture College Assiut University and from a private farms in Assiut Province. Both dead and those showing signs of respiratory disease were included.

1- Swabbing:

Lung, Trachea and air sacs were swabbed. Swabs were sown onto Mycoplasma medium.

2- Mycoplasma medium:

Mycoplasma broth and agar media were prepared as described by Yoder (1980) which composed of brain heart infusion broth and agar (Difco), 20% fresh Horse serum, 5% yeast extract, 2% thallium acetate, penicillin G. sodium 1000 u/mL and its pH was adjusted to 7.8.

3-Standard antisera:

Standard antisera against different mycoplasma speciese were kindly obtained from prof. Dr. Adel Mohamed Soliman, Dept.of poultry diseases, fac. Vet. Med. Assiut university

a-Pathogenicity test:

Forty, two-weeks old Coturnix quails were divided into 4 equal groups, these quails were proved to be free from mycoplasma infection by bacteriological and serological examination. The first 3 groups were used for experiment. 1st group infected with mycoplasma gallisepticum, 2nd group infected with mycoplasma gallinarum and 3rd group infected with mycoplasma pullorum.Birds of 4th group kept as noninfectd control. Each quail was inoculated intranasally with 0.2 mL of the local mycoplasma 10^7 CFU. Re-isolation was done on the 3rd week of quail age

according to Kuba *et al.* (1968). The clinical signs and post-mortem lesions were observed and recorded.

b-Sensitivity of the isolated strains to antimycoplasmal agents:

This test was done to determine the more effective antimycoplasmal drugs available in the field. Ten types of antibiotics were used: Lincospectin (100 ug) - Spectinomycin (100 ug) - Tylosin (100 ug) - Gentamycin(30 ug) - Neomycin (30 ug) - Tetracyclin (30 ug) - Oxytetracycline (30 ug) - Kanamycin (30 ug) - Ampicillin (10 ug) - Chloramphenicol (30 ug). The broth culture of strain with known colony forming unit (10^8/mL) was cultured on brain heart infusion agar by running drop technique. Plates were incubated at 37°C in moist candal jar for 3-4 days, then examined microscopically. The results were expressed by the method of Clyde (1964).

4-Isolation of mycoplasma:

The collected samples were cultured as described by Sabry and Ahmed (1975). Each swab sample was inoculated into 5 ml brain heart infusion broth, then incubated at 37°C for 3 days. 0.02 mL of broth culture was inoculated and streaked on brain heart infusion agar. The agar plate was incubated at 37°C 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 microscop. In case of mycoplasma growth on agar plates a single colony was picked up with an a gar-block and transplanted into fresh liquid medium and the growth was checked by regular plating of inoculated sample.

Purification and characterization:

It was done through the following steps :

(a) Purification and maintenance of isolates (Sabry, 1968).

(b) Genus determination using digitonin sensitivity test (Erno and Stipkovits, 1973).

(c) Biochemical characterization tests were carried out using glucose fermentation and arginin utilization (Freundt *et al.*, 1979).

5- Serological identification

It was carried out by growth inhibition test which recommended by Clyde (1964).

RESULTS

Microbiological studies:

Anumber of 40 isolates were recovered from 125 examined Coturnix quails, 80 (64%) clinically healthy and 45 (36%) dead birds

(Table 1). According to the biochemical patterns, the 40 recovered isolates of mycoplasma were classified into two groups (Table 2) Group I isolates fermented glucose but did not split arginine (Glucose +ve and Arginine -ve), Group II isolates did not ferment glucose but split arginine (Glucose -ve and Arginine +ve).

Serological identification

According to the results of the growth inhibition test which considered positive if the inhibitory zone was more than 2 mm. The recovered 40 isolates were classified serologically into M. gallisepticum 12, M. gallinarum 11, M. pullorum 8 and untyped isolates 9 (Table 3).

Results of the pathogenicity testing:

No mortalities were observed in all groups. The infected groups showed slight respiratory symptoms, watery nasal discharge, sneezing, depression, inappetence and some birds showed diarrhea. The control group revealed no apparent changes.

The post-mortum lesions observed in scarified birds was air sacculitis which was severe in 6 cases inoculated with M.gallisepticum. Affected air sacs were thickened and opaque. Congestion of lungs and liver was observed in some birds.

Re-isolation:

The inoculated mycoplasma were reisolated from respiratory organs of living and slaughtered birds (Table 4).

In vitro-sensitivity:

The senstivety testing of the isolated strains to antimycoplasma agents showed that all strains were highly sensitive to Lincospectin, Spectinomycin and Tylosin but moderate senstive to Gentamycin, Neomycin and Kanamycin. On the other hand, they were resistance toTetracycline, Oxytetracycline, Ampicilline and Chloramphenicol (Table 5).

DISCUSSION

Mycoplasma infection accounts for major economic losses to the poultry industry due to downgrading of meat, reduced feed utilization and egg production efficiency as well as increased medication costs (Bencina *et al.*, 1988). Moreover, the conduction of adequate prevention and control programmes are also expensive (Stipkovits, 1979).

The present study was carried out to investigate, the incidence and possible role of mycoplasma in quails, which acts as a reservoir and carrier of microbial agents for birds and human (David *et al.*, 1967 and

El-Attar *et al.*, 1996). All this support the importance of sanitation and sound management in poultry farms against such infection. Out of 125 samples 40 isolates, were recovered from Coturnix quails. The isolates were identified according to the growth inhibition test which recommended by Kleven (1975), who was reported that this test is of great value in identifecation of mycoplasma isolates. In Egypt, El-Ebeedy *et al.* (1977) and Soliman (1984) typed their isolates into 12 isolates M. gallisepticum (30%), 11 isolates M. gallinarum (27.5%), 8 isolates M. pullorum (20%) and 9 isolates untyped (22.5%). These results are in agreement with those reported by Tiong (1978), Reece *et al.* (1986) and Cookson and Shiraprasad (1994) who isolated M. gallisepticum from two flocks of quails with an incidence of 10% and 13%. These results are also similar to previous studies done in Egypt by Ahmed (1996) who isolated M. gallisepticum, M. gallinarum, M. gallinaccum and M. pullorum from quails.

The experimental infection of quails with the isolated strains showed that no mortalities were recorded from infected quails at two weeks after infection,although some infected birds showed clinical signs as slight respiratory symptoms, watery nasal discharge, sneezing, depression, inappetence and some birds showed diarrhea. The postmortem lesions detected in quails including air sacculitis, congestion of lungs and liver. These findings are closely similar to those observed by Yagihashi *et al.* (1988) and Soeripto *et al.* (1989).

Reisolation of mycoplasma from experimentally infected birds was successful after two weeks post-infection. Similar to El-Ebeedy (1976), Ahmed (1996) and Mahmoud (1999).

In vitro sensitivity test of the isolated strains to antimycoplasmal agents showed that these strains were highly sensitive to Lincospectin, Spectinomycin, and Tylosin. These results similar to those reported by Sinclair (1980), Soliman (1984), and Recce *et al.* (1986). On the other hand, isolates were resistance to Tetracycline, Oxytetracyclin, Ampicilline and Chloramphenicol. This resistance may be attributed to miss use of antibiotics in the field which resulted to devolopment of acquired resistance of field isolates to these antibiotics .

According to our results it can be concluded that Mycoplasma infection in quails should be considered as an important disease which act as a source of transmission of the disease to the different species of birds.

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Table 1: Recovery rate of mycoplasma from Q. quails.

No. of samples	No. of positive	Percentage %	Site of isolation (No)		
			Lung	Trachea	Air sacs
125	40	32	12	18	10

Table 2: Biochemical reactions of Mycoplasma isolates

No. of positive isolates	Biochemical reactions	
	Group I Glucose +ve Arginin –ve	Group 2 Glucose -ve Arginin +ve
40	24	16

Table 3: Serological properties of isolated strains.

Species	No. of +ve	Percentage %
M.gallisepticum	12	30
M. gallinarum	11	27.5
M. pullorum	8	20
Untyped	9	22.5
Total	40	

Table 4: Results of experimentally infected quails with the isolated strains of mycoplasmas and percentage of reisolation.

Groups	Strain	No. of inoculated birds	Rout	Reisolation after experimental infection*
1	M. gallisepticum	10	intranasal	8/10(80%)
2	M. gallinarum	10	Intranasal	9/10(90%)
3	M. pullorum	10	Intranasal	5/10(50%)
4	Control	10		0/10-

*No. of positive / No. of examined.

Table 5: Results in-vitro sensitivity of Mycoplasma isolates to different antibiotics

Antibiotics	Concentration	Species		
		M. gallisepticum	M. gallinarum	M. pullorum
Lincospectin	100 µg	+++	+++	+++
Spectinomycin	100 µg	+++	+++	+++
Tylosin	100 µg	+++	+++	+++
Gentamycin	30 µg	++	++	++
Neomycin	30 µg	+	+	+
Tetracyclin	30 µg	-	-	-
Oxytetracyclin	30 µg	-	-	-
Kanamycin	30 µg	+	+	+
Ampicillin	10 µg	-	-	-
Chloramphinicol	30 µg	-	-	-