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Some Studies on *Mycoplasma* of Ostrich

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Abstract

Mycoplasmas in ostriches are the primary causative agents of respiratory diseases, resulting in significant stock losses, reduced production and hatchability, and downgrading of carcasses, leading to substantial economic losses in the industry. The present study is considered the first record for the isolation of three *Mycoplasma* isolates from ostriches experiencing respiratory signs, arthritis, and conjunctivitis with identification accomplished through PCR using common *Mycoplasma* primers and 16SrRNAgene for sequencing. Ostriches between 5-6 months of age showed the highest prevalence of isolates with respiratory signs, arthritis, and conjunctivitis (90%), while those aged 12 months showed 60% prevalence. 90 tracheal and ocular dry swabs were examined for the presence of *Mycoplasma* infection, from an ostrich farm in AL Obour city in Egypt, where sheep were also being raised nearby. The extracted DNA from each swab were pooled in 18 groups, each consists of five pooled DNA samples. These groups were negative *for Mycoplasma gallisepticum* and *Mycoplasma synoviae* by PCR. The three sequenced 16SrRNAgenes represent strains from male, female and young age ostriches. They were submitted to GenBank under accession no. OR511890, OR511891 with96% similarity to *M.struthionis*, and *M. falcons* respectively. While, the isolate (OR 511889) showed high nucleotide identity (99%) for *M. arginini*. In conclusion, three new *Mycoplasmas* were isolated from Ostrich farm with respiratory manifestation for the first time in Egypt. Which is a foundation, for further studies on a large number of ostrich farms for a wide scale investigation and control programs.

Keywords: *M. struthionis, M.Arginini, M. Falconis*, PCR, Sequencing.

Introduction

Mycoplasmas are cell wall-less bacteria known to be the smallest cellular organisms capable of self-reproduction due to its small genome [1]. They are commensals as well as parasites of a wide range of hosts, in many cases causing disease [2]. In 1967, *mycoplasmas* were classified under the class *Mollicutes*, a name derived from the Latin words; "soft skin." highlighting their lack of a cell wall [3]. Today, mycoplasmas are recognized as a distinct group of eubacteria that evolved from Gram-positive bacteria and are considered the smallest self-replicating prokaryotes without a cell wall [4-5-6].

Mycoplasmosis is a respiratory infectious disease that poses a significant threat to ostrich production, leading to high mortality rates and the downgrading of carcasses in slaughter ostriches [7]. Mycoplasma gallisepticum (Mg) is the most pathogenic and economically important bacterial respiratory pathogen of poultry. [8].

In ostriches, mycoplasmosis primarily manifests as respiratory disease, leading to inflammation of the upper respiratory tract [9]. Several *Mycoplasma* species have been identified in ostriches including three ostrich-specific mycoplasmas, Ms01, Ms02 and Ms03 (Ms, *Mycoplasma struthionis*, the host is *Struthio camelus*) were identified to be associated with respiratory disease in ostriches in South Africa [10].

Recently, *Mycoplasma nasistruthionis* sp. nov. and *Mycoplasma struthionis* sp were detected in South Africa by [11] using advanced molecular techniques such as 16S rRNA gene sequencing and MALDI-ToF mass spectrometry.

A study conducted in Iran revealed that 21.05% of tested samples were positive for *mycoplasma* via PCR, with 7.89% identified as Mycoplasma *gallisepticum* and 14% *as Mycoplasma synoviae* [12]. The first isolation of *Mycoplasma gallisepticum* from ostriches in Egypt's Matrouh Governorate was

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reported in 2003, where it was detected alongside *Staphylococcus aureus* [13].

Although serological tests like Serum Plate Agglutination (SPA) have been used to detect *M. gallisepticum* antibodies, these tests are known for their low specificity, while PCR proved to be more sensitive and faster [14]. Recent studies have been continued to underscore the significance of *mycoplasma* infections in ostriches, particularly in relation to poultry. A study by [15] reported severe respiratory outbreaks in ostrich farms caused by *Mycoplasma gallisepticum*, resulting in significant morbidity and occasional mortality. Another study highlighted the role of *Mycoplasma synoviae* in causing chronic respiratory symptoms and joint infections in ostriches, emphasizing the need for early detection and effective management strategies [16]. Emerging *Mycoplasma* species in ostriches have also been identified, prompting continuous surveillance and research to understand the evolving dynamics of *Mycoplasma* infections [12]. These findings underscore the importance of maintaining strict biosecurity measures, along with the use of medication and vaccination, to control pathogenic *mycoplasmas* in ostrich flocks.

Material and Methods

Sample collection: -90 tracheal and ocular samples were collected from January 2022 to March 2022 from an ostrich farm in AL Obour city in Egypt with a density of 200 ostriches. Samples were collected from ostriches showing symptoms of respiratory manifestation of respiratory infection. forty-five tracheal and forty-five ocular dry swabs were collected from ostriches with age ranges from three months to three years. The swabs were placed into ice and then transported to the mycoplasma laboratory Animal Health Research Institute, GIZA, EGYPT for examination within twenty- four hours.

Isolation and Biochemical Identification

The dry swabs were inoculated with 300ul PBS(Phosphate Buffer Saline) then 100ul of the PBS were subcultured in Pleuropneumonia-like organism PPLO broth, then cultured on PPLO agar media as described by [17].

Pleuropneumonia-like organism(PPLO) media consists of (PPLO broth or PPLO agar, Swine serum, Yeast extract 5% solution, (deoxyribonucleic acid) DNA 0.2% w/v solution, Penicillin G-Sodium , Thallium acetate 2% w/v solution) and supplemented with arginine as mentioned by WOAH 2021. [18].

The plates were incubated in candle jars at 37°C for (2-21) days and examined every two days using dark field inverted microscope. A single colony that has the typical form of mycoplasma (fried egg) of primary cultures of each of the isolates was picked and propagated to the log phase and subsequently used for DNA extraction and mycoplasma identification. *Mycoplasma* was distinguished from *Ahcoleplasma* using the digitonin test to differentiate between (*Mycoplasma* and *Achloeplasma* [19] and Biochemical identification as described by[20] and was performed by using glucose fermentation and arginine deamination tests.

To make a pure culture, a single colony was selected as agar block, then transferred into a broth media and the purified isolates were stored as agar blocks at -20°C.

Molecular detection of mycoplasma

DNA Extraction:

DNA extraction was applied on swabs by using Genomic DNA extraction kit (Gene Direx.Taiwan) .A DNA extraction is used according to the kit manufacturer's instructions. Finally, the purified DNA was stored at −20 °C until amplification. PCR was applied using three primers first is common for *Mycoplasma* according to [21], the second primer is for 16S rRNA gene for *Mycoplasma Synovaie* (MS) according to [22], and the third primer is for 16SrRNA gene for avian *Mycoplasma* according to +++++8[23].

PCR amplification and cycling protocol

PCR was carried out using (S1000) thermo cycler (Bio-RAD). The total reaction volume is 50 µL. PCR mix consisted of (25 µl of PCR master mix 2x,1µL MGSO con.10 pmoles,1µLGPO con.10pmoles, 5µL DNA and 18µLwater). [24].

Electrophoresis

PCR products were run on a 1.5% agarose gel using Tris-boric acid-EDTA buffer pH 8.0 for 45 min at 1.7 volts using appropriate size markers (100bp DNA ladder H3 RTU BIO-HEILXand seen using an ultraviolet transilluminator [25].

Gene sequencing

The three sequenced 16SrRNAgenes represent strains from male, female and young age ostriches were sequenced by Macrogen® Company, using Applied Biosystems 3130 genetic analyzer (ABI, USA). The sequencing was carried out using forward and reverse primers, and a combination of the Sanger method [26] and new 454 technology. The sequencing data was verified by NCBI Blast search, and chromate graphs were compiled and edited using BioEdit software version 7.1.5. Nucleotide analysis was carried out using BLAST n (http://www.ncbi.nlm.nih.gov/BLAST/) to characterize the edited sequences of the *Mycoplasma* isolate. The sequence was compared with other gene bank sequences by using the CLUSTAL W multiple sequence alignment algorithm, version 1.83 of Meg Align module of Laser gene DNA Star software Pairwise, mentioned by [27].

A phylogenetic tree was created using MEGA version 11 and the neighbor-joining (NJ) method to determine the genetic relatedness of the *Mycoplasma* spp. based on distance.

Results

Clinical signs: -the examined ostriches showed coughing, nasal exudates, swollen sinuses, foamy eyes and rattle sounds in the throat.

Biochemical Examination:

Table (2) shows the biochemical examination:

Thirty- three samples out of 90 samples that showed a change in colour (red color of PPLO broth media).

Ten samples showed a color change into slightly yellow; these samples are weak glucose -positive. Table (2)

Two samples showed no color change; these samples hydrolyse arginine they are Arginine positive.

Twenty- one samples showed no color change into violet; these samples also, don't ferment glucose (negative for glucose) so, suspected to be similar to *Mycoplasma falconis. M. falconis* does not ferment glucose,nor hydrolyzes arginine

The samples showed negative *Acholeplasma* by digitonin sensitivity test

Bacterial Culture: The samples were cultured on PPLO media showed typical fried egg colony shape within 3-21 days as shown in fig 1,2 and 3

The sequenced genes were submitted to GenBank under accession no.OR 511889 for *M. arginini*, and OR511890, OR511891 for 16S rRNA for *Mycoplasma* spp.

M. arginini (OR 511889)) showed high nucleotide identity (99%) with all other *M. arginini* detected in sheep fig (6) and Table (3). *Mycoplasma* spp (OR511890) showed high nucleotide identity (96%) with *M.falconis and M. struthionis* (was detected from ostrich). In addition, *Mycoplasma* spp (OR511891) showed 96% identity with *M. falconis, M. struthionis* and *Mycoplasma* spp. Detected in golden eagles and white-tailed eagles.

Nucleotide identity between *M. arginini* and other Genebank isolates as shown in table (3).

Discussion

In our study, the suspected ostriches exhibited clinical signs consistent with *Mycoplasma* infection, including coughing, nasal exudates, swollen sinuses, foamy eyes, and rattle sounds in the throat. These symptoms are indicative of a respiratory infection commonly associated with *Mycoplasma* species, which are known to cause significant respiratory distress in avian species [28, 29]. Out of 90 samples collected, 33 samples showed a color change in PPLO broth media, suggesting a biochemical reaction indicative of Mycoplasma. The digitonin sensitivity test confirmed that these samples were negative for *Acholeplasma,* as they were not lysed by digitonin. The samples that showed negative reactions are suspected to be *Mycoplasma falconis*. It is important to note that *M. falconis* does not ferment glucose, does not hydrolyzes arginine, and does not hydrolyze urea [11]. Biochemical test results are summarized in Table 1, where 10 samples were positive for glucose fermentation, 2 samples were positive for arginine hydrolysis, and 21 samples exhibited negative reactions. These results align with the known biochemical characteristics of *Mycoplasma falconis* and provide a basis for suspecting its presence in the ostriches studied [30]. The samples were cultured on PPLO media, and within 3-21 days, they exhibited the characteristic "fried egg" colony morphology typical of *Mycoplasma* species [11,3]. This colony morphology is a hallmark of *Mycoplasma*, further supporting the identification of these isolates as belonging to this genus. The appearance of these colonies is shown in Fig. 1, where the typical "fried egg" colonies are clearly visible. Molecular detection was performed using PCR, targeting the common 16S rRNA gene for *Mollicutes* and Avian *Mycoplasma*. Agarose gel electrophoresis of the PCR products is shown in Fig.4 and Fig. 5. In Fig. 4, the 280 bp band corresponding to the *Mollicutes* 16S rRNA gene is visible in the positive clinical samples (lanes 2-11, 14, 15, 18, 20, 21, 22), with the *Mycoplasma* positive control in lane 13 and the negative control in lane 12 [23]. Fig. 4 illustrates the amplification of the 700 bp band specific to Avian *Mycoplasma* 16S rRNA, visible in lanes 2-9, with the *Mycoplasma* positive control in lane 10 and the negative control in lane 11. The presence of these bands in the clinical samples confirms the presence of *Mycoplasma* species in the ostriches tested [11].

The sequenced genes were submitted to GenBank under accession no. OR 511889 for *M. arginini*, and OR511890, OR511891 for *M.struthionis* and *M.falconis.*

M. arginini (OR 511889) showed high nucleotide identity (99%) with all other *M. arginini* detected in sheep as sampels that isolated in Turky in 2019, these sampels isolated from lung in sheep as shown in table (7). Other strains isolated from goat and sheep that were nasal swaps in Egypt in 2015 showed 99% similarity to our strain while In Pakistan in 2022 they were able to isolate two strains and they used vaginal swaps; the 2 strains were 99% similar to our strain. The *Mycoplasma* isolate (OR511890) showed high nucleotide identity (100%) with *M.falconis* and *M. struthionis*(was detected in ostrich) table (5). *Mycoplasma* isolate (OR511891) showed 96% identity with *M. falconis, M. struthionis* and

Mycoplasma species detected in golden eagles and white-tailed eagles.

These sequences OR511890, OR511891 are completely identical to strains that isolated from falcons in USA in 2019 as shown in table (6) and another 4 strains those were isolated from lung of ostrich in Austeria 2019. Another strain was isolated in 2021 from lung of ostrich was 100% identical to our 2 strains.

Conclusion

There are three new *Mycoplasmas* were isolated from Ostrich farm with respiratory manifestation for the first time in Egypt. Which is a foundation, for further studies on a large number of ostrich farms for a wide scale investigation and control

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Not applicable.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; BUFVTM 03-01-25).

TABLE 1. The primers used for detection of *Mycoplasma*

Primer designation	('3-Sequence (5'	Refere nce	Amplified Product Size	Cycle
C ommon *mycoplasma* primer	MGSO 5'TGC ACC ATC TGT 'CAC TCT GTT ACC CTC3 GGG AGC AAA CAG '5 3-GPO 'GAT TAG ATA CCC T3	**[21]**	**bp**280	Initial Denaturation: at 95°C for min5. Denaturation: at94°C for 30 sec Annealing: at 55°C for 30 sec at72°C for 1 min :Extension Final Extension: at 72 °C for 10 min
MS primer	f (5'GAG AAG CAA AAT -MS ('AGT GAT ATC A3 r (5'CAG TCG TCT CCG AAG -MS ('TTA ACA A3	**[22]**	bp 211	for **C°**Initial Denaturation: at 95 .min5 for 1min **C°**Denaturation: at94 for 45 sec **C°**Annealing: at 58 for 60 sec **C°**Extension: at72 for 20 **C°** Final Extension: at 72 min
MG primer	MG-14F: 5'-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C-3' MG-13R: 5'-GCT-TCC-TTG-CGG-TTA-GCA-AC-3	**[22]**	bp 185	for **C°**Initial Denaturation: at 95 .min5 for 1min **C°**Denaturation: at94 for 30 sec **C°** Annealing: at 58 for 45sec **C°**Extension: at72 for 20 **C°** Final Extension: at 72 min
SrRNA for 16 Avian *Mycoplasma*	F5'-CGT TCT CGG GTC TTG TAC AC-3' R5'-CGC AGG TTT GCA CGT CCT TCA TCG-3'	**[23]**	**Different levels of bp**	for **C°**naturation: at 94Initial De .min3 for 20 sec **C°**Denaturation: at94 for40 sec **C°**Annealing: at 58 for 30sec **C°**Extension: at72 for **C°**Final Extension: at 72 min5

TABLE 2. The Biochemical Identification

Type of samples		weak+Ve G	+Ve Arg	-Ve G -Ve Arg	Total
Tracheal swabs 45	8		1	12	21
Ocular swabs 45	2		1	9	12
Total 90 swabs	10		2	21	33

All the isolated Mollicutes showing Fried egg appearance on PPLO agar media and were sensitive for Digitonin so, there were no *Acholeplasma* species.

+Ve G positive glucose fermentation +Ve Arg positive arginini hydrolysis

TABLE 3. The phylogenetic tree of *Mycoplasma arginini*. 16s gene red circle: this research isolate, and other reference *Mycoplasma arginini* strains. Nucleotide identity between *M. arginini* and other Genebank isolates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		Seq->
1		99%	99%	99%	99%	99%	99%	99%	99%	99%	99%	99%	99%	99%	99%	1	OR511889.1 M. arginini/S.19/Ostrich
2	99%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	2	MK789491.1 M.arginini/MYCO17/Sheep
3	99%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	3	MK789487.1 M.arginini/MYCO13/Sheep
4	99%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	4	MK789486.1 M.arginini/MYCO12/Sheep
5	99%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	5	MK789485.1 M.arginini/MYCO11/Sheep
6	99%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	100%	6	MK789481.1 M.arginini/MYCO7/Sheep
7	99%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	100%	7	MK789476.1 M.arginini/MYCO2/Sheep
8	99%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	100%	8	KP972459.1 M.arginini/Dak-2/M.arg/EG014/Sheep
9	99%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	100%	9	OQ221770.1 M.arginini/18/3-C15/Sheep
10	99%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	100%	10	HQ661823.1 M.arginini/C1/Sheep
11	99%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	100%	11	HQ661822.1 M.arginini/D7/Sheep
12	99%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	100%	12	OQ221769.1 M.arginini/18/3-A15/Sheep
13	99%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	100%	13	OQ221768.1 M.arginini/18/3-D15/Sheep
14	99%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		100%	14	HQ661820.1 M.arginini/E3.5/Sheep
15	99%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%		15	HQ661819.1 M.arginini/D1/Sheep
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

TABLE 4. *Mycoplasma* sp. phylogenetic tree red circle's, this research isolates, and other reference *Mycoplasma* sp. Strains

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		Seq->
1		96%	100%	100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	99%	98%	1	OR511890.1 Mycoplasma sp. S.20/ Ostrich
2	96%		96%	95%	95%	95%	95%	95%	95%	96%	96%	96%	96%	96%	96%	2	OR511891.1 Mycoplasma sp. S.5/ Ostrich
3	100%	96%		100%	100%	100%	100%	100%	100%	99%	99%	99%	99%	99%	98%	3	NR_024984.1 M.falconis H/T1
4	100%	95%	100%		100%	100%	100%	100%	100%	99%	99%	99%	99%	99%	98%	4	MF769011.1 M.struthionis VIB/Ostrich
5	100%	95%	100%	100%		100%	100%	100%	100%	99%	99%	99%	99%	99%	98%	5	MF769010.1 M.struthionis VIA/Ostrich
6	100%	95%	100%	100%	100%		100%	100%	100%	99%	99%	99%	99%	99%	98%	6	MF769009.1 M.struthionis 238B/Ostrich
7	100%	95%	100%	100%	100%	100%		100%	100%	99%	99%	99%	99%	99%	98%	7	MF769008.1 M.struthionis 238A/Ostrich
8	100%	95%	100%	100%	100%	100%	100%		100%	99%	99%	99%	99%	99%	98%	8	NR_178373.1 M.struthionis 237IA/Ostrich
9	100%	95%	100%	100%	100%	100%	100%	100%		99%	99%	99%	99%	99%	98%	9	DQ223545.1 M.struthionis Ms01/Ostrich
10	99%	96%	99%	99%	99%	99%	99%	99%	99%		100%	100%	100%	100%	99%	10	KM485612.1 Mycoplasma sp./ GoldenEagle
11	99%	96%	99%	99%	99%	99%	99%	99%	99%	100%		100%	100%	100%	99%	11	GQ150568.1 Mycoplasma sp. Sgv2e/White-tailed eagle
12	99%	96%	99%	99%	99%	99%	99%	99%	99%	100%	100%		100%	100%	99%	12	GQ150567.1 Mycoplasma sp. Sgv2d/White-tailed eagle
13	99%	96%	99%	99%	99%	99%	99%	99%	99%	100%	100%	100%		100%	99%	13	GQ150566.1 Mycoplasma sp. Sgv2c/White-tailed eagle
14	99%	96%	99%	99%	99%	99%	99%	99%	99%	100%	100%	100%	100%		99%	14	GQ150565.1 Mycoplasma sp. Sgv2b/White-tailed eagle
15	98%	96%	98%	98%	98%	98%	98%	98%	98%	99%	99%	99%	99%	99%		15	MK615063.1 Mycoplasma sp. MV122/White-tailed eagle
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		

TABLE 5. Strain of OR11890 *Mycoplasma* SP.S.20\Ostrich

	Accession NO	eciesSp	Sample	Country	Year	Similarity
	OR11890	OSTRICH	Tracheal and occular	Egypt	2022	100%
1	NR_024984	Falcon	Tracheal swab	USA	2019	%100
2	MF769011	Ostrich	Lung	Austria	2019	%100
3	MF769010	Ostrich	Lung	Austria	2019	%100
4	MF769009	Ostrich	Lung	Austria	2019	%100
5	MF769008	Ostrich	Lung	Austria	2019	%100
6	NR_178373	Ostrich	Lung	Vienna	2022	%100
7	DQ223545	Ostrich	Respiratory system	South Africa	2005	%99
8	KM485612	Golden Eagle	Pharyngeal swab	Italy	2015	%99
9	GQ150568	Griffon vultures	Tracheal swab	Italy	2010	%99
10	GQ150567	*Gypus fulvus*	Tracheal swab	Italy	2010	%99
11	GQ150566	*Gypus fulvus*	Tracheal swab	Italy	2010	%99
12	GQ150565	*Gypus fulvus*	Tracheal swab	Italy	2010	%99
13	MK615063	White tailed eagle	Choana	Austria	2019	%99

TABLE 6. Strain of OR11890 *Mycoplasma* SP.S.20\Ostrich

	Accession NO	Species	Sample	Country	Year	Similarity
	OR511891	Ostrich	Tracheal and occular	Egypt	2020	100%
1	NR_024984	Falcon	Tracheal swab	USA	2019	%96
2	MF769011	Ostrich	Lung	Austria	2019	%95
3	MF769010	Ostrich	Lung	Austria	2019	%95
4	MF769009	Ostrich	Lung	Austria	2019	%95
5	MF769008	Ostrich	Lung	Austria	2019	%95
6	NR_178373	Ostrich	Lung	Vienna	2022	%95
7	DQ223545	Ostrich	Respiratory system	South Africa	2005	%95
8	KM485612	Golden Eagle	Pharyngeal swab	Italy	2015	%96
9	0568GQ15	*Griffon vultures*	Tracheal swab	Italy	2010	%96
10	GQ150567	*Gypus fulvus*	Tracheal swab	Italy	2010	%96
11	GQ150566	*Gypus fulvus*	Tracheal swab	Italy	2010	%96
12	GQ150565	*Gypus fulvus*	Tracheal swab	Italy	2010	%96
13	MK615063	White tailed eagle	Choana	ustriaA	2019	%96

TABLE 7. Strain of Strain of OR 511889 *Mycoplasma* arginini

	Accession NO	Species	Sample	Country	Year	Similarity
	OR511889	Ostrich	Trracheal and occular	Egypt	2020	100%
1	MK7894981	Sheep	Lung	Turkey	2019	%99
2	MK789487	Sheep	Lung	yTurke	2019	%99
3	MK789486	Sheep	Lung	Turkey	2019	%99
4	MK789485	Sheep	Lung	Turkey	2019	%99
5	MK789481	Sheep	Lung	Turkey	2019	%99
6	MK789476	Sheep	Lung	Turkey	2019	%99
7	KP972459	Sheep and goat	Nasal swab	EGYPT	2018	%99
8	OQ221770	Goat	Nasal swab	Pakistan	2022	%99
9	HQ661823	Droper sheep	Vaginal swab	South Africa	2011	%99
10	HQ661822	Droper sheep	Vaginal swab	South Africa	2011	%99
11	OQ221769	Sheep	Vaginal swab	Pakistan	2022	%99
12	OQ221768	Sheep	Vaginal swab	Pakistan	2022	%99
13	HQ661820	Goat	Vswab aginal	South Africa	2011	%99
14	HQ661819	Droper sheep	Vaginal swab	South Africa	2011	%99

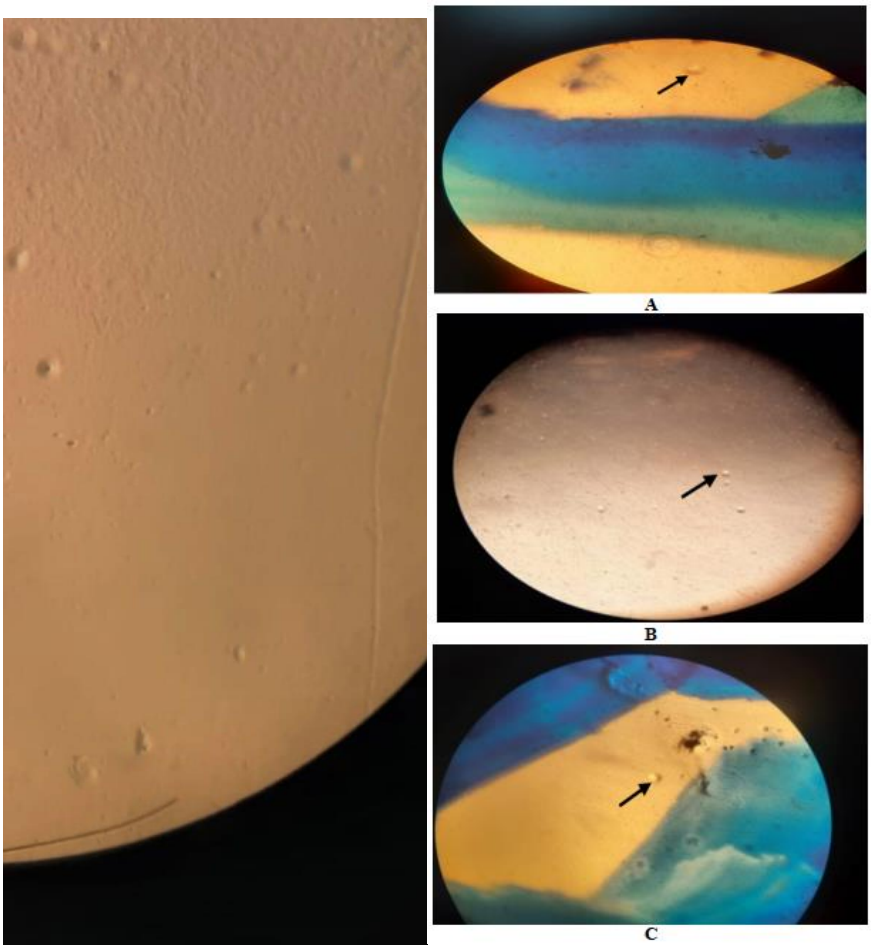

Fig. 1. Fried egg appearance of *mycoplasma* colonies on PPLO agar from positive samples

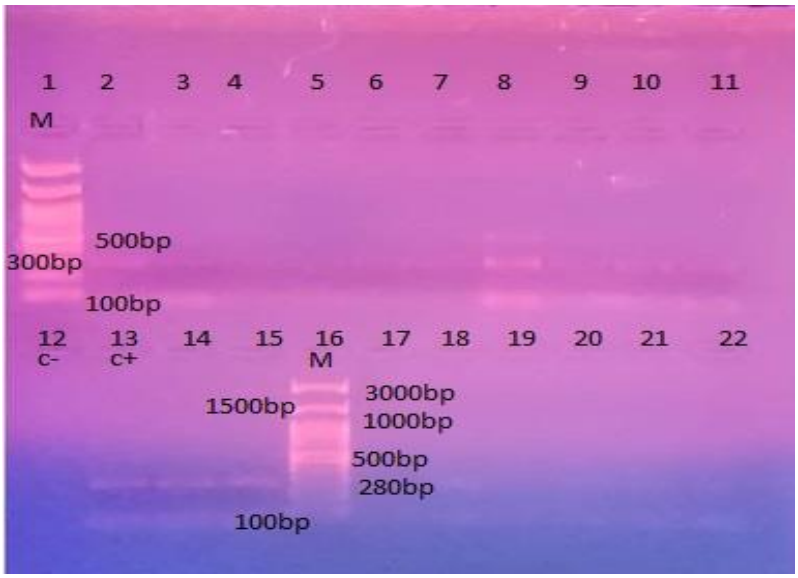

Fig. 2. Agarose gel electrophoresis of common 16SrRNA gene for Mollicutes at 280bp Lanes 1&16 :100bp DNA ladder. Lanes 2- 11,14 ,15,18,20 21,22: positive clinical samples lane 12: control Negative Lane 13: *Mycoplasma* Control Positive. lanes 17 and 19: Negative samples

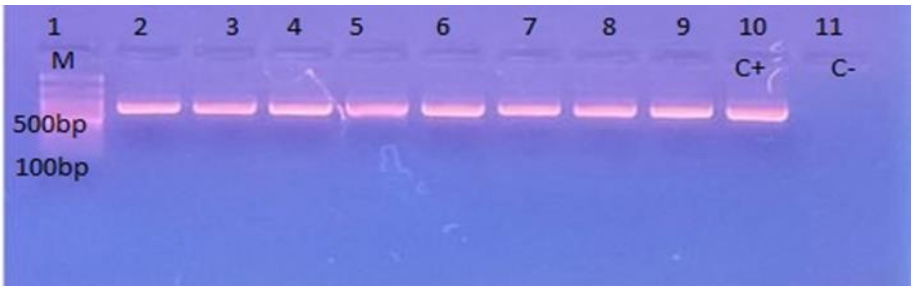

Fig. 3. Agarose gel electrophoresis of common 16SrRNA gene for Avian *Mycoplasma* at 700 bp

Lane1: 100bp DNA ladder; Lane 2-9: positive clinical samples; Lane 10: *Mycoplasma* Control Positive Lane 11: control Negative

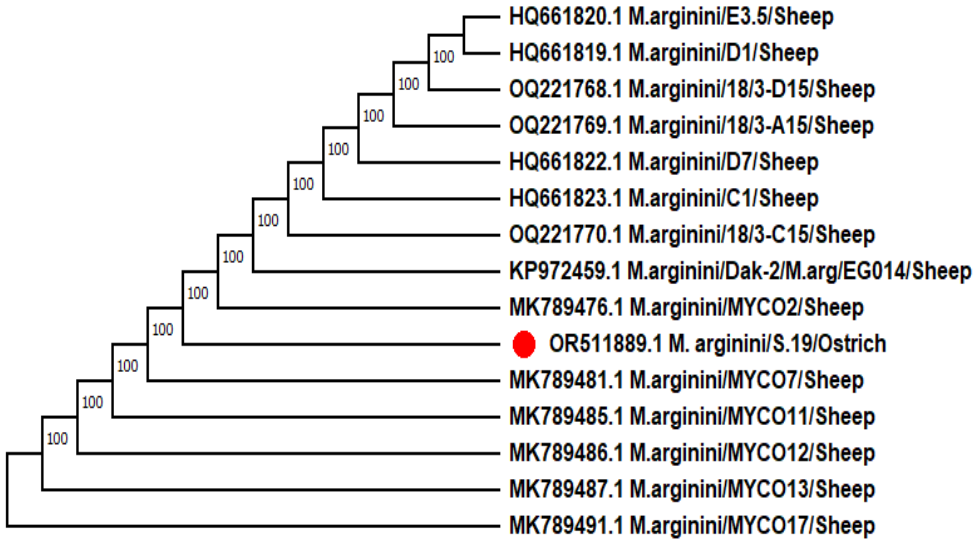

Fig. 4. The phylogenetic tree of *Mycoplasma arginini.* 16s gene red circle: this research isolate, and other reference *Mycoplasma arginini* strains

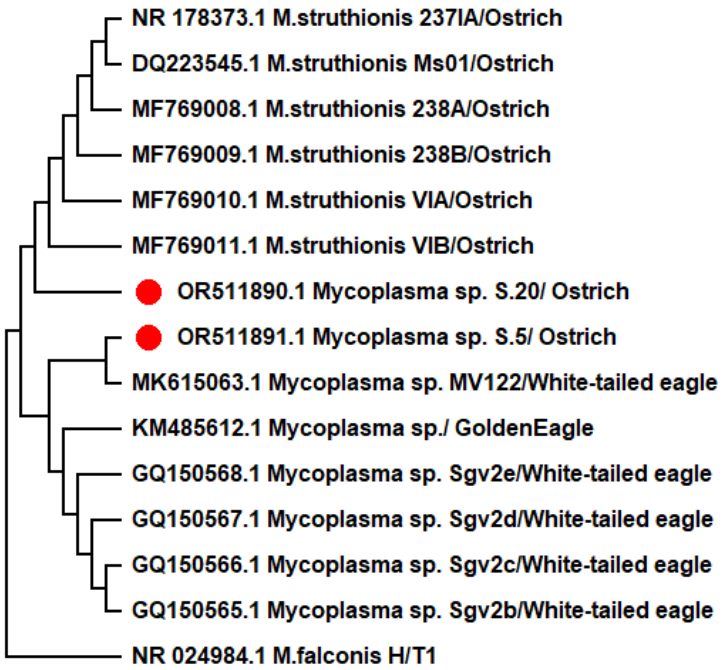

Fig. 5. *Mycoplasma* sp. phylogenetic tree red circle's, this research isolates, and other reference *Mycoplasma* sp.

References

1. Matteau, D., Duval, A., Baby, V. and Rodrigue, S. Mesoplasma florum: a near-minimal model organism for systems and synthetic biology. *Frontiers in Genetics*, **15**, 1346707. (2024). https://doi.org/10.3389/fgene.2024.1346707
2. Razin, S Molecular biology and genetics of mycoplasmas (Mollicutes). *Microbiol. Rev.*, **49**,419-455(1985).
3. Razin, S., Yogev, D. and Naot, Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.*, **62**,1094-1156 (1998)
4. Razin, S. and Jacobs, E., Mycoplasma adhesion. J. *Gen. Microbiol*., **138**,407-422 (1992)
5. Rottem, S. and Barile, M.F. Beware of mycoplasmas. *Trends Biotechnol.*, **11**,143-151 (1993).
6. Dybvig, K..and Voelker, L.L. Molecular biology of mycoplasmas. *Annual Review of Microbiology*. **50**, 25-57 (1996)
7. Verwoerd, D.J. Ostrich diseases. *Rev. Sci. Tech.*, **19**,638-661(2000). DOI: veterinaryworld.org/Vol.14.2021/35.pdf
8. Abdelhassieb, H. S., Attia, S. and Ahmed, S. O. Comparison of different NAT assays for the detectionof microorganisms belonging to the class Mollicutes. *BMC Veterinary Research*, **13**, 195 (2024).
9. Van der Merwe, E. F. Preliminary investigations into ostrich mycoplasmas: identification of vaccine candidate genes and immunity elicited by poultry mycoplasma vaccines (Doctoral dissertation, Stellenbosch: Stellenbosch University). (2006).
10. Botes, A., Peyrot, B.M., Olivier, A.J., Burger, W.P. and Bellstedt, D.U. Identification of three novel mycoplasma species from ostriches in South Africa. *Vet. Microbiol*., **111**,159-169 (2005)
11. Spergser, J., Botes, A., Nel, T., Ruppitsch, W., Lepuschitz, S., Langer, S., Ries, S., Dinhopl, N., Szostak, M., Loncaric, I. and Busse, H.J. Mycoplasma nasistruthionis sp. nov. and Mycoplasma struthionis sp. nov. isolated from ostriches with respiratory disease. *Syst. Appl. Microbiol*., **43**,126047 (2020)
12. Moomivand, H., Pourbakhsh, S. and Jamshidian, M. Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran. *Journal of the Hellenic Veterinary Medical Society*. **68**, 647-652 (2017)
13. Oraby, F.A.I. and Hassan, A.M. Mycoplasma gallisepticum and staphylococcus aureus infection in ostrich (struthio camelus) in matrouh governorate. *Kafrelsheikh Veterinary Medical Journal*, **1**,645-652 (2003)
14. Sprygin, A.V., Andreychuk, D.B., Kolotilov, A.N., Volkov, M.S., Runina, I.A., Mudrak, N.S., Borisov, A.V., Irza, V.N., Drygin, V.V. and Perevozchikova, N.A. Development of a duplex real-time TaqMan PCR assay with an internal control for the detection of Mycoplasma gallisepticum and Mycoplasma synoviae in clinical samples from commercial and backyard poultry. *Avian Pathol.*, **39**,99-109 (2010)
15. McAuliffe, L., Ellis, R.J., Lawes, J.R., Ayling, R.D. and Nicholas, R.A. 16S rDNA PCR and denaturing gradient gel electrophoresis; a single generic test for detecting and differentiating Mycoplasma species. *J. Med. Microbiol.*, **54**,731-739 (2005)
16. Tebyanian, H., Mirhosseiny, S.H., Kheirkhah, B., Hassanshahian, M. and Farhadian, H. Isolation and Identification of Mycoplasma synoviae From Suspected Ostriches by Polymerase Chain Reaction, in Kerman Province, Iran. *Jundishapur J. Microbiol*., **7**,e19262 (2014)
17. Frey, M.L., Hanson, R.P. and Andrson, D.P. A medium for the isolation of avian mycoplasmas. *Am. J. Vet. Res.*, **29**,2163-2171 (1968)
18. World Organisation for Animal Health WOAH Terrestrial Manual. Chapter 3.3.5. – Avian mycoplasmosis (Mycoplasma gallisepticum, M. synoviae) 2021.
19. Freundt, E.A., Ernø, H. and Lemcke, R.M. Chapter IX Identification of Mycoplasmas. In: Bergan T, Norris JR, editors. *Methods in Microbiology*. **13**, *Academic Press*; p. 377-434(1979).
20. Freundt, E. A. Principles of Mycoplasma Classification. Annals of the *New York Academy of Sciences*, **225**(1),7-13(1973). https://doi.org/10.111/j.1749-6632. 1973.tb45630.x
21. Van Kuppeveld, F.J., Johansson, K.E., Galama, J.M., Kissing, J., Bölske, G., van der, L,ogt, J.T. and Melchers, W.J. Detection of mycoplasma contamination in cell cultures by a mycoplasma group-specific PCR. *Appl. Environ. Microbiol*., **60**, 149-152 (1994)
22. WOAH 2023 Chapter 3.3.5 (Lauerman, L.H., Hoerr, F.J., Sharpton, A.R., Shah, S.M. and van Santen, V.L. Development and application of a polymerase chain reaction assay for Mycoplasma synoviae. *Avian Dis*., **37**,829-834 (1993)
23. Ramírez, A. S., Naylor, C. J., Yavari, C. A., Dare, C. M. and Bradbury, J. M. Analysis of the 16S to 23S rRNA intergenic spacer region of Mycoplasma synoviae field strains. *Avian Pathology*, **40**(1), 79–86 (2008). https://doi.org/10.1080/03079457.2010. 537305

24. Sprygin, A. V., Andreychuk, D. B., Kolotilov, A. N., Volkov, M. S., Runina, I. A., Mudrak, N. S., Borisov, A. V., Irza, V. N., Drygin, V. V. and Perevozchikova, N. A. Development of a duplex real-time TaqMan PCR assay with an internal control for the detection of Mycoplasma gallisepticum and Mycoplasma synoviae in clinical samples from commercial and backyard poultry. *Avian Pathology: Journal of the W.V.P.A*, **39(**2), 99–109(2010). https://doi.org/10.1080/03079451003604621

25. Abdelhassieb,H. S., Attia, S. and Ouda, S. E. Molecular Typing of Mycoplasma gallisepticum (Mg) In Egypt Using Lipoprotein Gene. *Egyptian Journal of Veterinary Sciences*, **55**(4),1037-1045 (2024).

26. Crossley, B.M., Bai, J., Glaser, A., Maes, R., Porter, E., Killian, M.L., Clement, T. and Toohey-Kurth, K. Guidelines for Sanger sequencing and molecular assay monitoring. *J. Vet. Diagn. Invest*., **32**,767-775 (2020)

27. Thompson, C.C., Vieira, N.M., Vicente, A.C. and Thompson, F.L. Towards a genome-based taxonomy of Mycoplasmas. *Infect. Genet. Evol*., **11**,1798-1804 (2011)

28. Bradbury, J.M. Recovery of mycoplasmas from birds. *Methods Mol, Biol*., **104**,45-51 (1998)

29. Kleven, S.H. Control of avian mycoplasma infections in commercial poultry. *Avian Dis.*, **374**-52,367 (2008)

30. Poveda, J.B., Giebel, J., Kirchhoff, H. and Fernandez, A. Isolation of mycoplasmas from a buzzard, falcons and vultures. *Avian Pathol*., **19**,779-783 (1990)

بعض الدراسات على الميكوبلازما في النعام

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الملخص

الدراسه الحاليه تعتبر اول توثيق لعزل ثلاثه عزلات من *الميكوبلازما* من النعام في مصر، تم تحديد هوية تسعين مسحة جافة مأخوذه من القصبة الهوائيه والعين باستخدام تقنيه تفاعل البوليميراز المتسلسل متبوعه بعمليه تسلسل الحمض النووي. تم جمع هذه المسحات الجافة 16S rRNA لاستهداف جين PCR من مزرعه نعام في مدينه العبور بمصر من نعام يظهر اعراض الجهاز التنفسي.

اظهرت نتائج تحليل التسلسل ان إحدى العزلات الثلاث *للميكوبلازما* كانت متطابقه بنسبه 99% مع *ميكوبلازما ارجينيني*. اما العزله الثانيه كانت متطابقه بنسبه 100%مع *ميكوبلازما ستراثيونيس*. اما العزله الثالثه فكانت متشابهه مع *ميكوبلازما فالكونيس*.

تم ايداع الجينات التي تم تسلسلها في قاعده بيانات GenBank لكل من ***M. struthionis*** و ***M. falconis*** تحت ارقام الوصول OR511890و OR511891. في حين اظهرت العزله المسجله تحت رقم OR 511889 تطابقا عاليا في النيوكليتيدات بنسبه 99% مع *ميكوبلازما ارجينيني*.

اظهرت النتائج ان 90% من النعام الذي يعاني من اعراض تنفسيه والتهاب مفاصل والتهاب الملتحمه كان في عمر خمسه الي سته أشهر بينما 60% من النعام بعمر 12 شهر ظهرت عليه الاعراض ذاتها. كما لوحظ ارتفاع معدلات الاصابه باعراض الجهاز التنفسي خلال فصل الشتاء.

يوصي باجراء دراسات اضافيه على عدد أكبر من مزارع النعام لفهم مدي انتشار وتاثير عدوي *الميكوبلازما* في مصر.

الكلمات الدالة: النعام، *ميكوبلازما ستراثيونيس، السلمونيلا، ميكوبلازما ارجنيني، ميكوبلازما فالكونيس*، تفاعل PCR البوليميراز المتسلسل.