

PREVALENCE OF *MYCOPLASMA GALLISEPTICUM* IN THE COMMERCIAL LAYER FLOCK

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Abstract

Avian mycoplasmosis have been considered a severe problem in poultry diseases; *Mycoplasma gallisepticum* being one of the most important. This study was conducted in Joint-Stock Company Balticovo, Latvia, to determine the prevalence of *M. gallisepticum* infection in hen's flocks in the farm. A total of 904 serum and 335 swab samples from non-vaccinated birds against *M. gallisepticum* from 65 chicken flocks of different age, from day 1 to 75 weeks old, were all tested. The commercially available enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests were used. Results revealed that the seropositive flock rate, based on ELISA tests and, according to ratio that represents the extent to which a light source effectively stimulates the rods: S/P ratios and antibodies titer higher 1.076 were 22/904 (2.43%) and 4/904 (0.44%), respectively, while PCR-positive flock rates were not confirmed. Seroprevalence of *M. gallisepticum* in commercial layer flock in Latvia was more common seen in birds from 17 to 30 weeks of age.

Key words: poultry, *Mycoplasma gallisepticum*, seroprevalence, ELISA, PCR.

Introduction

Outbreaks of infectious disease are a constant risk for the agricultural industry and *Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of gallinaceous and certain non-gallinaceous avian species (Osman et al., 2009). Mycoplasmas are ubiquitous throughout the animal kingdom and virtually every mammal, bird, reptile, amphibian and fish that has been tested for mycoplasmas has revealed unique species (Pitcher and Nicholas, 2005).

Mycoplasma gallisepticum is an avian pathogen most frequently associated with chronic respiratory disease in chickens (*Gallus gallus domesticus*) and infectious sinusitis in turkeys (*Meleagris gallopavo*). It is a major problem in the commercial poultry industry worldwide causing significant economic losses (Levisohn and Kleven, 2000). The most common economic impacts of *M. gallisepticum* are decreased egg production in layers (Mohammed et al., 1987; Levisohn and Kleven, 2000a; Bradbury, 2007).

Transmission of *M. gallisepticum* infection to new hosts can occur vertically in ovo from infected breeders (Levisohn and Kleven, 2000; Bradbury, 2005). Horizontal bird-to-bird transmission occurs within flocks through close contact, probably via respiratory tract excretions. The rate of spread through a flock will be influenced by management systems (e.g., stocking density, type of drinker and feeder). Between flock spread can also occur through fomite carriage (Racicot et al., 2011). *M. gallisepticum* can survive in different reservoirs within a poultry farm and the fact it can weaken the immune system to other diseases, occasionally also respiratory, is a world concern. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common (Marois et al., 2002).

Mycoplasmosis is one of the most important disease in poultry production nowadays under intensive production conditions and in most countries (Netherlands, Germany and others). Therefore, control programs for *M. gallisepticum* are based on maintaining commercial breeding stock free of infection. There has never been *M. gallisepticum* research in Latvia.

This study was undertaken to determine the prevalence of *M. gallisepticum* infection in hen flocks in Joint-Stock Company Balticovo.

Materials and Methods

Clinical samples

Research was carried out in Joint-Stock Company Balticovo from 2012 to 2014. Number of samples tested (n=1239) are summarized in Table 1.

Blood samples (n=904) from pullets and layers in different ages (1 day to 75 weeks) and two different breeds (Lohman Brown, Hy-Line) were collected aseptically from wing vein of individual birds with 1.5 mL sterilized disposable plastic syringe without anticoagulant and allowed to clot for 1 h in the syringe. Blood containing syringes were kept in the room at 20 °C for 4-5 h. The serum (liquid portion) was decanted in centrifuge tube and centrifuge at 1500 rpm for 10 min to have clear serum. The serum was collected in sterile Eppendorf tube and preserved at -20 °C until further processing for the serological study. Blood was collected to perform sero-analyses to detect antibodies against *M. Gallisepticum* using enzyme-linked immunosorbent assay (ELISA). None of the chickens had been vaccinated with any *M. Gallisepticum* vaccine.

Swab samples (n=335) were taken as described from both clinically healthy and sick birds, both from fallen birds (n=163) (Table 1) to detect

Table 1

Samples from laying hen flock, 2012-2014

Samples	Samples tested
Sera from chickens and layers	904
Swab samples from live and fallen birds, including	163
Trachea	185
Cloaca	60
Joints	9
Ovarium	6
Air sac	6
Nostrils	1
Swab samples from surroundings, including	172
Birds shipping transport	30
Different parts in henhouses (ventilator, cages, floor, food tray, water nipples)	110
Stuff (clothes, shoes, hands)	30
Air filter	2
Total numbers of swab samples	335
Total numbers of samples	1239

M. gallisepticum using PCR method. In sick and fallen birds the clinical signs of diseases of upper respiratory tract (discharge from nostrils, inflammation of the air sacs and other) was observed. Samples from surroundings (n=172) (Table 1) were taken with sterile transport swab (Sarsted, DE) from birds shipping transport, stuff and henhouses in different parts according to the requirements of standard (LVS ISO 18593:2007).

Serology

Antibodies to *Mycoplasma gallisepticum* were detected with ELISA assay, tested in World's Poultry Science Association Latvia department using commercial kit (BioChek, UK) following the manufacturer's instructions. To read the result, a spectrometer with length of the wave 405 nm was used. In case of the positive reaction in microplates, yellow coloring whose intensity depends directly on presence of anti-MG immunoglobulins forms.

Results were expressed as S/P ratios relative to a standard positive control. Serum samples with S/P ratios equal to or greater than 0.5 were considered positive.

PCR method

Samples were tested in World's Poultry Science Association Latvia department using Polymerase chain reaction (PCR), which was used for detection of *Mycoplasma gallisepticum* in organs (trachea,

lungs and air sacs) of infected birds. For isolation of bacterial DNA from tissue, swabs were dipped in PBS for several hours at room temperature (15 – 25 °C), centrifugated of pellet bacteria at 5000 rpm for 10 min and supernatant containing DNA was placed on the QIAcube-shaker (QIAcube Protocol Sheet).

Real-time PCR for identification of *Mycoplasma gallisepticum* the bacto type *Mycoplasma* Mg/Ms PCR Kit (96) (QIAGEN, DE) was used. Amplification was performed in a Rotor-Gene Q.

Cycling parameters were as follows: initial denaturation at 90 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 15 sec, primer annealing at 60 °C for 20 sec, extension at 75 °C for 15 sec, was completed by one cycle of denaturation at 95 °C for 15 sec, primer annealing at 60 °C for 45 sec and extension at 75 °C for 5 min. The amplified products were separated as previously described by C. Marois et al. (2000).

Results and Discussion

In most countries, control programs of the *M. gallisepticum* are based on maintaining commercial breeding stock free of infection. Monitoring programs for the detection of *Mycoplasma spp.* infection are based mainly on serological tests. Regular serological monitoring of commercial poultry is essential for the detection of an infection, provided that representative sample sizes and tests with appropriate sensitivity and specificity are used (Landman, 2014).

The results of the ELISA test in our study (Table 2) showed that 22 from 904 samples of the birds are *M. gallisepticum* serologically positive (2.43%), if we count S/P ratios. The analysis of the results of other scientists testify that the number of hens which are infected with *M. gallisepticum* compared to our results are much higher, for example, in Algeria 69.9% (Heleili et al., 2012), in Bangladesh 64.47% (Zulfekar et al., 2015), in Serbia 19.05% (Kapetanov et al., 2010) positive birds, whereas in France (Dufour-Gesber et al., 2006) and in Netherlands (Landman, 2014) the positive *M. gallisepticum* cases were not detected.

Comparing frequency of infection in different breeds (Table 2), results showed that during the time of monitoring the most of the Hy-Line breed birds were infected (79.17%) with *M. gallisepticum* although other authors (Kapetanov et al., 2010) reported about higher frequency of infection just in Lohman Brown breed hens (76.6%). Our finding testifies that Hy-Line breed hens could be infected already vertically from parents flock.

Assessment of the dynamics of prevalence of *M. gallisepticum* in hen flocks in 3 years' time confirms that the occurrence of *M. gallisepticum* significantly decreased in recent years. Our analysis shows that in 2012 there were 13.51% (20/148) infected birds, in 2013 – 0.49% (2/412), but in 2014 - none (0/342) positive case within examined birds. This beneficial situation in commercial layer flock can be explained by a strong control of the parent's flock and by managing a good biosecurity plan in the hen flock.

During the last years in Europe strong supervision and control plans with the aim to avoid the horizontal and vertical prevalence of MG in the parent's hen flocks and in the commercial poultry flocks were established in accordance with Council Directive 2009/158/EC and Commission Decision 2011/214/EU. Therefore, most of the commercial laying flocks are trying to be free from *M. gallisepticum*, however, frequently the problem with other infectious diseases like Infectious bronchitis virus, Newcastle disease, the positive MG (Landman, 2014) in the flocks could be observed.

Table 2

Anti-MG-ELISA antibody (IgG) status of poultry

Number of samples	year	age	S/P Ratio	Titer	Breed
1	2012	28	0.601	818	Lohman Brown
2	2012	20	0.579	785	Hy-Line
3	2012	20	0.603	821	Hy-Line
4	2012	20	0.722	1001	Hy-Line
5	2012	20	0.563	761	Hy-Line
6	2012	20	0.519	696	Hy-Line
7	2012	20	0.669	920	Hy-Line
8	2012	20	0.545	735	Hy-Line
9	2012	20	0.770	1074	Hy-Line
10	2012	20	0.635	869	Hy-Line
11	2012	20	0.559	755	Hy-Line
12	2012	20	0.561	758	Hy-Line
13	2012	20	0.529	711	Hy-Line
14	2012	20	0.525	705	Hy-Line
15	2012	20	0.888	1257	Hy-Line
16	2012	20	0.872	1232	Hy-Line
17	2012	20	0.830	1167	Hy-Line
18	2012	20	0.780	1090	Hy-Line
19	2012	20	0.639	875	Hy-Line
20	2012	20	0.601	818	Hy-Line
21	2013	29	0.767	1070	Lohman Brown
22	2013	25	0.528	709	Lohman Brown

Table 3

***Mycoplasma gallisepticum* in hen flocks**

Age of the birds Type of sample	ELISA Tested samples / positive samples (%)			PCR Tested samples / positive samples	
	till 16 th week	from 17 th till 30 th week	from 31 st till 75 th week	till 16 th week	from 17 th till 30 th week
Serum	399 / 0	266 / 4 (1.5%)	239 / 0	NT	NT
Swab samples from live poultries (trachea, cloaca)	NT	NT	NT	76 / 0	62 / 0
Swab samples from died poultries (trachea, articulation, uterine duct, air sacs, nostils)	NT	NT	NT	3 / 0	22 / 0
Samples from environment, personel	NT	NT	NT	140 / 0	32 / 0

NT – not tested

The evaluation of 22 cases with serologically positive *M. gallisepticum* (according to S/P ratio) (Table 2) showed that in our study only four birds had the titer of antibodies above 1076. According to the recommendation of the producer of the ELISA test kit, only *M. gallisepticum* antibodies titer higher 1076 confirms the positive case. Therefore, the results of the current study have shown that *M. gallisepticum* practically (4/904 or 0.44%) was not observed in the hen flock. Findings of sera results with antibodies titer below 1076 we can explain with cross-reaction that can give false positive results (Kemp et al., 1994) because results can be affected by antibodies of other infectious diseases (*M. sinoviae*, Newcastle disease, Infectious Laryngotracheitis) (Adair et al., 1990). Other researchers (Stipkovits, 1993) also have found out that the presence of *M. gallisepticum* infection in flocks could cause cross reacting of the antibodies in serological tests. According to data of A.Ahmad et al. (2008), the sensitivity and specificity of ELISA test for the detection of *M. gallisepticum* was 74.60%.

Screening programs that are only based on seroconversion may be inadequate for diagnostic and control of mycoplasmosis. The authors suggest the adoption of other techniques to confirm the presence of the agent (*M. synoviae*), such as DNA detection by molecular assays (PCR), because antibodies based tests are uninformative about the active infection (Ewing et al., 1996). PCR represents a rapid and

sensitive alternative for the traditional mycoplasma culture methods, which require specialized media, reagents for serotyping of the isolates and are time-consuming (Kemp fetal.,1994; Levisohn and Kleven, 2000; Arshad et al., 2013).

The studies of other authors on naturally infected birds the most positive number of samples in air sac 23.3%, trachea 11.6%, lung 8.3% (Reda et al., 2012) were found. Also, M. Rauf with co-authors (Rauf et al., 2013) have reported that the highest detection was in trachea (39.2%) followed by air sac (27.4%) and lowest in lungs (15.92%). In the present study in 2014 for control of prevalence of *M. gallisepticum* 439 samples (Table 3) were taken. Although birds in every age are sensitive to mycoplasmosis, new birds are much more sensitive to infection than grown-up birds (Kleven and Ferguson-Noel, 2008). Therefore, significantly more samples to find the MG we took straight from birds at the age up to 16th week.

Our data without any PCR-positive case (Table 3) confirm the results of strong biosecurity procedures during the rearing of young pullets and MG control in parents flock.

Conclusion

This study confirmed the low seroprevalence of *M. gallinarum* in commercial layer flock in Joint-Stock Company Balticovo and that it was more common diagnosed in birds from 17 to 30 weeks old.

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