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Article

Compared the effect of indirect ELISA and serum plate agglutination (SPA) test for the detection of *Mycoplasma gallisepticum* in chicken

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Abstract

Mycoplasma gallisepticum (MG) is a highly economical and persistent threat of poultry industry in Bangladesh. Indirect ELISA (iELISA) and Serum plate agglutination test (SPA) is available serological test for diagnosis of MG antibodies. The aim of this research was conducted on the basis of comparison on diagnosis results between iELISA and SPA test for MG antibody in same sample in layer chicken. Total 563 serum samples were collected and tested for MG antibody by both iELISA and SPA test. Out of 563 samples 363 (64.48%) samples were positive by iELISA and 316 (56.13%) samples were positive in SPA test. The higher incidence of MG antibody was found in chicken at 50-56 weeks and flock size was 3000-4200 as 69.63% by iELISA and 61.21% by SPA and in Sonali breeds 69.08% by iELISA and 60.64% by SPA. The results showed the comparatively higher number of positive results in iELISA test than SPA test. So the findings of the study demonstrated that a significant ($p<0.05$) difference between iELISA and SPA test present. The study may helpful for screening the flock for MG and small-holding farmers may use SPA test rather than iELISA test due to rapid, easy and cost effective.

KEYWORDS

Mycoplasma gallisepticum, iELISA, SPA, serum, chicken.

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1 Introduction

Mycoplasma gallisepticum (MG) an organism under family Mycoplasmataceae, affect chicken and turkey causes chronic respiratory disease (CRD) resulting decrease food conversion ratio (FCR), dropping egg production with hatchability as well as increase production cost (Kempf and Gesbert, 1998; Yoder, 1991). MG is the most common, constant and also an economical threat of poultry industry of Bangladesh (Arefin et al., 2011; Ali et al., 2015). The degree of severity of mycoplasmosis depends on secondary bacterial infection mainly *Escherichia coli* (OIE, 2015) and also some viruses infection such as Newcastle Disease Virus and Infectious Bronchitis Virus. Diagnosis of MG can be done by culture test, different biochemical test, serological test and molecular test. The most common and specific serological test for detection of subclinical also clinical MG in flock is indirect Enzyme Linked Immunosorbent Assay (iELISA) and Serum Plate Agglutination (SPA) test (Avakian et al., 1988; Jalilnia et al., 2011). The ELISA test is more specific can detect up to very minute level of antibody by the help of ELISA reader on the other hand the SPA test is a rapid test where agglutination of antigen-antibody reaction can detect by necked eyes (Kempf and Gesbert, 1998). Both serological tests were used for detection of specific antibodies against MG although there were differences in sensitivity and specificity of both methods (Ley, 2008; Arefin et al., 2011; Feizi et al., 2013). The experiment was designed to know the comparison of results between iELISA and SPA test and draw a conclusion between findings of two serological tests.

2 2. Materials and Methods

2.1 Collection and preparation of samples:

A total of 563 (2% of total population) blood samples were collected from 28,150 layer chickens rearing in 12 layer farms at Northern region of Bangladesh. The selected farms were on three layer breeds (Sonali, ISA Brown and White leg horn) and the birds were not vaccinated against *Mycoplasma gallisepticum* (MG). One ml blood samples were collected aseptically from wing vain by 3ml disposable plastic syringe with sterile needle without anticoagulant. Individual syringe and needle was used for individual chicken. The syringe with blood samples were kept 1 hour at 450 angles in room temperature for clot the blood within syringe. The supernatant serum was decanted in a centrifuge tube for centrifuge at 2000rpm for 5minutes and straw color clear serum was collected in a sterile eppendorf tube. The serum samples were stored at -200C until the moment of serological test was performed.

2.2 Indirect Enzyme-Linked Immunosorbent Assay (iELISA):

The all collected serum samples were kept for iELISA test for detection of specific antibodies against MG by using commercially available indirect ELISA test kit (BioChek) manufactured by BioChek UK Ltd. Co. UK (Stipkovits et al., 1993; Kempf et al., 1994). The ELISA

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test was performed according to manufacturer directions. Briefly, serum samples were diluted with sample diluents as 1:500 ratio and dispended in the respective wells of antigen coated plate. Then 100 µl of each negative control, positive control and reference control were added in A1-B1, C1-D1 and E1-D1 wells respectively. Incubated at 220C temperature for 30 minutes and then washed by wash buffer. Added 100 µl of conjugate in all wells and incubated at 220C temperature for 30 minutes then washed by same way. Finally, added 100 µl substrate solutions in all wells and kept 15 minutes and then added 100 µl stop solutions in all wells to stop reaction. Then the optical densities (OD) were read by ELISA reader (Multiskan® EX, USA) at 405nm light absorbance. The positive and negative results were interpreted by putting the OD values in BioChek ELISA software.

2.3 Serum Plate Agglutination (SPA) test:

The all serum samples were kept for SPA test by using commercially available methylene blue stained antigen (Lilli test MG RSA Antigen, UK). The SPA test of serum was performed according to standard protocol of Kempf and Gesbert, 1998. In brief, 50µl of serum was taken in white tiles and then added 50µl of methylene blue stained antigen on serum and then mixed homogenously by sterile tooth pick. Then the results of agglutination for positive results observed under light source and negative result judged by no agglutination.

2.4 Statistical Analysis:

At first the collected raw data were entered in Microsoft Excel and analyzed with SAS software (SAS, 1996). The confidence interval for comparative study between iELISA and SPA were calculated according to the formula given in SAS software and data were analyzed by χ^2-test. P-value was considered significant if $P<0.05$.

3 Results

A total of 563 serum samples were collected from 3 layer breeds at 38-61 weeks of age. Both serological tests were performed in all 563 samples for detection of specific antibodies against MG. The 363 samples were positive in iELISA test and 316 samples positive in SPA test and the percentages were 64.48% and 56.13% respectively (Table 1). By both serological tests the highest incidence shown in farm ID 1 that was 71.43% and 63.10% for iELISA and SPA test respectively. Whereas the farm ID 12 shown the lowest incidence as 50.00% by iELISA and 42.31% by SPA test (Table 1).

The figure 1 shown comparisons between iELISA and SPA test on age of layer chicken. In both tests higher positive was found in farms under 50-55 weeks of age that was 69.63% (149/214) and 61.21% (131/214) respectively. On the other hand lowest positive was found in farms under 56-61 weeks of age and results were 53.26% (49/92) and 44.57% (41/92).

The highest and lowest positive of MG antibodies was found in Sonali and White leg horn respectively in both tests among three layer breeds (Figure 2). The MG antibodies were 69.08%

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(172/249) by iELISA and 60.64% (151/172) by SPA in Sonali, 64.77% (114/176) by iELISA and 56.82% (100/172) by SPA in ISA brown, 55.80% (77/138) by iELISA and 47.10% (65/138) by SPA in White leg horn (Figure 2). The incidence of MG is related to flock size and highest incidence was found in larger flock size than smaller flock size by both iELISA and SPA test. The lowest incidence was found 56.82% (50/88) and 48.86% (43/88) in flock size 1300-1600 by iELISA and SPA test respectively and highest incidence was 69.63% (149/214) and 61.21% (131/214) by iELISA and SPA test respectively in flock size 3000-4200 (Figure 3).

Table 1: Comparison between iELISA and SPA test

Farm ID	Age (Weeks)	No. of serum tested	No. chicken/breed			iELISA results		SPA results	
			Sonali	ISA Brown	White leg horn	Positive	%	Positive	%
1	55	84				60	71.43	53	63.10
2	59	30				16	53.33	14	46.67
3	61	36				20	55.56	16	44.44
4	46	54				35	64.81	31	57.41
5	50	70				48	68.57	42	60.00
6	49	46	221	182	160	28	60.87	24	52.17
7	38	40				27	67.50	24	60.00
8	53	60				41	68.33	36	60.00
9	43	35				23	65.71	20	57.14
10	40	32				21	65.63	18	56.25
11	44	50				31	62.00	27	54.00
12	56	26				13	50.00	11	42.31
	Total:			**563**		**363**	**64.48**	**316**	**56.13**

Figure 1: Comparison between iELISA and SPA test on the basis of age of chicken.

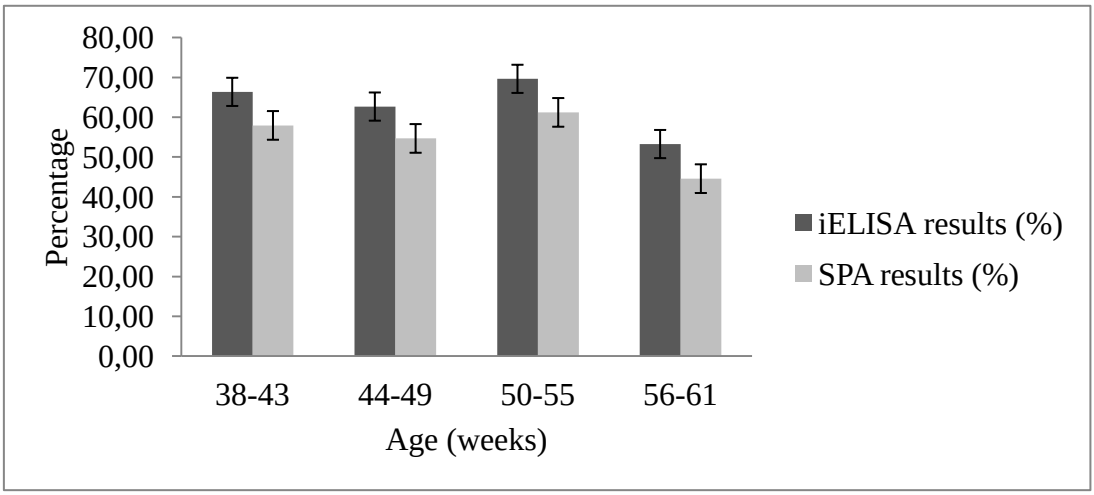

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Figure 2: Comparison between iELISA and SPA test on the basis of breeds of chicken.

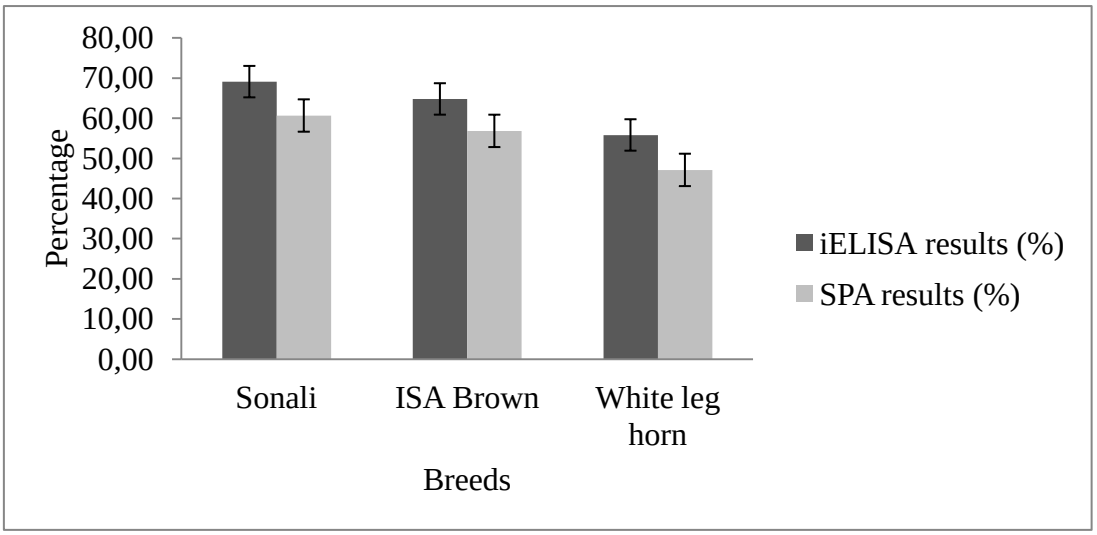
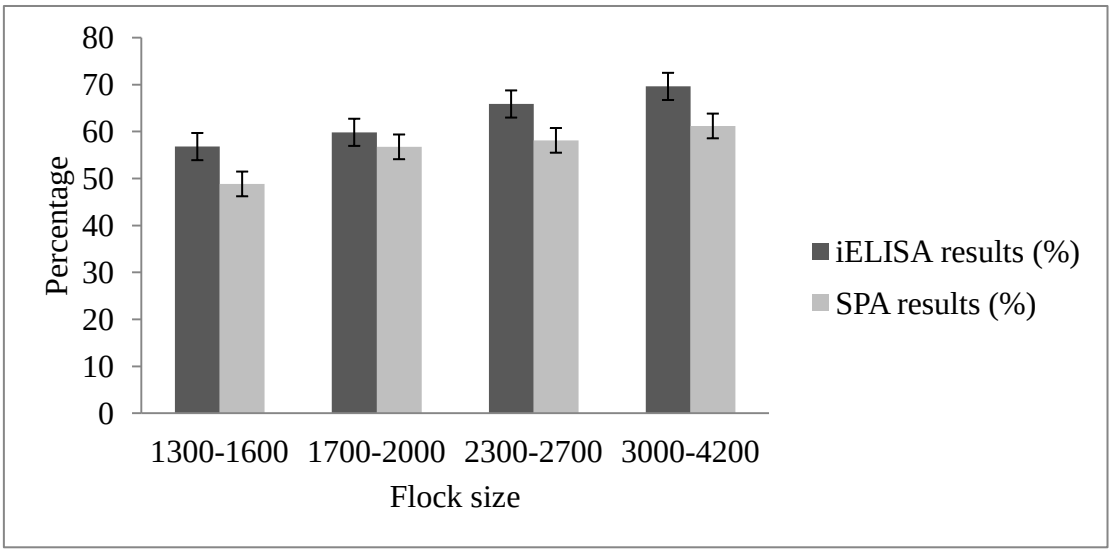


Figure 3: Comparison between iELISA and SPA test on the basis of flock size.



4 Discussion

The objective of this study was comparison between two serological tests (iELISA and SPA) for the diagnosis of MG antibody in commercial layer chicken in Bangladesh. Total 563 serum samples were collected during study period (2014-2016) from selected 12 commercial layer chicken farms in Northern region of Bangladesh. This study showed the comparative of results in between two serological tests. The overall sero-positive of MG was found 64.48% by iELISA and 56.13% by SPA test. The comparatively number of positive sample was higher in

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iELISA test than SPA test in same samples of the selected farms. Similar findings were revealed by Hossain et al. (2007) who reported overall seroprevalence of MG was 55.13% in chicken, higher prevalence was in young (72.72%) than adult (44.00%) and higher in larger flocks (62.86%) than smaller flocks (52.00%) by SPA test in Rajshahi district of Bangladesh. These results also agreed with the findings of Sikder et al. (2005) who reported overall seropositive of MG by SPA test was 56.86% and 58.90% in layer chicken and breeder farm respectively in southern part of Bangladesh.

Our results shown the number of seropositive samples were higher in iELISA (64.48%) than SPA (56.13%) test and these results disagreed with the Feizi et al. (2013), that reported seroprevalence of MG was higher in SPA (42.22%) than ELISA (33.33%) test in broiler breeder in Iran. The breed variation, biosecurity, management practices and geographical location may the cause of disagreement with findings of Feizi et al. (2013).

The flock size is a factor that influences the spread of MG within flock. The results of this study showed the higher incidence of MG in larger flock than smaller flock which is strongly agreed with the findings of Heleili et al. (2012) that revealed 76.97% incidence in large flock (18000 birds) and 20% in small flock (500-100 birds) by SPA test of commercial poultry.

The study showed significant ($p<0.05$) differences in results of both tests. Feberwee et al. (2005) demonstrated a comparative study among serological tests for MG and found a high number of false positive results in both ELISA and SPA test. The authors also explained the factors that affect directly or indirectly to test results is cross reaction, lack of inactivation, age of chickens and applications of vaccines that are very similar with the present study.

Butcher (2002) indicated that the factors which play a partial role for high number of false positive results in SPA test for MG is serum collected from vaccinated flock, contaminated serum, repeated thawing the serum before test, cross-reaction with MG antigen in MS affected flock. The use of ELISA and SPA test for MG antibody detection is only a screening tool for flocks but not for individual birds recommended by the World Organization for Animal Health (OIE).

Nascimento et al. (2000) carried out the detection of antibody by serological test are different, while SPA test detect circulating IgM antibody that develop 3-5 days of post infection (DPI) and persist 70-80 days but ELISA and HI tests detect only IgG antibodies develop 7-10 DPI and persists 180 days. Luciano et al. (2011) reported the serological test- SPA, HI and ELISA should be used only for screening tools of avian mycoplasmosis in poultry breeder flock.

This recommendation based on different sensitivity and specificity of both tests (OIE, 2015; Pourbakhsh et al. 2010). Researchers recommend that heating the serum at 56°C for 30 minutes could reduce false positive reactions (Butcher, 2002).

5 Conclusion

Although these tests were given different results so that it should be used as screening tests of MG routine monitoring for poultry flock health status. Positive results obtained of SPA test should be confirmed by additional tests such as HI, culture or molecular assays (PCR) because of the lack of specificity observed in SPA. However, the iELISA and SPA test may be

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helpful for poultry farmers to know the MG status of the flock as well as pay an important role in use of anti-mycoplasma drugs, vaccination program and biosecurity of the farm.

Conflict of interest: The authors declare that there is no conflict of interest.

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