

MYCOPLASMA BOVIS WAS NOT DETECTED IN MILK FROM DAIRY CATTLE IN THE CZECH REPUBLIC

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Abstract

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The aim of this study was to screen selected Czech dairy cattle farms for presence of *Mycoplasma bovis*. Between 2012 and 2014, 160 individual samples of cow milk, 60 colostrum samples, and 105 bulk-tank milk samples collected on 21 dairy farms were analyzed in our laboratory. DNA was extracted from samples and tested by quantitative PCR assay for *M. bovis*. All analyzed samples were negative. Results support long-term trend of very low prevalence of *M. bovis* in the Czech Republic.

Keywords: *Mycoplasma bovis*, respiratory disease, mastitis, cattle, qPCR

INTRODUCTION

Mycoplasma bovis can be found in the upper respiratory and reproductive tracts in both young and old cattle (Pfutzner *et al.*, 1983). *M. bovis* is primary pathogen of cattle; it is capable of causing acute to subacute forms of bovine pneumonia, arthritis, and mastitis and has also been associated with keratoconjunctivitis, otitis, meningitis, infertility, and abortion (Pfutzner and Sachse, 1996). It occurs in many countries of the world including European countries and the USA. Estimation of real prevalence of *M. bovis* in these countries reaches value of 20% (Maunsell *et al.*, 2011). Although clinical signs tend to be most severe following recent infection, disease may persist in herds over a prolonged period (Byrne *et al.*, 2005), with clinically healthy cows shedding the agent in their milk (Sachse *et al.*, 2010).

M. bovis causes huge economic losses in cattle and milk production in both Europe and the USA. The losses caused by respiratory diseases in cattle are approximately a sum of €576 million per year in Europe. *M. bovis* is estimated to be responsible at least for the quarter or third of these losses. In the USA, this organism causes a loss of \$32 million per

year as a result of the loss of the weight gain and the diminished carcass value. The expenses due to *M. bovis* mastitis are estimated to be \$108 million per year (McAuliffe *et al.*, 2004).

Since *M. bovis* infection is difficult to treat by chemotherapy (Ghadersohi *et al.*, 1997; Stipkovits *et al.*, 1999), current methods to control the disease include preventive vaccination, culling of diseased animals (Ghadersohi *et al.*, 1997; Stipkovits *et al.*, 1999) and measures such as increased hygienic standards, disinfections and control or restrictions of animal movements (Sachse *et al.*, 1993). Eradication of *M. bovis* infection can only be achieved by the complete replacement of all cattle in infected populations with animals from *M. bovis*-free herd (Pfutzner and Sachse, 1996).

In former Czechoslovakia *M. bovis* was first isolated from sporadic cases of disease in 1975 (Jurmanová *et al.*, 1975) and then not until 2007 (Zendulková *et al.*, 2007). *M. bovis* is not systematically monitored in the Czech Republic, so up-to-date epidemiological data are not available. We established study to assess current state of *M. bovis* dissemination in Czech dairy herds showing with signs of clinical or subclinical mastitis and in bulk-tank milk.

MATERIAL AND METHODS

Samples

Three types of samples were used in this study:

- 1) individual milk samples,
- 2) colostrum samples, and
- 3) bulk-tank milk samples.

Total of 160 milk samples from individual cows were submitted to our laboratory in 2012 and 2013. They came from 21 Czech dairy farms, where repeated problems with mastitis were reported. Individual samples originated from cows showing with clinical signs of mastitis and/or altered milk consistency. Sum of 60 colostrum samples were collected in 2014. They originated from two dairy farms in Vysočina Region in the Czech Republic. Mastitis occurred on these farms in the time of sampling. Total of 105 bulk-tank milk samples coming from 85 dairy farms were collected in 2013 and 2014. Sampled farms have been chosen randomly, with no regard to mastitis occurrence on the farms.

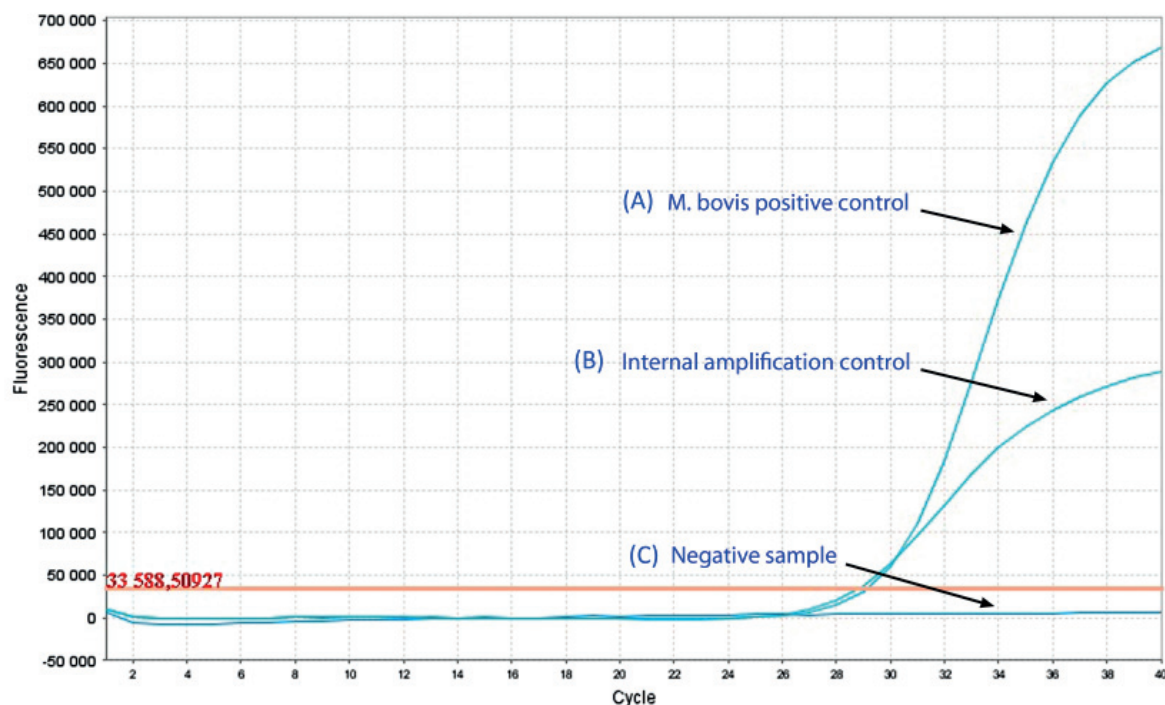
DNA Extraction Procedures

DNA of milk samples was extracted using the PathoProof DNA Extraction Kit (Thermo Scientific, Vantaa, Finland). Extraction was done according to the manufacturer's protocol. In order to eliminate fat, colostrum samples were incubated at 50 °C for 15 min and then centrifuged at 5000×g for 5 min.

M. bovis qPCR Detection

PMB996-F and PMB1066-R primers and the Mbovis1016 hydrolysis probe (FAM/BHQ1) previously published in study of Sachse *et al.* (2010) were used for *M. bovis* detection in individual milk samples and colostrum samples. Sachse *et al.* (2010) state that the assay sensitivity is $1 \cdot 10^0$ copies/ μ l. qPCR protocol was followed. An exogenous internal amplification control (IAC), i.e. synthetic DNA strand, complementary primers, and Cy5-labeled probe (Generi Biotech, Hradec Králové, Czech Republic) was added to each reaction according to the manufacturer's protocol, in order to monitor possible inhibitory effects. As a positive control, DNA extracted from milk spiked with *M. bovis* culture was used (Fig. 1). Briefly, amplifications were carried out in 96-well plates (Applied Biosystems, Foster City, CA, USA) using the ABI 7500 real-time PCR instrument (Applied Biosystems, Foster City, CA, USA) and the PCR conditions were as follows: uracil DNA-glycosylase (UDG) pre-treatment at 50 °C for 2 min, initial denaturation/activation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s and extension at 60 °C for 45 s. Each 20- μ l reaction contained 10 μ l of TaqMan Gene Expression Master Mix [Applied Biosystems, Foster City, CA, USA, (contains UDG)], 900 nM of each primer, 250 nM of the probe and 2 μ l of template DNA. Reactions were run in duplicates.

Bulk tank milk samples were analyzed for *M. bovis* using commercial PathoProof Major-3 assay (Thermo Fisher Scientific, Vantaa, Finland)



1: Demonstration of amplification reaction specificity (individual milk samples). (A) *M. bovis* DNA gives positive result, (B) IAC is co-amplified with *M. bovis* DNA, (C) and sample not containing *M. bovis* DNA gives negative result (DNA of *Staphylococcus aureus* CCM 4246 was used)

according to the manufacturer's protocol. Amplifications were carried out in 96-well plates (Applied Biosystems, Foster City, CA, USA) using the ABI 7500 real-time PCR instrument (Applied Biosystems, Foster City, CA, USA).

RESULTS

Individual Milk Samples and Colostrum Samples

Total of 160 individual milk samples and 60 colostrum samples were tested using qPCR method described by Sachse *et al.* (2010). All these samples were negative for *M. bovis*. Reactions were not inhibited, since amplification of the internal amplification control was not impaired. Control sample containing *M. bovis* DNA was positive.

Bulk-tank Milk Samples

Sum of 105 bulk-tank milk samples were analyzed for *M. bovis* using commercial PathoProof Major-3 assay. This assay contains its own internal amplification control reaction. Internal control was not impaired and no *M. bovis* DNA was detected in these samples.

DISCUSSION

Upper respiratory tract mucosa and the mammary gland appear to be the most important sites of persistence and shedding of *M. bovis* (Punyapornwithaya *et al.*, 2010). *M. bovis* is regarded as a contagious mastitis pathogen, with udder-to-udder spread being major mean of transmission (Gonzales and Wilson, 2003). So it is generally accepted that milk samples are suitable material for *M. bovis* detection and they are being used by laboratories for this purpose.

Mycoplasma mastitis is especially problem in hot and dry areas of the world (Blowey and Edmondson, 2010). Individual cow prevalence and incidence of clinical mycoplasma mastitis vary widely between herds and studies (Maunsell *et al.*, 2011). Sachse *et al.* (2010) have demonstrated that while the *M. bovis* load in herds with clinical mastitis was significantly higher than in disease-free animals, infection persisted in the latter. Our results indicate that *M. bovis*-induced mastitis is not significantly spreaded in Czech dairy herds. Results are in congruence with findings of Jozefová *et al.* (2014), who also did not identified any mycoplasma in individual milk samples. However, they did identified *M. bovis* in lungs and nasal cavity of calves, but on very low level (2% of samples).

Cases of mastitis in studied animals was not of mycoplasma origin and neither infection persisted in these animals. We can hypothesize, that *M. bovis* prevalence and incidence in the Czech Republic is significantly lower than in other European countries (Chazel *et al.*, 2010; Sachse *et al.*, 2010) and the USA (Maunsell *et al.*, 2011). Reasons for this remarkable fact may include geographical isolation of Czech dairy herds, high hygienic standard on farms, good milking routine and welfare of animals. Considering limited scale of our survey, our results possibly do not reflect true prevalence of *M. bovis*-induced mastitis. Additional reasons are that mycoplasmas may be shed intermittently (Pfutzner and Sachse, 1996) and mastitic milk is withheld from the bulk tank.

Despite low prevalence of *M. bovis* in the Czech Republic, attention should be paid to this known but currently non-notifiable bovine pathogen. Nationwide study on mycoplasmoses could provide detailed information about extent of disease and about its impact on economy of cattle farming and welfare of animals.

CONCLUSION

This study has employed qPCR method of detecting pathogenic bacterium *M. bovis* in milk, colostrum, and bulk-tank samples. Presence of *M. bovis* was not proven in analyzed samples. Negative findings have supported long-term trend of low prevalence of mycoplasma mastitis in the Czech Republic.

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