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SEROPREVALENCE OF *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* ANTIBODIES BY RAPID PLATE AGGLUTINATION TEST IN BROILER CHICKEN FLOCKS OF HARYANA

Piyush Tomar, Y. Singh, P. Kundu, G. Narang & Neelam Rani
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar- 125 004

ABSTRACT
Avian Mycoplasmosis has become one of the most serious and frequently reported diseases being faced by the poultry industry throughout the world causing considerable economic losses.The *M. gallisepticum* (MG) and *M. synoviae* (MS) are the two most pathogenic avian Mycoplasmas. During present study, rapid plate agglutination (RPA) test was conducted with coloured antigen (Soleil Biovac, France) to know the prevalence of MG and MS antibodies in 382 serum samples of broiler chicken. Out of 382 samples, 98 were from 6 to 8 weeks old broiler chicken brought for disease diagnosis in Disease Investigation (DI) Laboratory of Department of Veterinary Public Health and Epidemiology, LUVAS, Hisar and from local meat market whereas 284 serum samples were from day old chicks from 18 different hatcheries of Haryana. Out of 98, 6 to 8 weeks old broiler chicken serum samples, 38/98 (38.77%) were found positive for either MG and/or MS by RPA test. However, a lower seroprevalence of 22/98 (22.44%) and18/98 (18.36%) was observed for individual infection of MG and MS respectively. Two samples, out of 98 samples showed presence of antibodies of both MG and MS. Higher prevalence rate of 22.44% was recorded with MG as compared to MS (18.98%) in six to eight weeks old broiler chickens. Whereas, seroprevalence data of 284 day old chicks revealed 100/284 (35.21%) positive for either MG and/or MS by RPA test. However, a lower seroprevalence of 82/284 (28.87%) and 30/284 (10.56%) was observed for individual infection of MG and MS respectively. Twelve samples, out of 284 samples showed presence of antibodies of both MG and MS. Higher prevalence rate of 28.87% was recorded with MG as compared to MS (10.56%) in day old broiler chickens. Seroprevalence in day old chicks reflects presence of maternal antibodies indicating that parent flocks were infected with MG and MS infection. From this study it might be suggested that the commercial broiler farms and parent stock should be routinely screened to monitor MG and MS infection and the reactor birds should be culled as MG and MS organism has the potential to transmit vertically.

KEY WORDS: Rapid plate agglutination test, *M. gallisepticum, M. synoviae*, broiler chicken seroprevalence.

INTRODUCTION

Poultry industry in India has made remarkable progress during the last few decades from backyard poultry to a considerably organized commercial industry. Despite rapid growth of poultry industry, it is still prone to certain infectious agents which may cause great economic losses. Different viral and bacterial diseases are the major health hazards being faced by poultry industry, among which mycoplasmosis is one of the most important. *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the OIE listed pathogen (OIE 2008) which cause disease both in poultry and turkey where as *M. meleagridis* and *M. iowae* are pathogenic to turkeys only (Bradbury, 2001; Bibank*et al*., 2013). *Mycoplasma synoviae* causes infectious synovitis or mild upper respiratory disease whereas; *Mycoplasma gallisepticum* can cause chronic respiratory disease (CRD) in chickens and have been reported to cause serious economic losses (Osman *et al*., 2009). Exacerbation of clinical signs and increase in mortality in birds with MG infection caused by concurrent bacterial and/or viral infection have been widely reported (Bradbury, 2005, Ravi*et al*., 2009). *Mycoplasma gallisepticum* infection has tropism primarily for mucosal membranes of respiratory tract, conjunctiva and sinuses (Levisohn and Klevin, 2000). The avian mycoplasmas usually enter the host via the respiratory tract except the vertical transmission. Upper airways and trachea are the preferred sites of infection for most of strains of MG and MS. In routine, usually the primary screening of flock for MG and MS is done by serological assays namely rapid plate agglutination (RPA) and/or ELISA and their confirmation by culture and/ or molecular methods (Ramadass *et al*., 2006, Zhang *et al*., 2011). Several serological tests are used to detect MG or MS antibodies, but due to variations in specificity and sensitivity, they are recommended for flock screening rather than for testing individuals (OIE, 2008). Rapid plate agglutination test is the only test which detects both IgM and IgG antibodies. This allows an earlier detection of the infection from a few days up to one week in advance compared to ELISA test.This is probably the first study in Haryana statewherein seroprevalence of MG and MS in different broiler chicken flocks has been conducted by RPA test.

MATERIALS & METHODS

The present study was undertaken on 382 serum samples collected from broiler chicken. Out of 382 samples, 98 were from six to eight weeks old broiler chicken brought

for disease diagnosis in DI lab, VPHE, LUVAS, Hisar as well as sold in local meat market. However, 284 samples were from day old broiler chicks, collected from 18 different hatcheries of Haryana. Blood was collected either from jugular vein or directly from heart, and serum was separated aseptically within 24 h and stored at -20°C until use. None of the chicken had a history of vaccination with any of MG or MS vaccine. The serum and antigen were brought to room temperature (20 -25°C) before performing the test.The RPA test was conducted with coloured antigen of MG and MS (Soleil Biovac, Animal health, France) as per method described by Shadmanesh and Mokhtari (2013). The test was performed by placing 25 µl of undiluted serum on a clean perspex glass plate and mixed with equal volume of coloured MG/MS antigen with the help of micropipette tips. The plate was rotated gently to swirl the mixture and results were recorded within two minutes. Presence of clear blue violet agglutinates (clumps) within two minutes were considered positive. Reaction was considered negative if no agglutinate was detected after two minutes of mixing.

The results of RPA test to know the seroprevalence of MG and MS in 382 broiler chicken of Haryana are shown in table 1. The clumps usually started appearing in the middle and became concentrated at the periphery of the mixture. Negative reaction was judged by the absence of agglutination reaction. Positive reaction was characterized by the formation of definite clumps within 2 min after mixing the test serum with antigen. The strength of the agglutination reaction was measured according to the following criteria (Fig. 1):

- = Absence of clumps without background clearing
+ + = Small clumps without background clearing
+++= Medium sized clumps with almost complete background clearing
++++ =Large clumps with complete background clearing

RESULTS & DISCUSSION

The results revealed 38.77% (38/98) seroprevalence for *Mycoplasma* (MG and/or MS) infection in broiler flocks of 6-8 weeks old. Comparable seroprevalence was also noticed by Srinivassan *et al.* (2014) who reported seroprevalence of 50% (30/60) *Mycoplasma* (MG and MS) infection in Tamil Nadu. The seroprevalence of individual MG and MS infection observed was 22.4% and 18.36% respectively. However Helleli *et al*. (2012) reported 69% MG seroprevalence by RPA in broiler and layer chicken at Batna commercial farms in Algeria. Shadmanesh and Mokhtari (2013) also reported very high prevalence of MG (85%) by RPA in native hens of Eghlid, Iran. Serovalence of MS by RPA recorded in 6-8 weeks broiler chicken in the present study was 18.36%. On the other hand, Helelli, (2012) reported high seroprevelance of MS (66%) in broiler and layer chicken at Batna commercial farms in Algeria. On the contrary, Shadmanesh and Mokhtari (2013) found very less seroprevalence of MS (2%) by RPA in broiler poultry flocks of Iran. Seroprevalence data of 284 day old chicks revealed 100/284 (35.21%) positive for either MG and/or MS by RPA test. However, a lower seroprevalence of 82/284 (28.87%) and 30/284 (10.56%) was observed for individual infection of MG and MS respectively. Twelve samples, out of 284 samples showed presence of antibodies of both MG and MS. Higher prevalence rate of 28.37% was recorded with MG as compared to MS (10.56%). However, Elgnay and Azwai (2013) detected very low MG (3.4%) and MS (6.4%) antibodies in 1 day old broiler chickens in Libya. In one study, slightly higher seroprevalence (57%) of MG by RPA was reported by Sarkar *et al*. (2005) in selected model breeder poultry farms of Bangladesh as compared to 28.87% seroprevalence of present study in day old broiler chicks which reflects maternal antibodies from breeder stock.Day old chicks have only IgG immunoglobulin in blood because IgM is not transferred via maternal circulation. The transfer starts from day 7 of embryonic development and reaches its maximum rate 3 to 4 days before hatch. The amount of IgG transferred to the egg yolk and from the egg yolk to the embryo has been reported to be proportional to maternal serum IgG concentrations (Soares, 2008). Since RPA test detects both IgM and IgG immunoglobulin, it can be used for detecting maternal antibodies (IgG) in 1 to 3 day-old chicks specific for MG and MS infection.

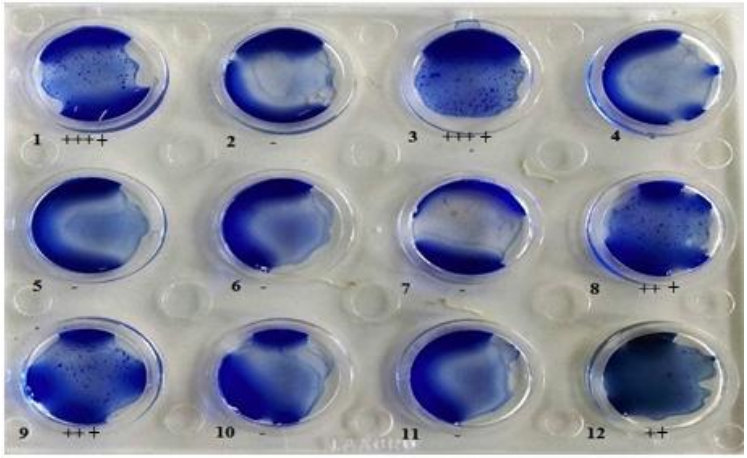

FIGURE1:Photograph showing the results of rapid plate agglutination test using MG antigen and sera collected from broiler chicken
Well 1= Positive control, Well 2 =Negative control, Wells 3-12= Field sera
++ = Weak, +++ = Moderate, ++++ = Marked, - = Negative

In this study Rapid plate agglutination (RPA) test was conducted in day old chicks which reflect maternal antibodies, so it indicates that parent flocks were infected with MG and MS infection. The lower seroprevalence of both MG and MS recorded in present study may be due to collection of samples from apparently healthy flocks. The presence of antibodies in 6-8 weeks old broiler chickens for MG and MS in healthy as well as birds affected with respiratory infection indicates that avian mycoplasmosis is prevalent in Haryana. Efforts needs be made towards educating the poultry farmers for the effective control of MG and MS in breeder farm, broiler farm and the hatcheries through good management practices, regular screening and culling of positive parent stock and adoption of appropriate prophylactic or therapeutic remedies. Vaccines may also be used in breeding stock to reduce transmission through egg or to aid MG eradication on multi-age sites (OIE, 2008).

TABLE 1: Seroprevalence of MG and MS antibodies by RPA test in broiler chicken

Result of serum samples from Broiler Chicken Flock (6-8 weeks) of Haryana						
Location of poultry flock	No. of sera tested	Age group	No. of samples positive for		No. of samples positive for both MG and MS (%)	Total no. of positive Samples (%)
			MG(%)	MS(%)		
DI lab, Hisar	27	6-8 weeks	6 (22.22)*	4 (14.81)	1 (3.7)	9 (33.33)
Dabra	12	7 weeks	2 (16.66)	2 (16.66)	-	4 (33.33)
Hansi	16	8 weeks	3 (18.75)	2(16.66)	-	5 (31.25)
Satrod	15	7 weeks	1 (06.66)	5 (33.33)	-	6 (40)
Balsamand	15	6 weeks	1 (06.66)	4 (26.66)	-	5 (40)
Rawalwas	13	7 weeks	9 (69.23)	1 (07.69)	1 (7.69)	9 (69.23)
Total	98	6-8 weeks	22 (22.44)	18 (18.36)	2 (2.04)	38 (38.77)
Result of serum samples of 1-2 days old broiler chicken from different hatcheries						
Location of Hatchery	No. of sera tested	Age group	No. of samples positive for		No. of samples positive for both MG and MS (%)	Total no. of Positive Samples (%)
			MG(%)	MS (%)		
Hansi	24	1 day	3 (12.5)	3 (12.5)	-	6 (25)
Jind	16	1 day	10 (62.5)*	7 (43.75)	5 (31.25)	12 (75)
-do-	17	1 day	4 (23.5)	10 (58.8)	3 (17.74)	11 (64.7)
-do-	20	1 day	10 (50)	1 (5)	1 (5)	10 (50)
-do-	13	2 days	8 (61.5)	-	-	8 (61.5)
-do-	19	1 day	8 (42.1)	-	-	8 (42.1)
Safidon	15	1 day	1 (6.66)	1 (6.66)	-	2 (13.33)
-do-	14	1 day	1 (7.14)	-	-	1 (7.14)
Jind	14	1 day	4 (28.57)	-	-	4 (28.57)
-do-	16	1 day	3 (18.75)	3 (18.75)	1	5 (31.25)
-do-	12	1 day	4 (33.33)	1 (8.33)	-	5 (41.66)
-do-	16	1 day	5 (31.25)	1 (6.25)	-	6 (37.5)
Panipat	15	1 day	-	-	-	-
-do-	13	1 day	6 (46.15)	1 (7.69)	1 (7.69)	6 (46.15)
-do-	16	1 day	-	-	-	-
-do-	16	1 day	1 (6.25)	1 (6.25)	1 (6.25)	1 (6.25)
-do-	9	1 day	1 (11.11)	1 (11.11)	-	2 (22.22)
-do-	19	1 day	13 (68)	-	-	13 (68)
Total	284	1-2 days	82 (28.87)	30 (10.56)	12 (4.225)	100 (35.21)

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REFERENCES

Bibank, F., Kalidari, G.A., Razmyar, J. and Rad, M., (2013) Isolation of Mycoplasma spp from broiler flocks with respiratory syndrome in Mashhad, Iran. Iranian J Vet. Sci. Tech, 5 (1), 11-118.

Bradbury, J.M. (2005) Poultry mycoplasmas: sophisticated pathogens in simple guise. British Poultry Science, 46, 125-136.

Bradbury, J.M., Yavari, C.A. and Dare, C.M., (2001) Detection of Mycoplasma synoviae in clinically normal pheasants. *Vet Rec*, 148, 72-74.

Elgnay, F.S. and Azwai, S.M. (2013) Seroprevalence of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* in one day old broiler chickens in Libya.Journal of Animal and Poultry Sciences, 2(1), 11-18

Helelli, N., Ayachi, A., Mamache, B. and Chelihi, A.J., (2012) Seroprevalence of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* at Batna commercial poultry farms in Algeria. Vet. World, 5 (12), 709-712.

Levisohn, S. & Kleven, S.H. (2000) Avian mycoplasmosis (*Mycoplasma gallisepticum*). Rev. sci. tech. Off. int. Epiz, 19, 425-442.

OIE (2008) Manual of Diagnostic tests and Vaccines for Terrestrial Animals. Chapter 2.3.5: Avian mycoplasmosis (*Mycoplasma gallisepticum,* M. synoviae), pp. 482-496

Osman, K.M, Aly, M.M., Amin. Z.M. and Hasan, B.S., (2009) *Mycoplasma gallisepticum*: an emerging challenge to the poultry industry in Egypt. Rev. Sci. Tech. O.I.E, 28(3): 1015-1023.

Ramadass, Ananthi, R., Senthilkumar, T.M.A., Venkatesh, G.& Ramaswamy, V. (2006) Isolation and characterization of Mycoplasma gallisepticum and *Mycoplasma synoviae* from poultry. Indian J. Anim. Sci, 76 (10), 796-798.

Raviv, Z., Ferguson-Noel, N., Laibinis, V., Wooten, R. and Kleven, S.H., (2009).Role of *Mycoplasma synoviae* in commercial layer *Escherichia coli* peritonitis syndrome. Avian Diseases, 51, 685-690.

Sarkar, S. K., Rahman, M.B., Rahman, M., Amin, K.M.R., Khan, M.F.R., and Rahman, M.M. (2005) Seroprevalence of*Mycoplasma gallisepticum* in chickens from a model breeder poultry farm in Bangladesh *International Journal of Poultry Science*, 4, 32-5.

Shadmanesh, A and Mokhtari, M.M. (2013) Serological investigation of five diseases; influenza, newcastle, salmonella, *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in native hens of Eghlid, Iran, Vet. World, 6 (3), 126-130.

Soares Roberto. (2008) In: Passive immunity. Cevasenta animale, Issue No.18

Srinivassan, P., Balasubramaniam, G.A., Gopala, Krishna Murthy, T.R. and Balachandran, P. (2014) Prevalence and pathology of oviduct impaction in commercial white leghorn layer chicken in Namakkal region of India. Veterinary World.org, 7, 553-558.

Zhang, J, Guo., Y, HuS, B., Wang, X., Xiao, Y., Gao, X., LiZ, L.M., Peng, F. and Xu, Q., (2011). An adhesion molecule based colloidal gold immune chromatography assays trip for rapidly and specifically detecting chicken antibodies against *Mycoplasma gallisepticum*. Res. Vet. Sci, 90(3), 379-384.