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Seroprevalence of *Mycoplasma* Infection in Broiler Population of Assam

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ABSTRACT

The experiment was conducted in the Department of Veterinary Pathology, C.V.Sc, A.A.U., Khanapara, Guuwahati, Assam, India during September, 2020–August, 2021. Avian Mycoplasmosis is a severe threat to the poultry industry. *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) represent the most important Avian Mycoplasma spp. worldwide in the poultry industry. All age groups of chickens and turkeys are susceptible to infection. Most of the outbreaks were recorded between 3rd and 6th weeks of age. The disease is characterized by respiratory rales, coughing, nasal discharge, conjunctivitis, sinusitis and air sac lesions. In the present study, a total of 400 sera were tested for detection of antibodies from 29 farms from 11 different locations of Kamrup (M) and Kamrup (R) district, Assam. For detection of *Mycoplasma* antibodies, indirect ELISA was performed by using commercially available kits for MG and MS (IDEXX Laboratories). Out of the total samples tested, 13.25% showed sero-positivity for MG and 7.25% showed sero-positivity for MS. Among different age groups, highest sero-positivity was recorded in age group of above 5 weeks (8.5%) and lowest sero-positivity was recorded in birds of age 3–4 weeks (0.75%) for MG. For MS, the highest sero-positivity was recorded in birds of age group of above 5 weeks (5.25%) and lowest sero-positivity was recorded from age 3–4 weeks (0.5%). Season-wise highest sero-positivity was recorded in winter (6.5%), followed by post-monsoon (4.25%), monsoon (1.75%) and pre-monsoon (0.75%) for MG. In case of MS, highest sero-positivity was recorded in post-monsoon (3%), followed by winter (2.75%), monsoon (1%) and pre-monsoon (0.5%).

KEYWORDS: Broiler, chronic respiratory disease, elisa, mycoplasma, seroprevalence

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Poultry farming is one of the fastest-growing segments of the agricultural sector today in India with an average growth of 8–10% annum^{-1}. Poultry farming in India has transformed into a techno-commercial industry from the status of backyard farming since last three decades. It plays a vital role in the socio-economic development of poor and landless farmers. Assam is one of the major chicken rearing states in India. In Assam, chicken fulfill a significant proportion of animal protein like any other states of the country in the form of meat and eggs. India is the third largest producer of eggs and fifth major producer of broiler chicken meat in the world (Anonymous, 2019).

Among the different diseases, Avian Mycoplasmosis is an economically important disease and poses severe threat to the poultry industry (Anonymous, 2008, Sarika et al., 2019). In the early 1900s, Mycoplasmosis was described for the first time as a respiratory disease of poultry (Sawicka et al., 2020). Avian mycoplasmosis was first described in turkeys in 1926 and in chickens in 1936 (Umar et al., 2017). *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) represent the most important Avian Mycoplasma spp. worldwide in the poultry industry (Felice et al., 2020). Avian Mycoplasmosis is also prevalent in other countries, i.e. 45.1% broilers in Bangladesh (Hossain et al., 2010), 0.9% Layers and 2.7% broilers in Belgium (Michiels et al., 2016) and Egypt, China, Malaysia, Vietnam, Thailand, etc. (Osman et al, 2009). They are minute in size and there is total lack of cell wall (Atalla et al., 2015). Respiratory tract mucosa is the main target tissue for *Mycoplasma* though other tissues are also affected (Ley, 2003). Both horizontal (by close contact, contaminated dust particles, infectious aerosols or droplets) and vertical (through infected eggs) transmission has been observed in Mycoplasma infection (Kleven, 2008, Rajkumar et al., 2018).

CRD is an important respiratory disease of chickens and turkey which affects both the grower and adult birds (Peebles and Branton, 2012). The disease is characterized by respiratory rales, coughing, nasal discharge, conjunctivitis, sinusitis and air sac lesions (Bradbury, 2005, Brown et al., 2007, Rajkumar et al., 2018). Brar et al. (2017) observed various degrees of haemorrhages in trachea and lungs. Severe congestion and consolidation seen in lungs and in some cases, white colored necrotic foci or zones of necrosis were observed (Rajukumar et al., 2017). Rajkumar et al. (2017) noted various gross lesions like sinusitis, conjunctivitis, tracheitis with yellowish cheesy material in trachea. In addition to the commercial poultry flocks, the disease has been reported from the backyard poultry (Yadav et al., 2021). All age groups of chickens and turkeys are susceptible to *Mycoplasma gallisepticum*, but young birds are considered to be affected severely than older birds (Singh et al., 2016). MS infection is mostly considered to occur as a subclinical upper respiratory infection, which can progress to respiratory disease with air sac lesions, when combined with Newcastle disease or Infectious Bronchitis, and to Infectious Synovitis when it becomes systemic (Soeripto et al., 1989, Felice et al., 2020). The bacteria multiplies in trachea, lungs and air sacs and rarely in sinuses (Gondal et al., 2015).

ELISA is more specific and sensitive as compared to SPAT (serum plate agglutination test) and HI (Haemagglutination Inhibition) tests (Czifra et al., 1993, Yadav et al., 2021). Although Mycoplasma infection is very common in the chicken population of Assam, the reports on seroprevalence of Avian Mycoplasmosis is meager in available literature. The present communication deals with the sero-epidemiology of Mycoplasma infection in broiler population of Kamrup (M) and Kamrup (R) districts of Assam by using indirect ELISA.

2. MATERIALS AND METHODS

The present study was conducted in the Department of Veterinary Pathology, C.V.Sc, A.A.U., Khanapara, Guuwahati, Assam, India.

2.1. Ethical approval

The research work was approved by the Institutional Animal Ethics Committee (IAEC) vide letter No: 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/20-21/897, dated 31.07.2021.

2.2. Study area

The study was conducted from September, 2020–August, 2021 in two districts of Assam viz. Kamrup (Metro) and Kamrup (Rural). The region is located between 91°14'60.00" E Longitude and 26°19'60.00" N latitude and shares common state boundary with Meghalaya in southern side (Figure 1).

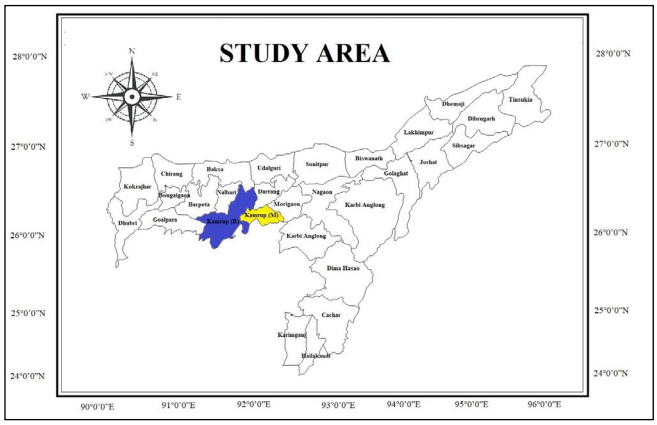

Figure 1: Map of Assam indicating study districts

2.3. Study populations

The study populations were unvaccinated broiler chicken populations above 3 weeks of age from 11 different locations of Kamrup (M) and Kamrup (R) districts of Assam.

2.4. Study design

A cross sectional study was conducted to estimate the seroprevalence of Avian Mycoplasmosis in Kamrup (M) and Kamrup (R) districts and a total of 29 broiler farms were included in the present study. A structured questionnaire was prepared to collect data such as age, health status, previous occurrence of any other diseases etc.

2.5. Design of study and sampling

The sampling methods followed in the study was simple random sampling to select the study population based on access to transportation, history of previous occurrence of disease, no vaccination etc. The bird from individual flock was selected randomly to reached the required sample size. Since there was no previous study Avian Mycoplasmosis in the selected areas, the present study considered 50% expected prevalence (P), 95% confidence level and 5% absolute precision or Marginal error. Based on these assumptions, the total number of animals to be included in the investigation was determined by using the following formulae described by Daniel (1999).

Sample size (n)=(Z^2 Pexp (1-Pexp)/d^2 ……(1)

Where n=required sample size, P_{exp}= expected prevalence, d^2=desired absolute precision and Z is multiplier from normal distribution at 95% confidence interval (1.962).

Thus, the calculated value with the formula comes out as 384, however in order to improve the precision, the sample size was increased up to 400.

2.6. Association of different factors

2.6.1. Season

A calendar year was divided into four (4) seasons viz. pre-monsoon (March–May), monsoon (June–September), post-monsoon (October–November) and winter (December–February) as per Regional Meterological Centre, Barjhar, Guwahati, Assam.

2.6.2. Age

To study the effect of age on Seroprevalence and occurrence of the disease, the birds were divided into 3 age groups, viz. 3–4 weeks age, 4–5 weeks age and above 5 weeks age.

2.7. Sample collection and transport

The blood samples (about 2 ml) were collected from the medial metatarsal vein in clot activator vial. After proper labeling and transportation to the laboratory, serum was separated by centrifuging at 4000 rpm for 10 m. Then the separated serum was collected in a screw capped plastic vial and stored at -20°C for further use.

2.8. Serological analysis of the samples

For detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies, indirect ELISA was performed by using commercially available kits for MG and MS (IDEXX Laboratories) as per manufacturer's protocol. Optical density (O.D.) of the wells were measured at 650 nm in ELISA reader.

2.9. Interpretation

A test sample is considered positive if the S/P ratio is >0.50 and a test sample is considered negative if the S/P ratio is ≤0.50. S/P value was calculated as

S/P value=(Mean OD of test sample-Mean OD of negative control)/(Mean OD of positive control-Mean OD of negative control) ……(2)

2.10. Data analysis

The data generated from the laboratory analysis and questionarie survey were recorded and analyzed by standard procedures by using R-software (Version 3.5.0) (R Core Team, 2018) for statistical representation. The alpha level was set at 0.05 and 95% confidence interval (CI 95%) was calculated. Chi-square test was done and *p*-value was calculated to look for any significant association between effects and factors. If the probability value (*p* value) is less than or equal to set alpha level (0.05) then the result was considered as statistically significant.

3. RESULTS AND DISCUSSION

In the current study, the Seroprevalence of Avian Mycoplasmosis was carried out in 11 different locations of Kamrup (M) and Kamrup (R) districts of Assam. The spatial distribution of *M. gallisepticum* (MG) *and M. synoviae* (MS) in different locations has been shown in Table 1 and Figure 2.

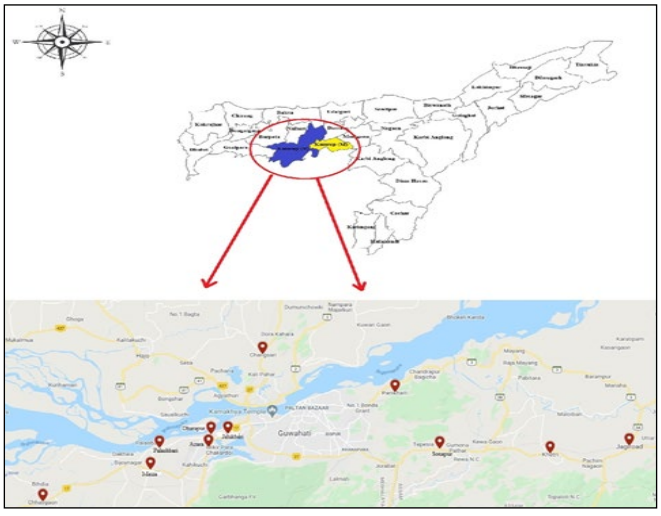

Figure 2: The spatial distribution of Avian Mycoplasma antibodies in different locations of undivided Kamrup district, Assam

Table 1: Seroprevalence of Avian Mycoplasma antibodies in different locations

Location	Total samples	Positive samples		Positivity percentage	
		MG	MS	MG	MS
Chhaygaon	32	5	2	1.25	0.5
Mirza	43	11	4	2.75	1
Sonapur	46	7	3	1.75	0.75
Jagiroad	44	3	2	0.75	0.5
Jalukbari	27	2	3	0.5	0.75
Changsari	32	4	1	1	0.25
Dharapur	34	2	2	0.5	0.5
Azara	36	5	3	1.25	0.75
Palashbari	47	7	4	1.75	1
Panikhaiti	26	3	1	0.75	0.25
Khetri	33	4	4	1	1
Total	400	53	29	13.25	7.25
Chi-square value		10.2334	4.12113		
p-value [Sig=*p*<0.05; NS=*p*>0.05]		NS	NS		

Out of the total of 400 serum samples were tested, 53 (13.25%) showed positivity for *Mycoplasma gallisepticum* (MG) and 29 (7.25%) showed positivity for *Mycoplasma synoviae* (MS) by i-ELISA. Highest (2.75%) seropositivity of MG was recorded from Mirza and lowest (0.5%) seropositivity from Jalukbari and Dharapur. Similarly, highest (1%) sero-positivity of MS could be recorded from Mirza, Palashbari and Khetri and lowest (0.25%) from Changsari and Panikhaiti. Seroprevalence of the antibodies of MG and MS have insignificant association (*p*=ns) between different locations. Rajkumar et al. (2018) reported a Seroprevalence rate of *Mycoplasma gallisepticum* as 52.1% from 5 States viz. Telangana, Karnataka, Gujrat, Himachal Pradesh and West Bengal based on ELISA. There has been a Seroprevalence rate of 21.4% based on an ELISA study in Rewa (Madhya Pradesh) (Singh et al., 2016). However, Rajkumar et al. (2018) recorded a Seroprevalence of *M. synoviae* as 32.6% from 5 different states of India. A comparatively lower prevalence of MS (6.4%) was reported by Ramadass et al. (2006) in poultry flocks of Tamil Nadu, India. The wide range of sero-positivity might be due to the virulence, quantum of infection, bacterial load and health status of the birds at the time of infection. The use of ELISA in diagnosis is strongly recommended for monitoring flocks rather than for testing individual birds (Anonymous, 2018).

Age is a very important parameter influencing the Seroprevalence of Mycoplasmosis. Age-wise highest sero-positivity, i.e. 8.5% and 5.25% for MG and MS respectively could be recorded in the age group of above 5 weeks (Table 2). However, lowest could be seen in the age group of 3–4 weeks with a sero-positivity of 0.75% and 0.5% for MG and MS respectively. Statistical analysis revealed no significant association (*p*=NS) of age group with the Seroprevalence of MG and MS antibodies. Variation in sero-positivity might be associated with other environmental factors.

Table 2: Seroprevalence of Avian Mycoplasma antibodies at different age groups

Age group	Total sample	Nos. of positive sample		% positivity	
	Tested	MG	MS	MG	MS
3–4 weeks	59	3	2	0.75	0.5
4–5 weeks	127	16	6	4	1.5
Above 5 weeks	214	34	21	8.5	5.25
Total	400	53	29	13.25	7.25
Chi-square value		4.76459	4.60279		
p-value [Sig=*p*<0.05; NS=*p*>0.05]		NS	NS		

Season-wise, for MG, highest sero-positivity was recorded in winter (6.5%) followed by post-monsoon (4.25%), monsoon (1.75%) and pre-monsoon (0.75%) (Table 3). In case of MS, highest prevalence was recorded in post-monsoon (3%) followed by winter (2.75%), monsoon (1%)

Table 3: Seroprevalence of Avian Mycoplasma antibodies in different seasons

Season	Total sample tested	No. of positive samples		% Positivity	
		MG	MS	MG	MS
Pre-monsoon (March–May)	47	3	2	0.75	0.5
Monsoon (June–September)	79	7	4	1.75	1
Post-monsoon (October–November)	121	17	12	4.25	3
Winter (December–February)	153	26	11	6.5	2.75
Total	400	53	29	13.25	7.25
Chi-square value		5.18491	2.46968		
p-value [Sig=*p*<0.05; NS=*p*>0.05]		NS	NS		

and pre-monsoon (0.5%). No significant association (p=ns) of season and sero- positivity of MG and MS antibodies could be observed. Present finding were in close agreement with the previous results reported by Hossain et al. (2010) where highest sero-positivity of MG and MS was found in the winter season. It was found that cold weather and high relative humidity could influence the Seroprevalence of MG and MS. Similar findings was also recorded by Heleili et al. (2012). However, almost similar sero-positivity i.e. 3% and 2.75% could be seen in post-monsoon and winter respectively in the present study.

4. CONCLUSION

The overall sero-positivity was recorded as 13.25% for *Mycoplasma gallisepticum* and 7.25% for *Mycoplasma synoviae*. The i-ELISA kit (IDEXX laboratories) has good potential to monitor the presence of *Mycoplasma* antibodies and its presence indicates exposure to infection naturally, either directly or indirectly. Birds of all age groups were susceptible to infection. Season plays an important role in prevalence of the disease. Low temperature, dusty environment during winter season, act as a predisposing factor for the occurrence of the disease.

6. REFERENCES

Anonymous, 2008. Avian mycoplasmosis (*Mycoplasma gallisepticum, M. synoviae*). In: Manual of Diagnostic tests and Vaccines for Terrestrial Animals. OIE, 482–496.

Anonymous, 2018. OIE- World Organization for Animal Health. Avian mycoplasmosis (*Mycoplasma gallisepticum*, *Mycoplasma synoviae*). In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE Reference Laboratory, 844–859.

Anonymous, 2019. 20th Livestock Census. All India Report, Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi. Available from https://www.dahd.nic.in/division/provisional-key-results-20th-livestock-census. Accessed on 15th December, 2019.

Atalla, H., Lysnyansky, I., Raviv, Y., Rottem, S., 2015. *Mycoplasma gallisepticum* inactivated by targeting the hydrophobic domain of the membrane preserves surface lipoproteins and induces a strong immune response. PLoS ONE 10(3), e0120462.

Bradbury, J.M., 2005. Poultry mycoplasmas: sophisticated pathogens in simple guise. British Poultry Science 46, 25–136.

Brar, R.S., Leishangthem, G.D., Gadhave, P.D., Singh, N.D., Banga, H.S., Sodhi, S., 2017. Histopathological and immunohistochemical studies on cases of chronic respiratory disease in poultry. Indian Journal of Veterinary Pathology 41(3), 232–234.

Brown, D.R., Whitcomb, R.F., Bradbury, J.M., 2007. Revised minimal standards for description of new species of the class mollicutes (Division Tenericutes). International Journal of Systematic and Evolutionary Microbiology 57(11), 2703–2719.

Czifra, G., Sundquist, B., Tuboly, T., Stipkovits, L., 1993. Evaluation of a monoclonal blocking enzyme-linked immunosorbent assay for the detection of *Mycoplasma gallisepticum* specific antibodies. Avian Diseases 37(3), 680–688.

Daniel, W.W., 1999. Biostatistics: A Foundation for Analysis in The Health Sciences (7th Edn.). John Wiley and Sons, New York, 322–328.

Felice, V., Lupini, C., Mescolini, G., Silveira, F., Guerrini, A., Catelli, E., Di Francesco, A., 2020. Molecular detection and characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* strains in backyard poultry in Italy. Poultry Science 99(2), 719–724.

Gondal, M.A., Rabbani, M., Muhammad, K., Yaqub, T., Babar, M.E., Sheikh, A.A., Ahmad, A., Shabbir, M.Z., Khan, M.I., 2015. Characterization of *Mycoplasma gallisepticum* isolated from commercial poultry flocks. The Journal of Animal & Plant Sciences 25(1), 108–113.

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

Hossain, K.M.M., Hossain, M.T., Yamato, I., 2010. Seroprevalence of *Salmonella* and *Mycoplasma gallisepticum* infection in chickens in Rajshahi and surrounding districts of Bangladesh. International Journal of Biology 2(2), 74–80.

Kleven, S.H., 2008. Control of avian mycoplasma infections in commercial poultry. Avian Diseases 52, 367–374.

Ley, D.H., 2003. *Mycoplasma gallisepticum* Infection. In: Saif, Y.M. (Ed.). Diseases of Poultry (11th Edn.). Iowa State University Press, Ames, Iowa, 722–744.

Michiels, T., Welby, S., Vanrobaeys, M., Quinet, C., Rouffaer, L., Lens, L., Martel, A., Butaye, P., 2016. Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial poultry, racing pigeons and wild birds in Belgium. Avian Pathology 45(2), 244–252.

Osman, K.M., Aly, M.M., Amin, Z.M.S., Hasan, B.S., 2009. *Mycoplasma gallisepticum*: an emerging challenge to the poultry industry in Egypt. Review of Scientific and Technical OIE 28(3), 1015–1023.

Peebles, E.D., Branton, S.L., 2012. *Mycoplasma gallisepticum* in the commercial egg-laying hen: A historical

perspective considering the effects of pathogen strain, age of the bird at inoculation, and diet on performance and physiology. Journal of Applied Poultry Research 21(4), 897–914.

Rajkumar, R., Reddy, M.R., Somvanshi, R., 2018. Molecular typing of Indian *Mycoplasma gallisepticum* isolates. Indian Journal of Animal Research 53(12), B-3715. DOI 10.18805/ijar.B-3715.

Rajkumar, S., Reddy, M.R., Somvanshi, R., 2017. Molecular detection and pathology of spontaneous cases of chronic respiratory disease in chicken. Indian Journal of Veterinary Pathology 41(4), 277–282.

Rajkumar, S., Reddy, M.R., Somvanshi, R., 2018. Molecular prevalence and seroprevalence of *Mycoplasma gallisepticum* and *M. synoviae* in Indian poultry flocks. Indian Journal of Animal Research 8(1), 15–19.

Ramadass, P., Ananthi, R., Senthilkumar, T.M.A., Venkatesh, G., Ramaswamy, V., 2006. Isolation and characterization of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* from poultry. Indian Journal of Animal Sciences 76(10), 796–798.

Sarika, N., Devigasri, C., Sankar, S., Mini, M., 2019. A report of natural concurrent infection with *Avibacterium paragallinarum* and *Mycoplasma gallisepticum* in chicken. The Pharma Innovation Journal 8(1), 16–18.

Sawicka, A., Durkalec, M., Tomczyk, G., Kursa, O., 2020. Occurrence of *Mycoplasma gallisepticum* in wild birds: A systematic review and meta-analysis. PLoS ONE 15(4), e0231545.

Singh, N., Shukla, S., Sharma, V., 2016. Detection of Anti *Mycoplasma gallisepticum* antibodies in different age group of chicken by enzyme linked immunosorbant assay. Journal of Animal Research 6(1), 49–51.

Soeripto, W.K.G., Cottew, G.S., Harrigan, K.E., 1989. Virulence and transmissibility of *Mycoplasma gallisepticum*. Australian Veterinary Journal 66, 65–72.

Umar, S., Munir, M.T., Ur-Rehman, Z., Subhan, S., Azam, T., Shah, M.A., 2017. Mycoplasmosis in poultry: Update on diagnosis and preventive measures. Worlds Poultry Science Journal 73(1), 12–17.

Yadav, J.P., Tomar, P., Singh, Y., Khurana, S.K., 2021. Insights on *M. gallisepticum* and *M. synoviae* infection in poultry: A systematic review. Animal Biotechnology 2021, 1–10. DOI 10.1080/10495398.2021.1908316.