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Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* Infection in the Six Model Breeder Poultry Farms at Patuakhali District in Bangladesh

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Abstract: The present study was undertaken to know the seroprevalence of Salmonella and Mycoplasma gallisepticum (MG) infection in six model breeder poultry farms (MBPFs) located at kalapara Upazilla under Patuakhali district, Bangladesh. A total of 364 sera samples were collected from chickens belonging to six MBPFs. All sera samples were examined by rapid serum plate agglutination (SPA) test using commercial Salmonella (SP) and MG antigens to determine the presence of Salmonella and MG specific antibodies in different age and sex of birds belonging to MBPFs. In addition to that prevalence of Salmonella and Mycoplasma infection in MBPFs during rainy and winter seasons were also recorded. The results of serological tests were analyzed statistically. The overall prevalence of Salmonella and Mycoplasma infection in six MBPFs were recorded as 23.46% and 46.88% respectively. Prevalence of salmonella was recorded highest in rainy season (25.00%) than the winter season (21.88). On the contrary, Mycoplasma infection was recorded highest in winter season (61.45%) than the rainy season (51.74%). Both Salmonella and Mycoplasma infections were recorded highest in female birds (24.10%) than the male birds (15.62%). The prevalence of MG infection decreased with the increase of age. MG infection recorded highest 71.42% at 18 weeks of age and lowest 50% at 22 weeks of age. On the other hand, the prevalence Salmonella infection was increased with the increase age. Salmonella infection was found highest 30.76% at 39 weeks of age and lowest 13.33% at 32 weeks of age. It was concluded from the present study that both Salmonella and MG infection were significantly present in all six MBPFs and SPA test could be used as a tool for quick detection of Salmonella and MG infection.

Key words: Seroprevalence, salmonella, mycoplasma gallisepticum, model breeder poultry farms

Introduction

Poultry rearing may play a very important role for income generation and poverty reduction particularly for the distressed women, unemployed youths in Bangladesh by means of self-employment. For this purpose, a model of semi scavenging poultry rearing system known as MBPF has been developed under the Poultry Management Technology Improvement Projects (PMTIP), Partnership Livestock Development Project (PLDP) and Small Holder Livestock Development Project (SLDP-2). In model farming, Sonali breed of chickens has been reared instead of indigenous local chicken because of their high productivity and increased resistant to diseases (Rahman et al., 1997). In the recent years poultry farming has been hampered by the outbreak of fatal infectious diseases caused by bacteria, viral, Mycoplasmal and other causal agents (Ahmed and Hamid, 1991). Among bacterial diseases Salmonellosis is one of the most important diseases in poultry that cause serious economic loss due to mortality and reduced egg production (Khan et al., 1998). Salmonellosis is caused by Salmonella gallinarum and Salmonella pullorum which are responsible for fowl typhoid and pullorum diseases, respectively

(Snoeyenbos, 1994). An indirect enzyme linked immunosorbant assay (ELISA), double ELISA, whole blood agglutination test and rapid plate agglutination test under field conditions are performed for the diagnosis of salmonellosis (Feberwee *et al.*, 2001). With the expansion of poultry rearing and farming, *S. pullorum* and *S. gallinarum* have become wide spread problem in Bangladesh (Rahman *et al.*, 1979). In order to control *Salmonella* infection of poultry in Bangladesh detailed epidemiological investigation and strain identification is prerequisite.

Mycoplasmosis is also an important disease in poultry. It is caused by four commonly recognized pathogenic Mycoplasma namely *Mycoplasma* gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis and Mycoplasma iowae (Bradbury, 2001). Mycoplasma belongs to the class: Molicutes, order Mycoplasmatales, under Mycoplasmataceae family. Mycoplasma gallisepticum causes chronic respiratory disease (CRD) in chicken, which is characterized by decreased egg production in layer. It is the most economically important Avian Mycoplasma (AM) (Ley and Yoder, 1997), M. gallisepticum can be diagnosed by studying their different properties, such as morphological, cultural

characteristics, physical, bio-chemical and serological properties of the causal agent (Ley and Yoder, 1997). Therefore, it is important to know the status of Salmonellosis and Mycoplasmosis in chickens of MBPFs in order to take an effective control measure.

Materials and Methods

The research work was conducted in six MBPFs during the period from January 2004 to August 2004. Of six MBPFs three were located at Tiakhali union and rest of the three were located at Nilganj union under Kalapara Upazilla of Patuakhali district, Bangladesh. Each MBPF consisted of 54 Fayoumi and 6 Rhode Island Red (RIR). The ratio of hen and cock was 9: 1 in a MBPF.

Collection of blood from chickens for preparation of sera: A total of 364 blood samples were collected from the birds belonging to six MBPF during winter (January-February) and rainy (July-August) seasons for the preparation of sera. Blood samples were collected aseptically from the wing vein of birds using 5ml sterile disposable syringes and needles. Then the samples were kept at room temperature for two hours to clot blood inside the syringe. After clotting, fluid portion of blood were placed in graduated centrifuge tubes and centrifuged at 1500 rpm for 30 minutes. The clear sera samples were poured in sterile vials which was labeled and transferred to the Laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh in iceboxes for the detection of Salmonella and MG infection by SPA test.

Salmonella antigen: Standard *Salmonella* (Nobilis^(R) SP) antigen manufactured by Intervet International, Holland was used for SPA test for the detection of *Salmonella* antibodies in the sera samples.

Mycoplasma antigen: Standard *Mycoplasma* gallisepticum (Nobilis^(R) (MG) antigen manufactured by Intervet International, Holland was used for SPA test for the detection of M. gallisepticum antibodies in the sera samples.

Detection of Salmonella infection by SPA test: The SPA test was performed according to the procedure described in OIE manual, 2000, with crystal violet stained Salmonella antigen (Noblis^(R) SP antigen). For this test 0.02 ml of antigen and 0.02 ml of chicken sera were placed side by side with a micropipette on a glass plate. Then the antigen and the sera were mixed thoroughly by stirring with small tooth pick followed by rocking. Results of SPA test were read within 2 minutes. In positive cases granules were formed slowly indicating that sera samples contained antibody against Salmonella infection. In negative case granules were not formed within 2 minutes indicating that antibody against

Salmonella were absent in the sera samples. The results of SPA test were recorded.

Detection of Mycoplasma gallisepticum infection by SPA test: In order to determine Mycoplasma gallisepticum infection the SPA test was conducted according to the instruction of OIE Manual (2000). For this test 0.02 ml antigen and 0.02 ml of chicken sera were placed side by side with a micropipette on a glass plate. Then antigen and sera samples were mixed properly by stirring with small tooth pick. Results of SPA tests were recorded within 2 minutes. In positive cases granules were formed slowly within 2 minutes. In negative case, no such granules were formed within two minutes indicating that antibody against Mycoplasma gallisepticum were absent in the test sera samples. The results of SPA test were recorded.

Statistical analysis of serological data: The results of serological test were analyzed statistically which was based on geographic location of MBPFs, age and sex of the birds belonging to MBPFs and seasonal incidence of *Salmonella* and *Mycoplasma* infection.

Results and Discussion

Seroprevalence study for detection of Salmonella and MG infection: In order to determine seroprevalence of Salmonella and MG infection a total of 364 sera samples were collected from six MBPF during winter and rainy season. All sera samples were tested by SPA test. The results of seroprevalence of Salmonella and Mycoplama infection in MBPF are presented in Table 1. In flock no.1, the prevalence of Salmonella infection was found to be 13.33% at first sampling but it increased to 14.70% at second sampling (5 months after first sampling). The prevalence of Salmonella infection also increased from first sampling to second sampling from 21.87% to 24% in flock no. 2, 19.44% to 27.58% in flock no. 3. 14.28% to 25% in flock no. 4 and 28.57% to 30% in flock no. 5 and 30.25% to 30.76% in flock no.6. The prevalence rate was recorded the highest (30.76%) in flock no. 6, during second sampling at the age of 39 weeks. On the other hand prevalence rate was the lowest (13.33%) in flock no. 1, during first sampling at the age of 32 weeks. However, the over all seroprevalence of Salmonella infection in six MBPFs was 23.46%. The overall seroprevalence of MG infection was found to be 56.86%. The other species of Avian Mycoplasma were not considered in this study. In flock no. 1, seroprevalence of MG infection was 50% at first sampling but it decreased to 41.17% at second sampling (5 months after first sampling). The prevalence of MG infection was also decreased from 65.62% to 52% in flock no. 2: 61.11% to 55.17% in flock no. 3; 57.14% to 50% in flock no. 4; 71.42% to 60% in flock no. 5 and 61.29 to 53.84% in flock no. 6 during first

Table 1: Seroprevalence of Salmonella and MG infection in birds of different ages in six MBPFs

Sampling	Study area	No. of flock	Age of birds (WKS)	Total serum tested	Prevalence (%) of Salmonella	Prevalence (%) of Mycoplasma
First	Tiakhali	1	32	30	13.33	50.00
sampling	union	2	22	32	21.87	65.62
		3	42	36	19.44	61.11
	Nilgonj	4	28	28	14.28	57.14
	union	5	18	35	28.57	71.42
		6	25	31	30.25	61.29
Second	Tiakhali	1	53	34	14.70	41.17
sampling	union	2	43	25	24.00	52.00
		3	63	29	27.58	55.17
	Nilgonj	4	49	28	25.00	50.00
	union	5	39	30	30.00	60.00
		6	46	26	30.76	53.84

Table 2: Seroprevalence of Salmonella and MG infection in six MBPFs during winter and rainy season

Seasons	study area	Total	Positive case (%)	Positive case (%)	Overall prevalence	Overall prevalence
		serum	of Salmonella	of Mycoplasma	of Mycoplasma	Mycoplasma
		tested				
Winter	Tiakhali union	98	18 (18.36)	58 (59.18)		61.45
	Nilgonj union	94	24 (25.53)	60 (63.82)	21.88	
Rainy seasons	Tiakhali union	88	19 (21.59)	43 (48.86)		51.74
	Nilgonj union	84	24 (28.57)	46 (54.76)	25.00	

to second sampling. The prevalence of MG was recorded highest (71.42%) at 18 weeks of age in flock no.5, whereas, the prevalence was the lowest (50%) in flock no.1 at the age of 22 weeks. The prevalence of MG infection decreased with the increase of age in all flock.

Seasonal incidence of Salmonella and MG infection in MBPFs: The results of seasonal seroprevalence of Salmonella and mycoplasma infection are presented in Table 2.

It was observed that the prevalence rate of *Salmonella* infection was higher (25.00%) in rainy season and lower (21.88%) in winter season. The seasonal seroprevalence was analyzed by one way ANOVA method (F-test) and variation found to be non-significant (p>0.05) between the two seasons. It was observed that higher prevalence of *Mycoplasma* infection was recorded in winter (61.45%) and the lower in summer (51.74%). The seasonal variation of seroprevalence was analyzed by One-way ANOVA method (F-test). The value of the test indicated non- significant (p>0.05) variation between two seasons.

Seroprevalence of Salmonella and MG infection in male and female birds in MBPFs: The prevalence rate of Salmonella infection was found to be higher (24.10%) in female birds and lower (15.62%) in male birds. MG infection was recorded higher (52.71%) in female birds and lower (46.88%) in male birds. The results were analyzed by One-way ANOVA method (F-test) and found non significant (p>0.05) association between male and

female birds and the results were presented in Table 3.

Seroprevalence of Salmonella and MG infection on the basis of geographical location

The prevalence of *Salmonella* infection was also observed on the basis of study areas and the results were presented in Table 4.

From Table 4 it is clear that the prevalence of Salmonella infection was higher (26.96%) at Nilgonj Union than that of Tiakhali Union (19.89%). MG infection in MBPF was also recorded highest (63.28%) in Nilgonj Union than Tiakhali union (58.73%).

In the present study, SPA test was performed to determine seroprevalence of Salmonellae and MG infection in chickens in MBPF. ELISA and HI test can also be used for detection of antibodies against Salmonella and MG infection of chickens. Avakian et al., (1988) stated that SPA test is very simple, sensitive and it can be used for the detection of both Salmonellae and MG infection. SPA a test is suitable for detection of MG antibody as early as 10 days after infection (Pradhan, 2002). The ELISA is considered expensive (Bradbury and Kleven, 1997) and the HI test is considered to be highly specific but less sensitive than SPA test (Avakian et al., 1988). However SPA test may sometimes show false positive reaction. To overcome the false positive reactions the test sera were inactivated by heating at 56°C for 30 minutes in a water bath and retested according to the instruction of OIE Manual (2000) for the confirmation of the results. Farmers of MBPFs did not vaccinate chickens against Salmonella and MG. As a

Table 3: Seroprevalence of Salmonella and MG infection in female and male birds belonging to six MBPFs

Sex of	Study area	No. of	Positive cases (%)	Positive cases (%)	Overall prevalence	Overall prevalence		
birds		tested	of Salmonella	of Mycoplasma	(%) of Salmonella	(%) of Mycoplasma		
		sera						
Female	Tiakhali union	168	34 (20.23)	88(52.38)	24.096	52.71		
	Nilgonj union	164	46 (28.04)	87(53.04)				
Male	Tiakhali union	18	3 (16.66)	8 (44.44)	15.62	46.88		
	Nilgonj union	14	2 (14.28)	7(50)				

Table 4: Seroprevalence of Salmonella and MG in MBPFs located at Tiakhali and Nilgani union

Study area	No. of	Salmonella	Mycoplasma	Salmonella	Mycoplasma
	tested	Positive	positive	Prevalence	Prevalence
	sera	case	case	(%)	among tested
					cases (%)
Tiakhali union	186	37	98	19.89	58.73
Nilgonj union	178	48	94	26.96	63.28

results chance of false positive reaction was very less. In this experiment no serum sample gave false positive reaction by SPA test.

In the present study it was revealed that all the experimental flocks were positive to Salmonella infection. The highest prevalence of Salmonella infection was 30.76% and the lowest prevalence was 13.33%. The overall prevalence of Salmonella infection in MBPF was 23.46% (Table 1). This was strongly supported by Alam et al. (2003) and Sarker (2004) who observed 23.8% and 22.77% prevalence of Salmonella infection in Dinajpur district and in Noakhali district of Bangladesh. Whereas Jha et al. (1994) observed 21.8-25.7% prevalence of Salmonella infection in 51-61 week old birds in Eastern Nepal. But Terzolo et al. (1977); Ghosh (1988); Muneer et al. (1988); Waltman and Home (1993); Yang et al. (1996) Hasegawa et al. (1999) reported 9%, 13.9%, 19.60%, 7.5%, 15%, 10% and 16% prevalence of Salmonella infection, respectively. The present finding was also higher than the report of Sarker (2004) who reported 22.77% seroprevalence of Salmonella infection in SLDP-2 area Noakhali district in Bangladesh.

In this study, the prevalence of Salmonella infection was found higher (25.00%) in rainy season than in winter season (21.88%) (Table 2), which was supported by Saleque et al. (2003). It might be due to the influence of hot weather and rain that might reduced the immune status of the birds and there by made vulnerable them to infection. The prevalence of Salmonella infection was also increased with age (Truong and Tieu, 2003) The seasonal variation of prevalence of Salmonella infection was significant on statistical analysis by one-way ANOVA method (F-test), similar report was given by Saleque et al. (2003). The prevalence was higher (24.096%) in female than in male (15.63%) (Table 3) indicating that female birds were more susceptible to Salmonella infection than male birds. The statistical analysis by one way ANOVA method (F-test) between the prevalence of male and female birds showed non significant

relationship. The prevalence of *Salmonella* infection was also higher (26.96%) in Nilgonj union than in Tiakhali union (19.89%) (Table 4). The variation might be due to faults in management, (biosecurity, degree of infection in litter, feed etc.) and rearing system.

The highest prevalence (71.42%) of MG infection was found in the present study in flock no.5, and the overall prevalence was 56.86% (Table 1) which was strongly supported by the previous investigations of Bencina (1987), Wieliczki et al. (2000), Godoy (2001), Pradhan (2002), Biswas et al. (2003) and Dulali (2003). They reported 56.54%, 57.15%, 59.10%, 54.90% and 52% seroprevalence of MG infection in chickens, respectively. The prevalence of MG infection was higher in the present study than Biswas et al. 1992 and Amin et al. (1992). They reported 13-32% seroprevalence of MG infection in the selected farms of the selected area. The overall prevalence of MG infection was higher than Bencina et al. (1987), Wieliczki et al. (2000), Biswas et al. (2003) and Dulali (2003) which might be due to the maintenance of breeder stock for a long period of time and 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 to MG infection are poor ventilation, contamination of litters and no restriction on movement of technical personnel, visitors and such other persons as well as other bio-security measures (Dulali, 2003).

In the present study seasonal variation for prevalence of MG infection was observed. The prevalence was higher (61.45%) in winter season and lower (51.74%) in rainy season (Table 2) which was in agreement with the result of David *et al.* 1997 and Pradhan *et al.* 2000. It might be due to the influence of cold weather. The statistical analysis by one way ANOVA method (F-test) showed non significant variation between the prevalence of two seasons. It was also found that the prevalence of

MG infection was higher (52.71%) in female than in male (46.88%) (Table 3), indicating that female birds were more susceptible than male birds to MG infection but the fact was not established. The statistical analysis by one way ANOVA method (F-test) revealed that there was no significant difference in the prevalence of MG infection between the sex of birds. Prevalence was also decreased with the increase of age. It might be due to the seasonal influence as during the winter the birds were younger than in rainy season (Nunoya et al., 1995 and David et al., 1997). The prevalence of MG infection was higher (63.28%) in Nilgonj union than Tiakhali union (58.73%) (Table 4). The variation might be due to faulty in management and bio-security (Chandiramani et al., 1966). The present findings were in close agreement with the previous results reported by Kelly et al., 1994 in Zimbebwe, Chrysostome et al., 1995 in Benin, Shah-Majiid, 1996 in Malaysia, Pandey and Hasegawa, 1998 in Zambia, Mushi et al., 1999 in Botswana, Chakrabortv et al., 2001 in India, Alam et al. (2003) and Talha (2003) in Bangladesh.

From the present research work, it may be suggested that the MBPF should be periodically checked to know the status of *Salmonella* and MG infection. The positive birds should be culled and strict bio-security measures should be undertaken in order to take an effective control measures against them. It may be concluded from the study that SPA test can be used for quick detection of *Salmonella* and MG infection.

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