Pathogen dependent effects of high amounts of oxytocin on the blood-milk barrier integrity during mastitis in dairy cows

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Pathogen abhängige Wirkung der Mastitisbehandlung mit hohen Mengen an Oxytocin auf die Integrität der Blut-Milch-Schranke bei Milchkühen

Die Reduzierung des Antibiotikakonsums bei Tieren in der Lebensmittelproduktion wird immer wichtiger. Daher müssen geeignete Alternativen zur Mastitisbehandlung bei Milchkühen geprüft werden. Oxytocin (OT) induziert das Einschiessen der Milch in das Milchgangsystem und unterstützt somit das Ausmelken der Milch aus infizierten Vierteln der Milchdrüse. Über die Euterentleerung hinaus führt die Injektion sehr hoher OT-Dosierungen durch eine verringerte Integrität der Blut-Milch-Schranke zu einer erhöhten Anzahl somatischer Zellen (SCC) in der Milch und ermöglicht den Transfer von Immunglobulinen (Ig) aus der Blutbahn in die Milch.

Das Ziel der vorliegenden Studie war es, bei an Mastitis erkrankten und mit zwei hohen Dosen Oxytocin (100 IE iv.) behandelten Kühen, in Abhängigkeit des Pathogens die Veränderungen von SCC, den aus Blut stammenden Milchkomponenten Lactatdehydrogenase (LDH), Serumalbumin (SA) und IgG in Milch zu untersuchen. Milchproben von 184 Milchkühen aus verschiedenen Betrieben wurden am Tag 1 (Tag der klinischen Untersuchung und Mastitisdiagnose) und an den Tagen 2, 3, 14 und 28 entnommen. Der Nachweis der Krankheitserreger erfolgte mittels einer bakteriologischen Untersuchung (Tag 1). Die Kühe wurden zufällig der Behandlungs- (OT-Injektionen an den Tagen 1 und 2) oder der Kontrollgruppe (kein OT) zugeordnet. Unabhängig von der zugewiesenen Versuchsgruppe wurden die Kühe mit reduziertem Allgemeinzustand nach der Probenentnahme gemäss dem Therapieprotokoll der Tierarztpraxis behandelt.

Milch-SCC, LDH, SA und IgG änderten sich spezifisch in Abhängigkeit der beteiligten Pathogene. Die höchsten

Abstract

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The reduction of antibiotic use in food producing animals becomes increasingly important. Therefore, suitable alternatives for mastitis treatment in dairy cows have to be considered. Oxytocin (OT) induces milk ejection and hence supports milk removal from infected mammary quarters. Beyond udder emptying, the injection of very high dosages of OT causes increased somatic cell counts (SCC) in milk and enables the transfer of immunoglobulins (Ig) from blood into milk through a reduced blood-milk barrier integrity.

The aim of the present study was to investigate pathogen-specific changes of SCC, the blood derived milk components lactate dehydrogenase (LDH), serum albumin (SA), and IgG in milk of cows suffering from mastitis caused by different pathogens treated with two intravenous injections of high dosages of OT (100 IU). Milk samples from 184 dairy cows from different farms were collected on day 1 (day of clinical examination and mastitis diagnosis) and on days 2, 3, 14, and 28. Bacteriological examination (day 1) identified involved pathogens. Cows were randomly assigned to treatment (OT injections on days 1 and 2) or control group (no OT). Independently of the assigned experimental group, cows received the common therapy protocol of the veterinary practice after sample collection if the general condition was affected.

Milk SCC, LDH, SA, and IgG changed specifically depending on involved pathogens. Highest values of all three parameters were measured in mastitis caused by *Streptococcus uberis*. Changes were less pronounced with other *Streptococci* spp., *Staphylococci* spp. or *Corynebacterium bovis*. Oxytocin treatment did not affect any of the studied parameters independent of the involved pathogen. Only in quarters infected with *Staphylococci* other than *Staphylococcus aureus* a decreased SCC and in-

Werte aller drei Parameter wurden bei durch *Streptococcus uberis* verursachten Mastitiden gemessen. Die Veränderungen waren bei *Streptococci* spp., *Staphylococci* spp. oder *Corynebacterium bovis* weniger ausgeprägt.

Unabhängig vom verursachenden Pathogen hatte die Oxytocin-Behandlung keinen Einfluss auf einen der untersuchten Parameter. Einzig bei Mastitiden verursacht durch andere Staphylokokken als *Staphylococcus aureus* wurden eine verminderte SCC gemessen und bei Mastitiden ohne Nachweis eines Pathogens konnte eine erhöhte IgG-Konzentration beobachtet werden. Daher ist eine hochdosierte OT-Verabreichung nicht als eigenständige Mastitisbehandlung bei Milchkühen geeignet.

Schlüsselwörter: Mastitis, Oxytocin, Blut-Milch-Schranke, Kuh

creased IgG concentrations in quarters, where no pathogens were detected, were observed. Thus, high dosage OT administration is obviously not suitable as a standalone mastitis treatment in dairy cows.

Key words: mastitis, oxytocin, blood-milk barrier, cow

Introduction

In dairy cows, different pathogens can cause subclinical and clinical mastitis, which usually results in changes of milk composition.[1] To achieve high standards of milk quality in dairy farming, mastitis is commonly treated with antibiotics. As the use of antimicrobial treatment in food producing animals is progressively losing acceptance due to the possible development of resistant pathogens as well as the possible occurrence of drug residues, alternative treatment strategies have to be considered and investigated. The use of high amounts of injected oxytocin (OT) has been discussed as a potential alternative to antimicrobial treatment. Oxytocin is a neuropeptide hormone released from the posterior pituitary in response to teat stimulation by the calf or the milking machine.[2,3] It induces myoepithelial contraction in the alveolar parenchyma. At a physiological level OT induces milk ejection.[4] The treatment with a high supraphysiological dosage of OT is considered to allow a maximum udder emptying to remove the pathogens from the infected quarter together with the milk.[5]

Intravenous OT administration up to 100 IU has been shown to increase the permeability of the blood-milk barrier and hence supports the transfer of immunoactive blood components into milk.[6,7] Blood constituents that are transferred from blood into milk include the enzyme lactate dehydrogenase (LDH), the protein serum albumin (SA), and immunoglobulins (Ig).[8,9] A recent study showed that the administration of OT in supraphysiological amounts causes an increase of the somatic cell count (SCC) and Ig concentrations in milk of healthy cows and also in cows with experimentally induced mastitis.[7]

Based on experimental models with components of Gram-positive and Gram-negative bacteria it is known that these effects of OT are pathogen-specific, i. e. only if the blood-milk barrier is already impaired in response to the pathogen this opening is enhanced by supraphysiological OT.[7] However, knowledge about effects of OT on changes of the blood-milk barrier integrity and SCC during mastitis induced by different live pathogens is lacking. Therefore, the aim of the present study was to investigate effects of intravenous administration of high dosages of OT on blood-derived components like SCC, LDH, SA, and IgG in milk of cows with naturally occurring mastitis caused by different pathogens. Results should help to estimate potential positive effects of high OT dosages on mastitis cure in dairy cows.

Materials and Methods

Animals

The animal trials followed the German and Austrian laws on animal protection, and were approved by the District Government of Upper Bavaria, Germany (registration number: AZ 55.2-1-54-2532-4-2017) and the Office of the Upper Austrian State Government, Austria (registration number: Ges-2016-409034/5-Ho), respectively. Lactating German and Austrian Fleckvieh cows (n = 184) with signs of mastitis in at least one udder quarter were presented to two veterinary practices in Bavaria, Germany, and Upper Austria. Both practices were close (< 100 km) to each other, therefore climatic conditions and feeds were comparable between the visited 59 farms. Different housing types were equally distributed between treatment groups: all year tie stall housing, all year free stall housing, free stall and pasture, tie stall and pasture, or a combination of tie and free stall. Cows were enrolled in this study if symptoms like clots or flakes in milk, pain, swelling, impaired general condition, or only an increased SCC without further

symptoms were detected. Cows were included in the study if they were less than 250 days in milk and had no mastitis treatment in the previous four weeks. Cows have not been vaccinated against any mastitis pathogens.

Experimental Design

Cows were randomly assigned to the treatment group (OT; n=101) or to the control group (n=83). A careful clinical examination was performed on days 1, 2, 3, 14, and 28, respectively, and included the recording of general condition, body temperature, changes in udder condition or milk quality, and other significant findings.

Milk samples from all four quarters were collected on days 1 (prior to the treatment), 2 (immediately before the second treatment), 3, 14, and 28. Immediately after sampling, cows of the treatment group received 100 IU of oxytocin intravenously on days 1 and 2. These cows were not milked before, or immediately after the injections. If the mastitis was acute, i.e. cows showed clots or flakes in the milk and the general condition of animals was affected, cows were treated independently of the assigned experimental group according to the common therapy protocol of the veterinary practice (including antibiotic treatment), directly after collecting the samples on day 1. The number of quarters receiving antibiotic treatment were equally distributed between groups (41 and 43% of all quarters in the OT treatment group and non OT treated group, respectively).

Laboratory Analyses

On days 1, 2, 14, and 28 the SCC was measured in the milk samples with a DeLaval cell counter (DCC; DeLaval, Tumba, Sweden) immediately after sampling. Results above 3×106 cells/mL were recorded as $>3 \times 106$ cells/mL as the detection limit for the counting device is between $3-4 \times 106$ cells/mL. Results were log-transformed (log10) to assure normal distribution and to take into account that higher cell counts above the detection limit could not be captured. On day 3 SCC was solely estimated with a California Mastitis Test (CMT).[10] After SCC measurement milk samples were stored for further analyses at –20 °C.

To measure LDH activity, milk was thawed and milk serum was obtained by centrifugation at 1 900 x g for 15 min at 4 °C and then at 20 800 x g for 30 min at 4 °C. In the milk serum LDH activity was analyzed using a commercial kit AXON00025 (Axon-Lab AG, Baden, Switzerland) in an automated analyzer (Cobas Mira, Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol.

Milk samples from 299 quarters of 97 cows with (n=57) or without OT treatment (n=40), and from Gram-positive infected and non-infected quarters on day 1, 2, and 3 were selected for the measurement of SA and IgG concentrations. This selection was necessary due to the limited budget. Concentrations of SA and IgG were analyzed using commercially available ELISA kits (No. E10-113 and E10-118, respectively; Bethyl Laboratories, Montgomery, TX), according to manufacturer's instructions. The inter- and intra-assay coefficient of variation was 4,4% and 6,3%, respectively, for SA. The inter- and intra-assay coefficients of variation for IgG were 4,8% and 8,9%, respectively.

Bacteriological examinations of the milk samples from days 1 and 28 were performed according to standard procedures.[11]

Statistical Analyses

Quarters were grouped by pathogens based on their quarter milk bacteriological results on day 1. Results are presented as least square means ± SEM. Statistical analyses were performed by using SAS (SAS version 9.4 SAS Institute Inc., Cary, NC). Differences were considered significant when $P<0,05$. The general linear models (GLM) procedure was used to test the effects of treatment, day, and pathogen on SCC, LDH activity, and on IgG and SA concentrations in milk within quarter. Differences of least square means of IgG and SA concentrations, SCC, and LDH activity in milk between treatment (with or without OT treatment), treatment days, and pathogens were tested for significance using paired t-test. Correlation between LDH and SCC, and IgG and LDH were evaluated by the CORR procedure. If SCC values were at or above detection limit the SCC was considered to be 3×106 cells/mL. SCC was logarithmized (log10) for mathematical-statistical calculations.

Table 1: Pathogen, number of quarters (n), percentage of all measured quarters, and mean somatic cell count (SCC) on day 1 (before treatment) of infected udder quarters and number of quarters from animals treated with oxytocin (OT); bacteria were detected in 161 out of 700 examined quarters of 184 cows.

Pathogen	n	%	OT (n)	SCC (cells×1000/mL)
Corynebacterium bovis	14	2,0	10	1,210±308
Escherichia coli	7	1,0	2	>3,000
Staphylococcus aureus	35	5,0	18	1,389±185
other *Staphylococcus*	39	5,6	25	691±123
Streptococcus dysgalactiae	17	2,4	6	1,957±289
Streptococcus uberis	44	6,3	12	1,676±186
Klebsiella	1	0,1	0	2,400
Yeast	1	0,1	1	>3,000
Streptococcus agalactiae	1	0,1	0	>3,000
Trueperella pyogenes	1	0,1	0	>3,000
Gram-positive rods	1	0,1	0	253
no detected pathogen	539	77	295	791±49

Results

Pathogens and SCC

Detected pathogens and the related SCC in affected quarters on day 1 (before treatment) are shown on table 1. Results were obtained from 700 quarter samples from 184 cows. In 539 quarters, there was no pathogen detectable. In 61% of these quarters, the SCC was above 100000/mL and in 14% of these quarters the SCC was above the detection limit of the DCC system (3 × 106/mL). Pathogens were detected in 161 quarters, including 74 quarters of cows treated with oxytocin. The mean SCC within pathogen groups before treatment was similar in treatment and control groups except in *Streptococcus uberis* infection in which the group that was not treated with OT started already with a significantly higher SCC.

The SCC on days 1, 2, 14, and 28 in quarters infected with different pathogens with and without OT treatments are shown on table 2. The number of samples with SCC at or above 3 × 106 cells/mL in each group is also shown on table 2. Oxytocin treatment, day, and pathogen affected SCC ($P<0,05$, $<0,001$, $<0,001$, respectively). From the first to the second day SCC did not change in any pathogen group with or without OT treatment. An OT effect was seen solely on day 14 in quarters infected with other *Staphylococci* than *Staphylococcus aureus* were SCC was significantly decreased compared to the values on day 2, which was not seen in non-treated quarters. In addition, OT had no effect on SCC in quarters were no pathogen was detected.

The CMT results on day 3 were between ++ and +++ in quarters infected with the different pathogens. The results of CMT within pathogens were not different between groups with or without OT treatment.

LDH activity

Mean LDH activity in milk from quarters infected with different pathogens within treatment group on the different days are shown on table 3. The activity of LDH

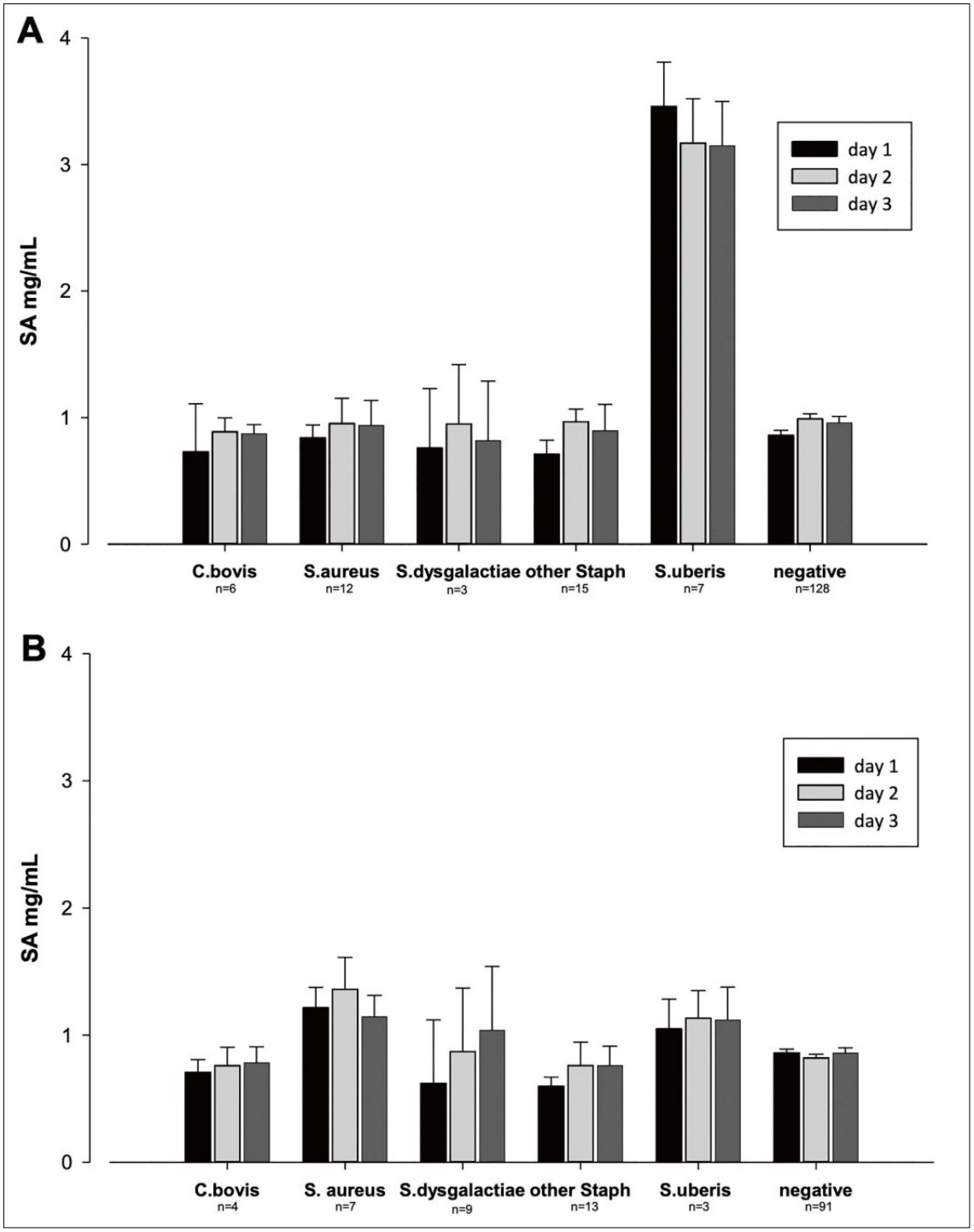
Table 2: Somatic cell count (cells × 1000/mL; means ± SEM) in milk of quarters (n) in different pathogen and treatment groups (with oxytocin; without oxytocin) on different days relative to clinical diagnosis of mastitis (= day 1); *: number of quarters with SCC > 3000000/mL.

	with oxytocin					without oxytocin				
Pathogen	n	day 1	day 2	day 14	day 28	n	day 1	day 2	day 14	day 28
Corynebacterium bovis	10	5,95±0,17 *2	5,91±0,17 *0	5,86±0,19 *1	5,26±0,30 *0	4	5,63±0,22 *1	5,55±0,28 *1	5,37±0,14 *0	4,97±0,24 *0
Staphylococcus aureus	18	5,81±0,17 *4	5,71±0,10 *3	5,81±0,13 *0	5,97±0,13 *3	17	5,90±0,16 *1	5,89±0,15 *2	5,79±0,16 *1	5,65±0,22 *0
other *Staphylococcus spp.*	25	5,54±0,10 *1	5,81±0,09 *1	5,37±0,13 *0	5,46±0,15 *3	14	5,64±0,17 *1	5,96±0,10 *2	5,57±0,13 *1	5,58±0,15 *0
Streptococcus uberis	12	6,06±0,17 *12	6,08±0,16 *8	5,75±0,20 *4	5,94±0,27 *2	25	5,86±0,12 *6	5,94±0,10 *4	5,75±0,11 *1	5,91±0,13 *1
Streptococcus dysgalcatiae	6	5,73±0,38 *2	5,81±0,22 *1	5,62±0,25 *0	5,46±0,26 *0	11	6,23±0,12 *6	6,28±0,17 *4	5,80±0,18 *1	6,15±0,13 *2
Gram-negative	2	– *2	– *1	– *0	–	6	6,46±0,02 *5	6,35±0,08 *4	5,65±0,29 *1	–

*: number of quarters with SCC > 3000000/mL

Table 3: Lactate dehydrogenase activity (U × 1000/L; means ± SEM) in mastitis milk of udder quarters (n) in different pathogen and treatment groups (with oxytocin; without oxytocin) on different days relative to clinical diagnosis of mastitis (= day 1).

	with oxytocin						without oxytocin					
Pathogen	n	day 1	day 2	day 3	day 14	day 28	n	day 1	day 2	day 3	day 14	day 28
Corynebacterium bovis	10	0,77±0,46	3,28±2,07	2,01±1,37	–	–	4	0,20±0,09	0,23±0,11	0,20±0,09	–	–
Staphylococcus aureus	19	0,62±0,18	0,77±0,25	0,61±0,17	1,48±1,25	0,54±0,23	17	1,13±0,68	3,19±2,81	0,38±0,12	0,23±0,09	0,43±0,15
other *Staphylococcus spp.*	17	0,39±0,14	0,73±0,29	0,49±0,17	0,13±0,03	0,12±0,04	10	0,58±0,31	0,47±0,22	0,55±0,25	0,10±0,01	0,26±0,05
Streptococcus uberis	12	2,85±0,75	3,30±0,83	1,88±0,48	0,67±0,25	0,87±0,30	25	1,32±0,43	1,37±0,40	1,16±0,31	1,77±0,90	–
Streptococcus dysgalcatiae	6	1,55±0,15	1,71±0,91	1,36±0,51	0,37±0,05	–	11	4,04±1,75	5,48±2,11	5,72±2,36	1,58±0,70	0,47±0,13
Gram-negative	2	–	–	–	–	–	6	3,21±1,55	7,79±3,10	8,33±2,46	4,93±2,82	–



Pathogen dependent effects of high amounts of oxytocin on the blood milk barrier integrity during mastitis in dairy cows

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Figure 1: Serum albumin concentrations in milk on days 1 (=day of clinical diagnosis of mastitis), 2, and 3 in udder quarters infected with different pathogens (*C. bovis*= *Corynebacterium bovis, S. aureus*= *Staphylococcus aureus, S. dysgalactiae*= *Streptococcus dysgalactiae,* other *Staph*=other *Staphylococcus* than *Staphylococcus aureus, S. uberis*= *Staphylococcus uberis*) with (A) or without (B) oxytocin treatment; values are means±SEM; n=number of quarters

was positively correlated (P<0,05) with SCC in infected quarters with all different pathogens except in quarters infected with *Staphylococcus aureus*. Highest LDH activities were found in milk samples from glands infected with Gram-negative bacteria and with *Streptococcus dysgalactiae* and *Streptococcus uberis*. The OT treatment had no effect on LDH concentrations, whereas the day and the pathogen had significant effects on LDH activities in milk (P<0,001). In *Streptococcus dysgalactiae* infected quarters a significant decrease of LDH activity was seen between day 14 and 28 in both, OT treated and non-treated quarters.

The Pearson correlation coefficients between LDH activity and IgG concentrations in milk for all samples on the first three days were r=0,54 (P=0,007), r=0,61 (P<0,001), 0,97 (P<0,001), and r=0,89 (P<0,001), in quarters with *Corynebacterium bovis*, *Staphylococcus au-*

reus, other Staphylococcus, and *Streptococcus uberis*, respectively, whereas no significant correlation was found in quarters where no pathogen was detected (r=0,01; P=0,295).

SA concentrations

Mean SA concentrations in milk of quarters infected with different pathogens within treatment group on day 1 to 3 are shown in figure 1. Concentrations of SA were only increased in *Streptococcus uberis* infected quarters of the treatment group on all three days including the first day before treatment.

IgG concentrations

Mean IgG concentrations in milk of quarters infected with different pathogens within treatment group on day 1 to 3 are shown in figure 2. If cows were treated with OT the IgG concentration in the 128 quarters with

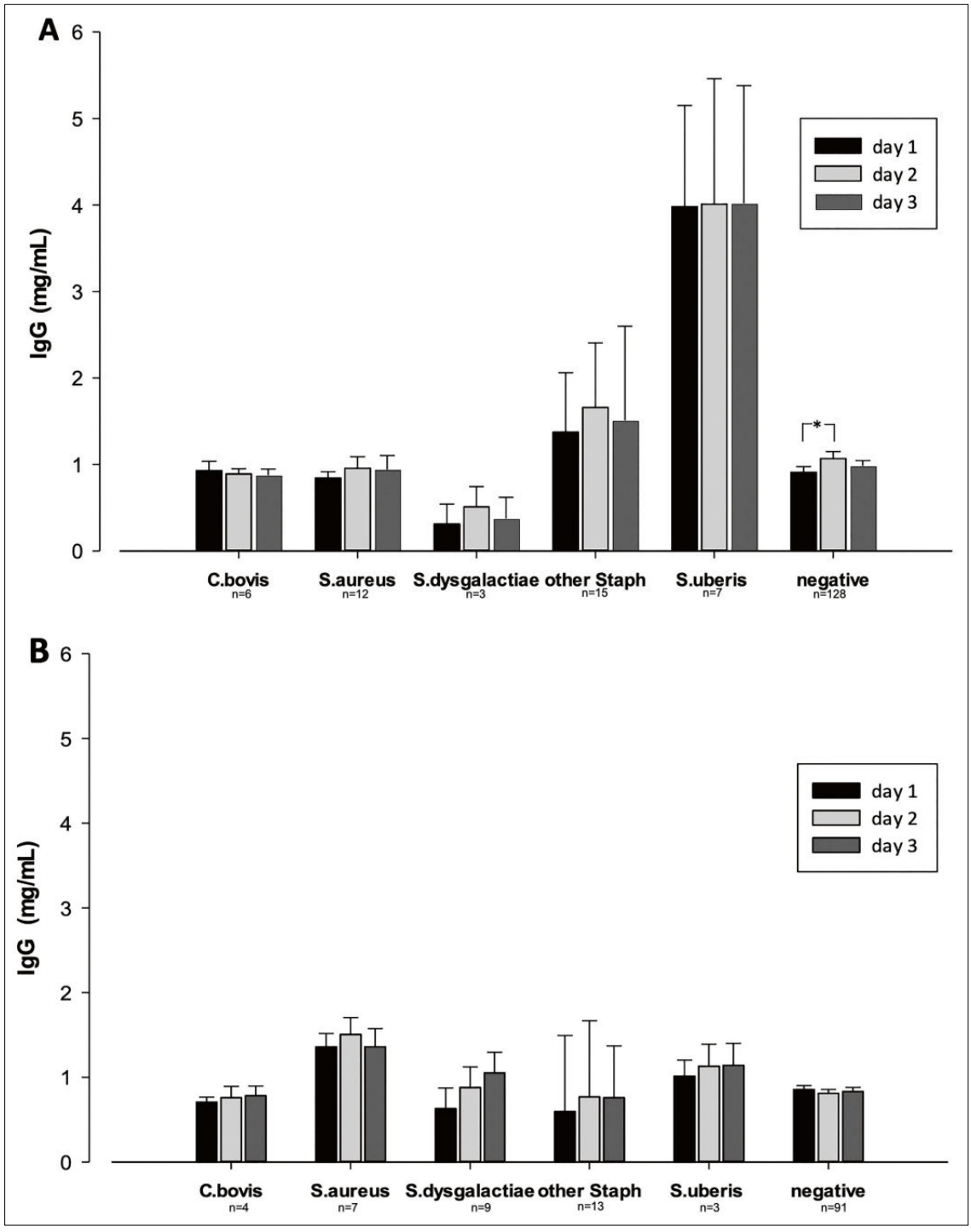

Figure 2: Immunoglobulin G concentrations in milk on day 1 (=day of clinical diagnosis of mastitis), 2, and 3, in udder quarters infected with different pathogens (*C. bovis*= *Corynebacterium bovis, S. aureus*= *Staphylococcus aureus, S. dysgalactiae*= *Streptococcus dysgalactiae,* other *Staph*=other *Staphylococcus* than *Staphylococcus aureus, S. uberis*= *Staphylococcus uberis*) with (A) or without (B) oxytocin treatment; values are means±SEM; n=number of quarters; * indicates significant difference between day within treatment group

negative bacteriological results increased from day 1 to day 2 ($P<0,05$). There was no further change of IgG concentrations on day 3. In quarters of OT treated cows that were diagnosed with Gram-positive bacteria the IgG concentrations ranged from 0.16 mg / mL and 11m24 mg/ mL on day 1 and did not significantly change on day 2 or day 3.

Highest IgG concentration were measured in a quarter that was infected with Staphylococcus spp. (11,24 mg/ml and 11,68 mg/mL on day 1 and day 2, respectively). Three of the quarters infected with *Streptococcus uberis* were from one cow, which was in the 9th lactation. These three quarters had high IgG concentrations up to 10,99 mg/mL. For technical reasons, IgG concentrations are not available in quarters infected with *Escherichia coli*. OT treatment did not have an effect on IgG concentrations on days 2 and 3 within groups of involved pathogens.

Other symptoms

All cows with *Escherichia coli* infection had increased rectal temperature above 39,5 °C. Furthermore, 22,7% of cows infected with *Streptococcus uberis*, and 17,6% of cows infected with *Streptococcus dysgalactiae* developed this temperature increase. In all other infections less than 12% of cows developed a rectal temperature above 39,5 °C.

Clots and flakes were found in milk from all *Escherichia coli* infected quarters. Fewer quarters with flakes were found in *Streptococcus uberis* (57,7%), *Streptococcus dysgalactiae* (52,9%) and other *Staphylococci* (44,4%), and lowest numbers of quarters with flakes were found in *Staphylococcus aureus* infections (31,4%).

In the OT treatment group 9, 10, 3, 6, and 3 quarters and in the control group 4, 3, 5, 5, and 11 quarters with detected *Corynebacterium bovis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, other Staphylococcus spp., *Streptococcus uberis*, respectively, were treated with antibiotics.

Discussion

The most abundant bacteria that caused mastitis in the present study were *Streptococcus uberis*, *Staphylococcus aureus*, and other *Staphylococci*. This represents the expected distribution of mastitis pathogens in dairy farming throughout Europe.[12] It also shows that *Streptococcus uberis* is increasingly a significant inducer of mastitis compared to older studies where coagulase-negative *Staphylococci* and *Staphylococcus aureus* were more often detected than *Streptococcus uberis*.[13] Interestingly, 61% of the quarters in which no pathogens were detected had an SCC above 100 000 cells/mL, and 14% of these bacteriologically negative quarters had even an SCC above the detection limit of the used device (3×106 cells/mL). According to the German Veterinary Medical Society[14] an SCC below 100 000 cells/mL in quarter foremilk samples is in the physiological range and with increasing SCC above 100 000 cells/mL the likelihood of an existing infection increases.

The somatic cells in milk are mainly cells of the innate immune system[15], which have significant effects in eliminating udder pathogens. Immunoglobulins can facilitate bacterial phagocytosis by opsonization.[16] Therefore, a pronounced increase of SCC and Ig concentrations in milk during mastitis through OT application could improve the elimination of pathogens.

As only standard methods for bacterial detection were used in this study, it is possible that quarters were infected with pathogens that are not captured with these methods, e. g. Mycoplasma. On the other hand, bacteria may have already been eliminated from the gland. It was shown that in severe mastitis induced by *Escherichia coli* occasionally only endotoxin and no live bacteria can be found in mastitic milk.[17] Furthermore, Fuenzalida and Ruegg showed that the mammary immune system is capable of eliminating *Escherichia coli* in mild to moderate clinical mastitis without any treatment.[18] Therefore, in milk from quarters with no bacteria detected, Gram-negative bacteria might have had induced an inflammation, but they were already eliminated by the immune system of the mammary gland. Other reasons for not detecting bacteria in milk from infected mammary glands could be the varying shedding of bacteria into the milk as it is seen for example in *Staphylococcus aureus* infections.[19]

The treatment with high dosages of OT did not influence SCC independently from the involved pathogen. In a previous study, we have shown that the SCC increase within hours in healthy and immunologically stimulated quarters after injection of 100 IU OT.[7] However, this study was realized under clinical conditions. It is likely that a pronounced increase of SCC could not be detected in the present study because the cows in this study were presented to the veterinarians after symptoms were detected by the farmer, and, therefore, the first sampling did not occur before the SCC was already considerably increased or other symptoms like flakes were detectable. At that time the infection was already established and the increase of SCC was already triggered and in progress. Is not known when exactly the invasion of the pathogens occurred, and the first sampling and OT treatment was, therefore, not performed at a defined stage of mastitis. In addition, the difference to earlier experiments may be due to the fact that in endotoxin induced mastitis the applied stimulus loses gradu-

ally its activity through dilution by produced milk, whereas live bacteria are continuously multiplying, which represents an ongoing and increasing immune challenge.

The intravenous application of 100 IU oxytocin to dairy cows was expected to pronounce the opening of the blood milk barrier, and to increase, therefore, not only the SCC, but also the LDH activity and SA and IgG concentration in milk.[9,7] In milk SA has no immunological function but is a marker of the barrier integrity. Antibodies, however, can bind to specific bacteria and opsonize the pathogens to facilitate the phagocytosis by leukocytes.[20] Therefore, the increase of IgG in milk during mastitis could support the combat against the involved pathogens if specific antibodies would be available in the blood, e. g. after previous contact with the pathogen or after vaccination.[16] In this field study, the treatment of dairy cow mastitis with 100 IU oxytocin did not show significant effects on the increase of IgG after the treatment. Only in healthy quarters, an increase of IgG content in milk by OT treatment was detected. The varying concentrations of IgG on day 1 in this study was not surprising, as concentrations of IgG in milk during an ongoing mastitis are very individual.[7] Furthermore, as already discussed for SCC, the first sampling was performed at different stages of mastitis, which may mask a further increase of IgG transfer from the blood.

It is known that different bacteria induce the mammary immune response and the opening of the blood-milk barrier differently, which leads to different concentrations of several immune factors and blood components in milk.[21,1] In the present study highest values of SCC, LDH, and IgG were measured in *Streptococcus uberis* infected quarters. Very high concentrations of IgG in milk of *Streptococcus uberis* infected quarters of one cow in the 9th lactation were found. Caffin et al.[22] showed that IgG1 concentrations in serum increase after the third lactation. Therefore, the age of this cow could be one of the reasons for the very high IgG concentrations in milk. It is also known that IgG decreases during the course of lactation.[23] This cow was only 40 days in milk. Furthermore, it has been shown that cows with mastitis induced by *Streptococcus uberis* have higher IgG concentrations in serum than cows with mastitis induced by *Staphylococcus aureus*.[24]

The enzyme LDH in milk increases during a mammary immune response and originates partially from soluble LDH in the blood through an opening of the blood milk barrier.[9] However, LDH is also released by damaged cells.[25,9] Thus, the origin of LDH in milk shows the opening of the blood-milk barrier on the one hand and the increased number of leukocytes in milk and cell damage in the udder on the other hand.[26,27,28] This leads to correlations between the LDH activity in milk and SCC during mastitis, which was shown in this field study. Similar results were found by Hernàndez-Castellano et al.[29] in *Corynebacterium bovis*, *Staphylococcus aureus*, and *Streptococcus uberis* infected quarters. In quarters infected with Gram-negative bacteria, highest SCC were expected.[30] Together with a greater impairment of the blood-milk barrier of Gram-negative compared to Gram-positive bacteria[31], this led to the very high LDH activity in milk that was found in milk from quarters infected with Gram-negative bacteria. Interestingly, also in quarters infected with *Streptococcus uberis* und *Streptococcus dysgalactiae* high LDH concentrations were found which is likely associated with the strong increase of SCC but may also indicate a relevant disruption of the blood milk barrier and/or tissue damage. An effect of OT injections on LDH concentration could not be detected, indicating lacking effects on the blood-milk barrier and tissue degradation by the pathogens.

Clots and flakes in milk were mainly found in quarters that were infected with *Streptococcus uberis* and *Escherichia coli*. These bacteria are known to induce severe mastitis. Therefore, the treatment with high dosages of OT does not seem to be a sufficient therapy to treat mastitis induced by these bacteria; however, supportive effects on cure rates are possible.

Conclusion

The present study confirms that the transfer of blood components into milk during mastitis of dairy cows is pathogen dependent. The treatment of mastitis in the field with high dosages of OT alone does not sufficiently increase the IgG concentrations in milk to expect a positive effect on mastitis cure rates even if specific antibodies against the respective pathogen are available in circulation. Solely in healthy quarters an increase of IgG can be induced by OT. Furthermore, an increase of SCC in response to injection of high dosages of OT, which could have a positive effect on the elimination of bacteria, cannot be seen in already ongoing mastitis. Obviously, the effects of OT in supraphysiological dosages on mastitis depend on the stage of the disease at which the injections are applied. Therefore, the use of high dosages of OT as a stand-alone mastitis therapy in the field does not appear to be a suitable alternative to antimicrobial treatment.

Effets sur l'intégrité de la barrière sang-lait lors d'un traitement de mammites avec des quantités élevées d'ocytocine chez les vaches laitières en fonction des agents pathogènes

La réduction de l'utilisation d'antibiotiques chez les animaux destinés à l'alimentation devient de plus en plus importante. Par conséquent, des alternatives appropriées au traitement des mammites chez les vaches laitières doivent être envisagées. L'ocytocine (OT) induit l'éjection du lait et favorise donc l'élimination du lait des quartiers infectés. Au-delà de la vidange de la mamelle, l'injection de doses très élevées d'OT entraîne une augmentation du nombre de cellules somatiques (CSC) dans le lait et permet le transfert d'immunoglobulines (Ig) du sang vers le lait grâce à une réduction de l'intégrité de la barrière sang-lait.

Le but de la présente étude était d'étudier les changements spécifiques aux agents pathogènes du CSC, les composants du lait dérivés du sang que sont la lactate déshydrogénase (LDH) et l'albumine sérique (SA) ainsi que les IgG dans le lait de vaches souffrant de mammites causées par différents agents pathogènes traités par deux injections intraveineuses de doses élevées d'OT (100 UI). Des échantillons de lait de 184 vaches laitières de différentes exploitations ont été prélevés au jour 1 (jour de l'examen clinique et diagnostic de mammite) et aux jours 2, 3, 14 et 28. L'examen bactériologique (jour 1) a identifié les agents pathogènes impliqués. Les vaches ont été assignées au hasard au traitement (injections d'OT les jours 1 et 2) ou au groupe témoin (pas d'OT). Indépendamment du groupe auquel elles étaient attribuées, les vaches ont reçu le protocole thérapeutique usuel du cabinet vétérinaire après le prélèvement de l'échantillon si leur état général était affecté.

Le CSC, la LDH, la SA et les IgG du lait ont varié spécifiquement en fonction des agents pathogènes impliqués. Les valeurs les plus élevées des trois paramètres ont été mesurées dans les mammites causées par *Streptococcus uberis*. Les changements étaient moins prononcés avec d'autres *Streptococci* spp., *Staphylococci spp.* ou *Corynebacterium bovis*.

Le traitement à l'ocytocine n'a affecté aucun des paramètres étudiés indépendamment de l'agent pathogène impliqué. On a uniquement observé, dans les mammites causées par des staphylocoques autres que *Staphylococcus aureus*, une diminution du CSC et, dans les mammites où aucun agent pathogène n'a été détecté, une augmentation des concentrations d'IgG dans les quartiers. Ainsi, l'administration d'OT à forte dose n'est pas appropriée comme traitement unique des mammites chez les vaches laitières.

Mots clés: mammite, l'ocytocine, barrière sang-lait, vache

Effetto patogeno-dipendente durante il trattamento della mastite con elevate dosi di ossitocina sull'integrità della barriera emato-mammaria nelle vacche da latte

La riduzione dell'uso di antibiotici negli animali per la produzione alimentare sta assumendo un'importanza sempre maggiore. Di conseguenza, è opportuno considerare delle alternative adeguate per il trattamento della mastite nelle vacche da latte. L'ossitocina (OT) induce l'eiezione del latte e quindi aiuta la rimozione di questo dai quarti mammari infetti. L'iniezione di dosi molto elevate di OT provoca non solo lo svuotamento della mammella, ma pure un aumento del numero di cellule somatiche (SCC) nel latte permettendo quindi il trasferimento di immunoglobuline (Ig) dal sangue al latte attraverso una ridotta integrità della barriera tra sangue e latte.

Lo scopo del presente studio era di esaminare i cambiamenti patogeno-dipendenti dal SCC, i componenti del latte derivati dal sangue del lattato deidrogenasi (LDH), l'albumina del siero (SA) e le IgG nel latte, delle vacche affette da mastite causata da diversi patogeni e trattate con due iniezioni intravenose ad alti dosaggi di OT (100 UI). I campioni di latte provenienti da 184 vacche da latte di diverse aziende sono stati raccolti il giorno 1 (giorno dell'esame clinico e della diagnosi di mastite) e nei giorni 2, 3, 14 e 28. L'esame batteriologico (giorno 1) ha identificato gli agenti patogeni coinvolti. Le vacche sono state assegnate in modo casuale al gruppo con il trattamento (iniezioni di OT il giorno 1 e 2) o al gruppo di controllo (nessuna OT). Indipendentemente dal gruppo sperimentale assegnato, se dopo la raccolta del campione le condizioni generali dell'animale risultavano compromesse, le vacche ricevevano un comune protocollo terapeutico dello studio veterinario.

Si è rilevato che a seconda degli agenti patogeni coinvolti, l'SCC del latte, la LDH, la SA e le IgG cambiavano in modo specifico. I valori più alti fra tutti e tre i parametri sono stati misurati in caso di mastite causata da *Streptococcus uberis*. I cambiamenti erano meno pronunciati con gli *Streptococchi* spp., *Staphylococci* spp. o *Corynebacterium bovis*.

Si è notato che il trattamento con ossitocina non influenzava nessuno dei parametri studiati, indipendentemente dal patogeno coinvolto. Si è osservato nei quarti infettati da *stafilococchi* diversi dallo *Staphylococcus aureus* una diminuzione della SCC mentre nei quarti dove non venivano rilevati agenti patogeni si evidenziava un aumento delle concentrazioni di IgG. Pertanto, la somministrazione di OT ad alto dosaggio non è ovviamente adatta al trattamento stand-alone della mastite nelle vacche da latte.

Parole chiave: mastite, ossitocina, barriera sangue-latte, vacca

Pathogen dependent effects of high amounts of oxytocin on the blood milk barrier integrity during mastitis in dairy cows

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