VÚŽV
Institute of Animal Science

Workshop

Immunogenetics and Livestock Breeding for Disease Resistance

Virtual, November 23 - 24, 2021

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Department of Genetics and Breeding of Livestock Species
Institute of Animal Science
Prague

Czech Republic

Workshop schedule

Tuesday, November 23, 2021, CET	Presenter	Affiliation	Title
14.00 - 14.10	Karel Novák	Institute of Animal Science, Prague	Introductory comments
14.10 - 14.50	Dr Sabine Hammer	Institute of Immunology, University of Veterinary Medicine, Vienna	MHC in livestock species and the IPD-MHC database
14.50 – 15.30	Prof Petr Hořín	Faculty of Veterinary Medicine, University of Veterinary Sciences, Brno	Coronaviruses and antiviral resistance in *Felidae*
15.30 – 15.40			break
15.40 - 15.55	Prof Ottmar Distl	University Hannover	Genomic tools to develop footrot resistant sheep
15.55 - 16.35	Prof Anastasia Vlasova	Department of Veterinary Preventive Medicine, Ohio State University, Columbus, USA	Bovine coronaviruses and the associated diseases
16.35 - 16.50	Dr Nidhi Sukhija	National Dairy Research Institute, Karnal, India	Immunological consequences of selective sweeps in Indian Gir and Tharparkar cattle
16.50 - 17.20			Discussion, conclusions

Wednesday, November 24, 2021, CET	Presenter	Affiliation	Title
14.00 - 14.10	Karel Novák	IAS, Prague	Introductory comments
14.10 - 14.50	Prof Romi Pena	University of Lleida	The role of immune-related genes in general resilience in pigs
14.50 – 15.30	Prof Michal Vinkler	Department of Zoology, Charles University, Prague	Overview of the avian TLR
15.30 – 15.40			break
15.40 – 15.55	Karel Novak	IAS Prague	Effects of TLR diversity in Czech Simmental cattle
15.55 – 16.35	Dr Wioleta Drobik-Czwarno	Department of Animal Breeding and Production, Warsaw University of Life Sciences, Warsaw	Mapping of the avian flu resistance
16.35 – 17.15	Prof Seyed Abbas Rafat	Faculty of Agriculture, University of Tabriz	Multi-locus methods in genetic analysis to discover the relationship between phenotype and genotype in resistance to gastrointestinal nematodes in sheep
17.15 – 18.00			Discussion, conclusions

Abstracts

Introductory comments

Karel Novák*

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Why do we think that the link between immunogenetics and breeding is important and is worth our efforts to improve it? The workshop was intended to summarize the current state in this area and to contribute to the relevant knowledge.

In the first place, the organization of the workshop was related to the activities of the International Society for Animal Genetics (ISAG). The society originated from a series of conferences on the animal blood groups and histocompatibility antigens held since 1954. Notably, it was in Prague in 1964 when the society was established and accepted the name European Society for Animal Blood Group Research (E.S.A.B.R.). Subsequently, the scope and name were extended to the International Society for Animal Blood Group Research (I.S.A.B.R.) in Vienna 1972. In Torino in 1988, the society was transformed to the present ISAG. The fact that two key conferences, in 1964 and in 1994, were held in Prague illustrates the perceived importance of animal immunogenetics in the country throughout this period.

From the same beginning of ISAG, the efforts were aimed at the improvement of resistance to the animal diseases. The most straightforward way is to apply the immunogenetic knowledge in breeding. In parallel, the commercial breeding in livestock has evolved into a branch based mostly on statistical models and formal genomic markers. The actual question is whether the efficiency of the formalized system can be increased by adding the functional knowledge. The situation was characterized as a black box strategy in the paper by Boichard et al. (2016) and integrating of biological knowledge with genomic evaluation was suggested.

One of the reactions to the inherent gaps in genomic selection was a systemic search for causative gene variants. Genome-wide association studies have been applied on a large scale, however, only a limited number of causal variants had been identified using GWAS in cattle by the middle of the decade. Projects reflecting this situation were initiated in the Animal Genomics and Improvement Laboratory of USDA in 2017. This switch was paralleled by the development in other teams, as illustrated in the paper by Cai et al. (2018). The authors suggested to prioritize the candidate genes by taking additional information about the biological role of the neighbouring genes. In this way, pure genomic and functional data can be integrated to identify the causal genes. In contrast to increasing the extent of experimental work in the traditional strategy, the new approach is based rather on bioinformatics. It implies improvement in the use of the molecular data on the formation of complex traits, including the immune traits as an important subset.

The present workshop also included the role of animal coronaviruses as a model for the study of antiviral resistance. The last findings confirm the role of host genetic determinants in

the progress of coronavirus infections. Among other, a haplotype on human chromosome 3 has been shown to be associated with critical development upon infection with the respiratory coronavirus 2 (SARS-CoV-2) origin (Ellinghaus et al., 2020) and subsequently assigned to the Neanderthal genome (Zeberg and Pääbo, 2020). In general, one of the earliest observations of coronavirus resistance was published for chicken congenic lines with different susceptibility to the avian bronchitis (Cook et al., 1990). The resistance was due to different MHC-B haplotypes, as shown in 2013 by Banat et al. Obviously, polymorphism in any member of the coronavirus sensing pathway is of interest for the livestock science and the knowledge can be translated into the human immunogenetics, and vice versa. An exhausting review on bovine coronaviruses of cattle as the main livestock species was published by Vlasova and Saif (2021). Although bovine respiratory disease is associated with a number of causal viruses, the contribution of coronaviruses is still not clearly differentiated in veterinary practice.

The genetics of disease resistance in poultry is another topic treated in the workshop, which importance is given by a limited number of causal polymorphisms identified in poultry compared to mammalian species. The exceptions comprise the effect of the *TLR4* variants on salmonellosis in chicken, published already by Leveque et al. (2003), and the effect of *TLR5* and *TLR21* variants (Shaughnessy et al., 2009). There is also a remarkable finding of the role of variation in the interferon-induced transmembrane protein 1 (IFITM1), described in a series of publications spanning from the initial paper by Smith et al. (2015) through the description of the long-term evolution of the locus (Bassano er al., 2019). Moreover, the principal change in the possibilities to control the avian influenza could be provided by the ongoing search for the resistance genes in chicken.

Summing up, the lectures on the workshop demonstrate that there is no simple, ready-to-use solution for the effective utilization of the results of immunogenetics in breeding. Nevertheless, new tools in genomics allow to efficiently integrate the results of functional genetics to the genomic breeding schemes. The necessity to improve the resilience of livestock using genetic tools is taken into consideration in the grant calls of the EU and national project calls. The potential collaboration and coordination of activities can be facilitated on the platform of the ISAG society, due to its tradition and a general scope. To continue the animal health vs. breeding research, a subsequent meeting is to be carried out in 2022, hopefully in person.

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MHC in livestock species and the IPD-MHC database

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Livestock species are a major source of animal protein worldwide. To ensure animal health and food safety, it is essential to prevent infectious diseases via biosecurity and use of well-designed vaccines and therapeutics. Advances in genomics have informed our understanding of the complexity of the immune system and the genes that influence disease and vaccine responses, with the most important being the major histocompatibility complex (MHC). Viral, bacterial and parasitic infections have severe influences on animal welfare and livestock economy. Development of an adaptive immune system to fight off these infections relies on effective activation of T lymphocytes and their recognition of pathogen-derived peptides presented by MHC molecules to T cell receptors (TCR). The highly polymorphic nature of the MHC allows

for the presentation of a wide panel of antigenic peptides and thus influences disease resistance and vaccine responsiveness.

The IPD-MHC Database represents the official repository for non-human major MHC sequences, overseen and supported by the Comparative MHC Nomenclature Committee, providing access to curated MHC data and associated analysis tools. The databases are centred around humans as well as animals important for food security, for companionship and as disease models. IPD-MHC gathers allelic MHC class I and class II sequences from classical and non-classical MHC loci from various non-human animals including pets, farmed and experimental model animals. The IPD project works with specialist groups or nomenclature committees who provide and manually curate individual sections before they are submitted for online publication. Since 2015, the IPD-MHC project has been through a structured programme of improvement, aimed at expanding the content and improving the utility of the database in line with improved sequencing methods and community demand. As a result, the IPD-MHC database has been redesigned to provide a unified resource for the inter- and intra- species comparison of genomic and non-genomic data from different taxonomic groups. This work has been performed in synergy with the Comparative MHC Nomenclature Committee to draft a unified and improved set of guidelines for the allele nomenclature to cover MHC variation at genomic level. The project has grown in both content and impact, now hosting 95 different species and almost 12,000 alleles.

The increasing availability of high quality, manually curated data spurred the need for advanced tools for the analysis and interpretation of allele variation. This included the redesign of existing tools to provide new pathways to access and consume the expanded volume of data. The IPD project has recently introduced a centralized API allowing the programmatic interrogation of the databases, and a more sophisticated extrapolation of the available data. A primer design and virtual PCR tool has recently been integrated, providing a resource for the ad-hoc design of inter- or intra-species, locus-specific or allele-specific probes. Because of the improving the bioinformatic framework, additional species-specific metadata is hosted, including a taxonomic-specific haplotype section. Structural information from homology models will be included for class I MHC alleles, providing insight on the overall structure and the specific impact of variation, including the ability to allow users to analyse and compare peptide-binding pockets. With the latest improvements and expansions, the future of the IPD-MHC database has been secured by increasing the utility of the high-quality data being hosted. To reflect the recent advance of allele sequencing technologies and the increasing demands of novel tools for the analysis of genomic variation, the IPD project is constantly undergoing a progressive redesign and reorganisation.

Key Words:
Livestock, MHC, Comparative Genomics, Polymorphism, IPD, Database, Nomenclature

Genetic susceptibility to viral infections: a feline example

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Host factors can significantly contribute to the pathogenesis of infectious diseases. The host reaction to the presence of pathogens is highly variable. One part of the observed variation is caused by variation of genes underlying host defense mechanisms. Pathogens are considered to be a driving force of evolution, and pathogens leave signatures of purifying as well as diversifying selection in the host genomes. Polymorphisms of immunity-related genes thus reflect evolutionary host and pathogen interactions. As such, they also represent markers of genetic susceptibility and/or resistance to infections. The range of variability of phenotypes related to infectious diseases comprises not only susceptible and resistant individuals, but also those that developed tolerance to the pathogen presence in their organism without clinical symptoms of disease. If they shed the pathogen to their environment, they represent an important epidemiological risk. Like for clinical disease, host genes contribute to the shedder status.

Coronaviruses represent an important group of human and animal pathogens. Some of them are similar but not identical to the human SARS-CoV2. The feline coronavirus (FCoV) is a complex pathogen of domestic cats and other felids. In infected cats, the widespread enteric pathotype (formerly FECV) circulating in population undergoes mutations to a virulent pathotype (formerly FIPV) followed by the activation of immune responses. This is a critical event because susceptible cats develop a fatal clinical disease, feline infectious peritonitis (FIP). While several candidate genes and GWAS regions were associated with FIP, very little is known on the genetics of the shedder status for the enteric pathotype. We have shown that persistent fecal shedding and shedding of high amounts of FCoV particles are associated with two IR genes, *NCR1* and *SLX4IP*. The strongest association was observed for the natural cytotoxicity triggering receptor 1 (NKp46) gene *NCR1*, which is a plausible functional candidate (Bubenikova et al. 2020, 2021). Since FIP also affects other felids and is a cause of deaths in zoos, we have characterized this gene in a panel of felid species. In the proximity of the intronic SNP previously associated with fecal shedding, we have identified codons under negative and positive selection as well as substitutions potentially altering the protein function.

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Genomic tools to develop footrot resistant sheep

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Ovine footrot is a complex disease caused by *Dichelobacter nodosus*, which is clinically characterized by interdigital dermatitis and under-running footrot. The objectives of our research are to exploit genomics to develop a molecular testing system for resistance to footrot in sheep. In a large number of flocks in Germany, we recorded the prevalence of footrot using clinical data and the load of benign and virulent strains of *D. nodosus*. On farm data recording in 208 flocks comprising >12,500 sheep was done using a mobile electronic hand-held system for individual animal ear tags and data input. We employed qRT-PCR to differentiate benign and virulent *D. nodosus* strains for classification of the footrot status of flocks. Based on flock prevalences for *D. nodosus*, we were able to distinguish resistant, tolerant and susceptible animals. Genotyping was done for approximately 4500 sheep on ovine SNP50, ovine GGP50 and ovine Infinium HD SNP beadchips in Merino, Leine, Suffolk and East Friesian for 250-650 animals each and for at least 50-100 animals each for Bentheim, Dorper, Grey Heath, Forest sheep, Romney Marsh, Texel, White Polled Heath, White Hornless Heath and Pomeranian coarsewool. We found heritabilities for resistance to footrot using mixed models with genomic relationship matrices at h2=0.6-0.8 for the different breeds. Heritabilities for footrot scores were at 0.20-0.40. Genome wide association studies (GWAS) with mixed models showed significantly associated regions within breeds. Breeds with highest similarities in genomic relationship matrices were simultaneously analysed using across-breed GWAS. Whole genome sequencing data from 385 individuals were employed to filter for highly associated variants. On whole genome sequences imputed genotyping data allowed us to validate highly associated regions and variants within and across breeds. Characterisation of associated genes was using whole genome sequencing data. Finally, we were able to develop a molecular testing system for footrot resistent sheep.

Bovine coronavirus and the associated diseases

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Bovine coronaviruses (BCoVs) cause respiratory and enteric infections in cattle and different species of farmed and wild ruminants. BCoV is a pneumoenteric betacoronavirus that infects epithelium of the upper and lower respiratory tract and intestine. Variable tissue tropism and the ability to easily cross interspecies barriers are the hallmarks of multiple CoV infections. The major clinical syndromes associated with BCoV include winter dysentery and shipping fever in

older cattle as well as neonatal calf diarrhea. So far, no distinct genetic or antigenic markers have been identified in BCoVs associated with these distinct clinical syndromes. In contrast, like other CoVs, BCoVs exist as quasispecies. High prevalence, occurrence of asymptomatic carriers, high virus titers excreted, and exceptional genomic plasticity are among the major challenges for the control and prevention of BCoV infections. Besides cattle, BCoVs and bovine-like CoVs were identified in various domestic and wild ruminant species (water buffalo, sheep, goat, dromedary camel, llama, alpaca, deer, wild cattle, antelopes, giraffes, and wild goats), dogs and humans. Surprisingly, bovine-like CoVs also cannot be reliably distinguished from BCoVs using comparative genomics. We will summarize the up-to-date information on BCoV pathogenesis, epidemiology, interspecies transmission, immune responses, vaccines, and diagnostics.

Immunological consequences of selective sweeps in Indian Gir and Tharparkar cattle

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The inheritance of neutral mutations with the beneficial mutations is termed as selective sweep. Gir and Tharparkar breeds represent the arid and semi-arid ecotypes of the Indian sub-continent. The search for such sweeps navigated our study on Gir and Tharparkar breeds of cattle at LRC, NDRI, Karnal (India). DNA samples (n=7,7) were sequenced using ddRAD (Double Digest Restriction-site Associated DNA) approach. A total of 13.4 and 12.1 million reads passed the quality control, respectively and showed an alignment of 99.7%, 93.5% and 91.6% in Gir with reference genome of *Bos taurus*, *Bos indicus* and Gir, respectively while 99.87% and 92.13% in Tharparkar with reference assemblies of *Bos taurus* and *Bos indicus*. In Gir, a total of 198952, 182917 and 163349 SNPs while in Tharparkar, a total of 185682, 167092 and 144417 SNPs as compared to *Bos taurus* reference assembly, were identified. A total of 19,127 SNPs, passed the quality control. In all, 191 Selective sweep regions were found by CLR approach in Gir and Tharparkar as well as F_{ST} approach in top 1 percentile of the empirical distribution. A total of 86 and 73 genes can be considered Gir and Thar-specific, respectively. Wright's statistic (F_{ST}) between Gir and Tharparkar was 0.055, i.e., moderate. Gene pathway analysis using PANTHER portal revealed enrichment of genes in pathways for chemokine and cytokine signaling pathway, Wnt signaling pathways and seven other pathways related to immunity. In all, three QTLs were intersected, pertaining to immune role. A total of five genes were traced with role in biological processes of immune system. This study gives an insight into immunologically meaningful variants that shaped the robust bovine genome. These findings may be applied for GWAS after validation in larger population.

The role of immune-related genes in general resilience in pigs

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The concept of resilience refers to the ability of animals to withstand stressors and rapidly recover their production. Although resilience has a genetic component, it cannot be directly measured, a fact that is essential to improve it through selective breeding. However, resilience can be indirectly measured based on productivity-related traits and immunophenotypes. In a current line of research, we aim to elaborate resilience indicators in pigs and to use them to describe genetic variation associated with resilient responses. The ultimate goal of our research is to contribute to the sustainability of production systems by selecting pigs less likely to suffer from external stressors resulting in more robust phenotypes.

In a first experiment, we explored new indicators of resilience in growing pigs. Five batches of commercial Duroc pigs were challenged with an attenuated Aujeszky vaccine at 12 weeks of age. The vaccine was used as a proxy of an infectious challenge, with the advantage of knowing the time and infectious load of each challenge. Under a phase of immune stress, animals redirect nutrients destined for muscle synthesis and growth to the immune system to support increased functionality. Therefore, two resilience indicators were measured: deviation from the expected body weight at 16 weeks of age and the increment of the acute-phase protein haptoglobin at four days post-vaccination. We assumed that resilient pigs would quickly recover their production and show minor deviations of body weight and a minor activation of haptoglobin following the challenge. In contrast, pigs with a depressed production and a high activation of haptoglobin were deemed susceptible. The classification of pigs as resilient or susceptible did not depend on the body weight of the animals and resulted in significant differences also at the end of fattening. A genome-wide association study (GWAS) carried out with resilience indicators data from 445 pigs and 41,165 SNPs identified genomic regions at pig chromosomes 2, 8, 9, 11 (body weight deviation) and 8, 9, 13 (haptoglobilin increments) which explained high proportions of the genetic variance for these traits. Importantly, there was no overlap between these regions and those obtained for the observed body weight at 16 weeks of age. These genomic regions harbour promising candidate genes (*CD6*, *PTGDR2*, *IKZF1*, *RNASEL* and *MYD88*) involved in immune and stress pathways, and in growth signals (*GRB10* and *LCORL)*, which emphasises the strong relationship between resilience and the immune response. The genome sequence of 80 pigs with extreme phenotypes for the resilient indicators is currenty being inspected for variability in the most prominent GWAS regions.

In a second experiment, we are studying the resilience to viral infections in sows. The porcine reproductive and respiratory syndrome virus (PRRSV) causes serious health and productivity problems both in growing pigs and sows. In the later, PRRSV infection raises abortion rate and piglet losses at farrowing. Genetic markers can be useful to identify resilient

sows that can successfully cope with a PRRSV outbreak without compromising their health status or productivity. Using data from a farm of 305 Landrace x Large White sows where a PRRSV outbreak occurred, we investigated the stability of reproductive performance of the sows by comparing the number of losses before and during the PRRSV outbreak. A GWAS study identified 13 genomic regions that contained 44 variants associated with the stable phenotype. These regions showed very little overlap with the genomic regions detected in growing pigs. A preliminary analysis of the 304 genes located in the relevant regions highlighted the relationship of these genes with molecular functions such as binding to RAGE receptors, involved in inflammatory processes, and ephrin receptors, related to embryonic development, angiogenesis and binding to growth factors.

Evolution in avian TLRs: evidence for convergent adaptations

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Many immune genes show prominent coding sequence variation between or within species. But how does this variation shape the immune defence? To answer this question, we can use predictive tools combining evolutionary analysis with structural bioinformatics. In our research we focus on the molecular convergent evolution which may provide insights into functional similarities between different protein variants. Given their structural conservatism, we focus on innate immune receptors, the Toll-like receptors (TLRs), that are involved in the detection of danger signals at the host-pathogen interface. Since birds represent an evolutionary parallel to mammals, and (similar to bats) they serve as key vectors of zoonotic infections, we use namely avian models. In passerine birds as well as in the domestic chicken we show that part of the TLR phenotypic diversity likely evolved in convergence between species and populations. This evidence indicates the functional significance of the molecular variants, opening the possibility for targeted prediction-based testing of relevant genetic variants

Association of variants in anti-bacterial *TLR* with health, milk utility and reproductive traits in Czech Simmental cattle

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Screening was performed in Czech Red Pied (Czech Simmental) cattle for the association of the sequence variants in the anti-bacterial series of *TLR* genes (*TLR1*, *-2*, *-4*, *-5* and -6) with

phenotypic traits comprising milk fat percentage, fat yield, protein percentage and protein yield, total milk yield, somatic cell score, udder health index, milkability, lactation persistency, incidence of cystic ovaries, early reproductive disorders, calving ease, maternal calving ease, production longevity, and calf vitality index. Gene variants were discovered by hybrid resequencing using WGS with Hiseq X-Ten technology and amplicon panel with PacBio RSII. Found polymorphisms were genotyped in 164 bulls using primer extension assays. Associations were detected using one-way ANOVA with subsequent Benjamini-Hochberg tests. Limited or no association with udder health and milk utility traits were observed for 30 polymorphisms tested, in contrast to the expectation. On the other hand, associations were observed between variants in all *TLR*s and reproductive traits, namely for incidence of cystic ovaries and index of early disorders. Two variants of *TLR4* and one of *TLR5* were associated with calving ease. Two SNPs in *TLR4* were associated with production longevity. The association of rs43578094 in *TLR4* with calf vitality index might reflect perinatal risks. Only three SNPs in *TLR5* were associated with milk production traits. The presumed causality was corroborated by supporting evidence. The shared pattern of associations for *TLR1*, *-2* and *-6* can be explained by the interaction of the products in TLR1/TLR2 and TLR6/TLR2 heterodimers. There is a good positional match with the known QTLs for calving ease, namely, (#1681 on chr. 6, #43837 on chr. 8 and #48258 on chr. 16, with *TLR1*/*TLR6*, *TLR4* and *TLR5*, respectively. Consistently, the effects of non-immune functions of *TLR*s on reproductive traits are documented in model species.

Genomics for highly pathogenic avian influenza resistance in chicken

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The influenza virus belongs to the *Orthomyxovirodae* family and consists of negative-stranded RNA, a few proteins, and a lipid envelope composed of two types of glycoproteins, haemagglutinin (H) and neuraminidase (N). So far, several strands of the virus have been identified, with two main groups responsible for avian influenza, LPAI (Low Pathogenic Avian Influenza) and HPAI (Highly Pathogenic Avian Influenza). Especially HPAI, which imposes a huge threat on poultry production and introduces the risk of epidemic in human population is under intense investigation in the recent years. So far it has been controlled mainly with widespread implementation of biosecurity, and in the case of an outbreak, liquidation of flocks and establishment of protection zones. Alternative strategies of combating HPAI include use of vaccines, genetic modification and genetic selection to increase general and specific immunity of birds. These kinds of strategies often require knowledge on the genes involved in immune response to the pathogen. So far, number of genes which may be associated with differences in

response to HPAI between species of poultry and between individuals have been identified. The main attention was focused on genes taking part in innate immune response, which is responsible for preventing the infection, restricting virus replication and spread. At present, Interferon Stimulating Genes (ISG) and RIG-I-like receptors were indicated as the main candidate genes in layer chicken. Proteins coded by genes belonging to BTLN family, defensins and proteins involved in apoptosis were also associated with differences in response to HPAI. In recent years, studies on genetic basis of resistance to HPAI was conducted in layers, focusing on genome-wide detection of differences between survivors and controls. Data from US 2015 and Mexico 2012-2016 HPAI outbreaks enabled better analysis of this problem. So far number of immune response genes have been identified as associated with survival under HPAI, however their specific role in birds response to the virus requires further studies. Results obtained so far showed that resistance to HPAI is a complex trait and can vary between different virus strains and its genetic background can differ depending on virus strains and genetic lines of birds.

Multi-locus methods in genetic analysis to discover the relationship between phenotype and genotype in resistance to gastrointestinal nematodes in sheep

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Multi-locus methods in genetic analysis to discover the relationship between phenotype and genotype in resistance to gastrointestinal nematodes in sheep. One of the strategies against GIN is anthelmintic treatment, but there are some reports about drug resistance of nematodes. Among GIN parasite control strategies, there is use of host genetic resistance. Some hosts have genetic mechanisms to resist GIN. There are three major reasons for selective breeding for parasite resistance: a) helminthiasis, the disease caused by GIN is the most important livestock disease worldwide, b) use of anthelmintic drugs is regarded as unsustainable due to emergence of multiple drug resistant parasites and, c) integrated parasite management strategies including selective breeding is an important long term component objective to reduce the dependence on drugs for control of GIN. In the face of ongoing spread of anthelmintic resistance, there is increasing failure of existing chemical control methods against GIN. McMahon et al., (2017) detected the reduced efficacy of benzimidazole, avermectins and moxidectin treatment in Northern Iceland. So, there are some issues about efficiency of existing anthelmintic methods for control of GIN. Drug resistance to levamisole and albandazole in sheep flocks of Iran have been reported as 66 and 27%, respectively. Moreover, nowadays the environmental problems associated with chemical control of parasites is notably important for consumers (Charlier et al., 2017). Breeding for genetic resistance of the host is among complementary investigated solutions to anthelmintic use (Pretson et al., 2014). The selective breeding for resistant animals

is one of the successful strategies against GIN. The most utilized trait that has been used in classic selection of sheep is faecal egg count (FEC). However, the use of FEC as an effective trait in sheep breeding programs has some limitations. One of the limitations is the necessity of animal infection by GIN and thereafter recording the FEC. Also, measurement of FEC is not very easy. So, interest in marker assisted selection for genetic resistance to GIN is increasing (Atlija et al., 2016). In this lecture, we compare the multi-locus method with the single-molecule method using the same data. Three GWAS methods, General Linear Model (GLM), fixed and random model circulating probability unification (FarmCPU), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) will be presented to identify marker-trait association. GLM is single locus model. Farmcpu and Blink are multi locus models. (Kemper et al., 2011) demonstrated successful prediction for resistance to worms in sheep by multi-locus methods. They demonstrated that methods that use all markers simultaneously can successfully predict the genetic merit of worm resistance. We propose to try more improved genome wide association analysis method such as FarmCPU (Fixed and random model Circulating Probability Unification) and Blink (Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway) in the future studies. Single-locus methods seem to have more false positives than multi-locus methods.