STATUS OF IGG ANTIBODIES AGAINST MYCOPLASMA GALLISEPTICUM IN NON-VACCINATED COMMERCIAL POULTRY BREEDER FLOCKS

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ABSTRACT

The age wise status of *Mycoplasma gallisepticum* (MG) infection of broiler breeding stock (MG nonvaccinated) in and around Lahore district was determined during the period of December, 2006 to November 2007. In this study, a total of 2777 serum samples were referred from different areas surrounding Lahore. History revealed that all the samples were from MG non vaccinated flocks and subjected to Indirect Enzyme Linked Immunosorbent assay (i-ELISA) to detect the status of IgG antibodies produced due to MG field exposure. The highest positive percentage(74.60%) of MG infection was found in breeding stock aging from 6 to 23 weeks while lowest (33.17%) in flocks of 60 to 76 th weeks of age. The result further revealed that the younger birds are more prone to MG infection compared to aged ones and presence of MG antibodies may not associate with clinical signs except in complicated cases.

Key words: *Mycoplasma gallisepticum,* breeding stock, enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Chornic Respiratory Disease (CRD) *is* one of the major constraints among other avian diseases in fastly growing poultry industry of Pakistan. It is caused by an important pathogen of poultry*, Mycoplasma gallisepticum*. Primarily, this is a disease of chicken and turkeys but also infects many other domestic and wild birds all over the world (Jordan and Amin, 1980; Bradbury *et al.,* 1993). All ages of chickens and turkeys are susceptible to this disease but young birds are more prone to infection than adults (Nunoya *et al.,* 1995).

The organisms mostly colonize in respiratory tract especially tracheal epithelium and air sacs. It is transmitted vertically via egg yolk through infected breeder flock and horizontally by close contact. The disease is characterized by respiratory signs including conjunctivitis with frothy ocular exudates, sneezing, coughing, nasal discharge, breathing through partially opened beak and closing of eye lids, etc. The organism is recognized as distinct pathogenic strain among the pleuropneumonia like organisms (PPLO) of avian origin.

Due to the infection, poultry business does not remain feasible in terms of high rate of morbidity, low hatchability, reduced growth rate, poor feed conversion, decline in egg production and ban on the export of *M. gallisepticum* infected eggs and chicks to other countries. Economic losses in the poultry industry caused by this infection are significant. Production losses between 10 and 20% have been reported in layers (Bradbury, 2001). In USA, 15.7 fewer eggs are reported in MG infected breeder than a healthy one, contributing to a loss of 127 million eggs in 1984, which corresponded to an annual loss of 125 million dollars (Mohammed *et al*., 1987). Current measures to combat MG infection have included flock testing and eradication programs and in some part of the world by vaccinating multiple age laying units with low virulent F-strain of MG (Carpenter *et al* 1981, Kleven *et al* 1984)

Limited data are available about the prevalence of MG infection in breeding stock in Pakistan. In routine detection of MG infection may be achieved by detecting antibodies against MG in the host. For this purpose various tests including serum plate agglutination (SPA), haemagglutination-inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) are used (Mohammed *et al*., 1986; Parker *et al*., 2002; Kang *et al*., 2002). In Pakistan mainly SPA is used for screening MG infected flocks which detects only the early stage of infection (*IgM*) .Due to this routinely used test, the antibodies status in the late infection is still to be monitored in Pakistan.

Keeping in view the economic importance of the infection the study is designed to determine the status of MG infection in non vaccinated breeding stock in different parts of Lahore division, Pakistan.

MATERIALS AND METHODS

The age wise status of *IgG* antibodies against MG was determined through Indirect ELISA in and around Lahore district during a period of one year from December 2006-November 2007. A total of 2777 clotted blood samples of 128 flocks were received from over 6 weeks of age, in sterile disposable syringe. The total population of these flocks was 804060 and all the birds were MG non-vaccinated. The sera were separated in labeled sterile vials and processed in University

Diagnostic Laboratory (UDL), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. The samples were stored at -20°C till further use and divided into four groups (A-6 to 23 weeks,B-24 to 41 weeks ,C-42 to 59 weeks and D- 60 to 76 weeks).Each group was further subdivided into three sub groups on the basis of age of the birds (Table-1).

ELISA TEST: The ProFLOK. MG ELISA kit (Synbiotics Corporation, USA) was used in this study according to the manufacturer's instructions. Briefly, 50ul (1:50 diluted in dilution buffer) of unknown serum samples, normal and positive control were added to MG antigen coated plate along with 50ul dilution buffer followed by incubation for 30 minutes at room temperature. After three times washing with washing solution (1:20 diluted in distilled water), 100ul of Goat anti-chicken Ig G peroxidase conjugate (1:100 diluted in dilution buffer) is added to the respective wells and incubated at room temperature for 30 minutes. Upon repeating the washing procedure again, 100ul of substrate solution was dispensed into each well. After incubation for 15 minutes at room temperature and addition of 100ul (1:5 diluted in distilled water) into each well, the absorbance was read at 405 nm on a Thermo micro plate reader. In order for result to be valid, the mean negative control absorbance should be below 0.200 and the corrected positive control value range should be between 0.250 and 0.900. The serum/positive ratio (SP) was calculated as: (mean of test sample–mean of negative control) / (mean of positive control–mean of negative control). An MG titer was calculated by the following suggested equation: Log 10 titer= (1.464 X Log 10 SP) +3.197. The SP value less than 0.200 is taken as negative while SP value 0.200 to 0.599 is considered as probable or not. Conclusively SP greater or equal to 0.600 is considered positive for MG infection.

RESULTS AND DISCUSSION

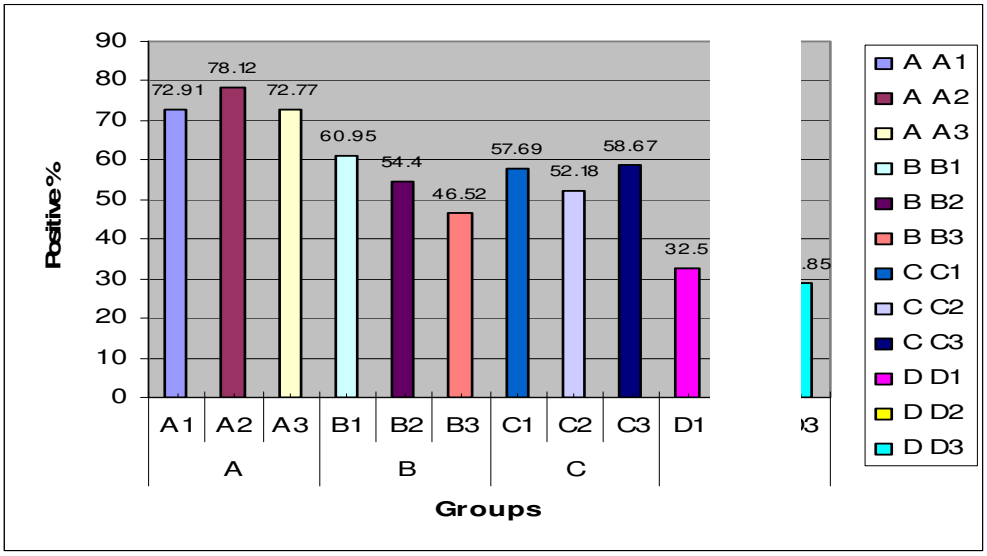
The highest sero conversion against *MG* was seen in group A (74.60%) aging from 6 to 23 weeks while lowest (33.17%) in birds of 60 to 76 weeks of age (group D). However, within these sub groups, highest percentage of positive samples was seen in A2 (12-17 weeks) and lowest in D3 (72-76 weeks). (Table: 1).These findings indicate the decreasing pattern of MG infection with the increase of age in all flock (from 74.60 to 33.17%) fig (I). These results are in line with the observation of Sarkar (2004), as younger birds are more prone to *MG* infection than adult (Nunoya *et al*.,1995) but in this study, group C (42-59 weeks) showed a slight deviating pattern. It might be due to the seasonal influence as during the winter, the birds were younger than in summer season (Nunoya *et al*., 19953, David *et al*., 1997). The study further revealed that in winter season birds are more susceptible to infection as compared to summer. As for as the routinely used SPA test is concerned, the ELISA is considered expensive and is suitable to detect IgG produced in later stages of infection while SPA is restricted to early stages of infection to detect *IgM* antibodies, so ELISA test should be used to detect *MG* antibodies to avoid the chances of false positive results and to determine the status of infection in latter stages of infection. The data collected indicated that presence of MG antibodies may or may not associate with clinical signs which are in agreement with the work of Stipkovits and Kempf, (1996) as birds may show no clinical signs in uncomplicated cases. The emphasis should be focused on the seroprevalence of *MG* throughout the country and efforts be made on the isolation and characterization of local isolates and design vaccination schedule of locally prepared vaccine along with good bio-security measures to safeguard the economic losses to poultry industry in Pakistan.

Table-1: Anti-*MG*-ELISA antibody (IgG) Status of Poultry breeding Stocks at Various ages.

Groups	subgroups	Age (wks)	Population	No of Flocks tested	No of birds tested	ELISA SP Positive % age	Groupwise Positive % age
	A1	6-11	68000	10	288	72.91	
A	A2	12-17	55000	08	192	78.12	74.60
	A3	18-23	77100	12	360	72.77	
	B1	24-29	39000	07	210	60.95	
B	B2	30-35	44060	11	244	54.40	53.95
	B3	36-41	51500	09	144	46.52	
	C1	42-47	107000	13	208	57.69	
C	C2	48-53	82700	16	320	52.18	56.18
	C3	54-59	58000	12	196	58.67	
	D1	60-65	62300	14	280	32.50	
D	D2	66-71	71900	10	186	38.17	33.17
	D3	72-76	87500	06	149	28.85	

Fig: (i) **AGE WISE STATUS OF MG**

- A1 06 – 11 weeks
- A2 12 – 17 weeks
- A3 18 – 23 weeks
- B1 24 – 29 weeks
- B2 30 – 35 weeks
- B3 36 – 41 weeks
- C1 42 – 47 weeks
- C2 48 – 53 weeks
- C3 54 – 59 weeks
- D1 60 – 65 weeks
- D2 66 – 71 weeks
- D3 72 – 76 weeks



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