ABSTRACTS
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POSTER PRESENTATIONS

Animal Health: Mastitis

T1 Dairy herd size and herd expansion are related to dairy cow mortality in Southeastern US dairy herds. G. W. Rogers[1], J. B. Cooper*[1], and J. S. Clay[2], [1]*The University of Tennessee*, *Knoxville*, [2]*Dairy Records Management Systems*, *Raleigh, NC*.

Lactation records from dairy herds in 9 Southeastern states processed through DRMS were utilized to determine the relationship between dairy cow mortality and herd size and between mortality and herd expansion. Data analyzed were from herds with a minimum of 10 years of continuous recording between 1982 and 2005. Binary mortality traits (1=CAR code 6 indicating lactation ending in death versus 0=all other CAR codes) were developed separately for 1816 Holstein herds (2,292,630 lactations) and 268 Jersey herds (282,123 lactations) for lactations 1, 2 and 3 or later. Binary traits were analyzed using a logistic regression model including herd, year of calving, season of calving, age at calving and herd expansion [(maximum average herd size for 4 consecutive years)/(average herd size for first 4 years), as %] or herd size or both herd expansion and herd size. Herd size for Holsteins was categorized as: <100, 100 to 200, 200 to 400, 400 to 700 and >700 cows. Herd size for Jersey herds was categorized as: <100, 100 to 200 and >200 cows. Smaller herd size was associated with lower mortality risk except Holstein herd sizes of 200 to 700 had higher mortality risk than herds with >700 cows. Four herd expansion categories were constructed for Holsteins: <30%, 30 to 200%, 200 to 350% and >350%. In Holstein herds, herd expansion <30% had significantly higher mortality risk than herds that expanded >350%; herds with expansion from 30 to 350% tended to have higher mortality risk than herds expanding >350%. Jersey herd expansion categories were <30%, 30 to 200% and >200%. Jersey herd expansion <200% was associated with lower mortality risk compared with expansion >200%. Intermediate-scale expansion was associated with increased risk of mortality in Holstein herds (compared to >350%) but decreased risk of mortality in Jersey herds (compared to >200%). Including herd size and herd expansion in the model resulted in a more dramatic impact of Holstein herd expansion on mortality with expansion >350% favorably associated with mortality.

Key Words: Dairy Cow Mortality, Herd Size, Herd Expansion

T2 Genetic polymorphism of lactoferrin gene and association with mastitis in Holstein cows. J. B. Cheng[1], J. Q. Wang*[1], D. P. Bu[1], G. L. Liu[1], C. G. Zhang[1,2], X. L. Dong[1,2], H. Y. Wei[1], L. Y. Zhou[1], and K. L. Liu[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*College of Animal Science and Technology of Yangzhou University*, *Yangzhou, China*.

Whether lactoferrin (LF) is a candidate genetic marker for mastitis resistance needs to be studied. In this study, by using the method of sequencing to detect the polymorphisms, we found 6 SNPs in a region of 602bp promoter from 128 dairy cows. In the -241bp and -190bp, there were a T to C mutation and a G to A mutation, and those mutations were first discovered. The others mutations were in -28 bp (A/C), +33 bp (C/G) -131bp (T/C) and -156 bp (A/G), respectively. Analysis of association between the genotypes of LF gene and SCS that reflects mastitis traits was carried out with GLM procedure using the SAS software, and the lactation number and lactation month of cow were also be taken into consideration. Statistical results indicated that SCS was significantly correlated with the month of lactation（$r = 0.222$, Spearman correlation test; $P < 0.05$), and with no strong relationships to the LF genotype and lactation number ($P > 0.05$). Yet, the level of SCS tended to be higher as the lactation number increased. The month of lactation could strongly affect the level of SCS in the milk ($P < 0.05$). In the test group cows, the SCS level increased significantly ($P < 0.01$) from month 1, values of 1.707 to a peak of 3.592 on month 4. A dramatic decline of SCS occurred from month 5 to the month 6 with the value of 2.506, followed by an increase from month 7 to the month 9 ($P < 0.05$). The genotypes and diplotypes all did not have significant effect on SCS. Whether LF gene could be used as a genetic marker of mastitis resistance needs further studies to validate.
Acknowledgement: Research funded by Ministry of Science and Technology (2006BAD12B03).

Key Words: Genetic Polymorphism, Lactoferrin, Mastitis

T3 Photonic plasmid stability of transformed *Salmonella typhimurium* using Stanford Photonic imaging and three plasmid types. K. Moulton*[1], P. Ryan[1], D. Moore[1], S. Laird[1], J. Curbelo[1], D. Lay[2], and S. Willard[1], [1]*Mississippi State University*, *Mississippi State*, [2]*USDA-ARS, Livestock Behavior Research Unit*, *West Lafayette, IN*.

Acquiring a highly stable photonic plasmid in transformed *Salmonella typhimurium* for use in biophotonic studies of bacterial tracking *in vivo* is critical to experimental paradigm development. The objective of this study was to determine the stability of transformed *Salmonella typhimurium*(*S. typh*-lux) using three different plasmids and their respective photonic properties. *S. typh*-lux isolates (porcine) were electroporated with either pCGLS-1, pAK1-lux or pXEN-1 plasmids. *S. typh*-lux was then grown over a 24 h period in LB broth or LB broth (10 ml) plus ampicillin (AMP; 50 μg/ml) and placed in an incubated shaker (37° C). After 24 h, inoculums were placed into a black 96-well plate for imaging (100 μl; n = 8 replicates). Photonic imaging was conducted for 5 sec and emissions quantified (RLU/sec). Inoculums were plated on Brilliant Green agar plates (with and without AMP), colony numbers counted (CFU) and plates imaged for the ratio of non-emitting to emitting colonies. Inoculums were sub-cultured daily from d 0 to 10 with and without AMP selection to determine photonic stability over time for the three plasmids. In the presence of AMP, *S. typh*-lux with the pCGLS-1, pAK1-lux and pXEN-1 plasmids remained 100% emitting over the entire 10-d study. Photon emitters of *S. typh*-lux with pCGLS-1, pAK1-lux and pXEN-1 without AMP selection decreased over time ($P < 0.05$), representing only 11 ± 1%, 35 ± 1% and 43 ± 1%, respectively, of the bacterial population by d 10. Photonic emissions were positively correlated with bacterial concentration ($P < 0.05$) for pAK1lux, pCGLS-1 and pXEN-1 ($r = 0.96$, 0.98 and 0.82, respectively). These data characterize the photon stability properties for *S. typh*-lux transformed with three different photon generating plasmids that may permit real-time *Salmonella* tracking using *in vivo* or *in situ* biophotonic paradigms. [USDA-ARS Biophotonics Initiative # 58-6402-3-0120].

Key Words: *Salmonella*, Biophotonics, Plasmid

T4 Seasonal variation of mortality rate in dairy cows of the Po Valley (Italy). A retrospective study from 2001 to 2006. A. Vitali[1], L. Bertocchi[2], N. Lacetera*[1], U. Bernabucci[1], A. Cuteri[1], M. Guerini[3], and A. Nardone[1], [1]*Dipartimento di Produzioni Animali*, *Viterbo, Italy*, [2]*Istituto Zooprofilattico Sperimentale Lombardia-Emilia Romagna*, *Brescia, Italy*, [3]*Osservatorio Epidemiologico Veterinario Regione Lombardia*, *Brescia, Italy*.

The present study is aimed to analyze seasonal variations of mortality rate in dairy cows. The analysis was carried out in years 2001-2006 and in the geographic area comprised between 44°- 46°.15' latitude north and 8°.30'- 12°.30' longitude east, known as the area of the Po Valley including the regions Lombardia and Emilia Romagna, Italy. This area is characterized by a subcontinental-temperate climate and a high density of dairy herds (approximately 900,000 dairy cows in 45,000 square kilometres). Data were extracted from the Italian Bovine Spongiform Encephalopathy (BSE) database, which provided data of daily mortality of cows older than 24 months. Data on cow populations were provided by the Italian National Institute of Statistics. The standardized mortality ratio (SMR) for each season and for each year was calculated by the ratio of observed and expected deaths (OD and ED, respectively). The ED for each of the 6 years considered in the study were calculated by the product between annual mortality rate and the population of the area, stratified by 4 age classes. Under the hypothesis of Poisson distribution, a 95% confidence interval (CI) was calculated for the SMR and its value was considered statistically significant if the value 1 was outside the confidence range. For all years and regions, the analysis of SMR showed that during summer season the OD was significantly higher than the ED. In summer season OD overcame ED by values ranging from +21% (year 2005, Lombardia) to +60% (year 2003, Lombardia); the corresponding 95% CI were 1.17-1.24 and 1.57-1.64 for the years 2005 and 2003, respectively. Results reported herein indicate the relevance to develop appropriate strategies, including management, feeding, genetic selection and insurance plans, to limit economic losses associated with heat stress in dairy cows.

Key Words: Dairy Cows, Season, Mortality Rate

T5 Monitoring body temperature of postpartum dairy cows using an intravaginal device. R. R. Peters[1], B. Erez*[1], L. A. Bornt[1], F. Siewerdt[1], and M. E. Iager[2], [1]*University of Maryland*, *College Park*, [2]*Mid-Maryland Dairy Veterinarians*, *Hagerstown, MD*.

The first objective of this research was to build a system to continuously monitor body temperature (BT) within vagina of postpartum cows with ability to transfer temperature data cow-side to a palm pilot. A Pfizer EAZI-BREED™ Controlled Internal Drug Release (CIDR®) Cattle Insert was modified to hold a data logger button that monitors BT and wired with electrical connections that could be accessed and connected to palm pilot near the vulva. A second objective of this research was to ascertain that system under study provides BT data similar to rectal temperature (RT). Cows (n=12) were vaginally fitted with a CIDR no earlier than 1 day postpartum. ibuttons were programmed to record BT every 15 min. RT of these cows was taken twice daily at milking time in milking parlor for 10 days. BT and ambient temperature (AT) were collected from Jan to Feb, 2007. AT was from weather station 10 km away. In the initial statistical analysis, temperature data from CIDR and RT taken at similar times were compared. BT (degrees C) averaged 38.6 and 38.9 from ibutton mounted on a CIDR and rectal thermometer, respectively. Because AT and RT had a significant ($P < .0001$) positive correlation (.30) AT was included in regression model. AT did not significantly affect intravaginal temperature. Regression coefficient of RT over CIDR is .71±.04. Correlation coefficient and r-square value between CIDR and rectal thermometer were .75±.05 and .56, respectively. Regression coefficient (.71±.05) indicates that ibutton overestimates BT when temperatures are minimal and underestimates BT when temperatures are maximal. A third objective was to determine efficiency of the CIDR in detecting BT over 39.4 C. With CIDR temperatures uncorrected for AT, CIDR found only 30% of temperatures exceeding 39.4 C. When temperature from CIDR was corrected for AT, threshold temperature of 39.4 C decreased to 39.2 C. With adjusted temperature of 39.2 C, efficiency for detecting fever increased to 65%.

Key Words: Body Temperature, Intravaginal Device, Postpartum Cow

T6 A data exchange format and national database for producer-recorded health event data from on-farm management software. J. B. Cole, D. J. Null*, and L. R. Bacheller, *USDA-ARS-BA-ANRI-AIPL*, *Beltsville, MD*.

There is great interest in producing dairy cows that are healthy and remain in the herd longer. Direct and indirect costs associated with

disease represent a significant expense to producers, and selection for improved health may reduce these costs significantly. Genetic response to selection for improved health based upon breeding values from genetic evaluations of field-recorded traits has been well-documented. That genetic variation is not now being directly utilized for genetic improvement, and several challenges must be overcome before useful tools to this end can be provided. The Animal Improvement Programs Laboratory, in conjunction with industry partners and veterinary experts, has developed a data exchange format (Format 6) for the transfer of health and management data from on-farm record-keeping systems to a national database. A Format 6 record includes detailed cow identification, a health event code, an event date, and an optional detail field. This format can provide the necessary data for research into, and implementation of, genetic evaluations for economically-important health traits. Format 6 was designed to be easily extensible, as demonstrated by the addition of a locomotion score event to the specification in January, 2008. The database and editing systems were tested using 63,423 health events from 23,332 cows provided by Dairy Record Management Systems (Raleigh, NC). The most common disorders reported were mastitis (38%), metritis (16%), and other reproductive problems (15%). Primiparous cows accounted for 38% of records, and rates of occurrence differed by age for some disorders. For example, 61% of dystocia events were for first parity cows, while only 79% of the cows with milk fever were older cows. A total of 3920 individual events were flagged by the edits system, and the most common data errors were calving dates that did not match event dates for dystocia (26%) and calving dates with no matching test day data (72%). Format 6 records are stored in the national dairy database with cow test day data, and may provide valuable information for genetic improvement.

Key Words: Database, Genetics, Health

T7 Dexamethasone administration increased bovine lymphocyte clock gene expression *in vitro* and *in vivo*. S. S. Pozzo*, M. K. Rankin, and T. F. Gressley, *University of Delaware*, *Newark*.

Regulation of circadian rhythms in the brain and peripheral tissues occurs via differential expression of clock genes in response to signals including hormonal profiles. Two experiments were conducted to explore the impact of administration of the synthetic glucocorticoid dexamethasone (dex) on clock gene expression in bovine lymphocytes. In the first experiment, lymphocytes were isolated from 5 Holstein cows and treated *in vitro* with 0 or 1.5 nM dex. The 1.5 nM dex dose suppressed *in vitro* lymphocyte proliferation by approximately 50%. Lymphocyte RNA was harvested after 0, 4, 8, 12, 16, 20 and 24h. Quantitative real-time RT-PCR was used to determine mRNA expression of 8 clock genes (*Bmal1, Clock, Per1, Per2, CK1ε, Cry1, Cry2* and *Rev-erbα*) relative to housekeeping genes *RPS9* and β-*actin*. Expression of *Per1* and *Per2* were affected by time (P<0.01), with peak expression found for both at 4h. *Per1* expression was up-regulated 67% in response to 1.5 nM dex (P<0.01). In the second experiment, 6 Holstein steers averaging 225 kg were injected with either saline or dex (0.1 mg/kg BW) followed by the opposite treatment 1 wk later. Lymphocytes were harvested from blood sampled 0, 4, 8, 12, 16, 20 and 24h following treatment, and PCR was conducted as described for the first experiment. Dex increased expression of *Bmal1* 53% (P=0.06), *Per1* 70% (P<0.01), *Per2* 32% (P=0.09), *CK1ε* 23% (P=0.07) and *Cry1* 47% (P<0.01). Time effects were found for *Clock* (P<0.01) and *Cry1* (P=0.09), peaking at 12 and 16h respectively. Interactions between treatment and time were found for *Per2* (P=0.06), *CK1ε* (P<0.01) and *Cry2* (P=0.03). Relative to the saline treatment, dex increased *Per2* expression 8, 12 and 16h after treatment (P<0.05), *CK1ε* expression 8, 16 and 24h after treatment (P<0.05), and *Cry2* expression 12 and 24h after treatment (P<0.05). Our research demonstrates that dex alters bovine lymphocyte clock gene expression patterns both *in vitro* and *in vivo*. Changes in circadian rhythms may be important in regulating response of the bovine immune system to glucocorticoids.

Key Words: Clock Genes, Lymphocytes, Dexamethasone

T8 Negative energy balance (NEB) alters neutrophil (PMN) gene expression in response to a *Streptococcus uberis* (*S. uberis*) mastitis challenge in lactating dairy cows. K. M. Moyes*, J. K. Drackley, D. E. Morin, R. E. Everts, H. A. Lewin, and J. J. Loor, *University of Illinois*, *Urbana*.

Our objectives were to compare gene expression profiles in PMN during a *S. uberis* mastitis challenge between lactating cows subjected to feed restriction to induce NEB (n=2) and cows fed ad libitum to maintain positive energy balance (PEB; n=5). All cows had composite SCC <200,000 cells/mL prior to the study, and milk from all quarters was bacteriologically negative. NEB cows were feed-restricted to 60% of calculated NE_L requirements for 7 d, whereas PEB cows were fed the same diet for ad libitum intake. After 5 d of feed restriction, one rear mammary quarter of each cow was inoculated with 5,000 cfu of *S. uberis* (strain O140J). Blood PMN were isolated before inoculation and pooled within level of energy balance (NEB and PEB). PMN also were isolated at 12 and 20 h post-inoculation and pooled within cow (i.e., 12 plus 20 h), and then cows were pooled within level of energy balance. A 13,257 oligonucleotide (70-mers) array was used for transcript profiling. Cy3- and Cy5 labelled cDNA from PMN and a reference standard were used for hybridizations (8 microarrays total). Genes with > 2.0-fold change in expression were considered significantly different. In PMN before inoculation, NEB resulted in 76 down-regulated genes by >2.0-fold versus PEB cows. Genes up-regulated by NEB (n=29) were involved in protein metabolism (*USP21* and *FSCN1*); down-regulated genes (n=47) were involved in antigen presentation (*HLA-DXA1*) and elongation of fatty acids (*ELOVL6*). During infection, 175 genes were differentially expressed in NEB versus PEB cows. Genes up-regulated by NEB (n=103) included *OAS1* and *SERTINB4*, which are involved in the immune response. Genes involved in protein metabolism (*PTPRK* and *DPYSL3*), oxidative stress (*GSTA2*), and immune response (*MAP4K4/NIK*) were down-regulated (n=72) in NEB versus PEB cows during infection. Energy balance alters gene expression profiles in blood PMN from cows, both before and during mastitis challenge.

Key Words: Genomics, Energy Balance, Neutrophil

T9 Comparison of minimum inhibitory concentrations of *Staphylococcus aureus* obtained from clinical and subclinical cases of mastitis. L. Oliveira*[1], P. Ruegg[1], H. Langoni[2], and M. D. Apparao[1], [1]*University of Wisconsin*, *Madison*, [2]*FMVZ - UNESP*, *Botucatu, SP, Brazil*.

The objective was to compare minimum inhibitory concentrations (MIC) of selected antimicrobials for *Staphylococcus aureus* isolated from clinical and subclinical mastitis. Duplicate aseptic milk samples were collected from all quarters of cows (n = 381) on commercial

dairy herds, with somatic cell counts (SCC) that exceeded 200,000 cell/ml. Additional duplicate quarter milk samples were obtained from cows identified with clinical mastitis. Pathogens were identified using laboratory procedures as defined by the NMC (1999). Minimum inhibitory concentrations were determined for 12 antimicrobial agents using broth microdilution with a custom extended dilution range (Trek Diagnostics,). MIC values were determined for *Staph aureus* obtained from clinical (n = 48) and subclinical (n = 68) cases of mastitis. SAS 9.1 was used to perform survival analysis based on type of mastitis (clinical or subclinical). Antimicrobial concentrations present in wells of the panel of the susceptibility test were used as "time" in the survival analysis. The event was defined as inhibition of bacterial growth, and isolates that not inhibited at the highest concentration tested were right censored. Kaplan-Meier survival curves of the each antimicrobial were performed for each strata of mastitis (subclinical or clinical). *Staph aureus* were resistant to at least 1 antimicrobial obtained from 9 of the 12 enrolled farms. Of isolates (n = 116), 29 (25%) were resistant to one or more antimicrobial agents. Of isolates, 33.8% (subclinical) and 40.0% (clinical) exhibited resistance to at least 1 antimicrobial. The greatest proportions of resistant isolates (11%) were detected for tetracycline. No resistance was observed for ceftiofur or cephalothin. Mastitis type was associated with MIC of erythromycin and tetracycline. Mastitis type was not associated with the MIC of the other antimicrobials. In this study, antimicrobial resistance was uncommon among the *S. aureus* isolated from bovine mastitis and there was homogeneity among clinical and subclinical isolates.

Key Words: Mastitis, *Staphylococcus aureus*, Sensitivity Test

T10 Comparison of in-vitro MIC's of gram positive pathogens isolated from cases of subclinical and clinical mastitis. M. D. Apparao[1], P. L. Ruegg*[1], A. Lago[2], S. Godden[2], R. Bey[2], R. Dingwell[3], and K. Leslie[3], [1]*University of Wisconsin*, *Madison*, [2]*University of Minnesota*, *St. Paul*, [3]*University of Guelph*, *Guelph, ON, Canada*.

The objective of this study was to compare the in-vitro minimum inhibitory concentrations (MIC) of Gram positive pathogens isolated from cases of subclinical and clinical mastitis. Isolates for this study were obtained from cases of subclinical and clinical mastitis that were enrolled in a multi-site, multi-herd controlled field study conducted in Minnesota, Ontario and Wisconsin. Laboratory procedures were as defined by the NMC (1999) and target pathogens (Gram positive cocci) isolated from milk samples were identified to the species level using the API test (bioMerieux). A broth micro dilution technique employing custom designed panels containing extended dilutions of 12 antimicrobial agents were used to determine the MIC. Statistical analysis was done using Proc Lifetest (SAS 9.1). Gram positive pathogens (n = 187) were isolated from quarter milk samples obtained from clinical (n = 51) and subclinical (n = 136) cases of mastitis. The MIC's were different for isolates obtained from subclinical and clinical mastitis cases for ampicillin, ceftiofur, enrofloxacin, penicillin-novobiocin and tetracycline ($P < 0.05$). Pathogen specific differences in the MIC's of subclinical and clinical mastitis isolates were also observed. The MIC's of penicillin, penicillin-novobiocin and spiramycin were different for cases of subclinical and clinical mastitis caused by coagulase negative staphylococci (n = 121). The MIC's of enrofloxacin, sulphadimethoxine and tetracycline were different for subclinical and clinical mastitis caused by streptococci (n = 26). The MIC's of tetracycline and oxacillin were different for subclinical and clinical cases caused by Staphylococcus aureus (n = 22) and "other" Gram positive mastitis pathogens (n = 18), respectively. The overall and pathogen specific differences in MIC's between subclinical and clinical isolates suggests that nature of infection (subclinical or clinical) should be taken into account while comparing MIC profiles or monitoring resistance of mastitis pathogens.

Key Words: Mastitis, Sensitivity Test, Antimicrobials

T11 Nystatin, pathogen-associated molecular patterns and bovine neutrophil activation. M. Worku* and A. Morris, *North Carolina A&T State University*, *Greensboro*.

Nystatin is an antifungal compound with potent proinflammatory properties, shown to inhibit lipid rafts, Lipid rafts are putative microdomains in the plasma membrane rich in lipids and shown to mediate many signaling events including TLR-4 signaling. A TLR2- and TLR1-dependent process serves as the molecular basis for the pro-inflammatory properties of Nystatin. The objective of this study was to evaluate the effect of Nystatin in LPS mediated TLR signaling and the subsequent proinflammatory cytokine response. Further to determine its effect as a lipid raft inhibitor in bovine neutrophils. Blood was collected from a clinically healthy Holstein Friesian cow. Neutrophils were isolated by differential centrifugation. Isolated Neutrophils were treated with LPS (100ng/Î¼l) in the presence or absence of Nystatin (30Î¼g) for 15 min controls were maintained in PBS. RNA was then isolated using Tri-reagent (SIGMA). The quality and quantity of RNA was determined using the 2100 Agilent Bioanalyzer. Specific primers for CD14, IL1-B, IL-8, TNFa, NRAMP-1 and TLR-4 were used for reverse transcriptase PCR. Amplified products were run on a 2% agarose gel and visualized following staining with ethidium bromide. LPS treated Neutrophils had low levels of IL-1B expression. Expression of TNF-Î¬ or TLR-4 or CD14 genes was not observed. Nystatin did not inhibit the expression of IL-8 and Nramp1 genes. Treatment with Nystatin increased expression of the gene encoding IL-8 in both LPS treated and control PMN. Nystatin might have acted through the TLR found on the neutrophil and increased the expression of the gene encoding IL-8. Further studies are needed to ascertain if Nystatin is behaving as pathogen-associated molecular patterns (PAMP) to induce cell activation through TLR, signaling and secretion of cytokines to mediate biologic effects in bovine PMN. Further the results may have implications for the use of "nonimmunologic" drugs that serve as agonists for TLR. The associated activation can result in unintended beneficial and detrimental effects on animal health and well being.

Key Words: Nystatin, Neutrophil, Pro-Inflammatory Cytokine

T12 Macrolide and lincosamide resistance in staphylococci and streptococci isolated from quarters with persistent subclincal mastitis. M. D. Apparao, P. L. Ruegg*, and H. Khatib, *University of Wisconsin*, *Madison*.

The objective of this study was to examine the relationship between occurrence of ermB and ermC and in vitro susceptibility of staphylococci and streptococci isolated from subclinical mastitis. Cows with subclinical mastitis were randomly allocated to a "treatment" or "control" group. CMT positive quarters (n = 213) of cows in the "treatment" group received intramammary treatments using pirlimycin. No treatment was given to quarters (n = 208) of cows in the control group. Aseptic milk samples were collected pretreatment and 3 weeks later. A

"persistent" infection was defined as isolation of the same bacterial species from both pretreatment and post treatment milk samples. Persistent infections (54 infections; 108 pathogens) were confirmed using a PCR based methodology. Susceptibility of persistently infected pathogens was determined using broth micro-dilution. Identification of ermB and ermC was performed using a published PCR protocol. Statistical analysis was carried out using SAS 9.1. An association between presence of ermC and in vitro resistance was observed for both erythromycin and pirlimycin ($P < 0.05$). Of 16 isolates that demonstrated phenotypic resistance to erythromycin, 10 (63%) were positive for ermC whereas 3 (3%) of 92 erythromycin susceptible isolates were positive for this gene. Of 18 isolates that demonstrated in vitro resistance to pirlimycin, 9 (50%) were positive for ermC gene whereas only 4 (4%) of 90 susceptible isolates were positive. No ermB were detected. Of 10 isolates positive for ermC that demonstrated in vitro resistance to erythromycin, 2 were susceptible to pirlimycin, indicating probable induction of ermC gene expression. No association was observed between either treatment or sampling period and presence of ermC. In conclusion, the association between the presence of ermC and in vitro resistance to macrolide and lincosamide antimicrobials suggests that routine screening for ermC may serve as an important tool for surveillance of antimicrobial resistance in bovine mastitis pathogens.

Key Words: Mastitis, Susceptibility Tests, Antimicrobials