Epidemiological and Diagnosis Status of *Mycoplasma* gallisepticum in Chickens around the globe

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Abstract

Chickens are suffered with several diseases in which Mycoplasma gallisepticum diseases is one of great importance. Infected chickens can be diagnosed on the basis of history, clinical signs, postmortem and microscopic examination and diagnostic methods. After observation of clinical signs, necropsy practice is the best tool to differentiate the disease on the basis of type of lesions. On postmortem examination airsacculitis is regarded as characteristic lesion. Microscopic changes can be observed in trachea, lungs and air sacs. Diagnosis of *Mycoplasma* can be done by several tests including serological tests and molecular tests. Serological tests are used for early diagnosis of the disease. Different disease status was observed in various countries like Bangladesh, India, Iran, Egypt, China and some other countries. Infection can be controlled by proper screening the breeder flock, vaccination of layer chicks and culling of infected flocks because if flock is infected, it remains for whole life, so eliminate all the flock to avoid the further losses due to *Mycoplasma*.

Keywords: Mycoplasma gallisepticum; MG; Diagnosis; Epidemiology; Chickens; Mycoplasmosis

Background

Poultry industry is fast growing sector in Pakistan but beyond this fast growth of poultry industry, it is prone to lot of infectious and non-infectious threats which causes huge economic losses. It is actually a very risky business due to less measures adopted by the farmers to prevent the disease. Great number of viral and bacterial pathogen effect the birds in which mycoplasmosis is of great importance. Birds of all ages are prone to *Mycoplasma* infection but young birds are more effected than mature ones [1]. *Mycoplasma* pathogens are gram negative and belong to class Mollicutes, order-I Mycoplasmatales, family-I Mycoplasmataceae and genus *Mycoplasma*. Mycoplasmas are small size bacteria, without cell wall and having a triple layer plasma membrane [2]. Mycoplasmosis includes four major infectious, highly prevalent species including *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), *M. meleagridis* (MM) and *M. iowae* (MI). MG and MS mostly effect the chickens but MM and MI are produce disease in turkeys [3]. It is also known as chronic respiratory disease (CRD) of poultry. *Mycoplasma* colonies have a typical fried egg appearance on culture media [4]. MG can be transmitted by both ways either horizontal or vertical. Horizontal transmission can be possible within a flock by direct or indirect contact, direct contact by feces or respiratory secretions and indirect contact through contaminated feed, water, equipment and human cloths etc., while transmission of MG between the flocks is possible through wind. MG is transmitted vertically from parents to offspring through eggs [5,6]. Disease is distributed worldwide and affects both the broiler and layer birds. Significant economic losses occurring due to decrease in egg production and egg quality, embryonic mortality, poor hatchability, high morbidity, decrease weight gain and medication costs [7]. Disease status varies in different areas depends on age, sex, flock size and season [8]. Present study will explain the current MG status can be seen around the

MG diagnosis

There are different serological techniques to detect MG at initial stages i.e. Serum plate agglutination (SPA) test [9], Haemagglutination inhibition (HI) test [10] and Enzyme linked-Immunosorbant assay (ELISA) [11]. Molecular techniques are good alternatives for

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conventional diagnostic methods. Specific primers target specific genes of MG [12]. Real time PCR is considered as fast and sensitive test to detect MG infected flocks [13]. Loop-mediated isothermal amplification (LAMP), is another method to diagnose MG, was developed in 2000. LAMP depends on an auto-cycling strand displacement DNA synthesis performed by *Bst* DNA polymerase large fragment under isothermal conditions of 60 - 65°C. This assay is highly specific, and the amplification efficiency is equal to that of PCR based methods. Moreover, LAMP method synthesizes large amounts of DNA that can be easily detected by turbidity or fluorescence so, gel electrophoresis is not required [14].

MG prevalence in different countries

MG prevalence was different in various countries, even at different regions in same country, like Bangladesh, India, Iran, Egypt, China and some other countries table 1-6 respectively.

City/State/Region	Bird's type	Samples collected	Diagnostic tests	MG prevalence or incidence (%)	Paper references
Feni Sadar thana and Chhagoalnaiya thana districts	Breeder poultry farms	382 sera samples	SPAT	58.90% overall disease but 62.44% and 53.10% in winter and summer season while 59.90% and 48.57% in female and male birds respectively. 62.80% in Feni sadar and 53.45% in Chhagoalnaiya thana districts.	[15]
Lohagara and Satkania Upazila of Chittagong district	Chickens	400	SPAT	73% in layer and 53% in broiler birds at Lohagara whereas 60% in layer and 465 in broiler chickens at Satkania Upazilla region.	[16]
Rajshahi district	Chickens	575 sera samples	SPAT	55.13% overall disease while 61.48% in winter and 47.74% in summer. 62.86% in large flocks and 52.00% in small flocks.	[17]
Rajshahi and surrounding districts	Layer chicken	605 sera samples	SPAT	71.7% in young and 50.4% in adult birds. 68.6% in large flocks and 50% in small flocks. 61.6% in winter (autumn 56.9%, 55% rainy) and 49.6% in summer	[18]
Bhola district, Bangladesh	Chicken (backyard and commercial layer	480 blood samples	SPAT	Overall 55.83% (268/480) while 62.5% in backyard chickens and 53.61% in commercial farming system. 60.63% in pullets, 55.63% in adults and 51.25% in old birds. 60.42% in winter and 51.25% in summer season.	[19]
Five upazilas (Sadar, Sariakandi, Gabtoli and Sherpur) of Bogra District	commercial layer chickens	563 blood samples	SPAT and iELISA	Overall 64.47% and 56.13% by iELISA and SPAT. 70.13% in December followed by November (68%), October (65.67%), August (63.46%), September (58.54%) and July (51.78%). 69.63% in large flocks and 56.82% in small flocks.	[20]
kotwali thana in Chittagong	Chickens	455 including live, sick and dead birds	SPAT	Overall mycoplasmosis was 15.38%. 73% in broiler and 27% in layer chickens. 49% in 8-21 days age group followed by 21% in 22-35 days age group, 21% in 0-7 days age group, 6% in 36-60 days age group and 3% in above 60 days age group. 33% in January, 30 February, 23 March, 13 December and 1% in November.	[21]

 Table 1: MG prevalence in Bangladesh.

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City/State/Region	Samples collected	Diagnostic tests	MG status (%)	Paper references
Different regions of India	1715 Choanal cleft swabs	Isolation of MG, PCR	10.38%	[22]
Namakkal (Tamil Nadu)	103 sera samples	iELISA	53.40%	[23]
Rewa region of Madhya Pradesh	98 sera samples	ELISA	21.40%	[24]
Seven states of India	1285 serum samples	ELISA	32.06% MG	[25]
Hyderabad	13,394 dead chickens	Necropsy examination	11.50%	[26]
Haryana	98	SPAT	22.40%	[27]
Haryana	92	PCR	27%	[28]
Different Districts of Tamil Nadu	1350 sera samples	SPAT ELISA	55.5% (SPAT) 42.1% (ELISA)	[29]
Telangana, Karnataka, Tamilnadu, Gujarat, Odisha Himachal Pradesh, West Bengal	309 Choanal swabs	PCR	11.6%	[30]

Table 2: MG prevalence in India.

City/State/Region	No. of Samples	Diagnostic tests	MG status (%)	Paper references
Center north of Iran	2000 serum samples	SPAT	6.25%	[31]
Chaharmahal Va Bakhtiari province	324	PCR	30.50%, 38.55%, 22.81% and 18.60% MG in Shahrekord, Borujen, Farsan and Lordegan areas, respectively	[32]
Mazandaran province, north of Iran	From 2002 to 2008 different flocks	SPAT and ELISA	Positive agreement between these two methods was 33%, depending upon age, sex, flock size and season with different positive rates and risk factors	[33]
Kerman Iran	88 isolates	Culture and nested PCR	69% by culture and 100% by nested PCR.	[34]

Table 3: MG prevalence in Iran.

City/State/Region	Diagnostic tests	MG status (%)	Paper references
Egypt	Culture and PCR	20% by culture and 33.3% by PCR	[34]
Egypt	SPAT and ELISA	69.9% layers were positive by ELISA and 58.3% by SPAT. 60% broiler farms were positive by ELISA and 48.7% by SPAT.	[35]
Egypt	Culture and PCR	40% by both	[36]
El-Sharkia governorate, Egypt	rkia norate, Egypt Culture, GIT, ELISA 23.3%, 11.6% and 8.3% samples were positive by culturing air sacs, trachea and lungs respectively. 16.5% by GIT and 48.3% by ELISA.		[37]
Egypt	PCR, conventional cultivation method and direct PCR.	70.9% by PCR, 65.45% by direct PCR and 17.66 by conventional cultivation method.	[38]

Table 4: MG prevalence in Egypt.

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Country/Province/ Region	Bird's type	Samples No.	Tests used	MG status (%)	References
Chongqing	Chickens	907 and 882 in 2012 and 2013 respectively	ELISA	62.62% AND 72.45% in 2012 and 2013 respectively. 59.08% and 68.97% in spring and 65.89 and 75.65% in winter season in 2012 and 2013 respectively. 50.28%, 68.51 and 17.5% in 2012 and 57.89%, 78.14% and 20% in 2013 in broilers, breeders and backyard farms respectively.	[39]
China (Shanghai)	Chicken	183 swab samples	Loop-mediated isothermal amplification (LAMP), PCR	20.2% by LAMP and 13.1% by PCR.	[14]
Guizhou province	Poultry birds	801 serum samples	ELISA	43.07 %	[40]

Table 5: MG in China.

Country/ State/Region	Bird's type	No. of samples	Tests used	MG %	References
Nakornpathom province, Thailand.	Chickens	30 flocks, 15 birds from one flock (450 birds)	SPAT, ELISA and PCR	18.2-40%, 16.7-40% and 18.2- 40% MG +ve samples by SPAT, ELISA and PCR at the age of 1 month, 1-2, 2-3 and 3-4 months respectively.	[41]
Northern and Central Jordan	Chickens	115 flocks	PCR for MG, RT- PCR for NDV, APV and IBV	14.8% positive flocks for MG+IBV, 5.2% for MG+NDV and 6% for MG+APV.	[42]
São Paulo, Paraná and Pernambuco states of Brazil	Chickens	1046	Multiplex PCR	72.7%	[43]
Argentina (for 3 time period in different counties)	Chicken	2411 sera samples	SPAT	32.8%, 55.1%, and 76.2% MG for 1 st , 2 nd and 3 rd study period respectively.	[44]
Algeria	Chicken	505 serum samples	SPAT	Overall MG % was 69.90%. 61.48% in winter and 47.74% in summer.	[45]
Algeria	Chicken	18 tracheal swab	Culture SPAT PCR	72.22%, 61.11% and 63.63% by culture, SPAT and PCR respectively	[46]
Few states of Peninsular Malaysia	Chickens	300	Real time RCR	Out of total, 94 samples were tested positive for MG.	[47]
Niger State, Nigeria	Chickens	552 blood and 138 swab samples	MG/MS ELISA, Culture	91.83% by MG/MS ELISA. Out of total, 126 swab samples showed fried egg appearance on mycoplasma agar plate.	[48]
Triângulo Mineiro, Brazil, State of Minas Gerais	Chicken	120 samples	SPAT HI	18% by SPAT and 0 % by HI.	[49]
Valenciana (Spain)	Broilers	7363 samples	ELISA	One region showed an average ELISA titer values of more than 500 in the study period, which indicate any previous infection of MG in broiler birds.	[50]

Table 6: MG prevalence in other countries.

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Conclusion

MG is prevalent in many countries time to time. It causes great economic losses to the poultry industry. It should be controlled at hatchery level and on other hand horizontal transmission can be controlled by good biosecurity measures. First, monitoring of whole flock should be done to know the disease condition time to time and to remove the diseased birds, otherwise all the flock should be removed to control the further spread of this disease. Serological tests are very fast to detect MG status in any flock. Timely vaccination and detection is helpful to control the disease at early stages. There is also need to educate the farmer regarding biosecurity and preventive measures to reduce the economic losses occurring due to this disease. However, more epidemiological studies should be done to check the disease status at specific regions.

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