

Original Research Article

Seroprevalence of *Mycoplasma gallisepticum* in Broiler Chicken by ELISA

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ABSTRACT

Background: This study aimed to determine the sero-prevalence of *Mycoplasma gallisepticum* (MG) which causes chronic respiratory disease in poultry. It causes huge economic loss to the poultry industry. **Materials and Methods:** A total of 464 sera samples were collected and tested ELISA test using commercially *Mycoplasma gallisepticum* ELISA kit (Idexx, USA) to detect the presence of antibodies against MG. **Results:** A total of 464 commercial broiler sera samples from 5 different geographical regions were subjected to ELISA. From 464 samples tested, 105 samples were positive in ELISA. Our results indicated that the prevalence of MG in broiler farms was 22.62 % during study period. **Conclusion:** It can be concluded that MG in broiler breeder farms has potency of great economical losses so detailed study prevalence of MG should be carried out for control and prevention of disease.

INTRODUCTION

Indian poultry industry has been achieved remarkable progress during last recent years with playing a major role in poverty alleviation and income generation. Despite of rapid growth in poultry industry it is still prone to infectious diseases and in the recent years poultry farming has been hampered by the outbreak of fatal infectious diseases among which avian mycoplasmosis mentioned as potential constraint to health status and productivity of chicken.

Mycoplasmosis is one of the major emerging avian diseases in poultry industry caused due to *Mycoplasma gallisepticum* and *Mycoplasma synoviae* organisms. *Mycoplasma synoviae* causes infectious synovitis or mild upper respiratory disease whereas *Mycoplasma gallisepticum* can

cause chronic respiratory disease (CRD) in chickens and have been reported to cause serious economic losses. [1]

The losses caused by *Mycoplasma* infections are due to increase in embryo and early chick mortality (upto10-20%), poor feed conversion ratio and reduction in weight gain and egg production (up to 10-20%) together with a high medication cost. [2]

Control programs for Mycoplasmosis are based on maintaining commercial broiler and breeding stock free of mycoplasma infection. Prevention of infection and precise diagnosis of poultry mycoplasmosis is an important element in planning control strategies. Diagnosis of mycoplasmosis is currently done by microbial culture, serological tests and molecular methods. [3]

But reliable and rapid diagnosis is needed to prevent dissemination of infection for that detecting the subclinical infection in the flock serology is the best tool. [4] Flock testing and culling is the best control measure for MG infection. [5] Thus, the present study has been undertaken to know the seroprevalence of Mycoplasmosis in commercial broiler chicken in different geographical areas of Maharashtra state.

MATERIALS AND METHODS

The investigation has been carried out to know the sero-prevalence of MG in commercial broiler in different geographical regions of Maharashtra, India during period of March 2017 to January 2018.

This study was conducted in the Department of Veterinary Microbiology, Bombay Veterinary College Mumbai. A total of 464 serum samples were collected from 2 to 6 weeks old, birds which had not been previously vaccinated against *Mycoplasma gallisepticum*.

Blood samples were aseptically collected from the wing veins using 5ml sterile disposable syringes and needles. Blood was allowed to clot in the syringe and was kept for about 1 hour at room temperature. After this, serum of each sample was separated, centrifuged, and transferred to sterile microtubes kept at 4°C until use.

Enzyme-Linked Immunosorbent Assay (ELISA)

Sera analyzed for antibodies against MG using a commercially available ELISA antibody test kit (*Mycoplasma gallisepticum* antibody Test Kit—Idexx Laboratories, Inc., Maine, USA) according to the manufacturer's instructions.

Briefly, samples were diluted five-hundredfold (1:500) with the diluent, and 0.1ml of each sample was dispensed in a well of a plate previously coated with MG antigen. Plates were incubated for about 30 minutes at room temperature. [Fig.1&2]

After that, plates were washed with deionized water, and 0.1ml of the conjugate was placed in each well (Goat antichickens: horseradish peroxidases conjugate HRPO). Plates were incubated for about 30 minutes and washed again. Finally, 0.1ml of the substrate solution

(tetramethylbenzidine or TMB) was dispensed into each well and incubated for 15 minutes at room temperature.

The reaction was blocked with 0.1ml of stop solution. Absorbance was measured at 650nm. Results were expressed as serum-to-positive ratios (S/P ratios) relative to a standard positive control. Serum samples, with S/P ratios greater than 0.5 (titers greater than 1,076) were considered as positive.

RESULTS AND DISCUSSION

The seroseroprevalence of MG antibodies in commercial broiler farms of different geographical regions of Maharashtra was studied by ELISA. A total of 464 sera samples were collected from commercial broiler farms and antibodies were detected with commercially provided ELISA kit. Out of these 464 sera samples, 105 showed S/P ratio higher than 0.50 which was considered as positive. [Table 1]

Thus overall seroprevalence recorded was 22.62% of MG in different geographical areas of Maharashtra state ranging from 12.69% to 26.08%. Comparable seroprevalence was noted by Singh et al. [6] who investigated a total of 98 sera samples of different age groups and observed 21.40% overall seroprevalence. Similar observation was also recorded by Luciano et al. [7] who investigated 712 samples by ELISA and observed overall seroseroprevalence of 21.06%.

Messa Junior et al. [8] screened a total 459 backyard chicken serum samples and observed overall MG seroprevalence of 48.8% ranging from 28.5% to 72.4% in four different villages in Southern Mozambique. They investigated the status of MG antibody by ELISA on broiler breed stock as highest (74.60%) in Lahore district of Pakistan. Feizi et al. [9] recorded 33.3% in Northwest of Iran. Previous survey from India, France, Italy, Egypt and Jordan reported varying seroprevalence of 53.40%, 84%, 31%, 60% and 73.5% of MG by ELISA respectively in chicken (Udhayavel et al. [10]; Mary et al. [11]; Osman et al. [12]; Saad and Dirgham [13]).

The variation in seroprevalence of mycoplasmosis in poultry birds might be due to the replacement of breeding stock with the progeny of the same flock,

seasonal influence, poor ventilation, contamination of litters and no restriction on them movement of the technical personnel, visitors and such other persons as well as other bio-security measures. [14]

CONCLUSION

Mycoplasmosis is prevalent among chickens in different geographical regions of Maharashtra state and can cause severe economic losses. The efforts need to be made regarding awareness of the poultry farmers for the effective control of mycoplasmosis in breeder farm, broiler farm and the hatcheries. Good management practices, regular screening and culling of positive parent stock and adoption of appropriate prophylactic or therapeutic remedies can help in controlling the infection in poultry. A detailed study regarding seroprevalence of mycoplasmosis must be carried out to establish the current status of this disease in Maharashtra state.

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Table 1: Collection of samples from different geographical regions

| Geographical Region | Total sample tested | MG ELISA Result | |
|----------------------|---------------------|-----------------|--------|
| | | No. | % |
| Western Maharashtra | 115 | 30 | 26.08% |
| Kokan | 105 | 27 | 25.71% |
| Northern Maharashtra | 99 | 26 | 26.26% |
| Marathwada | 82 | 14 | 17.07% |
| Vidharbha | 63 | 8 | 12.69% |
| Total | 464 | 105 | 22.62% |



Fig.1. Showing ELISA kit.



Fig.2. Showing ELISA plate

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