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SEROEPIDEMIOLOGICAL INVESTIGATION OF *MYCOPLASMA BOVIS* IN CALVES

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Abstract

Within the multifactorial etiology of respiratory infections in cattle, *Mycoplasmae* play an important role. Most of the *Mycoplasma* microorganisms play a minor role in the development of the infections in cattle, contrary to *Mycoplasma bovis* (*M. bovis*) that is commonly the primary agent of the disease. Respiratory tract mucosa is the primary colonization site of *M. bovis* in cattle. Colonization of the upper respiratory tract of calves with *M. bovis* often occurs within the first few weeks of life. Animals with chronic infection and without clinical symptoms occasionally shed *M. bovis* and they are highly important for the epidemiology of the infection. Stress factors such as transportation, entrance into the feeding object, coldness, etc. are associated with the secretion of *M. bovis* from a nose. Diagnostic procedure relies on clinical symptoms and detection of causative agent, regardless of whether the infection is found in individual animals or in the entire herd. Serological detection of *M. bovis* antibodies is often a reliable diagnostic method. The most used indirect method is ELISA test. During a two-year period, blood serum samples from calves (beef cattle) were examined and analyzed. Calves originated from cattle farms (big and small) of Holstein- Friesian and Simmental breed. The total number of 3777 samples was examined applying ELISA (Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek[®] *M. bovis*). Positive findings were obtained in 182 animals, i.e. 4.81%. The obtained results confirmed the presence of *M. bovis* in cattle herds. Further research from epizootical aspect and the role of *M. bovis* in the occurrence of health problems in cattle population is necessary.

Key words: *Mycoplasma bovis*, calves, antibodies, ELISA

SEROEPIDEMIOLOŠKA ISPITIVANJA *MYCOPLASMA BOVIS* KOD TELADI

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Kratki sadržaj

U multifaktorijalnoj etiologiji respiratornih infekcija goveda, mikoplazme imaju značajno mesto. Većina vrsta mikoplazma imaju sekundarnu ulogu u nastajanju infekciji kod goveda, za razliku od *Mycoplasma bovis* (*M. bovis*) koja ima primarnu ulogu. Sluzokoža gornjih respiratornih puteva je primarno mesto za kolonizaciju *M. bovis* kod goveda. Naseljavanje *M. bovis* gornjeg respiratornog trakta kod telade nastaje već u prvim nedeljama života. Hronično inficirane jedinke bez kliničkih simptoma, koje povremeno izlučuju *M. bovis*, su značajna u epidemiologiji infekcije. Stresni događaji kao što su transport, ulazak u tovilište, hladnoća i drugo su povezani sa izlučivanjem *M. bovis* iz nosa. Na osnovu kliničke slike se postavlja sumnja na mikoplazmozu, a definitivna dijagnoza se postavlja dokazivanjem uzročnika, bilo da se radi o pojedinačnim grlima ili na nivou zapata. Dokazivanje infekcije *M. bovis* serološkim testovima se uspešno primenjuju. Danas se najčešće koristi indirektni metoda ELISA test. U toku dvogodišnjih ispitivanja pregledali smo krvne serume teladi koja su namanjena za tov. Telad su poticala sa farmi goveda (većih i manjih) i pripadali rasi Holštajn-Frizijskoj i Simentalskoj. U toku ispitivanja pregledali smo ukupno 3777 krvnih seruma teladi. Koristili smo metod ELISA (Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek® *M. bovis*). Pozitivne rezultate utvrdili smo kod 182 životinja, što predstavlja 4,81%. Dobijeni rezultati ukazuju da je *M. bovis* prisutna u zapatima krava i da je potrebno vršiti dalja ispitivanja sa epizootiološkog aspekta i njene uloge u nastajanju zdravstvenih problema u populaciji krava.

Ključne reči: *Mycoplasma bovis*, telad, antitela, ELISA

INTRODUCTION

Mycoplasmas is recognized as the cause of some of the most severe and economically most costly diseases in cattle (Gonzalez et al, 1993; Gonzalez and Wilson 2003). More than 20 different *Mycoplasma* species have been isolated from cattle with different clinical symptoms of a disease (Henderson and Ball 1999). Most of the *Mycoplasma* microorganisms have a secondary role in cattle infection, contrary to *Mycoplasma bovis* (*M. bovis*), which has a primary role in the occurrence of the disease. *M. bovis* was first isolated back in 1961 in the US, as a disease with clinical symptoms of severe mastitis in cattle (Hale et al, 1962), and then during the next 40 years, it has spread to Europe, Asia and the rest of the world (Filioussis et al, 2007). So far, the presence of mycoplasmosis in cattle has been reported in most European countries. The prevalence of *M. bovis* is underestimated and other bacteria are often isolated in calves with pneumonia or cattle with mastitis, where actually *M. bovis* is the primary cause. Only a few laboratories in the world routinely perform the monitoring of mycoplasmas. The occurrence of *M. bovis* in the herd is always associated with the cases of pneumonia, mastitis and arthritis (Pfutzner and Sachse, 1996). As compared to economic losses from respiratory diseases, the losses associated with mycoplasmosis in the cattle industry of US or UK are very high, tending to increase due to mycoplasmatic mastitis cases (Rosengarten and Citti, 1999).

M. bovis is widely spread among bovine population in enzootically infected areas. The infection is usually introduced into the new herds by clinically healthy calves or young cattle shedding the causative agents. Infected cattle shed mycoplasmas via the respiratory tract for many months or years representing the permanent reservoir of the infection. Respiratory tract mucosa is the primary site for the colonisation of *M. bovis* in cattle. Respiratory tract mucosa

and mammary gland are the most important locations for the maintenance and secretion of *M. bovis*, which can persist even several months. Stress factors such as transportation, entrance into the feeding object, coldness, etc. are associated with the secretion of *M. bovis* from a nose. Animals with chronic infection and no clinical symptoms are occasionally shedding *M. bovis* and they are very important factor in the epidemiology of the infection. *M. bovis* has been proved a frequent causative agent of pneumonia, mastitis and arthritis in cattle (Nicholas et al, 2000). *M. bovis* can also be transmitted from an infected cow to a foetus or post partum to a newborn calf. The causative agent infects respiratory tract and stays there live and infectious until the pubescence or even the first calving (Bobos and Vidic, 2005)

Diagnostic procedure relies on clinical symptoms and detection of causative agent, regardless of whether the infection is found in individual animals or in the entire herd. ELISA is the mostly used indirect method. Serological detection of *M. bovis* antibodies is often a highly reliable diagnostic method. The level of antibodies detected by ELISA method persists for many months, especially in case of preceding month-long antibiotic therapy at herd level. In such cases, the isolation of the agent is very difficult. All other serological tests, such as possible indirect haemagglutination or film inhibition, are not as successful as indirect ELISA and thus not widely used. Commercial diagnostic tests are available in the market used worldwide.

MATERIALS AND METHOD

During a two-year period, blood serum samples from calves (beef cattle) were collected, examined and analyzed. The samples were taken from animals originating and living in different regions in the territory of Serbia. The calves of Holstein- Friesian and Simmental breed originated from several cattle farms (big and small. The total number of 3777 samples was collected and examined.

The diagnostic was performed using ELISA method. The diagnostic kit used in this research was a commercial Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek[®] *M. bovis*, which is used in a routine laboratory work. The blood sera were analyzed using indirect ELISA test according to manufacturer's instructions. ELISA (Enzyme – linked immunosorbent assay) is a sensitive and specific method for detection of specific antibodies against certain infectious agent from blood sera. Antibodies from the serum bind with the antigen contained in a layer coating the wells of the test and an antigen-antibody complex is formed. Subsequently, the complex is stained to enable better visualisation.

RESULTS AND DISCUSSION

In total, 3777 blood serum samples of calves from different farms were analyzed for the presence of specific antibodies against *Mycoplasma bovis*. The analysis was performed using indirect ELISA method, a commercial kit. Positive findings were detected in 182 animals, i.e. 4.81% of the total population examined.

The finding of specific antibodies against *Mycoplasma bovis* is presented in Table 1.

Table 1. Findings of antibody against *M. bovis* in blood sera of calves

Farm	No. of examined calves	Positive	%
1	576	11	1,90%

2	234	18	7,69%
3	482	33	6,84%
4	675	27	4,00%
5	785	49	6,24%
6	311	8	2,57%
7	714	36	5,04%
Total	3777	182	4,81%

Similar results were obtained in another study performed in Serbia that included different regions and different diagnostic laboratory. In this research, 2.74% of calves proved positive to specific antibodies against *Mycoplasma bovis* in an indirect ELISA method (Vojinovic et al, 2012).

Mycoplasmae play an important role in the multifactorial etiology of respiratory infections in cattle. Several *Mycoplasma spp.* can cause severe mastitis in cattle, but *M.bovis* is the the predominant one. The disease spreads rapidly, that is, many cows manifest clinical signs of mastitis in one or more udder quarters in a very short period. In lactating cows, the infection mostly affects the entire udder. On farms with history of cattle mycoplasmosis, problems with joints, reproductive failures, pneumonia in calves and respiratory problems in adult cattle were recorded (Stokka et al, 2001). Dairy cows with mycoplasmatic mastitis show a drastic drop of milk production. Considering the infectious nature of the disease, clinical symptoms spread within the herd very fast, thus appropriate control measures have to be implemented (Vidic et al, 2012).

Unlike the majority of bacterial infections, the therapy of *Mycoplasma* infections is highly demanding, which is due to organism's resistance to mostly used antibiotics. Vaccination is a potential strategy to control *M.bovis* infection; however, the efforts to develop effective vaccine for use in young calves have been problematic so far. An effective program for the control of *M. bovis* infections includes a number of factors such as early detection of carriers and their removal from the heard, appropriate vaccination schedule, breeding conditions providing minimum environmental stress, housing with good air circulation, "all in all out" management practice to prevent infection transmission from older animals to younger ones or at least separating the calves from adult animals as early as possible in case of the occurrence of endemic disease exists, etc (Nicholas and Ayling, 2003).

CONCLUSION

Despite the great number of clinical cases and significant economical losses, *M.bovis* is still considered an unimportant pathogen among veterinarians. Mycoplasmosis in cattle may have severe socio-economic impact from the aspect of export and international trade. The obtained results demonstrated wide distribution of *M.bovis* in cattle herds, thus further research of epizootical features and role of *M. bovis* in the occurrence of health problems in cattle population is necessary. The research of *M.bovis* should be extended to a wider cattle

population of different age, with a particular focus on older animals. Considering the possible economical losses, more comprehensive research should be taken into consideration.

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