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**Determination of *Mycoplasma bovis* specific antibodies in blood sera of asymptomatic carriers-calves in three farms in the Republic of Serbia by using indirect ELISA assay**

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**ABSTRACT.** Blood serum samples of asymptomatic carriers-calves were collected from three farms in the territory of the Republic of Serbia during 2011 and 2012. Commercial *Mycoplasma bovis* ELISA kit (Bio-X Diagnostics, Belgique) for serological diagnosis from cattle blood sera and milk was used in this research. Calves' blood sera were tested using immunoenzymatic indirect ELISA assay as described by manufacturer's instructions. From 5603 blood sera of asymptomatic carriers-calves 144 (2,57%) samples were tested positive for the presence of specific *Mycoplasma bovis* antibodies. In three different farms proportions of seropositive samples varied from 0,32% to 10,6% in regard to total number of tested samples from the individual farms. In this paper we present the results of *Mycoplasma bovis* prevalence in asymptomatic carriers-calves.

**Keywords:** antibodies, cattle, *Mycoplasma bovis*, Serbia

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## INTRODUCTION

*Mycoplasma bovis* is the smallest prokaryotic microorganism classified in classis *Mollicutes*, family *Mycoplasmataceae*, genus *Mycoplasma*. *Mycoplasma bovis* was isolated for the first time in milk of mastitic cow in 1961 (Hale et al., 1962). Furthermore, experimental investigation of microbial presence in calves has shown that *Mycoplasma* species are present and can cause pneumonia (Gourlay and Thomas, 1969; Gourlay et al., 1976). *M. bovis* is widespread and causes great economic losses worldwide (Nicholas, 2004; Tenk, 2005). Reported prevalence from 10% up to 70% varies depending on the region of the world (Wiggins et al., 2011). Authors from France report extremely high prevalence of 100% (Byrne et al., 2001; Nicholas et al. 2006), while in Hungary Tenk et al. (2004) reported seropositivity of 64.7%. *M. bovis* is becoming an emerging pathogen in subtropical regions of Asia where authors in two separated studies reported prevalence of 8% and 6% respectively (Fu et al., 2011; Zhao et al., 2012), and in Africa where occurrence of disease was reported in Zambia, Rwanda, Namibia and Nigeria (Nicholas et al., 2006; Tambuwal et al., 2011).

*M. bovis* causes pneumonia, mastitis and arthritis in cattle (Razin, 1978; Gonzales et al., 1993), but it can also be causative agent of meningitis, otitis media, keratoconjunctivitis and abortion (Maeda et al., 2003; Levisohn et al., 2004; Gagea et al., 2006., Arcangioli et al., 2012). In asymptomatic cows, *M. bovis* can be found in colostrum and milk which is main infection source for calves (Pfutzner, 1990).

Recommended tests for serological diagnostics are: complement fixation test, direct and indirect fluorescence method, ELISA assay, immunoblotting test, rapid agglutination test. Hence, the aim of this study was to estimate prevalence of *M. bovis* in asymptomatic young calves in the Republic of Serbia.

## MATERIALS AND METHODS

A total of 5603 blood sera samples of asymptomatic calves were collected and tested from three farms in the territory of the Republic of Serbia during 2011 and 2012. Number of samples from three farms was 1058 (farm 1), 3715 (farm 2), 830 (farm 3) according to their size and production. Animals were not vaccinated against *Mycoplasma bovis*. They were not previously treated with any medication.

Commercial indirect *M. bovis* ELISA kit (Bio-X Diagnostics, Belgique) for serological diagnostic from cattle blood sera and milk was used in our research. Automatic washing was performed using PW 41 Microplate washer Bio- Rad Laboratories, France. Reading of optical density was done on spectrophotometer TEKAN, Austria ELISA reader, with the use of 450nm filter as recommended in manufacturer's instructions. Interpretation of results was done by manufacturer's instructions for validation and interpretation of the results.

## RESULTS

During 2011 and 2012, a total of 5603 blood sera of asymptomatic carriers-calves from three different farms were tested in our research using immunoenzymatic indirect ELISA assay. A total of 144 (3%) samples were found to be positive for the specific *M. bovis* antibodies. Number of seropositive samples varied from 0.32% to 10.6% with regard to total number of tested samples from the individual farm (Table 1).

## DISCUSSION

At the veterinary laboratory in Weybridge UK, during 1990-2000, *M. bovis* was the most frequently isolated pathogenic microorganism in cattle with pneumonia, as well as occasionally isolated pathogen in cattle with mastitis and arthritis (Ayling et al., 2004). The results of testings for *M. bovis* specific antibodies in asymptomatic carriers-calves using indirect ELISA

**Table 1.** Number of tested and seropositive samples of calves for presence of *Mycoplasma bovis* specific antibodies.

Farm	No. of samples	No. of positive samples	Frequency
1	1058	44	4
2	3715	12	<1
3	830	88	11
Total	5603	144	3

assay proved that seroconversion exists in tested population at 3%. Results of similar research in Hungary, which is a neighbouring country to the Republic of Serbia, showed that the infection was present in cattle population (Tenk et al., 2005). Authors from France showed that *M. bovis* specific antibodies in clinically healthy calves varies from 2 to 13% (Le Grand et al., 2002), while a prevalence of <1% was reported for the population of clinically healthy dairy cows (Arcangioli et al., 2011). The results in our research show that in Serbia, *M. bovis* prevalence in calves (3%) is about that in other European regions.

Determination of presence of *M. bovis* specific antibodies using indirect ELISA assay is recognised as reliable method since antibodies against *M. bovis* are long lasting and can be detected months after the infection (Nicholas, 2004). Knowledge of exact prevalence in herd is essential for the prevention of disease in order to avoid direct and indirect economic losses in production (Pfutzner and Sachse, 1996). It is also nec-

essary to develop a program of measures to be applied in herds with a high prevalence in order to control the disease.

### CONCLUDING REMARKS

In this paper we present for the first time results on prevalence of *M. bovis* in asymptomatic carriers-calves in the Republic of Serbia. As *M. bovis* is one of the major causative agents of pneumonia in cattle, the research should be extended to heifers and adult cattle in order to accurately determine the prevalence of the disease in our cattle population.

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### CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest. ■

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