Research Article

Surveillance of *Mycoplasma* spp. Among Cases of Viral Respiratory Affections in Broiler Chickens in Egypt

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Abstract |In poultry, respiratory tract affections are caused by various pathogens, including bacterial and viral agents. In Egypt, the prevalences of avian influenza virus (AIV), infectious bronchitis virus (IBV), and *Mycoplasma* spp. are high. Thus, co-infection with one or both viruses and *Mycoplasma gallisepticum* (MG) or *Mycoplasma synovaie* (MS) is thought to play an important role in the clinical severity of respiratory infections. In this study, we conducted a passive surveillance study for MG and MS among cases of viral respiratory affections in broilers. Samples were collected from 68 broiler chicken flocks with variable mortality rates in four Egyptian governorates (Fayoum, Beni-Suef, Giza, Minia). All samples were cultivated on PPLO media and detected under stereo-microscope for fried egg colonies. Forty-three samples were detected as *Mycoplasma* spp., using PCR for species identification, MG, MS, and Untyped strains, with a high prevalence of MG (27.9%) and lowest for MS (8.8%). An rRT-PCR used for detection of AIV-H9N2 and IBV showed high prevalence for IBV (88.2%) and AIV-H9N2 (52.9%), the highest rates of co-infection were MG-H9N2-IBV, Mycoplasma other than MG/MS, and H9-IBV with rates of (17.6%), (14.7%), and (13.2%) respectively, While for single infection were IBV (14.7%) and H9N2 (7.4%). The highest mortality rate was (30%) of co-infection with MG, IBV, and H9N2. The current study highlights the role of co-infection between the *Mycoplasma* spp. and other respiratory viruses, especially LPAIV (H9N2 subtype) and IBV, in exaggerating the clinical outcome after natural infection in broiler chickens. More field and experimental investigations are crucial to highlight the pathogenesis and the interaction between different respiratory pathogens in chickens.

Keywords | Avian influenza, Broiler chickens, Co-infection, H9N2, Infectious bronchitis *Mycoplasma gallisepticum*, *Mycoplasma synovaie*.

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INTRODUCTION

Poultry farms in Egypt have been suffering from various problems during the last decades that led to severe economic losses, with respiratory diseases being the most common cause of mortalities in a poultry flock (Taher et al., 2017; Wigle, 2000). Respiratory tract affections are caused by many agents, including but not limited to bacterial pathogens, such as MG and MS and viral agents, such as AIV and IBV. These pathogens, on many occasions, af-

fect the chicken's respiratory tract leading to severe clinical disease and subsequent significant economic losses (Hassan et al., 2016; Rim et al., 2019; Shehata et al., 2019).

Avian mycoplasmosis is caused by several pathogenic mycoplasmas, of which MG and MS are known to be the most impactful. Both MG and MS infections cause economic losses in the poultry industry by reducing weight gain and meat quality in broilers, causing drop in egg production in laying birds and increasing embryo mortality in eggs laid by infected breeders (Bagal, 2019; El-Ashram et al., 2021; El-Naggar et al., 2022; Marouf et al., 2022; Messa Júnior et al., 2017). Outbreaks with high morbidity and mortality in broilers are frequently observed due to concurrent infections and environmental factors (Sid et al., 2016). MG infection is one of the pathogens commonly incriminated in chronic respiratory disorders (Ferguson-Noel et al., 2020; Gharibi et al., 2018). Infection with *Mycoplasma* predisposes the host to other pathogens such as *Escherichia coli, Haemophilus gallinarum*, and various respiratory viruses (Stipkovits et al., 2012b). MS frequently causes subclinical upper respiratory tract infection, air sac disease, and acute to chronic infectious synovitis in chickens and turkeys (Abdelrahman et al., 2021; Emam et al., 2020; Gharibi et al., 2018; Houta et al., 2021). MG and MS can display little to no clinical signs in avian species unless complicated by other respiratory pathogens (Ferguson-Noel et al., 2020). Climate is a particularly important environmental factor that may seriously affect respiratory diseases in poultry (Rajkumar et al., 2017).

Nowadays, viral respiratory diseases represent a significant problem in Egyptian poultry flocks. They are frequently caused by AIV, velogenic NDV, and IBV (Awad et al., 2016; Dewidar et al., 2021; Taher et al., 2017). Although low pathogenic, infection with H9N2 in the poultry populations results in significant economic losses when complicated with other pathogens (Naeem et al., 1999; Shehata et al., 2019) Chickens, simultaneously infected with low pathogenic avian influenza virus (LPAIV) of H9N2 (AIV-H9) subtype and IBV, either field or vaccinal strains, display severe clinical signs, obvious gross lesions, and significantly higher mortality rates than chickens infected with a single pathogen (Hassan et al., 2017). This is attributed to the role of IBV in enhancing the pathogenicity of AIV-H9, which could be explained by the protease-like action of IBV spike protein (S1) that exaggerates the replication of AIV-H9 in infected birds (Samy and Naguib, 2018a; Seifi et al., 2012).

The prevalence of AIV-H9N2 and IBV viruses in Egypt is high, even in apparently healthy broilers, layers, and breeders chicken flocks (El-Sayed et al., 2021), making the poultry farms more liable to co-infection with multiple pathogens. However, few studies have been published focusing on avian mycoplasmas in aggravating respiratory viral outbreaks in Egypt. In this study, we conducted a passive surveillance study for MG and MS among cases of viral respiratory affections in broilers.

MATERIAL AND METHODS

Samples

Flocks investigated: Samples were collected from 68 broiler chicken flocks suffered from respiratory manifestations, including rales, sneezing, and nasal and ocular discharges and variable mortality rates in four Egyptian governorates, Fayoum (no. 28), Beni-Suef (no. 19), Giza (no. 12), and Minia (no. 9). The primary gross lesions were tracheitis, pneumonia, airsaculitis, and bronchial casts at the tracheal bifurcation. Nasopharyngeal and tracheal swabs and tissue specimens from trachea, lung, and air sacs were collected. The collected samples were divided into two parts; one for mycoplasma isolation and molecular identification and the other for detection of AIV-H9 and IBV by real-time RT-PCR (rRT-PCR). The protocol we followed in examining the collected samples is illustrated in Figure 1.

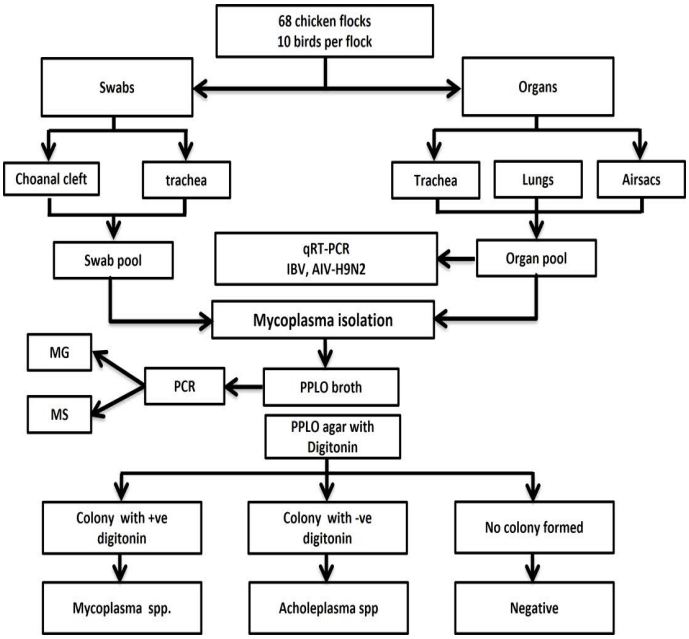

Figure 1: Diagnostic scheme of collected field samples. Abbreviations: IBV; infectious bronchitis, AIV-H9N2: avian influenza H9N2, MG; *Mycoplasma gallisepticum, MS; Mycoplasma synovaie*, PCR; polymerase chain reaction, PPLO; pleuropneumonia-like organisms, +ve; positive, -ve; negative.

Mycoplasma spp. isolation

All *Mycoplasma* spp. cultivation and purification procedures were performed according to the instruction of the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Catania and Riveroi, 2021). Briefly, the collected

Table 1: Oligonucleotide primers used for viral pathogens, *M. gallisepticum* and *Mycoplasma synovaie* molecular diagnosis

Pathogen[1]	Oligonucleotide	Primer and probe sequence (5`-3`)	Amplicon size (Shankar)	Reference
MG	F	GAGCTAATCTGTAAAGTTGGTCGCT	185	(Catania and Riveroi, 2021)
	R	TCCTTGCGGTTAGCAAC		
MS	F	GAGAAGCAAAATAGTGATATCACAG	205-210	
	R	TCGTCTCCGAAGTTAACA A		
IBV (S1)	GU391-F	GCTTTTGAGCCTAGCGTT	NA	(Callison et al., 2006)
	GL533-R	GCCATGTTGTCACTGTCTATTG		
	Probe	FAM-CACCACCAGAACCTGT-CACCTC-BHQ1		
AI-H9	RITA2-H9-F1	CAATGGGGTTYGCTGCCT	NA	(Hassan et al., 2019)
	RITA2-H9-F2	CAATGGGRKTTGCTGCCT		
	RITA2 –H9-R	TTATATACARATGTTGCAYCTG		
	RITA2 –H9-FAM	FAM-TTYTGGGCCATGTCIAATG-GRTC-BHQ1		

[1] MG; *Mycoplasma gallisepticum*, MS; *Mycoplasma synovaie*, IBV; infectious bronchitis virus, S1; spike protein-1 gene, AIV-H9; avian influenza virus (H9-subtype), HA; haemagglutinin gene, bp: base pair, NA; not applicable.

samples, including organs and swabs, were suspended in a 5 mL of pleuropneumonia-like organisms (PPLO) broth, incubated at 37˚C for 24-48 hours, and observed for color change. After the broth color changed into orange, 0.2ml of the broth was transferred by running drop technique into PPLO agar plate prepared according to (Razin, 1978). The plates were incubated under reduced oxygen tension in CO_2 jars at 37˚C. After 48 hours of incubation, the plates were examined with an inverted microscope for the characteristic fried egg appearance of *Mycoplasma spp.* colonies. The plates were daily examined for *Mycoplasma* spp. colonies for up to 3 weeks of incubation.

Mycoplasma spp. identification

Bacterial DNA was extracted from PPLO broth according to (Fan et al., 1995). A set of primers targeting the MG and MS 16S rRNA were used (Table 1) (Catania and Riveroi, 2021). The reaction mixture consisted of 25µl of 2×DreamTaq™ Green Master Mix (*Fermentas, Burlington, Ontario, Canada*), 1µl of each forward and reverse primers, 5µl of template DNA, and the mixture was completed by nuclease-free water to 50µl. Amplification of the target gene was accomplished with one cycle of denaturation at 94˚C for 3 min., followed by 35 cycles of denaturation 94˚C for 20 sec., annealing at 58˚C for 40 sec., and extension at 72˚C for 60 sec., and the final extension step was at 72˚C for 15 min. PCR products were electrophoresed in 2% agarose gels in tris borate EDTA (TBE) buffer containing 0.5% ethidium bromide. The DNA was visualized under an ultraviolet illuminator.

Detection of AIV-H9N2 and IB viruses using rRT-PCR

Viral RNA was extracted using Bioflux® viral RNA Mini-Spin column extraction kit (Hangzhou Bioer Technology Co., Ltd, Hangzhou, China) following the manufacturer's instructions. Oligonucleotide primers and Taqman probes targeting the AIV-H9N2 hemagglutinin "HA" and the IBV S1 genes were used (Table 1). The rRT-PCR reaction volume was 20 µl consisted of 10 µl of 2x SensiFAST Probe Lo-ROX One-Step Mix (Bioline, UK), 0.8 µl of 10 µM of each forward and reverse primers, 0.2 µl of the 10 µM probe, 0.2 µl of the reverse transcriptase enzyme, 0.4 µl of RiboSafe RNase inhibitor, 5 µl of the template RNA, and 2.6 µl of RNase free water. The rRT-PCR thermal profile consisted of 1 cycle of reverse transcription at 45˚C for 10 min., 1 cycle of initial denaturation at 95˚C for 2 min., 40 cycles of denaturation at 95˚C for 5 sec., annealing/extension at 60˚C (for AIV-H9) or 54˚C (for IBV) for 60 sec.

RESULTS

Mycoplasma spp. isolation from field cases of viral respiratory affections

A total of 68 samples from broiler chicken flocks were subjected to Mycoplasma isolation on PPLO agar (supplemented with digitonin discs). DNA extracts from PPLO broth were examined for the presence of MG and MS genes using conventional PCR. The successful isolation rate of *Mycoplasma* and *Acholeplasma* spp. from the respiratory tract of broiler chickens was indicated by the characteristic colonial morphology (fried egg appearance colonies which are tiny, circular, and smooth colonies with a dense raised center) (Figure 2) and the microbial growth inhibition by digitonin (at least 5 mm growth inhibition measured from the edge of the disc). Out of 68 samples, successful isolation of *Mycoplasma* spp. was evident in 35

Table 2: Summary of *Mycoplasma* spp. isolation and molecular detection of AIV-H9 and IBV in different samples according to the geographical distribution.

Item			Fayoum	Beni-Suef	Giza	Minia	Total No (%)[4]
Number of flocks			28	19	12	9	68
Microbial isolation on PPLO agar[1]	*Mycoplasma* spp.		13	10	8	4	35 (51.47%)
	Acholeplasma		5	3	0	0	8 (11.76%)
	Negative	Subtotal	10	6	4	5	25 (36.76%)
		Total[2]	15	9	4	5	33 (48.53%)
Molecular subtyping of Mycoplasmas by PCR	MG[3]		6	5	5	3	19 (27.94%)
	MS		2	2	0	2	6 (8.82%)
	MG+MS		0	1	1	1	3 (4.41%)
	None-MG None-MS		8	3	3	1	15 (22.06%)
	Subtotal		16	11	9	7	43 (63.24%)
	Negative		12	8	3	2	25 (36.76%)
	Total		28	19	12	9	68 (100%)
IBV and AIV-H9 detection by rRT-PCR	IBV		14	2	9	5	30 (44.12%)
	AIV-H9		3	2	0	1	6 (8.82%)
	IBV and AIV-H9		10	15	2	3	30 (44.12%)
	Negative		1	0	1	0	2 (2.94%)

[1]PPLO agar; pleuropneumonia-like organisms agar media, PCR; polymerase chain reaction, rRT-PCR; real-time reverse transcription PCR; [2]Including *Acholeplasma*; [3]MG; *Mycoplasma gallisepticum*, MS; *Mycoplasma synoviae*, None-MG None-MS; *Mycoplasma* spp. other than MG or MS, IBV; Infectious Bronchitis Virus, AIV-H9; Avian influenza virus of H9 HA subtype; [4]Percentages were calculated to the total number of samples.

samples (51.47% isolation rate), *Acholeplasma spp.* was detected in 8 samples (11.76%), while 25 samples were negative for both spp. (36.76%) (Table 2).

Molecular identification of *Mycoplasma* spp.

DNA extracts from PPLO broth were tested for the presence of MG 16S rRNA and MS 16S rRNA. Out of 68 samples, MG was detected by PCR in 19 samples (27.94%), MS was detected in 6 samples (8.82%), both MG and MS were detected in 3 samples (4.41%). In the remaining 40 samples (58.82%), none of MG or MS genes was detected, including 15 samples (22.06%) positive for *Mycoplasma* spp. isolation (these samples were considered as nonMG-nonMS *Mycoplasma*), and 25 samples (36.76%) were negative samples either containing *Acholeplasmas* (8 samples) or none (17 samples) (Figure 3 and Table 2).

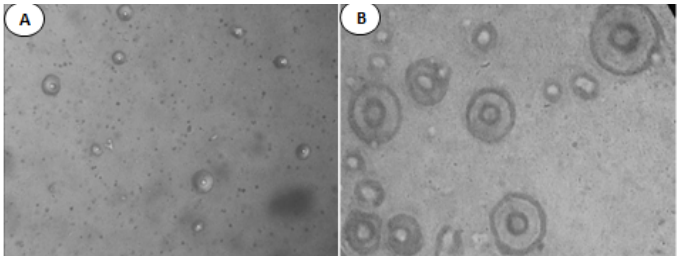

Figure 2: Characteristic colony morphology of *Mycoplasma* spp. isolated from broiler chickens showing fried egg appearance under inverted microscope at 4× magnification (A) and 10× magnification (B).

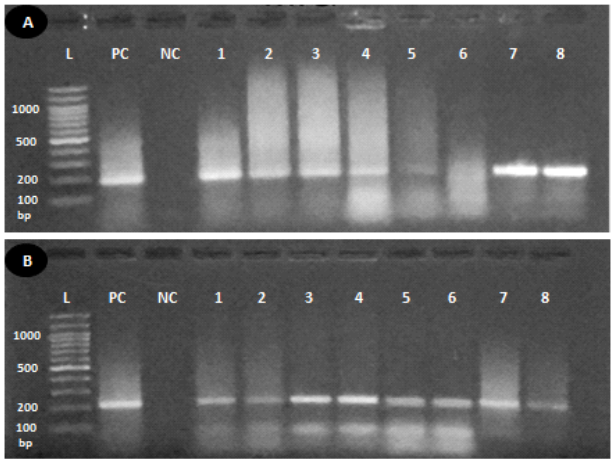

Figure 3: PCR detection of 16S rRNA gene of *Mycoplasma gallisepticum* (185 bp) (A) and *Mycoplasma synoviae* (205-210 bp) (B) in samples collected from broiler chicken flocks. L; DNA ladder, PC; positive control; NC; negative control, 1-8; field samples.

IBV and AIV-H9 detection in the collected samples

A pooled tissue samples (trachea, lung, and air sacs) of 68 broiler chicken flocks were subjected to rRT- PCR, and the results were as follow: AIV-H9 subtype 6 samples (8.8%), IBV 30 samples (44.1%), both IBV and AIV-H9 30 samples and 2 samples were negative for both viruses as shown in Table 2.

Table 3: Summary of the mortality rates in different flocks

Pathogen[1]	No (%)	Range of mortality rates (during samples collection)[2]		
		Minimum	Maximum	Mean
IBV	10 (14.7)	0.3	4.9	1.78
AIV-H9	5 (7.4)	0.8	2.6	1.54
AIV-H9, IBV	9 (13.2)	0.8	2.5	1.36
MG	1 (1.5)	NA	NA	NA
MG, IBV	6 (8.8)	1.3	6.8	3.37
MG, AIV-H9, IB	12 (17.6)	1.6	30	6.57
MS, IBV	3 (4.4)	2.6	11.2	6.63
MS, AIV-H9, IB	3 (4.4)	4.3	6.8	5.3
MG, MS, IBV	1 (1.5)	NA	NA	NA
MG, MS, AIV-H9, IB	2 (2.9)	3	5.6	4.3
None-MG none-MS, IBV	10 (14.7)	1.1	9.5	3.04
None-MG none-MS, AIV-H9	1 (1.5)	NA	NA	NA
None-MG none-MS, IBV, AIV-H9	4 (5.9)	2.2	3.3	2.88
Negative to all	1(1.5)	NA	NA	NA

[2]Note: the above mortalities were reported on the day of sample collection (i.e., noncumulative mortality rate of the flocks).
[2]MG; *Mycoplasma gallisepticum*, MS; *Mycoplasma synoviae*, None-MG None-MS; *Mycoplasma* spp. other than MG or MS, IBV; Infectious Bronchitis Virus, AIV-H9; Avian influenza virus of H9 HA subtype.

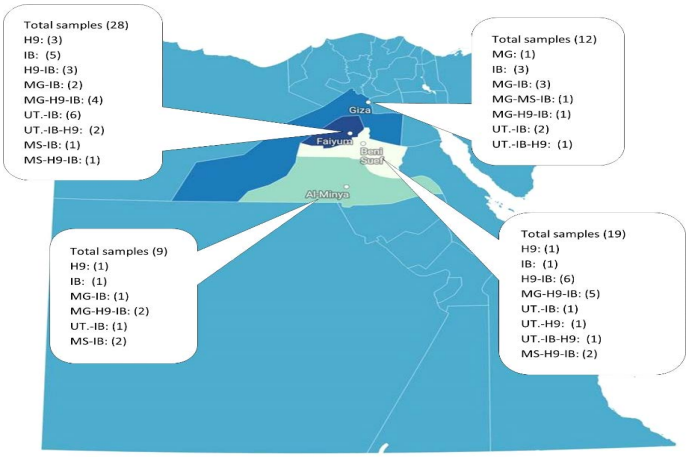

Figure 4: Geographical distribution of samples in northern upper Egypt

Geographical distribution and mortality rates associated with detected pathogens

The geographical distribution of the detected pathogens was different among the included governorates (Figure 4 and Table 2). For example, the IBV and none-MG none-MS *Mycoplasma* + IBV were the most prevalent infection in the Fayoum governorate (17.9% and 21.4%, respectively). However, in Beni-Suef governorate, the AIV-H9+IBV and MG+AIV-H9+IBV were more prevalent (31.6% and 26.3%, respectively). Furthermore, each IBV and MG+IBV represented 25% of the detected infections in Giza governorate. Finally, MG+AIV-H9+IBV and MS+IBV were 22% of the reported infections in Minia governorate. The mortality rates varied according to the detection of either single or mixed infection, where the lowest mortality (0.3%) was reported in a flock singly infected with IBV, while the highest mortality (30%) in a flock infected with MG-H9-IB (Table 3).

DISCUSSION

The poultry industry plays a vital role in the national economy by attracting investments, capital, and labor employments in Egypt (Attia et al., 2022). In addition, it is considered one of the most important relatively low-price animal protein sources (Beski et al., 2015; Marangoni et al., 2015). The industry has had rapid development and significant intensification during the last few decades. More than 850 million broilers were being fattened annually throughout the country during 2006 (Abdelwhab and Hafez, 2011). According to the Egyptian General Federation of Poultry Producers, the total number of reared broiler chickens increased exponentially to about 1.8 billion birds in 2019 (Galal, 2019). This great intensification in poultry production led to various problems, particularly respiratory problems in raised flocks (Abdelrahman et al., 2021; Gilbert et al., 2017).

The respiratory disease complex is one of the major problems facing the poultry industry in Egypt (Taher et al., 2017). MG, AIV, and IBV viruses are primary pathogens that may co-infect the avian respiratory tract leading to severe respiratory disease (Sid et al., 2015). The ecology and epidemiology of respiratory pathogens in broiler chickens are of worldwide concern, and screening their current epidemiological situation is urgently needed (El-Shall et al.,

2019). Therefore, a passive surveillance study has been conducted in 68 broiler chicken farms in four Egyptian governorates with apparent respiratory problems and variable levels of clinical severity and mortalities.

The obtained results indicate the high prevalence of *Mycoplasma* spp. in broiler chicken flocks with apparent respiratory manifestations parallel to previous reports (Ammar et al., 2016; Pflaum et al., 2016). PCR detection of mycoplasmas in field samples revealed a 63.24% positivity rate, while successful isolation of mycoplasmas on PPLO agar was evident in 51.47% of the samples. In eight samples, failure of *Mycoplasma* isolation was evident although of their positivity to PCR.

Isolation is time consuming and laborious compared to PCR, especially when dealing with field samples that are, undoubtedly, contaminated with other pathogens or commensal bacteria. Molecular detection of 16S rRNA in PPLO broth inoculated with field samples has a superior sensitivity for detecting Mycoplasma spp. compared to trials for isolation on PPLO agar. Moreover, PCR was able to identify the organism on the species level (Fraga et al., 2013; Kuo et al., 2017). MG was the most prevalent *Mycoplasma* with positivity in 51.2% of the *Mycoplasma* positive samples (22 samples [19 singular and 3 mixed with MS]), followed by the none-MG none-MS *Mycoplasma,* which represented 34.9% of the positive samples (15 samples) and MS that was detected in 9 samples representing 20.9% positivity. In 3 samples, mixed infection with MG and MS was confirmed (7% of the positive samples). These results are similar to those reported by (Marouf et al., 2020).

The rRT-PCR was used for examining all samples for AIV-H9 and IBV. Among 68 flocks, 30 (44.1%), 30 (44.1), and 6 (8.8%) flocks were infected with single IBV, mixed IBV/AIV-H9, and single AIV-H9 viruses, respectively. It is evident that IBV, either single or mixed infections, is highly prevalent in the respiratory disease complex in broilers in the examined governorates. Also, co-infection with both IBV and AIV-H9 predominate in broilers flocks with a 44.1% detection rate on the samples collected during 2020. Previous studies demonstrated more or less similar results between January 2012 and February 2014, where mixed infections were the most common cause of respiratory affection in Egypt. Additionally, the mixed infections with IBV and AIV-H9 viruses were the most common constellation in the investigated flocks (Hassan et al., 2016; Hegazy et al., 2019; Shehata et al., 2019).

The severity of clinical signs, postmortem lesions, and mortality rates of the tested flocks varied according to single and mixed infections. Clinical manifestations and postmortem lesions varied among naturally infected birds based on the infecting pathogen, the flock's immune status, and whether the disease is due to single or multiple infections. Mortality rates in the flocks under investigation ranged from 0.3% to 30%, with the highest mortality rate in a flock suffered from triple MG, IBV, AIV-H9 infection, although the flock was vaccinated against IBV and AIV-H9 at 2 days old. The presence of MG as a co-pathogen with the two respiratory viruses and the poor flock management could explain this high mortality rate. The role of mixed infection in exaggerating respiratory disease in different flocks has been extensively reported (Al-Dabhawe et al., 2013; Megahed et al., 2020; Nili and Asasi, 2002).

Co-infection with MG, IBV, and AIV-H9 has resulted in a 30% mortality rate even in flocks vaccinated against both viral pathogens. Also, the highest mortality in flocks singularly infected with MG, IBV, or AIV-H9 did not exceed 1.6%, 4.9%, 2.6%, respectively compared to higher mortality rates (up to 30%) in co-infection with the three pathogens. Mixed infections of AIV-H9N2 with other respiratory pathogens (IBV, MG, *Staphylococcus aureus, Avibacterium paragallinarum, E. coli, Ornithobacterium rhinotracheale* or immune-suppressive agents) were reported as the leading cause of severe clinical disease with subsequent high mortality and significant economic losses (Abdelaziz et al., 2019; Pan et al., 2012; Perk et al., 2004). The role of *Mycoplasma* spp. in respiratory disease complex and the additive effect of bad management could also be highlighted in the light of the great variances in the sequels of disease manifestation between the natural and the experimental infections with AIV-H9. Thus, the secondary agents play an important role in the pathogenesis of viral respiratory problems in poultry (Aamir et al., 2007; Hassan et al., 2017). *Mycoplasma* can survive within the host cells and induce disease through evading the host immune response that can be achieved through adherence to host target cells, mediation of apoptosis, innocent bystander damage to host cell due to intimate membrane contact, molecular (antigen) mimicry that may lead to tolerance, and mitotic effect for B and/or T lymphocytes, which could suppress T-cell function and/or production of cytotoxic T cell (Arfi et al., 2021; Hoelzle et al., 2020; Yadav et al., 2021).

A tendency to higher mortality rates was observed in flocks co-infected with *Mycoplasma* and one or two of the tested viruses. The increased mortality rates in mixed infections may be due to the effect of *Mycoplasma* on the immune response of birds toward other pathogens. *Mycoplasma*, through the production of various molecules and interaction of its variable lipoprotein with the host cells, can cause significant damage to the host cells, inhibit phagocytosis, and affect the functions of B- and T-lymphocytes, which may lead to immunosuppression (Hassan et al., 2017; Pa-

pazisi et al., 2002; Rottem, 2003; Vogl et al., 2008). Thus, immunosuppression due to depletion of lymphoid organs and concurrent infections such as MG might increase the pathogenic potential of AIV-H9 subtype to chicken (Pazani et al., 2008) or predispose to several other infections (Stipkovits et al., 2012a; Stipkovits et al., 2012b). For example, co-infection of MG and low pathogenic avian influenza H8N3 in broiler caused obvious clinical signs, induced a significant reduction of body weights, and increased MG colonization of the lung and other inner organs (Stipkovits et al., 2012a). IBV also enhances air sacculitis in MS infection (Landman and Feberwee, 2004), which may explain the relatively high mortality rates in flocks infected with MS-IBV with an average mortality rate (6.6%). It is proposed that the increased pathogenicity of H9N2 during co-infection is associated with activation of HA cleavage by protease like activity produced from co-infecting pathogens. Other researchers hypothesised that the pathogenicity of H9N2 during co-infection could be exacerbated by immune system dysfunction or tissue damage caused by co-infecting pathogens (AboElkhair et al., 2021; Ismail et al., 2018). Many organisms, including *Staphylococcus aureus*, *H. paragallinarum*, *E. coli*, *O. rhinotracheale*, MG, MS, IBV, NDV, and even live IBV and NDV vaccines, have synergistic effects that increase H9N2 virulence and mortality in infected birds. Such synergistic effects may occur by increasing hyaluronic acid (HA) cleavage via trypsin-like protease secretion (Abdelaziz et al., 2019; Samy and Naguib, 2018b).

CONCLUSION

The current study demonstrates that MG, IBV, and AIV-H9 are highly prevalent in respiratory disease outbreaks in broilers. The co-infection of *Mycoplasma* spp. and respiratory viruses are the primary cause of severe clinical signs, postmortem lesions, and high mortality rates. The existence of none-MG none-MS *Mycoplasmas* in a relatively high number of the examined flocks is an interesting point of future research. Further studies are recommended for exploring the role of different avian *Mycoplasmas* in viral respiratory affections under controlled experimental conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

NOVELTY STATEMENT

Several studies have been conducted on the prevalence of viral infections in the respiratory disease problems in Egyptian poultry. However, bacterial pathogens, especially avian mycoplasma, are neglected in such problems. The current study sheds light on the prevalence of avian mycoplasma associated with the prevalent viral pathogens and their role in aggravating respiratory viral outbreaks in Egypt.

AUTHORS CONTRIBUTION

Abdelrahman A.A., Elkady M.F., Shany S.A.S., and Ali A.; conceptualization and study design. Abdelrahman A.A., Dardeer M. A. A. and Shany S.A.S.; sample collection and analysis. Abdelrahman, A.A., Shany, S.A.S., Hassan, K. E., and Ali A.; data analysis and initial draft writing. Shany, S.A.S., Dardeer M. A. A., and Elkady M.F.; supervision. Abdelrahman, A.A., Shany, S.A.S., Hassan, K. E., Dardeer M. A. A., Elkady M.F., and Ali A.; final draft revision and approval.

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