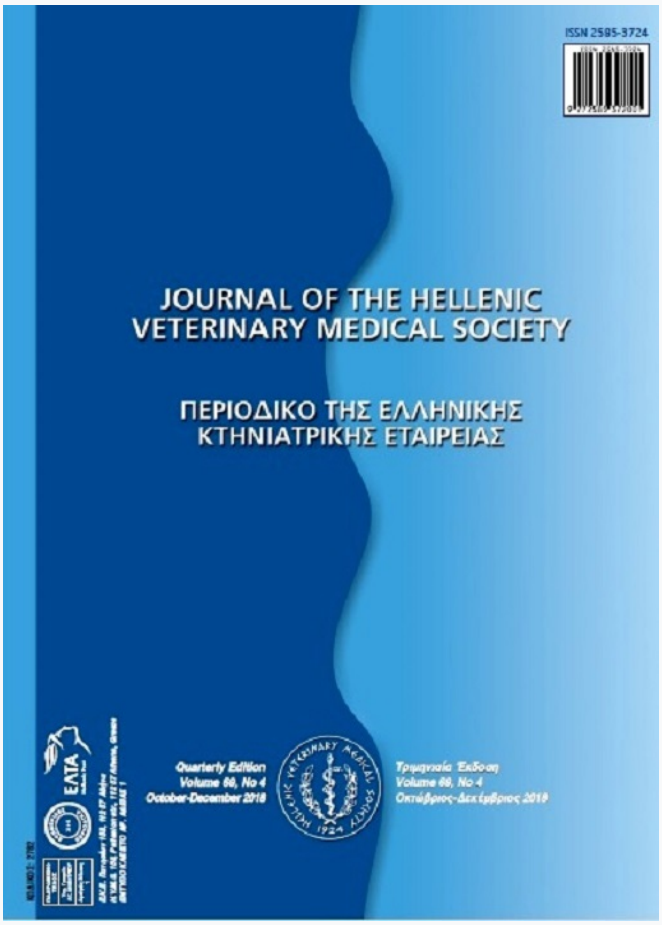
Journal of the Hellenic Veterinary Medical Society

Vol 69, No 4 (2018)



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doi: 10.12681/jhvms.16681

To cite this article:

Vojinovic, D., Zdravkovic, N., Prodanović, R., Vujanac, I., Nedić, S., Giadinis, N. D., Panuonis, N., Manic, M. M., Bugaraski, D., Palamarevic, M., Bogicevic, N., Dobrosavljevic, I., Spalević, L., Žutić, J., Prodanov-Radulović, J., & Bojkovski, J. (2019). Seroprevalence of Mycoplasma bovis in grazing dairy cows from five different areas in Serbia. *Journal of the Hellenic Veterinary Medical Society*, *69*(4), 1241–1246. https://doi.org/10.12681/jhvms.16681

J HELLENIC VET MED SOC 2017, 69(4): 1241-1245
ΠΕΚΕ 2017, 69(4): 1241-1245

Research article
Ερευνητικό άρθρο

Seroprevalence of *Mycoplasma bovis* in grazing dairy cows from five different areas in Serbia

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ABSTRACT. *Mycoplasma bovis* infection in grazing dairy cows has not been reported in the Republic of Serbia to date. It is important to monitor its seroprevalence on the field. The presence of specific antibodies against *M. bovis* in the blood serum of grazing dairy cows is investigated in the present study. A total of 131 blood serum samples of clinically healthy dairy cows were examined. Sampling was performed during 2013 from five different areas in Serbia: Zasavica, Pozarevac, Gruza, Novi Sad and Banatski Karlovac. A commercial ELISA kit for diagnosis of *M. bovis* antibodies in blood serum samples, manufactured by Bio-X Diagnostics, Belgium, was used. Specific antibodies against *M. bovis* were identified in 13 out of 131 samples (9.92%) from 4 locations; the only negative location was the most southern Gruza. The revealed seroprevalence is evidence for the presence of *M. bovis* in grazing dairy cows in different locations of Serbia.

Keywords: *Mycoplasma bovis*, ELISA, serology, grazing cows, Serbia

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Date of initial submission: 11-5-2018
Date of revised submission:27-5-2018
Date of acceptance: 9-6-2018

INTRODUCTION

The genus *Mycoplasma* (class *Mollicutes*, family *Mycoplasmataceae)* includes prokaryotic microorganisms that are capable of self-reproduction (Razin, 1998). Pathogenic species for cattle include *Mycoplasma bovis* (which is the main causative agent for infection) and *Mycoplasma mycoides* subsp. *mycoides SC*, causing contagious pleuropneumonia, which was included in the list for 2018 of the Office International des Epizooties (Pfutzner and Sachse, 1996; OIE, 2018). *Mycoplasma bovis* was first isolated in 1961 from the udder of cows suffering from mastitis (Hale et al., 1962).

In cattle, *M. bovis* can cause pneumonia, mastitis, arthritis, meningitis, otitis, keratoconjunctivitis and genital disorders (vaginitis and abortion) (Gonsales et al., 1983; Stipkovits, 1993; Pfutzner and Sachse, 1996; Gagea et al., 2006). The infection is transmitted mainly airborne, but can occur also via milk or sexually. Semen for artificial insemination can introduce the infection in a herd (Haapala et al., 2018). *Mycoplasma bovis* infection is of major importance in cattle production in European countries and the United States of America (USA), causing large economic losses (Tenk, 2005).In Europe, the damage caused annually by mycoplasmosis in general in cattle is estimated at 576 million Euros(Nicholas et al., 2000) and is responsible for one third of all pneumonia cases in calves. In the last decade *M. bovis* infection has been expanded to new geographical areas, including Ireland (Blackburn et al., 2007; Bell et al., 2012) and Greece (Filioussis et al., 2007), where it was not previously registered, as well as on other continents: parts of South America (Cerdá et al., 2000) and Australia (Al-Farha et al., 2017).

The methods used for serological diagnosis of *M. bovis* infection are indirect hemagglutination, film inhibition and indirect enzyme-linked immunoabsorbent assay (ELISA). The ELISA test, which uses the entire bacterial particle as the antigen, was applied for the first time in 1979 (Onoviran and Taylor-Robinson, 1979). Afterwards, ELISA test for the detection of antibodies against *M. bovis* present in milk of cows suffering from mastitis was developed (Byrne et al., 2000).

To the best of the authors' knowledge, there is no published data about *M. bovis* seroprevalence in grazing dairy cows in Serbia. Existing data indicated the presence of *M. bovis* antibodies (seropositivity of 2.57 and 4.81%), but only in calves so far (Vojinovic et al., 2014; Vidic et al., 2014).

Since the main route of *M. bovis* excretion is via milk, dairy cows were selected as the target animals for this study. Due to the close contact between animals from different farms and locations at grazing, animals are at increased risk to *M. bovis* infection compared to indoors intensively reared dairy cows. Extensive cattle breeding in pastures from early spring to late autumn is a re-emerging practice in Serbia, as a closer step to organic farming.

This study was performed in order to assess the exposure of grazing dairy cows to *M. bovis* in several parts of Serbia.

MATERIALS AND METHODS

The study was performed in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

Sampling material originated from 131 clinically healthy grazing dairy cows. Blood was collected during year 2013 from coccygeal vein, using 0.8mm needles (BD Vacutainer Precision Glide 0.8 x 38mm), into 5mL tubes with increased silica act clot activator, silicone-coated interior (BD Vacutainer serum tube). Cows were randomly selected from unvaccinated against *M. bovis* herds, in the age-range of 2 to 5 years and originated from 5 different areas of Serbia: Zasavica, Gruza, Pozarevac, Novi Sad and Banatski Karlovac (Fig. 1). Concerning cows' breeds, Simmental crossbreeds were sampled in Zasavica and Gruza, while Holstein-Friesians were sampled in the other 3 areas.

Blood samples were allowed to clot at room temperature. Serum was obtained, transferred into plastic vials and stored at +4°C until analysis. A commercial *M. bovis* ELISA indirect double well test kit for the serological (antibody) diagnosis of blood serum and milk was used (Bio-X Diagnostics, Belgium). ELISA plates washing was performed on ELISA PW41 Micro plate (Bio–Rad Laboratories, France) with optical values reading on ELISA reader (TEKAN, Austria), using a 450 nm filter. All procedures were carried out according to the manufacturer's instructions.

RESULTS

A total of 13 out of 131 (9.92%) sera samples were positive for the presence of specific *M. bovis* antibodies (Table 1). The number of seropositive samples ranged from 4.4% (Pozarevac) to 14.3% (Zasavica, Novi Sad). All the 15 cows from Gruza area were seronegative for antibodies against *M. bovis*.

Table 1. Number and percentage of examined and seropositive to specific *Mycoplasma bovis* antibodies blood sera samples from grazing dairy cows of 5 different areas of Serbia.

Area	Number of examined samples	Number of positive samples	Percentage %
Zasavica	14	2	14.3
Pozarevac	23	1	4.4
Gruza	15	-	-
B. Karlovac	58	7	12
Novi Sad	21	3	14.3
Total	**131**	**13**	**9.9**

DISCUSSION

Obtained results show, for the first time, preliminary data concerning specific *M. bovis* antibody prevalence in blood sera of clinically healthy grazing dairy cows in different locations of Serbia. Our previous reports showed seroprevalence of 2.57% in 5603serum samples of clinically healthy calves from 3 farms (Vojinovic et al., 2014). That emphasized the need for determination of seroprevalence in adult dairy cows, because the occurrence of clinical disease on the field would be expected (Vojinovic et al., 2014). Vidic et al.(2014) determined the seroprevalence to be 4.81% in 3,777 fattening calves during period 2012-2013 in the Republic of Serbia. The higher seroprevalence in the present study could be due to the lower number of examined samples, to different management systems and because the tested animals were older. It would be possible for older cattle to come in contact more times with carriers or infected cattle, having thus increased possibilities to be infected by *M. bovis*. Moreover, at pasture commingling of cattle from different herds could increase the possibility of cross-infection.

During a 10-year period, from 1990 to 2000 in the Weybridge Veterinary Laboratory, Great Britain, *M. bovis* was the most abundant microorganism isolated from cattle with pneumonia and occasionally in cattle with arthritis and mastitis (Ayling et al., 2004). Several studies suggest that synergisms between *M. bovis* and other microorganisms might exacerbate disease outcome of bovine mycoplasmosis, thus careful screening should routinely be performed (Burgi et al., 2018).

Le Grand et al. (2002) examined 32,197 cattle blood sera from 824 randomly selected herds, originating from 8 different French provinces. Using the ELISA method, they found 2 to 13% of positive herds within each province and an animal infection rate ranging between 10 to 20% within individual infected herds.

Mycoplasmatic infections are considered to be endemic in Poland. Examination of 3,670 cattle blood sera samples from 16 provinces in the period from 2007 to 2010 by ELISA showed high seroprevalence (76.1%), but there was no clinical manifestation of the disease. After correction of the test sensitivity, the prevalence was decreased to 28.2%, which was similar to other European countries. In Hungary, 595 cattle blood sera were examined and 10.9% were found seropositive to *M. bovis* (Tenk, 2005). In Bosnia and Herzegovina, from 2002 to 2010, *M. bovis* was isolated 8 times from nasal swabs and lung samples of cattle (Maksimovic and Rifatbegovic, 2012), indicating that there was active infection in Serbia`s neighboring country. Comparing available literature, we can conclude that the obtained results of 9.92% seropositivity are similar to surrounding and other European countries.

Apart from the fact that grazing cattle are an especially sensitive population to the development of bacterial and viral infections due to every day close contact between animals (many times from different farms), they are also more prone to parasitic infections. Those can affect nutrient intake, leading to deficiencies that compromise protective immunity against *Mycoplasma* spp. and other agents (Fekete and Kells, 2007). Thus, more intensive monitoring of these animals' health status and further studies are needed in order to establish measures for *M. bovis* infection control and eradication.

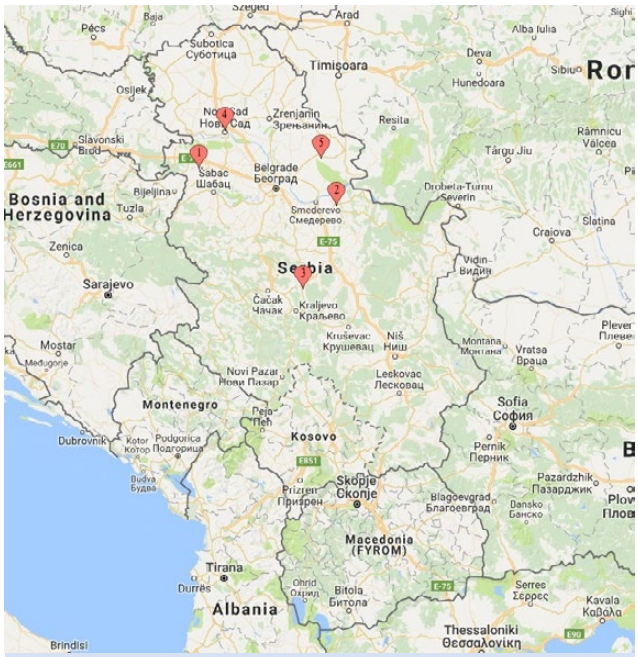

Figure 1: Location of the 5 different areas of Serbia where grazing cows were sampled for *Mycoplasma bovis* serology: 1 -Zasavica, 2 - Pozarevac, 3 - Gruza, 4 - Novi Sad and 5 - Banatski Karlovac (MapCustomizer, https://www.mapcustomizer.com/).

CONCLUSIONS

Specific antibodies against M. bovis were identified in 9.92% of the grazing cows from 4 areas; the only negative was the most southern region, Gruza. The revealed seroprevalence is the first evidence for the presence of M. bovis in grazing dairy cows in different locations of Serbia.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

ACKNOWLEDGMENTS

This research was financed by Ministry of Education, Science and Technological Development of Serbia, grants TR37015, TR 31071 and TR31079.

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