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Short Communication

RISK FACTORS FOR *MYCOPLASMA GALLISEPTICUM* SEROPREVALENCE IN CHICKENS

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ABSTRACT

Avian Mycoplasmosis is a chronic respiratory disease which causes very low mortality but it decreases egg production and causes carcass condemnation. A cross sectional study was conducted from September 2010 to October 2012 to identify the potential risk factors for *Mycoplasma gallisepticum* (Mg) seroprevalence in commercial chicken production in Chittagong area, Bangladesh. A total of 5589 serum samples were collected from one hundred chicken flocks of different production systems (commercial layer, broiler and layer breeder). Antibody against *Mycoplasma gallisepticum* was determined by commercial indirect Enzyme Linked Immunosorbent Assay (ELISA) kit. A multivariable logistic regression model was designed to evaluate the risk factors for Mg seroprevalence. The ELISA test results demonstrated that 32% of the birds were seropositive to Mg antibodies in the study area. A multivariable logistic regression model identified a strong effect of 'age' on the Mg antibody level where birds of more than 53 weeks of age showed the greatest risk of being positive for Mg (OR 419.27, 95% CI 294.67-596.54, P<0.0001) compared to birds less than 16 weeks of age. Moreover, monsoon season (OR 2.962, 95% CI 2.24-3.92, P<0.0001), male birds (OR 1.192, 95% CI 1.03-1.38, P=0.0196) and Commercial layer type of production system (OR 2.641, 95% CI 2.17-3.22, P<0.0001) were factors found to be associated the risk of attracting Mg in chickens.

Key words: Mycoplasmosis, Seroprevalence, ELISA, Antibody

INTRODUCTION

In Bangladesh, the poultry industry has been persistently growing over the last decade. There are three distinct types of FAO classified poultry production system namely industrial integrated, commercial poultry production, and village or backyard production system. It is one of the most lucrative agro-based industries in Bangladesh. There are approximately 227 breeder farms and 10,000 layer farms established in different parts of the country (BBS, 2010). Infectious diseases are of major concern in relation to the profit of poultry industry. Mycoplasmosis, commonly known as chronic respiratory disease (CRD) (Ley, 2008), is among the highly prevailing chronic diseases of chicken in Bangladesh, and represents a considerable economic threat to the poultry industry. The etiological agent of CRD is *Mycoplasma gallisepticum*. Poultry industry faces economic losses by CRD in terms of decline in egg production and poor hatchability in case of layers (Evans *et al.,* 2005). In broiler it causes inefficient feed conversion and reduced weight gain (Carpenter *et al.,* 1981). ELISA is routinely used for the monitoring of determining antibody against Mg (Ley and Yoder, 1997). The prevention and control of mycoplasmosis largely depends upon proper monitoring and prediction of its risk factors that are associated with the transmission dynamics of the agent (David, 2012). Thus, the seroprevalence of Mg in birds of different age groups, in different seasons, production status, sex, strain and flock sizes also vary (David *et al.,* 1997; Sarkar *et al.,* 2005; Sikder *et al.,* 2005). A considerable variation (13-60%) of seroprevalence against Mg was reported in Bangladesh (Biswas *et al.,* 1992; Amin *et al.,* 1992; Biswas *et al.,* 2003; Sarkar *et al.,* 2005; Sikder *et al.,* 2005). A variable prevalence of MG (26% to 62%) was reported from different parts of the world including India (Chakraborty et al, 2001), Malaysia (Shah-Majid, 1996) and Benin (Chrysostome et al, 1995). The aim of this study was to determine the seroprevalence of Mg antibodies and to investigate the associated risk factors for the introduction and spread of the Mg in layer and breeder chickens.

MATERIALS AND METHODS

Study area and sample collection: A total of 5589 serum samples were obtained from one hundred randomly selected chicken flocks from thirty farms of different production systems (Ten from each of commercial layer, broiler breeder and layer breeder) in

the Chittagong region, Bangladesh, during the period of September 2010 to October 2012.

Laboratory test procedure: The quantitative level of antibody in serum was measured by indirect ELISA using a commercial test kit, BioChek® (BioChek Ltd. London, UK) according to the manufacturer's instructions.

Statistical analysis: Data regarding possible risk factors of Mg infection were entered into an excel spread sheet program (Excel 2000, Microsoft) for data management purposes. Data were imported to SAS version 9.2 (SAS Institute Inc., Cary, NC) to perform further statistical analysis. Descriptive analysis was done by means of frequency (N, %) of positive and negative test results, general and stratified by different independent variables. Initially, a univariable logistic regression analysis was executed to determine the risk factors associated with Mg seroprevalence. The factors in the univariable analysis considered significant ($p < 0.10$) were selected for the multivariable logistic regression model. In this study a manual backward elimination procedure was made to construct the final model where a variable with the highest p-value is removed first from the model. The final model was consisted of variables with a p-value more than 0.05. Two-way interactions between independent variables were evaluated in the final model. Descriptive analysis was performed using the procedure PROC FREQ and univariable and multivariable logistic regression analyses was done by the procedure PROC GENMOD in SAS version 9.2 (SAS Institute Inc., Cary, NC). Finally a linear and quadratic polynomial regression model was fitted to evaluate the linear association between age of the birds and antibody level against Mg infection by SAS commands PROC REG, PROC GLM.

RESULTS AND DISCUSSION

The overall seroprevalence of Mg was estimated as 32%. The detailed prevalence for different levels of the independent variables is presented in Table 1.

Table 1. Frequency of variables identified as significant (≤0.05) in the univariate analysis as risk factor for *Mycoplasma* seroprevalence in chicken in Bangladesh.

Variable	Level	N (No. sampled)	Positive	Negative	*P*-value
			N (%)	N (%)	
Overall		5589	1808 (32)	3781 (68)	
Age	0-16 weeks	870	24 (3)	846 (97)	<0.0001
	17-34 weeks	2036	285 (14)	1751 (86)	
	34-53 weeks	1705	792 (46)	913 (54)	
	>53 weeks	978	707 (72)	271 (28)	
Sex	Male	1618	550 (34)	1068 (66)	0.0945
	Female	3971	1258 (32)	2713 (68)	
Strain	Ross	2411	948 (39)	1463 (61)	0.1970
	Hubbard classic	1014	194 (19)	820 (81)	
	Cobb-500	1135	293 (26)	842 (74)	
	Highline brown	406	71 (18)	335 (82)	
	High sex brown	623	302 (48)	321 (52)	
Type	Broiler breeder	4540	1434 (32)	3106 (68)	0.0008
	Layer breeder	330	95 (29)	235 (71)	
	Commercial layer	719	279 (39)	440 (61)	
Season	Winter	1362	275 (20)	1087 (80)	<0.0001
	Summer	2069	555 (27)	1514 (73)	
	Monsoon	2158	978 (45)	1180 (55)	

The finding of overall prevalence was supported by previous researcher (Biswas *et al.,* 1993; Alam *et al.,* 2003; and Amin *et al.,* 1992).The seroprevalence of Mg antibodies in this study is lower than reported by Hossain *et al.,* (2007) (55%), Sikder *et al.* (2005) (57%), Sarkar *et al.* (2005) (59%), and Biswas *et al.* (2003) (57%). Varying estimates of prevalence in different studies might be due to difference in the detection procedures used. An increasing trend in prevalence was recorded with the increasing age (Table 1). A significant correlation between birds (different flocks) being seropositive for *Mycoplasma gallisepticum* antibodies and age of the birds was demonstrated in this study. Avian mycoplasmosis is a chronic disease (Ley, 2008). Once the birds have contracted the infection they become carriers. It is difficult to eliminate the organism from birds. Consequently, in old birds the prevalence is higher as compared to younger birds (Botus et al., 2008). Male birds had a higher prevalence (34%) compared to females (32%). The prevalence of Mg in birds was found to vary

with seasons where the greater proportion (45%) of birds was positive during the monsoon and 20% prevalence was recorded in winter. This result disagree with the findings of Hossain *et al.* (2007), Pradhan *et al.* (2000) and Sikder et al. (2005) where a higher prevalence in winter season was reported. Sikder *et al.* (2005) reported 57.47% prevalence in summer season. It revealed that prevalence of Mg infection is highest (45%) and lowest (20%) during monsoon and winter, respectively. Based on the production type, the highest prevalence of Mg was observed in commercial layers (39%) and the lowest prevalence was recorded in layer breeders (29%). Sikder *et al.* (2005) and Sarkar *et al.* (2005) demonstrated 57% and 59% Mg seropositive layer chickens in Bangladesh, respectively, however they did not investigate other production systems namely broiler and layer breeder .The higher rate of prevalence in commercial layers over broiler breeders and layer breeders might be due to difference in the management systems. A strict biosecurity and a controlled environment maintained in the breeder flocks whereas biosecurity program in layer management is minimum than the breeders. They have limited space which allows the transmission of infectious organisms more efficiently than in other systems of rearing. Moreover, an exhaustive pattern of poultry rearing in a commercial layer management system may generate a potential for persisting pathogens due to population compactness.

Initially ten explanatory variables were included in the study; among them five were excluded from the analysis due to missing values (more than 20%) (Biosecurity, flock size, housing structure, mmanagement system) and the remaining five variables were included into the univariable analysis. A total of four variables (Table 1) were screened initially on the basis of inclusion criteria ($P<0.1$) to include in the multivariable logistic regression model. In the multivariable analysis, four variables namely season, sex, age and type were found to be associated with an increased risk of Mg infection in chickens (Table 2).

Table 2. Risk factors for *Mycoplasma* seropositive positive Chicken in Bangladesh, from a multivariable logistic regression model.

Variable	Level	Estimate	SE*	Odds ratio	CI (95%)	*P*-value
Intercept		-6.3391	0.1940			
Season	Winter	0				<0.0001
	Summer	0.7239	0.0951	2.062	1.71-2.48	
	Monsoon	1.0858	0.1426	2.962	2.24-3.92	
Sex	Female	0				0.0196
	Male	0.1752	0.0750	1.192	1.03-1.38	
Type	Broiler breeder	0				<0.0001
	Layer breeder	0.4857	0.0504	1.625	1.47-1.79	
	Commercial layer	0.9713	0.1008	2.641	2.17-3.22	
Age	0-16 weeks	0				<0.0001
	17-34 weeks	3.0193	0.0900	20.476	17.17-24.42	
	34-53 weeks	4.5289	0.1349	92.655	71.12-120.71	
	>53 weeks	6.0385	0.1799	419.27	294.67-596.54	

*SE, Standard Error

Among seasons, samples collected during monsoon showed a higher probability (OR: 2.9; 95% CI: 2.24-3.92) of being positive for Mg compared to winter (baseline).

Birds of more than 53 weeks of age had a higher risk (OR: 419.27; 95% CI: 294.67-596.54) of being positive compared to the baseline group (0-16 weeks old birds). Uneven levels of antibody and significant differences in seroprevalence among different age groups concerning Mg infection was reported by Hossain *et al.* (2007) and Sarkar *et al.* (2005). Previous studies, based on detection of antibodies by rapid serum agglutination test, demonstrated a decreasing trend of infection with increasing age (Hossain *et al.,* 2007; Sarkar *et al.,* 2005). In this study, an increasing trend of antibody level was observed with increasing age which is in agreement with Botus *et al.* (2008) where ELISA was used for the detection of Mg seropositivity. The minimum and maximum level of antibody was found in the birds younger than 16 weeks and older than 53 weeks of age, respectively. A similar result was reported by Botus *et al.* (2008) and Haghighi-Khoshkhoo *et al.* (2011). Among different production types, the risk of being positive for Mg infection was higher for commercial layer chickens (OR: 2.6; 95% CI: 2.17-3.22) in comparison to broiler breeders. Male birds had a slightly higher risk (OR: 1.19; 95% CI: 1.03-1.38) of being positive than females.

The findings from linear regression and quadratic polynomial regression equation of antibody titre against Mg seroprevalence in chicken are shown in

Table 3.

Table 3. Estimated model parameters (a, b and c) and fit statistics (R^2 and CV) of antibody titre against Mg seroprevalence associated with age of birds.

Measures	Linear regression	Polynomial regression
Intercept (a)	-866.02436 ± 78.76203	-118.2840591 ± 129.7590782
Slope (b)	71.60453 ± 2.02449	21.0778859 ± 7.2714266
Curve shape (c)	-	0.6630432 ± 0.0916824
R^2	0.1827	0.190247
CV	165.64336	164.8894

The quadratic term was more significant (p <0.0001) than the linear term (p= 0.0038) suggesting there is no straight-line relationship between age and titre; however a partial positive linearity was observed between titer and age. A probable logic behind this trend might be due to latent infection and for this reason once a bird is infected with Mg then the bird carry infection for a long time and continuously showed the antibody titer.

The finding of the study was based on blood samples submitted over a two year period. The blood sample was sent by different farmers of Chittagong districts to the lab for analysis of antibody level. We analyzed 5589 serum samples from thirty farms and antibody titers were detected by a highly sensitive indirect commercial ELISA kit.

However in this study some serum samples were from the same farm but collected at different times. We observed that among the different risk factors, age is the most important one in relation to Mg seroprevalence.

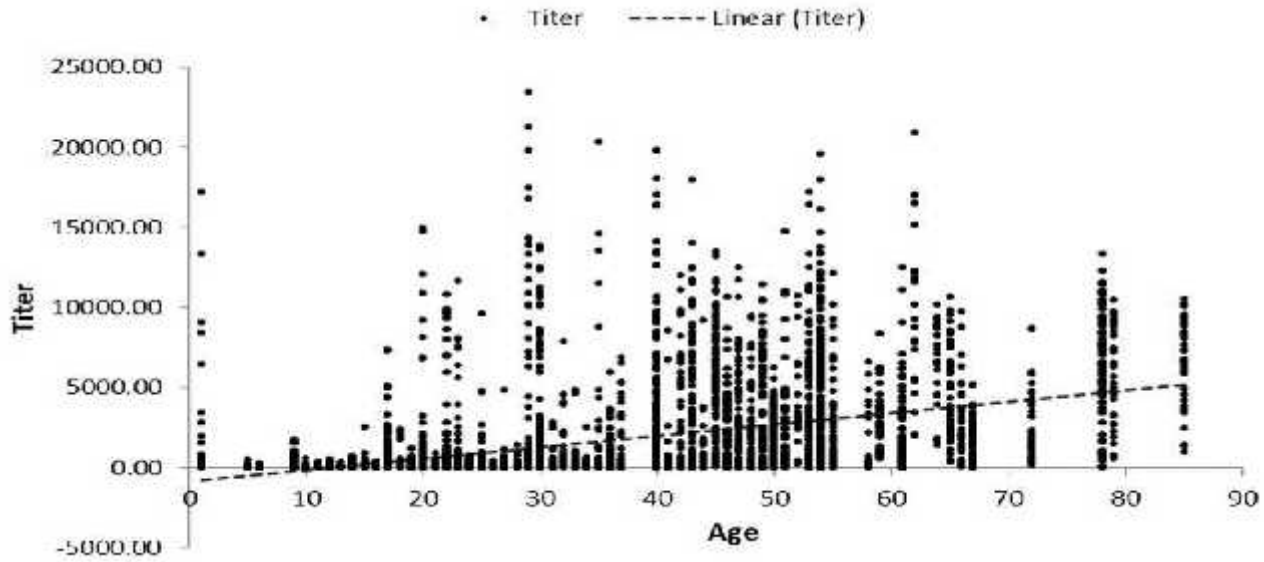

Fig 1. Observed and predicted level of antibody against *Mycoplasma gallisepticum* in chicken (by linear regression).

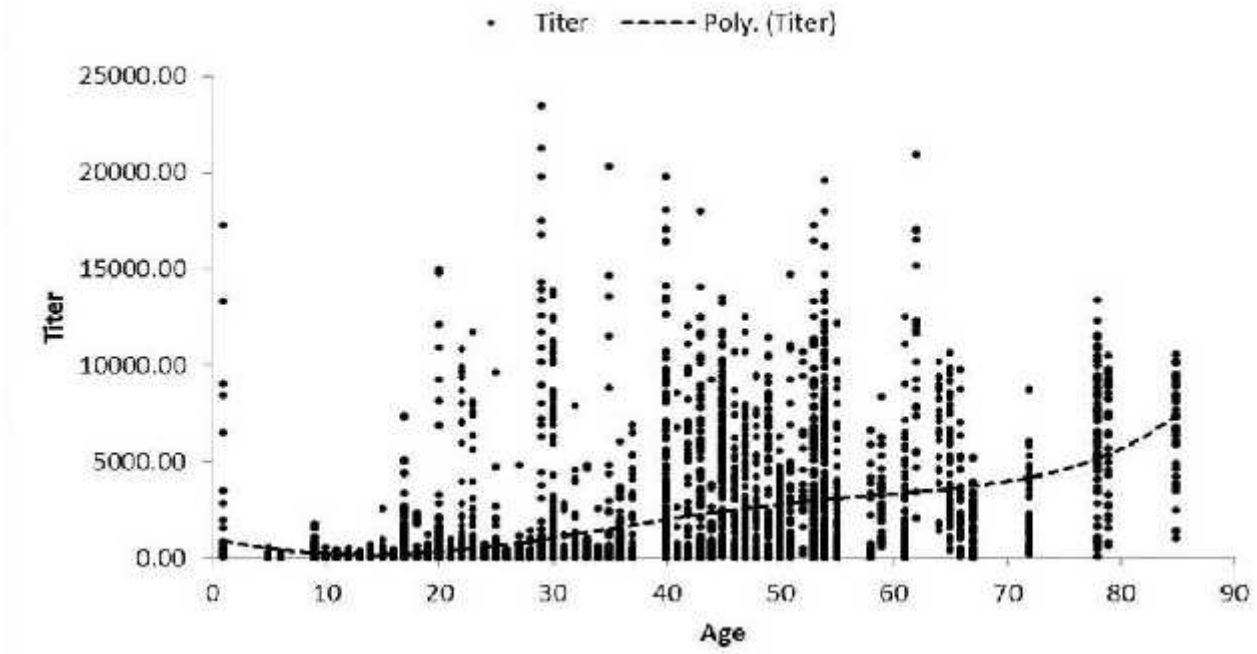

Fig 2. Observed and predicted level of antibody against *Mycoplasma gallisepticum* in chicken (by quadratic polynomial regression)

Conclusion: From this study we can conclude that birds should be reared in an "all in - all out system" to avoid mixing of birds of different ages. This means, avoiding different age groups of birds at a single farm. However, breeding flocks should be monitored routinely for controlling vertical transmission. On the other hand, a strict biosecurity measure should be employed such as limited movement of the farm personnel, visitors and other persons. The limitation of the study was the absence of sample from backyard poultry farm.

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