

Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* Infection in Chickens in Rajshahi and Surrounding Districts of Bangladesh

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Abstract

A serological survey on the prevalence of antibodies against *Salmonella* and *Mycoplasma gallisepticum* (MG) was carried out in layer chickens in Rajshahi and surrounding districts of Bangladesh. A total of 605 sera samples were examined by rapid plate agglutination (RPA) test using commercial *Salmonella* and MG antigens to determine the *Salmonella* and MG specific antibody. Out of 605 sera samples 14.1% showed single *Salmonella*, 45.1% showed single MG and 11.2% showed their concurrent infection. Prevalence of *Salmonella* was recorded the highest (37.6%) in adult compared to young (16.7%). On the contrary, MG and concurrent infections were recorded the highest (71.7% and 13.3%) in young compared to adult (50.4% and 10.4%). The prevalence of *Salmonella*, MG and concurrent infections were recorded the highest (34.3%, 68.6% and 17.1%) in large flocks compared to small flocks (21.3%, 50.0% and 8.8%). The prevalence of *Salmonella* infection was the highest (30.4%) in summer followed by winter (23.7%), rainy (25.0%) and autumn (23.3%). The prevalence of MG infection was the highest (61.6%) in winter followed by autumn (56.9%), rainy (55.0%) and summer (49.6%). Whereas, their concurrent infection was the highest (12.1%) in winter followed by summer (11.9%), rainy (10.8%) and autumn (10.0%).

Keywords: Seroprevalence, *Salmonella*, *Mycoplasma gallisepticum*, Chickens

1. Introduction

Salmonellosis is an important disease in chickens caused by *Salmonella pullorum* and *Salmonella gallinarum* and is referred to as pullorum disease and fowl typhoid, respectively. Pullorum disease occurs in chicks during their first few days of life and causes severe enteritis and bacteremia (Snoeyenbos *et al.*, 1994). Whereas, fowl typhoid is a disease of mature chickens and causes either acute enteritis with greenish diarrhea or a chronic disease of the genital tract that reduces egg production (Proux *et al.*, 2002). Salmonellosis in poultry causes significant economic loss due to mortality and reduced production (Khan *et al.*, 1998). With the expansion of poultry rearing and farming, pullorum disease and fowl typhoid have become the wide spread problem in Bangladesh (Rahman *et al.*, 1979). Among serological tests, the rapid plate agglutination (RPA) test is very simple, sensitive and used for the detection of *Salmonella* and MG antibodies under field condition (Avakian *et al.*, 1988). The application of a coordinated policy of hygienic measures together with serological testing and

slaughter of positive reactors has led to eradication of salmonellosis in many developed countries (Barrow, 1993).

Mycoplasmosis is also an important disease problem in chickens caused by four commonly recognized pathogens namely *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae* (Bradbury, 2001). MG infection is a chronic respiratory disease (CRD) in avian species (Ley, 2003) transmitted both horizontally and vertically and it remains in the flock continuously as a subclinical form (Bencina *et al.*, 1987). It causes decreased feed efficiency, poor carcass quality and sub-optimal egg production in layers (Ley and Yoder, 1997). The infection appears to be world wide spread and can be diagnosed by studying their different properties such as morphological, cultural, biochemical and serological properties of the causal agent (Ley and Yoder, 1997). 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). Therefore, it is important to know the status of salmonellosis, mycoplasmosis and their concurrent infections in chickens to take an effective control measure.

2. Materials and methods

This study was conducted at the Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi-6205, Bangladesh during the period from January to December 2009. A total of 121 layer chicken flocks were selected in Rajshahi and surrounding districts of Bangladesh.

2.1 Sampling procedure

From each flocks five chickens were randomly selected for blood collection. Two ml of blood was collected aseptically from wing vein using sterile syringe and needle. After that syringe with blood was kept in a cool box in a standing position for 6 hours, serum was harvested by decanting. The harvested sera were transferred to 1.5 ml micro centrifuge tubes and were kept in a cool box before shipping to the laboratory. Sera samples were stored at -20°C in the laboratory until use for RPA test (OIE, 2002).

2.2 Preparation of antigens

Antigens are the killed and colored *Salmonella* and *Mycoplasma* organisms. *Salmonella pullorum* antigens from standard (O: 1, 9,12₁ and 12₃) and variant (O: 1, 9,12₁ and 12₂) strains were used in this surveillance program for pullorum disease and fowl typhoid (Proux *et al.*, 2002). The MG antigen from an S-6 Adler strain of *Mycoplasma gallisepticum* organism was used in this surveillance program for avian mycoplasmosis. The *Salmonella* antigen (Nobilis[®] SP) and MG antigen (Nobilis[®] MG) used in this study were purchased from the Intervet International B.V. Boxmeer-Holland.

2.3 Rapid plate agglutination (RPA) test

The RPA test was conducted according to the instructions of OIE Manual (2002). For this test 0.02 ml of antigen and 0.02 ml of chicken serum were placed side by side with micropipettes on a glass plate. Then antigen and serum sample were mixed thoroughly by stirring with a small tooth pick. The glass plate was illuminated from below so 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 serum with antigen (Figure). The clumps usually started appearing and became concentrated at the periphery of the mixture. Negative reaction was judged by the absence of agglutination reaction. Care was taken so that the natural granulation of the antigen showed not to be taken as a positive reaction.

3. Results and discussion

3.1 Overall prevalence of *Salmonella* and MG infection

A total of 605 sera samples were collected from 121 commercial layer farms and were subjected to RPA test. Out of these, 85 (14.1%) were found positive for single *Salmonella* infection, 273 (45.1%) were found positive for single MG infection and 68 (11.2%) were found positive for their concurrent infection (Table1). The overall prevalence of *Salmonella* infection was 25.3% (14.1+11.2). Similar reports have been described by Alam *et al.* (2003) who reported 23.8% seropositive chickens for *Salmonella* infection in Dinajpur district of Bangladesh. Our finding supports the report of Bouzoubaa *et al.* (1992) who recorded 23.5% seropositive chickens for salmonellosis from Morocco. Our percentage (25.3%) is lower than that of Minga *et al.* (1987) and Bhattacharya *et al.* (2001) who reported 33.8% and 37.7% seropositive chickens for *Salmonella* infection in Tanzania and India, respectively. Whereas, Terzolo *et al.* (1977), Prukner (1987), Ghosh (1988), Muneer *et al.* (1988), Waltman and Home (1993), Yang *et al.* (1996), Hasegawa *et al.* (1999) reported 9.0%, 13.9%, 19.6%, 7.5%, 15.0%, 10.0% and 16.0% prevalence of *Salmonella* infection in chickens, respectively.

The overall prevalence of MG infection was 56.3% (45.1+11.2); other species of avian *Mycoplasma* were not considered in this study. Similar reports were demonstrated by Sikder *et al.* (2005) who reported 56.9% seropositive layer chickens for MG infection in Patuakhali district and by Sarkar *et al.* (2005) who reported 58.9% seropositive layer chickens for MG infection in some model breeder poultry farms in Feni district of Bangladesh. Our finding also is in agreement with previous reports of Bencina *et al.* (1987), Godoy *et al.* (2001), Biswas *et al.* (2003), Zhang *et al.* (2001) and Abdu *et al.* (1983) who reported 56.5%, 59.1%, 54.9%, 53.0% and 47.5% seroprevalence of MG infection in chickens, respectively.

3.2 Prevalence of *Salmonella* and MG infection in different ages

Concerning to the prevalence depending on the ages, the highest prevalence of *Salmonella* was 37.6% (27.2+10.4) at 64 weeks and above age group whereas the lowest prevalence was 16.6% (3.3+13.3) at 16-23 weeks age group. Similar report was demonstrated by Sikder *et al.* (2005) who reported the highest *Salmonella* infection was 30.8% at 39 weeks of age and the lowest was 13.3% at 32 weeks of age. Truong *et al.* (2003) reported that the prevalence of *Salmonella* infection increased with the increase of age. Whereas, the highest prevalence of MG infection was 71.6% (58.3+13.3) at 16-23 weeks age group and the lowest prevalence was 50.4% (40.0+10.4) at 64 weeks and above age group. Similar report was demonstrated by Sikder *et al.* (2005) who reported the highest (71.4%) MG infection at 18 weeks of age and the lowest (55.2%) at 63 weeks of age. Our finding also supports the report of Sarkar *et al.* (2005) who recorded 73.8% MG infection at 20 weeks of age in comparison to 45.2% at 55 weeks of age. Furthermore, the highest rate of concurrent infection was 13.3% at 16-23 weeks of age and the lowest was 10.4% at 64 weeks and above age group (Table 1). The highest rate of concurrent infection in the young chickens is probably due to the highest prevalence of MG infection at this age.

3.3 Prevalence of *Salmonella* and MG infection in relation to flock sizes

Serological investigation showed the highest (17.1+17.1=34.2%) *Salmonella* infection in large flocks (≥ 5000 birds) in comparison to small (≤ 1000 birds) flocks (12.5+8.8=21.3%). The present data were higher than those in the report of Skov *et al.* (1999) who recorded 16.8% *Salmonella* infection in a flock containing 30-40 thousand chickens in comparison to 11.9% in a flock containing 10-20 thousand and 9.7% in a flock containing less than 10 thousand chickens. Mdegela *et al.* (2000) recorded higher prevalence of *Salmonella* infection in commercial flocks (18.4%) than in scavenging chickens (6.3%) and reported that infection rate increased with the increase of flock size. Similarly, MG infection rate was the highest (51.4+17.1=68.5%) in large flocks in comparison to small flocks (41.3+8.8=50.1%). Furthermore, concurrent infection was the highest (17.1%) in large flocks in comparison to small flocks (8.8%) (Table 2). The highest infection rate in large flocks probably due to faulty in management and bio-security as well as the vertical transmission of the organisms.

3.4 Seasonal incidence of *Salmonella* and MG infection

The prevalence of *Salmonella* infection was the highest (18.5+11.9=30.4%) in summer followed by winter (11.6+12.1=23.7%), rainy (14.2+10.8=25.0%) and autumn (13.3+10.0=23.3%). Similar report was demonstrated by Rahman *et al.* (2004) who reported 48.1% prevalence of *Salmonella* infection in summer in comparison to 23.7% in winter. Our finding supports the report of Sikder *et al.* (2005) who recorded the highest (25.0%) prevalence of *Salmonella* infection in rainy season than in winter (21.9%). Bhattacharjee *et al.* (1996) reported the highest prevalence of salmonellosis during pre-monsoon (13.1%) in comparison to winter (10.4%), monsoon (6.8%) and post-monsoon (6.8%) period. The highest rate of *Salmonella* infection in summer season is probably due to the high growth rate of bacteria and the influence of hot weather that might reduce the immune status of the birds against infection. Whereas, the prevalence of MG infection was the highest (49.5+12.1=61.6%) in winter followed by autumn (46.9+10.0=56.9%), rainy (44.2+10.8=55.0%) and summer (37.8+11.9=49.7%). Similar report was demonstrated by Sarkar *et al.* (2005) who reported 62.4% prevalence of MG infection in winter in comparison to 53.1% in summer. Our finding also supports the report of Sikder *et al.* (2005) who recorded the highest (61.5%) prevalence of MG infection in winter than in rainy (57.5%) season. Similarly, the rate of concurrent infection was the highest (12.1%) in winter followed by summer (11.9%), rainy (10.8%) and autumn (10.0%) (Table 3). The highest rate of concurrent infection in winter is probably due to the highest prevalence of MG infection in that season. From the present study, we have seen that a significant number of birds carry concurrent infection which is more dangerous than single infection. Therefore, it is suggested that the poultry farms should be checked periodically to know the status of *Salmonella* and MG as well as their concurrent infection. The positive birds should be culled and strict bio-security should be undertaken in order to take an effective control measures.

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Table 1. Prevalence of *Salmonella* and MG infection in different ages

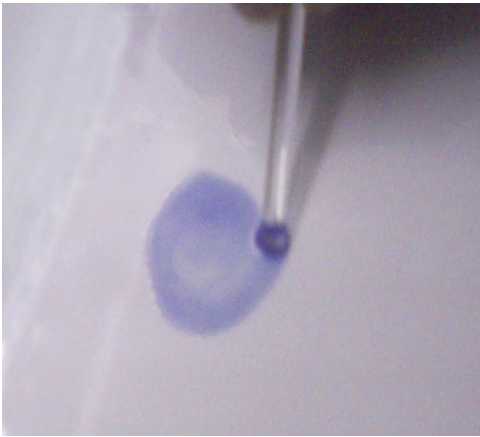
Age (weeks)	No. of flocks	Single <i>Salmonella</i> infection (%)	Single <i>Mycoplasma</i> infection (%)	Concurrent infection (%)	Total seropositive (%)	Total seronegative (%)	Total tested sample (N)
16-23	12	2 (3.3)	35 (58.3)	8 (13.3)	45 (75.0)	15 (25.0)	60
24-31	14	4 (5.7)	38 (54.3)	9 (12.9)	51 (72.9)	19 (27.1)	70
32-39	15	7 (9.3)	36 (48.0)	9 (12.0)	52 (69.3)	23 (30.7)	75
40-47	13	7 (10.8)	28 (43.1)	7 (10.8)	42 (64.6)	23 (35.4)	65
48-55	19	11 (11.6)	39 (41.1)	10 (10.5)	60 (63.2)	35 (36.8)	95
56-63	23	20 (17.4)	47 (40.9)	12 (10.4)	79 (68.7)	36 (31.3)	115
64-above	25	34 (27.2)	50 (40.0)	13 (10.4)	97 (77.6)	28 (22.4)	125
Total	121	85 (14.1)	273 (45.1)	68 (11.2)	426 (70.4)	179 (29.6)	605

Table 2. Prevalence of *Salmonella* and MG infection in relation to flock sizes

Flock size (No. of birds/flock)	No. of flocks	Single <i>Salmonella</i> infection (%)	Single <i>Mycoplasma</i> infection (%)	Concurrent infection (%)	Total seropositive (%)	Total seronegative (%)	Total tested sample (N)
500-1000	16	10 (12.5)	33 (41.3)	7 (8.8)	50 (62.5)	30 (37.5)	80
1001-1500	16	9 (11.3)	35 (43.8)	8 (10.0)	52 (65.0)	28 (35.0)	80
1501-2000	15	10 (13.3)	33 (44.0)	7 (9.3)	50 (66.7)	25 (33.3)	75
2001-2500	14	9 (12.9)	31 (44.3)	8 (11.4)	48 (68.6)	22 (31.4)	70
2501-3000	12	8 (13.3)	27 (45.0)	7 (11.7)	42 (70.0)	18 (30.0)	60
3001-3500	13	10 (15.4)	30 (46.2)	7 (10.8)	47 (72.3)	18 (27.7)	65
3501-4000	10	8 (16.0)	23 (46.0)	5 (10.0)	36 (72.0)	14 (28.0)	50
4001-4500	9	7 (15.6)	21 (46.7)	6 (13.3)	34 (75.6)	11 (24.4)	45
4501-5000	9	8 (17.8)	22 (48.9)	7 (16.6)	37 (82.2)	8 (17.8)	45
5001-above	7	6 (17.1)	18 (51.4)	6 (17.1)	30 (85.7)	5 (14.3)	35
Total	121	85 (14.1)	273 (45.1)	68 (11.2)	426 (70.4)	179 (29.6)	605

Table 3. Seasonal incidence of *Salmonella* and MG infection

Seasons	No. of flocks	Single <i>Salmonella</i> infection (%)	Single <i>Mycoplasma</i> infection (%)	Concurrent infection (%)	Total seropositive (%)	Total seronegative (%)	Total tested sample (N)
Winter (Dec-Feb)	38	22 (11.6)	94 (49.5)	23 (12.1)	139 (73.2)	51 (26.8)	190
Summer (Mar-May)	27	25 (18.5)	51 (37.8)	16 (11.9)	92 (68.2)	43 (31.9)	135
Rainy (Jun-Aug)	24	17 (14.2)	53 (44.2)	13 (10.8)	83 (69.2)	37 (30.8)	120
Autumn (Sep-Nov)	32	21 (13.1)	75 (46.9)	16 (10.0)	112 (70.0)	48 (30.0)	160
Total	121	85 (14.1)	273 (45.1)	68 (11.2)	426 (70.4)	179 (29.6)	605



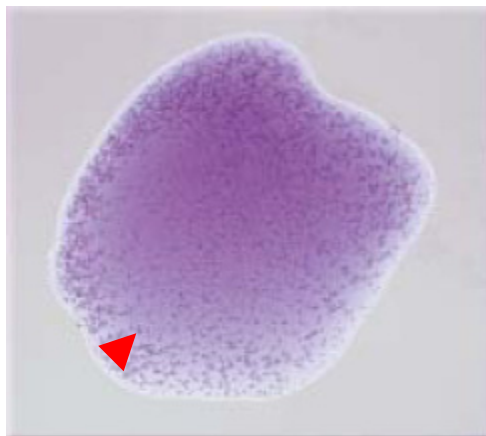
Mixing of antigen with test serum



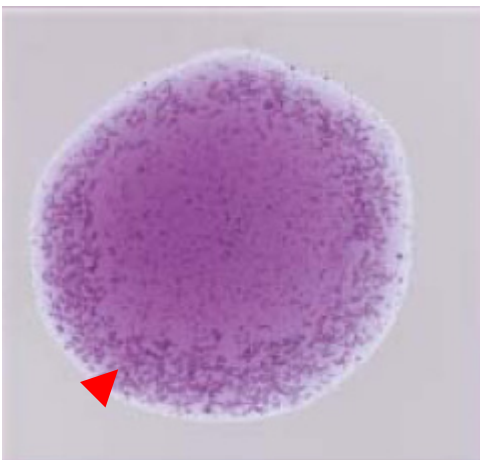
No clumps, no background clearing (-)



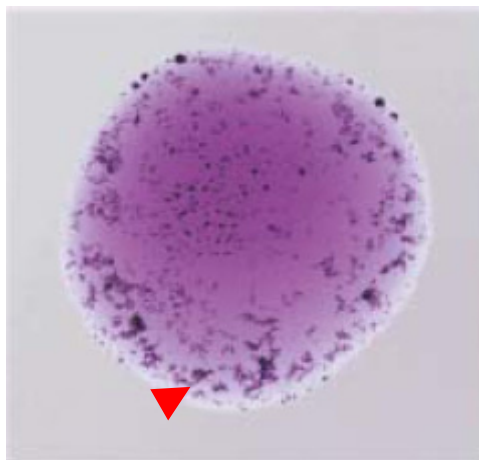
Small clumps, no background clearing (+)



Medium sized clumps almost complete background clearing (++)



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



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

Figure. Rapid plate agglutination (RPA) test, arrow indicates the agglutinated particles