



The Current Status of *Mycoplasma synoviae* in Broilers and Laying Chicken Farms in some Egyptian Governorates

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MYCOPLASMA *synoviae* (MS) has a major financial impact on poultry production since it can cause infectious synovitis, respiratory symptoms, and eggshell apex abnormalities in both turkeys and chickens. The current study aims to identify MS using bacteriological and molecular methods from cases suffering from arthritis in broilers and layer hens in certain Egyptian governorates. Between January 2020 and March 2022, one hundred twenty-five samples from 25 flocks/governorate representing 15 broiler chickens and 10 laying hens were from 5 Egyptian governorates (Giza, Cairo, AL-Dakahlia, AL-Qaliobia and AL-Fayoum). Conventional techniques were used to characterize the isolates, as well as 16S rRNA, and VLha specific MS primers were used for molecular identification by using PCR. Microscopic fried egg colony examination on the bacterial isolation samples showed 67/125 (53.6%) were suspected MS positive, and 58/125 (46.4%) were MS negative. Out of the 67 only 55 isolates were recognized as MS by conventional polymerase chain reaction (PCR) 55/67 (82%) with total rate of 55/125 (44%). Our research concluded that MS continues to be a significant avian pathogen, needed for immediate and regular monitoring of MS in chicken flocks, as well as future research to identify the best preventative and control approaches for this disease.

Keywords: Arthritis; *Mycoplasma synoviae*; PCR; 16S rRNA; VLha.

Introduction

Mycoplasma synoviae is the second-most significant species from a clinical and financial perspective, and it is recognized as a poultry disease among the mycoplasma species [1; 2]. In poultry production, this disease is to blame for huge financial losses [3]. This infection usually causes growth retardation, joint lesions, and respiratory symptoms in hens and turkeys [4]. Additionally, MS can cause abnormalities in eggshells in layers, which degrades egg quality and quantity and raises operational costs [5; 6]. The presence of co-infections such as infectious bronchitis virus, Newcastle disease virus, influenza A virus, *Escherichia coli*, or other Mycoplasmas accompanied with poor housing circumstances might exacerbate the clinical symptoms, which can vary in degree from sub-

clinical to severe forms [7]. Avian mycoplasmosis can be spread either horizontally, frequently by close contact between sick and healthy carriers, or vertically by breeders via eggs [8]. Additionally, Mycoplasma can continuously persist in the flock as forms [9]. MS is seen in flocks of layer, broiler, and breeder hens [10]. Identification of infected birds is crucial to preventing the risk of infection spreading to healthy birds, as well as focusing care and control actions in areas where MS is prevalent, to successfully limit the infections with MS [10]. Mycoplasma seroprevalence in chicken farms has been the subject of numerous reports throughout the world [11]. Vaccines and antibiotics are being used as control therapy for MS. Doxycycline, oxytetracycline, tylosin, and pleuromutilins are suitable antibacterial drugs for the treatment of MS; however, long-term drug use can result in drug resistance [7; 12]. Diagnostic techniques include

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isolation by culturing, serological examination, and molecular identification are most suitable tools for mycoplasma identification [11; 13]. Mycoplasma cultivation is a time-consuming, expensive, and labor-intensive laboratory technique that needs sterile surroundings. Other saprophytic organisms and first colonies on PPLO agar may not be detected for up to four weeks, and even then, mixed infections may cause a compromised result or a negative culture [14]. Amer et al. [15] used PCR for molecular diagnosis of MS infection from joints of chicken with clinical arthritis. So, the use of molecular technologies is particularly beneficial because conventional diagnosis methods have drawbacks. high sensitivity and specificity define the PCR as a quick test and this method enables the detection of Mycoplasma in clinical samples from treated birds or asymptomatic infected [16]. Moreover, PCR has the advantage of being able to identify co-infections with a variety of environmental and vaccine-derived respiratory microorganisms (*M. gallisepticum*, MS, IBV) [17]. The goal of the current study was to conduct a survey study on the current MS situation in commercial laying hens and broiler chicken in some Egyptian governorates using various mycoplasma diagnostic techniques culturing media and PCR technique as quick and accurate diagnosis are crucial for halting the spread of the diseases.

Material and Methods

Ethics Declaration

The Faculty of Veterinary Medicine, Cairo University's Institutional Animal Care and Use Committee (Vet. CU. IACUC), with reference number Vet CU 2009 2022519, has ethically approved this work.

Sampling

The samples were taken from suspected cases

showing signs of arthritis in different joints (**Fig.1**) from 5 governorates in Egypt (Giza, Cairo, AL-Dakahlia, AL-Qaliobia and AL-Fayoum) [15].

For detecting of MS infection between the seasons of January 2020 and March 2022, samples were taken from 25 flocks/governorate from each governorate that had locomotor problems but had not obtained a mycoplasma vaccination. These flocks included (15) broiler and (10) laying hen farms. From each farm provided a pooled sample of synovial fluid, which was taken from two recently deceased birds and used as one unit/sample for the purposes of the study (one sample). **Table 1** lists the total number of specimens obtained, the culture result, and the PCR result.

MS, Isolation, culture, and Bio-typing

The combined samples were inoculated into PPLO broth and incubated for 48 hours at 37°C with 5 to 10% CO₂ and humidity in accordance with Elbehiry et al.[18] standard protocols. Swine serum, yeast extract, and dextrose were added to the media, and growth inhibitors for bacteria and fungi, such as penicillin and thallium acetate, were used in the media in accordance with OIE [19] Most MS strains require the medium to be supplemented with cysteine hydrochloride and nicotinamide adenine dinucleotide (NAD) [20]. Using the drop technique, a loopful of each incubated sample was smeared on PPLO agar and incubated for 10 to 14 days at 37°C with 5 to 10% CO₂ and humidity [21]. the color changes from pink to yellow without any turbidity indicating the proliferation of Mycoplasmas in broth medium, although under the dissecting microscope, mycoplasma colonies like fried eggs are frequently seen on solid media [20]. The digitonin sensitivity test was employed to biotype the purified colonies, isolates of Mycoplasma and Acholeplasma and to differentiate isolates of Mycoplasma and Acholeplasma [22].



Fig.1. Postmortem of freshly dead chickens showing A, B 7 C: Severe arthritis including swelling with cardinal signs of inflammation in different joints.

TABLE 1. Total sample, MS culture, and PCR results for broilers chicken and layers suffering from locomotor disturbance in different Egyptian governorates.

Governorates	Total examined farm (No)	Type		Positive culture NO		Positive culture %		Positive PCR NO		Positive PCR %	
		Broiler	Layer	Broiler	Layer	Broiler	Layer	Broiler	Layer	Broiler	Layer
Giza	25	15	10	5	3	33.3	30	4	2	66.6	80
AL Qaliobia	25	15	10	9	5	60	50	7	4	77.7	80
AL Dakahlia	25	15	10	8	7	53.3	70	6	6	75	85.7
Al fayoum	25	15	10	4	5	26.6	50	3	4	80	66.6
AL Sharkia	25	15	10	12	9	80	90	10	9	83.3	100
Total	125	75	50	38	29	50.6	58	30	25	78.9	86.2

Purification and preservation of isolates

To create a pure culture, one fried egg-shaped colony was chosen, along with the agar block, and put into a broth media. The purified isolates were then stored in agar blocks at 20°C.

Molecular Detection

DNA extraction

Any presumed-positive samples from flocks without vaccinations that had fried egg-like colonies are subjected to confirmation testing using PCR. According to the manufacturer's instructions, DNA from samples containing typical mycoplasma colonies was extracted using a commercial genome extraction gene direx (simply) kit. DNA was collected in sterile Eppendorf and stored at -20°C until use. in accordance with the technique described by Cetinkaya et al.[23] we amplified a 278 bp region of the 16S rRNA gene using two forward and reverse Mycoplasma-specific primers. The sequence of 16S rRNA primer are (F:5' TGGGGAGCAAACAGGATTAGATACC3') and (R: 5' TGCACCATCTGTCACTCTGTTAAC CTC 3') an underlying cycling condition as seen in **Table 2**. According to Jeffery et al. [24] approach, additional primers to detect MS amplification a 392 bp region of the VLha gene include. The VLha primer's sequence are F 5 'TACTATTAGCAGCTAGTGC 3' R 5 'AGTAACCGATCCGCTTAAT 3 an underlying cycling condition as seen in **Table 3**. The

amplifications required a total volume of 25µL, consisting of 3 µL of DNA template, 2 µL of each primer (10 picomoles), 12.5 µL of Taq DNA polymerase, and the remainder sterile distilled water. Amplified PCR products were run on a 1.5% agarose gel with Tris-boric acid-EDTA buffer pH 8.0 for 45 min at 1.7 volts and observed with a UV transilluminator during the gel electrophoresis procedure.

Results

Field investigation and bacterial findings

The bacteriological study revealed that only 67 (53.6%) of the 125 samples were positive for MS isolation showed fried egg appearance on PPLO agar (Fig.2), and these samples represented 38/75 (50.6%) broiler chickens and 29/50 (58%) layer chickens, respectively. they were also positive for mycoplasma on the digitonin test, with a detection rate of 64/67 (95.5%).

The samples from the Al Sharkia governorates had the highest isolation percentage in broiler farms (80%), while the samples from the AL Fayoum governorates had the lowest isolation percentage (26.6%). in the other aspect of layer farms, AL Sharkia governorates recorded the highest isolation percentage (90%) and Giza governorates had the lowest isolation percentage (30%). layer farms have a greater isolation rate (58%) than broiler farms (50.5%).

Molecular findings

These positive samples were subjected to molecular identification using 16S rRNA and VLha genes and the percentage of positive samples was (78.9%) broiler farms and (86.7%) layer farms. Using MS-specific primers (16S rRNA and VLha gene), PCR was used to describe 67 suspect colonies, as shown in **Fig.(3)**. 55/67 (82%) colonies showed amplified fragments at

278 bp and 392 bp, respectively, while 30/38 (78.9%) were positive from broiler farms and 25/29 (86.2%) from layers, respectively. According to Table 1, The PCR 55 positive were representing 44% from total examined field samples, the highest ratio was observed in the broiler and layer in AL Sharkia out of the 67 isolates, and by conventional PCR, 55 of them were identified as MS.

TABLE 2. Shows the PCR condition of 16 srRNA primer.

Species	Initial denaturation	PCR conditions			Final extension	Reference
		Amplification (35 cycle of)				
		Denaturation	Annealing	Extension		
Mycoplasma genus	94°C for 2 min	94°C for 15 s	53°C for 15 s	72°C for 15 s	72°C for 5 min	[23]

TABLE 3. Shows the PCR condition of VLha primer.

Species	Initial denaturation	PCR conditions			Final extension	Reference
		Amplification (35 cycle of)				
		Denaturation	Annealing	Extension		
<i>Mycoplasma synoviae</i>	95 °C for 5 min	95°C for 45 sec	53°C for 30 sec	72 °C for 30 sec	72°C for 5 min	[24]

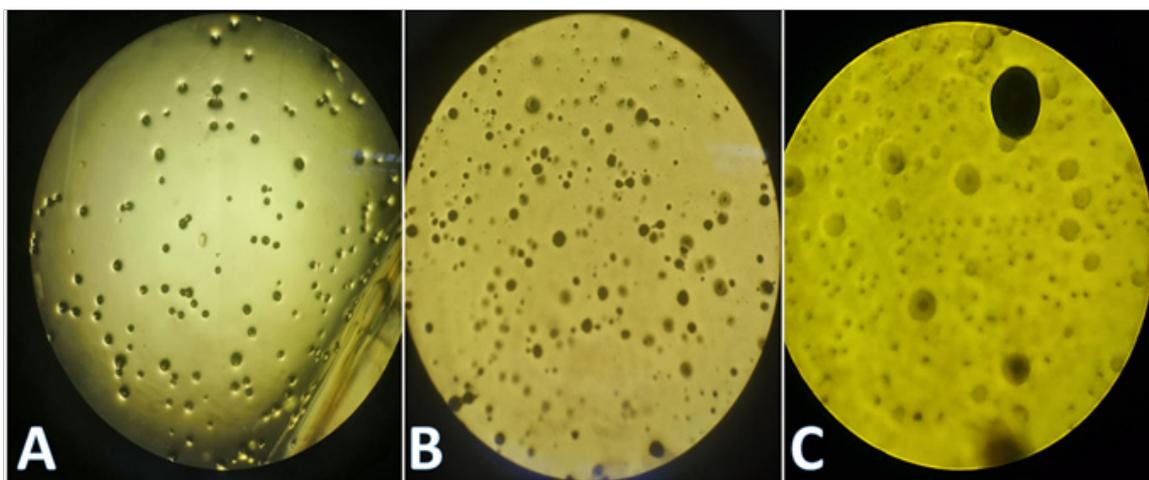


Fig.2. Microscopical appearance; A: Fried egg appearance of MS at 10x ; B: Fried egg appearance at 25x of MS; ; C: Fried egg appearance at 40x of MS.

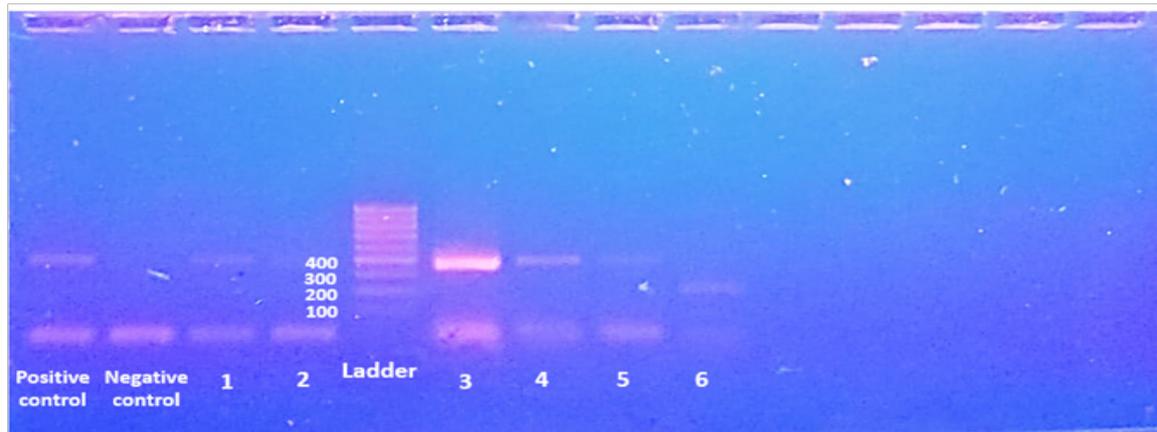


Fig.3. Ultraviolet transilluminator photo showed VLha gene, PCR results where lane from 1 to 5 were positive at 392 bp.

Discussion

Infections with avian mycoplasma are becoming more common, and this has resulted in significant economic losses all over the world [25]. MS is a major contributor to 33% chicken mortality, causing infectious synovitis and asymptomatic upper respiratory tract infection [26]. The current analysis shows the prevalence of mycoplasma in several Egyptian governorates using both conventional and modern methods. The detection rate of MS from specific farms with flocks of layers and broilers of different ages also showed that these organisms were vulnerable to a broad range of ages and governorates.

The diagnosis of the causative agent in *Mycoplasma* is regarded as the gold standard because a serological test cannot detect a subclinical or early infection [27]. Hence, the utilization of the culture and PCR techniques was based on the identification of *Mycoplasma* in the present investigation.

In this study, the isolation rate in various Egyptian governorates between March 2020 and January 2022 was (50.6%) for broiler chickens and (58%) for layer chickens. These isolation rates were quite like those noted by Marouf et al. [28]. However, according to studies by many investigators [29, 30, 15] broiler flocks are more common. These variations in isolation rates may be caused by differences in the time, place, management practices, treatments, and vaccination programs used when collecting the samples and because of the interest in immunizing breeders and the release of antibodies in young chicks. according to the MS

bio-typing feature, the ratio is 64/67 (95.5%). (Digitonin sensitive). Marouf et al. [31] reported a similar outcome. conventional methods for isolating and identifying MS are labor-intensive, time-consuming, and less sensitive, and they fail to distinguish *Mycoplasma* species from treated birds [10]. Meanwhile, the PCR method gives a quick, sensitive, and accurate means to distinguish MS from other infections [32].

Based on molecular detection in this study, 67 colonies produced PCR-amplified fragments at 279 based on the 16sr RNA gene and 392 bp based on the VLha gene, as seen in **Figures 3**. with a ratio of 82%, while PCR 55 positive were representing 44% from total examined field samples that largely concurs with those obtained by Khalifa et al. [33], who revealed the prevalence of *Mycoplasma* was 71.4%. The PCR has several advantages but is constrained and results in false-positive results due to contamination caused by poor sample management [34]. Hence, traditional cultural techniques ought to be used in addition to PCR [31; 35]. This variation in these percentages may result from the organism's fastidious nature and the great sensitivity of PCR [36].

Conclusions

PCR provides more accurate MS identification than other methods. MS infection rates in layers were greater than those in broilers. Therefore, to control this problem, rapid diagnosis, biosecurity, vaccination, and routine surveillance and prevention methods must be followed and reduce the probability that these subclinical, "hidden," and challenging-to-eradicate pathogens would spread throughout the chicken production chain.

List of abbreviations

Full term	Abbreviation
<i>Mycoplasma synoviae</i>	MS
Polymerase chain reaction	PCR
Infectious bronchitis virus	IBV
Carbon dioxide	CO ₂
Ribonucleic acid	RNA
Ethylenediaminetetraacetic acid	EDTA
Ultraviolet	UV

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Not applicable.

Authors' contributions

Amira M. Qoraa; Heba M. Salem collected data, wrote, and revised the original draft. M. Shakal supervised the manuscript. The authors read and approved the final manuscript.

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Declarations

All data included in this manuscript were adopted by the authors.

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Consent for publication:

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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الوضع الحالي للميكوبلازما سينوفي في دجاج التسمين ومزارع الدجاج البياض في بعض محافظات مصر

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للميكوبلازما سينوفي (MS) تأثير مالي كبير على إنتاج الدواجن حيث يمكن أن تسبب التهاب العشاء المفصلي المعدي وأعراض الجهاز التنفسي وتشوهات في قمة قشر البيض في كل من الطيور الرومية والدجاج. و تهدف الدراسة الحالية إلى التعرف على مرض الميكوبلازما سينوفي باستخدام الطرق البكتريولوجية والجزيئية في بعض الحالات التي تعاني من التهاب المفاصل في الدجاج اللحم والدجاج البياض في بعض المحافظات المصرية بين يناير ٢٠٢٠ ومارس ٢٠٢٢ حيث تم تجميع مئة وخمسة وعشرون عينة من ٢٥ قطع من كل محافظة تمثل ١٥ مزرعة دجاجة لاحم و ١٠ مزارع دجاج بياض والتي كانت من خمس محافظات مصرية ممثلة في (الجيزة، القاهرة، الدقهلية، القليوبية، الفيوم). تم استخدام تقنيات العزل التقليدية لتوصيف العزلات، وكذلك استخدام كل من الجينات 16S rRNA و VLha والمخصصة للميكوبلازما سينوفي لتحديد الجزيئات باستخدام تفاعل البلمرة المتسلسل. أظهر الفحص المجهرى لمستعمرة بمظهر البيض المقلي المميز لعينات العزل البكتيري أن ١٢٥/٦٧ (٥٣,٦٪) كانت إيجابية للميكوبلازما ال سينوفي ، و ١٢٥/٥٨ (٤٦,٤٪) كانت سلبية. من بين ٦٧ عزلة فقط، تم التعرف على ٥٥ عزلة على أنها MS عن طريق تفاعل البلمرة المتسلسل التقليدي (PCR) (55/67) (٨٢٪) بمعدل إجمالي ١٢٥/٥٥ (٤٤٪).

أظهرت نتائج البحث أن مرض الميكوبلازما سينوفي لا يزال أحد مسببات الأمراض المهمة التي تصيب الطيور، و من الضروري المراقبة الدورية والمنتظمة لمرض الميكوبلازما سينوفي في قطعان الدجاج، بالإضافة إلى ضرورة البحث المستقبلي لتحديد أفضل الأساليب الوقائية والمكافحة لهذا المرض.

الكلمات الأساسية: التهاب المفاصل , الميكوبلازما سينوفي , تفاعل البلمرة المتسلسل , VLha , 16S rRNA.