BREEDING AND GENETICS: APPLICATIONS AND METHODS— MOLECULAR BIOLOGY

0174 Variation in toll-like receptor genes and susceptibility to clinical mastitis in Holstein cows. C. M. Seabury[1], K. N. Galvao[2], K. Lager[3], and P. J. Pinedo[*4], *[1]Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University System, College Station, [2]Department of Large Animal Clinical Sciences and D. H. Barron Reproductive and Perinatal Biology Research Program, College of Veterinary Medicine, University of Florida, Gainesville, [3]Iowa State University, Extension and Outreach, Ames, [4]Texas A&M AgriLife Research, Amarillo.*

Toll-like receptor proteins (TLRs) recognize conserved pathogen-associated molecular patterns (PAMPS) and initiate signaling pathways that coordinate the innate immune response. The primary objective of this study was to investigate potential associations between bovine single nucleotide polymorphisms (SNPs) and insertion-deletion (indel) mutations occurring in 7 bovine *TLR* genes (*TLR*s 1, 2, 4, 5, 6, 9, and 10) and clinical mastitis (CM) in dairy cows. Clinical mastitis cases were diagnosed by herd personnel in the milking parlor if milk from one or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter, or with generalized illness, and all treatments were recorded in the on-farm software. Cows were considered as cases if they had at least two CM episodes in the current lactation with the first case occurring within the first 100 d after calving. Cows were included in the control group if they had no CM events during the complete lactation. Each case was matched with 3 control herd-mates in the same parity and with a calving date within 2 mo relative to the case cow. The final study population consisted of 686 Holstein cows (269 primiparae; 417 multiparae) in three farms located in Florida and Texas, including 510 cases and 176 controls. Custom allele-specific genotyping assays derived from multiple bovine *TLR* sequencing studies were utilized. Genotypes for 110 loci (SNPs and indels) that are known to be variable in domestic cattle were determined, resulting in 46 monomorphic loci and 64 loci with two alleles. Collectively, 35 loci did not meet our case-control inclusion criterion for minor allele frequency (MAF $\geq$ 0.10). The association between specific *TLR* genotypes and CM was evaluated by logistic regression with evaluation and correction for potentially confounding variables including: year and season of calving; parity; ME 305 d milk yield; and farm. Overall, five SNPs (*TLR2, TLR9*) produced uncorrected *P*-values $\leq$ 0.05 with respect to CM; four of these SNP associations (3 in *TLR2*, 1 in *TLR9*) endured corrections for multiple testing (*P*-values $\leq$ 0.05). Several confounding variables including year and season of calving, and milk yield remained significant after correction for multiple testing. Our analysis of these data suggests that naturally occurring bovine *TLR2* and *TLR9* variation may potentially elicit tangible effects on udder health in Holstein cows.

Key Words: mastitis, toll-like receptors, candidate gene

0175 Experimental intramammary challenge with *Staphylococcus chromogenes* in dairy heifers with specific *CXCR1* genotypes. J. Verbeke*, K. Piccart, S. Piepers, M. Van Poucke, L. Peelman, and S. De Vliegher, *Ghent University, Ghent, Belgium.*

The *CXCR1* gene encodes one of the two receptors for interleukin 8 (IL-8), named chemokine (C-X-C motif) receptor 1 (CXCR1). Foregoing experiments suggested that single nucleotide polymorphism (SNP) *CXCR1*c.980A > G (rs43323012) influences mammary gland immunity and mastitis resistance. To further investigate these findings, 8 mid-lactating Holstein heifers were challenged intramammarily with coagulase-negative staphylococci (CNS) species. Four heifers had genotype c.980AG and 4 heifers had genotype c.980GG. Each heifer was inoculated with 1.0×10^6 colony-forming units (CFU) of *Staphylococcus chromogenes*, originating from a chronically infected quarter. The quarter bacterial count (qBC) and quarter milk somatic cell count (qSCC) were measured at 4, 6, 9, 12, 18, 24, 28, 32, 36, 48, 54, 60, 72, and 78h post inoculation. Additionally, quarter milk production (qMP) was recorded at 12, 24, 36, 48, 60, and 72h post inoculation. Differences in the three outcome variables between c.980AG and c.980GG heifers were analyzed using linear mixed regression models with heifer as random effect and genotype, sampling time and their interaction as fixed effects. None of the quarters/heifers showed symptoms of clinical mastitis. All heifers cleared the inoculated *S. chromogenes* before the end of the trial. No significant differences in qBC and qMP were observed between c.980AG and c.980GG heifers. However, the change of qSCC over time tended to be associated with the heifer's genotype (interaction sampling time × genotype: $P = 0.06$). The increase in qSCC was more pronounced and persistent in c.980AG heifers compared to c.980GG heifers. In conclusion, increase in qSCC following an intramammary challenge with *S. chromogenes* tended to be associated with SNP *CXCR1*c.980A > G. Heifers expressing genotype c.980AG showed a higher immune response than heifers expressing genotype c.980GG.

Key Words: *CXCR1* genotype, *Staphylococcus chromogenes*, intramammary challenge

0176 Association of *CXCR1* gene polymorphisms with incidence rate of clinical mastitis, somatic cell count, and milk production in dairy cattle.
J. Verbeke*, M. Van Poucke, L. Peelman, S. Piepers, and S. De Vliegher, *Ghent University, Ghent, Belgium*.

The objective of this study was to analyze associations between single nucleotide polymorphisms (SNPs) in the *CXCR1* gene (NCBI Gene ID: 100125580), potential genetic markers for mastitis resistance, and the incidence rate of clinical mastitis (IRCM), test-day somatic cell count (SCC) and test-day milk production (MP). Clinical mastitis was monitored on 50 randomly selected Flemish dairy herds for a period of 1 yr. Each case was sampled and cultured according to NMC (National Mastitis Council) guidelines. Incidence rate of clinical mastitis (cases/days at risk) was calculated independently of the culture results and for *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli*, separately. Dairy herd improvement records including SCC and MP were available on 32 herds. A fluorescent multiprobe PCR assay was designed to genotype simultaneously SNP *CXCR1*c.735C > G (rs208795699) and SNP *CXCR1*c.980A > G (rs43323012). In total, 3107 cows were genotyped. Associations between the SNP and (pathogen-specific) IRCM were analyzed using mixed Poisson regression models. Linear mixed regression models were fit to test associations between the SNP and SCC and MP. In total, 681 CM samples were analyzed with *S. uberis* (23% of the culture positive samples), *E. coli* (20%), *S. aureus* (10%), and *S. dysgalactiae* (9%) being the most frequently isolated pathogens. Both SNPs were significantly associated with MP ($P < 0.05$) but not with (pathogen specific) IRCM or SCC. Milk production was higher in c.735GG cows (28.6 kg/day, $n = 571$) compared to c.735CG (28.1 kg/day, $n = 1043$) and c.735CC (28.0 kg/day, $n = 516$) cows. Additionally, MP was higher in c.980GG cows (28.4 kg/day, $n = 1277$) compared to c.980AG cows (27.9 kg/day, $n = 743$). In conclusion, SNP *CXCR1*c.735C > G and *CXCR1*c.980A > G were not associated with the studied udder health traits but were with test-day MP.

Key Words: *CXCR1* genotype, udder health, milk production

0177 *Calpastatin* and μ-*calpain* differ in their control of genotype specific residual variance of beef tenderness in Angus and MARC III steers.
R. G. Tait, Jr.*[1], S. D. Shackelford[2], T. L. Wheeler[2], D. A. King[2], E. Casas[3], T. P. L. Smith[1], and G. L. Bennett[1], *[1]USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, [2]USDA/ARS, Clay Center, NE, [3]USDA. ARS, National Animal Disease Center, Ames, IA*.

Genotype variant effects of *calpastatin* (*CAST*) and μ-*calpain* (*CAPN1*) on mean beef tenderness have been widely characterized. We have tested whether these genetic variants also control residual (non-genetic) variation, and subsequently total phenotypic variation, of tenderness. Observation of rare genotypes is important for understanding the role of these genetic markers on beef tenderness. Two populations at USMARC (Angus and MARC III) were subjected to marker-assisted selection for multiple years to equalize allele (*CAST*) and haplotype (*CAPN1*; CAPN1_316 with CAPN1_4751) frequencies. Within each population, analyses were conducted for 14-d slice shear force (SSF) in steers (Angus, $n = 199$; MARC III, $n = 254$) to estimate genotypic effect size, mode of inheritance, and polygenic effects utilizing 5-generation pedigrees. Beyond the traditional analyses with a single residual variance (Angus, $\sigma_e = 1.79$ kg; MARC III, $\sigma_e = 3.58$ kg), *CAST* and *CAPN1* genotype specific residual variance models were tested. In both populations, *CAST* genotype specific residual variance models fit significantly better (Angus, $P < 0.001$; MARC III, $P < 0.001$) than single residual variance models and were progressive in their effect. Across populations, *CAST* genotype specific residual variance models identified the homozygous tender genotype as having the smallest residual variance (Angus, $\sigma_{e\text{-}TT} = 1.22$ kg; MARC III, $\sigma_{e\text{-}TT} = 2.54$ kg), the heterozygous genotype had intermediate residual variance (Angus, $\sigma_{e\text{-}CT} = 1.99$ kg; MARC III, $\sigma_{e\text{-}CT} = 3.98$ kg), and the homozygous tough genotype had the largest residual variance (Angus, $\sigma_{e\text{-}CC} = 2.82$ kg; MARC III $\sigma_{e\text{-}CC} = 4.86$ kg). In comparison, *CAPN1* genotype specific residual variance models were not as well supported (Angus, $P = 0.05$; MARC III, $P = 0.03$) and in both populations the effects were not progressive, with a heterozygous *CAPN1* genotype (having an intermediate effect on mean) having the smallest residual variance. Effects of *CAST* and *CAPN1* on mean tenderness were maintained under all genotype specific residual variance analyses. These results indicate that beyond changes in the mean, *CAST* also influences phenotypic variation in beef tenderness, which may be important for management and marketing of beef. USDA is an equal opportunity provider and employer.

Key Words: carcass quality, marker effects, tenderness

0178 Investigation of polymorphisms at the *MUC4*, *MUC13*, *MUC20*, and *TFRC* candidate genes for *F4ab/ac* resistance in South African pig populations.
N. S. Chaora*, *Agricultural Research Council, Pretoria, South Africa*.

Selection for *E. coli* F4*ab/ac* resistance has become common due to the increasing resistance of the bacteria to antibiotics. Four candidate genes were studied in three South African breeds, Exotic (Large White, Landrace and Duroc), indigenous and crossbred (Exotic × indigenous), to identify polymorphisms conferring resistance to *E. coli* F4*ab/ac*. A total of 225 pigs aged 3-12 wk were genotyped to target restriction sites in *MUC4*, *MUC13*, *MUC20* and *TFRC* candidate genes. Four polymorphisms of c.8227G > C for *MUC4,* c.576C > T for

MUC13, g.191C > T for *MUC20* and c.291C > T for *TFRC* were detected. The susceptible allele *C* was close to fixation at over 90% in all three breeds for the *TFRC* and *MUC13* loci and there was a genic and genotypic significant difference ($P < 0.05$) amongst breeds for the *TFRC* loci. The resistant *TT* genotype was found in less than 2% of the entire population for the *TFRC* locus and was not found in any pigs for the *MUC13* locus. Both *TFRC* and *MUC13* were not polymorphic in the studied population. The *MUC4* and *MUC20* genes were polymorphic in the population. The resistant alleles *G* for *MUC4* and *C* for *MUC20* were present in the population with the highest frequency observed in the Exotic pigs. There was a significant difference in genotypic distribution amongst breeds at the *MUC20* and *MUC4* loci ($P < 0.05$). An excess of homozygotes in *TFRC* and *MUC20* was observed, leading to a deviation from HWE in the Exotic pigs at these loci. All three breeds were in HWE at the *MUC4* loci although an excess in heterozygotes was observed. The subpopulations at the *TFRC*, *MUC13* and *MUC20* loci were inbred and those at the *MUC4* locus were outbred. There was no significant linkage disequilibrium observed amongst the loci analyzed. The results showed that *MUC4* and *MUC20* were informative and the presence of the resistant alleles makes it possible to use them as markers for selection against susceptibility to F4 *E. coli*.

Key Words: pigs, polymorphisms, *E. coli* F4

0179 Buffalo and cattle sequence diversity and molecular evolution. M. Moaeen-ud-Din* and G. Bilal, *PMAS-Arid Agriculture University, Rawalpindi, Pakistan.*

Identification of genes of importance regarding production traits in buffalo is impaired by a paucity of genomic resources although buffalo genome is sequenced. An alternative to fill this gap is to exploit the plenty of data available for cow, including SNPchips. The cross-species application of comparative genomics tools, i.e., microarrays and comparative sequencing, to identify single nucleotide polymorphisms are potential gear to investigate the buffalo genome. However, both tools are dependent on nucleotide sequences similarity between the two species. Therefore, the objective of this study was to explicate the sequence diversity between cattle and buffalo for comprehending buffalo genome using available cattle genomic resources. In this study, gene diversity between buffalo and cattle was determined by applying 86 gene orthologues taken from NCBI consisting of over 273 kb of aligned sequences using MEGA program V6.0. Results for relative rate test were assessed with the chi-squared test using all available sites (over 273 kb) using MEGA program V6.0. There was approximately 3% difference in all genes in terms of nucleotide diversity; and 0.267 ± 0.134 in amino acids, indicating the possibility for successfully using cross-species strategies for genomic studies. There were significantly higher non synonymous substitutions both in cattle and buffalo. This higher rate of non-synonymous substitutions at similar level in buffalo and cattle indicates a similar positive selection pressure in both species. Results for relative rate test revealed no significant difference in unique mutations between cattle and buffalo lineages at synonymous sites. However, there was a significant difference in unique mutations for non synonymous sites. This indicated that the mutagenic process that generates substitutional mutation is taking place at approximately the same rate at silent sites in cattle and buffalo. However, there was greater variation in mutation rates at non-synonymous sites in both species. Moreover, despite common ancestry, our results indicate a different divergent time among genes of cattle and buffalo. The present study, for the first time, revealed that variable rates of molecular evolution may be present between cattle and buffalo suggesting usefulness in comparative genomics analysis.

Key Words: buffalo, cattle, gene diversity, molecular evolution