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Serology Study of *Mycoplasma gallisepticum* in Broiler Chickens in Chongqing

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Abstract: *Mycoplasma gallisepticum* (MG) is a bacterium that causes Chronic Respiratory Disease (CRD) a highly contagious disease that affects the respiratory system of the chicken leading to reduced egg production. In chickens, infection may be inapparent or result in varying degrees of respiratory distress with slight to marked rales, difficulty breathing, coughing and/or sneezing. Morbidity is high and mortality low in uncomplicated cases. The aim of this study was to determine the prevalence of *Mycoplasma gallisepticum* infection among chickens residing on chicken farms in Chongqing. To achieve this aim, field visits were made to chicken farms in WanZhou, ChangShou, HeChuan, JiangJing, QianJiang and WuLong. A total of 22 farms were investigated. A total of 1789 sera samples were tested using a commercial ELISA kit. The overall rate of antibody against *Mycoplasma gallisepticum* prevalnece was 62.62% (568/907) in 2012 and 72.45% (639/882) in 2013. The prevalence rates among broiler chickens from parental breeders, backyard farms and chicken farms were 50.28, 68.51 and 17.5%, respectively in 2012 and 57.89, 78.14 and 20%, respectively in 2013. When the data were evaluated according to season, the seroprevalence rates was 59.08% in the Spring and 65.89% in the Fall in 2012 and 68.96 and 75.65% in 2013. Seroprevalence rates of the anti-*Mycoplasma gallisepticum* in 2012 and 2013 varied significantly according to the breeding patterns of the animals ($p<0.001$). These results demonstrated that *M. gallisepticum* was widely distributed in chicken in Chongqing region and high stocking density and low air temperature may leading to a even more higher infection of *M. gallisepticum*.

Key words: Broiler chickens, *Mycoplasma gallisepticum*, serology, animals, Chongqing

INTRODUCTION

Mycoplasma gallisepticum (MG) is a bacteria belonging to the class Mollicutes and the family Mycoplasma taceae and the most pathogenic avian mycoplasma and the causative agent of Chronic Respiratory Disease (CRD) in chickens (Kleven, 1998). Infection with *Mycoplasma gallisepticum* is associated with slow onset, chronic respiratory disease in chickens. Clinical signs of infectious sinusitis for turkeys include watery eyes and nostrils and the area under the eye may be swollen. One may find airsacculitis with yellow exudates in the air sacs (Mercia, 2001). Chickens may have no outward symptoms or there may be a sticky nasal discharge, airsacculitis, coughing, difficult breathing, swelling of the face, sneezing, a foamy secretion in the eyes and a drop in food consumption. Egg production may be far below normal and there may be a drop in body weight as well. Once infected, birds may remain carriers for life (Hennigan *et al*., 2012).

Although, the mortality rate is low usually but these diseases can cause a significant loss to the poultry industry and especially to broiler breeders (Osman *et al*., 2009).

Several diagnostic methods such as virus isolation, serum plate agglutination test, Hemagglutination inhibition PCR real-time PCR have been used to study the serology typing and molecular character of *M. gallisepticum* (Osman *et al*., 2009; Jarquin *et al*., 2009; Carli and Eyigor, 2003; Xu *et al*., 2012). Some assays require a long time or specialized laboratory equipment with expertise. More recently, Enzyme-Linked Immunosorbent Assay (ELISA) with high sensitivity and specificity have become available (Wang *et al*., 1994; Osman *et al*., 2009).

Very little information regarding the prevalence of *Mycoplasma gallisepticum* in Chongqing is available. The aim of this study was to assess the level of anti-*M. gallisepticum* in broiler chickens if any using clinical and serologic approaches.

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MATERIALS AND METHODS

Blood samples collection: Different breeding patterns of chicken farms located in Hechuan, Wanzhou, Qianjiang, Jiangjing, Wulong and Changshou which have not vaccinated *Mycoplasma gallisepticum* were included in the study during the year of Spring and Fall in 2012 and 2013. Survey investigation of signs of *Mycoplasma gallisepticum* such as coughing, nasal and ocular discharge, poor productivity, stunting and slow growth was conducted with the owners. Blood samples were collected from 1789 chickens (Table 1) which were not presence of signs of *Mycoplasma gallisepticum* by examination. The serum was separated and stored at -20°C for future serologic research. Breeding pattern group were divided into parental breeders, chicken farms and backyard farms.

Serology: The concentration of anti-*Mycoplasma gallisepticum* antibody in the chickens was measured with a commercial Platelia™ *Mycoplasma gallisepticum* (IDEXX, USA) according to the manufacturer's instruction. Positive and negative control serum were provided with the kit. The examining wells were coated in triplicate with 0.025 μg rec SeM in 100 μL of 0.06 M carbonate buffer pH9.2 for approximately 1 h after which they were washed three times with PBS-T. The reaction was blocked with 4% non-fat milk then washed three times with PBS-T. The 100 μL serum diluted 1:200, 1:400 in PBS-T were added in triplicate and incubated for 1 h at 25°C (room temperature). Then, samples were washed three times with PBS-T. The chicken *Mycoplasma gallisepticum* peroxidase conjugated Protein G diluted in PBS-T was added and incubated for 1 h at 25°C (room temperature). After that samples were washed three times with PBS-T then 100 μL substrate solution were added to each well. The mixture was incubated 15 min at room temperature after which the reaction was stopped by adding 50 μL 2 M sulfuric acid. The optical density was read at wavelengths of 405 nm with an ELISA reader (infinite M200, Tecan, mannedorf, Switzerland).

Data analysis: The prevalence of *Mycoplasma gallisepticum* was calculated by dividing the number of positive subjects by the number of examined animals. Data were entered into a Microsoft Excel spreadsheet. A χ^2-test was used to analyze differences among the variables (Version 5.04; GraphPad Software Inc., La Jolla, CA, USA). The $p<0.05$ were considered statistically significant. Descriptive statistics was used to summarize the data. The model was fitted with likely risk factors associated with the *Mycoplasma gallisepticum* prevalence such as year and season and breeding patterns.

Table 1: Blood samples collected from different breeding patterns in Chongqing

	2012			2013		
Breeding pattern	No. of farms	Spring	Fall	No. of farms	Spring	Fall
Parental breeders	5	86	95	4	64	69
Chicken farms	7	329	357	7	338	371
Backyard farms	8	20	20	8	20	20
Total	20	435	472	19	422	460

RESULTS

Seroprevalence according to survey year and breeding pattern: A total of 1789 sera samples (907 in 2012 and 882 in 2013) were collected from brolie chickens located throughout Chongqing and analyzed with an indirect-ELISA. The overall prevalence rate of anti-*Mycoplasma gallisepticum* was 67.47% (1207/1789) form 2012 to 2013 (Table 2). The annual rates were 62.62% (568/907) in 2012 and 72.45% (639/882) in 2013. The prevalence rates among brolice chickens from parental breeders, backyard farms and chicken farms were 50.28% (91/181), 68.51% (470/686) and 17.5% (7/40), respectively in 2012 and 57.89% (77/133), 78.14% (554/709) and 20% (8/40), respectively in 2013. Seroprevalence rates of the anti-*Mycoplasma gallisepticum* in 2012 and 2013 varied significantly according to the breding patterns of the animals ($p<0.001$).

Seroprevalence according to survey year and season: When assessed according to season, the seroprevalence rates was 59.08% (257/435) in the Spring and 65.89% (311/472) in the Fall in 2012 (Table 3). In 2013, the rates was 68.96% (291/422) in the Spring and 75.65% (348/460) in the Fall in 2013. There was significant difference between different seasons ($p<0.05$) (Table 3).

Table 2: Prevalence of anti-MG antibodies in chicken in Chongqing according to survey breeding patterns

	2012		2013	
Breeding pattern	No. of tested	No. of positive (%)**	No. of tested	No. of positive (%)**
Parental breeders	181	91 (50.28)	133	77 (57.89)
Backyard farms	686	470 (68.51)	709	554 (78.14)
Chicken farms	40	7 (17.50)	40	8 (20)
Total	907	568 (62.62)	882	639 (72.45)

**Statistically significant difference ($p = 4.74787\times10^{-13}<0.001$ and $p = 2.93692\times10^{-18}<0.001$)

Table 3: Prevalence of anti-MG antibodies in chicken in Chongqing according to season

	2012		2013	
Seasons	No. of tested	No. of positive (%)*	No. of tested	No. of positive (%)*
Spring	435	257 (59.08)	422	291 (68.96)
Fall	472	311 (65.89)	460	348 (75.65)
Total	907	568 (62.62)	882	639 (72.45)

*Statistically significant difference ($p = 0.040<0.05$ and $p = 0.026<0.05$)

DISCUSSION

As one of the main pathogens harmful for the development of the poultry industry, Chronic Respiratory Disease (CRD) in chickens was caused by infecting of *Mycoplasma gallisepticum* is especially in intensively reared chickens is most likely to occur. Although, the mortality rate is low usually infection rates are high. Once birds have been infected, they become carriers and remain infectious for life. Transmission may be transovarian or by direct contact with birds, exudates, aerosols, airborne dust and feathers and to a lesser extent fomites. Fomites appear to a significant factor in transmission between farms. Recovered birds remain infected for life, subsequent stress may cause recurrence of disease.

There was several diagnostic methods which included virus isolation, serum plate agglutination test, Hemagglutination inhibition and PCR real-time PCR have been used to study prevalence of *M. gallisepticum*. As with high sensitivity and specificity, Enzyme-Linked Immunosorbent Assay (ELISA) was widely used. It was conducted to assess the *M. gallisepticum* antibody in Qinghai Province, results showed that the average infection rate of the disease was 67.15% and it was very differences between different fields which ranging from 4.76-92.41%. The differences between the fields is large, ranging from 4.76-92.41%. According to Xu (2012), infection rate of MG in Shandong Province was 51.47%. In the present study, high prevalence (67.47%) of anti-*M. gallisepticum* was obversved by using ELISA Method.

Poor air quality or crowding may facilitate infection, disease and transmission. There is a marked interaction (polymicrobial disease) between respiratory viruses, *Escherichia coli* and *M. gallisepticum* in the pathogenesis and severity of chronic respiratory disease. The analysis revealed that infection rate in commercial farms (75.16%) is higher than that in backyard farms (18.18%) (Quan *et al*., 2007). A survey on epidemiological characteristics of *M. gallisepticum* in XinJiang was conducted by Wang *et al*. (2009), the ELISA results demonstrated that the positive rate of MG was 55.9%. MG positive rate of chicken from breeding farms, middle to small-scale commercial farms and farmer-raised groups were 15.3, 71.3 and 36.6%, respectively. The results also showed that the MG positive rate increased with the stocking density increased.

Chongqing is a middle-West area of china and the temperature varies greatly between difference seasons. The seroprevalence rates of *M. gallisepticum* was 65.89% vs. 59.08% (in 2012) and 75.65% vs. 68.96% (in 2013) in different season. These results of seroprevalence rates according to season showed that there was significant difference between different seasons as the weather become colder, the infection rate become higher.

CONCLUSION

The overall high prevalence (67.47%) of anti-*M. gallisepticum* obversved in the present study demonstrated that *M. gallisepticum* was widely distributed in chicken in Chongqing region. There was a increasing trend year by year and the larger scale of the farming or the higer stocking density and the lower air temperature may leading to a even more higher infection of *M. gallisepticum*.

Good management and biosecurity practices are necessary to ensure that *M. gallisepticum* infections are not introduced from many sources. It is known that chicken travel very frequently in the country for many reasons such as breeding and sale. Therefore, additional efforts is required to educate owners in prevention measurements of *M. gallisepticum*.

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REFERENCES

Carli, K.T. and A. Eyigor, 2003. Real-time polymerase chain reaction for detection of Mycoplasma gallisepticum in chicken trachea. Avian Dis., 47: 712-717.

Hennigan, S.L., J.D. Driskell, N. Ferguson-Noel, R.A. Dluhy, Y. Zhao, R.A. Tripp and D.C. Krause, 2012. Detection and differentiation of avian mycoplasmas by surface-enhanced raman spectroscopy based on a silver nanorod array. Applied Environ. Microbial., 78: 1930-1935.

Jarquin, R., J. Schultz, I. Hanning and S.C. Ricke, 2009. Development of a real-time polymerase chain reaction assay for the simultaneous detection of Mycoplasma gallisepticum and Mycoplasma synoviae under industry conditions. Avian Dis., 53: 73-77.

Kleven, S.H., 1998. Mycoplasmas in the etology of multifactorial respiratory disease. Poult. Sci., 77: 1146-1149.

Mercia, L.S., 2001. Storey's Guide to Raising Poultry. Storey Books, North Adams, MA., ISBN: 9781580 172639, pp: 272-273.

Osman, K.M., M.M. Aly, Z.M.S. Amin and B.S. Hasan, 2009. Mycoplasma gallisepticum: An emerging challenge to the poultry industry in Egypt. Revue Scientifique et Technique-Office International Des Epizooties, 28: 1015-1023.

Quan, H., Z.H. Han, X.L. He and Y. Wen, 2007. Serology detection of *Mycoplasma gallisepticum* in chicken. Anim. Quarantine China, 24: 37-37.

Wang, L.Y. and M.H. Gand, 1994. A research on *Mycoplasma gallisepticum* (MG) antigen by Indirect Dot-ELISA Method. Acta Vet. Zootechnica Sin., 25: 533-538.

Wang, T., D.L. Ran and L. Lan, 2009. Serology prevalence survey and control of *Mycoplasma gallisepticum* disease in Xingjiang province. Xinjiang Anim. Husbandry, 1: 37-38.

Xu, J.Y., M.C. Yang, X. Sun and B. Xu, 2012. Comparative test of drug prevention and vaccine inoculation on prevention infection of *Mycoplasma gallisepticum* in broiler breeding chickens. Shandong Anim. Husbandry Vet., 33: 10-12.