

Animal Health: Disease Assessment

292 Effect of intrauterine dextrose or iodine infusions on clinical cure and reproductive performance of lactating dairy cows with clinical metritis under certified organic management. M. G. Maquivar*^{1,2}, A. Barragan², J. Velez², H. Bothe², and G. M. Schuenemann¹, ¹Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, ²Aurora Organic Dairy, Platteville, CO.

The objective was to assess the effect of intrauterine infusion of 50% dextrose in water (DEX) or 0.18% povidone-iodine in water (IOD) on clinical cure and pregnancy per AI (P/AI) of lactating dairy cows diagnosed with clinical metritis (MET). Cows (n = 303) from 1 organic dairy herd were screened for MET at 7 ± 3 DIM. Cows with MET (foul-smelling brown-red watery vaginal discharge) were stratified by parity and randomly allocated into 1 of 2 treatments: (1) 1 L of DEX (n = 71) or (2) 1 L of IOD (n = 74). At 26 ± 3 DIM post-therapy, treated cows were re-examined for clinical endometritis (CE; scale 0–3) to assess treatment responses (cured = vaginoscopy score 0 or 1). All cows were subjected to the same reproductive program (estrous detection with tail chalking) and cows displaying signs of standing estrus any time after the voluntary waiting period (45 d) were AI. Body condition score (BCS) was recorded at 7 ± 3 and 26 ± 3 DIM. Pregnancy diagnosis was performed via ultrasonography at 39 ± 3 d post-AI. The proportions of cows that were cured and P/AI to first services were assessed using GLIMMIX. Non-MET cows (n = 158) were included in the analysis. DIM at first service, cervix diameter, BCS at treatment, and rectal temperature at treatment were not different between IOD and DEX groups. The proportion of cured cows tended to be greater for IOD (48.5%) than DEX cows (31.3%; *P* = 0.1). The DIM to first AI was shorter (*P* < 0.05) for non-MET cows (70.3 d) compared with DEX (76 d) or IOD cows (82 d). The DIM to conception was shorter (*P* < 0.05) for non-MET cows (90 d) compared with DEX (105 d) or IOD cows (109 d). IOD treatment tended to improve clinical cure of MET cows. Although non-MET cows had greater P/AI (37.6%; *P* < 0.05), the proportion of P/AI was similar between DEX (16%) and IOD cows (15%; *P* > 0.05). Further investigation is needed to determine the efficacy of intrauterine DEX and IOD therapies for the treatment of MET in organic dairy herds.

Key Words: metritis, dairy cow, organic management

293 Identification of biomarkers in milk for degree of physiological imbalance for lactating dairy cows. K. M. Moyes*¹, J. S. Osorio², V. Bjerre-Harpoth¹, B. M. Damgaard¹, V. M. Thorup¹, T. Larsen¹, J. J. Loor², and K. L. Ingvarsten¹, ¹Aarhus University, Tjele, Denmark, ²University of Illinois, Urbana.

Identification of biomarkers in milk for degree of physiological imbalance (PI), a situation in which physiological parameters deviate from the normal, is needed for early detection of cows at risk for disease thereby improving animal welfare and performance and economic outcome for farmers. A meta-analysis was conducted using 3 separate studies consisting of 265 cows of 2 breeds (Holstein and Jersey) ranging from parities 1 to 5 extending over an entire lactation period (i.e., 0–69 weeks in milk). An index for PI was generated based on weekly plasma glucose, NEFA and BHBA where PI index = [ln(NEFA)] + [ln(BHBA)] – [glucose]. Composite milk samples were collected and analyzed for fat (%), protein (%), lactose (%), somatic cells (cells/mL), isocitrate (mM), free glucose (mM), BHBA (mM), and glucose-6-phosphate (mM). Pearson correlations were generated among the PI index and milk components using SAS. Regression analysis using maximum R-squared was used to identify milk components that best explain variations in degree of PI. PROC MIXED

was used to determine the effect of stage of lactation, parity and breed on variations in milk components. Free glucose in milk explained most of the variation in PI for cows in early lactation whereas isocitrate in milk explained most of the variation in PI for cows in later lactation (i.e., >13 wk in milk). We identified free glucose and isocitrate in milk as potential biomarkers for degree of PI for cows throughout lactation. Cows with a greater degree of PI experienced higher isocitrate and lower free glucose in milk when compared with cows with lower degree of PI. Breed and stage of lactation altered concentrations of free glucose and isocitrate; and free glucose in milk was affected by parity. Further investigations of in-line and real-time measurement of these indicators of degree of PI and the use in individual cow and herd management is warranted.

Key Words: physiological imbalance, biomarker, milk

294 Use of digital infrared thermography and oxidative stress biomarkers as a diagnostic tool to diagnose interdigital dermatitis in sheep. S. Talukder*¹ and P. Celi^{1,2}, ¹The University of Sydney, Narellan, NSW, Australia, ²The University of Melbourne, Parkville, VIC, Australia.

This study reports preliminary data on the use of digital infrared thermography (IRT) and biomarkers of oxidative stress (OS) to diagnose the degree of interdigital dermatitis (ID) lesions in sheep. Interdigital space skin temperatures were obtained from healthy (n = 6) and ID affected (n = 11) crossbred rams with a FLIR T620 series infrared camera and images were analyzed using ThermoCAM Researcher Professional 2.9 software. Interdigital space lesions were scored using a 5 point scoring system (0–4). Blood were sampled from 17 rams and plasma was analyzed for reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and advanced oxidation protein products (AOPP). The degree of OS was estimated by the ratio of ROMs/BAP (U.Carr./μmol/L) multiplied by 100 to give an OS index (OSI). Footrot scores were used to stratify the rams in 3 groups: Group A (0 > 0.5), B (0.5 > 1.5) and C (≥1.5). Changes in OS biomarkers and skin temperature were analyzed by a linear mixed model using Genstat version 14. We observed that skin temperature was significantly higher in rams with ID lesions (*P* < 0.05; Table 1). It was noted that ROMs and OSI biomarkers did not differ significantly between feet with ID lesions; a trend for higher BAP and AOPP concentrations in rams with footrot score > 1.5 was noted. In conclusion, IRT was reliable in detecting elevated temperature associated with ID in sheep.

Table 1. Oxidative stress biomarkers and interdigital space skin temperature in sheep

	Group			SE	<i>P</i> -value
	A	B	C		
ROMs (U.Carr.)	119.7	148.1	118.9	25.5	0.60
BAP (μmol/L)	4012.0	4523.0	3688.0	315.0	0.09
OSI (arbitrary units)	3.0	3.3	3.5	0.1	0.59
AOPP (μmol/L)	17.3	11.9	19.2	2.6	0.08
Max Temp (°C)	35.7 ^b	36.6 ^b	37.0 ^a	0.7	0.04
Min Temp (°C)	30.2 ^b	31.5 ^a	31.2 ^a	0.5	0.02
Average Temp (°C)	33.7 ^b	34.9 ^a	35.1 ^a	0.7	0.05
Footrot score	0.06 ^c	1.3 ^b	2.2 ^a	0.3	0.01

^{a-c}Within rows, means followed by different letters are different at *P* = 0.05.

Key Words: infrared thermography, oxidative stress, footrot

295 A clinical diagnostic scoring system for bovine respiratory disease in dairy calves. W. J. Love*¹, S. S. Aly^{1,2}, P. H. Kass¹, C. M. Drake³, T. B. Farver¹, H. E. Crockford², J. H. Davis², A. L. Van Eenennaam⁴, and T. W. Lehenbauer^{1,2}, ¹*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis*, ²*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, Tulare*, ³*Department of Statistics, University of California, Davis*, ⁴*Department of Animal Science, University of California, Davis*.

Bovine respiratory disease (BRD) is an important source of economic loss in dairy and beef calf-raising operations, but reliable clinical diagnosis in calves remains challenging. The objective of this study was to develop a standardized clinical scoring system to identify BRD cases preweaning. A matched case-control study was performed on a large calf ranch in the San Joaquin Valley. Clinical signs were recorded and pharyngeal and nasal swabs were collected from 1774 Holstein calves between July 2011 and January 2012. Clinically ill calves were pair-matched to healthy calves based on age, source farm, and date. Calves ranged from 23 to 69 d of age. Observed clinical signs included ocular and nasal discharge, cough, head tilt, ear droop, fever, increased respiratory rate and effort, decreased appetite, and diarrhea. Viral PCR tests were performed to detect infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, bovine respiratory syncytial virus (BRSV), and bovine coronavirus. Aerobic bacteria and mycoplasma cultures were also performed. Cases were clinically ill and culture- or PCR-positive for one of the following pathogens: BRSV, unpigmented *Mycoplasma* spp., *B. trehalosi*, *H. somnus*, *M. haemolytica*, or *P. multocida*. Controls were clinically healthy and negative for pathogens. Conditional logistic regression (CLR) models were used to determine the importance of each clinical sign for classifying calves as diseased or healthy while accounting for the matched study design. Ocular and nasal discharge, head tilt, ear droop, cough, and fever were forced in the model. Scores were weighted by their CLR coefficients and summed. Nasal discharge, eye discharge, and fever above 39.2°C were each assigned 2 points, and cough, ear droop, and head tilt were each assigned 4 points. A score of 4 or higher was identified as the optimal cut-off for a BRD case. The scoring system correctly classified 89.0% of the cases and controls with 91.7% sensitivity and 86.9% specificity. While neither clinical signs or culture are reference tests for BRD, this system offers a standardized tool to rapidly identify calves at high risk for BRD.

Key Words: bovine respiratory disease, clinical scoring system

296 Assessment of work shift transition of calving personnel on stillbirth in Holstein dairy cows. A. Hunter*, M. G. Maquivar, S. Bas, J. D. Workman, and G. M. Schuenemann, *Department of Veterinary Preventive Medicine, The Ohio State University, Columbus*.

Risk factors affecting stillbirth varies from farm-to-farm and blanket recommendations often fail when applied to a variety of different herds. The objective was to assess the time of birth around the work shift transition (1 h before and 1 h after) of calving personnel (n = 3) on stillbirth in Holstein cows. Prepartum dairy cows (PRIM, n = 1403; MULT, n = 2467) from one commercial dairy herd were monitored (close-up pen) for imminent signs of birth [appearance of amniotic sac (AS) outside the vulva] and moved to a contiguous maternity pen until birth. Calving ease (scale 1–4), time of birth, single or multiple calves, calf sex, calf presentations (forward or backward), time spent in labor, and stillbirth (born dead or died within 24 h after birth) were recorded. According to farm protocol, assistance was provided to cows without calving progress 80 min after AS appearance or earlier (e.g., to correct malposture). The effect of time of birth around the work shift transition

of calving personnel on stillbirth was estimated using GLIMMIX. Births were characterized for forward (96%) or backward (4%) presentations, breech (1%), and multiple calves (5.1%). The overall proportion of stillbirth was 8%. PRIM cows had greater proportion of stillbirth (11%) compared with MULT cows (6%; $P < 0.05$). PRIM cows had greater proportion of stillbirth (11.4%) around the shift change compared with MULT cows (5%; $P < 0.05$). Cows experiencing difficult birth, calves born in backward presentations, and the time around the shift change of personnel increased the proportion of stillbirth ($P < 0.05$). Dairymen, consultants, and veterinarians often trouble-shoot high prevalence of stillbirth in dairy herds and this process requires constant monitoring and comprehensive assessment of several events. Proactive communication practices among calving personnel, especially around the work shift transition should be considered to reduce the risk of stillbirth under field conditions.

Key Words: stillbirth, calving management, dairy personnel

297 Validation of two diagnostic methods for endometritis in postpartum dairy cows. J. Denis-Robichaud* and J. Dubuc, *Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada*.

The objectives of this observational study were to determine diagnostic criteria for 2 endometritis diagnostic methods and to quantify their effect on subsequent reproductive performance in postpartum dairy cows. Data from 558 Holstein cows (25 herds) enrolled in a randomized clinical trial were used. Cows were examined 34 (± 7) days after parturition for endometritis using a standard cytobrush technique. After collection of the endometrial sample, the cytobrush was rolled on a microscope slide for subsequent cytological evaluation using a microscope and was dipped into 1 mL of sterile water for leukocyte esterase (LE) colorimetric testing (Multistix, Bayer Corporation, Elkhart, IN). Cytology results were recorded as the proportion of polymorphonuclear cells and endometrial cells while LE results were recorded in 5 categories: negative, trace, + (small), ++ (moderate), and +++ (large). The voluntary waiting period for breeding was 50 d. Subsequent reproductive events were recorded up to 250 d after parturition. Diagnostic criteria for endometritis were determined based on the maximal sum of sensitivity and specificity for predicting the risk of pregnancy at 120 d after parturition. The effect of these diagnostic criteria on reproductive performance were quantified using logistic regression and Cox proportional hazard models adjusted for herd clustering effect. Cytological endometritis (CYTO) was defined as $\geq 6\%$ polymorphonuclear cells in endometrial cytology. Endometritis determined by LE testing was defined as colorimetric categories $\geq ++$ (moderate). Prevalence of endometritis based on CYTO and LE disease definitions were 41 and 32%, respectively. Both diagnostic methods were associated with a detrimental impact on first service conception risk (CYTO: Yes = 19.3%, No = 35.4%, $P < 0.01$; LE: Yes = 24.5%, No = 35.9%, $P < 0.01$) and on median time to pregnancy (CYTO: Yes = 158 d, No = 113 d, hazard ratio = 1.27, $P < 0.01$; LE: Yes = 136 d, No = 105 d, hazard ratio = 1.28, $P < 0.01$). These findings suggest that CYTO and LE results can be used to diagnose endometritis in postpartum dairy cows.

Key Words: cytology, endometritis, leukocyte esterase

298 The effects of calving assistance on health, reproduction, and survival of Holstein dairy cows. M. Villettaz Robichaud*¹, D. L. Pearl¹, J. Rushen², A. M. de Passillé², S. M. Godden³, S. J. LeBlanc¹, and D. B. Haley¹, ¹*University of Guelph, Guelph, ON, Canada*, ²*Agriculture and Agri-Food Canada, Agassiz, BC, Canada*, ³*University of Minnesota, St-Paul*.

Difficult calvings are known to have negative effects on dairy cows' uterine health, subsequent reproductive performance, and survival. To avoid calving difficulties, some farms, as a matter of practice, assist all cows at calving. We investigated the effects of giving early obstetrical assistance to cows on their health, behavior and survival. On one large commercial dairy farm, out of 257 Holstein dairy cows (primiparous $n = 84$; multiparous $n = 173$) enrolled in this study, 134 were randomly assigned to receive early assistance and 123 did not receive any assistance for 1 h. All cows had a normally positioned single fetus and pulling using human force alone given 15 min after the first sight of both hooves was provided for cows that received early assistance. Cows that were not assigned for early assistance that had not delivered within 1 h after the first sight of both hooves were assisted. Of the cows intended to not receive assistance, 26% were assisted after 1 h and 23% of the intended early assisted cows, calved before the 15 min waiting time, e.g., without assistance. The incidence of metritis ($n = 53$ cases) and the odds of pregnancy ($n = 134$) or death or culling before 120 DIM ($n = 37$) and odds of calf stillbirth ($n = 7$) did not differ significantly between treatment groups (Multivariable logistic regression: $P > 0.05$). However, when looking the time spent standing measured with pedometers, cows in the early assisted group spent 1 h (± 26 min) more time standing per day during the 10 d following calving compared with unassisted cows (Mixed model: $P = 0.016$). In uncomplicated calvings, there was no difference in health or pregnancy outcomes between cows with systematically given prompt obstetrical assistance and cows given 1 h to calve undisturbed after initial assessment.

Key Words: calving assistance, calving difficulties, metritis

299 Feeding behavior, milk yield, activity, and insulin sensitivity in lame dairy cows. S. Janssen¹, M. Heppelmann¹, U. Meyer², S. Daenicke², and J. Rehage^{*1}, ¹*Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany*, ²*Dept. Animal Nutrition, Friedrich-Loeffler-Institute, Braunschweig, Germany*.

Lame dairy cows often show increased plasma levels of nonesterified fatty acids (NEFA) which may be caused by reduced insulin sensitivity. Thus, aim of the study was to investigate feeding behavior, activity and insulin sensitivity in lame cows. Twenty-one pluriparous lame dairy cows with sole ulcers or white line disease of one hind limb were detected by biweekly lameness scoring (score 2 or 3; scale 0–5) and matched with healthy herd mates (controls) according to parity and days in milk. All cows received functional claw trimming and lame cows claw treatment. Feeding behavior (dry matter intake (DMI), number of trough visits, feeding rate), body weight and milk yield were recorded daily and milk constituents twice weekly from d –7 to d 7 related to the day of lameness detection (d 0). From d0 to d7 pedometers recorded activity and blood samples were collected for analysis of glucose, NEFA, insulin (from which RQUICKI was calculated as a surrogate insulin sensitivity index) and cortisol. Data were analyzed using the GLM procedure for repeated measurements (factors group (G), time and their interaction) of the SAS package. Compared with controls lame cows showed in average longer lying periods (11 vs. 13 h/d, resp., G: $P < 0.01$), spent less time feeding (188 vs. 155 min/d, resp., G: $P < 0.01$), had less trough visits (48 vs. 31 /d; G: $P < 0.05$), and increased feeding rates (116 vs. 143 g/min, resp., G: $P < 0.05$). Daily mean DMI, milk yield and calculated energy balance as well as mean plasma concentrations of glucose, insulin, and cortisol did not differ between control and lame cows. In control compared with lame cows mean plasma levels of NEFA (175 vs. 340 $\mu\text{mol/L}$, resp., G: $P < 0.05$) were lower and RQUICKI significantly higher (0.58 vs. 0.44, resp., G: $P < 0.05$). Increased plasma NEFA levels in cows with early detected mild lameness appear not to be caused by reduced DMI. They

may be an expression of increased fat mobilization induced by reduced insulin sensitivity or of reduced activity and muscular NEFA utilization. It may be also possible that cows with reduced insulin sensitivity are disposed for claw defects.

Key Words: dairy, insulin, lameness

300 Evaluation of milk leucocyte differential diagnosis for selective dry cow therapy. M. Hockett^{*1} and R. Rodriguez^{1,2}, ¹*Advanced Animal Diagnostics, Morrisville, NC*, ²*North Carolina State University, Raleigh*.

This study compared selective dry cow therapy after diagnosis of sub-clinical mastitis by milk leucocyte differential (MLD; Advanced Animal Diagnostics) to blanket treatment. First and second lactation, Holstein cows ($n = 300$) at dryoff (223 ± 3 d in gestation) were assigned to CON ($n = 151$) or SELECT ($n = 149$) by random number and groups were balanced for lactation number. Quarter milk was aseptically collected < 24 h from dryoff (DRY) and analyzed by MLD. All cows in CON group and cows in SELECT group with subclinical infection by MLD in any quarter (TRT) had all quarters treated with cephalixin benzathine. Uninfected cows received no antibiotics (NOTRT). Teats were sealed by Orbeseal and barrier postdip. Quarter milk was aseptically collected from cows 3 (D3) and 10 (D10) days postpartum. At each sampling, SCC was determined and 0.01 mL was plated for culture analysis. Samples were interpreted as infected if: ≥ 1 cfu of major pathogen, ≥ 2 cfu of CNS, or ≥ 5 cfu of *Bacillus* were observed. Statistical analysis was performed using GLIMMIX procedure of SAS to determine differences in quarter-level infection rates and differences in SCC at DRY, D3, D10. SCC did not differ between CON or Select groups at any time or between TRT and NOTRT groups postpartum. Culture positive rates were similar for CON and SELECT at DRY and D10 and infection rates were higher at DRY and D3, but not D10 for TRT compared with NOTRT (Figure 1). This study indicates the use of MLD to diagnose infected quarters and selective treatment of only infected cows reduced the use of antibiotics and resulted in similar infection rates and SCC 10 d post-calving compared with blanket antibiotic therapy.

Table 1. SCC and culture infection rates of CON, SELECT, TRT and NOTRT quarters at DRY, D3, and D10

Group	SCC (cells/mL)			Culture infection rate (%)		
	DRY	D3	D10	DRY	D3	D10
CON	204,396 ^a \pm 20,712	307,230 \pm 31,265	118,394 \pm 17,759	18.5 ^d	13.7 ^e	14.6
SELECT	238,383 ^a \pm 23,373	324,927 \pm 31,183	79,161 \pm 17,930	20.4 ^d	17.0 ^b	11.9
TRT	411,388 ^b \pm 28,634	327,462 \pm 42,608	81,858 \pm 24,157	12.9 ^e	13.3 ^e	10.9
NOTRT	43,214 ^c \pm 30,404	322,747 \pm 45,929	77,238 \pm 25,732	27.1 ^f	20.3 ^b	12.9

Values with different superscripts differ: ^{a,b,c} $P < 0.0001$; ^{d,e,f} $P = 0.01$; ^{g,h} $P = 0.01$.

Key Words: leucocyte, dryoff, selective

301 Development of a statistical model to predict metritis. G. U. Maier^{*1}, J. H. Bittar¹, C. A. Risco¹, N. Martinez², F. S. Lima², L. F. Greco², E. S. Ribeiro², J. E. Santos², M. M. Vercouteren³, and K. N. Galvão¹, ¹*Department of Clinical Sciences, University of Florida, Gainesville*, ²*Department of Animal Sciences, University of Florida, Gainesville*, ³*Department of Animal Health, Utrecht University, Utrecht, the Netherlands*.

Metritis is an important disease of the transition period in dairy cows with an estimated incidence of 10–30%. Cows that develop metritis have

decreased milk yields and fertility, and increased odds of being culled from the herd. An accurate model that can predict incidence of metritis may allow for targeted monitoring and/or implementation of preventive strategies to diminish the negative effects of metritis on performance. The objective of this study was to identify risk factors for metritis and to develop a statistical model to predict metritis in dairy cows from data that can be collected within the first week postpartum. Variables housing type (pasture vs. freestall), breed (Holstein, Jersey or Holstein Jersey Cross), dystocia, retained placenta, twins, stillbirth, and blood serum concentrations of calcium, NEFA, and BHBA at 4 ± 3 DIM were collected from 928 dairy cows from 3 dairies. Probabilities for each animal to develop metritis based on 2 logistic regression models [with = Model1 or without blood metabolites = Model2] were calculated and receiver operating characteristics (ROC) curves constructed. Metritis incidence was 13.1%. Variables retained ($P \leq 0.1$) in both models that

increased the odds of metritis were freestall housing, twin calvings, retained placenta, dystocia, primiparity, Jersey breed and low body condition score as well as increasing blood levels of NEFA for Model 1. The ROC-derived cut-off probabilities to predict metritis were 0.11 (area under the curve (AUC) = 0.87; $P < 0.001$) and 0.12 (AUC = 0.86; $P < 0.001$) for Model 1 and Model 2, respectively. Model 1 and Model 2 had similar ($P = 0.08$) AUC. Model 1 resulted in Sensitivity (Se) and Specificity (Sp) of metritis diagnosis of 82 and 78% (78% accuracy), and Model2 resulted in Se and Sp of 80 and 75% (75% accuracy). Applying Model 2 to a second data set with 309 cows from another dairy resulted in overall accuracy of 71% (Se = 63% Sp = 71%). We concluded that using a probability cut-off derived from a logistic regression model could accurately predict metritis in our data set; however, accuracy was only moderate using a different data set.

Key Words: dairy cow, metritis, statistical prediction model