P5049 Quantitative trait loci associated with osteochondrosis in Standardbred trotters

Sigrid Lykkjen[1], Nils I. Dolvik[2], Molly E. McCue[3], Aaron K. Rendahl[3,4], James R. Mickelson[5] Knut H. Roed[1].
[1]The Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, Post-box 8146 Dep., N-0033 Oslo, Norway.
[2]The Norwegian School of Veterinary Science, Department of Companion Animal Clinical Sciences, Section for Equine Medicine and Surgery, Oslo, Norway.
[3]University of Minnesota, Department of Veterinary Population Medicine, St Paul, USA.
[4]University of Minnesota, School of Statistics, Minneapolis, USA.
[5]University of Minnesota, Department of Veterinary and Biomedical Sciences, St Paul, USA.
Corresponding author: sigrid.lykkjen@nvh.no

Osteochondrosis (OC), a disturbance in the process of endochondral ossification, is a common and clinically important disease that affects developing joints in animals and humans. The purpose of this study was to identify quantitative trait loci (QTL) associated with osteochondrosis dissecans (OCD) at the intermediate ridge of the distal tibia in Norwegian Standardbred trotters (NST) using the *Illumina* Equine SNP50 BeadChip whole genome single nucleotide polymorphism (SNP) assay. Radiographic data and blood samples were obtained from 464 NST yearlings. Based on the radiographic examination 162 horses were selected for genotyping; 80 cases with an OCD at the intermediate ridge of the distal tibia, and 82 controls without any developmental lesions in the examined joints. When conducting a case-control genome wide association study (GWAS), regions on chromosomes (ECA) 5, 10, 27 and 28 showed moderate evidence of association ($p < 5 \times 10\text{-}5$) with OCD in the tibiotarsal joint. Two SNPs on ECA10 represent the most significant hits ($p = 9.31 \times 10\text{-}7$). Putative QTLs on ECA 5, 10, 27 and 28 represent interesting areas for future research, validation studies and fine mapping of candidate regions. Results presented here represent the first GWAS of OC in horses using the recently released *Illumina* Equine SNP50 BeadChip.

P5050 A simple and efficient method for DNA extraction from low amounts of animal bones

María M. Martinez[1], Lucila Giordana[1], Mónica Costa[1], María Cecilia Ratti[1], María M. Cerruti[1].
[1]Laboratorio de Genética Aplicada, Sociedad Rural Argentina, Juncal 4431, 2º. Buenos Aires, Argentine.
Corresponding author: mmartinez@sra.org.ar

DNA extraction from bones is a routine procedure in service labs doing parentage testing as well as in forensics when there is no other biological material available. In bones, the DNA is present in low amounts, it can also be degraded and the presence of inhibitors has been frequently reported. Therefore, many methods have been described to extract DNA based on processing quite a lot of bone powder to overcome these limitations. We tried different techniques focused on scaling-down the whole procedure. The chosen method uses Dextran-blue as a carrier for removing the PCR inhibitors through a selective ethanol precipitation. It was initially assayed on cattle bone remains. Due to the good quality and high rate of recovered DNA, the starting material can be less than 0.25 ml of powder bone and sample handling can be sized to 1,5 mL tubes. The whole procedure can be completed in a few hours and the DNA is suited for STR amplification. The protocol worked very well for all cattle bones tested, included one boiled for several hours. On equine bones, however, DNA recovery is much lower and highly dependent of the bone. Thus, when Dextran-blue performance is low, we tried another approach starting from thin pieces of bone that were exhaustively decalcificated until material was soft enough to be treated as a cartilage tissue. DNA was then extracted using the DNeasy Blood and Tissue Kit (Qiagen). This technique can also be handled on a reduced scale and completed in a short time.

P5051 A mutation in the mitofusin 2 gene (*MFN2*) is perfectly associated with inherited neuropathy in Tyrolean Grey cattle

Tosso Leeb[1], Birgit Gredler[2], Ursula Reichart[3], Simone Müller[3], Claudia Syring[4], Torsten Seuberlich[5], Anna Oevermann[5], Michael H. Stoffel[6], Mireille Meylan[4], Mathias Müller[3], Johann Sölkner[2] and Cord Drögemüller[1].
[1]Institute of Genetics, University of Berne, Switzerland.
[2]Division of Livestock Sciences, University of Natural Resources and Applied Life Sciences, Vienna, Austria.
[3]Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria.
[4]Clinic for Ruminants, University of Berne, Switzerland.
[5]NeuroCentre, Division of Experimental Clinical Research, University of Berne, Switzerland.
[6]Division of Veterinary Anatomy, University of Berne, Switzerland.
Corresponding author: Tosso.Leeb@itz.unibe.ch

Tyrolean Grey cattle represent a local breed with a population size of ~5000 registered cows. In 2003, a previously unknown neurological disorder was recognized in Tyrolean Grey cattle. The clinical signs of the disorder are similar to those of Weaver in Brown Swiss cattle but occur much earlier in life. The pedigrees of the affected calves suggest monogenic autosomal recessive inheritance and nearly all affected animals trace back to a single female. We studied the neuropathology of this disease and histologic examination of peripheral nerves showed an axonal degeneration. Genotyping 14 cases and 30 controls using the illumina BovineSNP50 BeadChip allowed us to localize the causative mutation to a 3 Mb interval on cattle chromosome 16 by association and homozygosity mapping. Haplotype analysis of 14 additional carriers that were not parents of the genotyped cases narrowed the interval further down to 2 Mb. The *MFN2* gene is located within this interval and encodes a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network. A recessive inherited human axonal neuropathy (Charcot-Marie-Tooth disease-2A2) is caused by *MFN2* mutations. Therefore, we considered *MFN2* a positional and functional candidate gene and performed mutation analysis in affected and control Tyrolean Grey cattle. We did not find any non-synonymous variants in the coding sequence. However, we identified a perfectly associated silent SNP in the coding region of exon 19 of bovine the *MFN2* gene. Marker assisted selection can now be used to eliminate this neuropathy from Tyrolean Grey cattle.

P5052 T_H1 and T_H2 cytokines mRNA levels in Brazilian Somalis crossbreed sheep resistant and suceptible to *Trichostrongylus colubriformis* infection

Lilian G. Zaros[1], Luiz L. Coutinho[2], Andrine M. C. Navarro[3], Maria Rosalba M. Neves[3], Camila L. Benvenuti[3], Lucia H. Sider[4] and Luiz da S. Vieira[4].
[1]Universidade Federal do Rio Grande do Norte – UFRN, Natal, RN, Brazil.
[2]Escola Superior de Agricultura Luiz de Quieroz – ESALQ/USP, Piracicaba, SP, Brazil.
[3]Universidade Estadual Vale do Acaraú – UVA, Sobral, CE, Brazil.
[4]Embrapa Caprinos e Ovinos, Sobral, CE, Brazil.
Corresponding author: lgzaros@gmail.com

Cytokines are proteins that play a central role in immune mechanisms involved in defense against gastrointestinal nematodes infections. The present study used the real-time PCR methodology to quantify Brazilian Somalis crossbreed sheep cytokines (IL-4, IL-13, TNF-α and IFN-γ) in two groups: one resistant and other susceptible to *Trichostrongylus colubriformis* infection. From a Somalis sheep herd, 75 young animals were kept together on pasture without anthelmintic treatment for 4 months. The eight most resistant and the eight most susceptible animals were chosen based on the mean of fecal egg counts and slaughtered for recover the parasites and small intestine tissue samples collection. RT-PCR was performed using the LightCycler PCR and SYBR Green I dye. SDHA (succinate dehydrogenase complex subunit A) was used for normalization and the relative quantification of genes was calculated by REST software. Resistant animals presented lower EPG counts than susceptible animals (1312,5 and 5081,6, respectively; $P<0.0001$) and 3 fold less specimens of *Trichostrongylus colubriformis* ($P<0.05$). Only IL-13 was up-regulated in resistant animals ($P<0.02$) and the other three genes analyzed, IL-4, TNF-α and IFN-γ were down-regulated in this group, although not significantly ($P>0.05$). IL-13 is a cytokine that stimulates the T_H2 response, leading the host to quickly and efficiently respond to the infection and contributes to the parasite expulsion. Although IL-4 acts together with IL-13 in this process, IL-13 is independent to eliminate the parasite for itself. It can be inferred that in the resistant animals a bias of T_H2 type response was activated.