J. Dairy Sci. 96:1661–1667
http://dx.doi.org/10.3168/jds.2012-5669

Short communication: Role of *Mycoplasma arginini* in mastitis caused by *Streptococcus dysgalactiae*

Laszlo Stipkovits,*†[1] Maria Somogyi,†‡ Balazs Asvanyi,‡ Agnes Toth,‡ and Susan Szathmary†‡§
*Carlsbad Research Organization, Lucsony u. 15-17, 9200 Mosonmagyarovar, Hungary
†RT-Europe Research Center, Pozsonyi ut 88, 9200 Mosonmagyarovar, Hungary
‡GalenBio Kft, Lucsony u.15-17, 9200 Mosonmagyarovar, Hungary
§GalenBio Inc., 1949 Kellogg Ave., Carlsbad, CA 92008-6582

ABSTRACT

We performed a comparative study on the development of mastitis induced by *Mycoplasma arginini* or *Streptococcus dysgalactiae* after challenging the cows. *Mycoplasma arginini* did not cause any clinical symptoms on its own, resulting in only a transient increase of somatic cell count (SCC; increase ranging from 0.5 $\times$ 10^6 to 0.8 $\times$ 10^6 cells/mL) and a slight decrease of milk production (10%) for 5 d. In contrast, *Strep. dysgalactiae* induced more severe clinical signs in animals and SCC increased to 1.60 $\times$ 10^6 to 2.11 $\times$ 10^6 cells/mL for 10 d. In addition, milk production decreased (22.9 to 27.0%) for 10 d. After 3 mo (2 mo after the first challenge), animals that were challenged previously with *M. arginini* were rechallenged with *Strep. dysgalactiae*. Severe clinical mastitis developed, with very high SCC (5.00 $\times$ 10^6 to 21.5 $\times$ 10^6 cells/mL), and a very significant reduction of milk production (28.6 to 68.7%), which lasted more than 4 wk, was observed. The severe clinical mastitis developed not only in cows inoculated with *Strep. dysgalactiae* and *M. arginini* in the same udder quarter but also in cows infected in the quarter previously not challenged with mycoplasma. Cows challenged first with *Strep. dysgalactiae* and rechallenged with *M. arginini* 2 mo later developed only slight changes in both SCC and milk production, similar to those when the cows were challenged with *M. arginini* alone. We conclude that *M. arginini* infection does not cause remarkable mastitis (characterized by decrease in milk production and increase of SCC) but it significantly predisposes animals to infection with *Strep. dysgalactiae*, leading to severe clinical mastitis.
Key words: challenge, *Mycoplasma arginini*, *Streptococcus dysgalactiae*, mastitis

Short Communication

The prevalence of mammary gland inflammation is relatively high in dairy cattle. Mastitis causes considerable economic losses through decreased milk yield, impaired milk quality, and increased cost of treatment and veterinary services (Seegers et al., 2003). Mastitis occurs in clinical and subclinical forms. Several bacterial species including *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Corynebacterium bovis*, and *Staphylococcus aureus* (Lee et al., 2008) are responsible for causing mastitis (Zhao and Lacasse, 2008). Among the *Mycoplasma* species, *Mycoplasma bovis* is reported very frequently as a causative agent of mastitis worldwide (Nicholas et al., 2007). Other *Mycoplasma* species, such as *Mycoplasma alkalescens* (Hirose et al., 2001), *Mycoplasma leachii* (Alexander et al., 1985), *Mycoplasma bovigenitalium* (Roy et al., 2008), *Mycoplasma californicum* (Mackie et al., 1982), *Mycoplasma canadense* (Infante-Martínez et al., 1999), and *Mycoplasma verecundum* (Higuchi et al., 2011) less frequently cause mastitis. Other *Mycoplasma* species (e.g., *Mycoplasma arginini*) are not regarded as etiological agents of mastitis; nevertheless, they are frequently recovered from bulk tank milk (Fox et al., 2005). Therefore, it is important to study the direct effect of *M. arginini* on the udders of cows and the influence of *M. arginini* infection on subsequent *Strep. dysgalactiae* challenge.

The experimental cows originated from a herd consisting of 500 Holstein-Friesian cows. All animals were examined serologically for the presence of *M. bovis* antibodies using an *M. bovis* ELISA kit (Chekit-*M. bovis* sero; Bommeli Diagnostics, Liebefeld-Bern, Switzerland). In addition to this, nasal swabs, milk samples collected from all quarters of the udder, and vaginal swabs taken from 20 cows were cultured in medium B (Erno and Stipkovits, 1973) for the presence of mycoplasmas. Tubes containing 2 mL of broth inoculated with samples were incubated for 10 d at 37°C. On d 3, 7, and 10, 10 μL of broth culture was spread on agar plates that were also incubated for 10 d at 37°C. Agar

Received April 29, 2012.
Accepted November 26, 2012.
[1]Corresponding author: stipkovits@earthlink.net

plates were regularly examined under the microscope for the presence of mycoplasma colonies. Inoculated broth samples were tested after 24 h of incubation by PCR using primers specific for mycoplasma and primers specific for *M. bovis* (Tenk et al., 2006).

Twelve cows in middle of second lactation were selected for the experiment. Before the study, all cows were retested for the presence of mycoplasmas as described earlier; no mycoplasmas were detected. Cows were divided into 3 groups of 4 animals each in such a way that the average milk production of a group did not differ statistically from that of the other groups during the 5 d before challenge. The cows were labeled A, B, C, and D in group 1; E, F, G, and H in group 2; and I, J, K, and L in group 3.

The cows of group 1 were challenged with 5 mL of a 48-h-old *M. arginini* culture (strain 65), which was isolated from the serum of a 1-d-old calf (Stipkovits et al., 1975). The strain was cloned 3 times, lyophilized, and stored at −70°C; it was cultured every 4 to 5 yr. Before challenge, the broth culture was diluted 1:100 with PBS. The concentration of the inoculum was 1.3×10^6 cfu/mL, and cows were inoculated in the milk cistern of the left rear (**LR**) quarter of the udder after the morning milking using a 20-cm-long catheter 3 mm in diameter. After inoculation, the udder was massaged gently. The protocol of the animal experiment was approved by the Animal Research and Care Committee of the Carlsbad Research Organization Ltd. (Mosonmagyarovar, Hungary), and animal care and experimentation were carried out in accordance with the institutional and national guidelines. Cows of group 2 were inoculated in the cistern of the LR quarter with 5.0 mL of a 24-h-old broth culture of *Strep. dysgalactiae* diluted 1:100 in PBS (5.3×10^6 cfu/mL). Animals in group 3 (control group) were inoculated with 5 mL of sterile PBS into the LR quarter. The groups were kept in separate air spaces. Feeding and maintenance were the same in all 3 groups, but care and milking of cows in different groups were performed by different personnel.

For 5 d before and 15 d after challenge, the cows were examined clinically every day after the morning milking. The presence of clinical signs was scored as follows: 1 = udder was swollen or painful or the local temperature on the udder surface was increased. Milk production of cows was measured in the morning and evening. The SCC of milk samples obtained from each quarter at the morning milking was checked. Milk quality was recorded every morning and scored as follows: score 1 = color of the milk had become yellowish or brownish; score 2 = milk contained flakes, and score 3 = milk coagulated. In group 1, the mycoplasma counts of milk samples obtained during the morning milking from each quarter were checked. Similarly, in group 2 the bacterial count of milk samples was tested. Milk samples from cows of group 3 were also tested for the presence of mycoplasma and bacteria.

Two months after the end of the first experiment (first challenge), the cows were rechallenged as follows: In group 1, cows A and B were challenged in the LR quarter and cows C and D were challenged in the right rear (**RR**) quarter with 5 mL of 1:100 diluted 24-h-old broth culture of *Strep. dysgalactiae* (6.5×10^6 cfu/mL. In group 2, cows E and F were challenged in the LR quarter and cows G and H in the RR quarter with a 1:100 diluted 49-h-old *M. arginini* broth culture (3.9×10^6 cfu/mL) in the same way as in the previous experiment. Clinical examination, milk production recording, and the checking of milk SCC and milk quality were performed as described previously.

Two months after the start of the rechallenge experiment, the animals were euthanized and examined for pathological lesions in the internal organs and udder. Lung lesions were scored as follows: score 1 = area of lesions was <50% of the lobe, and score 2 = lesions extended to >50% of the lobe. The udder quarters were scored in a similar manner. Attempts were made to isolate *M. arginini* and *Strep. dysgalactiae* from all lobes of the lung, from the liver, spleen, and kidney, and from all udder quarters.

The clinical scores, scores of milk changes, and the isolation of mycoplasmas from the organs of animals in the different groups were compared by using the Chi-squared test. The quantities of milk produced in the groups during 5-d periods were compared by Student's *t*-test.

The cows used for the studies originated from a herd that was serologically negative for *M. bovis* by ELISA test and negative for *M. bovis* and *M. arginini* by culture and PCR. Nasal swabs, milk samples collected from all quarters of the udder, and vaginal swabs taken from 20 cows selected for studies proved to be negative by culturing and by PCR using primers specific for mycoplasmas and primers specific for *M. bovis*.

In group 1, we observed no swelling or pain of the udder or increased local temperature of the udder surface during the 15-d observation period after challenge with *M. arginini*. In group 2, 3 animals showed slight swelling of the udder with mild pain and increased local temperature of the udder surface for 2 to 3 d between d 6 and 10; scores varied between 5 and 9 (Table 1). No clinical symptoms were present in animals of group 3.

Milk production of cows was assessed from d −5 to d −1 (prechallenge milk production), and no differences were recorded between groups 1, 2, and 3 based on Student's *t*-test. Following challenge, however, milk production in group 1 was 3.6 and 8.4% lower rom d 1 to