Comparative Studies on *Mycoplasma Gallisepticum* and *Mycoplasma Synoviae* in Migratory and Captive Quails

Khafagy, A. A.*, Eid, H. M. *, Hassan, A. M. **, and Mona, E. M. ***
**Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Suez Canal University.*
***Mycoplasma Research Department, Animal Health Research Institute, Dokki, Giza.*
****B.V.SC 2003-Cairo Univeresity*

Abstract

This study was conducted to compare between the *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in migratory and captive quails. Fifty four (13.5%) and 87 (21.75%) *Mycoplasma* strain isolates were isolated from 400 samples from migratory and captive quails respectively. Ten (2.5%) *Mycoplasma gallisepticum* strains were isolated from captive quails by culture method and identified by **PCR** while, 9 (2.25%) *Mycoplasma gallisepticum* strains were isolated from migratory quails by culture method but 11 (2.75%) strains were identified by **PCR**. *Mycoplasma synoviae* couldn't be isolated by culture method or **PCR**. The most effective antibiotic was Tylosin which could inhibit the growth of 88.88% of *Mycoplasma gallisepticum* isolated in case of migratory quails and 80% in case of captive quails using **MIC** Technique.

Introduction

Mycoplasma is the causative agent of Chronic Respiratory Disease **(CRD)** in chickens and infectious sinusitis in turkeys, chickens, game birds (as quails), pigeons, and passerine birds of all ages ***(Hennigana et al., 2012)***. *Mycoplasma gallisepticum* **(MG)** and *Mycoplasma synoviae* **(MS)** are considered to be the most important of the pathogenic Mycoplasmas, and both occur in world-wide ***(OIE Terrestrial Manual, 2004)***. During the 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 reservoir and carrier of microbial agents for domestic birds and human ***(Fatma, 2004)***.

Culture method represented the performance standard for direct detection of the organism ***(Maricarmen et al., 2005)***, but **PCR** represent rapid and

sensitive method, which is able to provide accuracy results in the presence of mixed *Mycoplasma* infection and bacterial contamination or inhibition of growth by antibiotics, antibodies and other host factors ***(Stipkovitz, 2001)***; so the aim of this work was to evaluate the using of recent techniques in confirming the identification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* isolated from quails to classical methods and also to determine the most effective antimicrobials.

Materials & Methods

1- Sampling: A total of 800 samples were collected from quails (400 samples from migratory quails and 400 from captive quails). These samples were collected during different months of the year in different governorates from different organs; as shown in (Table 1).

2- Isolation of *Mycoplasma*: The samples were cultured in Frey's broth and on Frey's agar medium ***(Frey et al., 1968)*** as described by ***(Sabry and Ahmed, 1975)***. Digitonin sensitivity test ***(Freundt et al., 1973)*** was done to differentiate between *Mycoplasma* and *Acholeplasma* genera, also urea hydrolysis test **(Razin, 1978)** for differentiation between *Mycoplasma* and *Ureaplasma* genera.

3- Biochemical characterization tests: To identify the purified *Mycoplasma* isolates using different tests as glucose fermentation, arginine deamination, tetrazolium reduction tests ***(Erno and Stipkovits, 1973)***, and film and spot formation medium ***(Fabricant and Freundt, 1967)***.

4- List of Antibiotics used for Minimum Inhibitory Concentration MIC Technique:

MIC applied on the 10 *Mycoplasma gallisepticum* samples isolated from captive quails and 9 *Mycoplasma gallisepticum* samples isolated from migratory quails using culture method by the following antibiotics:

- Doxycycline produced by GMP (certified Spain -EU).
- Erythromycin produced by Pantex-Holland.
- Tilmicosin produced by ELA-Geneva.
- Tiamutin produced by Sandos GmbH (Basale Switzerland).
- Tylosin produced by Elanco-USA.
- Enrofloxacin produced by Invesa-Spain.

The best method for interpretation of the results which compare the **MIC** results to C-max (maximum plasma concentration of the drug at the use by optimum dose) according to ***(Burch and Valks, 2002; El-Soud et al., 2004, and Abu-Basha et al., 2007)*** to determine the sensitive isolates (the antibiotic will be sensitive if **MIC** for it was less than C-max). The following (Table 2) shows C-max for different antibiotic used:

5- Polymerase Chain Reaction PCR:

PCR applied on the 800 samples (400 captive and 400 migratory quail samples) for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

a) DNA Extraction and purification:

DNA extraction by rapid method ***(Fan et al., 1995)***.

DNA extraction using QIA amp.

b) Oligonucleotide Primers Selection

Two sets of primers were used for detection of mgc2 gene of the isolated *Mycoplasma gallisepticum*. The selected primers were prepared as described by **Garcia et al., 2005** and the sequences of the primers were:

mgc2F 5` - CGCAATTTGGTCCTAATCCCCAACA - 3`.

mgc2R 5` - TAAACCCACCTCCAGCTTTATTTCC - 3`.

Two sets of primers were used for detection of 16S gene of *Mycoplasma synoviae.* The selected primers were prepared as described by ***Lauerman (1998)*** and the sequences of the primers were:

16SF 5` - GAGAAGCAAAATAGTGATATCA - 3`.

16SR 5` - CAGTCGTCTCCGAAGTTAACAA - 3`.

c) PCR Amplification and Cycling Protocols

6-Identification of the PCR Products (Electrophoresis) *(Sambrook, 1989):*

Identification of the **PCR** products (Electrophoresis) following amplification, a 5µl of the **PCR** product was mixed with 2µl of loading buffer and taken for electrophoresis on a 2% (weight/volume) agarose gel (Biometra USA). Gel was stained with ethidium bromide and inspected under UV lamp (Biometra USA). A visible band being sized by **DNA** molecular marker was considered as positive sample.

Table (1): *Migratory and Captive Quail Samples collected during Different Months of the Year in Different Governorates from Different Organs*

Bird Type	Total Number of Birds	Months							Governorates					Sample Types				Samples Total
Migratory Quails	100	200	200	0	0	0	0	0	80	80	80	80	80	100	100	100	100	400
Captive Quails	100	60	60	60	60	60	60	40	80	80	80	80	80	100	100	100	100	400
Grand Total	200	260	260	60	60	60	60	40	160	160	160	160	160	200	200	200	200	800

Table (2): *Different Antimicrobial Agents used in Minimum Inhibitory Concentration (MIC) Method and Interpretation of their Sensitivity by C-max*

Antibiotics	Doxycycline	Erythromycin	Tylosin	Tilmicosin	Tiamutin	Enrofloxacin
C-max (µg)	54.58	6.9	4.2	2.46	3.56	1.88

Table (3): *MG mgc2 Gene (one of the cytadhesin genes that play an important role in the virulence of MG) PCR Cycling Protocol (Garcia et al., 2005)*

Initial Denaturation	Actual Cycles Temperature/Second	Final Extension
95°C for 3 minutes	35 cycles of: Denaturation: 94/20 Annealing: 58/40 Extension: 72/60	72°C for 15 minutes

Table (4): *MS 16S Gene (subunit from ribosomal RNA responsible for the synthesis of essential proteins) PCR Cycling Protocol (Lauerman, 1998)*

Initial Denaturation	Actual Cycles Temperature/Second	Final Extension
94°C for 5 minutes	35 cycles of: Denaturation: 94/60 Annealing: 55/60 Extension: 72/120	72°C for 10 minutes

Results

In (Table 5) out of 19 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails during different months of the year from different organs using culture method was the highest in September from nasal swabs (3 isolates = 15.79%), followed by lung tissues and trachea as the same (2 isolates = 10.53%), and finally air-sacs the percent was (0%); in October the highest incidence was from nasal swabs and lung tissues as the same (2 isolates = 10.53%), and finally trachea and air- sacs the percent was (0%); in November the highest incidence was from nasal swabs and trachea with (1 isolate = 5.26%); in December the highest incidence was from trachea and air-sacs with (1 isolate = 5.26%); in January the highest incidence was from nasal swabs and lung tissues with (1 isolate = 5.26%); in February the highest incidence was from lung tissues with (2 isolates = 10.53%); and finally in March the percent was (0%). On the other hand **MG** isolation during the migration months of the year from different organs of migratory and captive quails using culture method was highest at September from nasal swabs which was 3 isolates (by both culture and **PCR**) in case of migratory quails, and 2 isolates at February from lung tissues in case of captive quails (by both culture and **PCR** methods). Also out of 19 **MG** positive isolates the incidence during different months of the year from different organs of migratory and captive quails using culture method was the most higher at September (7 isolates = 36.84%), followed by October (4 isolates = 21.05%), then November, December, January, and February as the same with (2 isolates = 10.53%), and finally March with (0%). In (Table 6) out of 19 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails in different governorates during different months of the year using culture method was the highest from North Sinai in September with (3 isolates = 15.79%), followed by October, November, and December as the same with (1 isolate = 5.26%); from Kafr El-Sheikh the highest incidence was in September with (3 isolates = 15.79%), then October and December as the same with (1 isolate = 5.26%); from El-Fayoum the highest incidence was in February with (2 isolates = 10.53%), followed by September, October, and January as the same with (1 isolate = 5.26%); from Matrouh the highest incidence was in November, and January as the same with (1 isolate = 5.26%); and finally; from Port Said the highest incidence was in October with (1 isolate = 5.26%). On the other hand *Mycoplasma gallisepticum* isolation was the highest from North Sinai governorate at September which was 3 isolates (4 by **PCR**) in case of migratory quails; and from El-

Fayoum governorate at February 2 isolates in case of captive quails. Also out of 19 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails in different governorates during different months of the year using culture method was the most higher in North Sinai (6 isolates = 31.58%), followed by Kafr El-Sheikh, and El-Fayoum as the same with (5 isolates = 26.32%), then Matrouh with (2 isolates = 10.53%), and finally Port Said with (1 isolate = 5.26%).

In (Table7) out of 19 *Mycoplasma gallisepticum* positive isolates the incidence from different organs of migratory and captive quails in different governorates using culture method was the highest from nasal swabs in North Sinai (3 isolates = 15.8%) followed by Port Said, Kafr El-Sheikh , Matrouh and El-Fayoum as the same with (1 isolate = 5.3%); from lung tissues the highest incidence was in El-Fayoum (3 isolates = 15.8%), followed by Kafr El-Sheikh and North Sinai with (2 isolates = 10.5%); from trachea the highest incidence was in Kafr El-Sheikh (2 isolates = 10.5%), followed by Matrouh and El-Fayoum as the same with (1 isolate = 5.3%); from air-sacs there were (1 isolate = 5.3%) in North Sinai from captive quails only. The highest incidence of **MG** was from lung tissues in El-Fayoum governorate which was 3 isolates (in both culture and **PCR** techniques) in case of captive quails, also from nasal swabs and lung tissues in North Sinai governorate in case of migratory quails as 2 isolates (in both culture and **PCR** techniques).

Also out of 19 **MG** positive isolates the incidence from different organs of migratory and captive quails in different governorates using culture method was the highest from nasal swabs and lung tissues as the same with (7 isolates = 36.84%), followed by trachea with (4 isolates = 21.1%), and finally air-sacs with (1 isolate = 5.3%). In (Table7) out of 19 *Mycoplasma gallisepticum* positive isolates the incidence from different organs of migratory and captive quails in different governorates using culture method was the highest from nasal swabs in North Sinai (3 isolates = 15.8%) followed by Port Said, Kafr El-Sheikh , Matrouh and El-Fayoum as the same with (1 isolate = 5.3%); from lung tissues the highest incidence was in El-Fayoum (3 isolates = 15.8%), followed by Kafr El-Sheikh and North Sinai with (2 isolates = 10.5%); from trachea the highest incidence was in Kafr El-Sheikh (2 isolates = 10.5%), followed by Matrouh and El-Fayoum as the same with (1 isolate = 5.3%); from air-sacs there were (1 isolate = 5.3%) in North Sinai from captive quails only. The highest incidence of **MG** was from lung tissues in El-Fayoum governorate which was 3 isolates (in both culture and **PCR** techniques) in case of captive quails, also from

nasal swabs and lung tissues in North Sinai governorate in case of migratory quails as 2 isolates (in both culture and **PCR** techniques).

Also out of 19 **MG** positive isolates the incidence from different organs of migratory and captive quails in different governorates using culture method was the highest from nasal swabs and lung tissues as the same with (7 isolates = 36.84%), followed by trachea with (4 isolates = 21.1%), and finally air-sacs with (1 isolate = 5.3%).

In (Table 8) Out of 400 samples in the migratory quails 23 *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation tests positive; and arginine deamination, tetrazolium reduction, film & spot formation medium tests were negative which suggested being *Mycoplasma gallinaceum*.

Fourteen *Mycoplasma* isolates were digitonin sensitivity, arginine deamination, tetrazolium reduction, film & spot formation medium tests positive; and glucose fermentation test was negative which suggested being *Mycoplasma gallinarum.*

Nine *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation and tetrazolium reduction tests positive; film & spot formation medium, and arginine deamination tests were negative which suggested being *Mycoplasma gallisepticum.*

Eight *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation, and film & spot formation medium tests positive; arginine deamination, tetrazolium reduction tests were negative which suggested being *Mycoplasma iners.*

On the other hand out of 400 samples in the captive quails, 34 *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation tests positive; and arginine deamination, tetrazolium reduction; film & spot formation medium tests were negative which suggested being *Mycoplasma gallinaceum*.

Twenty tow *Mycoplasma* isolates were digitonin sensitivity, arginine deamination, tetrazolium reduction, film & spot formation medium tests positive; and glucose fermentation test was negative which suggested being *Mycoplasma gallinarum.*

Ten *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation, and tetrazolium reduction tests positive; and arginine deamination, film & spot formation medium tests were negative which suggested being *Mycoplasma gallisepticum*.

Sixteen *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation, and film & spot formation medium tests positive; arginine deamination, tetrazolium reduction tests were negative which suggested being *Mycoplasma iners.*

Five *Mycoplasma* isolates were digitonin sensitivity, glucose fermentation, arginine deamination, and tetrazolium reduction tests positive; film & spot formation medium test was negative which suggested being *Mycoplasma iowae*.

In (Tables 9 & 10) showed that the most effective antibiotic was Tylosin which could inhibit the growth of 88.88% of *Mycoplasma gallisepticum* positive isolates in case of migratory quails, and 80% of *Mycoplasma gallisepticum* positive isolates in case of captive quails, on the other hand Doxycycline was the least effective antibiotic it inhibit only the growth of 44.44% of isolated *Mycoplasma gallisepticum* positive isolates in case of migratory quails and 40% in case of captive quails.

Mycoplasma gallisepticum isolates from migratory quails was more sensitive than the strain isolated from captive quails.

In (Table 11) out of 21 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails during different months of the year from different organs using **PCR** technique was the highest in September from nasal swabs and trachea as the same (3 isolates = 14.29%), followed by lung tissues (2 isolates = 9.52%), and finally air-sacs the percent was (0%); in October the highest incidence was from nasal swabs (3 isolates = 14.29%), followed by lung tissues (2 isolates = 9.52%), and finally trachea and air- sacs the percent was (0%); in November the highest incidence was from nasal swabs and trachea with (1 isolate = 4.76%); in December the highest incidence was from trachea and air-sacs with (1 isolate = 4.76%); in January the highest incidence was from nasal swabs and lung tissues with (1 isolate = 4.76%); in February the highest incidence was from lung tissues with (2 isolates = 9.52%); and finally in March the percent was (0%). On the other hand **MG** isolation during the migration months of the year from different organs of migratory and captive quails using culture method was highest at September from nasal and tracheal swabs as the same which was 3 isolates in case of migratory quails, and 2 isolates at February from lung tissues in case of captive quails.

Also out of 21 **MG** positive isolates the incidence during different months of the year from different organs of migratory and captive quails using **PCR** technique was the most higher at September (8 isolates = 38.095%), followed by October (5 isolates = 23.81%), then November, December, January, and February as the same with (2 isolates = 9.52%), and finally March with (0%).

In (Table 12) out of 21 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails in different governorates during different months of the year using **PCR** technique was the highest from North Sinai in September with (4 isolates = 19.05%), followed by October, November, and December as the same with (1 isolate = 4.76%); from Kafr El-Sheikh the highest incidence was in September with (3 isolates = 14.29%), then

October and December as the same with (1 isolate = 4.76%); from El-Fayoum the highest incidence was in February with (2 isolates = 9.52%), followed by September, October, and January as the same with (1 isolate = 4.76%); from Matrouh the highest incidence was in October, November, and January as the same with (1 isolate = 4.76%); and finally from Port Said the highest incidence was in October with (1 isolate = 4.76%), followed by Kafr El-Sheikh, North Sinai, Matrouh, and El-Fayoum as the same (0%). On the other hand *Mycoplasma gallisepticum* isolation was the highest at September from North Sinai governorate which was 4 isolates in case of migratory quails; and at February from El-Fayoum governorate 2 isolates in case of captive quails.

Also out of 21 *Mycoplasma gallisepticum* positive isolates the incidence from migratory and captive quails in different governorates during different months of the year using **PCR** technique was the most higher in North Sinai (7 isolates = 33.33%, followed by Kafr El-Sheikh, and El-Fayoum as the same with (5 isolates = 23.81%), then Matrouh with (3 isolates = 14.29%), and finally Port Said with (1 isolate = 4.76%).

In (Table 13) out of 21 *Mycoplasma gallisepticum* positive isolates the incidence from different organs of migratory and captive quails in different governorates using **PCR** technique was the highest from nasal swabs in North Sinai (3 isolates = 15.8%) followed by Matrouh (2 isolate = 9.52%); then Port Said, Kafr El-Sheikh , and El-Fayoum as the same (1 isolate = 4.76%); from lung tissues the highest incidence was in El-Fayoum (3 isolates = 14.29%), followed by Kafr El-Sheikh and North Sinai with (2 isolates = 9.52%); from trachea the highest incidence was in Kafr El-Sheikh (2 isolates = 9.52%), followed by North Sinai, Matrouh, and El-Fayoum as the same (1 isolate = 4.76%); from air-sacs there was (1 isolate = 4.76%) in North Sinai from captive quails only. The highest incidence of **MG** was from lung tissues in El-Fayoum governorate which was 3 isolates (in both culture and **PCR** techniques) in case of captive quails, also from nasal swabs and lung tissues in North Sinai governorate in case of migratory quails as 2 isolates (in both culture and **PCR** techniques). Also out of 21 **MG** positive isolates the incidence from different organs of migratory and captive quails in different governorates using **PCR** technique was the highest from nasal swabs (8 isolates = 38.095%), followed by lung tissues with (7 isolates = 33.33%), then trachea with (5 isolates = 23.81%), and finally air-sacs with (1 isolate = 4.76%).

Table (5): *The Incidence of Mycoplasma gallisepticum isolates from Migratory and Captive Quails during Different Months of the Year from Different Organs using Culture Method*

Months	Sample Types																Total No. of +ve	%
	Nasal Swabs				Lung Tissues				Trachea				Air-Sacs					
	Migratory quails	%	Captive quails	%	Migratory Quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%		
September	3	15.79	0	0	1	5.26	1	5.26	2	10.53	0	0	0	0	0	0	7	36.84
October	1	5.26	1	5.26	2	10.53	0	0	0	0	0	0	0	0	0	0	4	21.05
November	0	0	1	5.26	0	0	0	0	0	0	1	5.26	0	0	0	0	2	10.53
December	0	0	0	0	0	0	0	0	0	0	1	5.26	0	0	1	5.26	2	10.53
January	0	0	1	5.26	0	0	1	5.26	0	0	0	0	0	0	0	0	2	10.53
February	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
March	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total No.	4	21.05	3	15.79	3	15.79	4	21.05	2	10.53	2	10.53	0	0	1	5.26	19	100

Table (6): *The Incidence of Mycoplasma gallisepticum isolates from Migratory and Captive Quails in Different Governorates during Different Months of the Year using Culture Method*

Gover-norates	Months																								Total No. of +ve	%
	September				October				November				December				January				February				March			
	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory Quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%		
[illegible]	3	15.79	0	0	1	5.26	0	0	0	0	1	5.26	0	0	1	5.26	0	0	0	0	0	0	0	0	0	0	0	0	6	31.58
afr El-[illegible]	2	10.53	1	5.26	1	5.26	0	0	0	0	0	0	0	0	1	5.26	0	0	0	0	0	0	0	0	0	0	0	0	5	26.32
El- Fayoum	1	5.26	0	0	0	0	1	5.26	0	0	0	0	0	0	0	0	0	0	1	5.26	0	0	2	10.53	0	0	0	0	5	26.32
Matrouh	0	0	0	0	0	0	0	0	0	0	1	5.26	0	0	0	0	0	0	1	5.26	0	0	0	0	0	0	0	0	2	10.53
Port Said	0	0	0	0	1	5.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5.26
Total No.	6	31.58	1	5.26	3	15.79	1	5.26	0	0	2	10.53	0	0	2	10.53	0	0	2	10.53	0	0	2	10.53	0	0	0	0	19	100

Table (7): *The Incidence of Mycoplasma gallisepticum isolates from Different Organs of Migratory and Captive Quails in Different Governorates using Culture Method*

Sample Types	Governorates																Total No. of positive	% of positive
	North Sinai				Kafr El-Sheikh				El-Fayoum				Matrouh				Port Said					
	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%		
Nasal Swabs	2	10.5	1	5.3	1	5.3	0	0	0	0	1	5.3	0	0	1	5.3	1	5.3	0	0	7	36.84
Lung Tissues	2	10.5	0	0	1	5.3	1	5.3	0	0	3	15.8	0	0	0	0	0	0	0	0	7	36.84
Trachea	0	0	0	0	1	5.3	1	5.3	1	5.3	0	0	0	0	1	5.3	0	0	0	0	4	21.1
Air-Sacs	0	0	1	5.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	5.3
Total No.	4	21.1	2	10.5	3	15.8	2	10.5	1	5.3	4	21.1	0	0	2	10.5	1	5.3	0	0	19	100

Table (8): *Biochemical Characterization of Mycoplasma Species Isolated from Migratory and Captive Quails*

Bird Types	Biochemical Tests						Suggested Isolates	No. of *Mycoplasma* Isolates	Total No. of +ve
	Digitonin Sensitivity	Urease Test	Glucose Fermentation	Arginine Deamination	Tetrazolium Reduction	Film and Spot Formation Medium			
Four-hundred Migratory Quail Samples	+	-	+	-	-	-	*M. gallinaceum*	23	54
	+	-	-	+	+	+	*M. gallinarum*	14	
	+	-	+	-	+	-	*M. gallisepticum*	9	
	+	-	+	-	-	+	*M. iners*	8	

Four-hundred Captive Quail Samples	+	-	+	-	-	-	*M. gallinaceum*	34	87
	+	-	-	+	+	+	*M. gallinarum*	22	
	+	-	+	-	-	+	*M. iners*	16	
	+	-	+	-	+	–	*M. gallisepticum*	10	
	+	-	+	+	+	-	*M. iowae*	5	

Table (9): *Minimum Inhibitory Concentration (MIC) Results of Mycoplasma gallisepticum Isolates from Migratory Quails*

Antibiotics	Minimum inhibitory concentration mg/µl									C-max	Number of sensitive isolates
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9		
Doxycycline	32	64	64	64	32	16	128	64	32	54.58	4/9 (44.44)
Erythromycin	4	8	4	8	8	16	16	1	2	6.9	4/9 (44.44)
Tylosin	0.25	0.5	0.06	0.06	0.13	1	0.5	8	2	4.2	8/9 (88.88)
Tilmicosin	0.5	4	0.13	0.13	1	16	0.5	4	1	2.46	6/9 (66.66)
Tiamutin	0.5	2	0.25	1	4	4	0.5	1	0.5	3.56	7/9 (77.77)
Enrofloxacin	4	4	1	0.5	1	8	0.5	4	0.5	1.88	5/9 (55.55)

Table (10): *Minimum Inhibitory Concentration (MIC) Results of Mycoplasma gallisepticum Isolates from Captive Quails*

Antibiotics	Minimum inhibitory concentration mg/µl										C-max	Number of sensitive isolates
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10		
Doxycycline	128	64	32	32	128	32	32	64	64	64	54.58	4/10 (40%)

Erythromycin	**16**	**8**	**1**	**4**	**16**	**4**	**8**	**1**	**8**	**4**	**6.9**	**5/10 (50%)**
Tylosin	**1**	**0.5**	**8**	**0.13**	**0.5**	**0.25**	**0.13**	**8**	**2**	**0.06**	**4.2**	**8/10 (80%)**
Tilmicosin	**16**	**2**	**8**	**0.13**	**0.5**	**0.5**	**4**	**4**	**1**	**0.13**	**2.46**	**6/10 (60%)**
Tiamutin	**4**	**2**	**16**	**1**	**2**	**1**	**4**	**1**	**0.25**	**0.25**	**3.56**	**7/10 (70%)**
Enrofloxacin	**8**	**4**	**16**	**0.5**	**1**	**4**	**1**	**4**	**0.5**	**1**	**1.88**	**5/10 (50%)**

Table (11): *The Incidence of Mycoplasma gallisepticum isolates from Migratory and Captive Quails during Different Months of the Year from Different Organs using PCR Technique*

Months	**Sample Types**												**Total No. of +ve**	**%**
	Nasal Swabs				**Lung Tissues**				**Trachea**				**Air-Sacs**					
	Migratory quails	**%**	**Captive quails**	**%**	**Migratory Quails**	**%**	**Captive quails**	**%**	**Migratory quails**	**%**	**Captive quails**	**%**	**Migratory quails**	**%**	**Captive quails**	**%**		
September	3	14.29	0	0	1	4.76	1	4.76	3	14.29	0	0	0	0	0	0	8	38.095
October	2	9.52	1	4.76	2	9.52	0	0	0	0	0	0	0	0	0	0	5	23.81
November	0	0	1	4.76	0	0	0	0	0	0	1	4.76	0	0	0	0	2	9.52
December	0	0	0	0	0	0	0	0	0	0	1	4.76	0	0	1	4.76	2	9.52
January	0	0	1	4.76	0	0	1	4.76	0	0	0	0	0	0	0	0	2	9.52

Total No.	March	February
5	0	0
23.81	0	0
3	0	0
14.29	0	0
3	0	0
14.29	0	0
4	0	2
19.05	0	9.52
3	0	0
14.29	0	0
2	0	0
9.52	0	0
0	0	0
0	0	0
1	0	0
4.76	0	0
21	0	2
100	0	9.52

Table (12): *The Incidence of Mycoplasma gallisepticum isolates from Migratory and Captive Quails in Different Governorates during Different Months of the Year using PCR Technique*

Type of Samples	Months																		Total No. of +ve	
	September			October			November			December			January			February			March				
	Migratory quails	%	Captive quails	Migratory quails	%	Captive quails	Migratory Quails	%	Captive quails	Migratory quails	%	Captive quails	Migratory quails	%	Captive quails	Migratory quails	%	Captive quails	Migratory quails	%	Captive quails		
North Sinai	4	19.05	0	1	4.76	0	0	0	1	0	4.76	1	0	0	0	0	0	0	0	0	0	7	33.33
Kafr El-Sheikh	2	9.52	1	1	4.76	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	5	23.81
El- Fayoum	1	4.76	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	5	23.81
Matrouh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	14.29
Port Said	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4.76
Total No.	7	33.33	1	4.76	4	19.05	3	14.29	4	19.05	3	14.29	2	9.52	0	0	0	0	1	4.76	21	100	

Table (13): *The Incidence of Mycoplasma gallisepticum isolates from Different Organs of Migratory and Captive Quails in Different Governorates using PCR Technique*

Type of Samples	Governorates																Total No. of +ve	%
	North Sinai				Kafr El-Sheikh				El Fayoum				Matrouh				Port said					
	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%	Migratory quails	%	Captive quails	%		
Nasal swabs	2	9.52	1	4.76	1	4.76	0	0	0	0	1	4.76	1	4.76	1	4.76	1	4.76	0	0	8	38.095
Lung tissues	2	9.52	0	0	1	4.76	1	4.76	0	0	3	14.29	0	0	0	0	0	0	0	0	7	33.33
Trachea	1	4.76	0	0	1	4.76	1	4.76	1	4.76	0	0	0	0	1	4.76	0	0	0	0	5	23.81
Air-sacs	0	0	1	4.76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4.76
Total No.	5	23.81	2	9.52	3	14.29	2	9.52	1	4.76	4	19.05	1	4.76	2	9.52	1	4.76	0	0	21	100

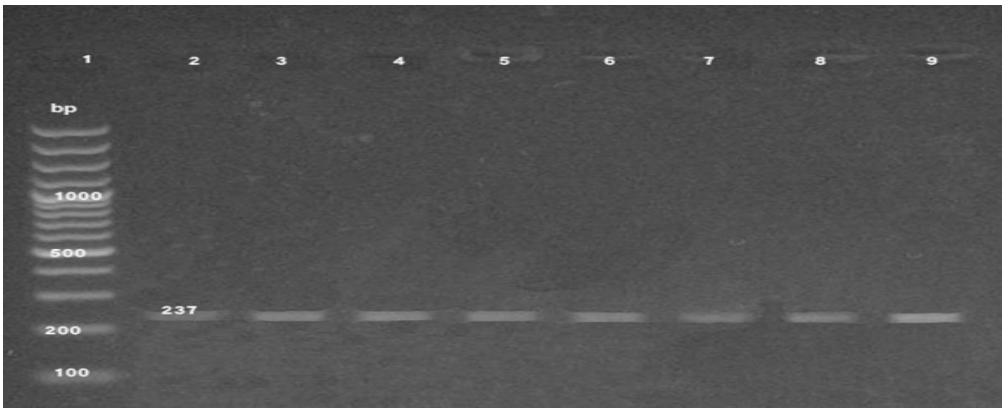

Fig. (1): *Electrophoresis pattern of the PCR products of Mycoplasma gallisepticum Isolates from Migratory and Captive Quail Different Organs using mgc2 Gene*

Lane 1: 100 bp DNA ladder.
Lane 2 : *Mycoplasma gallisepticum* isolate from migratory quail nasal swabs.
Lane 3 : *Mycoplasma gallisepticum* isolate from captive quail nasal swabs.
Lane 4: *Mycoplasma gallisepticum* isolate from migratory quail trachea.
Lane5: *Mycoplasma gallisepticum* isolate from captive quail trachea.
Lane 6: *Mycoplasma gallisepticum* isolate from migratory quail lungs.
Lane 7: *Mycoplasma gallisepticum* isolate from captive quail lungs.
Lane 8: *Mycoplasma gallisepticum* isolate from migratory quail air-sacs.
Lane 9: *Mycoplasma gallisepticum* isolate from captive quail air-sacs.

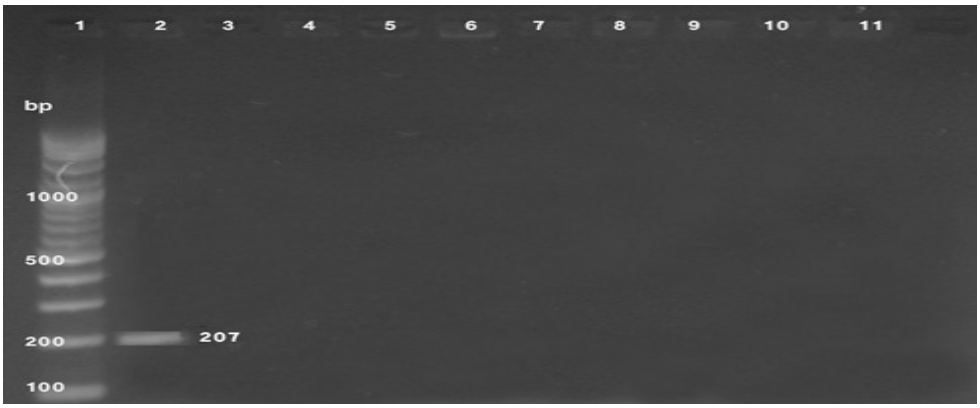

Fig. (2): *Electrophoresis pattern of the PCR products of Mycoplasma synoviae Isolates from Migratory and Captive Quail Different Organs using 16S Gene*
Lane 1: 100 bp DNA ladder.
Lane 2: *Mycoplasma synoviae* positive control.
Lane 3: *Mycoplasma synoviae* negative control.
Lane 4 : *Mycoplasma synoviae* isolate from migratory quail nasal swabs.
Lane 5 : *Mycoplasma synoviae* isolate from captive quail nasal swabs.
Lane 6: *Mycoplasma synoviae* isolate from migratory quail trachea.
Lane7: *Mycoplasma synoviae* isolate from captive quail trachea.
Lane 8: *Mycoplasma synoviae* isolate from migratory quail lungs.
Lane 9: *Mycoplasma synoviae* isolate from captive quail lungs.
Lane 10: *Mycoplasma synoviae* isolate from migratory quail air-sacs.
Lane 11: *Mycoplasma synoviae* isolate from captive quail air-sacs.

Discussion

The data in (Table 5 & 11) revealed that the increase of *Mycoplasma gallisepticum* in September was in coordination with results achieved by ***(Baha El-Din, 1993; El-Naenaeey et al., 2000; and Ibrahim and Busse, 2012)****;* this is due to the stress associated with migration which increase the bird's susceptibility to pathogens or enhance their shedding rate ***(Dhama et al., 2008)***. On the other side the increment of the incidence in captive quails during December and other cold months is in agreement with ***(Ley and Yoder, 1997)*** it tends to be more severe and of longer duration in the cold months and affects younger birds more severely than mature birds.

In (Table 6 & 12) the increment of the incidence from North Sinai governorate may be due to transmission of *Mycoplasma* through the east northern Egyptian borders which may happen by air transmission or by the migratory birds themselves; this may explain the high incidence during September in North Sinai governorate (3 isolates = 15.79%) and this was in agreement with ***(Baha El-Din, 1993;*** *and* ***Ibrahim and Busse, 2012)***.

In (Table 7 & 13) increase incidence from nasal swabs indicated that the primary habitats of Mycoplasmas are the mucosal membranes of the respiratory tract, and/or the urogenital tract, eyes and joints. Adhesion of Mycoplasmas to host cells is a prerequisite for successful colonization, and ensuing pathogenesis ***(Levisohn and Kleven, 2000)***. Also Mycoplasmas constitute part of the normal flora in small migratory birds' respiratory tracts. This would suggest that migratory birds could carry disease-causing Mycoplasmas over large distances and spread disease through wild and domesticated populations ***(Christine, 2010)***, and these results were in coordination with the results achieved by ***(El-Shater 1986; Bencina et al., 1987; El-Naenaeey et al., 2000; and Vitula et al., 2011)***.

In (Table 8) the isolation of different types of Mycoplasma species from quails was conceded by ***(Bogomolova et al., 1978; Tiong, 1978; Nascimento & Nascimento, 1986; Reece et al., 1986; Bencina et al., 1987; El-Naenaeey et al., 2000; Murakami et al., 2002 and Fatma, 2004)*** and referred that Mycoplasma infection in quails should be considered as an important disease which act as a source of transmission to different species of birds ***(Fatma, 2004)***.

In (Table 9 & 10) the most effective antibiotic detected by minimum inhibitory concentration **(MIC)** was Tylosin which could inhibit the growth of 88.88% of *Mycoplasma gallisepticum*

strain isolated in case of migratory quails and 80% in case of captive quails. *Mycoplasma gallisepticum* strain isolated from migratory quails was more sensitive than the strain isolated from captive quails, these results were in agreement with ***(Kempf et al., 1989; Ching et al., 1997; Hannan, 2000 and Reda and Abd El-Samie, 2012)***; and were in disagreement with **Gerchman et al., 2011**. On the other hand, the resistant to Doxycycline may be attributed to miss use of it in the field which resulted to development of acquired resistance of field isolates to this antibiotic.

On the other hand the positive **PCR** products amplified at 237bp this was in agreement with ***(Hnatow et al., 1998; Garcia et al., 2005; Lysnyansky et al., 2005)***. The mgc2 gene, which encodes a cytadhesin protein ***(Hnatow et al., 1998)***, is currently the one of the preferred gene targets for this assay, due to its specificity for *Mycoplasma gallisepticum* ***(Garcia et al., 2005)***. Also they suggested that the mgc2 **PCR** is the method of choice for *Mycoplasma gallisepticum* in the field. The expected size for amplification products with mgc2 based **PCR** was varied in range of 236-302bp for *Mycoplasma gallisepticum*. Also *Mycoplasma synoviae* couldn't be isolated from both migratory and captive quails by culture method or **PCR** and these results were disagreement with ***(Bencina et al., 1987)***.

References

Abu-Basha, E.A.; Idkaidek, N.M.; and Al-Shunnaq, A.F.; (2007): Pharmacokinetics of Tilmicosin (Provitil Powder and Pulmotil Liquid AC) Oral Formulations in Chickens Veterinary Research Communications, 31: 477–485.

Baha El Din, S. M.; (1993): Notes on recent changes in the status of breeding herons in the Egyptian Nile Valley and Delta. Ornithological Society of the Middle East. Bulletin, 29, 12-15.

Bencina, D.; Dorrer, D.; and Tadina, T. (1987): *Mycoplasma* species isolated from six avian species. Avian Pathology, 16(1987) 653-664.

Bogomolova, N. N.; Izotova, N. V.; Boriskin, I. S.; Lotte, V. D.; and Kolianova, I. S.; (1978): Isolation and study of several properties of *Mycoplasma* from Japanese quail embryos. Vopr. Virusol. 1978:560–564.

Burch, D.G.S.; and Valks, M.; (2002): Comparison of Minimal Inhibitory Concentrations **(MIC)** against Chicken *Mycoplasma* of Tiamulin and Other Antimicrobials and their Concentrations in the Blood. Proseeding of the 12th World Veterinary Poultry Association, Cairo, Egypt, January 2002 P: 322.

Ching Wu, DVM, PhD; Thomas R. Shryock, PhD; Tsang Long Lin, DVM, PhD; and Melissa Fleck Veenhuizen, DVM, MS, CCW, TLL (1997):
Testing antimicrobial susceptibility against *Mycoplasma hyopneumoniae* in vitro. Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, Indiana, 47907; TRS, MFV: Elanco Animal Health.

Christine R., (2010):
The Detection of Mycoplasmas in Migratory Birds. Thesis submitted by Zoology Department at Miami University.

Dhama, K., Mahendran M., and Tomar S., (2008):
Pathogens Transmitted by Migratory Birds: Threat Perceptions to Poultry Health and Production, International Journal of Poultry Science 7 (6): 516-525.

El-Naenaeey EY.; El-Messalmy M.; El-Sheikh S.; Hamouda A.; (2000):
Mycoplasmas prevalent in quails and some wild birds in Egypt, Veterinary Medical Journal Giza, 48: 91 - 110.

El-Shater, S. S. A.; (1986):
Some studies on chronic respiratory disease in fowls. Ph.D. Thesis (Poultry Diseases), Faculty of Vet. Med., Assiut University.

El-Soud, K. A.; Al-Tarazi Y. H. & Al-Batineh M. M.; (2004):
Comparative pharmacokinetics and bioavailability of amoxicillin in chickens after intravenous, intramuscular and oral administrations. Veterinary Research Communication, 28, 599–607.

Erno H., and Stipkovits L., (1973):
Bovine Mycoplasmas, cultural and biochemical studies. Acta Vet Scared., 14: 450- 463.

Fabricant, J.; and Freundt, E. A.; (1967):
Importance of extension and standardization of laboratory tests for the identification and classification of *Mycoplasma*. Ann. N. Y. Acad. Sci., 143: 50 - 58.

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.

Fatma A.M., (2004):
Further studies on quail mycoplasmosis in Assuit governorate Assiut Veterinary Medical Journal, 50 (102):228-236.

Freundt E.A., Anderson B.E., Erno N., Kunze M., and Black F.T., (1973):
The sensitivity of mycoplasmatales to sodium polyanethol. Sulphate and digitonin. Zb1. Back. Parasite infehtkr, Hyg. L.Aby. Drig. A. (225): 104- 112.

Frey M., Hanson, R. and Anderson, P., (1968):
A medium for the isolation of avian *Mycoplasmas*. Americ. Vet. J., 29:2163-2171.

Garcia N., Ikuta N., Levisohn S., and Keleven S.H., (2005):
Evaluation and comparison of various PCR methods for detection of *Mycoplasma gallisepticum* infection in chickens. Avian Dis. 49 (1): 125-132.

Gerchman I., Levisohn S., Mikula I., Manso-Silván L. and Lysnyansky I., (2011):
Characterization of in vivo-acquired resistance to macrolides of *Mycoplasma gallisepticum* strains isolated from poultry Veterinary Research, 42:90.

Hannan P. C.T., (2000):
Guidelines and recommendations for antimicrobial Minimum Inhibitory Concentration **(MIC)** testing against veterinary Mycoplasmas. Vet. Res. 31, 373-395.

Hnatow L.L., Keeler C.L, Tessmer L.L., Czymmek K. & Dohms J.E. (1998):
Characterization of MGC2, a *Mycoplasma gallisepticum* cytadhesin with homology to the *Mycoplasma pneumonia* 30-kilodalton protein P30 and *Mycoplasma genitalium* P32. *Infect. Immun.,* Vol. 66(7): 3426-3442.

Hennigana, Suzanne L., Jeremy D. Driskellb, Naola Ferguson-Noelc, Richard A. Dluhyd, Yiping Zhaoe, Ralph A. Trippb, and Duncan C. K., (2012):
"Detection and Differentiation of Avian Mycoplasmas by Surface-Enhanced Raman Spectroscopy Based on a Silver Nanorod Array". Applied and Environmental Microbiology 78 (6): 1930–1935.

Ibrahim W. A., and Busse P., (2012):
Migration of passerines through some protected areas in the eastern part of Egypt. Ring 34: 69-205.

Kempf I., Ollivier C., Morin M., Hospitalier R., Guittet M., and Bennejean, G., (1989):
Minimum inhibitory concentration of 13 antibiotics against 21 strains of avian *Mycoplasma***.** The vet. Point 20:935-940.

Lauerman, L.H., (1998):
Mycoplasma PCR assays. In L.H. Lauerman (Ed.), Nucleic Acid Amplification Assays for Diagnosis of Animal Diseases (pp. 41 _52). Davis, CA: American Association of Veterinary Laboratory Diagnosticians.

Levisohn, S. and Kleven, S.H. (2000):
Avian mycoplasmosis (*Mycoplasma gallisepticum*). In C.W. Beard & S. McNulty (Eds.), Diseases of Poultry: World Trade and Public Health Implications, Vol. 19 (pp. 425 _442). Paris, France: Office International des Epizooties.

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.

Lysnyansky, I.; Garcia, M.; & Levisohn, S.; (2005):
Use of mgc2- polymerase chain reaction-restriction fragment length polymorphism for rapid differentiation between field isolates and vaccine strains of *Mycoplasma gallisepticum* in Israel. Avian Dis., 49: 238–245.

Maricarmen G., Nilo I., Sharon L., and Keleven S.H., (2005):
Evaluation and comparison of various **PCR** methods for detection of *M. gallisepticum* infection in chickens. Avian Dis., 49: 125-132.

Murakami S., Miyama M., Ogawa A., Shimada J., & Nakane T., (2002):
Occurrence of conjunctivitis, sinusitis and upper region tracheitis in Japanese quail (Coturnix coturnix japonica), possibly caused by *Mycoplasma gallisepticum* accompanied by Cryptosporidium sp. infection, Avian Pathology, 31:4, 363-370.

Nascimento, M.G.F.; and Nascimento, E.R.; (1986):
Infections sinusitis in cortunix quails in Brazil. Avian Dis., v.30, n.1, p.228-230.

OIE, (2004):
Avian mycoplasmosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition.

Razin, S.; (1978):
The mycoplasmas. Microbiol. Rev.42:414-70.

Reda, L. M.; and Abd El-Samie., L.K.; (2012):
Some Studies on the Diagnosis of *Mycoplasma gallisepticum* in Chicken. Nat Sci. ;10(12):247-251.

Reece, R.L.; Ireland, L., and Scott, P.C.; (1986):
Mycoplasmosis in racing pigeons. Australian Vet. J., 63 (5): 166-167.

Sabry, M. Z.; and Ahmed, A. A.; (1975):
Evaluation of culture procedure for primary isolation of Mycoplasmas from female genitalia of farm animals. J. Egypt. Vet. Med. Ass. , 35: 18 - 34.

Sambrook, J.; Fritsch, E.F; and Maniatis, T; (1989):
Gel electrophoresis of DNA. Molecular Cloning: a Laboratory Manual. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, chapter 6.

Stipkovits, L.; (2001):
Development in *Mycoplasma* infection: diagnosis and control, In: Proceedings XII International Congress of the World Veterinary Poultry Association. Edited by Sami Ahmed, A.A.; Sabry, Y.I.; Uousef, A.A.; El-Gohary; and Hashim, M.M. The Egyptian

Branch of the World Veterinary Poultry Association. pp. 50-58.

Tiong, S.K.; (1978): Isolation of *Mycoplasma gallisepticum* from sinuses of three quails (Coturnix coturnix japonica). Veterinary Record, 103, 539.

Vitula, F.; Peckova, L.; Bandouchova, H.; Pohanka, M.; Novotny, A.; Jira, D.; Kral1, J.; Ondracek, K.; Osickova, J.; Zendulkova, D.; Rosenbergova, K.; Treml, F.; and Pikula, J.; (2011):

الملخص العربى

أجريت هذه الدراسة للمقارنة بين ميكروب الميكوبلازما جاليسبتيكم و الميكوبلازما سينوفي في طيور السمان المهاجرة و المحبوسة، ٥٤ (١٣,٥%) و ٨٧ (٢١,٧٥%) من عترات الميكوبلازما تم عزلها من ٤٠٠ طائر سمان لكل من الطيور المهاجرة و المحبوسة على التوالي. تم عزل ١٠ عترات للميكوبلازما جاليسبتيكم بواسطة العزل الأولي و اختبار تفاعل عديد البلمرة المتسلسل من طيور السمان المحبوسة، بينما تم عزل ٩ عترات للميكوبلازما جاليسبتيكم بواسطة العزل الأولي و ١١ عترة بواسطة اختبار تفاعل عديد البلمرة المتسلسل من طيور السمان المهاجرة. و لم يتم عزل الميكوبلازما سينوفي بواسطة العزل الأولي و اختبار تفاعل عديد البلمرة المتسلسل. كان التايلوزين أكثر المضادات الحيوية فعالية حيث ثبط ٨٨,٨٨% من معزولات الميكوبلازما جاليسبتيكم في حالة طيور السمان المهاجرة و ٨٠% في حالة طيور السمان المحبوسة باستخدام اختبار أقل تركيز مثبط لنمو الميكروبات.