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INVESTIGATION OF *MYCOPLASMA SYNOVIAE* SEROPREVALENCE IN BROILER BREEDER FARMS IN SOUTH BAČKA REGION

Milena Samojlović^{1*}, Marko Pajić¹, Biljana Božić¹, Slobodan
Knežević¹, Miloš Pelić¹, Dalibor Todorović¹, Sava Lazić¹

¹Scientific Veterinary Institute "Novi Sad", Rumenački put 20, Novi Sad, Serbia

Abstract

Mycoplasma synoviae is known to cause respiratory disorders, synovitis, subclinical infections, air sacculitis and eggshell apex abnormalities in domestic poultry worldwide. The aim of this study was to determine *M. synoviae* seroprevalence in 5 different broiler breeder farms in South Bačka from 2014 to 2017. A total of 1511 samples were tested using commercial indirect enzyme linked immunosorbent assay (ELISA) for detection of antibodies in the blood sera. In this study, the seroprevalence of 25.21% was found and 47 (40.87%) flocks out of 115 tested were positive to *M. synoviae*. Seroprevalence varied between 31.02% in 2015 and 16.78% in 2016. Flock prevalence ranged from 31.03% in 2014 to 55.88% in 2015. These results suggest that *M. synoviae* infection is present in broiler breeder farms in South Bačka, and that is necessary to conduct further research, systematic monitoring and to improve biosecurity measures on broiler breeder farms.

Key words: *Mycoplasma synoviae*, seroprevalence, broiler breeder

^{1*} Corresponding author: milena.s@niv.ns.ac.rs

SEROPREVALENCA *MYCOPLASMA SYNOVIAE* NA FARMAMA RODITELJSKIH JATA TEŠKIH LINIJA U JUŽNOBAČKOM OKRUGU

Milena Samojlović¹, Marko Pajić¹, Biljana Božić¹, Slobodan
Knežević¹, Miloš Pelić¹, Dalibor Todorović¹, Sava Lazić¹

¹ Naučni institut za veterinarstvo "Novi Sad", Rumenački put 20, Novi Sad, Srbija

Kratak sadržaj

Poznato je da *Mycoplasma synoviae* uzrokuje respiratorna oboljenja, sinovitis, subkliničke infekcije, zapaljenje vazdušnih vreća i deformitete ljuske jata kod domaće živine širom sveta. Cilj ovog istraživanja bio je da se utvrdi seroprevalenca *M. synoviae* na 5 različitih farmi roditeljskih jata teških linija od 2014. do 2017. godine. Indirektnom ELISA tehnikom ukupno je testirano 1511 krvnih seruma. Utvrđena je seroprevalenca od 25.21%, a od 115 testiranih jata 47 (40.87%) je bilo pozitivno na antitela protiv *M. synoviae*. Seroprevalenca se kretala od 16.78% u 2016. godini do 31.02% u 2015. godini. Prevalenca jata je iznosila od 31.03% u 2014. godini do 55.88% u 2015. godini. Rezultati dobijeni u ovom istraživanju ukazuju da je infekcija *M. synoviae* prisutna na farmama roditeljskih jata teških linija u Južnobačkom okrugu i da je neophodno sprovesti dalja istraživanja, sistemski monitoring i poboljšati mere biosigurnosti na farmama.

Ključne reči: *Mycoplasma synoviae*, seroprevalenca, roditeljska jata teških linija

INTRODUCTION

Mycoplasma synoviae (Ms) is one of the most important pathogens in domestic poultry worldwide, including breeders, broilers and layers (Vardaman et al., 1973; Kleven and Ferguson – Noeln, 2008). Ms infections in poultry have always been regarded as less important than infections with *Mycoplasma gallisepticum*, but during the last decade, the importance of infections with Ms have been highlighted in several researches and there is an increased consciousness to produce Ms free poultry (Feberwee et al., 2008; Landman, 2014). In chickens, Ms infections have been associated with the respiratory disorders, synovitis, subclinical infections, air sacculitis (Vardaman et al., 1973; Macowan

et al., 1984), as well as eggshell apex abnormalities and egg production losses (Landman and Feberwee, 2001; Van Beek et al., 2002; Landman and Feberwee, 2004; Feberwee et al., 2007).

Mycoplasma synoviae can cause significant economic losses due to decrease of egg production rate, growth and hatchability rates, poor eggshell quality, and condemnation of carcasses at slaughter because of air sacculitis and arthritis (Fiorentin et al., 2003; Kleven 2003a; Peebles et al., 2011). An additional contribution to the importance of Ms infection in domestic poultry is brought by the high seroprevalence of Ms in countries with developed poultry industry (Feberwee et al., 2008; Feberwee and Landman, 2012) and its ability to interact with other pathogens such as Newcastle disease virus and infectious bronchitis virus (Kleven et al., 1972; Hopkins and Yoder, 1982; Feberwee et al., 2009). Reduction in egg production of 5 to 10 %, a reduction in hatchability of 5 to 7 % and more than 5 % increased mortality in the young chickens were reported in infected breeder flocks (Stipkovits and Kempf, 1996). Broiler flocks from the seropositive broiler breeders showed higher rate of mortality from air sacculitis (King et al., 1973; Macowan et al., 1984). The economic impact of Ms infection is mainly focused on increased condemnation of carcasses due to air sacculitis (Senties-Cué et al., 2005). Reduced weight gain and poorer feed conversion are also the result of Ms infection in broilers (King et al., 1973). *Mycoplasma synoviae* can be transmitted horizontally and vertically (Jordan, 1975). Vertical transmission is considered to be a major way of spreading of Ms in domestic chickens, therefore the most efficient method of control would be regular monitoring and elimination of positive breeder flocks (Kleven and Ferguson-Noeln, 2008; Lockaby et al., 1998). Although various live and attenuated Ms vaccines have been developed and used worldwide (Morrow et al., 1998), the Ms vaccine is not yet available in Serbia.

In Serbia, serological monitoring of Ms is still on voluntary basis and data about seroprevalence of Ms in poultry farms are lacking. It is necessary to identify Ms seropositive farms, to assess the epidemiological risks in different poultry categories and to improve biosecurity measures on farms. The aim of this study was to investigate Ms seroprevalence on different broiler breeder farms and to perceive the epidemiological situation of Ms infection in South Bačka Region.

MATERIAL AND METHODS

A total of 1511 blood samples were taken from 115 broiler breeder flocks from 5 different farms in South Bačka region. The samples were taken from

2014 to 2017, following the voluntary *M. synoviae* control program established by Scientific Veterinary Institute "Novi Sad". Blood samples were aseptically taken from non-vaccinated broiler breeders that had no clinical symptoms.

Sera were tested for the presence of antibodies to *M. synoviae* by a commercial ELISA test kit (IDEXX 99-06728, IDEXX Laboratories, Westbrook, ME) according to manufacturer's instructions. Briefly, 100 µl of each diluted serum sample (1:500) were added to the wells previously coated with Ms antigen. Undiluted positive and negative controls were tested in duplicate wells. Samples were incubated at 18-26 °C for 30 minutes. After washing the plates with distilled water 3 to 5 times, 100 µl of conjugate (Goat antichicken antibodies: Horseradish peroxidase conjugate) were added to each well and incubated at 18-26 °C for 30 minutes. Plates were washed again 3 to 5 times with distilled water and 100 µl of TMB (tetramethyl benzidine) substrate were dispensed into each well of the plate. The substrate solution was incubated at 18-26 °C for 15 minutes. The reaction was quenched with 100 µl of stopping solution. The absorbance was measured at 650 nm. Serum samples with S/P values greater than 0.5 (titer greater than 1076) were considered positive.

RESULTS

The obtained results are presented in Table 1 and Table 2. In general, 381 samples were found positive for the presence of anti-Ms antibodies, with the overall prevalence of 25.21%. The highest – 31.02% and the lowest – 16.78% seroprevalence was found in 2015 and 2016, respectively (Table 1). It was found that 47 (40.87%) out of the 115 tested flocks were positive. Positive flock rates in different years ranged between 31.03% and 55.88% (Table 2).

Table 1. Seroprevalence of Ms from 2014 to 2017

Year	Number of tested samples	Number of positive samples	Prevalence (%)
2014	361	100	27.70
2015	432	134	31.02
2016	453	76	16.78
2017	265	71	26.79
Total:	1511	381	25.21

Table 2. Results of the presence of Ms in poultry flocks from 2014 to 2017

Year	Number of tested flocks	Number of positive flocks	Positive rates (%)
2014	29	9	31.03
2015	34	19	55.88
2016	33	12	36.36
2017	19	7	36.84
Total	115	47	40.87

DISCUSSION

Mycoplasma synoviae is one of the most important mycoplasma species that affect domestic poultry during laying period. Our results revealed Ms infection seroprevalence of 25.21% in broiler breeders. Another study conducted in Serbia showed seroprevalence of 36.66% in 2000 and 22.60% in 2009. The decrease in seropositivity in 2009 compared to 2000 was due to specific measures that were applied in layer and broiler breeder flocks (Kapetanov et al., 2010). Similar serological survey in China demonstrated an overall seroprevalence of 41.19 %, which is twice as high in comparison to our results. Seroprevalence in China varied in different provinces from 5.10% to 100%. Commercial vaccine is not yet available in China, so the authors concluded that detected antibodies originated from the natural infection (Xue et al., 2017). In Serbia, the situation in view of the availability of vaccine against Ms is the same as the one in China, so our results indicated seroconversion following natural infection as well. A study in Portugal revealed Ms seroprevalence of 40.3% (Moreira et al., 2015), which is similar to the one found in China.

Survey in Portuguese broiler breeder flocks revealed high prevalence of Ms infection, with 483 positive samples and 24 (66.7%) positive flocks out of 36 tested (Moreira et al., 2015). In the Netherlands, flock prevalence was 35% (Ferberwee et al., 2008), in South America 15% (Buim et al., 2009) and in Middle East 27% (Amer et al., 2012). In our study, flock prevalence was 41%, which is lower in comparison to those from Portuguese flocks, and higher in comparison to other above-mentioned results. These results suggest worldwide distribution of Ms infection in breeder flocks. Another study addressing the Ms flock prevalence was carried out in Serbia by Kapetanov et al. (2010), and the authors reported flock prevalence of 40.48 in 2000 and 20.74% in 2009. Flock prevalence in our study varied from 31.03% in 2014 to 55.88% in 2015. It can

be concluded that Ms flock prevalence is different from year to year, because the occurrence of infection is influenced by numerous factors.

Since Ms can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996), it can cause infection in broilers with increased mortality, feed conversion and condemnation. (Xue et al., 2017; Moreira et al., 2015). Feberwee et al. (2008) reported lower seroprevalence in meat rearing breeder stock (6%) than in meat-type grandparents stock (10%), due to reduced risk of vertical transmission by elimination of Ms-positive flocks. Authors also found higher seroprevalence in meat production breeder stock (35%) than in meat rearing breeder stock. The accuracy of the estimated seroprevalence could have been influenced by frequency and number of birds sampled per flock, which was different for different poultry categories (Feberwee et al., 2008). In order to establish reliable Ms control and prevention program sampling methods, sample size and poultry categories must be determined in advance. High prevalence of Ms infection was found in Portuguese breeder farms, so the authors suggested that culling Ms-positive flocks with such a high prevalence is not an option (Moreira et al., 2015). On the other hand, elimination of positive breeder flocks is recommended, if possible, as one of the best solutions when there is a low Ms prevalence (Buim et al., 2009; Feberwee et al., 2008). The elimination of positive flocks is not carried out in Serbia, but infected flocks are treated with appropriate medications. The use of medications to treat Ms infection increases the production costs. Although vaccines and medication can reduce clinical signs and economic impact of Ms infection, medication cannot completely eliminate mycoplasma infections (Whithear, 1996; Kleven 2003a) and vaccines are not yet available in all countries, such as China and Portugal (Moreira et al., 2015; Xue et al., 2017) as well as Serbia. Ms are capable of establishing lifelong infections in poultry, and that can be one of the reasons for inability to eradicate Ms in commercial poultry flocks, especially in breeders that stay a long period in rearing and production sites.

CONCLUSION

It is evident that Ms is present on broiler breeder farms in South Bačka. In the absence of systematic monitoring of Ms infection in Serbia, further studies need to be done to obtain an overall picture of Ms prevalence in our country. Serological monitoring of Ms infection plays an important role in the prevention and control of this infection in poultry. Besides, it is also important to improve biosecurity measures on farms and to import broiler breeder chickens only from the farms that conduct vaccination against Ms. It is recommendable

to introduce vaccination against Ms in all broiler breeder farms in Serbia, as one of the most significant prevention measures.

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