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SEROPREVALENCE OF *MYCOPLASMA GALLISEPTICUM* INFECTION IN CHICKEN IN THE GREATER RAJSHAHI DISTRICT OF BANGLADESH

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ABSTRACT

A serological investigation of *Mycoplasma gallisepticum* (MG) infection in chicken was conducted in the greater Rajshahi district of Bangladesh on 115 flocks during the period from July 2006 to June 2007. A total of 575 sera samples were collected and tested by serum plate agglutination (SPA) test using *Mycoplasma gallisepticum* antigen (Nobilis®MG, Intervet International B.V. Boxmeer-Holland) to determine specific antibodies in different flocks. The overall seroprevalence of MG infection in different flocks was recorded as 55.13%. Seroprevalence of MG infection was found significantly ($p<0.05$) higher during winter season (61.48%) than in summer (47.74%). Again this was recorded in different age groups, with significantly ($p<0.01$) higher occurrence in young (72.72%) compared to adult (44.00%). On the other hand, the seroprevalence of MG infection was found little ($p>0.05$) higher in large flocks (62.86%) in comparison to small flocks (52.00%). It has been found that MG infection is still an important disease problem in chickens in Bangladesh.

Key words: Seroprevalence, *Mycoplasma gallisepticum*, chicken, serum plate agglutination (SPA) test

INTRODUCTION

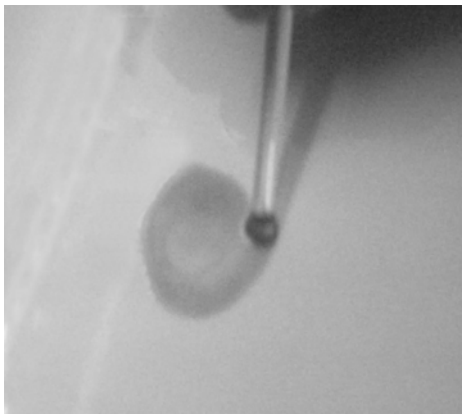
Mycoplasmosis is an important disease problem in chickens caused by four commonly recognized pathogens namely *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae* (Bradbury, 2001). *Mycoplasma gallisepticum* (MG) infection is a chronic respiratory disease (CRD) in avian species (Ley, 2003). MG infection causes decreased egg production in chickens, turkeys and other avian species. In addition to the overt disease, the infection causes decreased feed efficiency, poor carcass quality and sub-optimal egg production in layers (Carpenter *et al*., 1981; Ley and Yoder, 1997; Luginbuhl *el al*., 1976). The infection appears to be world wide in distribution (Ley and Yoder, 1997). MG infections are transmitted both horizontally and vertically and it remains in the flock constantly as a subclinical form (Bencina *et al*., 1988). MG can be diagnosed by studying their different properties such as morphological, cultural characteristics, biochemical and serological properties of the causal agent (Ley and Yoder, 1997). Among serological tests the serum plate agglutination (SPA) test could be used as a tool for quick detection of MG infection. The control of avian mycoplasmosis by vaccination is limited since only few vaccines are available. Total eradication through test and slaughter is the most effective control method (Yoder, 1991). But in practical this is expensive but also the emergence of multiage complexes in the commercial layer industry makes this approach impractical (Evans and Hafez, 1992; Levisohn and Kleven, 2000). Due to economic importance diagnosis and prophylaxis of avian mycoplasmosis have received attention. Reports on seroprevalence of mycoplasmosis in chickens are very much limited in the greater Rajshahi district of Bangladesh. Therefore, the aim of the present study was to determine the seroprevalence of mycoplasmosis in chickens to undertake an effective control measure.

MATERIALS AND METHODS

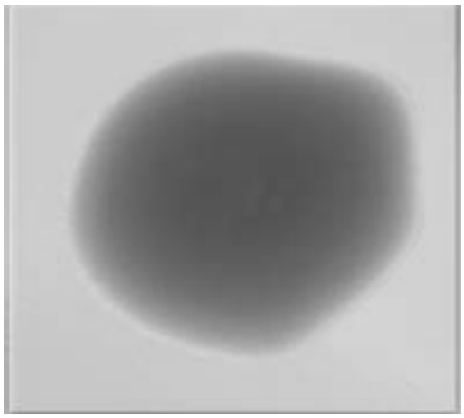
This study was conducted jointly in the "Lab Avian" laboratory, Rajshahi and Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Bangladesh during the period from July 2006 to June 2007. A total of one hundred and fifteen layer chicken (18-71 week age) flocks were selected in different upazilas of the greater Rajshahi district.

From each flock five chickens (a total of 575 adult chickens) were randomly selected for blood collection. 2 ml of blood was collected aseptically from wing vein of each bird and then sera were separated and stored at -21°C until use for serum plate agglutination test. The MG antigen (Nobilis® MG) used here in this study was purchased from the Intervet International B.V. Boxmeer-Holland (the antigen was a suspension of killed and stained S-6 Adler strain of *Mycoplasma gallisepticum* organism). The SPA test was conducted according to the instructions of antigen manufacturer (Intervet International B. V. Boxmeer-Holland). One drop (0.05 ml) of test serum was mixed with one drop (0.05 ml) of antigen on a clean glass plate at room temperature (25^0C). The glass plate was illuminated from below as to facilitate observing the reaction, avoiding excessive heat from the light source. Positive reaction was characterized by the formation of definite clumps within 2 minutes after mixing the test sera with the antigen (Fig. 1). The clumps usually started and concentrated at the periphery of the mixture. Negative reaction was indicated by absence of agglutination reaction (Fig. 1). Care was taken so that the natural granulation of the antigen (due to the presence of whole cells) was not taken as a positive reaction. The strength of the agglutination reaction was measured according to the following scheme:

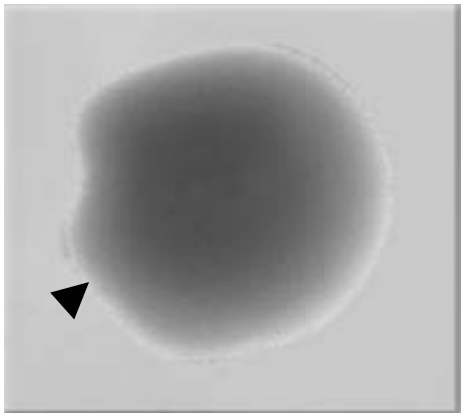
– = No clumps, no background clearing
\+ = Small clumps, no background clearing
++ = Medium sized clumps, almost complete background clearing
+++ = Large clumps, almost complete background clearing
++++ = Very large clumps, mostly in the periphery, complete background clearing



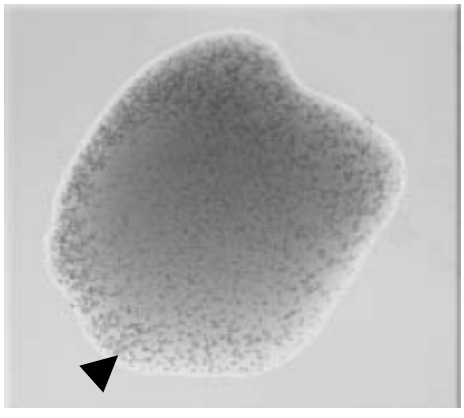
Mixing of antigen with test serum



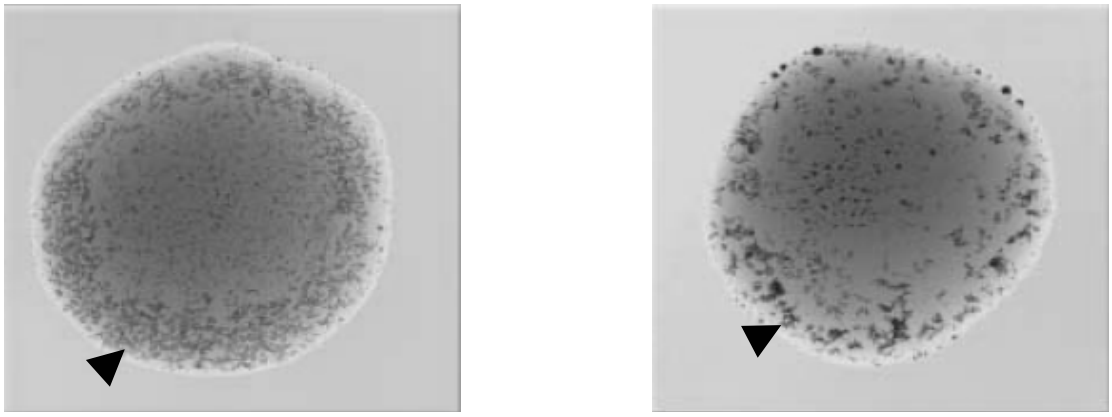
No clumps, no background clearing (−)



Small clumps, no background clearing (+)



Medium sized clumps, almost complete background clearing (++)



Large clumps, almost complete background clearing (+++)

Very large clumps, mostly in the periphery, complete background clearing (++++)

Fig. 1. Serum plate agglutination (SPA) test. Arrow indicates the agglutinated particles

Statistical analysis

The seroprevalence of MG infection with season of year, age of birds and flock size were compared by means of the Chi-square test. A significant level of 5% was used.

RESULTS AND DISCUSSION

Three hundred and seventeen (55.13%) chickens out of 575 were seropositive to MG infection (Table 1) in the present study. Similar reports were demonstrated by Sikder *et al.* (2005) who reported 56.86% seropositive layer chickens for MG infection in Patuakhali district of Bangladesh and Sarkar *et al.* (2005) who reported 58.90% seropositive layer chickens for MG infection in some model breeder poultry farms in Feni district of Bangladesh. This finding also is in agreement with previous reports of Bencina *et al.* (1987), Godoy *et al.* (2001), Pradhan (2002), Biswas *et al.* (2003), Zhang *et al.* (2001), Dulali (2003) and Abdu *et al.* (1983) who reported 56.54%, 59.10%, 57.15%, 54.90%, 53%, 52% and 47.54% seroprevalence of MG infection in chickens respectively. The figure of MG infection in the present study was higher than Biswas *et al.* (1992) and Amin *et al.* (1992) who recorded 13-32% seroprevalence of MG infection in some selected poultry farms in the southern part of Bangladesh. The higher prevalence might be due to the replacement of breeding stock with the progeny of the same flock. However, intensive nature of poultry farming provided opportunity for recycling of the pathogens due to population density (Pradhan, 2002). The other factors that contribute MG infection are poor ventilation, contamination of litters and no restriction on the movement of the technical personnel, visitors and such other persons as well as other bio-security measures (Dulali, 2003).

Table 1. Seroprevalence of *Mycoplasma gallisepticum* in chickens in relation to seasons

Seasons	No. of samples tested	Seropositive No. (%)	Total seropositive No. (%)		Seronegative No. (%)	Total seronegative No. (%)		χ^2
			Summer	Winter		Summer	Winter	
July-September 2006	130	62 (47.69)	127 (47.74)	190 (61.49)	68 (52.30)	139 (52.26)	119 (38.51)	10.75*
October-December 2006	154	93 (60.38)			61 (39.61)			
January-March 2007	155	97 (62.58)			58 (37.41)			
April-June 2007	136	65 (47.79)			71 (52.20)			
Total	**575**	**317 (55.13)**			**258 (44.87)**			

*Indicates Significant at $p < 0.05$; Summer: April-September; Winter: October-March.

Among 317 seropositive chickens, 190 were found during winter (October to March) and 127 were found during summer (April to September) season (Table 1). The seropositivity in chickens was 61.49% in winter compared to 47.74% in summer which supports the finding of David *et al.* (1997) and Pradhan *et al.* (2000). Similar report was demonstrated by Sarkar *et al.* (2005) who reported 62.44% prevalence in winter in comparison to 53.10% in summer. This finding also supports the report of Sikder *et al.* (2005) who recorded highest prevalence of MG infection in winter (61.45%) than in rainy (57.47%) season. This seasonal variation in prevalence of MG infection might be due to the influence of cold weather.

According to the age, the highest prevalence of MG infection was 72.72% in 18-25 weeks age group whereas lowest prevalence was 44.00% in 66 weeks and above age group (Table 2). Similar report was demonstrated by Sikder *et al.* (2005) who reported highest MG infection (71.42%) at 18 weeks of age and lowest (55.17%) at 63 weeks of age. This finding also supports the report of Sarkar *et al.* (2005) who recorded 73.80% MG infection at 20 weeks of age in comparison to 45.16% at 55 weeks of age. Similar report was demonstrated by Talha (2003) who reported the prevalence of MG infection significantly decreased with the increase of age. Highest infection in the young chickens is due to the vertical transmission of the organisms.

Table 2. Seroprevalence of *Mycoplasma gallisepticum* in chickens in relation to age

Age of chickens (Week)	No. of flocks	Seropositive (%)	Seronegative (%)	Total tested sample	χ^2
18-25	11	40 (72.72)	15 (27.27)	55	22.36**
26-33	13	45 (69.23)	20 (30.76)	65	
34-41	14	43 (61.43)	27 (38.57)	70	
42-49	15	42 (56.00)	33 (44.00)	75	
50-57	19	50 (52.63)	45 (47.36)	95	
58-65	18	42 (46.67)	48 (53.33)	90	
66-above	25	55 (44.00)	70 (56.00)	125	
Total	**115**	**317 (55.13)**	**258 (44.87)**	**575**	

** Indicates Significant at $p < 0.01$.

Table 3. Seroprevalence of *Mycoplasma gallisepticum* in chickens in relation to flock size

Flock size (No. of chickens /flock)	No. of flocks	Seropositive (%)	Seronegative (%)	Total tested sample	χ^2
500-1000	15	39 (52.00)	36 (48.00)	75	2.16
1001-1500	14	37 (52.85)	33 (47.14)	70	
1501-2000	13	34 (52.30)	31 (47.69)	65	
2001-2500	12	32 (53.33)	28 (46.66)	60	
2501-3000	12	33 (55.00)	27 (45.00)	60	
3001-3500	13	36 (55.38)	29 (44.61)	65	
3501-4000	11	31 (56.36)	24 (43.63)	55	
4001-4500	10	29 (58.00)	21 (42.00)	50	
4501-5000	8	24 (60.00)	16 (40.00)	40	
5001-above	7	22 (62.86)	13 (37.14)	35	
Total	**115**	**317 (55.13)**	**258 (44.87)**	**575**	

Serological investigation in the greater Rajshahi district showed the highest infection rate (62.86%) in large scale flocks (≥5001 birds) in comparison (52.00%) to small (500-1000 birds) flocks (Table 3). Similar report was demonstrated by Talha (2003) who recorded 36% MG infection in a flock containing 300 chickens in comparison to 33% in a flock containing 250 chickens. Highest infection rate in large scale flocks probably due to faulty in management and bio-security (Chandiramani *et al.,* 1966).

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