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Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synovae* in Poultry- India Perspective

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A B S T R A C T

Mycoplasma gallisepticum and *Mycoplasma synoviae* are important poultry pathogens responsible for high economic losses to the poultry industry in term of mortality, poor weight gain and decrease of feed efficiency in broiler chickens and reduction of egg production in layer chickens and turkeys. Serum plate agglutination (SPA), hemagglutination inhibition (HI) and ELISA are the most common serological techniques used for rapid detection of infection and the adoption of control measures of the diseases. Application of molecular techniques like PCR, real time PCR is currently used for the demonstration of the DNA of the pathogen. Different studies based on detection of anti antibodies and DNA of organism show that disease is quiet prevalent in both broiler and layer in India. Both killed and live vaccines are available for vaccination against *Mycoplasma gallisepticum* or *M. synoviae* which can be a useful to control vertical transmission from breeder to chicks. Eradication of disease is possible through strict biosecuity measure at farm, early detection of new infections and prevention of vertically transmission of agents.

Introduction

Poultry mycoplasmosis is an important a respiratory diseases of chickens and turkey. Disease is of worldwide distributions and affects both the broiler grower and the layer birds. Poultry industry are facing significant economic losses occurring due to drop in egg production, decrease in egg quality, high embryonic mortality, poor hatchability, high morbidity, poor weight gain and medication costs occur in treatment (Peebles and Branton, 2012).

Etiology

Avian mycoplasmosis is mainly caused by *Mycoplasma gallisepticum* and *Mycoplasma synoviae* species of mycoplasma (OIE, 2008) and less commonly by *Mycoplasma meleagridis* and *Mycoplasma iowae*. Organism classified into the class Mollicutes

Order Mycoplasmatales and Family Mycoplasmataceae. Mycoplasmas are the smallest free living eubacteria but differ from other bacteria not having cell wall. The complete genome sequence of *Mycoplasma gallisepticum* strain reveal the presence of 996,422 bp with an overall G+C content of 31 mol% (Papazisi *et al.,* 2003).

Species affected

Presently mycoplasmosis is one of the most important economic diseases of poultry and particularly affect intensive production systems in most of countries. Of different pathogenic mycoplasma species *M. gallisepticum* is most economically significant and affects mainly gallinaceous avian species. It causes chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. *M. synoviae* is also pathogenic for both chickens and turkeys. All the age groups of turkeys and chickens are susceptible but disease is more common in upto 32 weeks old commercial layer chicken (Udhayavel *et al.,* 2016; Singh *et al.,* 2016). *Mycoplasma meleagridis* and *Mycoplasma iowae* is pathogenic primarily for turkeys only.

Transmission

Poultry mycoplasmas are transmitted through both direct and indirect contact with infected birds and fomites (Kleven, 2008). Horizontal bird-to-bird transmission occurs within flocks through close contact. Infectious aerosols generated after coughing and sneezing of infected birds carry the organism and transmitted it other birds through direct inhalation or contact with infected bird or infected fomites (NneomaOkwara, 2016).

Contaminate feed and water can also transmit the disease. Infected breeders can also transmit the mycoplasma vertically to chicks through *in ovo* resulting hatched chickens carry the mycoplasma infection (Peebles and Branton, 2012).

Risk factors

Immune status of birds, litter conditions, stocking density, climate, type of drinker and feeder affects the rate of spread within the flock. Infected fomites and personal carry the organism play important role in between flock transmission (Nneoma Okwara, 2016). Co-infection of *M. gallisepticum* and low pathogenic avian influenza virus (H3N8) in chickens has been reported (Stipkovits *et al.,* 2012) which suggested that LPAI may predispose the birds to *M. gallisepticum* infection and vice versa (Sid *et al.,* 2016). Presence of concurrent infection with ranikhet disease virus, infectious bronchitis virus, colibacillosis or other pathogens make disease more severe (Matilda *et al.,* 2018; Nneoma Okwara, 2016).

Indian scenario

Recent reports by various workers showed that *M. gallisepticum* and *M. synoviae* is quite prevalent in different states and geographical location of India and its prevalence varies from 10% to 55% and 2% to 52% respectively (Table 1 and 2). Seasonal wise study showed that incidence of CRD was observed highest during summer followed by winter and rainy season (Rajkumar *et al.,* 2017).

Prevalence in different countries

Systematic review of some recent publication showed that *M. gallisepticum* in poultry is highly prevalent in developing countries *i.e.* 43.50% layers and 63.5% broiler in Malaysia (Ching *et al.,* 2016), 45.1% in Bangladesh (Hossain *et al.,* 2010), 0.9% Layers and 2.7% broilers in Belgium (Michiels *et al.,* 2016), 53.40% in Pakistan (Hussain *et al.,* 2018) and 29.5% in Accra, Ghana (Matilda *et al.,* 2018).

Table.1 Prevalence of *M. gallisepticum* in poultry in India

State	Samples Size	Test	Prevalence/Incidence	References
Some Districts of Tamil Nadu	1350 Sera sample	ELISA and SPA	55.5% in SPA and 42.1% in ELISA	Vadivalagan *et al.*, 2018
Namakkal region of Tamil Nadu	103 layer sera samples	Indirect ELISA	53.40%	Udhayavel *et al.*, 2016
Different geographical regions in the country	1827 serum samples	ELISA	43.95%	Reddy, 2014
Different geographical regions in the country	1715 Choanal cleft swabs samples	Isolation and PCR	10.38%	Reddy, 2014
7 States (Telangana, Karnataka, Tamilnadu, Gujarat, Himachal Pradesh, West Bengal and Odisha)	309 Choanal swabs	PCR	11.65%	Rajkumar *et al.*, 2018
5 States (Telangana, Karnataka, Gujarat, Himachal Pradesh, West Bengal)	635 serum samples	ELISA	32.6%	Rajkumar *et al.*, 2018
Different States (n = 7) of India	1, 285 sera samples	ELISA	32.06%	Baksi *et al.*, 2016
Haryana	92 tissue samples	PCR	27%	Tomar *et al.*, 2017
Haryana	98 serum samples	RPA	22.44%	Tomar *et al.*, 2017
Haryana (18 different hatcheries)	284 serum samples from	RPA	28.87%	Tomar *et al.*, 2017
Hyderabad (Organized poultry farm)	13,394 dead birds	Post mortem examination	11.50% CRD	Rajkumar *et al.*, 2017
Rewa (Madhya Pradesh)	98 serum sample	ELISA	21.40%	Singh *et al.*, 2016

Table.2 Prevalence of *M. synovae* in poultry in India

State	Samples Size	Test	Prevalence/Incidence	References
7 States (Telangana, Karnataka, Tamilnadu, Gujarat, Himachal Pradesh, West Bengal and Odisha)	309 Choanal swabs	PCR	33.0%	Rajkumar *et al.,* 2018
5 States (Telangana, Karnataka, Gujrat, Himachal Pradesh, West Bengal)	635 serum samples	ELISA	52.1 %	Rajkumar *et al.,* 2018
Different states (n = 7) of India	1354	ELISA	41.1%	Baksi *et al.,* 2016
Haryana	92 tissue samples	PCR	2.1%	Tomar *et al.,* 2017
Haryana	98 serum samples	RPA	18.36%	Tomar *et al.,* 2017
Haryana (18 different hatcheries)	284 serum samples	RPA	10.56%	Tomar *et al.,* 2017
Tamilnadu	116 samples	PCR	36.2%	Senthilnathan *et al.,* 2015

Clinical signs

***M. gallisepticum* infection**

M. gallisepticum infection is characterized by respiratory manifestation. The incubation period of chronic respiratory disease varies ranging between 6-21 days. Infected chickens show sign of coughing, sneezing, rales, ocular and nasal discharges. There is decrease in feed consumption, decrease in egg production, increased mortality and poor hatchability. In broilers most outbreaks occur between 3rd and 6th weeks of age that lead to poor feed conversion, sharp decline in weight gain and low carcass quality. In turkeys infection is characterised by swelling of the infra orbital sinus, conjunctivitis and frothy exudates (Peebles and Branton, 2012).

***M. synoviae* infection**

M. synoviae infection are mostly subclinical in nature and is characterised by milder respiratory distress, lameness, pale comb, swollen hock and foot pad. Infections mainly involve synovial membranes of joints and tendon sheaths resulting exudative synovitis, tendovaginitis, or bursitis (Ferguson and Noormohammadi, 2013). In some cases associated with egg shell apex abnormalities, shell thinning with cracks and breaks (Feberwee *et al.,* 2009).

Diagnosis

Isolation and Identification of *Mycoplasma* spp

M. gallisepticum and *M. synoviae* are relatively fastidious micro-organisms that require special nutritional requirement (Kleven, 2008). Frey medium (Frey *et al.,* 1968), SP-4 medium (Bradbury, 1998), PPLO medium (Kleven, 2008) are some of the commercial available liquid and agar media used for *M. gallisepticum* / *M. synoviae*

isolation. *M. synoviae* requires extra addition of Nicotinamide adenine dinucleotide (NAD) in the media (Kleven, 2008). Due to lack of cell wall organism is not susceptible to antibiotic acting through cell wall inhibitions. Penicillin (2,000 IU/ml) and thallium acetate (up to 1:2.000) are added to the growth medium to control other bacterial and fungal contamination. Optimal growth is usually seen at 37°C with 5% CO2. Colonies are usually seen after 3–5 days post incubation and in solid media organism produce characteristic nipple shaped or fried egg appears colony. Both *M. gallisepticum* and *M. synoviae* ferment glucose and form acid which can be detected by the phenol red indicator (OIE, 2008).

Serological tests

Serological test like serum plate agglutination (SPA), hemagglutination inhibition
(HI) and enzyme-linked immunosorbent assays (ELISA) are the mostly used for the diagnosis and to study the sero epidemiology of disease (OIE, 2008).

Hemagglutination inhibition

Test is based on inhibition of haemagglutinating capability of *M. gallisepticum* and *M. synoviae* to avian red blood cells by specific antibodies in sera. HI has high specificity but the disadvantages are low sensitivity (Kleven, 2008).

Serum plate agglutination test

It is a simple, quick, and inexpensive test for the screening of *M. gallisepticum* and *M. synoviae* antibodies in serum. Positive sample agglutinate the coloured antigen after mixing equal amounts of tested serum sample and stained *M. gallisepticum* or *M. synoviae* antigen. The test has efficient sensitivity but of low specificity (Vadivalagan *et al.*, 2016).

ELISA

Enzyme-linked immunosorbent assays (ELISA) is sensitive and specific test for the detection of specific antibodies in serum (Ewing *et al.*, 1996)**.** Many commercial ELISA kits are available for the detection of *M. gallisepticum* and *M. synoviae*.

Molecular techniques

Accuracy, time saving, and cost effectively of molecular techniques made them to complementary or even alternative for conventional diagnostic methods. PCR assays proved to be sensitive and specific for *M. gallisepticum* detection. Various PCR assay are described by different worker and used for diagnosis and screening of samples (Rajkumar *et al.*, 2018; Reddy, 2014).

Several PCR primers targeting different genes of *M. gallisepticum* were described previously (Collett *et al.*, 2005). Besides selection of the mgc2 gene sequence has been used successfully for the differentiation of *M. gallisepticum* strains, including field and vaccine strains (Ferguson *et al.*, 2005). Real time PCR is also found a rapid and sensitive test to identify M. *gallisepticum*-infected flocks (Carli and Eyigor, 2003).

Prevention and control

Vaccines

Vaccination is an effective mean for controlling *M. gallisepticum* or *M. synoviae* in breeder and layer farm and it add to biosecurity measures of farm by enhancing the immunity of birds. Both killed and live vaccines are currently in commercial use. The commercial *M. gallisepticum* vaccine is used mainly in breeding flocks but also increasingly in laying flocks.

Killed vaccines

M. gallisepticum killed vaccines (bacterins) protect young birds from infection with virulent *M. gallisepticum* and commercial egg layers from *M. gallisepticum*-induced drops in egg production (Jacob, *et al.,* 2014). Vaccines have been shown to reduce but not eliminate colonization by *M. gallisepticum* following infection (OIE, 2008).

Live vaccines

Three types of live vaccine stain (F strain vaccine, 6/85 strain vaccine**,** and ts-11 vaccine) are available for commercial use in poultry for vaccination (Nicholas *et al.,* 2009). Both 6/85 and ts-11vaccines have greater safety and low potential for transmission to unvaccinated flocks in comparison to F strain (Abd-El-Motelib, and Kleven, 1993).

Even though these vaccines are generally safe, F strain may have the potential for infecting unvaccinated flocks transmissible and pathogenic to turkey (Evans, *et al.,* 2005).

Vaccination against *M. synoviae*

In comparison to *M. gallisepticum* vaccination live vaccination against *M. synoviae* is not frequent in practice and the only *M. snyoviae*-H vaccine is commercially available vaccine (Nicholas *et al.,* 2009).

Approved vaccine for poultry by Central Drugs Standard Control Organization, India

M. synoviae Vaccine, Live (strain MS-H)
M. gallisepticum Bacterin Vaccine, Inactivated, Oil Emulsion
M. gallisepticum Vaccine, Live
M. gallisepticum Vaccine, Live (strain ts-11).

Biosecurity

Biosecurity is important mean to prevent the entry and spread of disease in flock**.** It includes the acquisition of birds free from *M. gallisepticum* and *M. synoviae* antibodies and constant monitoring of breeder flocks. Proper hygiene in farm also play important role in control of avian mycoplasmosis (Racicot *et al.,* 2011). *M. gallisepticum* infection mainly is transmitted vertically through ovaries hence the preferred method for control is to maintain disease free flocks.

Use of antimicrobials in breeders and eggs

Mycoplasma is susceptible to antibiotics such as macrolides and lincosamides (tylosin), tiamulin, and fluoroquinolones. Use of antibiotics like tylosin and tiamulin in dipping solution of hatcheries egg have effective role in prevention of vertical transmission of mycoplasma.

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