Journal of Animal Research: v.6 n.1, p. 49-51. February 2016

DOI: 10.5958/2277-940X.2016.00009.7

Detection of Anti *Mycoplasma gallisepticum* Antibodies in Different Age Group of Chicken by Enzyme Linked Immunosorbant Assay

Namrata Singh[1], Sanjay Shukla[1]* and Varsha Sharma[2]

[1]Department of Veterinary Microbiology, COVS & A.H., Rewa, Madhya Pradesh, INDIA
[2]Department of Veterinary Microbiology, COVS & A.H. Jabalpur, Madhya Pradesh, INDIA

Corresponding Author: SK Shukla; Email: shukla_vet20@yahoo.co.in

Received: 13 January, 2015 **Accepted:** 30 January, 2016

ABSTRACT

Mycoplasma gallisepticum is an important *Mycoplasma* species, causes chronic respiratory disease in poultry. Present study was conducted with the aim of detection of anti *Mycoplasma gallisepticum* antibodies in different age group of chicken by enzyme linked immunosorbent assay. Chicken serum samples were collected from different areas and unorganized farm of district Rewa (Madhya Pradesh). A solid phase blocking ELISA test was performed in serum samples using SVANOVIR MG-Ab, Sweden kit. Around 98 serum samples of different age group of chicken were collected. Chicken were divided into four different age groups viz. group I (6-24 Wks), II (25-42 Wks), III (43-60 Wks) and IV (61-77 Wks), each group further divided into three subgroups according to age. Age wise study of *Mycoplasma gallisepticum* antibody detection revealed that age group I (6-24 wk age), II (25-42 Wk age), III (43-60 Wks) and IV(61-77 wk) were showing 30%, 20.83%, 20.83% and 12.50% seroconversion respectively. Study revealed that age group 6-24wks showing maximum antibody titer and age group 61-77 wks showing minimum antibody titer. Age subgroup 6-12 wks showing maximum 40% antibody titer. In overall study, Out of 98 samples tested 21.40% samples were positive by ELISA test. ELISA is used as a highly specific test for the detection of anti MG antibodies in chicken serum.

Keywords: *Mycoplasma gallisepticum,* Seroconversion, antibody titer

Mycoplasma gallisepticum is the most economically significant pathogenic *Mycoplasma* in poultry (Kleven and Levisohn 1996; Ley and Yoder 1997). This organism is the causative agent of chronic respiratory disease in chickens and is highly transmissible. Though, all ages of chickens are susceptible to mycoplasma infection but young birds are more prone to infection than adults (Nunoya *et al.* 1995). MG infection causes air sacculitis, sneezing, conjunctivitis, and decreased egg production in affected birds. Through direct contact, MG organisms from contaminated birds can be transmitted to the other birds, that is to say horizontal transmission, but affected breeder bird can spread MG organisms from their progeny which is, vertical transmission (Ley 2003).

In layers and broiler breeder chicken, flocks infected with MG there is production losses between 10 and 20% have been reported (Bradbury 2001). Many serological tests is performed to diagnose *Mycoplasma gallisepticum* infection viz. serum plate agglutination test, hemagglutination inhibition test and enzyme-linked immunosorbent assay. The screening test commonly used is serum plate agglutination test, but there is false-positive reactions reported in the plate test (Glisson *et al.* 1984; Yoder 1989), that's why positive sample is confirmed by either enzyme-linked immunosorbent assay or hemagglutination inhibition test. Prevention and control measure of Mycoplasmosis include surveillance (identification, isolation, culture and serology) and vaccination, account for additional costs (Mohammed *et al.* 1987; Saif *et al.* 2003).

MATERIALS AND METHODS

Study Area

Detection of anti *Mycoplasma gallisepticum* antibody was

carried out in the chicken population of in and around the Rewa district (Madhya Pradesh), India. Rewa district situated in the north eastern part of Madhya Pradesh. The climate of the district is humid subtropical with cold, misty winters, hot summer and humid monsoon season.

Samples

Total 98 numbers of serum samples of chickens were collected from Amahiya, Bichhiya, Bara, Indira Nagar and from small un-organized farm of in and around Rewa (MP). Birds were divided to four age group and within each group three subgroup divided. Blood collection carried out from wing vein of chicken and stored at sterile collection tube. Blood sample were kept in slanting position and allowed to clot for the separation of serum. Serum samples were stored at -20^{o}C until they were used. Serum samples divided into four group, Group I (6-24 Wks), II (25-42 Wks), III (43-60 Wks) and IV (61-77 Wks), each group further divided into three subgroups according to age (Table 1). All the serum samples tested for the presence of anti *Mycoplasma gallisepticum* antibodies by ELISA kit.

Procedure of ELISA Test

The SVANOVIR MG-Ab ELISA kit (Sweden) was used in the present study. In brief, 100µl positive control and 100 µl negative control serum were added to select well in duplicate. Then after, 50 µl of unknown serum samples were added to *Mycoplasma gallisepticum* antigen coated plate along with 50 µl of PBS-Tween buffer, followed by incubation for 30 minutes at room temperature. After washing of three times with PBS-Tween buffer, 100 µl of HRP conjugate was added to each well. Following incubation for 30 minutes at room temperature and repeating the washing procedure again, 100 µl of substrate solution was added to each antigen coated well. After incubation for 10 minutes at room temperature 50 µl of stop solution was added to each well and mixed thoroughly, the optical density of control and samples was read at 450 nm on a micro plate photometer.

In order for calculation of result mean O.D. value for each of control and samples were calculated. Percent inhibition value calculated by using formula, $OD_{Negative\ control} - OD_{samples\ or\ positive\ control} / OD_{Negative\ control} \times 100$. Interpretation made on the basis that PI>40 for positive, PI= 30-40 doubtful and PI<30 for negative samples.

RESULTS AND DISCUSSION

Age wise study of anti *Mycoplasma gallisepticum* antibody detection in chickens revealed that age group I (6-24 Wks age), II (25-42 Wks age), III (43-60 Wks) and group IV (61-77 Wks) was showing 30%, 20.83%, 20.83% and 12.50% sero-conversion respectively. Study revealed that age group 6-24 wks showing maximum antibody titer and age group 61-77 wks showing minimum antibody titer. Age group 6-12 wks showing 40% antibody titer. Overall 21.40% *Mycoplasma gallisepticum* antibody level was detected in 98 birds (Table 2) (Fig. 1).

Table 1: Collection of serum samples from different age group of chicken

Group	Sub groups	Age of birds (Weeks)	No. of bird tested
Group I	IA	6-12	10
	IB	13-18	8
	IC	19-24	8
Group II	IIA	25-30	8
	IIB	31-36	8
	IIC	37-42	8
Group III	IIIA	43-48	8
	IIIB	49-54	8
	IIIC	55-60	8
Group IV	IVA	61-66	8
	IVB	67-72	8
	IVC	73-77	8
	Total		98

Table 2: Status of anti-*Mycoplasma gallisepticum* antibody *by* ELISA in different age group of chicken

Group	Sub groups	Positive percentage of age by ELISA	Group wise positive percentage of age
Group I	IA	40.00	30.00
	IB	25.00	(Kempf *et al.* 1994)
	IC	25.00	
Group II	IIA	25.00	20.83
	IIB	25.00	
	IIC	12.50	
Group III	IIIA	12.50	20.83
	IIIB	25.00	
	IIIC	25.00	

Group IV	IVA	12.50	12.5
	IVB	12.50	
	IVC	12.50	
Overall positive		21.40%	

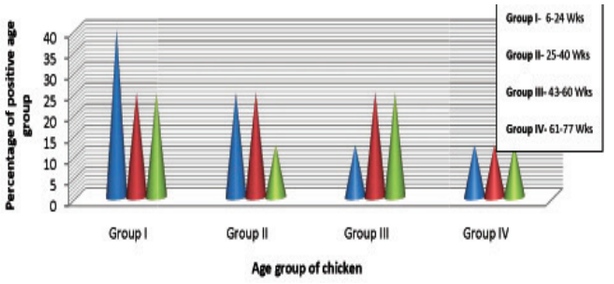

Fig. 1: Age wise status of anti *Mycoplasma gallisepticum* antibodies in chicken

The result findings are in accordance with Ahmed *et al.* 2008, where they carried out status of anti MG antibodies in chicken and found that younger birds are more prone to MG antibodies than older birds. Most positive reactors were seen in 6-7 week of age these result are similar to Asgharzade *et al*. 2013, where they experimentally infected chicken with *Mycoplasma gallisepticum* and observed the positive reactor at the age of 6-7 week by using commercial ELISA kit. Clinical findings of the results is correlated with findings of Nunoya *et al.*1995, after the detection of *Mycoplasma gallisepticum* antibodies in the chicken serum by ELISA, which showed the appearance of clinical symptom was seen in 10 week old chicken.

CONCLUSION

Mycoplasma gallisepticum antibody detection in chicken revealed that Mycoplasmosis can be a serious problem in chicken especially in the age group of 6-12 wks old chicken, so effective measure should be carried out to get rid of infection. Prevention and control measure should be carried out through vaccination and treatment of infection.

ACKNOWLEDGEMENTS

Authors are thankful Dr. S.P. Shukla, Dean College of Veterinary Science and Animal Husbandry, Rewa, NDVSU, Jabalpur for extending all the help required to carry out research work as and when needed.

REFERENCES

Ahmad, A., Rabbani, M., Yaqoob, T., Ahmad, A., Shabbir M.Z. and Akhtar., F. 2008. Status of igg antibodies against *mycoplasma gallisepticum* in nonvaccinated commercial poultry breeder flocks. *J. Anim. Pl. Sci.,* **18**: 2-3.

Asgharzade, S., Zaeri, S., Hasanzade, M., Ahmadi, M. and Talebi A.R. 2013. Detection of *Mycoplasma gallisepticum* in experimentally infected broiler chickens using Culture, S P A , ELISA, and PCR methods. *Com. Clin. Pathol.,* **6:** 1051-1055.

Bradbury, J.M. 2001. Avian Mycoplasmosis, In: Frank Jordan et al. (eds.) Poultry Diseases. 5th edn. W.B. Saunders Company, Iowa, pp: 178-193.

Glisson, J.R., Dawe, J.F. and Kleven, S.H. 1984. The effect of oil-emulsion vaccines on the occurrence of nonspecific plate agglutination reactions for *Mycoplasma gallisepticum* and *Mycoplasma synoviae. Avian Dis.*, **28:** 397-405.

Kempf, I., Gesbert, F., Guittet, M., Bennejean G. and Stipkovtts, L. 1994. Evaluation of two commercial enzyme-linked immunosorbent assay kits for the detection of *Mycoplasma gallisepticum* antibodies. *Avian Path.,* **23**: 329-338.

Kleven, S.H. and Levisohn S. 1996. *Mycoplasma* infections of poultry. In Molecular and diagnostic procedures in mycoplasmology, Vol. II (J.G. Tully and S. Razin, eds). Academic Press, New York, 283-292.

Ley, D.H. 2003. *Mycoplasma gallisepticum* infection. In: Diseases of Poultry. 11th ed. Y.M. Saif, H.J. Barnes, A.M. Fadly, J.R. Glisson, L.R. McDougald, and D.E. Swayne (eds.). Ames : Iowa State University Press 122 - 144.

Ley, D.H. and Yoder H.W. 1997. *Mycoplasma gallisepticum* infection. In Diseases of poultry, 10th Ed. (B.W. Calnek with H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, eds). Mosby-Wolfe, London, 194-207.

Mohammed, H.O., T.E. Carpenter and Yamamoto, R. 1987. Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma Synoviae* in commercial layer flocks. *Avian Dis*., **31:** 477-482.

Nunoya, T., Yagihashi, T., Tajima, M. and Nagasawa Y. 1995. Occurrence of keratoconjunctivitis apparently caused by *Mycoplasma gallisepticum* in layer chickens. *Vet. Patho.*, **32:** 11-18.

Saif, Y.M., H.J. Barnes, A.M. Fadly, J.R. Glisson and D.E. Swayne, 2003. Poultry Diseases, 11th Edition., Iowa State Press, Iowa, pp: 719-774.

Yoder, H.W. 1989. Nonspecific reactions to mycoplasma serum plate antigens induced by inactivated poultry disease vaccines. *Avian Dis.*, **33:** 60-68.