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Serosurvey for Infections with Poultry *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in Guizhou Province, Southwestern China

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Abstract: Serum samples from 801 poultry in Guizhou Province were examined for antibodies against *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) by Enzyme-Linked Immunosorbent Assay (ELISA). The results showed that total positive rates of MG, MS or MG/MS infection were 43.07% (345/801) 46.07% (369/801) and 30.59% (245/801) with the positive rates of herds at 84.62% (33/39) 82.05% (32/39) and 76.92% (30/39), respectively, meanwhile, farms and backyard households or duck and chicken/goose had significant difference ($p<0.01$) which showed the prevalence of infectious Mycoplasma in some herds of Guizhou Province. Further Pearson correlation coefficient analysis indicated that poultry sera had very strong or strong positive linear relationship between the positive numbers of single or double antibody of MG, MS or MG/MS and those of IBDV, IBV or IBDV/IBV, suggesting that avian Mycoplasma infection may be associated with vaccination against infectious bursal disease or infectious bronchitis.

Key words: Mycoplasma, infection, infectious bursal disease virus, infectious bronchitis virus, vaccine

INTRODUCTION

Avian mycoplasmosis is caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important and listed by the OIE. MG causes chronic respiratory disease of domestic poultry and results in loss of production and downgrading of meat-type birds, MS induces respiratory diseases, synovitis, eggshell apex abnormalities and egg production losses (Feberwee *et al*., 2008) or a silent infection, both strains vary in infectivity and virulence and infections may sometimes be unapparent (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.05_%20AVIAN_MYCO.pdf). There are many serological evidences of infection with mycoplasma at home (Ji and Ning, 1986) and abroad (Sarkar *et al*., 2005; Feberwee *et al*., 2008; Xavier *et al*., 2011), the prevention and control of mycoplasma infection is often a problem facing poultry industry. Since, the first report of mycoplasma contamination in cell culture (Robinson *et al*., 1956), mycoplasma in live virus vaccines has arisen great attention abroad (Kojima *et al*., 1997; Sampath *et al*., 2010; David *et al*., 2010; Volokhov *et al*., 2011) or at home (Ning and Ji, 1993; Jia *et al*., 2005). Recently, poultry farms or households generally lay emphasis on immune prevention and control of avian Infectious Bursal Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV), usually, the use of various kinds of vaccines made at home and abroad may pose a problem of side effect in practice. The economic significance of mycoplasma has been a subject of debate for many years but in recent years the situation among poultry in Southwestern China's Guizhou Province remained uncertain. So, it is necessary to determine the prevalence of antibodies against economically and socially significant infectious agents of *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) and to analyze possible reasons of circulation.

MATERIALS AND METHODS

Preparation of serum samples: A total of 801 sera were selected from blood samples submitted to Guizhou Animal Disease Control Center from 39 herds in 9 regions of Guizhou Province. All sera were harvested by centrifugation for 15 min at 3000 rpm and heat-inactivated at 56°C for 30 min.

Serological examination: Sera were tested using commercially available *M. gallisepticum*, *M. synoviae*, infectious bursal disease virus and infectious bronchitis virus antibody test ELISA kits (FLOCKTYPE®recMg, FLOCKTYPE®recMs, FLOCKTYPE®recIBDV and FLOCKTYPE®recIBV, Labor Diagnostik Leipzig, Germany).

Statistical analysis: χ^2-test calculator (Version 1.61) was downloaded (http://www.ziyuesoft.net) and used to test the difference between relevant samples. Pearson correlation coefficient analysis was carried out using Microsoft Excel statistical function.

RESULTS AND DISCUSSION

The 801 random serum samples from 14 township backyard households and 25 scaled farms were tested for seroprevalence for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) and the serologic results were presented in Table 1. MG and MS single and double antibody positive rates of herds were 84.62% (33/39), 82.05% (32/39) and 76.92% (30/39), respectively in which the scaled farms showed positive rates of 88% (22/25), 88% (22/25) and 84% (21/25) while backyard households of 85.71% (12/14), 71.43% (10/14) and 57.14% (8/14), meanwhile, the positive rates of chicken, duck and goose herds were 91.18% (31/34), 0% (0/3) and 100% (2/2) for MG; 94.12% (32/34), 0% (0/3) and 100% (2/2) for MS; 82.35% (28/34), 0% (0/3) and 100% (2/2) for MG/MS.

The total positive rates of MG and MS single or double antibodies were 43.07% (345/801), 46.07% (369/801) and 30.59% (245/801), respectively. Backyard households displayed lower seroprevalence of 33.53% (56/167), 35.33% (59/167) and 13.77% (23/167) when compared to 45.58% (289/634), 48.90% (310/634) and 35.02% (222/634) for scaled farms ($p<0.01$), meanwhile, the positive rates of chicken, duck and goose were 45.34% (336/741), 0% (0/40) and 45% (9/20) for MG; 48.58% (360/741), 0% (0/40) and 45% (9/20) for MS; 31.85% (236/741), 0% (0/40) and 45% (9/20) for MG/MS, ducks showed lower seroprevalence when compared with chickens and geese ($p<0.01$) while no difference was observed between chicken and goose ($p>0.05$).

To analyze thereasonof positive antibodies against *M. gallisepticum* and *M. synoviae*, 39 herds (farms/households) of serum samples were tested for antibodies against Infectious Bursal Disease Virus (IBDV) and Infectious Bronchitis Virus (IBV) at the same time (Table 2), statistical analysis showed that Pearson correlation coefficient of positive antibody numbers between MG and IBDV or IBV were 0.9, 0.8299 and 0.8959; MS and IBDV or IBV were 0.835, 0.7056 and 0.8378; MG/MS and IBDV or IBV were 0.82, 0.6819 and 0.8442, respectively. These reflected the existence of very strong or strong correlation between positive antibody numbers of Mycoplasma and IBDV or IBV.

The serological survey involved 39 poultry farms/households from 11 counties in 9 regions of Guizhou Province, sampling avian herds were not inoculated with Mycoplasma vaccine while inoculated with or without IBDV or IBV vaccine according to individual circumstances. *M. gallisepticum* and *M. synoviae* single or double antibody positive rates of herds were 84.62, 82.05 and 76.92% with the total positive rates at 43.07, 46.07 and 30.59%, respectively of which backyard households or ducks displayed obviously low positive rate ($p<0.01$) which provided the basis for prevention and control of these diseases.

In Bangladesh, overall sero-prevalence of MG infection was 58.90% (225/382) for exemplary breeder poultry farms (Sarkar *et al*., 2005). In Holland, the prevalence of *M. synoviae* serologically positive farms in commercial layer stock was high (73%) and significantly higher than all other poultry categories (layer and broiler parent farms was 25 and 35%, layer and broiler grandparent stock was 0 and 10%, respectively) which is in agreement with data of other research groups (Feberwee *et al*., 2008). More recently, Xavier *et al*. (2011) reported prevalence of MG and MS seropositive farms being 8.7~100% and 21.7~100%, respectively, high infection rates pose a huge threat to the poultry industry in Argentina. In China, Ji and Ning (1986) detected serum samples from 20 provinces and found that overall MG and MS antibody positive rates were 78.25% (313/400) and 20.07% (82/396), respectively; Lv *et al*. (2012) reported chicken MG antibody positive rate of 40.00% for 1245 sera from 12 regions in Yunnan Province using commercial enzyme-linked immunosorbent assay kits; reported overall goose sera MG and MS single or double antibody positive rates of 29.7% (55/185), 19.5% (36/185) and 14.6% (27/185), respectively, furthermore, *M. synoviae* was found in Henan, Shaanxi, Beijing, Guangdong, Guangxi, Hohhot and Shanghai areas, serological

Table 1: Seroprevalence of antibodies against *M gallisepticum* and *M synoviae*

			Positive No.				Positive No.	
Variables/Items	Herd No.	MG	MS	MG/MS	Sample No.	MG	MS	MG/MS
Location/herd								
Nine regions	39	33	32	30	801	345	369	245
Scaled farm	25	22	22	21	634	289	310	222
Raising mode								
Backyard households	14	12	10	8	167	56	59	23
Chicken	34	31	32	28	741	336	360	236
Breed								
Duck	3	0	0	0	40	0	0	0
Goose2	2	2	2	2	20	9	9	9

Table 2: Serum antibody test results of infection or immunization

			Positive No.					
Nine regions	Herd No.	Sample No.	MG	MS	MG/MS	IBDV	IBV	IBDV/IBV
Guiyang	1	20	7	4	2	16	16	13
	2	20	12	5	5	16	16	13
	3	50	20	14	8	22	30	14
	4	50	22	16	10	22	34	22
	5	50	20	28	18	34	40	32
	6	50	28	26	22	26	30	24
	7	48	20	10	8	26	46	20
	8	10	4	0	0	3	7	3
	9	15	2	2	2	4	10	4
	10	10	2	2	1	6	6	3
	11	20	4	1	0	15	13	10
Zunyi	12	30	0	12	0	10	10	4
	13	20	2	12	2	10	10	8
	14	10	4	10	4	6	4	4
Liupanshui	15	34	20	30	18	28	28	24
Anshun	16	10	0	0	0	0	0	0
	17	10	7	7	7	7	7	7
Bijie	18	40	38	40	38	40	36	36
	19	10	10	8	8	10	8	8
	20	20	4	6	4	18	20	18
	21	20	12	6	6	16	10	5
	22	10	10	4	4	8	8	8
Tongren	23	8	2	0	0	2	6	0
	24	12	2	0	0	2	10	0
	25	16	0	2	0	0	16	0
	26	4	0	0	0	0	4	0
	27	20	0	0	0	0	0	0
Qiannan	28	32	16	28	14	18	24	16
	29	20	10	18	10	16	18	14
	30	20	8	18	8	18	14	12
	31	12	8	12	8	12	12	12
Qiandongnan	32	10	10	10	10	10	10	10
	33	10	2	2	2	2	0	0
	34	10	0	0	0	0	0	0
	35	10	8	10	8	10	10	10
Qianxinan	36	10	8	10	8	8	10	8
	37	20	4	4	4	4	16	4
	38	20	15	6	4	20	20	20
	39	10	4	6	2	8	6	6

investigation indicated the presence of MS infection in many chicken farms. The 34 herds of chickens from 22 farms and 12 backyard households were selected based on an accepted error between 5 and 10%, the positive rate of MG antibody averaged 45.34% which was slightly higher than Yunnan ($0.01<p<0.05$) but significantly lower than Bangladesh, Holland and many regions reported in China (Ji and Ning, 1986) ($p<0.01$). The estimated prevalence of MS and MG/MS positive flocks in geese stock was unexpectedly higher than what Chen reported in the Pearl River Delta region of Guangdong Province, respectively ($0.01<p<0.05$ or $p<0.01$), however, the MG prevalence was probably showing no difference ($p>0.05$). The prevalence of MG or MS serologically positive ducks farms including one demonstration farm and two individuals were significantly lower than that of two goose farms or 34 chicken folks ($p<0.01$), additionally, the investigated ducks were found not vaccinated with IBDV or IBV vaccine as for the number of farms sampled, further test of IBDV and IBV serologically positive farms was carried out to analyze if there was any relationship between low Mycoplasma prevalence and IBDV or IBV vaccination. Statistical analysis showed that extremely strong or slightly strong linear relationship existed between positive antibody numbers of MG or MS and IBDV or IBV, suggesting that the observed avian Mycoplasma infection in Guizhou Province may be associated with IBDV or IBV vaccination. Robinson *et al.* (1956) reported Mycoplasma contamination in the process of cell culture as early as in 1956, many innovative methods were developed for monitoring or inactivating Mycoplasma in live virus vaccines abroad (Kojima *et al.*, 1997; Sampath *et al.*, 2010; David *et al.*, 2010; Volokhov *et al.*, 2011) or at home (Ning and Ji, 1993; Jia *et al.*, 2005). Mycoplasma can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996; Kleven, 2003), the high level of biosecurity, the stringent control of contact routes and the geographical isolation of grandparent farms minimize the risk of direct and indirect transmission. Mycoplasma contaminated eggs are bound to the poultry vaccine production, domestic poultry farms or households generally are liable to IBDV or IBV vaccination, this probably poses a significant epidemiological risk for infection of Mycoplasma (Ji and Ning, 1986; Ning *et al.*, 1993).

Although, there is linear relationship between infection and vaccination, considering the actual situation of the provincial herd management and benefit, it is necessary to take comprehensive measures to prevent and control avian mycoplasmosis.

CONCLUSION

The presence of antibodies against *M. gallisepticum* and *M. synoviae* in relevant samples demonstrated specific circulation among poultry in some regions of Guizhou Province and urgent need for further epidemiological surveillance.

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