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UH-01

Selective and deferred treatment of clinical mastitis in seven New Zealand dairy herds

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Objectives: This study focused on evaluating the ability of a novel on-farm diagnostic system for bovine mastitis (Mastatest©) to control antibiotic usage whilst achieving equivalent bacteriological and clinical cure rates alongside long term individual somatic cell count (**ISCC**) outcomes as conventional treatment choices.

Mastitis is the most frequent reason for antibiotic use in New Zealand dairy cattle and technologies reducing this use contribute to responsible product stewardship. Rapid identification of pathogen and antibiotic susceptibility facilitate targeted treatment but currently involve a minimum 24 hours delay. Studies from confinement systems where Gram-negative organisms are responsible for a significant proportion of mastitis, indicate selective treatment can reduce antibiotic use without reducing clinical or bacteriological cure. However, in New Zealand's seasonal, pastoral dairy system, mastitis is dominated by Gram-positive organisms and if treatment is deferred, it is vital both short- and long-term clinical health outcomes are not compromised.

Materials and methods: Mastatest© is an on-farm or clinic diagnostic system for bovine mastitis indicating the pathogen and its antibiotic sensitivity within 24 hours of sampling.

Mild to moderate mastitis cases in the 100 days after calving in 6,467 cows from 7 farms were milk sampled and randomly allocated to a positive control group (non-selective treatment) or a culture-based treatment using Mastatest©. All milk samples were processed on-farm.

For the positive control, the quarter was treated immediately with 3 treatments of procaine penicillin every 12 hours. For the selective treatment group, treatment was delayed for 24 hours and then informed by pathogen and antibiotic sensitivity from the Mastatest© result. Gram-negative and no-growth quarters were untreated. Gram-positive quarters were treated with the antibiotic for which the lowest in vitro antimicrobial sensitivity was reported.

Re-sampling was carried out from affected quarter(s) approximately 21 days after initial diagnosis and cultured for bacterial identification. Clinical recurrence within 60 days and ISCC data was recorded at herd tests over the duration of the lactation. Antimicrobial usage and days of milk withhold pending clearance of residues were also noted.

Results: There was no difference in bacteriological or clinical cure rate between the two treatment groups. Out of 535 quarter cases, 451 (84%) were bacteriologically cured at re-sampling and 43 (8%) were re-diagnosed with clinical mastitis within 60 days of the original diagnosis. Bayesian models predicted no difference in the cure proportion by treatment group but the probability of a bacteriological cure for cows infected with *Staph.aureus* was significantly less than that for *Strep. uberis* in both groups. There were numerical differences in the median bacteriological cure proportion by farm, but the coefficients spanned zero and overlapped. There was no evidence for a significant interaction between treatment group and farm, nor between treatment group and pathogen.

Final herd test ISCC - 225,000 cells/mL (95% predictive interval (PI) =25,000-4,543,145) - and days of milk withhold from supply - 5.7 days per quarter case (95% PI=1.0-6.5) - did not differ between groups. There were numeric differences in the median predicted ISCC by pathogen and farm, but the coefficients spanned zero and overlapped. There was no evidence for a significant interaction between treatment group and farm, nor between treatment group and pathogen.

Antibiotic usage was 24% less (95% PI = 12-47%) in the selective group with the model predicting that the there was a 98% chance that antibiotic usage in the selective group (1.3 daily doses per case, 95%PI=1.1-1.6) was less than in the non-selective group (1.7, 95%PI=1.4-1.9).

Conclusions: This study suggests that on farm decisions about deferred treatment of mastitis using Mastatest© to identify the intramammary pathogen can reduce the antimicrobial usage with no loss in bacterial or clinical cure and with no effect on ISCC over the lactation.

Keywords: Mastitis, deferred treatment, antibiotic sensitivity, Mastatest.

UH-02

Udder health in German dairy heifers – risk factors during first days of lactation

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Objectives: Heifer mastitis is of great importance in many German dairy farms. Intramammary infections (IMI) prior to calving and during the early lactation period may influence the development of the mammary glands, the future milk production, udder health and related culling hazard negatively (Piepers et al., 2009). Up to now, the exact time of infection has not been identified. However, knowing this time point would be of major importance in order to reduce the rate of new IMI by possibly switching off the main risk factors. The aim of this study was to evaluate the exact moment of IMI in dairy heifers during early lactation and to demonstrate the most important risk factors.

Material and methods: In total, 279 Holstein Frisian heifers reared on 3 German dairy farms were included in this study. The average bulk milk SCC during the test period was

150,000, 180,000 and 260,000 cells / ml, respectively. From September 2017 until March 2018 quarter milk samples were collected from all four udder quarters for cyto-microbiological diagnosis at two time points. The samples were obtained 3 and 17± 3 days after calving in order to define the postpartum IMI status. Isolated NAS strains were differentiated using MALDI-TOF-MS (Bruker, Bremen). The farmers observed the heifers during the first 100 days of lactation and documented all signs of clinical mastitis.

Results: No pathogen could be detected in 80.2% (n = 725) of the udder quarters three days after calving and in 85.8% (n = 776) of the udder quarters 17 days after calving. This means that on day 17 after calving an IMI could be detected in 129 udder quarters. Based on the infection status on day 3 after calving, 83.0% (n = 107) of these 129 quarters suffered from a new infection. A latent infection could be detected in 17% (n = 22) of these udder quarters. 80.4% of the udder quarters suffered from an infection caused by NAS at day 3 p.p. showed a somatic cell count of >100.000 cells/mL. The probability of IMI caused by contagious mastitis pathogens increased with elevated SCC. Heifers showed an increasing probability of IMI with NAS and coryneforms on day 17 postpartum when being at a higher age at calving. Heifers with high milk yields showed less IMI caused by environmental pathogens 17 days postpartum and fewer clinical mastitis in the first 100 days of lactation. However, animals with high milk yield showed increased IMI caused by NAS and coryneforms pathogens on day 3 postpartum. Udder edema was associated with an increased risk of IMI caused by NAS and coryneforms on day 3 postpartum. A strong association between udder health of dairy heifers and teat-cup fell-offs with the result of sudden air ingress could also be shown. Animals from farms with increased problems during milking showed an increased probability of IMI on days 3 and 17 postpartum.

Conclusion: This study reveals that an elimination of pathogens is possible during early lactation. However, there is a risk of new infection at the same time. In addition to the period before calving and calving itself, the period between the 3rd and 17th day of lactation plays an extremely important role for the udder health of dairy heifers. For this reason, the risk factors specific to this stage of early lactation must be taken into account in order to apply a modern udder health management to dairy farms.

Keywords: Mastitis, intramammary infections, dairy heifers.

UH-03

In vitro penicillin sensitivity of *Streptococcus ssp.* isolated from quarter milk samples of Bavarian dairy cows (2018 - 2019)

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Objective: The objective was to evaluate the *in vitro* antimicrobial resistance against penicillin of *Streptococcus (S.) uberis, S. agalactiae, S. dysgalactiae,* and *S. canis* of routine quarter milk samples.

Material and methods: In 2018 and 2019 the Bavarian Animal Health Services received quarter milk samples from whole dairy herd tests as well as individual cows. The laboratory used standard culture methods in accordance with guidelines of the German veterinary association to diagnose infections in quarter milk samples. The identification of *Streptococci* was based on colony morphology, hemolysis, esculin hydrolysis, camp factor as well as gram strain. Further differentiation of esculin-positive *Streptococci* was conducted with an in-house method that utilized *Enterococcus* selective agar plates and a disc test against Penicillin and Rifampicin. Strains of unclear results were identified by MALDI-TOF-MS (microflex™ MALDI Biotyper™, Bruker Daltonik, reference database V.3.3.1.0., Bruker Daltonik GmbH, Bremen Germany). A subset of aforementioned *Streptococci* was tested for sensitivity to common antibiotics using with the method of MIC (minimum inhibitory concentration) (mastitis 3 plat, Merlin Diagnostika GmbH). Breakpoints for sensitivity are based upon CLSI Vet01-A4 (2015) and CLSI Vet01S 5th edition. As a breakpoint for penicillin for the indication “mastitis cow” does not exist, an older breakpoint (CLSI M31-A2; AVID, 1998) was used instead. Furthermore, intermediate results were reported as resistant. Results were summarized by descriptive statistics.

Results: A total of 854,782 quarter milk samples from 218,336 cows of >7,000 individual dairy farms were analyzed in the laboratory in 2018-2019. These included samples from subclinical (based on California Mastitis Test (CMT), n= 213,174) and clinical cases (n= 17,642). Mastitis pathogens were found in 18.7 % (n= 159,900) of all submitted samples.

S. uberis was the most commonly isolated mastitis pathogen of clinical samples (2018: 35.0%, n= 2,566; 2019: 34.4%, n= 2,280). Additionally, *S. uberis* was the second most commonly isolated pathogen in samples of subclinical cases in both years (2018: 23.9%, n= 12,178; 2019: 23.1% n= 9,778) - only surpassed by Coagulase Negative *Staphylococci* (2018: 29.5%; 2019: 30.4%).

Of 7,345 *S. uberis* tested, exceedingly few isolates (n=47) had a breakpoint >0.125 for penicillin (2018: 0.8%, n= 33; 2019: 0.4%, n= 14). The vast majority had a breakpoint <0,125 for penicillin and thus was considered susceptible to penicillin *in vitro*. All tested isolates of *S. agalactiae* (n= 900) and *S. canis* (n= 355) had a breakpoint <0.125 for penicillin - i.e., none were considered resistant to penicillin. Only five (0.2%) of 2,684 tested *S. dysgalactiae* were considered resistant to penicillin as they had a penicillin breakpoint above >0.125 (2018: 0.1%, n= 1,444; 2019: 0.2%, n= 1,240).

Conclusion: Only sporadic *Streptococci* isolates had a breakpoint >0.125 and were resistant to penicillin *in vitro*. The vast majority of tested *Streptococci* had a breakpoint <0.125 for penicillin. Therefore, penicillin should continue to be the first choice for antibiotic therapy of *S. uberis* and esculin-negative Streptococci in Bavaria (Southern Germany).

Funding Source: Bavarian Ministry for Food, Agriculture and Forestry and the Bavarian Joint Founding Scheme for the Control and Eradication of contagious Livestock (Bayerische Tierseuchenkasse).

Keywords: Mastitis, streptococci ssp., penicillin sensitivity.

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UH-04

Monitoring Udder Health on Routinely Collected Census Data: Evaluating the Effects of a Changing Antimicrobial Policy in the Netherlands

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Objectives: Antibiotic resistance is becoming increasingly important and given its potential association with antimicrobial use (AMU), continuous actions are taken to reduce AMU in the livestock industry. One of the most important measures to avert preventive antibiotic usage was implementation of selective instead of blanket dry cow therapy in 2013. In the Netherlands, both AMU and udder health parameters are monitored in the Dutch cattle health surveillance system (CHSS) based on routinely collected census data (17,000 dairy herds). The aim of our study was to determine whether we could monitor the effect of changing AMU on udder health in Dutch dairy farms.

Material and methods: In total, six udder health indicators are monitored on a quarterly basis i.e. bulk milk somatic cell count (BMSCC), prevalence and incidence of high SCC cows (HSCC), herds with a >25% incidence of HSCC primi- and multiparous cows during the start of the lactation and herds with a >25% prevalence cows with a consistent HSCC before and after calving. The association between udder health key indicators and AMU was analysed using population average multivariable regression techniques with appropriate distributions and link functions. In addition, other variables that may be related to udder health were included in the models.

Results: With the implementation of general regulations aiming to decrease AMU, the Animal Defined Daily Dosage (DDDA) in dairy herds decreased from 5.78 in 2009 to 4.03 in 2013 (SDA, 2014). After implementation of SDCT, DDDA in dairy herds decreased further to 3.06 in 2017 (SDA, 2018). Our results showed that both implementation of regulations for AMU in general (2009) and implementing selective dry cow therapy rather than blanket treatment (2013), did not result in a deterioration in most udder health indicators (Fig. 1a). The only indicator that was somewhat negatively influenced by the implementation of SDCT was the percentage of herds with >25% new HSCC in multiparous cows during the start of lactation (Fig. 1b).

Even though most udder health parameters were not negatively influenced, the results of the multivariable models showed that not applying antimicrobials at all resulted in a higher BMSCC (+15.6 x10^3 cells/mL (95% CI: 15.1-16.1)), a higher incidence and prevalence of HSCC cows (OR=1.6) and a higher probability to have >25% cows with a new HSCC during the start of lactation.

Conclusion: The results indicate that even though AMU was decreased with 47% (from 5.78 DDDA in 2009 to 3.06 DDDA in 2017), a decrease in udder health indicators was not observed. Thus, application of SDCT did not seem to affect the general udder health in dairy herds. However, in dairy herds that did not use any antimicrobials the SCC of cows was significantly higher compared to the herds with an average AMU. In conclusion, it is advisable to closely monitor the effects on animal health when new policies to reduce AMU are implemented. The results provide confidence that AMU reducing measures lead to more prudent use of antimicrobials without jeopardizing animal health.

Keywords: Udder health, Antimicrobial use, Census Data, Selective Dry Cow Therapy.

UH-05

In vitro antimicrobial resistance of bacteria isolated from clinical mastitis cows in Germany

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Objectives: The goal of this study was to analyze the clinical mastitis pathogens resistance to routinely used antibiotics by analyzing samples collected across different regions in Germany.

Material and Methods: Quarter milk samples of cows with clinical mastitis were analyzed at a milk quality laboratory (bovicare GmbH, Germany). The samples were processed for bacteriology, SCC and resistance against the most frequently intramammary used antibiotics / antibiotic combinations: Amoxicillin and Clavulanic acid (AMC), Cefquinome (CEFQ), Cefoperazone (CFP), Cephapirin (CEPA), Cephalexin and Kanamycin (CKN) and Penicillin (P). The samples were submitted by 308 local veterinarians during September 2015 and February 2020. The median of samples per vet was 4. The samples were examined following NMC guidelines. Resistance was determined using agar diffusion method, evaluation of the inhibition-zone-results (S: susceptible, I: intermediate, R: resistant) was done using literature and companies' recommendations. The resistance results were statistically analyzed (IBM-SPSS; version 26.0) using Cochran-Q-test (S+I versus R) followed by Bonferroni-Dunn post hoc test. The Kruskall-Wallis-test with the Bonferroni-Dunn correction was used for analysis of the region´s effects. Differences consider statistically significant when P<0.05.

Results: 3,137 quarter bacteriological positive samples were tested for resistance. From East Germany were 488 (47%) collected, 853 (28%) from South and 796 (25%) from Northwest Germany. The most frequent pathogen was *S. uberis* (36.9%), followed by *S. aureus* (12.8%), *E. coli* (11.9%) and CNS (10.1%). This pathogen distribution is related in the literature. The results showed a significant difference of the in vitro susceptibility between the antibiotics tested (p=0.000). The resistance situation for the *S. uberis*-Isolates was: CKN^{a} (9%), $CEFQ^{ac}$ (7%), P^{bc} (6%), $CEPA^{bc}$ (6%), CFP^{d} (2%), AMC^{d} (1%). No differences across the regions were found. For *S. aureus* the distribution of resistant-isolates was: P^{a} (25%), CKN^{b} (7%), AMC^{b} (6%), $CEPA^{b}$ (5%), $CEFQ^{bc}$ (3%), CFP^{c} (2%). Regional differences were found only for P (less resistance in East

Germany). The frequency of resistant *E. coli*-isolates was: P[a] (100%), CEPA[b] (77%), CKN[c] (30%), CEFQ[d] (4%), CFP[d] (3%), AMC[d] (3%). Regional differences were found only for CNK (less resistance in Southern Germany). Considering all pathogens, the resistance situation was as follows: P[a] (32%), CEPA[b] (22%), CKN[c] (16%), CEFQ[d] (8%), CFP[e] (5%), AMC[e] (4%). There are differences between regions for P (more resistances in Northern) and CEPA (less resistances for the East Germany isolates).

Conclusion: Large number of isolates showed resistance against P, CEPA and CKN confirming the hypothesis that some gram positive and gram-negative mastitis isolates are not susceptible to often used antibiotics. AMC shows an activity against the most important gram-positive and gram-negative pathogens. AMC is an effective alternative to cephalosporines 3rd (CFP) / 4th generation (CEFQ). Similar results were published in Germany and other countries (Zieger et al. 2014, Leon et al. 2015, Leon et al. 2020, Bolte et al. 2020).

Keywords: Mastitis, resistance, pathogens, antibiotics, Germany.

UH-06

Analysis of udder health indexes of dairy operations from Galicia, Spain

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Objectives: The objective of this study was to process data from a dairy herd improvement (DHI) organisation of Galicia (Spain) to identify key risk factors of intramammary infection (IMI) and changes in these factors over a seven-year time series.

Material & Methods: Data were retrieved from the DHI organisation in Galicia (Federación Frisona Gallega, FEFRIGA) which monitors 2545 farms (July 2019). Base values of several udder health indexes (UHI) were estimated from this database. Then a sub-set of 254 farms was selected at random to establish individual time series for the UHI (01/2012 to 12/2019); this represents a total of 18563 control-days. Selected farms resorted to a consultancy service (veterinarian) to improve quality control. The Somatic Cell Count (SCC) decision-making threshold was 200 x103cells/ml (>200 x103=infected, ≤200 x103=healty). UHI considered in this study were:

- Prevalence
  - [PL1] PREVALENCE (first lactation cows: L1): L1 > 200 x103cells/mL in current control/total number L1 in current control
  - [PF] PREVALENCE (Flock level): Cows > 200 x103 cells/mL in current control/total number of cows in current control.
- Incidence rates
  - [IF] INCIDENCE (Flock level): cows previous control (C1) ≤ 200 x103 cells/mL and current control (C2)>200 x103 cells/mL /cows C1 ≤ 200 x103cells/mL and with presence in C2.
  - [IL1C1] INCIDENCE (First Lactation: L1) – First Control (C1): L1 C1 > 200 x103cells/mL current control /number of L1 C1 in current control.
  - [IDP] INCIDENCE (dry period): > 200 x103cells/mL first control after parturition with < 200 x103 cells/mL before dry period / < 200 x103cells/mL before dry period
- Cure rates
  - [CL] CURE (Lactation): number of cows with C1>200 x103 cells/mL and C2 (second control) ≤200 x103 cells/mL /number of cows C1>200 x103 cells/mL
  - [CDP] CURE DRY PERIOD (After dry period): number of cows C1 ≤200 x103 cells/mL and >200 x103cells/mL in previous control /number of cows C1, with >200 x103 cells/mL in previous control.
- **C**hronicity [CHR]
  - **C**ows >200 x103 cells/mL. in last two controls/Total infected in the previous month (>200 x103 cells/mL).

A selection of risk factors that can impact UHI were considered. These factors were:

- Average daily milk yield/cow/day (L/cow/day)
- Farm Size (<40, 40-65 or >65 heads)
- Bedding (organic/inorganic)
- Number of daily milkings/cow/day (2 or 3)
- Dry cow therapy (yes or no)
- Grazing (yes or no)
- Pre-dipping (yes or no)

Logistic regression was used as a statistical model for the analysis of factors to model the probability of a certain class. The autoregressive integrated moving average (ARIMA) analysis has been used for the study of the time series and to predict future points in the series (forecasting). ARIMA is actually a class of models that 'explains' a given time series based on its own past values, that is, its own lags and the lagged forecast errors, so that equation can be used to forecast future values. Data were processed with Statgraphics Centurion XVI software (Statgraphics Technologies, Inc., The Plains (VA), USA).

Results: The logistic regression enlightened the influence (either positive or negative) of every UHI (Table 1). Only significant associations (P<0.05) are presented.

The ARIMA analysis led to the conclusions that it does exist an over year tendency and a season effect for every single UHI with the exception of IDP.

Conclusions: Statistical studies on DHI data can verify the seasonability and trends in the evolution of data in time series. Also, this study of major factors that influence the udder health indexes can give guidance on the way to improve the udder health status.

Keywords: Udder health; cure rate; ARIMA.

Table 1. Significant (P<0.05) associations between risk factors and magnitude, and change (increase/decrease) in selected Udder Health Indexes (UHI).

Risk factors		**UHI**	
Item	**Magnitude**	**Decrease**	**Increase**
Milk yield	Higher production	PF, PL1, IF, IL1C1, IDP, CHR	CDP, CL
Farm size	Larger farms	CDP	PF, IL1C1, IDP
Bedding (small farms)	Inorganic	PF, PL1, IF, IDP	
Milking frequency	3x day	PF, PL1, CDP, CHR	IDP, CL
Dry cow therapy	No DCT	CDP, CL	PF, PL1, IF, IL1C1, CHR
Grazing	No grazing	PF, PL1	CDP
Pre-dipping	No teat dipping	CDP, CL, IL1C1, IDP	CHR

UH-07

Spread of livestock associated MRSA CC398 in German dairy herds

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Objectives: Livestock associated methicillin resistant Staphylococcus aureus (LA-MRSA) often belong to clonal complex (CC) 398 and may colonize and infect different animal species including humans (Cuny et al 2015). In both, animals and humans, LA-MRSA were rarely associated with clinical infections. In dairy cows, MRSA prevalence is low especially in comparison to pigs (Schnitt and Tenhagen 2019). However, LA-MRSA ST398 can cause mastitis in dairy cows (Falk 2018). Mastitis caused by MRSA is a problem in veterinary medicine, since most antibiotics approved for mastitis and dry cow therapy are β-lactams. Therefore, MRSA monitoring and prevention strategies are needed for dairy herds. This study aimed to determine the occurrence and spread of LA-MRSA on preselected German dairy farms.

Materials and methods: Based on previous phenotypic MRSA detection, 20 German dairy herds were selected and visited in 2018-19. Eight farms used automatic milking systems and 12 farms used milking parlours. From dairy cows, quarter milk samples (QMS) and bulk tank milk (BTM) was collected. Additionally, swab samples from calves, heifers, dust, teat liners, pigs, farm personnel and suckers from automatic calf feeders were analysed. In total, 3396 samples were screened for the presence of MRSA using a two-step selective enrichment protocol. All presumptive MRSA isolates were further analysed by MALDI-TOF, *mecA*/*mecC* PCR, *spa*-typing, SCC*mec*-typing and certain isolates were selected for next generation sequencing (NGS). A structured questionnaire and on farm observations were used to collect data on milking hygiene, biosecurity and general herd management.

Results: Prevalence: MRSA prevalence was 23.6% (47/199) in milk-fed calves, 13.6% (26/191) in samples from heifers, 8.6% (16/187) in post-weaning calves and 7.9% (47/595) of cows carried MRSA in QMS. In total, 68/2384 (2.9%) QMS were tested positive, indicating that multiple quarters were affected in some cows. As an indicator for mastitis, the average somatic cell count (geometric mean) was higher in QMS from MRSA affected quarters (357,000 cells/ml) in comparison to all quarters (114,000 cells/ml) (p<0.05). In BTM, MRSA was detected on 12 farms. In the environment, LA-MRSA was detected in dust, teat liners and automatic calf feeders. In samples from farm personnel, MRSA was detected on 4/7 farms.

Characterization of isolates: All MRSA isolates carried the *mecA* gene and belonged to CC398. Moreover, most isolates were identified as SCC*mec*-type V and *spa*-types t011 and t034. Results from NGS revealed additional genes that mediate resistance against aminoglycosides, macrolides, trimethoprim, tetracycline and lincosamides. Determination of virulence-associated genes in LA-MRSA isolates predicts low risk for human infections.

Potential risk factors: Improper milking hygiene procedures were observed on all farms with MRSA detection in milk (n=12). Two farms did not perform any udder cleaning and on one farm, one udder towel was used for all cows. Five MRSA-positive farms had no cluster disinfection and on one farm disinfection was only sporadically performed. Two farms did not do any post dipping and on three more farms post dipping was ineffective. Five MRSA affected farms kept both cattle and pigs at the same facility. However, MRSA was detected in only two pig barns and on one farm MRSA genotypes were different.

Conclusions: On 20 German dairy farms, the highest MRSA prevalence was detected in nasal swabs from young calves. Therefore, future MRSA monitoring programs should include samples from young stock. Improper milking hygiene as well as MRSA carrying humans and replacement heifers may enhance the spread of LA-MRSA CC398 within and between dairy herds. The role of pigs as a potential reservoir for LA-MRSA that may be transmitted to dairy cows needs to be further investigated.

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Keywords: Mastitis, antimicrobial resistance, MRSA, Staphylococcus aureus.

UH-08

Milk Somatic Cell Count in Buffaloes and its Relation to Intramammary Infections, Parity, and Stage of Lactation

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Objectives: Milk somatic cell count (SCC) is a key component of regulations for milk quality and is an indicator of udder health in dairy animals. The present study determines the physiological level of milk SCC in buffaloes and explores its relation to quarter infection, parity, and stage of lactation.

Materials and Methods: The study was conducted in two parts. The first part of the study determined the physiological levels and effect of quarter infection and parity on the milk SCC in buffaloes. In this, the quarter foremilk (QFM) and udder composite milk samples from ninety-one buffaloes of different parities were analyzed for bacteriology (Microbial Procedures of the National Mastitis Council) and SCC (using somatic cell counter SomaScope Smart, DELTA Instruments, The Netherland). The second part evaluated the effect of stage of lactation on the milk SCC. Here, 39 freshly calved buffaloes were enrolled and studied for quarter infection and milk SCC at four different stages of lactation viz., fresh calving (3-5 days of calving), early lactation (35-42 days), mid-lactation (120-135 days) and late lactation (210-225 days). The health status of quarters was defined as per International Dairy Federation criteria i.e. simultaneously considering the microbiology and SCC of QFM. The threshold value of QFM SCC was taken as ≤100×10^3 cells/ml for defining the healthy quarters The Data was stored in Microsoft Excel and analyzed by Chi-square test (Fisher's Exact Test), ANOVA with post HOC (Tuckey's method), Spearman's correlation, t-Test and Logistic regression with backward elimination using SAS version 9.3 (SAS Institute, Cary, USA). The level of significance was set at $p < 0.05$.

Results: The evaluation of quarter health in buffaloes under study revealed 7.99% specific subclinical mastitis, 11.57% nonspecific subclinical mastitis, and 7.71% latent infections. 72.73% of the quarters were found healthy. Animal wise, 20.88% of udders were positive for specific subclinical mastitis in at least one quarter. The average milk SCC in buffaloes was found 100×10^3 cells/ml (range 74-126) at the quarter level and 105×10^3 cells/ml (range 58-151) at udder level. The 77% of buffaloes had SCC ≤100×10^3 cells/ ml, with 7% showing >200×10^3 cells/ml. The milk SCC was found significantly ($p<0.05$) higher in the infected quarter (356×10^3 cells/ml) than in healthy quarters (52×10^3 cells/ml). The distribution of intramammary infections (IMI) to milk SCC showed 9.59%, 20.51% and 65.62% infections in quarters with QFM SCC of 0-100, 101-200 and > 200 ×10^3 cells/ml, respectively. The streptococci IMI resulted in highest milk SCC (mean 710×10^3 cells/ml) followed by *Staphylococcus aureus* (464×10^3 cells/ml) and coagulase-negative staphylococci (224×10^3 cells/ml) as compared to 52×10^3 cell/ml of healthy uninfected quarters ($p<0.01$). The *corynebacteria* infections initiated no significant reaction (mean SCC 54×10^3 cells/ml). The milk SCC increased with the advancing parity; average cell counts being 168×10^3 cells/ml for 3-4th parity buffaloes as compared to 84×10^3 cells/ml for 2nd parity and 48×10^3 cells/ml for 1st parity animals ($p<0.01$). The milk SCC showed a significant positive correlation of 0.30 with the 305-day lactation milk yield ($p< 0.01$). The evaluation of milk SCC *viz.-a-viz.* stage of lactation revealed the average milk SCC as 118×10^3 cells/ ml at calving, which decreased to 69×10^3 cells/ml at 35-42 d ($p<0.05$), and then increased to 80×10^3 cells/ml in mid- and 94×10^3 cells/m in late- lactation. The occurrence of quarter infections was minimum (10.25%) in freshly calved animals, which increased to 21.15% in early lactation, 22.91% in mid-lactation and 31.03% in late lactation (Chi-square = 58.00, df = 03, $p < 0.01$).

Conclusions: Assessment of present results in buffaloes in terms of our similar studies in HF×Sahiwal crossbred cows under parallel dairy management exposes that buffaloes relatively harbor fewer quarter infections and possess lower milk SCC. Thus, the physiological threshold of milk SCC in buffaloes probably may be defined at 100×10^3 cells/ml and considered pathological when >200×10^3 cells/ ml.

Keywords: Buffalo, Milk Somatic Cell Count, Intramammary Infection, Parity, Lactation.

UH-09

Selective dry cow therapy: the potential role of a novel biomarker panel

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Objectives: The aim of the study was to evaluate whether the combination of protein biomarkers will improve the diagnostic accuracy for selective dry cow therapy compared to somatic cell count (SCC) and California mastitis test (CMT).

Material and Methods: Two commercial dairy farms (900 and 600 milking cows respectively) were enrolled in the study. Quarters milk samples were collected aseptically from cows at the time of dry-off based on CMT results to enable a case-control study design. Each sample was tested for SCC, standard bacteriology combined with matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-ToF MS) and protein concentrations of five potential biomarkers*.

Standard bacteriology wwas performed using blood agar incubated at 37°C in aerobic conditions and examined after 24 hours. Plates without growth were considered negative for mastitis-associated pathogens (bacteria absent) and those with three or more morphotypes were considered contaminated and excluded from the analysis. Samples yielding one or two morphotypes were considered indicative of infection and isolates were submitted for species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). The results from the MALDI-ToF

MS were considered definitive.

The five biomarkers were combined in a classification tree model to predict bacteria presence. Its performance was compared to the SCC (≥ 200 000 cells/ml) and CMT results (> 0). Sensitivity (Se), specificity (Sp) and accuracy in distinguishing between bacteria presence and bacteria absent samples were calculated.

Results: Of 209 quarter milk samples, 14 (6.8%) were contaminated and excluded from the analysis. Of the remaining 195 samples, 108 (55%) had bacteria present and 87 (45%) did not.

The Sp of the biomarker classification tree model (63%; 95% confidence interval: 53%-73%) was significantly greater than of SCC (39%; 30%-50%) or CMT (25%; 17%-35%). The Se of the biomarker model (73%; 64%-81%) was similar to that of SCC (79%; 70%-85%) and significantly lower than for CMT (90%; 83%-94%). Accuracy of the biomarker model (69%; 62%-75%) was not significantly different compared to SCC (61%; 54%-68%) and CMT (61%; 54%-68%).

Conclusion: Selective dry cow therapy (DCT) is a treatment strategy designed to reduce antimicrobial usage, with selection largely based on SCC or clinical mastitis history, which are used as proxies for current infection status. There have been limited attempts to evaluate whether measuring inflammatory biomarkers in milk results in more accurate selection of infected cows for DCT.

We evaluated whether a combination of protein biomarkers in a classification tree model improved the diagnostic accuracy compared to SCC and CMT. The biomarker model has greater Sp at the cost of reduced Se.

Further investigations will show whether additional inflammatory biomarkers, used independently or in conjunction with other data such as SCC, could refine decision making at dry-off.

Keywords: Selective dry cow therapy, biomarkers, antimicrobial reduction.

UH-10

Diversity of selected staphylococci and streptococci isolated from milk samples in a dairy cow herd with low prevalences

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Background and objective: Most mastitis cases worldwide are caused by staphylococci and streptococci. Farm-specific strategies for the prevention and control of intramammary infections with these can be developed based on the results from the regular microbiological analysis of milk samples from clinical and subclinical mastitis cases. The dominant organisms on a farm determine if the focus should be on improving milking practices or the hygiene in the cows´ environment. This approach is based on the traditional categorization into contagious pathogens (e.g. *Staphylococcus (Staph.) aureus*) that are mainly transmitted during milking, and environmental pathogens (e.g. *Streptococcus* (*Strep*.) *uberis*) that have their reservoirs in the environment. The diversity of a bacterial species (i.e. number of different strains) in a herd is indicative of the sources of infections. If the diversity is low, a common source of infections is assumed (contagious transmission or an environmental hotspot). When the diversity is high, independent infection events are most probable. For some pathogens like *Strep*. *dysgalactiae,* it remains unclear if they spread rather contagiously or if cows become infected from environmental sources. Additionally, for some species (e.g. *Strep*. *uberis*) the traditional categorization has been challenged in recent decades. Furthermore, most studies have investigated the diversity of mastitis-causing pathogens in herds that are experiencing mastitis outbreaks or an unsatisfactory udder health situation. Therefore, the objective of the present study was to describe the diversity of selected staphylococci and streptococci isolated from milk samples in a herd with low prevalences.

Material and methods: From June to October 2020, one Swedish dairy cow herd was visited 10 times with 14-day intervals. At each visit, quarter foremilk samples from all lactating cows were collected aseptically according to DVG guidelines and transported immediately to the laboratory [1]. From each sample, 10µl were streaked onto esculin blood agar. Microbial growth was evaluated after 24 and 48 hours incubation at 37°C. Preliminary species identification was based on morphological and biochemical characteristics, and final species identification conducted by MALDI-TOF MS. For all isolates identified as *Staph*. *aureus*, *Staph. epidermidis*, *Strep*. *dysgalactiae* or *Strep. uberis*, strain typing based on RAPD-PCR was conducted. All isolates with the same banding pattern were considered to be the same RAPD-type. A quarter was considered infected if at least 100 colony-forming units per milliliter were detected and infections ended when at least two consecutive samples were negative for the respective strain type.

Results: In total, 8056 milk samples from 263 cows were collected. From 82 (1.0%), 91 (1.1%), 106 (1.3%) and 114 (1.4%) of all samples *Staph. aureus*, *Staph. epidermidis*, *Strep. dysgalactiae* and *Strep. uberis* were isolated, respectively. For *Staph*. *aureus,* five different RAPD-types were found. Of all *Staph. aureus* infections (n=48), 81% (n=39) were due to the same strain. All other strains caused maximum 8% of all *Staph*. *aureus* infections. For *Staph. epidermidis,* 45 different strains were isolated. None of these strains caused more than 5% of all infections (n=55). Seven different strains of *Strep. dysgalactiae* were isolated. One of these caused 50% (n=18) of all (n=36) observed infections. Two other strains were isolated from 19% (n=7) and 11% (n=4) of the *Strep*. *dysgalactiae* infections. All other strains caused maximum 8% of all *Strep*. *dysgalactiae* infections. The *Strep. uberis* isolates belonged to 18 different RAPD-types. 24% (n=10) of all *Strep. uberis* infections were caused by the same RAPD-type and another 15% (n=6) and 12% (n=5) were caused by two other RAPD-types. All other *Strep*. *uberis* strains caused less than 10% of all infections.

Conclusions: For *Staph*. *aureus*, *Strep*. *dysgalactiae* and *Strep*. *uberis*, dominating strains were isolated. This confirms the contagious nature of *Staph. aureus* even in this herd with a low occurrence and indicates that also *Strep*. *uberis* and *Strep*.

dysgalactiae infections can spread mainly contagiously or via environmental hotspots. In contrast, no dominating strain was found for *Staph*. *epidermidis*. Therefore, most infections with *Staph. epidermidis* seemed to have occurred due to independent infection events.

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Keywords: bovine mastitis, strain diversity, Staphylococcus spp., Streptococcus spp., mastitis pathogen categorization.

UH-12

Applying internal teat sealants at drying off; does full versus partial insertion of the tube cannula matter?

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Objectives: Internal teat sealant (ITS) and intramammary antibiotic application tubes can often be used with either a short or a long insertion cannula. Leelahapongsathon et al (2016) found that intramammary infection in early postpartum was significantly associated with full cannula insertion for the administration of antibiotic dry cow therapy (ADCT). There is a lack of published work on the effect of using full (FI) or partial (PI) cannula insertion for the administration of ITS. Our randomised control trial aimed to test the hypothesis that FI could increase the risk of introducing new infections into the udder leading to higher somatic cell counts (SCC) post-calving and a greater incidence of mastitis post calving comparing to PI.

Materials and methods: Three pedigree Holstein UK dairy farms were selected to take part in the study over a period of six months. The farmers selected which cows were to be dried off each week and dictated whether each cow would receive internal teat sealant only (ITS, Ubroseal® Boehringer Ingelheim Animal Health UK Ltd) or intramammary antibiotic and internal teat sealant (AB+ITS). Cows were then randomised to receive ITS or AB+ITS via either FI or PI of the cannula/e. The facilitator was blinded to the insertion type until the cow was enrolled. One farm opted to only allow enrolment of cows receiving ITS as the antibiotic tubes used at dry off did not have the option of partial cannula insertion. The facilitator was trained in best practise aseptic technique for drying-off cows by three different experts in the field. All farms milk recorded monthly and the SCC data collected was collated along with incidence of mastitis within 30 days of calving. Cure rates (cows with SCC>200K cells/ml before drying off having a first test of SCC<200k cells/ ml after calving) and new infection rates (cows with SCC<200K cells/ml before drying off having a first test of SCC>200k cells/ ml after calving) were calculated from these data. Univariable and multivariable regression analyses were employed for data analysis.

Results: 287 cows were included in the study, 47% of the cows received full insertion of the cannula/e (n = 135), 30% of the cows received AB+ITS as allocated by the farmers (n = 86). There was no evidence to allow us to reject the null hypothesis; there was no difference in post-calving SCC, new infection rates, cure rates, or mastitis incidence when comparing FI versus PI. With regards to cows with low SCC before drying off, cows receiving PI were 1.01 times as likely to have high SCC post calving as cows receiving FI (95% confidence interval (CI): 0.42 to 2.46, P = 0.98). Cows in their second or greater lactation and cows calving in the Spring or Summer were more likely to acquire a new infection compared to cows in their first lactation and cows calving in the Autumn respectively. Factors associated with a high SCC post calving were: calving season, infection status before drying off, and lactation group; treatment (PI vs FI) was not statistically significantly associated with this outcome either. PI versus FI was also not associated with the cure rate post calving (cows receiving PI were 1.45 times as likely to have low SCC post calving as cows receiving FI; 95% CI: 0.30 to 7.06, P = 0.65). Cows in their first lactation were 9.86 times more likely to cure an infection comparing to older cows (95% CI: 0.83 to 117.62, P = 0.07). Cows in their second or greater lactation were 5.23 times more likely to be diagnosed with clinical mastitis the first month after calving comparing to cows in their first lactation (95% CI: 1.34 to 20.31, P = 0.02). Treatment (PI vs FI) was not associated with mastitis incidence.

Conclusions: In conclusion this study showed that when the correct aseptic technique is used for drying cows off there is no difference in post-calving infection status or mastitis incidence when comparing FI versus PI.

Acknowledgements: This study was funded by Boehringer Ingelheim Animal Health UK Ltd. The funder was involved in the study design but not in data collection and analysis.

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Keywords: Udder health, drying off, internal teat sealants, mastitis, somatic cell count.

UH-13

Risk of clinical or subclinical mastitis following infusion of an internal teat sealant alone at the end of lactation in low somatic cell count cows

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Objectives: To identify risk factors for subclinical and clinical mastitis in dairy cows in the lactation following treatment with a bismuth subnitrate internal teat sealant (ITS) alone.

Methods: Cows (n=1,614 in total) from 36 herds were selected at random from within each herd from those cows with a maximum herd test somatic cell count (SCC) of <250,000 cells/mL and having had no history of clinical mastitis in the current lactation. In the last week of lactation, cows were assessed for the presence of teat end hyperkeratosis, milk samples were collected from each quarter following aseptic teat end preparation for microbiology, and a tube of ITS was infused into each quarter after the last milking. Herd owners (n=22) monitored for clinical mastitis in the first 60 days of the subsequent lactation. Herd-level risk factors including average bulk milk SCC in the last month of lactation, lactational cow-case clinical mastitis incidence, and cow-level risk factors including age, breed, presence of a major pathogen intramammary infection, milk yield at the last herd test of the lactation, and maximum SCC across lactation were considered as potential risk factors in multilevel binary logistic regression analyses.

Results: A total of 8.9 (95%CI=7.5-10.4) % of cows had subclinical mastitis as defined as a SCC >200,000 cells/mL at the first herd test in the subsequent lactation. Animals with a major pathogen infection at dry-off were more likely to have subclinical mastitis in the next lactation than those without a major pathogen (OR=4.7 (95%CI=2.29-9.65; p<0.001), animals >3-years-old were more like have a subclinical mastitis than 3-years-olds (OR=3.16 (95%CI=1.70-5.88), p<0.001), there was a positive association between cow-level milk yield at the last herd test of lactation and subclinical mastitis (OR=1.07 (95% CI=1.01-1.13), p=0.02), and there was a positive association between the natural log of the maximum SCC in the preceding lactation and subclinical mastitis in the next lactation (OR=1.54 (95%CI=1.13-2.10), p=0.01). The overall clinical mastitis incidence was 3.1 (95%CI=2.1-4.4)% in the first 60 days after calving in the next lactation. Cows from herds with a lactational cow-case incidence of >0.24 were at higher risk of clinical mastitis than cows from herds with a lactational cow-case incidence of <0.158, with cows from herds with cow case-clinical mastitis incidence intermediate not being different from these two categories. Cows yielding >15 L/day at the last herd test were more likely to be diagnosed with clinical mastitis than cows yielding <10 L/day, or 12.1 to 15 L/day, with those cows yielding 10 to 12 L/day intermediate between these categories. There was a positive linear association between the log maximum SCC in the preceding lactation and the risk of clinical mastitis (OR=1.96 (95%CI=1.09-3.54), p=0.03).

Conclusions: Both herd-and cow-level risk factors were identified for subclinical and clinical mastitis in the lactation following the infusion of an ITS. Hence cow selection criteria for infusion of ITS should consider herd level cow-case clinical mastitis incidence, cow-level milk yield at last herd test, as well as individual cow SCC.

Keywords: Internal teat sealant, mastitis, risk factors.

UH-14

Impact of clinical mastitis on the reproductive performance of eight Spanish dairy herds

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Objective: The objective of this study was to determine if cows experiencing clinical mastitis within the period calving-conception, had worse reproductive performance than cows with no clinical mastitis during this period.

Material and Methods: Data from dry off and pregnant cows were collected in eight commercial Spanish dairy herds from September-18 to January-20. Data collected for every animal was lactation number, calving date, conception date, mastitis occurrence and mastitis date, days in milk to first clinical mastitis case and number of inseminations per pregnancy. A total of 4763 animals were analyzed, 443 cows with mastitis before conception and 4320 with no clinical mastitis before conception as a control group.

Calving-conception interval (CCI) and number of inseminations (NI) per pregnancy were used as indicators to compare reproductive performance between the two groups.

The association between the presence of mastitis with continuous non-normally distributed variables (CCI and NI) was analyzed using a non-parametric test (Wilcoxon test) using SAS V.9.1.3 (SAS institute Inc., Cary, NC).

Results: The average calving conception interval was 39.7 days longer for cows suffering mastitis ($\geq$1 cases) before conception than non-affected ones (P<0.001).

Cows suffering at least one clinical mastitis case before conception need on average 1.1 more inseminations to get pregnant than cows with no clinical mastitis before conception (P<0.001).

Conclusions: As shown in previous scientific studies, results are indicating a strong association between the occurrence of clinical mastitis before conception and the overall reproductive performance in Spanish commercial dairy herds.

Keywords: Mastitis, reproduction.

UH-15

Study of the effects of the administration of a phytogenic core on the decrease of milk production at dry-off in dairy cows

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Objective: Drying off, defined as the cessation of milking at the end of lactation in dairy cows, is an essential step allowing mammary involution, renewal of mammary epithelial cells and even the treatment of persistent intramammary infections.

However, it can lead to complications such as mammary edema, source of pain and discomfort, and milk losses, which can lead to mastitis. One of the main risk factors for such complications is milk yield at drying-off. Reducing milk yield quickly should prevent these complications and improve welfare, especially for cows still producing more than 20 kg of milk at drying off.

Materials and methods: The study, approved by of an ethical committee, consisted of a randomized double-blind, multicenter trial to evaluate the effectiveness of a phytogenic complementary feed on the reduction of milk yield in cows during the two days following drying off, compared to a placebo. Based on the hypothesis of a 50% reduction in milk loss, the most visible factor for a farmer, for a statistical power of 90%, with a precision of 5%, one hundred and six Prim'Holstein cows producing an average of 21.9 kg of milk on the day of drying off, located in 4 farms in Sarthe (France), were randomly allocated into 2 groups. Cows in group A received orally after their last milking 100 mL of a placebo while cows in group B received 100 mL of phytogenic extracts composed of artichoke (Cynara scolimus) and chaste tree (Vitex agnus-castus) (Parlac®, Biodevas Laboratoires).

Milk yield was evaluated indirectly before the last milking (D0), then 24 (D1) and 48h (D2) afterwards by two parameters: the mechanical nociceptive threshold measured with an algometer reflecting the mammary engorgement and the morphology of the udder, evaluated by the distance between the teats. Milk loss and rumination (through rumination collar system) time were also described and compared. The statistical unit was the cow. A Student's t test was performed on nociceptive threshold and rumination time, a mixed model with random effect was performed on teat distance and a Chi² test was performed on the percentage of cows with milk loss.

Results: At D0, no difference was found in the characteristics of the animals of the 2 batches, confirming the randomization as effective. At D2, the nociceptive threshold was not significantly different between the 2 groups. At D1, cows in group B tended to have a lower rear teat distance than those in group A (50.56 mm vs. 60.67, p-value = 0.1). The variation in rear teat distance was +18.34 mm for cows in group A and +12.20 mm for those in group B (p-value = 0.071). The total distance between all teats was significantly lower for group B (403 mm vs. 445 mm for group A, p-value = 0.035) reflecting less mammary engorgement. The percentage of cows with milk loss was significantly lower for group B (23% vs. 47% for group A, p-value = 0.017). In both groups, rumination time decreased, but less for cows in group B compared to group A, with -18 min and -52 min of decrease respectively, p-value = 0.06.

Conclusion: This study showed that PARLAC® reduces milk loss in the 2 days following drying off, reducing then mammary engorgement, and improving the well-being of dairy cows (rumination less affected). It provides a natural solution for farmers who implement a sudden drying off and wish to reduce the milk production of high-yielding cows to limit the risk of complications.

Keywords: Dry-off, phytogenics, dairy, bovine.

UH-16

MALDI-TOF Bacterial Subtyping for the Rapid Detection of Methicillin-resistant *Staphylococcus aureus* in Milk Bacteriology

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Objectives: *Staphylococcus aureus* is one of the main pathogens involved in the development of contagious bovine mastitis and its presence is usually more associated with cases of subclinical than clinical mastitis. This pathogen shows low recovery rates, also due to antimicrobial resistance. A high priority has been given to the spread of methicillin-resistant *S. aureus* (MRSA), as it can have important implications for animal but also human health. Most of the MRSA strains present the *mecA* gene, held in a staphylococcal cassette chromosome (SCC*mec*) and acquired by horizontal gene transfer. The *mecA* gene is responsible for the synthesis of an alternative penicillin-binding protein (PBP2A), which is characterised by a low affinity for beta-lactam antibiotics, but also associated with a lower affinity to other antimicrobial classes. MRSA isolates are defined as multidrug-resistant. Recently, matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has become the reference method for the identification of microbial species and can also be applied for the detection of antibiotic resistance. The aim of this study was to assess the performance of the Bruker MALDI Biotyper (MBT) system's subtyping module for the rapid identification of MRSA isolated from milk. After the species identification of *S. aureus*, the subtyping module identifies MRSA by detecting a peak at 2413 ± 2 m/z, which corresponds to PSM-mec, a small phenol-soluble modulin that is encoded in the SCC*mec* locus. We compared phenotypic and genotypic analyses of *S. aureus* strains with results obtained with the MBT subtyping module to establish the diagnostic accuracy of this method in routine practice.

Materials and methods: A retrospective study was carried out on 63 isolates of *S. aureus* from 27 different farms, collected during the diagnostic activity in the microbiology laboratory of the Department of Veterinary Medicine at the University of Milan. These isolates were previously classified as phenotypically resistant to methicillin based on susceptibility to oxacillin, a specific active substance routinely used for screening beta-lactam antibiotic resistance in *S. aureus* isolates. *S. aureus* strains were genotypically investigated for the presence of *nuc* and *mecA* genes by PCR. Species-level identification of *S. aureus* was confirmed by MALDI-TOF MS with the MBT system using the direct transfer method; a log (score) ≥ 2.0 is the threshold for species-level identification. All identified *S. aureus* isolates with a score ≥ 2.0 were immediately processed by the subtyping module of the MBT Compass software to detect a specific peak at 2413 ± 2 m/z corresponding to the PSM-mec.

Results: Genotypic analysis of 63 *S. aureus* isolates showed that all were positive for *nuc*, which is recognised as highly specific for *S. aureus*. Phenotypic resistance to oxacillin was confirmed by *mecA* gene carriage. All the 63 original

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isolates were identified as *S. aureus* with a score ≥ 2.0 and therefore processed with the subtyping module, but only 11 strains were subtyped as 'presumptive PSM-positive MRSA'. The MBT detected only 17.5% of all MRSA identified by standard susceptibility tests and molecular analysis. The sensitivity reported was in agreement with previous results. In particular, the PSM-mec peptide detected by MALDI is only present in the SCC*mec* II, III and VIII cassette types.

Conclusion: MALDI-TOF MS is a rapid and accurate technique for bacterial identification, comparing to laborious and time-consuming biochemical tests and reliable but expensive PCR-based methods. On the other hand, its application to detect methicillin resistance in bovine *S. aureus* isolates is affected by limited sensitivity. However, it can be a valuable warning tool for the presence of MRSA in the herd, as this information is provided by the MBT subtyping module simultaneously with the identification of *S. aureus* without requiring further user intervention, sample processing, or additional materials. Future studies will be required to assess its specificity.

Keywords: Mastitis, Staphylococcus aureus, MRSA, MALDI-TOF.

UH-17

Test characteristics of differential cell counts to identify subclinical intramammary infections estimated by Bayesian latent class analysis

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Objectives: Prudent use of antibiotics requires that the treatment of subclinical mastitis is based on the treatment of cows with a confirmed intramammary infection (IMI). Because subclinical mastitis is defined as an infection in absence of visible changes in milk or udder appearance, the use of a diagnostic test is needed. The three most commonly used tests are: 1) somatic cell count (SCC), 2) bacteriological culture (BC) and 3) polymerase chain reaction (PCR). All these tests are imperfect, and lack either sensitivity (BC) or specificity (PCR) or both (SCC). In this study, we investigate the performance of VETSCAN DC-Q™, an automated differential leukocyte device, which uses the different leukocyte counts in combination with the total SCC to diagnose IMI. A model that accounts for classification error is the latent class model, which allows estimation of the sensitivity (Se) and specificity (Sp) of different tests assuming no gold standard test.

The objective of our study is to determine the test characteristics of the VETSCAN DC-Q™ to detect quarter level IMI in combination with quarter level SCC (QSCC) at a threshold of 100.000 cells/mL, BC and PCR in cows with a new elevated cow level SCC just after calving (≤ 30 days), early lactation (> 30 days) and in cows with an elevated SCC in the last 4 weeks before drying off.

Material & Methods: The VETSCAN DC-Q™ has three different settings based on DIM of the animals to be tested. To validate the three different settings of the VETSCAN DC-Q™ a total of 449 cows, i.e. 1796 milk samples on quarter level, were collected on 8 Dutch dairy herds. Inclusion criteria were a 4-weekly DHI- participation and high new IMI rate. Cows were selected on first elevated cow level SCC (≥ 200.000 cells/mL) in current lactation or with elevated SCC within 4 weeks before dry off. Quarter samples were taken aseptically before cluster attachment, within one week after DHI sampling. Within 6 hours, these samples were split and QSCC (Delaval DCC) and VETSCAN DC-Q™ test were performed, BC and PCR samples were stored at -18°C and these tests were later performed at two different laboratories. Bayesian latent class models were built to determine Se and Sp of the 4 tests, assuming no gold standard test.

Results: Results of BC and PCR were classified as minor or major pathogens. Of the 1794 samples analysed 23% tested positive by BC, 42% tested positive by QSCC at a threshold of 100.000 cells/mL (QSCC>100), 8% was positive for a major pathogen in PCR, 68% was positive for a major and/or a minor pathogen in PCR, and 41% tested positive by VETSCAN DC-Q™. The most commonly isolated pathogens by BC were *Streptococcus uberis* (8% of all samples) and *Coagulase negative Staphylococcus spp.* (6%).

Results from the latent class analysis model that estimated Se and Sp of identifying IMI caused by major or minor pathogens showed VETSCAN DC-Q™to have a Se varying from 0.64-0.90 and a Sp of 0.63-0.86 for the three different lactation periods. These estimates were almost similar to the test characteristics of standard QSCC>100. Overall test characteristics were substantially better than those from BC, which had very low Se (0.37) and PCR, which lacked Sp (0.51). For diagnosing IMI caused only by major pathogens, the Se of the VETSCAN DC-Q™ varied between 0.90-0.98, and Sp for “Early Lactation” and “30+ DIM” was 0.71 and 0.76, respectively, but Sp dropped to 0.27 in the category “Dry Off”.

Conclusion: Altogether, our results suggest that the VETSCAN DC-Q™ has good test characteristics for diagnosing intramammary infections, but the added value over an undifferentiated QSCC measurement remains to be demonstrated.

Usability of the VETSCAN DC-Q™ will depend on the goal of the test. For screening cows with new IMI in early lactation in order to treat infections before they become clinical or chronic, it is a useful and convenient tool, with fair to near excellent Se and Sp at a quarter-level. For selecting cows at the end of lactation for selective dry cow therapy (SDCT) based on the presence of only major pathogens, the results of the VETSCAN DC-Q™ might lead to a higher use of antibiotics than necessary, because of the low Sp.

Keywords: Differential cell count; dairy; subclinical mastitis; Bayesian latent class analysis.

UH-18

CellCheck Dry Cow Consults-supporting the adoption of selective dry cow therapy in Ireland

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Objectives: While blanket dry cow therapy has been commonplace in Ireland for many years, the recent European Veterinary Medicines Regulation (2019/6) means that preventive antibiotic usage in groups of animals, including dairy cows at the end of their lactation, is no longer acceptable. Hence, adopting a selective approach to dry cow treatment will require both a change in mindset and practice, for many Irish farmers and their prescribing veterinary practitioners. To facilitate this, CellCheck, the Irish national mastitis control programme, developed a Dry Cow Consult (DCC). The objective of this DCC was to enable farmers to engage with their nominated vet, to develop farm-specific selective dry cow treatment (SDCT) plans, where appropriate.

Materials and methods: DCCs have been delivered as part of the Targeted Advisory Service on Animal Health (TASAH) funded through the Rural Development Plan 2014-2022. Veterinary practitioners must first complete relevant training, delivered through the CellCheck programme, to be eligible to deliver a consult. To be considered eligible for the free service a dairy farmer must meet the following criteria:

- Average bulk milk tank somatic cell count (SCC) for the previous 12 months <200,000 cells/mL.
- At least 4 whole herd milk recordings in the previous 12 months.
- Members of HerdPlus, which is the Irish Cattle Breeding Federation's (ICBF) information service, providing herd-owners with performance data.

The purpose of the eligibility criteria was to identify farms that have some evidence of good mastitis control, as well as the necessary information and records to support decision-making and planning. During the three-hour consult, milk recording results and farm records are reviewed, as well as current practices when drying off cows and dry cow management, to help identify and resolve any potential risks associated with a selective dry cow strategy. Animals potentially suitable for receiving internal teat sealant only at drying off are identified. Participants of a DCC were invited to register for a Dry Cow Review the following year. Since 2018, 933 Consults and 268 Reviews have been completed.

Whole herd milk recording results, for the last recording before drying off and the first recording of the following lactation, were available for participating herds. Treatment records (both dry cow and in-lactation) were available, although were not complete for all participating herds. Telephone interviews were carried out with the participants of the consult service in 2018 (n=19). Audio recordings were professionally transcribed verbatim and analysed qualitatively using an inductive thematic analysis.

Results: Quantitative analysis showed that, although there was a slight increase in early lactation SCC in the group of cows treated exclusively with teat sealer at drying off, average SCC levels remained low (<200,000 cells/ml) and similar to those of cows treated with intramammary antibiotics at drying off. The CellCheck-recommended selection criteria for administering teat seal only at drying off were not always followed, with some farmers still including a small number of cows with a history of high SCC (>200,000 cells/ml) in this group. Results also showed that, while a considerable number of animals from each herd were eligible for SDCT, farmers were still reluctant to fully engage in this practice and maintained a cautious attitude.

The qualitative analysis identified 6 barriers and 6 facilitators to implementing SDCT. Barriers to SDCT included a significant fear of a resulting rise in mastitis incidence, infrastructural limitations, a perceived lack of availability of preventative advice, as well as peer influence. Facilitators to implementing SDCT included adopting a gradual approach to SDCT, regulatory pressure, high standards of farm hygiene, education and the use of targeted veterinary consults. Despite cited negative influences, peer influence can be utilised to encourage the farming community to change this particular behaviour.

Conclusions: SDCT has proven to be effective when used in cows with an adequate udder health status. While selective dry cow therapy is now a requirement under current Veterinary Medicines Regulations, for most dairy farmers in Ireland it will require a change in mindset and practice. Educating farmers on good drying-off routines is essential in order to build confidence and create awareness about the safety of moving to SDCT. Though there are challenges to face, engagement with professional support will be important and can be successfully facilitated through structures such as the Dry Cow Consult.

Keywords: Drying off, selective dry cow therapy, behaviour change.

UH-20

Gene expression of the teat canal epithelium in dry and lactating cows

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Objectives: A keratin plug is formed in the teat canal during the dry period and protects the mammary gland against infections. However, mastitis incidence during the early dry period is high. Information about the variation of gene expression in the teat canal epithelium could provide insights into the physiology of the keratin plug formation during the early dry period.

The objective of this study was to identify differences in

gene expression in teat canal biopsy samples obtained at different time points during the early dry period and early lactation.

Materials and methods: In a first study (Experiment 1), the gene expression of teat canal samples from five cows in late lactation to early dry period were analysed. Samples from Day 0 (dry off day) and Day 11 of the dry period were compared using RNA-Seq. Genes differentially expressed between Day 0 and Day 11 samples were selected for further analysis. The RNA-Seq results were used to select 42 relevant genes including nine keratin genes of interest that were analysed for variation of gene expression in Experiment 2 by Nanostring nCounter. Repeated teat canal biopsy samples obtained from eight cows on days 0, 7, and 21 of the dry period, and on approximately days 10 and 25 after calving (Day 70 and 90) were also analysed. Oral mucosa samples were taken at the same time-points to serve as controls.

Results: Experiment 1 identified 36 differentially expressed genes of the teat canal epithelium at dry off (Day 0) and Day 11. Twenty-two of the genes were downregulated. Gene ontology enrichment analyses revealed that these genes were mainly related to binding, adherence, and RNA transcription factors. Analysis of individual gene functions showed that they were genes involved in mitosis and immune response pathways. Results from Experiment 2 showed different patterns of expression of the genes between the teat canal and the oral tissue (control) over time. Genes NR4R, KRT17, CSRP2, KIF23, TRPS1, LOC107131159, and TFPI2 were downregulated 7 days after drying off whereas RBBP8NL, PKIB, MARKS, TLR5, BAZ1A, IRX3, KRT4, PADI1, PENK, RND3, SERPINA1, SYT17 only presented differences after calving ($p<0.05$) in the teat tissue. The expression levels of the genes in a comparison of the results of both methods (Illumina and Nanostring) coincided, suggesting a decrease in gene expression after drying off in the teat canal related to cellular functions such as binding, adhesion, mitosis and immune response.

Conclusion: Modern gene expression analysis of the experiments reported in this study suggests that cell proliferation and immune response of epithelial tissue in the teat canal decrease during the early dry period and there is a major reactivation of gene expression after calving. The pathways affected might contribute to the higher incidence of mastitis during the early dry period. To our knowledge, this is the first study to investigate changes in gene expression of the teat canal epithelium during the early dry and lactating period. Further detailed studies on the physiology of the teat canal and keratin plug formation would be required for developing strategies to improve the immune response of cows against mastitis in the early dry period with the aim of decreasing the incidence of mammary infections.

Keywords: Keratin plug, dry off period, gene expression, teat canal.

UH-21

Early detection of mastitits using ultrasonographic teat and udder tissue changes and measuring echogenicity using an innovative method

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Objective: Traditionally, the echogenicity of a tissue is expressed as anechoic, hypoechoic isoechoic and hyperechoic. However, this method is very subjective. So, a new method was devised to assess the level of echogenicity of a tissue with the help of software ImageJ. As mastitis is of high economic importance early diagnosis is necessary and USG can help in early diagnosis by measuring the changes in teat and udder tissue. So, the experiment was done to measure echogenicity of infected udder tissue and to evaluate the ultrasonographic changes due to intra-mammary infection of dairy cows.

Materials and Methods: Ultrasonography was conducted just before milking and quarter health status was determined based on milk SCC and bacteriological findings to determine the udder and teat tissue changes with respect to quarter health status. The ultrasound scans of teat structure were performed with a portable ultrasound scanning system (Sonosite M-Turbo) using a 10-5 MHz linear transducer by water bath technique or cup method. The teat tissue measurements were later done using the software ImageJ. The USG picture of udder tissue captured in RGB format was converted into greyscale (8-bit) format by using the same software. And in greyscale there are 256 shades ranging from pure black (0) to pure white (255). Division was done into 8 Grades(Grade1-8) , so that grade 1 has pixels ranging from 0 to 31, grade 2 had pixels ranging from shade 32-63 and so on till grade had shade 224 to 255.The software was used for the first time for the measurement of echogenicity.

A total of 32 apparently healthy lactating HF crossbred dairy cows, having 121 functional teats, were ultrasonographically scanned at university dairy farm. Quarter foremilk samples were aseptically collected and immediately transferred to the laboratory. Ultrasonographic readings such as teat canal length (TCL), teat cistern diameter (CD), teat wall thickness (TWT), overall teat diameter (OTD), the teat diameter at the level of the Furstenberg rosette (FTD) and associated changes in udder were recorded. The isolation and identification of microbial organisms from milk samples was done as per standard microbial procedures of National Mastitis Council (Brown *et al* 1969). Bromothymol blue (BTB) paper test, California Mastitis Test (CMT), electrical conductivity and pH of milk were performed. The analysis of milk samples for somatic cell count (SCC) (Delta Instruments, The Netherlands) was also done.

Results: Upon comparison of teat tissue measurements in relation to quarter health status, the TCL and CD were observed to be significantly increased in specific mastitis quarters compared to that of healthy ones. While TWT increased in quarters with latent infection and specific mastitis compared to healthy ones. The BTB score was significantly different in both non-specific mastitis and specific mastitis quarters, when

compared to both healthy and latent infection quarters. Similar observations were noted with CMT score, EC, pH and Log_{10}SCC values. The TWT was found to be significantly more in quarters with SCC 5-10 lac cells/ml of milk and in quarters with SCC > 10 lac, when compared with quarters having <2 lac and SCC 2-5 lac /ml of milk. The TCL, TWT and FTD were significantly more and CD less in infected teats in comparison to non-infected ones. Log_{10}SCC and CMT were significantly positively correlated with TWT and negatively with CD. During the measurement of echogenicity it was noted that pixels of higher grades (more echogenicity) i.e. G7 and G8 were absent from healthy quarters. And mean Log_{10}G1 was significantly lesser and Log_{10}G3 significantly higher for quarters with specific SCM thus suggesting that the amount of normal tissue (less echogenic) reduced due to infection and the echogenicity was also increased. The amount of pixels in G5 was significantly lesser in healthy and specific mastitic quarters as compared to quarters with latent infection.

Conclusion: It can be concluded from the results that **t**eat tissue changes can be detected in sub-clinically affected teats with the help of ultrasonography and Echographic measurements of teat were well correlated with milk inflammatory parameters so, they can be taken into consideration while assessing for infection. Grading, as indicated by our findings, can be used to measure echogenicity. The data is small, however with further valuation this technique can be standardized for better accuracy.

Keywords: Ultrasonography, teat, udder, tissue changes, echogenicity.

UH-22

The potential of intramammary administered cephapirin and cephalonium to select for ESBL-producing *E. coli* in the bovine gut and in dairy manure

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Objectives: Selection and spread of Extended Spectrum Beta-lactamase (ESBL)-producing Enterobacteriaceae within animal production systems and potential spill over to humans is a major concern. Orally applied cephalosporins can select for antimicrobial resistant organisms in the gastrointestinal tract. There is however limited research on the effect of non-oral, locally applied, antimicrobials on the selection of resistant organisms in the gastrointestinal tract. First generation cephalosporins are widely used to treat and prevent intramammary infections in dairy cows. We studied the potential effects of low doses of cephapirin (CP) and cephalonium (CL) to select for ESBL producing *E. coli* in fresh faecal fermentations and in the manure of adult dairy cows as a model for intramammary application of cephapirin, because the minimal concentration which still selects for resistance (MSC) is unknown in a complex environment like the gastrointestinal tract.

Materials and Methods: A literature search has been conducted, to determine the test conditions for the MSC testing and to predict the maximum expected concentrations of CP in the intestinal content and in the manure of dairy cows after intramammary treatment.

Three different laboratory experiments have been conducted on samples from The Netherlands, Germany, Belgium and the United Kingdom.

1): Competition assay in rich culture media at 37^{0}C. From every country, 3 ESBL and 3 non-ESBL *E. coli* isolates from dairy farms have been collected. A 1:3 ratio mixture of these ESBL and non-ESBL isolates under nine different conditions have been tested for their potential to select for Cefotaxime (CTX) resistance as an indicator for ESBL selection: no antibiotics baseline; no antibiotics (blank condition); 0.25 µg/ml CTX; 0.04, 0.4 and 4.0 µg/ml of CL; 0.08, 0.8 and 8.0 µg/ml of CP. After 6 h of incubation the percentage of resistant colonies to CTX in each of the different test conditions have been assessed to determine the MSC of CP and CL for ESBL producing *E. coli*.

2): Competition assay in fecal fermentations at 37^{0}C. Freshly derived manure samples from 10 different healthy dairy cows were pooled and were spiked with the 3 ESBL and 3 non-ESBL *E. coli* isolates at 10^7 CFU/ml in 9 different tubes containing anaerobic standard ileal efflux medium. A comparable approach as in experiment 1 has been followed to determine the MSCs for ESBL producing *E. coli* in freshly derived fecal fermentations of dairy cows.

3): Competition assay in dairy manure. Manure pit samples from each country where taken. The same procedures were being followed as in experiment 2, however at a temperature of 17^{0}C.

Results: In experiment 1, a significant (p=0.007) increase in CTX resistant colonies was seen between a 0.8 µg/ml (45% resistant) and a 8.0 µg/ml (96% resistant) concentration of CP. No significant increase from 0.08 (38%) to 0.8 µg/ml was observed. A significant (p=0.023) increase in CTX resistant colonies was seen between a 0.4 µg/ml (41% resistant) and a 4.0 µg/ml (90% resistant) concentration of cephalonium.

In experiment 2, a borderline significant (p=0.079) increase in CTX resistant colonies was seen between a 0.8 µg/ml (44% resistant) and a 8.0 µg/ml (75% resistant) concentration of CP. The results were skewed by the unexpectedly low number of CTX resistant colonies from the Belgian samples. In the experiment with different concentrations of CL (0.04, 0.4 and 4.0 µg/ml), no significant differences in CTX resistant colonies was observed (respectively 78%, 88% and 91%).

In experiment 3, no increases in CTX resistant colonies have been observed in the CP and CL group at each of the tested concentrations.

Based on available literature on pharmacodynamics and pharmacokinetics of CP and CL in dairy cows, the maximum expected concentration of CP and CL in the intestinal content of adult dairy cows after intramammary treatment according to the label will most probably not exceed 0.29 µg/ml. The expected maximum concentration of CP and CL in manure pits of

average dairy farms will not exceed 0.03 µg/ml.

Conclusions: We found that the expected concentrations of cephapirin in the gut and manure pits are at least 10 fold lower than the MSC of cephapirin to select for CTX resistance in the different environments. Thus, the potential of intramammary administered cephapirin to select for ESBL-producing *E. coli* in the bovine gut and in manure pits seems low.

Keywords: Antimicrobial resistance, intramammary, cephapirin, cephalonium, dairy cow.

UH-23

Comparison of endotoxin concentrations among commercially available Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis

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Objective: Lipopolysaccharide core-antigen vaccines have been developed to provide cross-protection against a variety of Gram-negative bacterial pathogens capable of causing clinical mastitis in dairy cows. Though a variety of products have been shown to reduce the severity and duration of coliform intramammary infections, these products differ in formulation including adjuvants and carrier solutions. A compositional difference of potential concern is a variation among products in endotoxin concentration. Adverse effects attributed to parenteral administration of endotoxin to cows include reproductive failures, anorexia, and decreased milk production. The purpose of this study was to quantify the endotoxin concentrations in Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis.

Materials & Methods: Four commercially available bacterins tested for endotoxin were vaccine A (Bovilis®-J5; Intervet, Inc.), vaccine B (Enviracor™ J-5; Zoetis, Inc.) vaccine C (Endovac-Dairy®; Endovac Animal Health) and vaccine D (J-Vac®; Boehringer Ingelheim Vetmedica, Inc.). Nine bottles of each vaccine, each bottle from a unique lot/serial number, were tested for endotoxin concentration by the *Limulus* amebocyte lysate assay using kinetic turbidimetric detection.

Data were analyzed using a linear mixed model for a complete block study design. A single sample from each vaccine lot/serial number was the experimental unit, and a random intercept term was included in the model to account for the lack of independence among samples within replicates. A fixed effect of vaccine, with four categories, was included in the model. The data were not normally distributed so they were transformed (log10) prior to analysis with a model fitted with a normal (Gaussian) and identity link. The model was fitted with restricted maximum likelihood estimation, Kenward-Roger degrees of freedom and Newton-Raphson and Ridging optimization procedures (Proc GLIMMIX SAS 9.4). When the main effect of vaccine product was significant (P <0.05), pair-wise comparisons were made using the Tukey-Kramer method to adjust for multiple comparisons.

Results: The overall effect of vaccine product was significant (P <0.05); rejecting the null hypothesis the endotoxin levels were equal among products. Similarly, pair-wise comparisons of all vaccine products indicated that all means differed significantly (P values <0.01). Model-adjusted mean for vaccine A was 1.43 $\log_{10}$ EU/ml (range 0.53 to 2.09 $\log_{10}$ EU/ml) vaccine B 3.77 $\log_{10}$ EU/ml (range 3.62 to 3.90 $\log_{10}$ EU/ml), vaccine C 4.90 $\log_{10}$ EU/ml (range 4.65 to 5.19 $\log_{10}$ EU/ml) and vaccine D 5.54 $\log_{10}$ EU/ml (range 5.34 to 5.66 $\log_{10}$ EU/ml).

Conclusion: Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis differed in endotoxin content with a difference greater than 13,000-fold in endotoxin units per milliliter between the lowest and highest concentrations.

Keywords: Endotoxins, vaccination, bovine mastitis, Gram-negative.

UH-24

Treatment of clinical mastitis: intramammary or in combination with parenteral administration of penicillin?

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Objectives: Antibiotics prescribed for dairy cows in Denmark are mainly penicillin products for parenteral mastitis treatment (DANMAP, 2020). However, it may be possible to reduce the amount of penicillin used, if intramammary (IMM) treatment is sufficient as an alternative to parenteral or combined IMM and parenteral treatment which is the first choice for Danish veterinarians (Wilm et al., 2021). This study aimed to investigate if IMM administration of penicillin was non-inferior to IMM combined with parenteral administration of penicillin regarding bacteriological cure of non-severe clinical mastitis cases caused by Gram-positive bacteria.

Materials and methods: The study was carried out as a non-inferiority longitudinal randomized clinical trial in 12 Danish dairy herds from May 2020 to June 2021. The treatment protocols evaluated were **A**) IMM treatment for three days, and **B**) both IMM and parenteral treatment for three days. The IMM treatment comprised 600,000 IE benzylpenicillinprocain administered in the affected quarter once per day for three days. The parenteral treatment comprised 15,000 IE penethamate-hydroiodid/kg body weight administered in the muscle (IM) once per day for three days. Treatment A and B were randomly assigned to cows with non-severe clinical mastitis caused by a Gram-positive bacteria. The farm personnel used an on-farm test to get an indication of this classification (Gram-positive/negative) within the first day of symptoms and before the onset of antibiotic treatment. In the meantime, all clinical mastitis cases were treated with ketoprofen in a dose of 3 mg/kg body

weight. Subsequently, the quarter milk samples were cultured and pathogens were identified using MALDI-TOF. A case was included if one or two bacterial species were identified, and at least one of those were Gram-positive. Bacteriological cure was evaluated on two follow-up samples, two and three weeks after ended treatment. A case was deemed to be cured if the pathogens present in the clinical sample were not detected in any of the follow-up samples. A non-inferiority analysis was carried out with a margin of 15%.

Results: Of almost 1,800 clinical mastitis cases registered, 347 were eligible for evaluation of bacteriological cure based on treatment A or B. The results showed that the cure rate depended on the pathogen. The overall cure for treatment A was 76% [95% CI: 69.2-82.5] and the overall cure for treatment B was 83% [95% CI: 76.8-88.3]. Treatment A was not non-inferior to treatment B with a margin of 15% across all bacterial species. However, the majority (183) of included mastitis cases involved *Streptococcus uberis* infection, either alone or in combination with another species. When including only these cases in the analysis, the cure rate for treatment A was 83% [95% CI: 74.1-90.1], and the cure rate for treatment B was 81% [95% CI: 70.9-88.3]. Thus, treatment A was non-inferior to treatment B for cases with *Streptococcus uberis*.

Conclusions: Penicillin administered IMM for three days is no worse than penicillin administered both IMM and parenteral for three days when it comes to bacteriological cure of non-severe clinical mastitis caused by *Streptococcus uberis*. Implementation of this result in Danish dairy herds can reduce antibiotics used for clinical mastitis treatment. However, the reduced treatment was not found non-inferior across all bacteria species. This underlines the importance of accurate mastitis diagnostics and the need to adjust treatment protocols to the herd's specific mastitis pathogens, in order to facilitate prudent use of antibiotics.

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Keywords: Mastitis, treatment, intramammary, cure.

UH-25

Identification of cows with delayed milk ejection using milk flow recordings

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Objectives: The objective of this study was to identify an interval of milk flow, from a parlor recording system, that would best identify individual cows with normal and delayed milk ejection and for that time interval, to determine the milk flow cutoff with the best specificity to identify cows with normal milk ejection.

Materials and methods: We connected digital recorders to measure vacuum (VaDias, BioControl) in the short milk tube and mouthpiece chamber of two liners during individual milking in 4 farms, and we collected milk flow dynamics from milking recording system software (DeLaval DelPro Software and SCR MC200 Series Milking Control Systems), for 529 cows. We obtained milk ejection time (seconds) after unit attachment by analyzing the milking vacuum data, as a proxy for milk flow, for each cow. Cows were categorized as either having delayed (> 30 s) or normal (≤ 30 s) milk ejection. Individual milk ejection time and milk ejection category were matched to milk flow during four time intervals: ≤ 15 s, 16-30 s, 31-60 s, and 61-120 s. We performed univariate and logistic regressions for each milk flow interval using milk flow as the dependent variable and milk ejection time and milk ejection category as independent variables, respectively, on SAS. Finally, we calculated sensitivity and specificity for milk flow cutoffs of 1.8, 2.3, and 2.7 kg/min (4, 5, and 6 lb/min) liters per minute.

Results: Univariate (table 1) and logistic regressions (table 2) of the four-time intervals on ejection time and milk ejection category, respectively, identified milk flow between 31-60 s as the best time interval to predict delayed milk ejection (R-square 0.33, P <.0001; LS means milk flow of 1.7 kg/min and 3.6 kg/min for delayed and normal milk ejection cows, respectively (P <.0001). For the 31-60 interval, a cut-off of 1.8 kg/min had the best specificity of 0.87, and a sensitivity of 0.64.

Conclusions: Delayed milk ejection lowers milking efficiency and can decrease milk production, impair teat health, and can negatively impact animal welfare. Producers and veterinarians can use of on-farm technology that measures individual milk flow dynamics to identify and monitor delayed milk ejection incidence in their herds, allowing them to look for underlying causes and intervene when needed to improve and maintain milking efficiency.

Table 1. Regressions of milk flow time intervals and milk let-down time.

Milk flow time interval (s)	R-Square	P-value
0-15	0.0125	0.01
16-30	0.0735	<.0001
31-60	0.3288	<.0001
61-120	0.0087	0.03

Table 2. ANOVA of milk flow time intervals and milk ejection category.

Milk flow time interval (s)	Delayed milk ejection LS Means	Normal LS Means	P-value
0-15	0.89	1.14	0.002
16-30	2.05	2.96	<.0001
31-60	1.67	3.6	<.0001
61-120	3.93	4.02	0.51

Keywords: Milk flow, delayed milk ejection.

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UH-26

Emergence of cloxacillin resistance in *Streptococcus uberis*

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Objective: To assess whether there has been a change in penicillin and cloxacillin minimum inhibitory concentrations (MIC) across time (2003 to 2018) in bovine *Streptococcus uberis* isolates.

Materials and Methods: *S. uberis* from clinical and subclinical mastitis cases from one farm during 2003/04 and 2018/19, were re-isolated from a preservation media (LSPQ Preservation Medium, Fort Richard Laboratories Ltd, Auckland, New Zealand). Isolates were confirmed as *S. uberis* based on Gram stain, catalase reaction, cleaving of esculin, SF broth, cleavage of inulin, and MALDI-TOF confirmation for non-conclusive reactions. MIC was determined using semi-quantitative penicillin and cloxacillin test strips (Liofilchem, Roseto degli Abruzzi, Italy) placed on a lawn of *S. uberis* grown on Mueller Hinton CLSI agar plates with sheep blood (Fort Richard Laboratories Ltd, Auckland, New Zealand). Differences in distribution of MIC for penicillin and cloxacillin between the 2003 and 2018 sample sets were assessed using frequency distributions and histograms, and visually assessed for a bimodal distribution. The MIC_{50} and MIC_{90} and their 95% confidence intervals were calculated. The key outcome variable was the epidemiological cut-off value (ECOFF), which was determined visually. Explanatory variables included: year 2003 (n=112) vs 2018 (n=44), clinical (n=46) vs subclinical (n=110) mastitis, spring (n=128) vs other (n=28) seasons, and timing of sampling relative to calving (≤4 days vs >4 days). Bivariate (chi-squared and univariate logistic) and then multivariable linear mixed regression models were used for analyses. Manual stepwise model building approach was used and variables with a $p > 0.05$ were removed from the final model, so long as the odds ratio for other variables in the model did not vary by greater than 20%.

Results: The MIC_{50} and MIC_{90} were 0.047 (interquartile range 0.03-0.09) and 0.19, respectively for penicillin, and 0.25 µg/mL (interquartile range 0.19-1), and 2 µg/mL respectively for cloxacillin. There was a bimodal distribution for the MIC values for penicillin and cloxacillin, and the ECOFF were defined as 0.064 and 0.38 µg/mL for penicillin and cloxacillin, respectively. A total of 28% of the isolates were above the ECOFF points for both penicillin and cloxacillin. The proportion of MIC values above the ECOFF increased from 23% to 40% for both penicillin and cloxacillin between 2003 and 2018. In the final model the proportion of isolates above the ECOFF increased over time (OR=1.06, CI 95% 1.08-1.11, p=0.02) and (OR=1.07, CI 95% 1.02-1.13, p=0.01) for penicillin and cloxacillin, respectively; and with a higher proportion above the ECOFF for samples collected outside of spring (OR= 2.95, CI 95% 1.03-10.7, p=0.06) and (OR= 3.02, CI 95% 1.05-11, p=0.059) for penicillin and cloxacillin, respectively.

Conclusion: The proportion of *S. uberis* isolates above the ECOFF for penicillin and cloxacillin increased between 2003 and 2018. While this data is from only one farm, it supports data from MIC estimates for *S. uberis* isolates from bulk tank milk across New Zealand which demonstrate increasing cloxacillin MICs (McDougall *et al.* 2018). In New Zealand, cloxacillin is widely used for therapeutic treatment during lactation, and prophylactically at dry off. It was commonly used in the herd from which these isolates were drawn. Isolates from later in lactation had higher MIC. Whether this is due to survival of these isolates following treatment or is associated with persistence and host adaptation remains to be determined. The clinical significance of the highest MICs observed i.e. 0.38 µg/mL for penicillin, and 4 µg/mL for cloxacillin remains unclear. However, a recent report suggests that MICs for oxacillin >0.5 µg/mL tended to be associated with reduced bacteriological cure following therapy of clinical *S. uberis* cases (McDougall *et al.* 2020).

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Keywords: Mastitis, S. uberis, MIC, Resistance.

UH-27

An Investigation into Optimising Antibiotic Use through Selective Use of Dry Cow Treatment at the Quarter Level

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Objectives: Blanket use of antibiotic dry cow therapy (DCT) was a cornerstone of the Five Point Plan. More recently, the selective use of antibiotic DCT in combination with blanket use of an internal teat sealant (ITS) has been successfully implemented in the UK. Historically, cow level application of antibiotic DCT has been advocated, primarily because quarters are not independent within cows and therefore an increased risk of infection has been perceived in 'uninfected' quarters in 'infected' cows. However, there is evidence that this lack of independence is less marked with 'environmental' than 'contagious' mastitis pathogens. The aim of this study was to investigate the utility of a quarter level approach to DCT in well managed low SCC herds with a low prevalence of contagious mastitis pathogens.

Materials and Methods: Farms with a history of low bulk

milk SCC were invited to participate in the study. Cows, within herds, were stratified as 'infected' or 'uninfected' (using SCC and clinical mastitis history), before being randomly allocated to one of three treatment groups: CLT, QLT0 and QLT1. The CLT (Cow Level Treatment) group were allocated, using SCC and clinical mastitis history, into animals eligible for the use of ITS alone (Cepralock™) or ITS in combination with antibiotic DCT (CEFA-SAFE™) - importantly this decision was applied at the cow level (all quarters within a cow receiving the same treatment). Within the QLT0 (Quarter Level Treatment - CMT>0) and QLT1 (Quarter Level Treatment - CMT>1) groups, quarters within cows were allocated (based on a California Mastitis Test (CMT) score of >0 or >1 respectively) to receive ITS alone (score below the threshold) or ITS in combination with antibiotic DCT (score above the threshold) depending on the quarter CMT score at drying off.

The bacteriological status and SCC of all quarters were assessed at drying off and within a week post calving and for SCC 7-14 days post calving. All cows were monitored for clinical mastitis from dry off until 100 days post calving.

Univariable and multivariable analysis were undertaken to assess the effectiveness of the three approaches for their impact on dry period outcomes including the likelihood of a quarter being pathogen free at calving. The impact on clinical mastitis was also assessed.

Results: 807 cows from six herds were recruited; 401 were defined as 'infected' and 406 as 'uninfected' by historic SCC and clinical mastitis data. The overall prevalence of infection at drying off was low with only 10.5% of quarters culturing a major pathogen. Minor pathogens were prevalent with 52.8% of quarters culturing positive for these species.

In cows defined as 'infected' at drying off, analyses suggested that there were no significant differences between treatment groups in the likelihood of a quarter being positive for a major pathogen or any pathogen post calving ($p>0.05$). In cows defined as 'uninfected' at drying off, whilst there was no difference in the likelihood of a quarter being culture positive for a major pathogen, quarters in QLT0 group (antibiotic DCT administered to quarters showing a 'trace' reaction on the CMT) were at significantly lower odds of being culture positive for a minor pathogen post calving than quarters in cows receiving a teat sealant alone (OR 0.658; 95%CI 0.488-0.889).

No significant differences in the proportion of quarters affected by clinical mastitis were observed between any of the treatment groups.

Antibiotic use was assessed with respect to the number of cures effected by treatment. In cows defined as 'infected' at drying off, cow level treatment achieved the highest 'cure' rate of major pathogens (97.7%) but was associated with the highest level of antibiotic tube usage/cure (13.66 tubes/major pathogen cure); the QLT1 group had the lowest tube usage (5.3 tubes/cure) but also the lowest cure rate (89.0%). In cows defined as 'uninfected' at drying off, the 'self-cure' was 100% in the CLT and QLT0 groups. Three quarters failed to 'cure' in the QLT1 group, though these were due to infections with pathogens against which antibiotic DCT would not have been effective.

Conclusions: In low SCC herds, with a low prevalence of contagious pathogens, there appears to be an opportunity to reduce antibiotic DCT use by selecting treatments at the quarter level without adversely affecting udder health.

Keywords: Udder Health, Dry Cow Therapy, Antibiotic, Mastitis.

UH-28

Impact of selective dry cow theraphy on farm udder health

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Objectives: The aim of the present retrospective observational study was to compare how the use of selective dry cow therapy (SDCT) influences udder health instead of blanket dry cow therapy (BDCT) in a set of dairy cattle farms impacts udder health.

Materials and methods: This study was carried out from January 2017 to December 2018, with the implementation of the SDCT in 25 selected dairy farms of Friesian cattle that traditionally applied BDCT. These farms were located in Galicia (North-western Spain) and associated with the Milk Quality Service.

Selection criteria

In order to be selected, farms must have had a sustained constant annual somatic cell counts(SCC) <250,000, percentage of monthly clinical mastitis <3% and no contagious isolates.

In cow selection, for the use of internal sealants only, no intramammary antibiotic therapy, the criteria were that the last three months of SCC <200,000, absence of clinical episode records at least in the last three months and negative result to the California mastitis test at the time of drying.

Furthermore, key factors for the selection of the farm were considered knowing their epidemiology, as well as good management practices around the dry period . At the cow level, it was considered important that the animals reach drying with the anatomical integrity of the sphincter in good condition.

Udder health parameters

After the SDCT implementation during the years 2017 and 2018, for evaluating herd udder health we used: changes in somatic cell count and percentage of monthly clinical mamitis, both parameters related to the subsequent lactation, comparing BDCT (2016) and SDCT (2018).

Also two rates of dry period performance were used: prevalence of infections at calving (percentage of cows with first postpartum SSC> 200.00 with respect to total calves) and rate of new infections at calving (percentage of animals that when drying had a SCC 200,000 and that in the first postpartum control had a SCC >200,000 with respect to the total cows that were dried with a control lower than SCC 200,000).

Data analysis

All data were stored in our own software "Gesgando". To

compare the monthly SCC of the 25 farms in 2016 and 2018, a linear regression model was developed. The Naperian logarithm of monthly SCC was used as a response variable and year, as an explanatory variable, taking into account month and farm as random effects. To compare the monthly percentage of clinical mastitis, a Poisson regression model with farm and month as random effects and year as an explanatory one has been performed.

Results: From January 2017 to December 2018, 2,210 cows were dried applying the selective dry cow therapy (SDCT), only 635 received intramammary antibiotic drying, that is, 71.27% of the cows only carried internal sealant, with the consequent antibiotic reduction.

The results obtained with respect to SCC were 159 cells/mL in tank (2016) to 166 cells/mL (2018). The percentage of average monthly clinical mastitis moved from 1.66% (2016) to 1.48% (2018). The effectiveness rates of drying therapy: prevalence of infection at calving 18.29% (2016) and 21.75% (2018), and rate of new infections at calving 16.4% (2016) and 17.76% (2018).

When analyzing the data, it is observed that the monthly SCC shows significant differences (pvalue <0.001) between years, being higher in 2018 (166.62 ± 42.59) than in 2016 (158.85 ± 38.68), on the contrary, percentage of monthly clinical mastitis shows a reduction (p- value <0.001) from 2016 (2.39 ± 2.93) to 2018 (1.57 ± 2.19).

Conclusion: The transition from BDCT to SDCT resulted in a huge reduction in the number of antimicrobials used on dairy herds without having a clear deleterious effect on udder health regarding general data obtained. When managed appropriately the dry and transition period and selected cows with optimal udder health can be successfully dried off without the use of intramammary antimicrobials.

The increase in the SCC after the new therapy implementation with a no clinical mastitis rate associated, maybe must be addressed with an improvement and even higher requirements in the conditions of dry cow management in a global context of antibiotic reduction.

Keywords: Cow, dry, selective, theraphy, health.

UH-29

Internal teat sealant retention after selective dry cow therapy in dairy cows

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Objectives: Internal teat sealants (ITS) are increasingly used in the EU for prophylaxis of new IMI in the dry period due to their efficacy and the concerns for prophylactic use of antibiotics (AB). ITS require no milk withdrawal time, and residues may enter the bulk tank and end up in cheese, affecting the image of the dairy industry. Current ITS residue data are mainly based on visual presence, and objective data of their weight in time after calving are lacking. The primary objective was to determine the quantity of ITS excreted in milk, when used alone or combined with AB, during the first week after calving.

Materials & Methods: The study was conducted on 3 farms in NL, and 4 in DE, selected on availability of DHI records, a conventional milking system, milking 2x/d and willingness to participate and comply to the protocol. Cows were treated with AB (Cefa-safe®, MSD Animal Health) in all 4 quarters only when the last monthly DHI test before dry-off (DO) showed a cow SCC > 200k cells/ml (high SCC). All cows were dried off with ITS (ShutOut®, MSD Animal Health). At DO, teat end callosity score, body condition score, milk leakage and udder pressure were measured.

Cow eligibility at DO included pregnancy, expected DO period > 32 d, > 3 functional quarters, milk yield > 5 L, good general health, and absence of clinical mastitis and concomitant treatment within 30 d of DO. During the 1st 7 DIM, 50 ml of milk was collected pre-milking at each milking, centrifuged, and the amount of remaining ITS and milk solids weighed. ITS in mg/milking was calculated by deducting the tubes total weight from the final 2 milkings, assuming no TS was left in the tubes (± 2SD), from the weight of the tube including all its content (ITS + milk solids). Quarter milk samples were collected aseptically prior to the final milking and DO treatment, and at 3 DIM, to determine etiology and SCC. Clinical mastitis events and general health were monitored from D0 to 30 DIM. ITS retention and treatment effects will be evaluated using generalized linear mixed models.

Results: A total of 103 cows were included in the preliminary analysis, 76 cows were 'high SCC' at DO, and treated with ITS+AB, and 27 cows were 'low SCC' at DO and treated with ITS alone. At DO, 63.5% of quarters were cultured positive and most identified pathogens were Non-aureus staphylococci (NAS, 20.8%), Staph. aureus (5.9%) and Lactococcus species (2.1%). At 3 DIM, 48% of quarters were cultured positive, and most identified pathogens were NAS (12.3%), Staph. aureus (1.4%), E. coli and yeast (both 1.2%). Bacteriological cure or new infection rate analysis is ongoing. Just prior to the 1st milking, 76.6% of quarters (n=423) contained ITS > minimum detection limit, quickly declining to 26.7% at the 2nd milking, 8.3% at the 3rd milking, and at 1.8% at the 14th milking. The ITS excretion curve analysis in g/milking is ongoing. Differences could be detected between the ITS retention when combined with AB or not.

Preliminary efficacy analysis of ITS was based on 219 quarters, 98 with a qSCC < 200k cells/ml and 121 qSCC > 200k cells/ml at DO. Comparing qSCC data (n=219) at DO and at d3 after calving, using a qSCC of 200k cells/ml as a threshold for imm infection, prevention of new infection rate, cure rate, new infection rate and rate of failure to cure, was 74.5%, 71.9%, 25.5%, and 28.1%, respectively. Generalized linear mixed models to identify risk factors for ITS retention and ITS efficacy are currently developed and the analysis is ongoing.

Conclusion: ITS residues were detected in 76.6% of quarters at the 1st milking after calving, declining to < 10% of quarters at the 3rd milking, and < 2% of quarters at the

14th milking. Differences in ITS excretion were detected when combined with AB or not. This study shows the importance for farmers to comply to EU regulations to not enter colostrum in the bulk milk tank and to install milk filters to prevent ITS residues from entering the bulk tank.

Keywords: Teat sealant, dry cow therapy, dairy cattle.

UH-30

The use of cabergoline at dry-off increased milk production in a commercial dairy herd in Brazil

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Cabergoline is a dry off facilitator that reduces milk production at dry-off by inhibiting prolactin secretion. It has been recently demonstrated that one single injection of cabergoline at dry-off effectively reduces milk leakage by 82% and new intramammary infections by 21% (Hop et al, 2019), accelerates mammary gland involution and improves its immune response (Boutinaud et al, 2016, 2017). Interestingly, recent literature has reported also an increase in milk production in the following lactation, in those cows where prolactin was inhibited in the first days after dry off (Lacasse, 2015). However, and to the authors knowledge, no studies to date have investigated the impact of the use of cabergoline at dry-off on dairy cow's milk production in the subsequent lactation and under Brazilian field conditions.

Objectives: The objective of this study was to investigate, under Brazilian field conditions, the benefits of reducing prolactin secretion at dry-off on the subsequent lactation performance with one single administration of cabergoline at dry-off (Velactis®, Ceva Santé Animale, Libourne, France).

Materials and methods: The trial was conducted from March 2019 to February 2020 in a commercial Holstein dairy herd in upper-Southwest Brazil in Minas Gerais State. A total of 417 Holstein cows, producing 27,9 ± 3.8 kg at drying off, were dried-off abruptly and treated with intramammary antibiotic therapy (Bovigan-VS®, Bayer Animal Health, Brazil) plus an internal teat seal (Ememast Selante®, Boehringer Ingelheim Animal Health, Brazil) at dry-off. Cows were randomized to two experimental treatments, as follows: 1) Cabergoline 5.6 mg (5mL, Velactis®, Ceva Santé Animale, France) given intramuscularly to cows with even ear tags, or 2) Control (saline, 5 mL) given intramuscularly to cows with odd ear tag numbers. Immediately after treatment, cows were moved to a new dry cow pen and kept in a far-off diet for approximately 4 weeks. Then, 3 to 4 weeks before calving all cows were moved to a close-up prepartum pen and fed an anionic-based diet until calving. Total daily milk production was recorded once within the week prior to drying off, and throughout lactation at 20-day intervals during milk testing performed from calving until 200 DIM, the records on incidence of retain placenta, clinical mastitis, and breeding records were retried from the herd's management software (Ideagri®, Brazil) and the edits in the final database included in the data analysis. Statistical analyses were performed using the SAS 9.4.

Results: There was a significant effect of treatment ($P < 0.05$) on milk production, in which cows treated with Cabergoline produced significantly more milk from calving to 200 DIM (Cabergoline: 7395 Kg vs Control: 7017 Kg; $P = 0.03$). There were no differences between experimental groups in the proportion of cows with clinical mastitis within 30 (Cabergoline: 6.0 ± 0.04 vs Control: 6.0 ± 0.05; $P = 0.95$) or 100 days post calving (Cabergoline: 13.9 ± 0.05 vs Control: 12.0 ± 0.04; $P = 0.71$). Similarly, treating cows with Cabergoline at dry off did not influence proportion of cows having retain placenta (Cabergoline: 11.4 ± 0.4 vs Control: 11.6 ± 0.3; $P = 0.97$) and proportion of cows conceiving by 150 DIM (Cabergoline: 69.0% vs Control: 65.5%; $P = 0.45$).

Conclusions: Treatment with Cabergoline at dry-off effectively improved milk production in Holstein cows. In addition, findings from the current trial indicate that cows treated with Cabergoline at dry-off produce in average 1.89 kg/day more milk than control cows. This represents extra income for the farmers and provide data to help producers evaluate best return on investment of differing technologies available that can be used at the time of dry-off.

Keywords: Dry-off, Mastitis, Dairy Herd, Milk Production, Cabergoline.

UH-31

Productive and reproductive impact of subclinical mastitis by prototheca in a commercial dairy farm in central Spain

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The genus *Prototheca* is a microalgae, unicellular, aerobic and achlorophyllic and ubiquitously distributed in commercial dairy farms. Although it is becoming an emerging and important disease in dairy cattle and despite of producing a fatal mastitis, this disease has been scarcely studied in terms of epidemiology, costs and specifically regarding the subclinical infections of this pathogen.

Objectives: Therefore, the aim of this study was to quantify the concrete consequences of the subclinical protothecal mastitis, regarding yield (predicted 305-day milk yield, fat and protein yields) and udder health (mean somatic cell count).

Matherial&methods: The retrospective and observational study was carried out 2016-2018 due to a natural outbreak at a commercial dairy cattle farm in central Spain, with 900 animals in milk and 500 heifers of which approximately 30% of the total herd was infected with the algae. Animals with clinical mastitis, without previous lactation to the outbreak (heifers) or cows that standed in the same lactation (due to a long lactation), or without available data were discarded from the study. A to-

tal of 418 cows were included in the study and based on the analysis of its milk (PCR t first screening and microbiological culture at periodical revisions) were distributed into the group of healthy or infected cows (39.23 and 60.77% of the animals, respectively). Yield, udder health and reproduction data during the previous lactation to the infection were compared within each individual cow to those to the lactation during the infection. The reproductive rates studied through collective farm data were the days in lactation at the first insemination as well as at the first heat, the average number of empty days and the interval between births. Absolute yield values during the lactation of the infection of infected and healthy cows were also compared. Saphiro-Wilk test determined the non-normality of the data and non-parametric tests (Mann Whitney U) were used to analyze the differences.

Results: The protothecal mastitis produced significantly lower projected milk yield to 305d in the infected cows (P<0.001) when compared to non infected cows (median of 8,847.26L vs 11,281.66 L) while non-infected cows showed no significant differences with respect to their previous lactation. The somatic cell count was 485.85 and 1,392.81 for healthy and infected cows (P<0.001). There was no significant difference in fat and protein yields with respect to the previous lactation (P=0.01).

In addition to the consequences due to the productive outcomes, we observed that collective reproductive results showed that the reproductive performance of the farm worsened between January 2016 and January 2017, when comparing the days to the 1st artificial insemination, the days to the first heat, the average number of open days and the interval between births. However, when analyzing the farm data for January 2018, these indicators showed an improvement that indicate that the measures taken were in the right way. Nevertheless, there were an obvious decrease in the number of total inseminations as well as in the intention to inseminate in the cows that were identified as positive.

Conclusions: In conclusion, our data indicate that the productive losses caused by subclinical mastitis produced by *Prototheca* in dairy cattle are very high, with a great impact on the quality of the milk produced and a possible impact on reproduction, which requires individual analysis of the data to be clarified. To all these consequences we must add the impact of this type of infection on the prevention and control measures used to control the outbreak. Finally, we consider of interest to continue with the analysis of the data regarding subsequent lactations in the positive surviving population to study their evolution.

Keywords: Mastitis, Prototheca.

UH-32

Procaine penicillin for mastitis - Have we forgotten how good it is and is it getting even better?

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Objectives: This study compares outcomes of clinical mastitis treated with intramammary procaine penicillin and the market leading cloxacillin and examines if MIC can explain differences in case cure rates.

Material & Methods: 16,500 cows from 35 seasonal calving dairy herds were monitored for clinical mastitis. Pre- and post-treatment samples were cultured, isolates identified, and MIC determined using Liofilchem® test strips. Cows were treated with either 1) cloxacillin (CL) 5 times s.i.d., 2) procaine penicillin (PP) 3 times b.i.d. ,or 3) the PP treatment followed by further treatment with a product containing both PP and CL (PC) 3 times s.i.d. starting 48 hours after initial case diagnosis. Treatment regimens were selected to conform with the New Zealand registered use. Case cure rate (CCR) (both clinical and bacteriological) was measured.

Results: Of the 767cases S. uberis (SU) was most commonly isolated (43.0%), followed by S. aureus (SA) (15.29%). In the case of SU, PP had a higher CCR than CL (76.83% vs 61.25%, p=0.01). In the case of SA, the combined PC treatment had a higher CCR than CL (40.96% vs 24.36%, p=0.02) and PP tended to be better than CL (CCR = 40%, p=0.08). The MIC50 and MIC90 of CL (0.125 and 0.250µg/ml) were higher than those of PP (0.023 and 0.190µg/ml) in SA, and much higher in SU (0.380 vs. 4.0, and 0.047 vs. 0.190µg/ml, for CL and PP, respectively). There was no difference in MIC50 for cases that cured vs. non-cured regarding either treatment. CCR for SU cases treated with CL, was lower though for isolates when MIC was ≥2µg/ml (p=0.04).

Conclusion: The MIC values for PP in this study were lower than previous studies in NZ and fit a trend of significant decrease in regards both SU and SA over the last 30 years. 32% of SU isolates had a MIC of CL ≥2µg/ml which is higher than the minimum inter-treatment concentration (ITCmin) achieved in milk. The MIC for all other isolate/treatment combinations was below the ITCmin. Individual MIC was only predictive of clinical outcome when it was >ITCmin. Better case outcome, supported by lower MIC (particularly in the predominant SU) and a New Zealand Vet Assoc. classification as a more responsible medicine class, support the intramammary use of procaine penicillin over cloxacillin for clinical mastitis.

Keywords: Procaine penicillin, cloxacillin, mastitis, treatment.

UH-33

Farmer and veterinarian perception on udder health management

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Objectives: Mastitis is the most costly disease in dairy herds in developed countries (Hogeveen et al., 2019) and a significant reason for compromised animal welfare (von Key-

serlingk et al., 2009). We have much knowledge on mastitis and udder health management, allowing for evidence-based improvement. In recent years, motivation and perception of the farmer have received increasing attention, with communication research (Jansen et al., 2010b; Jansen et al., 2010a), to successfully implement procedures such as the NMC 10-point plan(NMC, 2016). As a consultant, the veterinarian has a crucial role in udder health management, and their behavior has been described (McDermott et al., 2017). However, the effect of veterinary consultancy depends on the interaction between farmer and veterinarian. Because perceptions of udder health can differ between farmer and herd veterinarians, it is crucial to identify potential gaps in agreement to understand the impact on the outcome BMTSCC.

Material and methods: The participating dairy farmers and veterinarians were enrolled on the Danish National Cattle Data Base data. The criteria for participating were: DHI recording, > 90 % Holstein cows, parlor milking, conventional herd, herd size > 100 cows located in the western part of the country. The dairy farmers were contacted first and encouraged to participate in an anonymous survey conducted in a larger research project on udder health management. Next, the herd vet was contacted and encouraged to participate. The study enrolled a total of 88 herds with their herd veterinarian. The survey included questions regarding cooperation between farmer and herd veterinarian and the consulting methods by the vet. Veterinarians received the questionnaire by email and were reminded if not responding within two weeks. The dairy farmers filled out the questionnaire during the visit from the researcher. The answers were predominantly based on the Likert scale (Likert, 1932) to measure agreement between dairy farmers and herd veterinarians.

Results: The response percentage was 100 % for the dairy farmers and 94 % for the herd veterinarians. Initial analysis was an agreement based on Cohens Kappa and weighted Kappa value. Focusing on some of the results, the farmer regards the vet as most important in handling udder health in the dairy herd, with 91 % answering agree or strongly agreeing to this question. Also, in converting Evidence-Based Scientific Data to operational consulting, 74 % of the farmers agree or strongly agree about the vet`s effort. The data will be further analyzed, focusing on the agreement between the farmer and the vet, linked to the outcome BMTSCC as a proxy of udder health. This will high light where the herd veterinarian needs to focus to maintain the role as resource in the area udder health management in competition with other professionals and equipment supplier.

Conclusions: The initial results highlight differences in perception between farmer and herd veterinarians regarding udder health. Identifying potential obstacles is essential because the farmer sees the herd veterinarian as critical in udder health consulting. The vet translates evidence-based knowledge in udder health management to apply practical solutions for the farmer.

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Keywords: Management, udder health, social science.

UH-34

Fly repellency using deltamethrin reduces intramammary infections, stress and fatigue indicators of dairy ewes under intensive management

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Objective: The aim of the study was to assess the fly repellency effect of deltamethrin and link it to the occurrence of common bacterial intramammary infections (IMI), milk somatic cell counts (SCC), the serum cortisol (SC) and creatine kinase (CK) levels (stress and fatigue indicators, respectively) during the fly season.

Materials and Methods: The study was conducted in one intensively reared dairy sheep flock of Lacaune breed, located at Thessaloniki (Central Macedonia, Greece), between June and July 2020. Fifty multiparous ewes were randomly assigned in two similar groups (n=25 ewes per group); deltamethrin treated group (D group) and control group (C group). Group D ewes were individually dressed on their back with deltamethrin 10% (Deltanil® 10 mg/mL, Virbac Hellas, Greece) on day 0. The enumeration of the fly burden was carried out by direct observation of the animals on day 0 and two more times with 15-days intervals (i.e., day 15 and 30) to assess the repellency effect of deltamethrin. Moreover, 10 fly traps (5 per group) with sticky surface were set in predefined locations of equal distances within the pens at the level of the ewes. Individual blood samples were collected at the forementioned time points, while an electrochemilu-

minescence immunoassay method and spectrophotometry were used to estimate SC and CK concentrations, respectively. On every sampling occasion, milk samples were collected from each individual ewe and were transported to the laboratory to be microbiologically assayed, emphasizing on the isolation of *Staphylococcus aureus* and Coagulase-negative Staphylococci (CNS). Moreover, SCC were estimated using an automatic high-throughput analyzer. Mixed linear regression models were built to estimate the random effect of the ewe and the fixed effects of deltamethrin treatment and sampling occasion on the logarithmic values of flies, *S. aureus* CFU (Colony-forming units), CNS CFU, SCC, as well as SC and CK levels. All statistical analyses were performed using SPSS (v23).

Results: Deltamethrin treatment was associated with a reduced number of flies (mostly *Musca domestica*) landed on treated ewes, compared to untreated ones ($p<0.05$). Also, the application of deltamethrin was associated with decreased colony-forming units in the case of CNS IMI ($p<0.05$) and SCC in the milk ($p<0.001$). Likewise, SC and CK levels were significantly lower in the deltamethrin-treated ewes ($p<0.001$).

Conclusions: Deltamethrin application was associated with decreased colony forming units of CNS, isolated from IMI cases. Furthermore, the reduction in the logarithm of SCC in the deltamethrin-treated group implies that fly repellency using deltamethrin favours the health status of the mammary gland possibly via the reduction of pathogens transferred by flies and the inflammation caused by them. Finally, reduced SC and CK concentrations in the deltamethrin treated ewes support the assumption that fly-repellency effect of deltamethrin facilitates the control of flies and contributes to a more welfare-friendly environment for intensively reared dairy ewes.

Keywords: Dairy ewes, deltamethrin, fly, mastitis.

UH-35

Bulk-tank somatic-cell count as an indicator for mastitis-infection rates and fertility in the herd

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Objectives: Some dairies in Austria use stricter milk-quality parameters than other EU and non-EU countries. Farmers only receive enhanced payment for the highest milk quality, if the bulk-tank milk somatic-cell count (SCC) is <200,000 cells/ml, and the aerobic mesophilic count (AMC) is <50,000/ml. The background of more strict thresholds is that the regular 400,000 cells for shipping milk are rather high compared to the maximum SCC of 150,000 found in a healthy udder. The objective of this study was to show that a bulk tank SCC of <200,000 is an indicator for better udder health, in terms of mastitis-infection rates, and better herd-management, indicated by fertility and nutritional-related parameters, compared to higher SCC in the bulk tank.

Materials and methods: Data from 24 Austrian dairy farms (on average 36 cows with 8470 kg milk/cow/305 days) were collected for two years. Bulk-tank somatic-cell count was used to assign the farms to low SCC herds (LSCC; >200,000 cells/ml in maximum 10% of the tests), and high SCC herds (HSCC; >200,000 cells/ml in more than 10% of the tests). None of the farms ever exceeded the maximum threshold of 400.000 cells for shipping milk. From the herd data of at least 20 routine-milk tests, individual cow data (i.e., SCC, number of lactations, days in milk, protein-urea class, waiting time, days open, calving interval) were extracted. The individual cow SCC were assigned to SCC-classes (class1 ≤50,000; class2 ≤100,000; class3 ≤200,000; class4 ≤400,000; class5 >400,000 cells). Class 1 and 2 were considered as healthy, class3 as suspect, class4 and 5 as infected. Cows were considered chronically infected with being at least two consecutive tests infected, or three consecutive tests suspect or infected. The new-infection rate was calculated using the first milk-testing SCC of the lactation. SCC classes before and after the dry-off period of the same cow were used to calculate the infection or healing rate during the dry period. Statistical analysis was done using Mann-Whitney-U, Moods-Median, or chi²-Test, including posthoc tests, depending on the variables analyzed and data distribution. HSCC and LSCC were the fixed effects, and the evaluated herd data and mastitis indicators as dependent variables.

Results: LSCC had more cows in SCC-class1 (51%) and fewer cows in class3 (15%), 4 (7%), and 5 (6%) than HSCC (28, 23, 15, 13%, respectively; $P<0.01$). HSCC had more chronically infected cows (44%) than LSCC (20%; $P<0.01$). LSCC had more healthy cows and less infected cows before dry-off than HSCC (55 vs. 31%, 22 vs. 41%, respectively; $P<0.01$). New-infection rate and suspect animals at the beginning of the lactation were higher in HSCC than LSCC (24 vs. 12%, 15 vs. 8%, respectively; $P<0.001$). A higher new-infection rate on HSCC was seen for both primiparous ($P=0.06$) and multiparous ($P<0.01$) cows. The proportion of cows staying healthy during the dry period was twice as high in LSCC than in HSCC (48% vs. 24%; $P<0.01$), and of staying infected was higher in HSCC (15 vs. 5%; $P<0.01$). The waiting days, days open, and calving intervals were shorter in LSCC than HSCC ($P<0.01$). LSCC had more cows in the optimum protein-urea class, reflecting optimal feeding regimen than at HSCC (37 vs. 31%, $P<0.01$).

Conclusion: The study showed that a change to a bulk tank SCC of <200,000 is an indicator for lower mastitis disease rates in the individual cows. The more optimal fertility parameters and feeding regimen prove that LSCC farms also have better general herd management. Better udder health and optimal management are indicators for overall better animal health on these farms. Therefore, quality programs should consider lower thresholds for the bulk tank milk to value the work of LSCC farms and support the improvement of HSCC farms to contribute to better dairy-cow health.

Keywords: New infection rate, Healing rate, cow health, udder health.

UH-36

Dry period management and new high somatic cell count during the dry period in Dutch dairy herds under selective dry cow therapy

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Objective: The aim of this study was to describe the current management practices that could influence udder health during the dry period and to investigate the associations between management and new high SCC during the dry period. In 2008 the Netherlands started to improve transparency and decrease the use of antimicrobials in animal husbandry. Prevention of new high SCC during the dry period via antimicrobial dry cow treatment (DCT) was no longer allowed in animals with a low SCC before the dry period. An increase of new high SCC during the dry period was expected in dairy cows without antimicrobial protection, but was not observed.

Materials & Methods: In 2018 an online questionnaire was conducted among 1,942 Dutch dairy farms using 12 different veterinary clinics. The questionnaire asked about the management of dry cows at the start of and during the dry period, and around calving, considerations in the use of DCT, and knowledge of the 2012 guidelines for selective DCT. A total of 690 farmers (36%) responded to the questionnaire. Data on new high SCC during the dry period, use of antimicrobials for intramammary DCT and mastitis treatment, herd size, and milking system were available from other sources. Descriptive statistics were used to evaluate the associations between the different variables. For analysis of new high SCC on herd level, explanatory variables were analyzed using a generalized linear mixed model.

Conclusion: Respondents indicated that the most important management factor to reduce the risk of new high SCC was reduction of milk yield before dry-off. The variables associated with a lower proportion of new high SCC on herd level during the dry period were the use of dip or spray after drying off, a higher animal-defined daily dose of intramammary antimicrobials for DCT, the use of DCT in low-SCC cows based on SCC or mastitis history, correct knowledge of the guideline, and awareness of importance of low infection rate and good hygiene during dry-off. The variables associated with a higher proportion of new high SCC on herd level were dry cow housing other than cubicles and a higher animal-defined daily dose for intramammary antimicrobials for mastitis. This research clearly indicates that farmers can balance limited use of antimicrobials at dry-off with management measures to maintain good udder health during the dry period.

Keywords: Dry cow management, selective dry cow therapy, new high SCC.

UH-37

Efficacy of a ready-to-use udder care product based on lactic acid for daily teat disinfection after milking by spraying

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Objectives: The goal of the present trial was to assess the efficacy of a ready-to-use udder care disinfectant based on lactic acid (Kenolac® SD, PT3 biocidal product-type, from CID LINES, An Ecolab Company) for daily teat disinfection after milking by spraying.

Material & Methods: Two studies were designed to assess the efficacy of the product. In the laboratory study, Kenolac® SD disinfectant efficacy was tested according to the European Norms (EN 1040 and EN 1656) against mastitis causing bacteria (test conditions: in presence of organic matter, in 5 minutes contact time).

Test Organism	Concentration	Contact time	Temperature	Interfering substance
Bacteria (EN1656) Gram + *Staphylococcus aureus* (ATCC 6538) *Streptococcus ub*eris (ATCC 19436) Gram - *Escherichia coli* (ATCC 10536) **Yeast (EN1657)** *Candida albicans*	Ready to use	5 mins	30 °C	1% skim milk Dirty conditions

In the field study, the impact of two methods of application of Kenolac® SD on the microbiological contamination of the teats was evaluated: spray and dip. The samples are taken during milking. Kenolac® SD was used after each milking with a sprayer or a dip cup, respectively (recommended dosing regimen: 5 mL per cow per application). After dipping Kenolac® SD on the teats, waiting for 3 minutes contact time was required.

The reduction of bacteria growth was evaluated for the two application methods. The scoring system was based on bacteria count (colony-forming unit-cfu/ml): the bacterial reduction for Staphylococcus spp. and Coliforms count. These bacteria are a good indicator of the mastitis infection pressure, from contagious and from environmental sources.

Results: Laboratory trial showed that Kenolac® SD reduced the number of viable microorganisms tested by more than 5 log (bacteria) and 4 log (yeast) during 5 minutes of contact at 30°C under dirty conditions (1% skim milk).

Statistical analysis of the microbiological results obtained at the field trial showed similar results for the spray application than for the dip application regarding percentage of

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bacteria reduction on the teats after milking. A high microbiological reduction was achieved for spray application for total *Staphylococcus spp* (93,83%, n=114), *Staphylococcus aureus* (92,94%, n=114) and Coliforms (99,47%, n=114). For dip application, reduction was 90,76% (n=119), 91,4% (n=119) and 98,22% (n=119), respectively.

Conclusions: The efficacy of Kenolac® SD, ready-to-use udder care biocide based on lactic acid, has been demonstrated to be efficacious in reducing the amount of microorganisms involved in dairy cow mastitis, when applied by spraying the teats after milking.

Keywords: Mastitis, disinfection, teat dip, teat spraying, lactic acid.

UH-38

Bovine Ischaemic teat necrosis: an observational study investigating the potential farm level risk factors and economic impact on GB dairy farms.

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Bovine ischaemic teat necrosis (ITN) is an emerging disease affecting the teats of dairy cattle with many affected animals being culled due to the disease process. Little is known around the epidemiological and economic data that can inform control strategies.

Objectives: The aim of this observational study was to investigate farmer-reported experiences, identify potential farm-level risk factors ad provide an estimate on the economic impact of ITN on GB dairy farms.

Materials and methods: In January 2018, a postal questionnaire with an online and telephone option was sent to a random sample of 1855 Great Britain (GB) dairy farmers. Descriptive statistics were utilised to understand the main at-risk animals and the potential outcomes of the disease. The economic impact was estimated based on the different clinical outcomes. Univariable and multivariable logistic regression models were used to explore associations between the presence of ITN on farm and various risk factors.

Results: A usable response rate of 12.3% was obtained. Fifty-one percent (95% confidence interval (CI): 44.4 - 57.8%) of farmers reported having experienced ITN on their farm between 1985-2018. Rising numbers of farms indicated ITN is an emerging disease with 46.3% of farmers reporting the first case in the three years up to 2018. At the animal level, 47.3% (95% CI: 38.7-55.9%) of cases occurred during the first lactation and 78.9% (95% CI: 75.2-82.6%) within the first 90 days in milk. Only 20.8% (95% CI: 15.9-26.4%) of cases were reported to recover, whereas 22.8% (95% CI: 17.8-28.5%) of cases required culling. The remaining cases experienced complications such as loss of a teat and/or mastitis. From these data, the cost of ITN, through production losses and expenditure, was estimated to be £1121 per farm per year. The costs were estimated at £720, £860 and £2133 for recovered, complicated and culled cases, respectively. The presence of udder cleft dermatitis (UCD) (odds ratio 2.80; 95% CI: 1.54-5.07; p-value <0.01) and chapped teats (odds ratio 6.07; 95% CI: 1.96-18.76; p-value <0.01) in the milking herd were associated with the presence of ITN at farm-level.

Conclusions: This is the first national questionnaire of ITN within GB and highlights the association of UCD and chapped teats with ITN at farm-level. While there are many limitations and potential bias around farmer questionnaires these findings highlight several key areas for further disease investigation and possible intervention.

Keywords: Bovine; Ischaemia; Necrosis; Questionnaire; Risk factors; Dairy.

UH-39

The use of cabergoline at dry-off reduces the probability of milk leakage and dry-off stress-related behaviours in large commercial dairy herds in Torreón, Mexico

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Objectives: Cabergoline is a dry-off facilitator that reduces milk leakage by 81% and new intramammary infections by 21% (Hop et al, 2019), accelerates mammary gland involution and improves its immune response (Boutinaud et al, 2016, 2017), and improves lying time (Bach et al, 2015). However, no studies to date have investigated the benefits of the use of cabergoline at dry-off on dairy cows under Mexican field conditions. The objective of this study was to investigate, under Mexican field conditions, the effect of one single administration of cabergoline (Velactis®, Ceva Santé Animale, Libourne, France) at dry-off on milk leakage incidence and frequency of dry-off stress-related behaviours.

Materials and methods: The trial was conducted in 2018, and involved 322 Holstein- Frisian dairy cows from 3 modern commercial dairy farm in the area of Torreón. Cows were enrolled in the trial if they were 210 days in gestation and daily milk production was equal or more than 25kg the day before dry-off. All cows were dried off abruptly and randomly assigned to one of two groups: treatment group (n = 161) received a single intramuscular injection of 5.6 mg of cabergoline after last milking at 217 days of gestation (dry period length of 53 days) and the control group (n=161) was dried off at 210 days in gestation (dry period length of 60 days) and left untreated. Cows were observed at 24- and 48-hours following treatment for a period of 2 hours. The incidence of milk leakage and frequency of observation of dry-off stress-related behaviour (agitation, increased vocalization, waiting in front of the gates to the milking parlour, aggressive behaviour) were recorded during both periods. Chi-square test was used to determine if

relation between variables was significant.

Results: The results demonstrated that cows in cabergoline group were less likely to have milk leakage than cows in control group (1.9% vs. 13.4%, P < 0.01). Moreover, cows treated with cabergoline were less likely to express at least one dry-off stress-related behaviour than cows in control group (9.5% vs. 29.8%, P < 0.01).

Conclusions: It is the first time that the impact of cabergoline on milk leakage and dry-off stress-related behaviour is evaluated in Mexican commercial dairy farms. Our data provide evidence that a single injection of cabergoline at dry-off significatively reduces the incidence of milk leakage and stress-related behaviour in dairy cows and therefore improves management, udder health and welfare of the dairy cow.

Keywords: Cabergoline, dry-off, Milk leakage, Mexico, Cow.

UH-40

The impact of herd size and milking technology on milk production in dairy cattle units

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Objectives: The aim of the study was to survey the milking technology, and to assess the relationship between the milking technology, the herd size, and the milk production parameters on commercial dairy farms.

Material and Methods: The milking technology was surveyed by using a questionnaire on 417 Hungarian dairy herds with 177,514 cows in 2017, and it was compared with their official farm milk production data. The surveyed farms were categorized according to their size (Group 1: 1-50, Group 2: 51-300, Group 3: 301-600, and Group 4: >600 cows) and to their milking parlour types (herringbone, parallel, carousel, and others). The relationships were analysed by multivariate linear models, one-way ANOVA, and Fisher's exact test. Pairwise comparisons were performed by Tukey's post hoc tests.

Results: The number of farms was 40 (9.6%) in Group 1, 140 (33.6.0%) in Group 2, 142 (34.0%) in Group 3, and 95 (22.8%) in Group 4. The most commonly used type of milking parlours was the herringbone (296 farms, 71.0%), followed by the parallel (62 farms, 14.9%), the carousel (40 farms, 9.6%), and others (19 farms, 4.6%). Herds with different milking parlour types significantly differed in herd size (p<0.001), except for the parallel vs. other parlour types. The occurrence of parallel and carousel parlour systems decreased with the herd size.

The number of milking stalls per farm increased with the number of cows (p<0.001). Farms with herringbone parlour had significantly smaller number of milking stalls than the farms with parallel (p=0.022) and the carousel (p<0.001) parlours.

Herd size was significantly associated with the number of daily milkings (p<0.001). The number of daily milkings increased with the herd size, 60.0% of the farms with more than 600 cows milked the cows three or four times per day. The type of milking parlour was significantly related to the number of milkings per day (p<0.001). On dairy farms using herringbone parlours the cows were mostly milked two times a day, but where parallel or carousel milking parlour was installed the occurrence of this milking practice diminished.

Herd size was associated with average daily milk production per cow (p<0.001) and the average daily milk yield (p<0.001). As the herd size increased, so did the average daily milk yield (p<0.001) and the average daily milk production per cow (p<0.001). Herd size was significantly related to SCC (p<0.001), with the largest farms having the best quality milk in terms of somatic cell count.

The type of milking parlour was related to the average daily milk yield (p=0.039), and showed a tendency with average daily milk production per cow (p=0.062). Dairy farms using parallel milking parlours had significantly larger average daily milk production per cow (p=0.033) and average daily milk yield (p=0.019), than farms using herringbone parlour. The type of milking parlour tended to be associated with SCC (p=0.061). Herringbone parlours resulted in the highest average SCC, although no significant differences were found between the different parlour types.

Conclusion: The dairy herd size in Hungary has a huge range and large differences can also be seen in the milking technology (e.g. parlour type, number of milking stalls, and daily milkings) and milk production parameters (e.g. daily milk yield, SCC) between farms. However, majority of the surveyed farms still use herringbone parlours, but over 600 cows the parallel and carousel milking systems also play a significant role. Our findings show that the herd size has a greater impact on the milk production parameters than the type of milking parlour. The larger dairy farms may have better and newer housing, feeding conditions, and milking technologies, which could allow for higher milk production, however the average SCC was high in all size groups.

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Keywords: Dairy, milking technology, milking parlour, udder health, milk production.

UH-41

Determining the Cut-off value of Bovine Leukemia Virus proviral load based on the severity of clinical mastitis

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Objectives: Severe bovine mastitis causes considerable economic losses in the dairy industry due to significantly lowered milk production and an increase in cow mortality. Enzootic bovine leukosis (EBL) is caused by the bovine leukemia virus (BLV), and its prevalence in Japan has been steadily increasing. It has been reported that the proviral load (PVL), i.e., the proviral copy number of BLV in the blood of BLV-infected cows increases as the disease progresses, and that the prevalence of BLV tends to be higher on farms where the majority of cows have high PVL. In addition, previous studies have shown that the recurrence of bovine mastitis and morbidity rates were significantly higher on farms with a high prevalence of BLV compared to farms with uninfected cows. Further, other studies have demonstrated a strong association between the severity of clinical mastitis and PVL. Taken together, these findings imply that PVL may be associated with other diseases. Although methods for diagnosing and screening for PVL based on DNA concentration and cell number have been developed, the application of PVL as a criterion for evaluating the risk of clinical mastitis severity has not yet been examined. The purpose of this study was therefore to establish whether PVL values could be used to assess clinical mastitis severity using two quantitative polymerase chain reaction (qPCR) methods. The first method is targeting the BLV pol gene among evaluations based on DNA concentration (DNA-based qPCR), and the second method uses the Coordination of Common Motifs (CoCoMo) algorithm targeting the BLV long terminal repeat (BLV-CoCoMo-qPCR) among the evaluations based on cell number (Cell-based qPCR).

Materials and Methods: Milk and blood samples were collected from a total of 63 lactating Holstein-Friesian dairy cows with clinical mastitis from 13 dairy farms. The severity of clinical mastitis among all cases was classified as mild (milk abnormalities; n=32) or severe (milk abnormalities, udder abnormalities and general symptoms; n=31). DNA-based qPCR and cell-based qPCR were used to determine the PVL at the onset of clinical mastitis. Utilizing receiver operator characteristic curve analysis and the Youden index, and the severity of clinical mastitis as the dependent variable (outcome) and PVL as the independent variable, we estimated PVL cut-off values to estimate the onset of severe clinical mastitis for both the DNA-based qPCR and cell-based qPCR methods. Using cut-off values determined by DNA-based qPCR and cell-based qPCR as classification criteria, we classified PVL into the following three groups: High PVL (above cut-off value), Low PVL (below cut-off value), and Negative (PVL below detectable limits), and evaluated short- and long-term prognoses after the onset of clinical mastitis. Specifically, short-term prognosis was assessed by measuring milk somatic cell count in milk (SCC) on day 1, 7, 14 and 21, and long-term prognosis by estimating 6-year survival rates.

Results: The cut-off value was 18.4 (copies/10 ng DNA) (AUC (area under the curve): 0.731, sensitivity: 0.781, specificity: 0.71) using DNA-based qPCR, and 16,777 (copies/10^5 cells) (AUC: 0.731, sensitivity: 0.531, specificity: 0.903) using cell-based qPCR. Nosignificant differences were observed in either SCC or the 6-year survival rate among the three groups classified using the cut-off value; High-PVL group (DNA- based qPCR; n=34, Cell-based qPCR; n= 21), Low-PVL group (DNA-based qPCR; n=20, Cell-based qPCR; n=32) and Negative group (DNA-based qPCR; n=9, Cell-based qPCR; n=10).

Conclusions: Using DNA- and cell-based qPCR assays to determine PVL cut-off values and then estimate the severity of clinical mastitis was moderately accurate; no difference in accuracy was observed between the methods. The results implied that the cut-off value determined by both methods can be used to evaluate the risk of severe mastitis. In evaluating short- and long-term prognoses, no significant differences were observed among the High-PVL, Low-PVL, and Negative groups. The results suggested that the cut-off value may not be effective for predicting short- or long-term prognoses, but that it can be used as a criterion for evaluating the risk of severe clinical mastitis.

Keywords: Bovine leukemia virus, Cut-off value, Proviral load, Severity of mastitis.

UH-42

Comparison of various diagnostic tests for early diagnosis of mastitis in dairy goats

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Objectives: Subclinical mastitis (SCM) is one of the most important infectious diseases in goats that affect 5-30% goats. Early detection of mastitis followed by preventive activities in controlling the infections becomes of utmost importance. Parameters like somatic cell count (SCC), California Mastitis Test (CMT), electrical conductivity (EC), pH, milk composition and N-acetyl-β-D-glucosaminidase (NAGase) have been evaluated for diagnosis of SCM. SCC has been accepted as the most effective index of mammary inflammation in dairy herds to evaluate yield and quality of milk. The CMT roughly estimates the number of cells of the immune system and epithelial cells in a given milk sample. CMT levels correlate well with SCC levels found in caprine milk. During mastitis, NAGase activity has been found to be reliable for the detection of mastitis pathogen induced IMI. Measurement of electrical conductivity (EC) provides another way to detect mastitis. Concentration of lactose in milk decreases slightly in case of inflammation, making it promising to be applied as an indicator of IMI. If sensitivity and accuracy are the most important factors in mastitis diagnosis methods, the milk NAGase activity test and lactose content in milk together have the highest likelihood to be the most reliable. However, no single method is completely reliable in detecting subclinical mastitis.

Materials and methods: A cross sectional study was carried out involving 200 apparently healthy randomly selected lactating beetal crossbred goats from 10 loose dairy goat flocks across the state. The flocks were visited during morning hours and the Quarter foremilk (QFM) milk samples were aseptically collected. The analysis of milk samples for SCC was done using milk somatic cell counter (DELTA Instrument, The Netherland). Results were expressed in × 10^3cells/ ml. CMT was conducted by as per standard method described by Pandit and Mehta (1969). The EC using Electrical Conductivity Meter (Mettler Toledo), pH by digital pH meter and fat, protein and lactose content in milk using Milk analyser were recorded. The NAGase activity was measured using the spectrophotometric method of Kitchen *et al* (1978) with some modifications. The tests applied for diagnosis were correlated with each other. Sensitivity (SE), Specificity (SP), Likelihood Ratio (LR), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of these tests were evaluated. Discriminating Functional Analysis of the diagnostic tests was done at cut-off points where the tests are having maximum SE and SP to see the efficacy of test to diagnose subclinical mastitis.

Results and Conclusions: The pearson correlation test was applied to find out the correlation among different diagnostic tests. The CMT, lactose content, pH, NAGase and Log_{10}NAGase were significantly ($p < 0.01$) positively correlated with SCC. At cut-off value of 700 and 750 (×10^3 cells/ml), SCC was having comparatively high SE (51.40, 52.00), SP (89.45, 89.19), LR (4.87, 4.81), PPV (0.38 each) and NPV (0.63 each), respectively. The SE, SP, LR, PPV and NPV at 600 (×10^3 cells/ml) were same as those at 700 (×10^3 cells/ml). So, we can consider 650/700 or 750 as most suitable cutoff value to differentiate infected udder halves from non-infected ones. NAGase activity at cut-off value of 25 and 30 (nMoles/ml/min) showed moderately high SE, SP, LR , PPV and NPV. So, we can consider either of these cut-off values as most suitable to diagnose intra-mammary infection. For EC, pH and Lactose the cut-off values 6.4, 6.4 and 4.6, respectively were found most suitable to differentiate infected udder halves from non-infected ones.

At cut-off values of 750 **(**×10^3cells/ml) for SCC, 25nMoles/ml/min for NAGase, 6.4 for EC, 6.5 for pH and 4.4% for lactose, the Discriminating Functional Analysis showed that SCC, NAGase, EC, pH and lactose could be able to discriminate the positive cases with negative ones by 85.16%, 66.36%, 60.82%, 69.23% and 49.94%, respectively.

Keywords: Diagnosis, mastitis, goats, SCC, NAGase.

UH-43

Field trial to compare efficacy of two commercially available *Escherichia coli* J-5 vaccines against clinical coliform mastitis

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Objective: The first 100 days of lactation is a period of physiological transition for dairy cows, critical to the health and production for animals during the current and future lactations. Clinical coliform mastitis during this period has potentially negative impacts on the performance and longevity of a cow in the herd. The use of *Escherichia coli* J-5 bacterins before parturition and in early lactation has been shown to reduce the severity and duration of clinical coliform mastitis. The purpose of the current trial was to compare the efficacies of two commercially available *Escherichia coli* J-5 vaccines for reducing the incidence of clinical coliform mastitis during the first 100 days of lactation.

Materials & Methods: Cows enrolled in the trial were from a commercial herd of Holsteins. Within each projected treatment allocation week, cows were ranked based on their previous lactation milk production, within parity group, and then randomly assigned to one of the two treatment groups. Heifers were randomly allocated only by their electronic identification numbers. Experimental animals within a treatment group were immunized with one of two different commercially available bacterins: either vaccine 1 (Enviracor™ J-5; Zoetis Inc.) or vaccine 2 (Bovilis® J-5; MSD Animal Health Intervet Inc.). Both bacterins were administered at approximately 60 d prior to anticipated calving (day of drying off for cows), approximately 30 d after the primary immunization and on day 14 of lactation. All immunizations were 5 ml subcutaneous approximately 100 mm anterior of the scapula on the neck.

Clinical mastitis was diagnosed by farm personnel and quarter foremilk samples collected for bacteriological analyses prior to antibiotic therapy. The number of clinical cases was determined retrospectively by an investigator blinded to treatment codes using all reports of clinical signs and culture results of milk samples. Dairy-Comp 305 recording system was used to collect milk production following vaccination during lactation, first service pregnancy rates, percentage of pregnancies lost, and mortality and culling data for comparisons between treatment groups.

Results: Health and production data for 506 animals in the vaccine 1 group and 479 in the vaccine 2 group were analyzed. Rate of clinical mastitis caused by coliform bacteria during the first 100 days in milk for animals immunized with vaccine 1 was .0444 cases/ 100 cow-days compared with 0.0183 cases/ 100 cow-days for cows vaccinated with vaccine 2 ($P < 0.05$). Rate of total clinical mastitis also differed between treatments with 0.1138 cases/100 cow-days in vaccine 1 cows and .0690 cases/100 cow-days in vaccine 2 cows ($P < 0.05$). Rates of clinical cases caused by environmental streptococci, coagulase-negative staphylococci, other pathogens and bacteriologically- negative clinical cases did not differ between vaccine treatment groups. Percentage of animals that died or culled in the first 100 days in milk did not differ ($P > 0.05$) between cows immunized with vaccine 1 (4.4%) and vaccine 2 (3.8%). Percentage of cows pregnant after first artificial insemination service was 33.9% for vaccine 1 cows and 35.8% for vaccine 2 cows ($P > 0.05$). Pregnancy loss was 7.1% for cows in the vaccine 1 groups and 5.8% for those in the vaccine 2 group ($P > 0.05$). Daily milk production did not differ between vaccine groups for the two days prior to the immunization on day 14 of lactation, day 14 of lactation, or the three days after the immunization.

Conclusion: The efficacy of vaccines differed in reducing clinical mastitis caused by coliform bacteria during the first 100 days of lactation. Use of vaccine 2 reduced the rate of clinical coliform mastitis 2.4-fold greater than use of vaccine 1. Incidence of death and early culling, first service conception rate, and pregnancy losses did not differ between animals in the two vaccine groups. Milk production did not differ between groups on the days surrounding immunization on day 14 of lactation.

Keywords: Field efficacy trial, vaccination, coliform mastitis, E. coli, J-5 vaccines.

UH-44

Streptococcus agalactiae: new insights about a long-known mastitis pathogen

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Introduction and objectives: *Streptococcus agalactiae* is one of the first pathogens that was described as having harmful consequences for udder health in dairy cows. Until some decades ago it was considered a strictly cow-bound pathogen that was highly contagious within a herd, led to serious consequences with respect to subclinical and clinical mastitis, but was relatively easy to manage when adapting the traditional five point mastitis prevention scheme. At some point in time, in some regions *S. agalactiae* was almost considered eradicated. In recent years, however, several scientific publications indicated the pathogen was still present and even increased in prevalence. Voices from practice in the Netherlands endorse this, while indicating that the pathogen is not as harmful as it used to be in previous times. In our laboratory at GD we found that some *S. agalactiae* isolates had a surprising non-hemolytic character. This led to the question whether one of the most long-known pathogens in dairy mastitis, *S. agalactiae*, had changed over time, and whether enough attention is paid to this pathogen.

Material and methods: In the standard operating procedure (SOP) at GD, bulk milk samples from dairy farmers participating in the voluntary udder health surveillance program are examined on modified Edward's medium (EDW) for the presence of *S. agalactiae*. Based on the apparent changed hemolytic character of some *S. agalactiae* strains combined with the experience that EDW is sometimes difficult to read for the presence of *S. agalactiae*, it was decided to also use *Brilliance* GBS agar (SOP + GBS) for a one year period. Herd-level results on the prevalence of *S. agalactiae* based on the SOP and based on the SOP + GBS were compared. Additionally some management characteristics as well as the antimicrobial usage (AMU) in *S. agalactiae* positive herds based on the SOP, based on the SOP + GBS and *S. agalactiae* negative herds were compared.

Results: Bulk tank milk samples were collected during 10 sampling rounds from a one-year period from August 2020 until July 2021 in on average 1,896 herds, varying from 1,443 to 2,180 herds per sampling round. The prevalence of *S. agalactiae* per sampling round based on SOP + GBS varied from 2.4% to 2.8% of samples. By adding GBS to the SOP, 25% up to 72% more *S. agalactiae* positive samples per sampling round were found, indicating GBS has a significant added value in *S. agalactiae* diagnosis of bulk milk samples. Comparing 101 herds that were positive for *S. agalactiae* during a one-year period, the pathogen was found on GBS in 93 herds and on EDW in 84 herds at some point in time. In the 93 herds positive on GBS, *S. agalactiae* was also found on EDW in three or more samples in 28 herds. In the 84 herds positive on EDW, *S. agalactiae* was also found on GBS in three or more samples in 51 herds. This indicates GBS seems to have more added value in finding *S. agalactiae* than EDW, but that combining both media is needed to optimize sensitivity.

Disucsion and conclusion: Comparing udder health between herds positive for *S. agalactiae* in the SOP approach to those in the SOP + GBS approach during the first four rounds of the study did not reveal significant differences. Comparison of AMU revealed that total AMU in adult cows as well as intramammary AMU was higher for *S. agalactiae* positive herds than for negative controls, with a lower total and intramammary AMU for herds that were only positive in the SOP + GBS approach than those found in the SOP approach.

In conclusion it seems that, contrary to reports from the start of this century, *S. agalactiae* is still present in modern dairy farms, but may behave less aggressive. Specific attention should be given to its diagnostics, in which *Brilliance* GBS agar has an added value. It may be so that *S. agalactiae* cultured on GBS only behaves different from those also cultured on EDW. This needs further study, including analysis of culture results and possibly genome sequencing.

Keywords: Mastitis, streptococcus agalactiae, diagnosis.

UH-45

Presence of Antibiotic Resistance Genes in Staphylococci Isolated from Bovine Subclinical Mastitis

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Resistance to antimicrobial agents is an important problem in the dairy industry and poses a threat to human health. Antibacterial resistance in staphylococci, especially methicillin resistance, hampers the treatment and control of staphylococcal infections, which are prevalent in dairy cows. The aim of this study was to identify antibiotic resistance genes in staphylococci obtained from cases of bovine subclinical mastitis in three provinces (Burdur, Hatay and Van) in Turkey. In total, 283 isolates (Burdur, *n* = 36; Hatay, *n* = 47; Van, *n* = 200 iso-

lates) were studied. The isolates were first identified as *Staphylococcus aureus* and/or non-aureus staphylococci (NAS) by conventional phenotypic methods, and the species was then confirmed by a multiplex polymerase chain reaction (PCR). A simplex PCR assay was performed to detect antibiotic resistance genes (*mecA*, *mecC*, *aacA-aphD*, *ermA*, *ermB*, *ermC*, *tetK*, *tetM* and *blaZ*). Among the isolates from all three provinces, the *blaZ* gene was the most prevalent antibiotic resistance gene, present in 43 out of 156 (28%) NAS isolates, 27 out of 127 (21%) *S. aureus* isolates and 25% of the all isolates, whereas *tetM* was the most prevalent gene in the Hatay isolates, detected in 64% of all *Staphylococcus* isolates. The *mecA*-gene was present in 10% of the NAS, and in 3% of the *S. aureus* isolates. The *mecC* and *ermA* genes were not detected in any of the isolates. This shows that antimicrobial resistance, as determined by PCR, is common in *Staphylococcus* isolates from mastitis in Turkey, and warrants systematic treatment protocols as well as the implementation of preventative strategies to reduce antimicrobial usage.

Keywords: Bovine mastitis, antimicrobial resistance, Staphylococcus.

UH-46

Effects of selective dry cow therapy approach in an Italian commercial Dairy Farm

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Objective: The dry period in dairy cows is a crucial period for udder health. It provides an opportunity to treat pre-existing intramammary infections (IMI), using intramammary dry therapy, but leave the udder in a high-risk situation of exposure to new IMI. Blanket dry cow therapy (BDCT) consists in treating all quarters of all cows with antimicrobials at the dry off. This therapy was widely used by dairy farmers. Due to changes in epidemiology and increasing antibiotic resistance, efforts are being made to reduce the use of non-essential antibiotic treatments. The aim of this study was to investigate changing in milk's somatic cells count (SCC) values, IMI trend and milk yield between cows treated with selective dry cow therapy (SDCT) and cow treated with BDCT, in order to assess the usefulness of administration of antimicrobial treatment to seemingly healthy udders.

Materials and methods: The selected herd was monitored from October 2020 to September 2021. The enrolling criteria, as described in literature, were no clinical mastitis during previous lactation, average SCC value lower than 200.000 cell/ml along the lactation and no IMI from major pathogens. These data have been taken from dairy herd improvement (DHI) controls of the previous and actual lactation, while the presence of major pathogens was detected at dry-off milk sample. According to literature, as major pathogens, we considered *Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus agalactiae, Streptococcus uberis* and *Escherichia coli*. The enrolled cows were randomly assigned to SDCT group or BDCT group. The animals in SDCT group were treated only with internal teat sealant (Easiseal™, Fatro, Italy) while the cows in BDCT group were treated with intramammary antibiotic treatment (Orbenin extra™, Zoetis, Italy) and internal teat sealant. 10 days after calving, samples of milk from individual quarters was collected from all enrolled cows and the animals were monitored up to 100 lactation days for the onset of clinical mastitis. Data for milk yield have been taken from the first three DHI controls post-partum. Data were collected on a spreadsheet (Excel™) and, about bacteriological and SCC analyses, they were analyzed using SPSS 27.0 statistical software (IBM, SPSS, Armonk, USA). The frequencies of mammary infections, before dry off and post-partum, were compared by $\chi 2$ test, while the SCC, after the assessment of normal distribution of data by means of the Shapiro-Wilk test, was compared by means was compared by a non-parametric U-Mann Whitney test because the data were not normally distributed. Statistical significance was considered for $p<0.05$.

Results: Forty-six cows were included in the study due to meeting the inclusion criteria and 24 cows were assigned to SDTC group and 22 cows to the BDTC. Results showed a non-statistically difference of IMIs incidence in post-partum in the SDTC group in comparison with BDCT group. Within the BDCT group, there was a statistically decrease in post-partum IMIs ($p=0.033$) respect pre-partum IMIs, despite the presence of 3 quarters infected with major pathogens at post-partum milk sampling. No statistically difference was recorded for SCC in both treatments' groups before dry-off and post-partum. Using only SCC values and milk yield coming from DHI controls, we observe a non-significant difference in milk yield, both before dry-off and after calving, for two treatments, while SCC was significantly higher in BDCT group vs SDTC group ($p=0.036$) before dry-off, but non-significant differences were observed in SCC after calving in both experimental groups.

Conclusion: This study confirms that it is possible to apply selective dry off therapy without risk of new infections or increase of SCC at calving, considering cows without IMI from major pathogens and SCC values <200,000 cells/ml in the previous lactation period. Therefore, the use of selective dry-off has been confirmed as an effective method for reduction the use of antibiotics in dairy farms in a One-Health perspective. Moreover, milk yield was not affected by the absence of antibiotic treatment at dry-off.

Keywords: Cattle, Mastitis, Selective dry cow.

UH-47

Barrier Characteristics of Three External Teat Sealants to Prevent Bacterial Penetration Under In Vitro Conditions Using Rubber Calf Feeding Nipples

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The main objective of this study was to evaluate the barrier characteristics of a new external teat sealant for dry cows (Ubera® Dry, Inovet, Arendonk, Belgium) in preventing teat penetration by 3 common major mastitis pathogens (*Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis*) compared to two commercially available external teat sealants via a novel *in vitro* simulation model using rubber calf feeding nipples. All feeding nipples were filled with a sterile cotton plug soaked in sterile Broth Heart Infusion medium and were treated as follows: teat 1 and teat 5 were sealed with Ubera® Dry (Inovet, Arendonk, Belgium), teat 2 and teat 6 were sealed with T-Hexx® Dry (Huvepharma Livestock, Missouri, US), teat 3 and teat 7 were sealed with UDDERgold® Dry (Ecolab Food and Beverage Division, Minnesota, US), and teat 4 and 8 remained unsealed and served as positive and negative controls, respectively. After drying, teats 1 to 4 were immersed in a suspension of *E. coli* (i.e. experiment 1), *Staph. aureus* (i.e. experiment 2) or *Strep. uberis* (i.e. experiment 3) (≥ 1.5 x 10^8 CFU/mL) for 24 hrs whereas teats 5 to 8 were not exposed to one of the bacterial suspensions. All external teat sealants adhered well to the rubber teats. All cotton plugs collected from the teats that were not exposed to either *Escherichia coli, Staph. aureus* or *Strep. uberis* (teats 5 to 8) remained culture-negative except for one (due to contamination). Of the teats that were exposed to the major mastitis pathogens, all cotton plugs collected from the teats dipped with Ubera® Dry and T-Hexx® Dry remained also culture-negative for the mastitis pathogen they were exposed to. The cotton plugs of the teats that were sealed with UDDERgold® Dry while being exposed to *E. coli* and *Strep. uberis*, resulted in positive cultures for the respective bacteria as demonstrated using strain-typing. The cotton plugs collected from the teats that were not sealed with an external teat sealant and that served as positive controls became culture-positive for the mastitis pathogens they were exposed to. In conclusion, Ubera® Dry showed comparable and superior barrier performances against the penetration of *E. coli*, *Staph. aureus* and *Strep. uberis* compared to T-Hexx® Dry and UDDERgold® Dry, respectively, under *in vitro* conditions using a novel simulation model. A large-scale clinical trial is needed to evaluate the adherence, duration of adherence and barrier performances of Ubera® Dry against intramammary infection under field conditions.

Keywords: Transistion management, udder health, external teat sealant.

UH-48

Economic consequences of an evidence-based mastitis therapy concept

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Objectives: The economic significance of targeted evidence-based mastitis treatment protocols is unclear. On the one hand, a greater diagnostic effort is required before therapy, and on the other hand, certain treatments are dispensed with. In a field study in which an evidence-based mastitis therapy system was established on dairy farms, the main economic aspects were recorded to carry out not only a medical evaluation but also an economic assessment.

Material and methods: In a three-year field study, the effects of establishing a targeted mastitis therapy concept based on clinical scoring, the consideration of the animal's individual mastitis history and the application of an on-farm test before therapy selection were examined on 1392 mastitis cases in 5 dairy farms in Northern Germany. All clinical mastitis cases and treatment data were recorded over the entire duration of the study. After analyzing the existing conventional therapy concepts of the farms, the mastitis therapy concept and the on-farm culture mastDecide® were introduced. Three treatment groups were compared: the conventional treatment group – before introducing the new concept (n = 483), the targeted therapy group (n = 506) and the modified targeted therapy group (n = 403), including the cases in which farmers deviated from the therapy concept. The investigated approach did not lead to any change in bacteriological and cytological cure rates, new infection rates or recurrence rates. The two targeted treatment groups hardly differed in terms of cures and treatment effort (P> 0.05).

Results: The application of the test and the implementation of the targeted therapy concept as recommended resulted in a saving of 67.4 % of intramammary antibiotic doses and 63.8 % of systemic antibiotic doses compared to the conventional treatment. Furthermore, this procedure led to a lower application effort (- 56.3 %) and a reduction of milk that could not be delivered (-25.1 %). These savings were offset by increased costs due to the on-farm tests used and the time required for using the on-farm test. Included in the cost calculation are: the amount of milk discarded, the time spent on rapid diagnosis and treatment of the animals, the cost of the rapid test and the cost of pharmaceuticals. For the average case of mastitis treated with a targeted treatment concept, the costs in total were lower by 33.20 €. The reduction of costs depended on the pathogen group and amounted to 25.4 % of the costs of standard therapy for Gram-negative microorganisms, 22.9 % for Gram-positive microorganisms and 29.1 % for samples without bacterial growth, respectively.

Conclusion: With the help of the targeted evidence-based mastitis therapy concept, a reduction of antibiotic doses by more than 60 % and of costs by 25.7 % were achieved with unaffected cure rates.

Keywords: Mastitis, treatment, economics, targeted.

UH-49

Impact of mastitis degree and moment of occurrence in milk production in dairy cattle

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Objectives: Mastitis is the disease that causes the most loss in the dairy industry, even though there are already many studies regarding its prevention. Due to its gravity, mastitis can promote early culling animals, high costs with medicines, milk withdraw and decreased milk production and quality. Thus, we aimed to describe the impact of mastitis on milk production, based on mastitis degree and moment of occurrence.

Materials and methods: In this retrospective study, we used data from five dairies in the Netherlands and the databank consisted of 26,228 monthly milk test records from 1,230 cows, from September 2014 to January 2020. Lactations with less than five records were removed from the dataset. Mastitis was considered as affecting milk yields if observed at least 30 days before the milk test day, and was scored as 1) mild mastitis, and 2) acute mastitis. Then, we evaluated the effect of mastitis based on the drop in milk yield following three steps. Firstly, we removed all mastitis records from the data and fitted a wood's curve (WC) for each cow and lactation number. Secondly, we returned the mastitis data to the databank and tested the effect of mastitis in each WC parameters following the model: $MY = (1 + 1 \times Mast) \times Wk^{2 + 2 \times Mast} \times e^{-(3 + 3 \times Mast) \times Wk}$, where 1, 2 and 3 were parameters determined for each cow, 1, 2 and 3 were the effect of mastitis on MY, Mast is the binary occurrence of mastitis, and Wk is the week of lactation. The mastitis score (MS) was tested on all parameters. Lastly, a general WC was fit including in the general WC to estimate the MY loss throughout the lactation. Farm was included as random effect in all models. The model was run using PROC NLMIXED (SAS University edition) and parameters were considered different when $P < 0.05$.

Results: A total of 205 cows had, at some point of their life, a mastitis case recorded. There were 137 cows with mild mastitis and 68 with acute mastitis. Number of lactation when mastitis occurred varied from 1st lactation to 11th lactation. Also, there was a big variability of occurrence among week of lactation (2nd to 43th weeks). The mastitis level did not impact 1 ($P = 0.223$) but did affect 2 ($P = 0.026$) and 3 ($P = 0.047$). Additionally, Mast did impact 1 parameter ($P = 0.001$), thus one equation was fit for each mastitis degree as follows: Mast = 1) $MY = (31.860 + 0.966 \times Mast) \times Wk^{0.162 - 0.075 \times Mast} \times e^{-(0.023 - 0.0056 \times Mast) \times Wk}$; and Mast = 2) $MY = (31.860 + 0.966 \times Mast) \times Wk^{0.162 - 0.143 \times Mast} \times e^{-(0.023 - 0.012 \times Mast) \times Wk}$. Cows with mastitis had lower milk yield at lactation peak when compared with healthy cows. Cows with mastitis grade 1 had a peak production of 34.5 kg/d, and a peak of 32.0 kg/d with mastitis grade 2, while healthy cows had, on average, 37.1 kg/d of milk production at peak. Our analysis revealed that 50% of cows with mastitis grade 1 had a milk yield loss from 2.4 to 5.0 kg/day and cows with mastitis grade 2 had a milk yield loss from 4.1 to 8.0 kg/day. Week of lactation where mastitis occurred had a strong impact in milk production. Highest losses were detected between weeks 10th and 14th of lactation, and percentage of total milk loss during this period was up to 8.5% for mastitis grade 1 and 16.5% for mastitis grade 2.

Conclusions: In conclusion, despite a large variance in milk yield responses to mastitis, we demonstrated that mastitis results in a reduction in milk yield regardless of severity, but this drop is greater in acute mastitis cases. Additionally, the reduction in milk yield due to mastitis is related to the time of occurrence and is greater at weeks 10-14th of lactation, with losses up to 16,5% of milk yield.

Keywords: Mastitis, milk losses, dairy cattle.

UH-50

Negatively controlled, randomized clinical trial comparing label intramammary use of amoxicillin to ceftiofur hydrochloride for treatment of bovine clinical mastitis caused by Gram-positive pathogens

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Objectives: This negatively controlled field trial aimed to compare clinical, microbiological and performance outcomes of label use of a narrow spectrum antimicrobial (amoxicillin) with a wide spectrum antimicrobial (ceftiofur hydrochloride) for treatment of non-severe clinical mastitis (CM) caused by Gram-positive bacteria.

Material and methods: After microbiological culture results, lactating dairy cows with non-severe CM (without systemic symptoms) caused by Gram-positive bacteria were assigned to two protocols: AMOX - three infusions with 62.5 mg of amoxicillin (Amoxi-Mast®, Merck Animal Health) performed ~12 hours apart; CEFT - five infusions with 125 mg of ceftiofur hydrochloride (Spectramast®, Zoetis) performed ~24 hours apart; NEG-CTR – quarters assigned to this group did not receive any interventions until five days after diagnosis. Before the onset of the study a randomized list was created to pre-assign all pregnant animals to one of treatment groups; approximately 90% of cows were assigned to one of antibiotic treatment groups (~45% in each protocol) and 10% to the negative control.

Duplicate milk samples taken before treatment (day 0) and on days 3, 5, 8 and 14 ± 3 after enrollment for analysis of milk composition, somatic cell count (SCC), total bacterial count (TBC) and next-generation sequencing of 16S rRNA gene and quantitative PCR (qPCR). Multivariate logistic regression models were created to evaluate dichotomized outcomes such as clinical cure (CC) and bacteriological cure (BC) at 14 ± 3 days after enrollment and quarter-level CM recurrence by the same species up to 90 days after treatment. Repeated-measures analysis of ANOVA was conducted to analyze the effect of treatment on milk production, SCC, composition, TBC, bacterial relative abundance (RA; based on microbiota) and 16S rRNA gene copy numbers (based on qPCR).

Results: A total of 477 quarter-cases of CM were evaluated: 198 assigned to AMOX, 223 to CEFT and 56 to NEG-CTR. The most frequent isolated species were *Streptococcus*

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uberis (60.6%), *Strep. dysgalactiae* (19.3%), *Streptococcus* spp. (8.4%) and non-*aureus* staphylococci (6.1%). The overall CC (based on least square means) was 84.1% for AMOX and 89.0% for CEFT, and no significant statistical difference (*P*=0.15) was observed between groups. Likewise, no difference (*P*=0.10) between groups was observed on the evaluation of BC (AMOX = 58.2%; CEFT = 66.4%). Moreover, we found no statistical differences (*P*>0.05) between treatments when comparing CC and BC according to the mastitis-causing species (i.e., *Strep. uberis*, *Strep. dysgalactiae* or combination of other pathogens). Compared to antibiotic treated groups, quarters assigned to NEG-CTL had higher CFU, 16S rRNA gene copy numbers, and *Streptococcus* RA until day five after enrollment. Moreover, milk samples from NEG-CTL cows had lower fat and lactose contents, and higher total protein than antibiotic-treated cows up to the third test day after enrollment. A substantial reduction of bacterial load (CFU and qPCR) and *Streptococcus* RA was found for quarters receiving antibiotic therapy. Quarters treated with AMOX had higher CFU on days 5, 8 and 14 after enrollment compared to CEFT. In addition, the relative abundance of *Strep. uberis* was higher on day 14 after enrollment for AMOX-treated quarters than for those assigned to CEFT group, which may be related to the duration of treatment. Linear score of SCC (LSSCC) was higher for AMOX-treated cows than for those treated with CEFT in the first test day after CM, but no differences were observed in the second and third test days following CM. Higher milk production was observed for cows assigned to AMOX group compared with CEFT-treated cows until the third test day after enrollment. No significant differences between groups (*P*=0.92) were found on CM recurrence (9.0% for CEFT; 9.3% for AMOX), and on survival of cows in the herd after treatment (*P*=0.51).

Conclusion: Two-day protocol with three intramammary infusions of amoxicillin (narrow spectrum antimicrobial) had similar CC and BC than five administrations (once a day) with ceftiofur hydrochloride (wide spectrum), and no difference was observed on the risk of CM recurrence and cow survival. However, quarters treated with 5-day ceftiofur protocol had higher reduction of milk CFU than quarters treated with amoxicillin up to 14 days after treatment. Antibiotic use remains an indispensable strategy for treatment of Gram-positive bacteria, since untreated quarters remained with high CFU, gene copy numbers and *Streptococcus* spp. RA than quarters receiving any of the antimicrobial protocols.

Keywords: Treatment of clinical mastitis, dairy cows, microbiome, Gram-positive mastitis pathogens.

UH-51

Antibiogram of pathogens isolated from milk of mastitic dairy buffaloes (*Bubalus Bubalis*) of Punjab and association of California Mastitis Test Scores with intramammmary infection status

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Objectives: To determine the antibiogram of pathogens isolated from milk of mastitic dairy buffaloes and to evaluate the California Mastitis Test (CMT) as an indicator of intramammary infection (IMI) in lactating dairy buffaloes.

Materials and methods: A total of 1489 milk samples from 474 mastitic buffaloes were subjected to CMT and microbial culture in Mastitis Laboratory, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Isolates were confirmed by standard biochemical characterization and further subjected to antimicrobial sensitivity testing (AST). A total of 14 antibiotics belonging to 7 groups were used to study the drug resistance pattern. Further, the CMT was evaluated as an indicator of intramammary infection (IMI) in lactating dairy buffaloes using chi square test.

Results and conclusion: Of these, 643 (43.18%) quarters were culturally positive which comprises Coagulase-negative staphylococci (68.90%), Coagulase-positive staphylococci (27.06%), *Streptococcus spp.* (1.24 %) and Gram Negative organisms (2.80%). A total of 846 quarters revealed no growth. A total of 14 antibiotics belonging to 7 groups were used to study the drug resistance pattern. Overall antibiotic sensitivity test revealed highest susceptibility to Ceftriaxone+tazobactum (86.97%) followed by Ceftriaxone+sulbactum (78.91%) and Cefaperazone (73.13%), while least resistance was observed against penicillin (30.00%) followed by ampicillin (31.14%) and amoxicillin (38.81%). Coagulase-negative staphylococci (CNS) were the most frequently recovered bacterial species accounting for 68.90% of all isolates. Out of 54 CNS isolates antibiotic sensitivity testing was done only in 48 isolates, which revealed highest susceptibility to Ceftriaxone+sulbactu followed by Cefaperazone and gentamicin while resistance was observed against penicillin, amoxicillin and ampicillin. An IMI was identified in 43.18% of quarters. For buffaloes without evidence of clinical mastitis, the sensitivity of a CMT score more than or equal to trace in predicting an IMI on a quarter basis was assessed. CMT score with IMI was 1.21±0.076 and without IMI was 0.89±0.047. For quarters without evidence of clinical mastitis, the sensitivity of a CMT score ³ trace in predicting an IMI on a quarter basis was 0.48.

Keywords: Antibiogram, mastitis, buffaloes, AST, CMT.

UH-52

A Randomized Non-Inferiority Study Evaluating the Efficacy of Two Commercially Available Teat Sealants in Dairy Cows

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Minnesota, St. Paul, MN 55108, St. Paul, United States.

Objectives: The primary objective of this study was to compare the efficacy of a new internal teat sealant (ShutOut™, Merck Animal Health, Madison, NJ, USA) (SO) to the current US industry leader (Orbeseal®, Zoetis, Parsippany, NJ, USA) (ORB). This comparison was based on a non-inferiority evaluation of quarter-level new infection risk during the dry period, with secondary comparisons of cure risk, incidence of clinical mastitis at the cow level during the first 120 days in milk, as well as cow-level performance in early lactation based on milk production, somatic cell count (SCC), and risk for removal from the herd.

Materials & Methods: This study was conducted on six commercial dairy farms (two in Iowa and four in Minnesota) and one university dairy (Iowa State University). On all farms, cows were randomly assigned to treatment groups, blocked by farm on the day of enrollment. Eligibility criteria included an expected dry period of 30-90 days, at least three functional quarters, body condition score >2.0 out of 5, and a lameness score <4 out of 5. Cows designated to be culled early in the subsequent lactation were also ineligible. Cows were excluded from enrollment if they had received any antimicrobial treatment within 14 days of dry off.

Study personnel collected aseptic, quarter-level, duplicate samples for routine aerobic culture immediately prior final milking and treatment at dry-off, and again within 14 days of calving, to evaluate risk of new intramammary infection (NIMI) and cure of existing intramammary infection (CIMI). Following milking machine detachment, all quarters received 500 mg of cloxacillin benzathine (Orbenin DC, Merck Animal Health, Madison, NJ, USA) followed by their assigned sealant. Post-milking procedures and dry period care were executed per individual farm protocols. Animal performance was also monitored for 120 days post-calving using Dairy Herd Improvement Association (DHIA) electronic records and owner captured clinical mastitis events as well as culling and death loss. Effect of treatment on dry period CIMI and NIMI in the first 14 DIM were evaluated using generalized linear mixed models.

Results: At dry off, 65.1% of quarters were not infected with no difference between groups (P = 0.16). During the dry period, the least square means new infection risk was SO=0.266 [CI 0.233, 0.303] vs ORB=0.276 [CI 0.243, 0.313], with no difference identified between treatment groups (P = 0.646). In post-fresh samples, least square means of cured infections were also not different between treatment groups SO=0.966 [CI 0.927, 0.984] vs ORB=0.949 [CI 0.902, 0.974] (P=0.259). When evaluating cow-level events within the first 120 days, there was no difference in clinical mastitis risk with a probability of ORB cows developing an infection of 0.122 and 0.115 for SO cows (P=0.74). Risk of culling was also similar between treatment groups with ORB cows experiencing a probability of being affected of 0.111 and SO cows at 0.073 (P=0.699). Finally, death rate did not differ between treatment groups with a probability of 0.019 for ORB treated cows vs 0.022 SO cows (P=0.956).

Conclusions: No difference was identified in quarter-level new infection and cure risks during the dry period in our comparison of two commercially available internal teat sealants. Cow-level events including culling, clinical mastitis, and death within the first 120 DIM also showed no differences between treatment groups.

Keywords: Teat sealant, dry cow therapy, dairy, mastitis.

UH-53

Cabergoline as a tool to reduce the incidence of milk leakage and facilitate dry-off management in large dairy herds. Results in Saudi Arabia

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Objectives: The incidence of milk leakage (ML) has been proven to be a risk factor for acquiring new intramammary infections. Moreover, Zobel *et al*. (2013) have shown a relationship between the milk production at dry-off and the percentage of milk leakage. Usually many farms decide to perform a gradual dry-off procedure in order to decrease the milk production at the time of dry-off. However, this results in losses of milk production, extra time, labour costs and welfare issues due to nutrient restriction and social regrouping. In this study, dry-off procedure was done abruptly to evaluate if cabergoline treatment can facilitate the dry-off procedure and decrease milk leakage. The objective of this study was to investigate the effect of cabergoline (Velactis® Ceva Santé Animale, Libourne, France) on the incidence of milk leakage after dry-off in a large commercial dairy farm in Saudi Arabia.

Materials and methods: 861 dairy cows located in one commercial dairy farm (NADEC dairy farm) in Saudi Arabia were enrolled the day of the dry-off and followed up until 48-52 hours after. All cows were dried abruptly, without changing in feeding or milking frequency. 431 cows were treated with a single intramuscular injection of 5.6 mg cabergoline after last milking and 430 cows didn't receive a treatment and were considered as control group. The animal distribution was done in order to have similar proportion of primiparous and multiparous animals between the two groups (respectively 38.28% and 61.72% for the treated group and 38.37% and 61.67% for the control group). All levels of milk production cows were included if animals met the inclusion criteria. Milk leakage was observed at three different time-points after the dry-off: 20-24, 30-24 and 48-52 hours after. The study was randomized and blinded. The drug administrator was different from the person who did the milk leakage observations. The individual cow was the experimental unit and the quarter was the data collection. Data analysis was performed by using STATA® (version 14.0) software. Number of quarters leaking milk per cow in each group was estimated with corresponding 95% confidence interval.

Results: Overall, the percentage of cows with milk leakage was lower in cabergoline treated (12%) compared with control (49%) cows (P < 0.001). Cows in the control group

were 7.19 times more likely to show milk leakage compared with cabergoline treated cows When comparing the data by parity (adjusted on milk production, visit and teat score), the percentage of cows leaking milk was lower in cabergoline treated compared to control cows, both for primiparous (1% vs. 17%) and multiparous (5% vs. 35%) cows (P < 0.001). Also, the risk of having milk leakage was not statistically different between level of milk_production (adjusted on parity, visit and teat score): percentage of cows leaking milk was lower for cabergoline group compared to control group (P < 0.001) in cows producing <17kg (2% vs. 17%), 17-24.5kg (3% vs. 22%), 24.5-31kg (3% vs. 30%), and more than 31kg (3% vs. 33%).

Conclusions: It is the first time that milk leakage incidence is evaluated in a large and well managed commercial dairy farm in Saudi Arabia. Our data provide evidence that a single injection of cabergoline decreases significantly the percentage of milk leakage after dry-off, reducing risk factors for udder health in both primiparous and multiparous cows and for different levels of milk production. Considering these results, cabergoline can be a useful tool to be used in largest dairy farms to optimize the dry-off management, to decrease the milk production at the time of dry-off and consequently reduce the risk for new intramammary infections after the dry-off.

Keywords: Cabergoline, Dry-off, Cows, Milk leakage.

UH-54

Oral fully oxidized beta-carotene increases spontaneous bacteriological cure and reduces risk of subsequent clinical mastitis in cows with subclinical mastitis

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Objective: To determine if oral supplementation with fully oxidised beta-carotene (OxBC) would improve spontaneous bacteriological cure rate in lactating dairy cows with subclinical mastitis.

Materials and Methods: Cows (n=4 herds) with an elevated somatic cell count (SCC; >200,000 cells/mL) and which had not been treated with antimicrobials or nonsteroidal anti-inflammatory drugs in the preceding 14 days had milk samples collected following aseptic teat end preparation for culture and quarter-level SCC determination. Cows with one or more quarters that met the enrolment criteria (i.e. had quarter-level SCC >200,000 cells/mL and had one or two distinct bacterial species isolated upon culture) were blocked by lactation number (primiparous vs multiparous), ranked on herd test SCC, and randomly assigned to treatment (i.e. 0.3 g of OxBC, in the form of commercial product OxC-beta Livestock 10%, incorporated into 0.5 kg of a cereal based pelletised feed, and fed once daily for 42 days) or control (0.5 kg of the pelletised feed without the OxBC). Milk samples were collected from enrolled quarters at 21 and 42 days after initiation of treatment for microbiology and SCC determination. Cows were observed daily for evidence of clinical mastitis (i.e. presence of flecks or clots in the milk and/or heat and swelling of the mammary gland). Quarters were defined as having undergone spontaneous bacteriological cure if the bacterial species present prior to an initiation of treatment were not cultured in either of the post enrolment milk samples. A multilevel multivariable binary logistic regression model was used to determine the effect of treatment (i.e. OxBC vs control), and including potential covariates such as age, bacterial species (coded as major pathogens i.e. *Staphylococcus aureus* and Streptococcus spp. vs others), days in milk, quarter position within cow (i.e. fore vs rear glands), and pre-treatment quarter level SCC. Quarter level SCC were natural log (ln) transformed before analysis in a generalised linear mixed model with treatment and Day (i.e. Days 0, 21 and 42) and their interaction as fixed effects.

Results: Data from 77 vs 79 cows and 135 vs 128 quarters were available for analysis from the control vs treatment feeding groups, respectively. Treatment groups did not differ in terms of age, breed, cow-composite SCC prior to treatment, days in milk at enrolment, or frequency of bacteria associated with intramammary infection (all P>0.8). More quarters from cows fed OxBC underwent spontaneous resolution of bacterial infection compared with quarters from cows fed the control feed (13.9% (95% CI 4.1-23.7) vs 6.9% (95% CI 4.8-9.1) for quarters from OxBC fed vs control fed cows, respectively; odds ratio = 2.18 (95% CI 1.14-4.17); P=0.02). There was no effect of treatment (P=0.34) or day (P=0.12), nor was there a treatment by day interaction (P=0.17) for the quarter-level ln SCC. Fewer of the quarters in cows fed OxBC had clinical mastitis in the 42 days post initiation of feeding compared with quarters from control fed cows (1/129 (0.78% (95%CI 0.02-4.24)) vs 6/135 (4.44% (95%CI 1.65-9.42)). The odds ratio of mastitis diagnosis in quarters from cows fed OxBC was 0.17 (95%CI 0.03-0.82) relative to quarters from cows fed the control diet.

Conclusions: This study demonstrated that oral supplementation with fully oxidised beta-carotene (OxBC) of dairy cows with subclinical mastitis resulted in a higher spontaneous cure of existing intramammary infection and a reduced risk of subsequent clinical mastitis. The potential mechanism for this effect may be enhanced innate immune response associated with upregulating the numbers of pattern recognition receptors including toll -like receptors 2 and 4 (Johnston *et al.* 2014). This compound provides a non-antimicrobial approach to reducing the prevalence of intramammary infection in dairy herds.

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Keywords: Subclinical mastitis, control, beta-carotene.

UH-55

Farmer´s attitude, level of application and challenges to implement selective dry cow therapy on Spanish dairy farms

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Objectives: The dry period is crucial for udder health in the dairy cow. Indeed, this period is at the same time an opportunity to cure intramammary infections (IMI) and a risk for new IMI (Bradley and green 2004). For this reason, blanket dry cow therapy (BDCT), regardless the cow infection status, was included in the 5-point plan for mastitis control (Neave et al., 1969). However, in the last years, there is an increasing public health concern in EU about antimicrobial resistance. Consequently, a new European veterinary medicines regulation (EU 2019/6) has restricted the prophylactic use of antimicrobials. Hence, BDCT is not allowed anymore on European dairy farms since February 2022.

Selective dry cow therapy (SDCT) involves only treating infected cows with antibiotics at dry-off. Numerous studies (Kabera et al., 2021, Rowe et al., 2020, Swinkels et al., 2021) and previous experience (Vanhoudt et al., 2018) have shown that SDCT is an alternative strategy to reduce total antimicrobials usage (AMU) without negative consequences on udder health. Nevertheless, a good dry cow management (DCM) is crucial for a successful implementation of SDCT (Roodenburg et al, 2020).

In Spain, there is a lack of knowledge about DCM and SDCT implementation in the dairy farms.

Then the purpose of this study is to provide insight into (1) farmer´s knowledge, level of implementation and attitude towards SDCT in Spanish dairy farms just before new EU regulation becomes mandatory, (2) DCM and possible challenge to implement SDCT and (3) the relationship between SDCT, BDCT and the main key performance indicators related with dry cow udder health.

Materials and methods: The study was based on a survey performed to farmers belonging to Dairy Cow National Association (CONAFE) between August and December 2021. It consisted of fourteen questions to collect their knowledge about SDCT and the new regulation, their dry off strategy and DCM. To avoid bias, all the interviewers were previously trained and couldn't be involved in the milk quality of the farm.

A random sample of 450 farms representative of the different farm sizes and regions included in the DHI program were selected. At the end of the survey, we asked them for authorization to use their DHI data to calculate the main KPI related with the dry off period: cows infected at dry-off; new infections rate, cure rate and chronic rate at calving; and percentage of healthy cows at calving.

Results: A total of 401 questionnaires were completed, 390 agree to respond and 364 authorized to share their DHI data. Surprisingly, 39.7% of farmers have not previously hear about SDCT, shortly before it becomes mandatory. This highlights the lack of communication between the different stakeholders involved in the dairy segment. Furthermore, a 33.3% of farmers already knew about SDCT but didn't apply it, mainly due to their fear to worsen their udder health status.

On the other hand, 21.8% of farmers were already applying SDCT. More than a half for two or more years (51.8%) and only a very low percentage just starting in the last 12 months (9.4%). The AMU reduction was very high with a 45.9% of farms treating less than a 25% of cows with antibiotics at dry off.

One of the main challenges to implement SDCT, was related with the high level of production of cows at dry off. Farmers estimate around a 39.7% of cows still produce more than 25 litres at dry off. This feeling was confirmed by DHI data, with 40.7% of cows producing more than 25 liters of milk in the milk control previous to the dry off.

As expected, no statistical differences were found in the main KPI indicators of udder health related with the dry off between SDCT and BDCT farms (19.1 and 18.5% new IMI and 67.1 and 69% cure rate for SDCT and BDCT, respectively)

Conclusions: In conclusion, many Spanish dairy farms will have to get out of their comfort zone and move towards SDCT in a short time. It will be a great challenge for both dairy farmers and vets, with 78% of dairy farms still using BDCT in 2021. Hopefully, as those ones starting with SDCT they will also reach it successfully.

Keywords: Selective dry cow therapy, udder health, farmer attitude, dry cow management.

UH-56

Bacterial identification of milk samples from small family farms in the Pontal do Paranapanema region, State of São Paulo, Brazil

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Objectives: The objective of this study was to identify bacteria from milk samples from cows in rural São Paulo, in the Pontal do Paranapanema region.

Materials and methods: 2,431 samples were obtained from 616 animals from different small family milk productions in the Pontal do Paranapanema region, State of São Paulo, Brazil. The milk samples were cultured on blood agar for 48 hours to observe the growth of bacterial colonies, after which the colonies that grew were stained with the "Gram" staining technique for observation and identification under the microscope.

Results: We observed that from the 2,431 samples, 46.11% (1.121 / 2.431) did not show bacterial growth, 26.28% (639/2431) showed only growth of Staphylococcus sp., 12.58% (306/2431) showed only growth Bacillus sp., 3.82% (93/2431) showed only growth of Corynebacterium sp., 3.16% (77/2431) showed only growth of Enterobacteria, 2.5%

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(61/2431) showed growth of Bacillus and Staphylococcus sp., 0.98% (24/2431) showed only E.coli growth, 0.86% (21/2431) showed only Enterobacter gergoviae growth, 0.7% (17/2431) showed growth of Corynebacterium and Staphylococcus sp., 0.53% (13/2431) showed growth of Corynebacterium and Bacillus sp., 0.37% (9/2431) showed growth of Non-fermenting Gram Negative Bacteria (GNNF), 0.12% (3/2431) presented growth of Enterobacteria and Staphylococcus sp., 0.04% (1/2431) presented Yeasts and Bacillus and E.coli and Staphylococcus sp.

Conclusions: It concluded, we can say that Staphylococcus, Bacillus, Corynebacterium sp. and Enterobacteria are bacterial agents that cause mastitis and play an important role in the health of the mammary gland, in addition to the fact that studies of this type should continue to be carried out, in order to better understand what type of bacteria are affecting the production of family milk in Brazil .

Keywords: Production, bovine, cattle, hygiene.

UH-57

Antimicrobial susceptibility of Staphylococcus sp. isolated from bovine milk samples from family dairy farms in the Pontal do Paranapanema region, State of São Paulo, Brazil.

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Objectives: The objective of the study was to determine the antimicrobial susceptibility of Staphylococcus sp. isolated from samples of bovine milk production of small family dairy, located in the Pontal do Paranapanema region in São Paulo, Brazil.

Material and methods: 648 colonies of Staphylococcus sp. obtained from bovine milk samples from small family dairy farms were tested for antimicrobial sensitivity using the disk diffusion test, approved by the Clinical and Laboratory Standard Institute (CLSI). The following antibiotics were used: amoxicillin (25 µg), ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), enrofloxacin (10 µg), streptomycin (10 µg), gentamicin (10 µg), neomycin (30 µg), penicillin (10 IU), sulfazotrim (25 µg) and tetracycline (30 µg).

Results: We observed that 15.43% (100/648) of the colonies found were resistant to eight antibiotics simultaneously: amoxicillin, cephalothin, chloramphenicol, enrofloxacin, streptomycin, penicillin, sulfazotrim and tetracycline. In addition, 29.06% (186/648) of colonies were resistant to five antibiotics simultaneously: amoxicillin, ampicillin, cephalothin, chloramphenicol and penicillin. 9.72% (53/648) of the colonies were resistant to three antibiotics: amoxicillin, ampicillin and cephalothin. 2.62% (17/648) of colonies were resistant only to penciline, 2.31% (15/648) of colonies were resistant only to tetracycline, 1.08% (7/648) of colonies were resistant only to streptomycin , 1.08% (7/648) of colonies were resistant only to neomycin, 0.77% (5/648) of colonies were resistant only to enrofloxacin, 0.77% (5/648) of colonies were resistant only to gentamicin. 0.61% (4/648) of colonies were resistant only to ampicillin. 0.31% (2/648) of the colonies were resistant only to amoxicillin. 0.31% (2/648) of the colonies were resistant only to ceftiofur, 0.15% (1/648) of the colonies were resistant only to enrofloxacin, 0.15% (1/648) of the colonies were resistant only to chloramphenicol and 10.03% (65/648) of colonies were susceptible to all antibiotics tested.

Conclusions: These results indicate that multidrugs resistance to antibiotics is one great problem in Brazil and was caused by the prolonged and indiscriminate use of the same active principle, for this reason, more studies must to be made with the aim to describe a picture of the situation in Brazilian dairy farms.

Keywords: Production, bovine, cattle, hygiene, bacteria.