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Mycoplasma gallisepticum: an emerging challenge to the poultry industry in Egypt

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Summary

In this study, the authors examined the technical performance of culture methodology using specific media: *Mycoplasma* isolation media of pleuropneumonia-like organisms (PPLO) broth and PPLO agar. Digitonin sensitivity, growth inhibition, the serum plate agglutination test, a commercially available enzyme-linked immunosorbent assay (ELISA) and a commercially available simplex polymerase chain reaction (PCR) test were used to detect *Mycoplasma gallisepticum* infections in samples collected from the lungs, trachea and tracheal swabs of poultry. These samples were collected from broiler-breeder flocks, broiler flocks and layer flocks.

In addition, genomic bacterial deoxyribonucleic acid (DNA) was extracted and amplified, using a simplex PCR. The seroprevalence of *M. gallisepticum* antibodies in chickens and chicks was also investigated. The prevalence of *M. gallisepticum* was found to be highest in the layer flocks, at 33.3% (17/51), when the tracheal swab procedure was adopted. In young birds, the serum plate agglutination test and ELISA assay detected antibodies against *M. gallisepticum* in 69.9% (320/458) and 58.3% (267/458) of the chicken samples, respectively, and 48.7% (146/300) and 60% (180/300) of the samples from the chicks.

Keywords

Avian mycoplasmosis – Chickens – Conventional culture – Egypt – *Mycoplasma gallisepticum* – Mycoplasmosis – Poultry industry – Serology.

Introduction

The primary difference between mycoplasmas and other bacteria is that bacteria have a solid cell wall structure and can grow in the simplest culture media while mycoplasmas do not have a cell wall and can take on many different shapes. This makes them difficult to identify, even under a high-powered electron microscope. Mycoplasmas can also be very hard to grow in the laboratory and are often missed as pathogenic causes of disease for this reason.

Outbreaks of infectious disease are a constant risk for the agricultural industry and *Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of gallinaceous and certain non-gallinaceous avian species. It causes chronic respiratory disease (CRD) in chickens and other fowl (9). *Mycoplasma gallisepticum* can be found

worldwide (21), is a fragile (has no cell wall), host-adapted (to avian hosts only), fastidious (has specialised growth requirements) organism (15). Owing to the substantial losses caused in both performance and production, *M. gallisepticum* has been described as the most economically important of the four pathogenic *Mycoplasma* species affecting poultry (9). Losses attributed to mycoplasmosis, mainly *M. gallisepticum* infection, are due to:

– a decrease in egg production and quality
– poor hatchability (a high rate of embryonic mortality and culling of day-old birds)
– poor feed efficiency
– an increase in mortality and carcass condemnations
– medication costs (23).

Moreover, mycoplasmosis can spread quickly through an entire flock. The carcasses of birds sent to slaughter may also be downgraded (3, 38). Since *M. gallisepticum* is frequently complicated with other respiratory disease(s), including Newcastle disease, infectious bronchitis and *Escherichia coli* infection (12, 24, 34), *M. gallisepticum* must be differentiated from these common respiratory diseases in chickens. *Mycoplasma gallisepticum* infections are notifiable to the World Organisation for Animal Health (OIE) (38). Within the European Community, legislation (Directive 90/539/EEC) (8) governs the control of *M. gallisepticum* and *M. meleagridis* in intra-Community trade in poultry and hatching eggs. The legislation also applies to imports from third countries. In the United Kingdom (UK), the Poultry Health Scheme reflects the European Union (EU) Directive in laying down control measures for *M. gallisepticum* and *M. meleagridis* but not *M. synoviae*. On the other hand, the United States Department of Agriculture National Poultry Improvement Plan encompasses all three of these *Mycoplasma* species (4).

Since 1954, avian mycoplasmosis has been considered a significant problem in chicken flocks in Japan and other Asian countries. In Japan, *M. gallisepticum* and *M. synoviae* infections were confirmed aetiologically in chicken flocks affected with respiratory disease or synovitis in 1962 and 1973, respectively. In other Asian countries, including Bangladesh, India, Indonesia, the People's Republic of China, Korea, Malaysia, the Philippines, Taipei China, Thailand and Vietnam, as well as Israel, the occurrence of mycoplasmosis in chicken flocks has been recognised serologically or aetiologically (19, 33).

Mycoplasma can be detected in tissue fragments of affected organs, such as the trachea and lungs, as well as in swabs from the trachea (23). Previous studies have described alternative laboratory markers to conventional bacteriological culture for the detection of *M. gallisepticum* (14, 27). These markers have included serological testing of sera from cases of CRD infection for the presence of antibodies to *M. gallisepticum* (6, 11), as well as molecular detection of *M. gallisepticum* (13, 31, 36).

This study was undertaken to determine the prevalence of *M. gallisepticum* infection in chickens from selected model breeder poultry farms in Egypt, using conventional cultural and biochemical techniques, in addition to a simple, non-sophisticated polymerase chain reaction (PCR) to detect *M. gallisepticum* in field samples from healthy and diseased chickens.

Materials and methods

Samples

Samples from the lungs and trachea and tracheal swabs were collected from broiler-breeder flocks, broiler flocks and layer flocks. In addition, blood was collected from one-day-old chicks and four-week-old chickens to perform sero-analyses to detect antibodies against *M. gallisepticum*, using serum plate agglutination (SPA) and enzyme-linked immunosorbent assay (ELISA). None of the chickens had been vaccinated with any *M. gallisepticum* vaccine.

Qualitative processing for conventional detection of *Mycoplasma gallisepticum*

Culture and isolation

The specimens were inoculated onto several selectivemedia to isolate *M. gallisepticum*, including pleuropneumonia-like organisms (PPLO) broth and PPLO agar. All media were incubated at 37ºC in a moist 10% CO_2 incubator for three to five days (15). In addition, all the different phenotypes from each chicken were identified by using a combination of conventional identification methods (e.g. growth inhibition test, digitonin sensitivity, glucose fermentation, oxidase and arginine deamination).

Sensitivity to digitonin

Discs were soaked with 1.5% ethanolic solution of digitonin and incubated at 37ºC in a moist 10% CO_2 incubator. The running drop technique was employed. The discs were pressed gently onto the middle of the inoculated area. The inhibition zone was measured after three to five days of incubation.

Growth inhibition

The agar plates were inoculated with serially diluted, suspected, purified, viable *Mycoplasma* culture, using the running drop technique. After drying, a 6 mm disc, soaked with 20 µl of antiserum, was gently placed in the middle of

the *Mycoplasma* streak. The plates were incubated in a moist 10% CO_2 incubator at 37ºC for arginine-positive strains for three to five days (38).

Detection of *Mycoplasma gallisepticum* antibodies

Preparation of serum samples

The blood samples were obtained aseptically from the brachial vein of the selected birds, using 5 ml sterile disposable syringes and needles. The blood was allowed to clot in the syringe and kept for 1 to 2 h at room temperature. After clotting, sera were separated, centrifuged and poured into sterile vials, labelled individually and stored at 4ºC until use. These sera were then transported to the National Laboratory for Veterinary Quality Control on Poultry Production, packed in ice in a thermo flask, for further testing.

Serum plate agglutination test

The SPA test was conducted with crystal violet-stained *M. gallisepticum* antigen. Standard *M. gallisepticum* antigen was used in the rapid SPA test to detect antibodies against *M. gallisepticum* in the collected sera, to determine infection (21, 25). In brief, 30 µl of fresh serum was mixed with 30 µl of antigen and then incubated at room temperature for 1 to 2 min before the result was read.

Enzyme-linked immunosorbent assay

The serum samples were given a specific code number for each flock, and kept frozen at – 20ºC until analysis. A commercial test kit was used to detect specific antibodies against *M. gallisepticum*, based on indirect ELISA. A final serum dilution of 1:100 was used, according to the instructions of the manufacturer. All serum samples collected from each flock were run on the same test plate to prevent conclusion errors that could be due to day to day variation in the test (16, 17). Positive and negative reference controls provided by the manufacturer were also used in each test run, for quality control and to confirm the results. Optical density values were set at a wavelength of 405 nm, using an ELISA reader.

Molecular polymerase chain reaction to detect *Mycoplasma gallisepticum*

Deoxyribonucleic acid extraction

All deoxyribonucleic acid (DNA) isolation procedures were carried out in a class II biological safety cabinet (Nuaire, France), in a room physically separated from the room used to set up nucleic acid amplification reaction mixes and also from the 'post-PCR' room. This was in accordance with the good molecular diagnostic procedure guidelines provided by Millar *et al.* (22), to minimise contamination and thus the possibility of false positive results. Bacterial genomic DNA was extracted directly from the isolated strains, as well as from the reference strains *M. gallisepticum* (ATCC 19610), *M. synoviae* (WVU1853), *M. gallinarum* (ATCC 19708), *E. coli* (ATCC 11775) and *S.* Typhimurium (ATCC 14028), by using the standard OIE procedures (38). Extracted DNA was stored at – 20ºC before PCR amplification. For each batch of extractions, a negative extraction control was performed, containing all the reagents minus the sample, as well as a positive extraction control, using the *M. gallisepticum* strain S6.

Polymerase chain reaction amplification

Initially, PCR amplification conditions were optimised by separately varying the magnesium chloride concentration, annealing temperature, primer concentration and DNA template concentration. Following optimisation, reaction mixes (45 µl) were set up as follows:

– PCR 10 × buffer (75mM Tris HCl, 2 mM $MgCl_2$, 50mM KCl, 20mM $[NH_4]_2SO_4$)

– 10 mM deoxynucleoside triphosphate

– 5 units of *Taq* DNA polymerase

– 4 µl of DNA template

– 0.1 µM (each) of the set of primers.

The specific *M. gallisepticum* primers (18, 38) consisted of the following sequences:

– MG-14F: 5'-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C-3'

– MG-13R: 5'-GCT-TCC-TTG-CGG-TTA-GCA-AC-3'

Following a 'hot start', the reaction mixtures were subjected to the following empirically optimised thermal cycling parameters in a Biometra thermocycler (Germany):

– 94°C for 30 s, followed by 40 cycles of 55°C for 30 s

– 72°C for 60 s, followed by a final extension at 72°C for 5 min.

Positive (S6 and F strains of) *M. gallisepticum* DNA, *M. synoviae* and *M. gallinarum*, *E. coli* and *S.* Typhimurium controls were included in every set of PCR reactions.

Detection of amplicons

Following amplification, aliquots (15 µl) were removed from each reaction mixture and examined by electrophoresis (1.5 volts, 45 min), in gels composed of 2% (weight/volume) agarose in 1 × Tris-acetate-EDTA buffer (40 mM Tris, 20 mM acetic acid, 0.5 M Tris-

ethylenediamine tetra-acetic acid [EDTA], pH 8.0), stained with ethidium bromide (0.5 µg/ml). Gels were visualised under ultraviolet illumination using a gel image analysis system and all images were archived as digital graphic files. Where a band was visualised at the correct expected size of 185 base pairs (bp), the specimen was considered positive for *M. gallisepticum* (32).

Results and discussion

Mycoplasma detection has historically been a difficult and demanding task for many researchers and thus infection can go unnoticed. At present, routine bacteriological culture on selective/non-selective culture media is the cornerstone of microbiological detection. Cultural character and biochemical identification exhibited identical phenotypic cultural and biochemical properties. The cultured *M. gallisepticum* presented as 'fried egg' colonies, and were subcultured after filtration (35). These cultures grew in the absence of nicotinamide adenine dinucleotide and L-cysteine in the culture medium. They were sensitive to 1.5% digitonin and fermented glucose and did not hydrolyse arginine.

Those birds most affected came from the layer flocks, as shown in Table I. The highest prevalence of *M. gallisepticum*, 33.3% (17/51), was recorded in the layer flocks, using the tracheal swab procedure, proving that this is an excellent method for specimen collection, as indicated by Nascimento *et al*. (23).

In young birds, the results showed that the SPA test and ELISA assay detected antibodies against *M. gallisepticum* in 69.9% (320/458) and 58.3% (267/458) of the chicken samples and 48.7% (146/300) and 60.0% (180/300) of the samples from day-old-chicks, respectively (Table II). Serological tests are useful for monitoring *M. gallisepticum* infection in a flock and for taking prophylactic measures to control *M. gallisepticum* infection early in *M. gallisepticum*-free poultry. However, these tests showed a large number of cross-reactivities (1).

The chronic nature of *Mycoplasma* infections demonstrates a failure of the host immune system to deal effectively with these organisms (26). Chronic *Mycoplasma* colonisation of the major airways, leading to debilitating exacerbations of pulmonary infection, is the major cause of morbidity and mortality in chickens with CRD. Several authors have published their findings describing the pathophysiology and risk factors of *Mycoplasma* infection, which showed that early chronic lower airway infection related to *M. gallisepticum* in CRD was a major predictor of morbidity and mortality (5, 19, 20, 23, 37), especially when the birds were concurrently infected with other viruses or bacteria (20, 37). These authors suggested that early intervention may help decrease the associated morbidity and mortality of chickens with CRD. It is therefore important that primary diagnostic bacteriology laboratories have the ability to detect transient and early *M. gallisepticum* colonisation as early as possible, so that:

– aggressive antibiotic regimes may be considered

– the birds are managed optimally, in an attempt to avoid early biofilm formation and chronic colonisation with *M. gallisepticum*

– appropriate infection control precautions are put into place.

Polymerase chain reaction permits the detection of femtogram amounts of *Mycoplasma* DNA, corresponding to one bacterial cell (35). Tracheal swabs were therefore

Table II
The serum plate agglutination test and enzyme-linked immunosorbent assay in the detection of *Mycoplasma gallisepticum* antibodies in chicken flocks

Age of the birds	No. of serum samples	SPA test Positive samples No.	SPA test Positive samples %	ELISA Positive samples No.	ELISA Positive samples %
14 weeks	458	320	69.9%	267	58.3%
4 weeks	300	146	48.7%	180	60.0%

SPA: serum plate agglutination test
ELISA: enzyme-linked immunosorbent assay

Table I
Incidence of isolation of *Mycoplasma gallisepticum* from broiler-breeder, broiler and layer flocks

Types of flocks	Lung samples		Tracheal samples		Tracheal swab samples	
	No. of samples	No. of positive isolates	No. of samples	No. of positive isolates	No. of samples	No. of positive isolates
Broiler-breeder	105	6 (5.7%)	105	18 (17.1%)	105	32 (30.5%)
Broiler	123	0 (0%)	123	5 (4.1%)	123	6 (4.9%)
Layer	51	7 (13.7%)	51	13 (25.5%)	51	17 (33.3%)
Total	**279**	**13 (4.7%)**	**279**	**36 (12.9%)**	**279**	**55 (19.7%)**

submitted to PCR, using species-specific primers, and the sequences used enabled the detection of the target *M. gallisepticum* (Fig. 1). In this study, PCR detected more infected samples than either culture or serology (Table III). Of 279 samples of tracheal swabs collected from broiler-breeder flocks (n = 105), broiler flocks (n = 123) and layer flocks (n = 51), 131 tested positive using *M. gallisepticum*-specific PCR. Fifty-five of these samples also tested positive by culture.

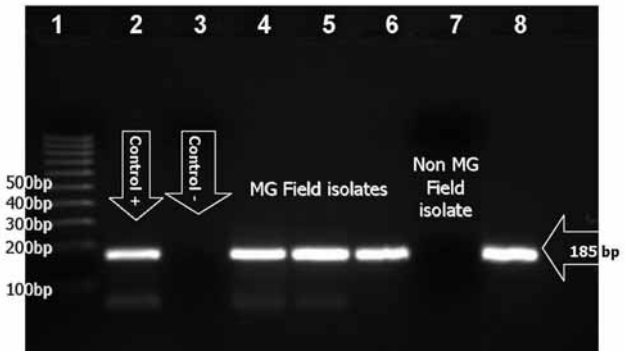

Fig. 1
Agarose gel electrophoresis using primers specific for *Mycoplasma gallisepticum*
Positive amplification of 185 base pair (bp) fragments using primers specific for *Mycoplasma gallisepticum* from extracted deoxyribonucleic acid (DNA) field isolates of *M. gallisepticum* was observed (Lanes 4, 5, 6 and 8), while no amplification could be observed from the extracted DNA of field isolates of *Mycoplasma* spp. (positive for arginine hydrolysis and negative for glucose fermentation test) (Lane 7). Lane 2 shows positive control (*M. gallisepticum* S6) and Lane 3 shows negative control (*Escherichia coli*). Lane 1 shows a 100 bp ladder

The serological data were not perfectly correlated to *M. gallisepticum* isolation and PCR assay. The SPA test primarily measures immunoglobulin M and is able to detect antibody in the serum within a week of infection. However, the SPA test is prone to false positive results and non-specific reactions may occasionally be high for a variety of reasons. For instance, non-specific reactions can be caused by the presence of antiglobulin-like factors (7), and sera from chickens infected with infectious bursal disease viruses were found to cross-react in *M. gallisepticum* SPA tests (30). In addition, different degrees of temporary immunosuppression might have allowed a more significant invasion of *M. gallisepticum*, and a consequent positive serological response *a posteriori* (10). However, it is often difficult to isolate *M. gallisepticum* in cultures from flocks that are concurrently infected with one or more non-pathogenic *Mycoplasma* species, such as: *M. gallinarum*, *M. gallinaceum*, *M. pullorum*, *M. gallopavonis* or *Acholeolasma laidlawii*. These non-pathogenic species usually overtake the slower-growing pathogenic species *in vitro*. In rare cases, *M. gallisepticum* isolates have taken as long as three weeks to show signs of growth in broth medium (2). Another cause of false positive reactions in SPA tests, as well as in ELISAs, stems from the antigenic relationship between *M. gallisepticum* and *M. synoviae*; some of the antigens of *M. gallisepticum* and *M. synoviae* share common epitopes (2). In addition to these cross-reactions, strong and frequent positive SPA and ELISA reactions occur to medium components, contributing to false positive SPA and ELISA serology in vaccinated birds (2).

Table III
The sensitivity of culture compared to polymerase chain reaction and serology methodologies

Types and number of flocks	Culture	PCR	Serology	
			SPA	ELISA
Broiler-breeder (105)	32 (30.5%)	68 (64.8%)	78 (74.3%)	61 (58.1%)
Broiler (123)	6 (4.9%)	21 (17.1%)	29 (23.6%)	19 (15.4%)
Layer (51)	17 (33.3%)	42 (82.4%)	46 (90.2%)	37 (72.5%)
Total (279)	**55 (19.7%)**	**131 (47.0%)**	**153 (54.8%)**	**117 (41.9%)**

PCR: polymerase chain reaction
SPA: serum plate agglutination test
ELISA: enzyme-linked immunosorbent assay

Not all laboratories employ molecular detection methods for *M. gallisepticum*, either from culture plates or chicken samples. Thus, small numbers of *Mycoplasma* colonies (e.g. 1 to 2) may be missed when they are present in the early stages of colonisation, preceding infection of the airways. This is particularly the case where such single colonies are mixed alongside other phenotypically similar genera on the primary culture plate (29). Pragmatic, practical and cost implications make it impossible to qualitatively identify the total bacterial microflora present on non-selective primary plates from specimens.

It is very important to pursue any such infected areas, which demonstrate a positive result by PCR but negative by culture, to establish whether there is transient CRD infection in the poultry which never results in established colonisation leading to chronic infection. Moreover, it is important to monitor poultry farms displaying such PCR+/culture– findings to enable optimal antibiotic management and infection control. Such discrepant results (PCR+/culture–) could reflect true *M. gallisepticum* colonisation with a false negative result from culture, due to:

– overgrowth of the sample by other bacteria

– the presence of non-cultivable or non-viable pathogens

– auxotrophic mutations in the organism (28)

– potential phenotypic misidentification of *M. gallisepticum*, which has been recently described (32).

Conclusion

Current methods of controlling *M. gallisepticum* infections among avian species in Egypt are limited. Only biosurveillance practices, through serological monitoring of flocks and *M. gallisepticum* isolation techniques, are used in all chicken and turkey industries.

The results of the serological tests in this study showed that a certain level of false positive results can be expected in virtually any serological test. Although the level of false positive results varied between the two serological tests, these results show that it is not advisable to rely completely on one test only.

These results indicate the disseminating capacity of *M. gallisepticum* and the possible use of simple PCR for epidemiological analysis and farm decontamination before the introduction of new birds.

The seroprevalence study revealed that *M. gallisepticum* infection is widespread in chickens. However, the losses caused by *M. gallisepticum* were not estimated in this study. Further research is necessary to assess the impact of *M. gallisepticum* on the village poultry production system. As the village poultry system is being used to alleviate poverty through increased production, losses due to *M. gallisepticum* infection may impede this goal.

It is unlikely that *M. gallisepticum* will be eradicated from the commercial poultry industry in the near future. However, through biosecurity programmes and effective use of vaccines, losses can be reduced.

Table IV
Comparison of *Mycoplasma gallisepticum* assay techniques

Comparison factors	Culture and identification	SPA	ELISA	PCR
Cost	Medium	Low	Medium	Medium
Ingredients	Medium	Low	Medium	Medium
Equipment	Low + high	Low	Medium	High
Labour	High	Low	Medium	Medium
Sample	Medium + high	Low	Medium	Medium + high*
Speed	5 to 14 days	Rapid	Moderate	24 h
Automation	Poor	Poor	Good	Good
Stage of infection	Sample-dependant	Early (7 to 10 days)	Intermediate	Sample-dependent
Sensitivity	Excellent	High (early)	Good	Excellent
Specificity	Excellent	Low	Good	Excellent
Antigen				
Availability	Limited	Good	Limited	Limited
Quality		Variable	Good	

* The cost can be reduced significantly by pooling samples
SPA: serum plate agglutination test
ELISA: enzyme-linked immunosorbent assay
PCR: polymerase chain reaction

All four methods (culture, SPA, ELISA and species-limited PCR) are effective in detecting the presence of *M. gallisepticum*, with varying degrees of success and discrepancy (Table IV).

Mycoplasma gallisepticum : un nouveau défi pour l'aviculture en Égypte

K.M. Osman, M.M. Aly, Z.M.S. Amin & B.S. Hasan

Résumé

Les auteurs ont réalisé une étude visant à déterminer les performances techniques de différents milieux de culture spécifiques pour l'isolement de *Mycoplasma* : bouillon de culture à base de *pleuropneumonia-like organisms* (PPLO) et gélose PPLO. Le test de sensibilité à la digitonine, le test d'agglutination rapide sur lame, une épreuve immuno-enzymatique (ELISA) commerciale et une épreuve d'amplification en chaîne par polymérase (PCR) commerciale ont été utilisés pour la détection des infections à *Mycoplasma gallisepticum* dans des échantillons de poumon et de trachée ainsi que dans des écouvillons trachéaux de volailles. Les prélèvements ont été réalisés dans des élevages de poulets de chair, de poulets reproducteurs et de poules pondeuses. En outre, l'acide désoxyribonucléique (ADN) génomique bactérien a été extrait et amplifié en employant une PCR simple. L'étude a également déterminé la prévalence sérologique des anticorps dirigés contre *M. gallisepticum* chez les poulets et les poussins. Le taux de prévalence le plus élevé (33,3 %, 17/51) a été relevé dans les bandes de poules pondeuses suite à l'analyse des écouvillons trachéaux. Dans les prélèvements issus de volailles jeunes, le test d'agglutination rapide sur lame et l'épreuve ELISA ont mis en évidence, respectivement, un taux de présence d'anticorps de 69,9 % (320/458) et de 58,3 % (267/458) chez les jeunes adultes, et de 48,7 % (146/300) et 60 % (180/300) respectivement chez les poussins.

Mots-clés

Aviculture – Culture classique – Égypte – *Mycoplasma gallisepticum* – Mycoplasmose – Mycoplasmose aviaire – Poulet – Sérologie.

Mycoplasma gallisepticum: un nuevo desafío para la avicultura en Egipto

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Resumen

Los autores realizaron este estudio a efectos de determinar los resultados técnicos de dos medios de cultivo – caldo de organismos semejantes a los de la pleuroneumonía (PPLO, por sus iniciales en inglés) y agar PPLO – para el aislamiento de *Mycoplasma*. Con ese fin, detectaron las infecciones por *M. gallisepticum* presentes en muestras de pulmón y tráquea, así como en hisopos traqueales de aves, mediante pruebas de sensibilidad a la digitonina, inhibición del crecimiento y aglutinación de suero en placa; también emplearon una prueba de inmunoabsorción enzimática (ELISA) y un ensayo de reacción en cadena de la polimerasa (PCR) simple adquiridas en el comercio. Las muestras provenían de parvadas de pollos para reproducción, pollos para carne y gallinas ponedoras.

Además, los autores extrajeron el ácido desoxirribonucleico (ADN) genómico bacteriano, que amplificaron con una PCR simple. También determinaron la

seroprevalencia de los anticuerpos contra *M. gallisepticum* en pollos y pollitos. En los hisopos traqueales, la mayor prevalencia (33,3%, 17/51) correspondió a las parvadas de gallinas ponedoras. En las muestras de aves adultas jóvenes, las pruebas de aglutinación de suero en placa y ELISA arrojaron una prevalencia de anticuerpos contra *M. gallisepticum* del 69,9% (320/458) y el 58,3% (267/458) respectivamente, y en las muestras de pollitos del 48,7% (146/300) y el 60% (180/300) respectivamente.

Palabras clave
Avicultura – Cría tradicional – Egipto – Micoplasmosis – Micoplasmosis aviar – *Mycoplasma gallisepticum* – Pollo – Serología.

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