A. Vollenweider^{1,2}, S. Corti³, M. Hochreutener³, B. Biner², R. Stephan³, U. Bleul¹

¹Department for Farm Animals, Clinic for Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Switzerland; ²Clinica Alpina SA, Tiermedizinisches Zentrum, Scuol, Switzerland; ³Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland

Mastitiserreger und Antibiotikaresistenz Albei Mastkühen in der Schweiz

Mastitiden bei Mutterkühen wurden bisher nicht so umfassend untersucht wie bei Milchkühen und Daten aus der Schweiz fehlen vollständig. In verschiedenen Studien wurde ein ähnliches Erregerspektrum wie bei Milchkühen nachgewiesen, was in dieser Untersuchung nicht bestätigt werden konnte. Um erste Daten aus der Schweiz zu erhalten, wurden von 297 Mutterkühen aus 31 Betrieben im Engadin (Graubünden) Milchproben entnommen und bakteriologisch untersucht. Bei 33 % der Kühe konnte in mindestens einer Einzel- oder Sammelmilchprobe ein major- oder minor-Erreger nachgewiesen werden. Die am häufigsten nachgewiesenen major-Erreger waren Staphylococcus aureus (8,4% der Kühe), Pasteurella multocida (4,1%), Streptococcus uberis (2%) und Streptococcus dysgalactiae (1,7%). 16% der Kühe hatten einen oder mehrere unproduktive Viertel, wobei nur 32% dieser Viertel den Landwirten zum Zeitpunkt der Untersuchung bekannt waren. In einem zweiten Studienteil wurden Milchproben von Mutterkühen mit Mastitiden bakteriologisch untersucht; die Kühe stammten aus verschiedenen Teilen der Schweiz und waren zur tierärztlichen Behandlung vorgestellt worden. Pasteurella multocida (22%) und Staphylococcus aureus (21%) konnten als häufigste Erreger von klinischen Mastitiden bei Mutterkühen identifiziert werden. Von den in beiden Studienteilen nachgewiesenen Staphylococcus aureus, Pasteurella multocida und Streptococcus uberis wurden mittels Mikrotitration und Disk-Diffusion Antibiogramme erstellt. Sechsundfünfzig Prozent der untersuchten Staphylococcus aureus-Stämme wurden als resistent gegen Penicillin G beurteilt. Unsere Ergebnisse zeigen, dass die bakteriologische Untersuchung von Milchproben bei Mastitiden bei Mutterkühen wichtig ist, um eine korrekte Diagnose und spezifische Therapie zu ermöglichen; dies kann durch die Erstellung von Antibiogrammen weiter verbessert werden. Zur Resistenzsituation von Mastitiserregern bei Mutterkühen wurden erste Daten erhoben, woraus wichtige Hinweise für eine evidenzbasierte Therapie abgeleitet werden können.

Schlüsselwörter: Eutergesundheit, Staphylococcus aureus, Pasteurella multocida, Atrophie, Zellzahl

Abstract

Mastitis in beef cows has not been studied as extensively as mastitis in dairy cows, and data from Switzerland are lacking. Various studies have shown a similar pathogen spectrum as in dairy cows, which could not be confirmed in this study. To gather initial data from Switzerland, milk samples from 297 lactating beef cows from 31 herds from the Engadin Valley in the Canton of Grisons were examined bacteriologically. At least one major or minor mastitis pathogen was recovered from at least one individual-quarter or composite sample from 33% of all cows. The most common major mastitis pathogens were Staphylococcus aureus (8,4% of cows), Pasteurella multocida (4,1%), Streptococcus uberis (2%) and Streptococcus dysgalactiae (1,7%). Sixteen percent of the cows had at least one blind quarter, but only 32 % of these had been previously detected by the owners. In the second part of the study, milk samples from beef cows with mastitis were examined bacteriologically; the cows originated from various parts of Switzerland and had been presented for veterinary treatment. Pasteurella multocida (22%) and Staphylococcus aureus (21%) were the most common pathogens isolated. Antibiograms using microtitration and disk diffusion testing were generated for the Staphylococcus aureus, Pasteurella multocida and Streptococcus uberis strains from both parts of the study. Fifty-six percent of the Staphylococcus aureus strains were resistant to penicillin G. Our results showed that bacteriological examination of a milk sample aids in the diagnosis and allows specific treatment of mastitis in beef cows; this may be further improved with antibacterial susceptibility testing. Our preliminary data for the resistance patterns of mastitis pathogens in beef cows will facilitate evidence-based treatment strategies.

Keywords: Udder health, *Staphylococcus aureus*, *Pasteurella multocida*, blind quarter, somatic cell count

https://doi.org/ 10.17236/sat00381

Eingereicht: 31.05.2022 Angenommen: 28.11.2022

A.Vollenweider et al.

Introduction

Although mastitis in beef cows is a common problem in veterinary practice, it has not received extensive scientific attention. In beef herds without clinically overt mastitis, the prevalence of intramammary infection has been shown to range from 9 to 66%.3,18,23,24,29,30,32,39,41 The spectrum of mastitis pathogens corresponded largely with that seen in dairy cows with a predominance of Staphylococcus spp. and Streptococcus spp. and Gram-negative pathogens were rare.^{3,18,23,24,29,30,32,39,41} To the authors' knowledge, studies on clinical mastitis in beef cows have not been carried out. The spectrum of pathogenic agents differs significantly between subclinical and clinical mastitis in suckling ewes and in dairy cows.^{16,42} Data specifically relating to udder health and mastitis in beef cows in Switzerland are lacking. Mastitis causes pain, may be lethal in some cows and can affect the growth rates of calves because of a decrease in the amount and quality of milk.^{8,14,18,23,26,29} For these reasons, studies of mastitis in beef cows that aim to optimise prophylaxis and treatment are important. The goal of this study was therefore to investigate udder health and mastitis in beef cows in Switzerland with particular emphasis on the prevalence and spectrum of pathogens associated with intramammary infection. Of particular interest were the related differences between data obtained in beef cow herds and at the level of individual cows that had mastitis and underwent veterinary examination and treatment at the owner's request. The antibiotic susceptibility of the most common and clinically relevant pathogens was examined.

Materials and Methods

Udder health in beef cows

The study population consisted of cows from farms located in the Engadin Valley in the Canton of Grisons. All beef cow farmers having a medical care contract with the Clinica Alpina SA (Scuol, Switzerland) were asked to participate. Thirty-one farmers with lactating beef cows during the study period agreed. Based on a questionnaire, information about the farm, husbandry conditions, general udder health and handling of udder diseases were collected. We specifically asked if the farmer regularly or sporadically checked the cows' udders visually or by manual palpation. The farms were located at 1,048 to 1,730 m above sea level, which corresponds to mountain zones III and IV. The first author visited all herds once from November 2018 to January 2019. The mean herd size was 23 cows (range, 5 to 46). Two herds were kept in tie-stalls and the remainder were housed in free-stall barns with access to an outdoor yard at the time of the visit. All cows were kept on alpine community pastures from May/June until September.

All lactating cows were restrained in a headlock for examination of the udder, and a kick-stop (a C-shaped device that was fixed between the flank fold and the top of the sacrum) was used in a few cases. Cows that required additional restraint were excluded from the study. The ear tag number, breed, date of birth, lactation number and last calving date were noted. The rectal temperature was measured, and the udder was inspected visually and palpated.² Quarters with palpable knots, diffuse induration, pain on palpation or swelling in combination with reddening or increased warmth of the udder skin were rated as abnormal. The positioning of the lowest teat apex (above or below the tarsus) was recorded. The hygiene score of the udder skin and the teats was assessed and recorded using a scale from 1 (no manure present) to 4 (confluent plaques of manure encrusted on and around the teats).7 A single stream of milk was expressed from each teat onto a black foremilk cup (Vormelkbecher 2-teilig, DeLaval, Tumba, Sweden) and assessed visually and olfactorily and blind quarters (no milk production) were noted. The secretion was rated as abnormal when watery, in presence of clots and/or blood and/or foul odour. When the milk appeared normal, the teat ends were cleaned and disinfected with swabs soaked in 70% isopropanol (BOVIVET Zitzentücher, Kruuse, Langeskov, Denmark), and a composite milk sample was collected from all teats into a sterile tube (Probenröhrchen 10 ml mit Schraubverschluss steril, medical solution, Wil, Switzerland). When palpation of the udder or inspection of the secretion produced abnormal results, individual-gland milk samples were collected from the affected quarters, and a composite sample was collected from the remaining quarters.

The milk samples were stored at 4 to 6°C, and the somatic cell count (SCC) of the composite samples was measured within 2 hours using a cell counter (DeLaval Cell Counter, DeLaval, Tumba, Sweden). This technique stained the nuclei of the somatic cells with a DNA-specific fluorescent reagent and the nuclei were counted one by one using an integrated digital camera.²² A cut-off value of 100,000 cells/ml was used to differentiate normal and abnormal mammary secretions.¹⁹ The individual-gland milk samples and all composite samples with an SCC >100,000 cells/ml were shipped uncooled and within 16 hours to the Institute for Food Safety and Hygiene, University of Zurich.

Mastitis cases

From November 2018 to November 2019, veterinarians from ten large-animal clinics located in various regions of Switzerland (Cantons of Aargau, Berne, Grisons, St.Gallen, Ticino and Zurich) collected 81 quarter milk samples from 49 beef cows with mastitis. The milk samples were submitted to the Institute for Food Safety and Hygiene, where culture and antibiotic susceptibility testing was done. The following data were recorded: Breed, results of the California Mastitis Test (CMT) and macroscopic assessment of the secretion (normal, watery, presence of clots and/or blood, foul odour), the stage of disease (acute, chronic, subclinical), the antibiotic used in pretreatment, the clinical appearance of the affected quarter, signs of systemic illness, lactation status (dry, nursing one or more calves) and the occurrence of pneumonia in the herd or in the calf.

Laboratory analysis

At the Institute for Food Safety and Hygiene the SCC of the mastitis milk samples was determined using a cell counter (DeLaval Cell Counter, DeLaval, Tumba, Sweden). Aliquots of all mastitis samples (individual quarter milk samples) and the samples sent in from Engadin Valley (individual quarter milk samples and composite samples with SCC >100 000 cells/ml) were streaked onto 5 % sheep blood agar plates and Bromothymol-blue Lactose Cystine (BROLACIN) agar plates, which were incubated for 48 h at 37°C. The morphology and Gram-staining properties of bacterial colonies were assessed after 24 and 48h of incubation. A catalase test was used for Gram-positive cocci, and catalase-positive cocci were assessed with the coagulase test. Coagulase-negative colonies were classified as coagulase-negative Staphylococcus spp. (CNS) and further differentiated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS, MALDI Biotyper®, Bruker Corporation, Bremen, Germany). MALDI-TOF-MS is based on the analysis of ribosomal proteins. The MALDI-TOF-MS spectrum of the microorganism is automatically compared with a data set of known organisms for identification.³⁷ Coagulase-positive colonies were identified as Staphylococcus aureus. Gram-positive cocci with a negative catalase test and Gram-negative colonies were further differentiated using MALDI-TOF-MS (MALDI Biotyper®). Based on their clinical importance, the pathogens were categorised as «major mastitis pathogens» (Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Streptococcus agalactiae, enterococci, Trueperella pyogenes, Pasteurella multocida, Mannheimia haemolytica, Mannheimia varigena, Escherichia coli), «minor mastitis pathogens» (CNS, Corynebacterium bovis, other Streptococcus spp.) and «others» (Aerococcus spp., Micrococcus spp., Arthrobacter arilaitensis, Citrobacter koseri, Bacillus cereus, Gram-negative rods).^{10,17} The category «others» included bacteria with unknown significance as intramammary pathogens. For samples with a positive culture result, the Mastitis pathogens and antibiotic resistance in beef cows in Switzerland

A. Vollenweider et al.



Figure 1: Bacteriological culture results from 97 clinically abnormal quarters from 60 beef cows. Red: Major mastitis pathogens, Blue: Minor mastitis pathogens.

	Antibiotic	Microtitration breakpoints in μg/ml	Disk Diffusion breakpoints in mm	Reference
	Penicillin G	R ≥ 0,25		CLSI 2020
	Ampicillin	$R \ge 0,5$		CLSI 2020
	Cefazolin	R ≥8		CLSI 2020
	Cefoperazone	$R \ge 8$		CLSI 2020
Ctan hula a saus surraus	Oxacillin	$R \ge 4$		CLSI 2020
Staphylococcus aureus	Pirlimycin	$R \ge 4$		CLSI 2020
	Erythromycin	R > 2		EUCAST 2021
	Marbofloxacin	$R \ge 4$		CLSI 2020
	Amoxicillin/Clavulanic acid	R ≥ 1/0,5		CLSI 2020
	Gentamicin 10µg		S ≥ 15, R ≤12	CLSI 2020
	Marbofloxacin	$R \ge 4$		CLSI 2020
Streptococcus	Cefazolin	$R \ge 8$		CLSI 2020
uberis	Penicillin G	$R \ge 2$		CLSI 2020
	Pirlimycin	$R \ge 4$		CLSI 2020
	Cefazolin	$R \ge 8$		CLSI 2020
Pasteurella multocida	Penicillin G	R ≥ 1		CLSI 2020
	Ampicillin	R >1		EUCAST 2021

Table 1: Breakpoints used for the interpretation of antibiograms of mastitis pathogens recovered from beef cows. R = resistant, S = susceptible

A. Vollenweider et al.

pathogen that appeared most likely to be responsible for a mastitis was referred to as the relevant pathogen for the respective cow. Samples that yielded more than three different bacterial species were considered contaminated.¹⁰

Of the antibiotics that are approved in Switzerland for the intramammary treatment of mastitis in cattle (www.vetpharm.uzh.ch), those that are commonly used in clinical practice were selected for susceptibility testing. The selected antibiotics and the respective testing mode are listed in table 1. After a bacteriological diagnosis had been made, the cultures were stored at -80°C until the antibiograms were generated. *Staphylococcus aureus* and *Streptococcus uberis* were re-cultured in brain heart infusion broth and *Pasteurella*





multocida were inoculated onto sheep blood agar overnight at 37°C. The minimum inhibitory concentrations (MIC) for penicillin G, ampicillin, cefazolin, cefoperazone, cefquinome, oxacillin, pirlimycin, erythromycin, marbofloxacin and the combinations amoxicillin/clavulanic acid and kanamycin/cephalexin were determined using a microdilution system (Micronaut-S microtitre plates, Merlin Diagnostika GmbH, Bornheim-Hersel, Germany). The growth of the microorganisms under investigation was assessed in the presence of increasing concentrations of antibiotics. The different concentrations of the antibiotics were predetermined by the Micronaut-S microtitre plates. The lowest concentration of an antibiotic that will inhibit the visual growth of a microorganism after overnight incubation is defined as the minimum inhibitory concentration (MIC). Calculations were made to determine the MIC that inhibits $\geq 50\%$ and $\geq 90\%$ of the tested bacterial strains.²¹ Additionally, the disk diffusion method was used for neomycin, gentamicin, kanamycin and spiramycin (according to the Clinical and Laboratory Standards Institute) because these antibiotics are not covered by the Micronaut-S system.⁵ Bacterial suspensions were prepared with 0,85 % NaCl resulting in a turbidity corresponding to the 0,5 McFarland Standard. The suspensions of Staphylococcus aureus were plated onto Mueller-Hinton agar and those of Streptococcus uberis and Pasteurella multocida onto Mueller-Hinton agar with 5% sheep blood. Disks containing neomycin 30µg, gentamicin 10µg, kanamycin 30µg or spiramycin 100µg were then placed onto the plates. Staphylococcus aureus was incubated aerobically for 16 to 18 h at 37°C, Streptococcus uberis for 20 to 24 h in 5% CO2 at 37°C and Pasteurella multocida for 18 to 24 h at 37°C. The inhibition zones were measured with a ruler and the results were digitised. The

Table 2: Minimal inhibitory concentrations (MIC) of 50 *Staphylococcus aureus* strains cultured from milk samples from beef cows. Light blue cells indicate the concentrations determined by microdilution using the Micronaut-S system. Numbers in the grey cells indicate strains with a minimal inhibitory concentration greater than the highest tested concentration. The bold black lines indicate the CSLI (2020) or EUCAST (2021) breakpoints. «n.i.» not interpretable.

Antibiotic	Minimal Inhibitory Concentration (MIC, μg/ml)										MIC	MIC	Resistant
	0,125	0,25	0,5	1	2	4	8	16	32	64	50% (µg/ml)	90% (µg/ml)	strains (%)
Penicillin G	22					4	13	11			4	≥ 16	56
Ampicillin						24	15	5	6		8	≥ 32	≥ 52
Cefazolin						50					≤ 4	≤ 4	0
Cefoperazone					31	19					≤ 2	4	0
Cefquinome				49	1						≤ 1	≤ 1	n.i.
Oxacillin				50							≤ 1	≤ 1	0
Pirlimycin				46	4						≤ 1	≤ 1	0
Erythromycin	1	3	46								0,5	0,5	0
Marbofloxacin		10	39	1							0,5	0,5	0
Amoxicillin/Clavulanic acid (2:1)						50					≤ 4/2	≤ 4/2	n.i.
Kanamycin/Cephalexin (10:1)						50					≤ 4/0,4	≤ 4/0,4	n.i.

MICs and disk diffusion results were interpreted using breakpoints published by the Clinical and Laboratory Standards Institute (CLSI) and by the European Committee on Antibiotic Susceptibility Testing (EUCAST).6,12 When breakpoints for bovine milk were not available, breakpoints derived from other animal species or human health data were adopted. An overview of the breakpoints is shown in Table 1. Bacterial strains without an inhibition zone in the disk diffusion were considered resistant even in the absence of published breakpoints for that antibiotic. The Micronaut-S system test had predetermined antibiotic concentrations, which in some cases were higher than the published breakpoints. This complicated the assessment, nevertheless the results are shown in Tables 2 to 4. When a published breakpoint is below the lowest tested concentration, it is not possible to determine whether a strain inhibited by the lowest tested concentration is resistant, or whether the strain has an even lower MIC, which could render the strain susceptible. This was taken into account by calculating the MIC50% and MIC90% (concentrations of the antibiotic agent that inhibit the growth of 50 and 90% of all tested strains, respectively) and by indicating the proportion of resistant strains in these cases as greater than or equal to (\geq) the number of strains with unequivocal interpretation.²¹ Several disk diffusion tests for Pasteurella multocida and Streptococcus uberis showed a putative inhibition zone with microcolonies growing along the concentration gradient. Only true inhibition zones were considered, and in their absence, a bacterial strain was categorised as resistant.

Data analysis and statistics

The data were transferred into Excel (Microsoft Corporation, Redmond, WA, USA) for analysis and descriptive statistics. For descriptive statistics, categorical variables were expressed as percentages. Excel add-in STATEL (ad Science, Paris, France) was used for all other calculations. The median and the lower and upper quartile (q25 and q75) were calculated for continuous variables. The normality of the data was examined using the Shapiro-Wilks W test. Paired and unpaired t-tests were used to compare normal data, and the Kruskal-Wallis test and Mann-Whitney U test were used to compare non-normal data. The relationship between two ranked variables was measured using Spearman's rank correlation coefficient. The distribution of the dichotomous dependent variables lactation number and hygiene score in relation to blind quarters was examined in a stepwise regres-

Mastitis pathogens and antibiotic resistance in beef cows in Switzerland

A. Vollenweider et al.



Figure 3: Distribution of mammary secretion changes (no changes, clots, serous) in 61 samples from beef cows with clinical or subclinical mastitis caused by major and minor mastitis pathogens.

Table 3: : Minimal inhibitory concentrations (MIC) of 22 Pasteurella multocida strains cultured from milk samples from beef cows. Light blue cells indicate the concentrations determined by microdilution using the Micronaut-S system. Numbers in the grey cells indicate strains with a minimal inhibitory concentration greater than the highest tested concentration. The bold black lines indicate the CSLI (2020) or EUCAST (2021) breakpoints. «n.i.» not interpretable.

Antibiotic		Minimal Inhibitory Concentration (MIC, µg/ml)											Resistant
	0,125	0,25	0,5	1	2	4	8	16	32	64	50% (µg/ml)	90% (µg/ml)	strains (%)
Penicillin G	21	1									≤ 0,125	≤ 0,125	0
Ampicillin						22					≤ 4	≤ 4	n.i.
Cefazolin						22					≤ 4	≤ 4	0
Cefoperazone					22						≤ 2	≤ 2	n.i.
Cefquinome				21				1			≤ 1	≤ 1	n.i.
Oxacillin				12	6	2	2				≤ 1	4	n.i.
Pirlimycin					1		21				≥ 8	≥ 8	n.i.
Erythromycin	1	1	6	4	9	1					1	2	n.i.
Marbofloxacin		21	1								≤ 0,25	≤ 0,25	n.i.
Amoxicillin/Clavulanic acid (2:1)						22					≤ 4/2	≤ 4/2	n.i.
Kanamycin/Cephalexin (10:1)						4	4	12	1	1	16/1,6	16/1,6	n.i.

A.Vollenweider et al.

sion model. Frequency distributions of categorical variables of two groups were compared using the chi-square test. Results are presented as bar charts and box plots. P values $\leq 0,05$ were considered significant. This study was approved by the Office for Food Safety and Animal Health of the Canton of Grisons, permit number GR 2018/31.

Results

Udder health in beef cows

A total of 297 lactating cows from 31 herds were used. The number of cows sampled per herd ranged from one to 24 (mean 10 cows), which corresponded to 4 % to 100 % (mean 42 %) of eligible cows per herd. There were 143 (48 %) cross-



Figure 4: Culture results from 57 milk samples from beef cows with clinical mastitis. Red: Major mastitis pathogens, Blue: Minor mastitis pathogens. bred cows (primarily Limousin x Braunvieh), 60 (20%) Braunvieh, 28 (9%) Limousin (9%), 25 (8%) Raetisches Grauvieh, 21 (7%) Simmental, 13 (4%) Angus and 7 (2%) cows from other breeds. One hundred and forty-four (48%) cows were in lactation 1 to 3, 128 (43%) were in lactation 4 to 9 and 19 (6%) were in lactation 10 to 14.

A total of 63% of owners inspected the udder daily, 10% inspected the udder randomly or carried out dry-cow monitoring and the remaining 27% did not inspect the udder. Only 7% of the owners carried out regular udder palpation. When changes indicative of udder inflammation were seen, 30% of the owners palpated the udder, 20% only palpated the udder when the cow was dry and 43% did not palpate the udder.

Blind quarters were detected in 48 (16%) cows; 41 had one and 7 had two blind quarters. The forequarters were more often affected (58%) than the hindquarters, but the difference was not significant. At the time of the veterinary visit, only 32% of the blind quarters were known to farmers. The occurrence of blind quarters was not related to the owners' udder inspection and palpation protocols, but it was associated with lactation number (p<0,0001) and hygiene score (p=0,02).

From 60 (20%) cows, 97 individual-gland milk samples were collected because of abnormal palpation findings or an abnormal secretion. Culture results from these samples are shown in figure 1.

In 34 of those 60 cows with abnormal findings, major or minor mastitis pathogens were cultured from one or several quarter milk samples. The hindquarters were significantly more often affected (61 %, p=0,031). When only infec-

Table 4: Minimal inhibitory concentrations (MIC) of 11 Streptococcus uberis strains cultured from milk samples from beef cows. Light blue cells indicate the concentrations determined by microdilution using the Micronaut-S system. Numbers in the grey cells indicate strains with a minimal inhibitory concentration greater than the highest tested concentration. The bold black lines indicate the CSLI (2020) or EUCAST (2021) breakpoints. «n.i.» not interpretable.

Antibiotic		Minimal Inhibitory Concentration (MIC, µg/ml)										MIC	Resistant
	0,125	0,25	0,5	1	2	4	8	16	32	64	50% (µg/ml)	90% (µg/ml)	strains (%)
Penicillin G	9							2			≤ 0,125	≥ 16	18
Ampicillin						9			2		≤ 4	≥ 32	n.i.
Cefazolin						11					≤ 4	≤ 4	0
Cefoperazone					9	2					≤ 2	4	n.i.
Cefquinome				11							≤ 1	≤ 1	n.i.
Oxacillin				11							≤ 1	≤ 1	n.i.
Pirlimycin				11							≤ 1	≤ 1	0
Erythromycin	9		2								≤ 0,125	0,5	n.i.
Marbofloxacin			3	8							1	1	0
Amoxicillin/Clavulanic acid (2:1)						11					≤ 4/2	≤ 4/2	n.i.
Kanamycin/Cephalexin (10:1)						11					≤ 4/0,4	≤ 4/0,4	n.i.

tions caused by major mastitis pathogens were considered, $71~\%~(p{<}0{,}00001)$ occurred in the hindquarters.

In 15 cows, all four quarters had abnormal palpation findings and/or secretion, and therefore, individual-quarter samples were collected from all quarters. Composite milk samples from two to four clinically normal quarters were collected from 282 (95%) cows, and the SCC was determined in 261 of these samples. Twenty-one cows yielded very small samples and therefore no SCC was determined. The SCC ranged from 3000 to 4689000 cells/ml with a median of 82000 cells/ml (q25: 39000 cells/ml, q75: 244000 cells/ml). One-hundred-and-sixteen samples (41%) had a SCC >100000/ml and were cultured. Results from the 137 cultured samples are shown in figure 2.

Samples with major mastitis pathogens had higher SCCs than samples with minor mastitis pathogens (p<0,00001). The former had a median SCC of 709,000 cells/ml (q25: 383 500 cells/ml, q75: 1466 000 cells/ml) and the latter had 236,000 cells/ml (q25: 142 500 cells/ml, q75: 375 500 cells/ ml). All samples that yielded Pasteurella multocida had SCCs >500,000 cells/ml (median 1,256,000 cells/ml, q25: 963 000 cells/ml, q75: 1770000 cells/ml). The maximum SCC of 4,689,000 cells/ml was measured in a sample with Staphylococcus aureus; the median SCC of Staphylococcus aureus-positive samples was 452,000 cells/ml (q25: 244000 cells/ml, q75: 709000 cells/ml. Cows in which the lowest teat apex was below the tarsus had significantly higher SCCs in the composite milk samples than cows in which the lowest teat apex was above the tarsus (p=0,006). The frequency of the lowest teat apex being below the tarsus increased with an increase in age of the cows (p<0,01), but there were no direct correlations between the SCC and age and lactation number of the cows.

Composite or individual-quarter samples from 98 (33%) cows yielded at least one major or minor mastitis pathogen. Eleven of these cows had different pathogens in the composite and individual-quarter samples; five had two different major mastitis pathogens, five others had a major and a minor mastitis pathogen and one cow had two different minor pathogens. Forty-eight (16%) cows had at least one sample (composite or individual-quarter) with a major mastitis pathogen.

Clinical signs of acute mastitis (fever, swelling, abnormal milk) did not correlate with the culture of major or minor mastitis pathogens. Daily inspection of the udder correlated with a decreased frequency of intramammary infections (p=0,049). Cows out of herds in which udders were monitored by means of palpation yielded fewer major mastitis pathogens than minor pathogens (p=0,02).

Staphylococcus aureus was the most common major mastitis pathogen at the cow level and was cultured from at least one sample in 25 (8,4 %) of the 297 cows. These cows originated

from 12 (39%) of the 31 sampled herds. One herd that consisted of two groups housed in two different barns was of particular interest; all *Staphylococcus aureus*-positive cows were kept in the same barn, which had an infection rate of 86% compared with 0% in the other barn. The overall infection rate was 27%.

Other common major mastitis pathogens were *Pasteurella multocida* (4,1 %), *Streptococcus uberis* (2 %) and *Streptococcus dysgalactiae* (1,7 %).

Mastitis cases

Eighty-one samples from 49 cows were sent in by ten large-animal clinics and examined bacteriologically; 54 (67 %) samples yielded a major mastitis pathogen and seven (9 %) yielded a minor pathogen. The macroscopic appearance of the secretion (rated by the veterinarians) correlated with the culture of major or minor mastitis pathogens (p<0,00001; Figure 3). A minor mastitis pathogen (other *Staphylococcus* spp.) was cultured from a serous secretion in only one cow, in which *Staphylococcus aureus* was cultured from the other three quarters. Major mastitis pathogens were cultured more frequently from the hindquarters than the forequarters (p=0,005).

Sixteen samples were rated as subclinical by the veterinarians, of which eight (50%) yielded major mastitis pathogens and five (31%) yielded minor pathogens. Eight samples yielded no growth because the quarters had been pretreated. The remaining 57 samples were from 42 cows with clinical mastitis, which was acute in 47 and chronic in 10 cases. Of these samples from clinical cases, 47 (82%) yielded major mastitis pathogens and two (4%) yielded minor pathogens. The culture results of the 57 clinical samples are shown in figure 4.



Figure 5: Box-and whisker plots of the somatic cell count (cells/ml) of 61 samples from suckler cows with clinical and subclinical mastitis caused by major and minor mastitis pathogens (p=0,011).

Mastitis pathogens and antibiotic resistance in beef cows in Switzerland

A. Vollenweider et al.

A. Vollenweider et al.

Quarters infected with major mastitis pathogens had a median SCC of 1,205,500 cells/ml (q25: 784000 cells/ml, q75: 2045000), which was higher than the median of 295,000 cells/ml (q25: 49000 cells/ml, q75: 831000 cells/ml) of quarters infected with minor pathogens (p=0,011; Figure 5). Conclusions about the type of mastitis pathogen could not be derived from the SCC (p=0,27).

Antibiotic susceptibility testing

For antibiotic susceptibility testing, bacterial isolates from both parts of the study were used. 50 *Staphylococcus aureus*, 22 *Pasteurella multocida* and 11 *Streptococcus uberis* isolates were analyzed.

The MICs for the *Staphylococcus aureus* strains are shown in Table 2, and the results of the disk diffusion test are shown in figure 6. Fifty-six percent of the *Staphylococcus aureus* strains were resistant to penicillin G and all strains were susceptible to cefazolin, cefoperazone, oxacillin, pirlimycin, erythromycin and marbofloxacin. Based on the knowledge that the susceptibility breakpoint of amoxicillin/clavulanic acid was below the tested concentrations, the only statement concerning the susceptibility of the tested *Staphylococcus aureus* strains is that at least no strain had an MIC that was higher than the lowest tested concentration. The ampicillin breakpoint was also below the tested concentrations, but 52% of the tested strains had MICs that were higher than the lowest tested concentration, and therefore they were



Figure 6: Interpretation of the antibiotic susceptibility of 50 *Staphylococcus aureus* isolates from beef cows with clinical and subclinical mastitis. Zone diameters were interpreted using CLSI (2020) and EUCAST (2021) breakpoints. Resistant (red) = no inhibition zone or zone diameter smaller than the breakpoint; intermediate (yellow) = inhibition zone between values for resistant and susceptible; susceptible (green) = zone diameter greater than the breakpoint. A bacterial strain was interpreted as resistant when there was no inhibition zone, and as unclear when there was an inhibition zone in the absence of an available breakpoint.

considered resistant. A statement concerning the susceptibility to the combination of kanamycin/cephalexin was not possible because breakpoints were not available (Table 1). Ninety-six percent of the *Staphylococcus aureus* strains were susceptible to gentamicin. No breakpoints were available for neomycin, kanamycin and spiramycin, but only one strain had no inhibition zone with all three drugs, and another strain had no inhibition zone with neomycin, which was interpreted as resistant.

The MICs for the *Pasteurella multocida* strains are shown in Table 3, and the results of the disk diffusion are shown in figure 7. All *Pasteurella multocida* strains were susceptible to penicillin G and cefazolin. The ampicillin breakpoint was below the tested concentrations, but no *Pasteurella multocida* strain had an MIC that was higher than the lowest tested concentration. No *Pasteurella multocida* breakpoints were available for the other antibiotics tested with the Micronaut-S and disk diffusion tests (Table 1). However, it was evident that at least 86% of the strains were resistant to neomycin and kanamycin and 45% against spiramycin because these strains did not have inhibition zones.

The MICs for the *Streptococcus uberis* strains are shown in Table 4 and the results of the disk diffusion are shown in figure 8. All *Streptococcus uberis* strains were susceptible to cefazolin, pirlimycin and marbofloxacin, and 18% were resistant to penicillin. No breakpoints were available for the other microbials tested (Table 1). All *Streptococcus uberis* strains were resistant to gentamicin, neomycin and kanamycin, and 64% had no inhibition zone with spiramycin.

Discussion

The prevalence of intramammary infections on cow level calculated in the present study (33 %) was within the broad range of values reported for beef cows.^{18,23,24,29,30,32,41} With the exception of one study, the large variation in published prevalences could be because data were generated from only one or two herds. A study of ten cow-calf herds in southern Sweden reported a prevalence of 40 %.³² In dairy cows, the prevalence was 17 % according to a study from Switzerland.²⁰ We assumed that the prevalence of intramammary infections calculated for the present study may be an underestimation, because only composite milk samples with a SCC >100 000 cells/ml were cultured.

Major mastitis pathogens are commonly associated with clinical mastitis that involves inflammation-related parenchymal damage and an increased SCC. In contrast, minor mastitis pathogens usually cause less tissue damage and a smaller increase in somatic cells and are often associated with subclinical mastitis.³⁴ In cows with clinical mastitis in our study, the major mastitis pathogens (82 to 4%). Sixteen percent of the cows examined in the study part on udder health of cows from Engadin Valley farms yielded a major mastitis pathogen in at least one sample.

In all samples cultured, Staphylococcus aureus was the most common major pathogen. This was in agreement with earlier studies of udder health in beef cows. 18,23,24,29,30,32,41 We did not genotype the Staphylococcus aureus strains due to our study design, but our results show that this could be of potential interest also in beef cows. Some genotypes have been associated with sporadic single-cow intramammary infections, whereas other types, such as genotype B, are highly contagious and pathogenic and have been associated with severe mastitis problems in dairy cows at the herd level.13 It is likely that the herd with a group in one barn in which 86% of the tested cows were Staphylococcus aureus-positive was infected with a more contagious genotype.¹³ The question arises as to how *Staphylococcus aureus* is transmitted in beef cow herds. Staphylococcus aureus is typically spread among dairy cows during milking.²⁵ Beef calves are commonly observed cross-suckling and can act as vectors in the transmission of pathogens among quarters of a cow or among different cows.²⁷ This particularly applies to strains that are adapted to the udder and detected on the teat skin and shed in the milk.²⁵ Other genotypes are opportunistic colonisers of normal and injured bovine skin and can cause sporadic and non-contagious intramammary infections in the presence of mitigating circumstances such as injuries.25

Gram-negative mastitis pathogens have so far only rarely been described in beef cows. There is only one study in which *Klebsiella* spp. were recovered from two of 92 (2,2%) cows and two studies cultured 0,2 to 2,3% «other bacteria», which were not further differentiated.^{18,29,32} Therefore, we were surprised that Pasteurella multocida was cultured from at least one quarter in 12 (4%) cows in the study part on udder health and was the most common pathogen in clinical mastitis cases (22%). It is not clear whether Pasteurellaceae would possibly be detected even more frequently with immediate processing in the laboratory; delaying the culture of nasal swabs taken from pigs with atrophic rhinitis by 48 hours reduced the recovery rate of Pasteurella multocida by 50%.4 We had a similar experience when a milk sample from a quarter with clinical mastitis yielded growth of Pasteurellaceae after being plated onto trypticase soy agar with 5% sheep blood shortly after collection and before shipment. When the sample was shipped and cultured the next day in the laboratory, it yielded no growth. It is therefore likely that processing immediately after sample collection yields a higher recovery rate for Pasteurellaceae. Furthermore, six (21%) Pasteurella multocida strains could not be re-cultured after storage at -80°C. These observations warrant further studies in other geographic regions to clarify, whether the prevalence of Pasteurella multocida mastitis in the region of our study is indeed higher than in other areas.

Nevertheless, our results showed that Pasteurella multocida was the pathogen with the highest prevalence in beef cows with clinical mastitis. Genotyping of Pasteurella multocida recovered from the nasopharynx of calves with pneumonia and from the milk of cows with clinical mastitis showed that the same strains can cause both conditions.⁴⁰ In Switzerland, the prevalence of pathogens of the Pasteurellaceae family in the nasopharynx of calves is 58 %.³⁶ It is therefore conceivable that Pasteurella multocida from the nasopharynx of calves is the cause of intramammary infections in cows. This mode of transmission could also explain why Pasteurella multocida mastitis is uncommon in dairy cows. In addition to Pasteurella multocida, Mannheimia haemolytica and Mannheimia varigena were each recovered from one case of clinical mastitis. Mannheimia haemolytica is the most common pathogen recovered from meat-producing sheep with acute mastitis, and both experimental and natural transmission of this pathogen from the upper respiratory tract of nursing lambs to the udder of their dams has been described.1,15

Our interpretation of the antibiograms was difficult, because breakpoints have not been established for all antibiotics used to treat bovine mastitis (Table 1). High resistance rates of *Streptococcus uberis* strains to gentamicin, neomycin, kanamycin and spiramycin were in agreement with the results of other studies on antimicrobial resistance of streptococci from bovine mastitis.^{31,33,35} Of note, 56% of the *Staphylococcus aureus* strains were resistant to penicillin, which was in contrast to a resistance rate of 14% in dairy



Figure 7: Interpretation of the antibiotic susceptibility of 50 *Pasteurella multocida* isolates from beef cows with clinical and subclinical mastitis. Zone diameters were interpreted using CLSI (2020) and EUCAST (2021) breakpoints. Resistant (red) = no inhibition zone or zone diameter smaller than the breakpoint; intermediate (yellow) = inhibition zone between values for resistant and susceptible; susceptible (green) = zone diameter greater than the breakpoint. A bacterial strain was interpreted as resistant when there was no inhibition zone, and as unclear when there was an inhibition zone in the absence of an available breakpoint.

Mastitis pathogens and antibiotic resistance in

beef cows in Switzerland

A. Vollenweider et al.

cows in a Swiss study.²¹ Since antibiotic udder preparations are probably used much less frequently in suckler cows than in dairy cows, a selection of resistant *Staphylococcus aureus* strains resulting from this is less likely. We suspect that our cultures yielded mostly skin and wound-associated genotypes of *Staphylococcus aureus* with a resistance pattern that may have differed from that of udder-associated genotypes. *Staphylococcus aureus* strains recovered from skin, wound and ear infections had resistance rates to penicillin of 75,8% in cats and 69,4% in dogs.⁹

Blind quarters occurred in 16% of the cows examined in the study part on udder health, but only about a third of these had been previously detected by their owners. This rate appears high compared with prevalences of 3 to 10% reported in other studies in beef cows.^{11,24,32} In two of these studies only one and then the same herd was examined. In



Figure 8: Interpretation of the antibiotic susceptibility of 50 *Streptococcus uberis* isolates from beef cows with clinical and subclinical mastitis. Zone diameters were interpreted using CLSI (2020) and EUCAST (2021) breakpoints. Resistant (red) = no inhibition zone or zone diameter smaller than the breakpoint; intermediate (yellow) = inhibition zone between values for resistant and susceptible; susceptible (green) = zone diameter greater than the breakpoint. A bacterial strain was interpreted as resistant when there was no inhibition zone, and as unclear when there was an inhibition zone in the absence of an available breakpoint.

a study of ten herds, in which the prevalence of cows with blind quarters was 10%, 64% of the examined cows had lactation numbers 1 to 3 compared with 48 % in the present study.³² We observed an increase in blind quarters with increasing age, and therefore believe, that the presence of older cows in our study accounted for the high blind-quarter rate. Blind quarters result from severe mastitis, teat injuries and other unknown factors.³ The blind-quarter rate was not associated with the udder inspection and palpation protocols used by the owners. However, the udder palpation protocol used by most owners was poor and limited to cows with a visually abnormal udder. In contrast, the blind-quarter rate in a well-managed dairy herd is much smaller and should not exceed 2%.38 Regular visual inspection of the udder of beef cows is a simple health measure that is associated with a decrease in intramammary infections. Therefore, regular palpation of the udder would likely have a positive effect on udder health because inflamed quarters would be detected earlier.

Conclusion

Pasteurella multocida (22%) and *Staphylococcus aureus* (21%) were the most common pathogens in samples from clinical mastitis in beef cows in Switzerland. Most (56%) of the *Staphylococcus aureus* strains were resistant to penicillin G. Similar to dairy cow management, bacteriological examination of milk samples and susceptibility testing are important tools for optimising treatment in beef cows. An antibiogram is of particular importance when *Staphylococcus aureus* is the mastitis-causing pathogen. The present study generated preliminary data on the resistance patterns of mastitis pathogens in beef cows in Switzerland, therefore facilitating evidence-based treatment strategies. The further establishment of breakpoints for susceptibility testing is of great importance to facilitate selective use of antibiotics.

Acknowledgements

We thank all the farmers and veterinarians who made this study possible.

Agents pathogènes des mammites et résistance aux antibiotiques chez les vaches mères en Suisse

Les mammites chez les vaches mères n'ont pas été étudiées de manière aussi approfondie que chez les vaches laitières et les données concernant la Suisse font défaut. Diverses études ont montré un spectre pathogène similaire à celui des vaches laitières, ce qui n'a pas pu être confirmé dans cette étude. Pour rassembler les premières données en Suisse, des échantillons de lait de 297 vaches mères provenant de 31 troupeaux de la vallée de l'Engadine dans le canton des Grisons ont été examinés bactério-logiquement. Au moins un agent pathogène majeur ou mineur de mammite a été retrouvé dans au moins un quartier ou dans un échantillon composite chez 33% de toutes les vaches. Les agents pathogènes majeurs de mammite les plus courants étaient Staphylococcus aureus (8,4 % des vaches), Pasteurella multocida (4,1 %), Streptococcus uberis (2%) et Streptococcus dysgalactiae (1,7%). Seize pour cent des vaches avaient au moins un quartier sec mais cela n'avait été détectés auparavant par les propriétaires que dans seulement 32 % des cas. Dans la deuxième partie de l'étude, des échantillons de lait provenant de vaches mères atteintes de mammites ont été examinés sur le plan bactériologique; les vaches provenaient de diverses régions de Suisse et avaient été présentées pour un traitement vétérinaire. Pasteurella multocida (22%) et Staphylococcus aureus (21 %) étaient les agents pathogènes les plus fréquemment isolés. Des antibiogrammes utilisant des tests de microtitration et de diffusion sur disque ont été générés pour les souches de Staphylococcus aureus, Pasteurella multocida et Streptococcus uberis des deux parties de l'étude. Cinquante-six pour cent des souches de Staphylococcus aureus étaient résistantes à la pénicilline G. Nos résultats montrent que l'examen bactériologique d'un échantillon de lait facilite le diagnostic et permet un traitement spécifique des mammites chez les vaches mères; ceci peut être encore amélioré par des tests de sensibilité aux antibactériens. Nos données préliminaires sur les profils de résistance des agents pathogènes de mammites chez les vaches de boucherie faciliteront les stratégies de traitement fondées sur des faits.

Mots clés: Santé de la mamelle, *Staphylococcus aureus*, *Pasteurella multocida*, quartier sec, nombre de cellules

Patogeni della mastite e resistenza agli antibiotici nelle vacche madri in Svizzera

Mastitis pathogens and antibiotic resistance in beef cows in Switzerland

A. Vollenweider et al.

La mastite delle vacche madri non è stata studiata in modo così approfondito come quella delle vacche da latte e inoltre mancano dati provenienti dalla Svizzera. Diversi studi hanno evidenziato uno spettro patogeno simile a quello delle vacche da latte, ma che purtroppo non ha potuto essere confermato in questo studio. Per raccogliere i primi dati dalla Svizzera, sono stati esaminati batteriologicamente i campioni di latte di 297 vacche madri in lattazione provenienti da 31 mandrie dell'Engadina, nel Cantone Grigioni. Almeno un patogeno major o minor della mastite è stato ritrovato in almeno un singolo quarto o da un campione misto nel 33% di tutte le vacche. I più comuni patogeni della mastite sono stati lo Staphylococcus aureus (8,4 % delle vacche), la Pasteurella multocida (4,1%), lo Streptococcus uberis (2%) e lo Streptococcus dysgalactiae (1,7%). Il 16% delle vacche aveva almeno un quarto atrofico, ma solo il 32% di questi era stato precedentemente individuato dai proprietari. Nella seconda parte dello studio, sono stati esaminati batteriologicamente campioni di latte di vacche madri affette da mastite; le vacche provenivano da diverse parti della Svizzera ed erano state sottoposte a trattamento veterinario. La Pasteurella multocida (22%) e lo Staphylococcus aureus (21%) sono stati i patogeni più frequentemente isolati. Gli antibiogrammi sono stati preparati per i ceppi di Staphylococcus aureus, di Pasteurella multocida e di Streptococcus uberis in entrambe le parti dello studio utilizzando tecniche di microdiluizione e test di diffusione su disco. Il 56 % dei ceppi di Staphylococcus aureus era resistente alla penicillina G. I nostri risultati hanno dimostrato che l'esame batteriologico di un campione di latte supporta la diagnosi e consente un trattamento specifico della mastite nelle vacche madri. Questo aspetto può essere ulteriormente migliorato con i test di suscettibilità antibatterica. I nostri dati preliminari sui modelli di resistenza degli agenti patogeni della mastite nelle vacche madri favoriranno delle strategie di trattamento basate su dati concreti.

Parole chiave: salute della mammella, *Staphylococcus* aureus, *Pasteurella multocida*, quarto atrofico, conteggio delle cellule somatiche

A. Vollenweider et al.

Literaturnachweis

- ¹ Arsenault J., Dubreuil P., Higgins R., Bélanger D.: Risk factors and impacts of clinical and subclinical mastitis in commercial meat-producing sheep flocks in Quebec, Canada. Prev. Vet. Med., 2008, 87:373–393
- ² Baumgartner W.: Untersuchung der Mamma. In: Baumgartner W. (Hrsg.): Klinische Propädeutik der Hausund Heimtiere, Enke Verlag, Stuttgart, D, 2014, 190–195
- ³ Beger M.: Eutergesundheitsstörungen bei Mutterkühen. Dissertation: Freie Universität Berlin, Fachbereich Veterinärmedizin, 2001
- ⁴ Chanter N., Goodwin R.F., Rutter J.M.: Comparison of methods for sampling and isolation of toxigenic Pasteurella multocida from nasal cavity of pigs. Res. Vet. Sci., 1989, 47:355–358
- ⁵ (CLSI) Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing. M100, 27th Edition, 2017
- ⁶ (CLSI) Clinical and Laboratory Standards Institute: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 5th Edition CLSI supplement VET01S, 2020
- ⁷ Cook N.B.: The influence of barn design on dairy cow hygiene, lameness, and udder health. Proceedings, 35th Annual Convention of the American Association of Bovine Practitioners, Madison, Wisconsin, USA, 2002, 97–103
- ⁸ de Boyer des Roches A., Faure M., Lussert A., Herry V., Rainard P., Durand D., Foucras G.: Behavioral and patho-physiological response as possible signs of pain in dairy cows during Escherichia coli mastitis: A pilot study. J. Dairy Sci., 2017, 100:8385–8397
- ⁹ de Jong A., Youala M., El Garch F., Simjee S., Rose M., Morrissey I., Moyaert H.: Antimicrobial susceptibility monitoring of canine and feline skin and ear pathogens isolated from European veterinary clinics: results of the ComPath Surveillance programme. Vet. Dermatol., 2020, 31:431-e114
- ¹⁰ Dingwell R.T., Leslie K.E., Schukken Y.H., Sargeant J.M., Timms L.L.: Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation in dairy cows. Can. Vet. J., 2003, 44:413–416
- ¹¹ Dueñas M.I., Paape M.J., Wettemann R.P., Douglass L.W.: Incidence of mastitis in beef cows after intramuscular administration of oxytetracycline. J. Anim. Sci., 2001, 79:1996–2005
- ¹² (EUCAST) European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021.
- ¹³ Fournier C., Kuhnert P., Frey J., Miserez R., Kirchhofer M., Kaufmann T., Steiner A., Graber H.U.: Bovine Staphylococcus aureus: Association of virulence genes, genotypes and clinical outcome. Res. Vet. Sci., 2008, 85:439–448
- ¹⁴ Foster R.A.: Erkrankungen der Milchdrüse bei Kühen. In McGavin M.D., Zachary J.F. (Hrsg.): Pathologie der Haustiere. Elsevier, München, D, 2007, 1176–1180
- ¹⁵ Fragkou I.A., Gougoulis D.A., Billinis C., Mavrogianni V.S., Bushnell M.J., Cripps P.J., Tzora A., Fthenakis G.C.: Transmission of Mannheimia haemolytica from the tonsils of lambs to the teat of ewes during sucking. Vet. Microbiol., 2011, 148:66–74

- ¹⁶ Gelasakis A.I., Mavrogianni V.S., Petridis I.G., Vasileiou N.G.C., Fthenakis G.C.: Mastitis in sheep – The last 10 years and the future of research. Vet. Microbiol., 2015, 181:136–146
- ¹⁷ Guélat-Brechbuehl M., Thomann A., Albini S., Moret-Stalder S., Reist M., Bodmer M., Michel A., Niederberger M.D., Kaufmann T.: Cross-sectional study of Streptococcus species in quarter milk samples of dairy cows in the canton of Bern, Switzerland. Vet. Rec., 2010, 167:211–215
- ¹⁸ Haggard D.L., Farnsworth R.J., Springer J.A.: Subclinical mastitis in beef cows.
 J. Am. Vet. Med. Assoc., 1983, 182:604–606
- ¹⁹ Hamann J.: Relationships between somatic cell count and milk composition. Bulletin of the International Dairy Federation, 2003, 372:56–59
- ²⁰ Ivemeyer S., Knierim U., Waiblinger S.: Effect of human-animal relationship and management on udder health in Swiss dairy herds.
 J. Dairy Sci., 2011, 94:5890–5902
- ²¹ Käppeli N., Morach M., Corti S., Eicher C., Stephan R., Johler S.: Staphylococcus aureus related to bovine mastitis in Switzerland: Clonal diversity, virulence gene profiles, and antimicrobial resistance of isolates collected throughout 2017. J. Dairy Sci., 2019, 102:3274–3281
- ²² Kawai K., Hayashi T., Kiku Y., Chiba T., Nagahata H., Higuchi H., Obayashi T., Itoh S., Onda K., Arai S., Sato R., Oshida T.: Reliability in somatic cell count measurement of clinical mastitis milk using DeLaval cell counter. Anim. Sci. J., 2013, 84:805–807

²³Lents C.A., Wettemann R.P., Paape M.J., Looper M.L., Buchanan D.S.: Effects of dry cow treatment of beef cows on pathogenic organisms, milk somatic cell counts, and calf growth during the subsequent lactation. J. Anim. Sci., 2008, 86:748–755

²⁴Lents C.A., Wettemann R.P., Paape M.J., Vizcarra J.A., Looper M.L., Buchanan D.S., Lusby K.S.: Efficacy of intramuscular treatment of beef cows with oxytetracycline to reduce mastitis and to increase calf growth. J. Anim. Sci., 2002, 80:1405–1412

²⁵Leuenberger A., Sartori C., Boss R., Resch G., Oechslin F., Steiner A., Moreillon P., Graber H.U.: Genotypes of Staphylococcus aureus: On-farm epidemiology and the consequences for prevention of intramammary infections. J. Dairy Sci., 2019, 102:3295–3309

²⁶Leslie K.E., Petersson-Wolfe Ch.S.: Assessment and management of pain in dairy cows with clinical mastitis. Vet. Clin. Food Anim., 2012, 28:289–305

²⁷Lewandrowski N.M., Hurnik J.F.: Nursing and cross-nursing behavior of beef cattle in confinement. Can. J. Anim. Sci., 1983, 63:849–853

- ²⁸ Merlin Gesellschaft für mikrobiologische Diagnostika GmbH: Gebrauchsanweisung zu Mircronaut-S Veterinär. Version 2016-04-18
- ²⁹ Newman M.A., Wilson L.L., Cash E.H., Eberhart R.J., Drake T.R.: Mastitis in beef cows and its effects on calf weight gain. J. Anim. Sci., 1991, 69:4259–4272
- ³⁰ Paape M.J., Duenas M.I., Wettemann R.P., Douglass L.W.: Effects of intramammary infection and parity on calf weaning weight and milk quality in beef cows. J. Anim. Sci., 2000, 78:2508–2514

- ³¹ Petrovski K.R., Grinberg A., Williamson N.B., Abdalla M.E., Lopez-Villalobos N., Parkinson T.J., Tucker I.G., Rapnicki P.: Susceptibility to antimicrobials of mastitis-causing Staphylococcus aureus, Streptococcus uberis and Str. dysgalactiae from New Zealand and the USA as assessed by the disk diffusion test. Aust. Vet. J., 2015, 93:227–233
- ³² Persson Waller K., Persson Y., Nyman A.-K., Stengärde L.: Udder health in beef cows and its association with calf growth. Acta Vet. Scan., 2014, 56:9
- ³³ Rato M.G., Bexiga R., Florindo C., Cavaco L.M., Vilela C.L., Santos-Sanches I.: Antimicrobial resistance and molecular epidemiology of streptococci from bovine mastitis. Vet. Microbiol., 2013, 161:286–294
- ³⁴ Reyher K.K., Dohoo I.R., Scholl D.T., Keefe G.P.: Evaluation of minor pathogen intramammary infection, susceptibility parameters and somatic cell counts on the development of new intramammary infections with major mastitis pathogens. J. Dairy Sci., 2012, 95:3766–3780
- ³⁵ Rüegsegger F., Ruf J., Tschuor A., Sigrist Y., Rosskopf M., Hässig M.: Antimicrobial susceptibility of mastitis pathogens of dairy cows in Switzerland. Schweiz. Arch. Tierheilkd., 2014, 156:483–488
- ³⁶ Schönecker L., Schnyder P., Schüpbach-Regula G., Meylan M., Overesch G.: Prevalence and antimicrobial resistance of opportunistic pathogens associated with bovine respiratory disease isolated from nasopharyngeal swabs of veal calves in Switzerland. Prev. Vet. Med., 2020, 185:105182
- ³⁷ Schubert S., Wieser A.: MALDI-TOF-MS in der mikrobiologischen Diagnostik. BIOspektrum, 2010, 07:760–762
- ³⁸ Schulz J.: Erkrankungen der Milchdrüse. In: Wendt K., Bostedt H., Mielke H., Fuchs H.-W.: Euter- und Gesäugekrankheiten. Gustav Fischer Verlag, Jena/Stuttgart, D, 1994, 226–238
- ³⁹ Simpson R.B., Nickerson S.C., Shockey J.D., Phillips J.M.: Prevalence of mastitis pathogens preceding and following parturition in primiparous beef cows. Prof. Anim. Sci., 2000, 16:188–195
- ⁴⁰ Turni C., Dayao D., Aduriz G., Cortabarria N., Tejero C., Ibabe J.C., Singh R., Blackall P.: A Pasteurella multocida strain affecting nulliparous heifers and calves in different ways. Vet. Microbiol., 2016, 195 :17–21
- ⁴¹ Watts J.L., Pankey J.W., Oliver W.M., Nickerson S.C., Lazarus A.W.: Prevalence and effects of intramammary infection in beef cows. J. Anim. Sci., 1986, 62:16–20
- ⁴² Wollowski L., Heuwieser W., Kossatz A., Addis M.F., Puggioni G.M.G., Meriaux L., Bertulat S.: The value of the biomarkers cathelicidin, milk amyloid A, and haptoglobin to diagnose and classify clinical and subclinical mastitis. J. Dairy Sci., 2021, 104:2106–2122

Korrespondenzadresse

Ulrich Bleul Clinic of Reproductive Medicine Department of Farm Animals Vetsuisse-Facultiy University Zurich Winterthurerstr. 260 CH-8057 Zurich E-Mail: ubleul@vetclinics.uzh.ch Mastitis pathogens and antibiotic resistance in beef cows in Switzerland

A. Vollenweider et al.