Workshop

Immunogenetics and Breeding in Livestock Species

Virtual, November 8-9, 2022

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

Workshop schedule

Tuesday, November 8, 2022, CET	Presenter	Affiliation	Title
14.00 – 14.10	Karel Novak	Institute of Animal Science, Prague	Introductory comments
14.10 – 14.50	Dr Sabine Hammer	Institute of Immunology, University of Veterinary Medicine, Vienna	The swine leukocyte antigen (SLA) complex: molecular genetics and importance in veterinary vaccine research
14.50 – 15.30	Prof Petr Hořín	Faculty of Veterinary Medicine, University of Veterinary Sciences, Brno	Genetics and comparative genomics of NK cell receptors
15.30 – 15.45	Thales Galdino Andrade	Ribeirão Preto School of Medicine, University of São Paulo	Insights on the diversity and distribution of cattle major histocompatibility complex *DRB3* alleles
15.45 – 16.00	Prof Abbas Rafat	Department of Animal Science, University of Tabriz	Identification of functional candidate genes associated with mastitis and milk production traits based on transcriptome-wide association and multi-tissue colocalization approaches using the available FarmGTEx dataset
16.00 – 16.10			break
16.10 – 16.50	Prof Dirk Werling	Royal Veterinary College, London	Species and breed-specific innate pattern recognition receptors: an immunologist’s plea to take genetics more into account when designing vaccines
16.50 – 17.30	Dr Terhi Iso-Touru	Natural Resources Institute - LUKE, Finland	Bovine mammary epithelial cells response to two different mastitis pathogens
17.30 – 17.45	Dr Nidhi Sukhija	ICAR-National Dairy Research Institute, Karnal, India	Genomic clues for selective sweep regions in Indian Gir and Tharparkar cattle on BTA6
17.45 – 18.00			Discussion, conclusions

Wednesday, November 9, 2021, CET	Presenter	Affiliation	Title
14.00 – 14.10	Karel Novak	Institute of Animal Science, Prague	Introductory comments
14.10 – 14.50	Prof Ottmar Distl	University of Hannover	Screening selection signatures associated with disease resistance in sheep using whole genome sequencing data
14.50 – 15.30	Dr Steven Fiddaman	Department of Biology, University of Oxford	Investigating the immunogenetics and function of Toll-like receptors in large genomic datasets
15.30 – 15.45	Prof Lior David	Department of Animal Sciences, The Hebrew University of Jerusalem	Multigenic resistance of common carp the viral CyHV-3 disease, infection and infectivity
15.45 – 16.00	Avon Augustin Nalpadan	Center for Integrated Genetics, Norwegian University of Life Sciences, Norway	Developing Cas9 expressing MDBK cell lines for GeCKO screening against bovine diseases
16.00 – 16.10			break
16.10 – 16.25	Karel Novák	Institute of Animal Science, Prague	Effects of *TLR* diversity on reproductive traits
16.25 – 17.05	Prof Yana Safonova	Department of Computer Science, John Hopkins University, Baltimore	Variations in cattle antibody repertoires correlate with immune responses to vaccines against the bovine respiratory disease
17.05 – 17.20	Kalifa Samaké	Faculty of Natural Sciences, Charles University, Prague	Balancing selection acting on haplotypes of bovine *TLR*s
17.20 – 17.35	Kanaka K.K.	ICAR-Indian Veterinary Research Institute, Bareilly, India	Characterization of ovalbumin coding sequence revealed altered antigenic index in predicted ovalbumin protein structure in two strains of WLH chicken
17.35 – 18.00			Discussion, conclusions

Abstracts

Introductory comments

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It is obvious that from the same beginning of the animal immunogenetics the efforts were aimed at the improvement of resistance to the animal diseases. The most straightforward way to this goal is to apply the immunogenetics knowledge in breeding. The importance of this topic can be exemplified by the health-oriented programs for cattle of the large breeding companies, e.g., Immunity+ (Semex) and DWP$ and CW$ breeding value indices (Zoetis).

A factor changing the situation is that the commercial breeding in livestock has developed into a self-standing discipline based on statistical models. Moreover, during the last decade tens of thousands of rather formal genomic markers started being used instead of approximately several tens before. Then the question arises whether the efficiency of the formalized breeding systems can be improved by the inclusion of the functional knowledge.

Some of the recent projects probably responded to the perceived low efficiency of the purely statistical approach for finding breeding values of formal markers. Among other, this situation was defined in the paper by Boichard et al. (2016) stating that, after the initial black box strategy, the genomic evaluation might be more accurate and more persistent by integrating biological knowledge.

A logical reaction to the perceived inherent gaps in the genomic selection was a systematic search for causative gene variants. However, although the genome-wide association studies have been applied on a large scale, only a limited number of causal variants had been identified using GWAS in cattle with this general method by 2015, i.e., in the middle of the last decade and five years after the introduction of whole-genome genotyping arrays.

In this situation, the integration of the functional knowledge from the bioinformatic databases and from the omics approaches to the gene expression became a realistic alternative approach. Generally, two strategies are applicable for the identification of the targets for health breeding. On one side, it is possible to increase the amount of experimental work, i.e., to collect more data from larger populations of animals, to increase the resolution of genotyping arrays, to extent the computing capacity etc. The second approach is based rather on bioinformatics, which means that the exploitation of the available molecular data on the formation of complex traits can be made more efficient. This approach is applicable to the immune traits as an important subset.

Among the first projects applying this approach were the projects initiated in the Animal Genomics and Improvement Laboratory of USDA since July 2017 (Fang, L. et al. 2019). Also

the projects in other leading labs included the incorporation of functional annotation data in cattle GWAS and genomic selection. This switch was paralleled by the development in other labs specialized in the genomics and advanced breeding in cattle. In the example of the paper by Cai et al. (2018) from the Aarhus laboratory, the authors suggested to prioritize the candidate genes that are identified using GWAS by taking additional information about the biological role of the neighbouring genes. In other words, this approach integrates pure genomic and functional data to identify the causal genes. By combining both approaches, the requirements of the Bayesian inference can be met. It can be reasonably assumed that the possibilities for the transfer of immunogenetic knowledge to breeding are wider and will be supported by the emerging bioinformatic approaches in future.

The importance of the inclusion of the immunity traits in breeding has always been reflected in the scope and the activities of the International Society of Animal Genetics (ISAG). Moreover, ISAG originated from the International Society for Animal Blood Group Research (I.S.A.B.R.) in Torino 1988. I.S.A.B.R. was in its turn derived from the European society E.S.A.B.R, established in 1964 in Prague. This development was preceded by a series of conferences on the animal blood groups and histocompatibility antigens held since 1954.

The current state of our possibilities to transfer the immunogenetic knowledge to breeding was surveyed in the presentations on the workshop, although it is not possible to cover fully such a complex topic in frame of a limited event. Nevertheless, we believe that the workshop will contribute to the determination of further productive directions of research and will be helpful in the identification of the possibilities for cooperation.

References:

Boichard D., Ducrocq V., Croiseau P., Fritz S., 2016. Genomic selection in domestic animals: Principles, applications and perspectives. *Comptes Rendus Biologies* 339:274–277.

Cai Z., Guldbrandtsen B., Lund M.S., Sahana G., 2018. Prioritizing candidate genes post-GWAS using multiple sources of data for mastitis resistance in dairy cattle. BMC Genomics 19:656.

Fang, L. et al. 2019. Comprehensive analyses of 723 transcriptomes enhance biological interpretation and genomic prediction for complex traits in cattle. Genome Research 30:790-801.

The swine leukocyte antigen (SLA) complex: Molecular genetics and importance in veterinary vaccine research

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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 Swine Leukocyte Antigen (SLA) system is among the most well characterized MHC systems in nonhuman animal species. A systematic nomenclature for the genes, alleles, and haplotypes of the SLA complex is critical to research in swine genetic diversity, immunology, health, and vaccinology, as well as organ and cell transplantation. Based on our new, detailed annotation of the Sscrofa11.1 genome assembly, the SLA complex encodes approximately 150 loci, with at least 120 genes predicted to be functional. Despite the ongoing domestication process, involving selection for favourable traits, pigs have still maintained a high degree of SLA diversity, as demonstrated by the presence of the 266 and 227 class I and class II alleles, respectively. Pig disease models provide better understanding of host-pathogen interactions. Pathogen effects on SLA gene expression drive the regulation of swine immune responses. Novel trait association data indicate that SLA alleles or haplotypes may be useful genetic markers for use in improving pig-breeding programs. Swine have become the preferred preclinical large animal model for biomedical studies, transplantation, xenotransplantation, and regenerative medicine research. Allogeneic transplantation research in pigs has improved understanding of rejection mechanisms of both host-versus-graft and graft-versus-host disease. Improved cross-matched genetically engineered pigs could reduce antibody-mediated rejection of pig xenografts in highly HLA-sensitized patients. Modifying SLA genes could improve pigs as donors for xenotransplantation.

Future perspective

(1) The impact of SLA genes on swine production and health traits needs to be attributed to individual SLA locus alleles and not just haplotypes.

(2) Renewed typing methods, from PCR SSP to NGS, will enable reliable typing of outbred pigs. To truly explore diversity, data based on large cohorts of pigs are necessary.

(3) Functional studies on MHC effects on cell interactions and on microbiota diversification are needed to understand the impact of SLA genes on the education of the porcine immune system.

(4) In-depth analysis of peptide presentation via major SLA genes will identify the broad range of functionally relevant vaccine targets.
(5) Identification and maintenance of important SLA-defined pig lines (e.g., NIH/MGH, Yucatan, or Babraham pigs) are essential as resources for pig biomedical models.
(6) Future tool development is needed for the swine biomedical model; this includes SLA class I and first SLA class II tetramers, T-cell receptor profiling, SLA-informed SNP chips, and panels of monoclonal antibody reagents to swine immune proteins.
(7) Availability of well-characterized, genetically engineered pigs for human disease models will lead to development and validation of novel therapeutics and improvements in xenotransplantation research.
(8) Human cross matching with SLA class I and II will facilitate xenotransplantation. Histocompatibility testing of pigs needs to be improved in analogy to human allogeneic transplantation.

Keywords: *Sus scrofa*, swine leukocyte antigen, SLA polymorphism, allogeneic, xenogeneic, vaccine responses

Genetics and comparative genomics of NK cell receptors

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Natural killer (NK) cells are an important part of innate immunity. They are able to directly recognize and kill virus-infected and/or tumor cells. It is a heterogeneous population consisting of subsets differing in their functional capacities and cell surface phenotypes. This heterogeneity is due to the differential expression of natural killer cell receptors (NKRs) representing a complex repertoire of germline-encoded receptors. Two types of NKRs regulating multiple NK cell activities can be distinguished: activating and inhibitory. After they bind an appropriate ligand, they can activate or inhibit, respectively, immune mechanisms mediated by NK cells. In these mechanisms, both qualitative and quantitative variability of NK cells and their functions are important. This variability is based on different types of polymorphism of NKR coding genes, including especially Single Nucleotide Polymorphisms (SNPs) and Copy Number Variation (CNV). Besides a number of individual genes belonging to various genes families, such as for example the Natural Cytotoxicity triggering Receptor (*NCR*) genes, two complex genomic regions encode NKRs playing crucial roles in disease,

infections and reproduction of placental mammals. The Leukocyte Receptor Complex (LRC) contains genes encoding killer immunoglobulin-like receptors (KIR), while the Natural Killer Complex (NKC) codes for NKRs with lectin-like structure (KLR, formerly Ly49). Both types of receptors, can bind Major Histocompatibility Complex (MHC) class I molecules as ligands, and in evolutionary terms, they can substitute each other. Similarly to MHC class I, *NKR* genes are highly polymorphic. Their allelic SNP as well as CNV variability underlies the overall avidity in the receptor-ligand interaction and as such is crucial for educating NK cells to distinguish between self, non-self, induced-self and missing self.

The repertoire of NKR genes differs between mammalian families with no single conservative model of NKR genes observed across all placental mammals. Even closely related taxons may differ in the LRC/NKC genomic structure and gene contents. In extreme cases, such as in humans and mice, genes for one type of receptors are missing or pseudogenized, while the other type is expanded. In humans, an expansion of KIR genes was observed within LRC, while a single Ly49 (*KLRA*) sequence identified in the NKC is a pseudogene. On the other hand, an expansion of Ly49 (*KLRA*) genes was reported in mice, while within LRC, no KIR gene was observed, and two KIR genes were relocated on the X chromosome. Various combinations of LRC and NKC gene contents were reported in different mammalian species. Therefore, analyses of mammalian families in which NKR genes have not been studied yet, and especially of domestic animal species, have the potential to bring to light new information on the evolution of NKRs and may also be of practical importance. In our work, we have studied NKR genes and complex genomic regions in three domestic animal families, the Equidae, Camelidae and Felidae. All three of them comprise species exposed to various selection pressures, exerted by different pathogens, especially in free-ranging species, and/or to strong and long-term pressure of artificial selection, such as in domestic animals. We have identified a new model of the NKC/LRC genomic structure in equids, provided a detailed annotation of these regions in camelids and started analyzing the so far unknown repertoire of NKRs in felids. For horses and camelids, we have also developed microsatellite-based tools for analyzing genetic diversity of their NKR and MHC regions. Currently, we study receptor-ligand (NKR-MHC) interactions in their evolutionary context.

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Insights on the diversity and distribution of cattle major histocompatibility complex DRB3 alleles

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The bovine Major Histocompatibility Complex (MHC), also known as Bovine Leucocyte Antigens (BoLA), is the genomic region that encodes the most important molecules for antigen presentation in immune responses. At first, the evidences of MHC in bovines pointed to a loci of two antigens, one detected by cytotoxic antiserum (MHC I) and another studied by mixed lymphocyte culture tests (MHC II). Nowadays, it is known that the bovine MHC is located in chromosome 23 with more than 150 genes and multiple alleles. The most studied gene in the BoLA region is the highly polymorphic BoLA-DRB3, which encodes a β chain with a peptide groove domain involved in antigen presentation for helper T cells to develop robust humoral responses. BoLA-DRB3 alleles also demonstrated to be associated with a diversity of infectious diseases and production traits, such as mastitis, trypanosomiasis, and tick burden. In order to catalogue these alleles, two nomenclature methods were proposed and the current use of both systems makes it difficult to list, comprehend and apply these data in an effective way. Despite this drawback, there are several reports of BoLA-DRB3 alleles frequencies in more than 26 countries evaluating many breeds, but lacking studies in Africa and Australia. We strongly encourage new studies addressing allelic frequencies, which can be incorporated in databases for practical approaches, such as: markers for infectious diseases resistance, production traits, and ectoparasitic interactions; genetic diversity studies; B and T cell epitope mapping; and the conservation of creole breeds.

Identification of functional candidate genes associated with mastitis and milk production traits based on transcriptome-wide association and multi-tissue colocalization approaches using the available FarmGTEx dataset

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Research on the simultaneous genetic assessment of milk production and mastitis has a long history. A challenging problem that arises in this area is the existence of a correlation between lactation performance and increased risk of mastitis due to selection. Understanding the complexity and genetic architecture of milk production and mastitis is critical to developing efficient breeding plans. Thus, previous studies show that some genes in different tissues have significantly different expression for mastitis and milk production. For example, *DGAT1* (Fang et al., 2017), *MAPK14, FRAP1, EIF4EBP2, GSK3A*, TSC1 (Bionaz and Loor, 2011) for milk production and *LOC515333, SAA3, CD14, NFKBIA, APOC2, LOC100335608* (Pereira et al., 2021), *SPEF2, FAM151A, IMMP2L, CCND2, NFKB1, KRT32, DHX58, KCNH4*, STAT5A, *STAT3* (Cai et al., 2018), *GLYCAM1, B2M, CD74, BoLA-DRA, FCER1G, SDS, NFKBIA*

(Asselstine et al., 2019) for mastitis identified as biomarkers. In recent years, researchers have shown increased interest in genome-wide association studies (GWAS) (Li et al., 2011; Benner et al., 2016). GWAS highlighted more than thousands of associated loci or variants with complex traits such as disease, production, reproduction and conformation in livestock (Freebern et al., 2019; Jiang et al., 2019a; Tian et al., 2020). Despite its long success, GWAS has a number of problems in use. For example, the majority of loci are located in the non-coding part of the genome (Maurano et al., 2012; Finucane et al., 2015; Visscher et al., 2017). Hence, it is remarkable that non-coding regions of the genome play an important role in the regulation of gene expression (Barbeira et al., 2021). To overcome this problem, some approaches have been developed, gene expression is the representation of the molecular phenotype and improves the functional explanation of GWAS results (Guo et al., 2015). A number of recent studies have shown that the integration of RNA-Seq data on intramammary infection of Holstein cattle and large-scale GWAS data from three dairy cattle breeds (Jersey, Nordic Red, and Holstein cattle) improves the biological interpretation of GWAS loci and improves genomic prediction for milk production and mastitis resistance (Fang et al., 2017). Recent methods focus on overcoming the problems by proposing the identification of variants involved in regulating gene expression levels through the expression of quantitative trait loci (eQTL). TWAS method attempts to combine the GWAS and eQTL approaches to control complex diseases or traits. With this motivation, we focus on previous bovine datasets from the FarmGTEx project proposed for milk yield and mastitis and mainly compared the results of TWAS and colocalization approaches to identify functional candidate genes associated with mastitis and milk production traits on multi-tissue Base - transcriptome records are associated. We used Gene Ontology (GO) to identify significant genes with DAVID and also STRING and GeneMANIA databases to identify interaction networks. In addition, to calculate the correlation between tissues were used available z-scores in TWAS results. Taken together, the present results confirm that *LYNX1*, *DGAT1*, *C14H8orf33* and *LY6E* are significant genes associated with milk production in 8, 6, 5, 5 and 5 tissues, respectively. And *FBLX1* identified as a significant gene for mastitis. We identified five significant terms for biological processes, three significant terms for cellular components, and four significant terms for molecular function. The following common genes emerge for both the TWAS and colocalization approaches: *CLN3* and *ZNF34* for milk production. In the present study, genes associated with mastitis and milk production traits were identified for different tissues in cattle and their function and interactions were investigated. On this basis, we conclude that TWAS, colocalization, appears to improve our understanding of the potential health status control mechanism in high-yielding dairy cows.

Species and breed-specific innate pattern recognition receptors: an immunologist's plea to take genetics more into account when designing vaccines

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Over the last years, the differences in the composition and function of cellular components of the immune system between various mammalian species haven been highlighted. Interestingly, whereas a lot of genetic information is available for different laboratory rodent strains and specific human geo-ethnic groups, we don't have yet a great understanding how similar differences identified in farmed animals affect their immune response. However, these genetic differences may not only affect how animals respond to pathogens, but may also affect how they respond to vaccines, and address differences in the adaptive as well as the innate immune system. Here, pattern recognition receptors play an important role. Indeed, many members of the C-type lectin family of glycan-binding receptors have been ascribed roles in the recognition of microorganisms and serve as key receptors in the innate immune response to pathogens. Other mammalian receptors have become targets through which pathogens enter target cells. These receptor roles have often been documented with binding studies involving individual pairs of receptors and microorganisms. Own studies using C-type lectin receptors in cattle show that the results are consistent with interactions previously ascribed to the receptors, but they also highlight binding to additional sugar targets that have not previously been recognized. Furthermore, recent evidence suggests that not only do cattle behave differently to an infection with Mycobacteria, but also that several cattle breeds may be more resistant to infection with the zoonotic pathogen *Mycobacterium bovis*. Whole genome sequencing of the Brown Swiss genome identified several potential candidate genes, in particular for Toll-like Receptor-2 (TLR2), a PRR that has previously been described to be involved in mycobacterial recognition. Cloning of the *TLR2* gene and subsequent gene-reporter and chemokine assays revealed that specific SNP, present in BS and *Bos indicus* breeds, resulted in a significantly higher response to mycobacterial antigens as well as tri-acylated lipopeptide ligands in general. This clearly indicates that we cannot even extrapolate data generated in one cattle breed to others on a 1:1 base, and we clearly have to take genotype to phenotype variations far more into account. However, this also potentially means that vaccines generated using „European" breeds may not work with the same efficacy in other, specifically *Bos indicus* breeds, and need to be tested far more rigorous before brought onto the target market

Genomic clues for selective sweep regions in Indian Gir and Tharparkar cattle on BTA6

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BTA6 harbours QTLs related to milk production and disease susceptibility. Selective sweeps are the regions of co-inheritance of neutral mutations along with the beneficial mutations. The search for intersections between selective sweep regions and QTLs navigated our study at LRC, NDRI, Karnal (n=7,7). A total of 191 Selective sweep regions were obtained by employing CLR approach in Gir and Tharparkar and FST approach, respectively. GRID2, SEC31A, PTPN13 and LDB2 genes were linked to Selective sweep regions in Gir cattle. STIM2 and UGT2A1 genes were linked to Selective sweep regions in Tharparkar cattle. FAM13A, RBPJ, PDS5A, MAPK10, HS3ST1 and SORCS2 genes were found by FST approach. FAM13A gene is related to production and adaptation while MAPK10 gene is related to reproduction and adaptation. A total of 12, 8 and 4 selective sweep regions overlapped with QTLs related to Milk protein percent, Milk unglycosylated kappa casein percentage and Milk kappa casein amount present on BTA6 by CLR approach in Gir and Tharparkar cattle and FST approach. Selective sweep studies are important because they serve as a validation platform for genomic selection strategies, helps in narrowing down data-set by 10 times from SNP level to sweep level and also serve as a pilot study for GWAS, to establish correlation with traits of interest.

Investigating the immunogenetics and function of Toll-like receptors in large genomic datasets

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Genomic data of domesticated animals are becoming increasingly ubiquitous and easy to generate. Coupled with the relative ease of computationally processing whole genomes, it is now possible to gather and jointly analyse many hundreds or even thousands of genomes. Leveraging datasets of this size brings with it analytical power that has scarcely been seen outside of human data. Motivated by a desire to better understand the domestic chicken, the Chicken Genomic Diversity Consortium was established in 2021, bringing together >25 researchers from >10 institutions. Currently, ~4,800 chicken and junglefowl genomes are being processed through a state-of-the art bioinformatic pipeline on the Leibniz Supercomputing facility, and it is expected that genomes will be continually added. The dataset comprises chickens from a diverse range of sources, including feral birds and local breeds from a variety of locations worldwide, European fancy breeds, commercial birds, experimental lines, and even ancient birds from archaeological sites. It is hoped that a sizeable fraction of extant chicken genomic diversity will be represented by this dataset. Alongside several other diverse goals, one of the aims of the Consortium is to analyse immune diversity at several key loci. Among the immune loci to be analysed are the Toll-like receptors (TLRs) which are pattern-recognition receptors at the front line of defence against pathogens. Genetic polymorphisms in TLRs are

known to influence host disease susceptibility, and thus an understanding of TLR diversity can be an important adjunct to studying infectious diseases of chickens. In a preliminary analysis of >1600 chickens and junglefowl, we found that chicken TLRs are highly diverse. Moreover, several TLRs deviated significantly from neutrality, and we found evidence of selective sweeps affecting *TLR2A*, *TLR2B*, *TLR5*, *TLR7* and *TLR21*. Further analysing sites under positive selection, we found that a significant fraction of these have known ligand-binding or dimerisation function, implying important functional changes. We also identified a number of high-impact variants such as internal stop codons, frameshift mutations, lost start codons, and splice site variants – some of which are found at high prevalence in certain breeds of chicken. These data therefore provide the foundation to catalogue chicken TLR diversity – benign, advantageous and disadvantageous – to inform future breeding efforts.

Multigenic resistance of common carp the viral CyHV-3 disease, infection and infectivity

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Infectious diseases stress animals and damage production of farmed animals. Challenges are even bigger under aquaculture conditions where disease control and prevention measures are limited. Breeding of genetically disease-resistant strains stands out as a sustainable solution to the problem. Common carp is a food fish cultured worldwide and it suffers from outbreaks of a major lethal disease caused by the cyprinid herpes virus 3. Our group have been developing genetically resistant and susceptible common carp strains. Although phenotypic measurements might be similar, several key aspects differentiate monogenic and multigenic resistances. Here, I will touch upon infection and infectivity differences between susceptible and resistant fish. Following infection, viral load in spleen of live susceptible fish was 100 times higher compared to resistant fish leading to four times higher cumulative mortality of the former. CyHV-3 resistance relies on improved immunity reflected in different signaling pathways and sets of cytokines. Interestingly, viral load levels in spleen and mortalities depended not only on type of infected fish, but also on type of infecting fish. Infection by susceptible fish led to higher viral loads and mortalities than infection by resistant fish. Therefore, we provide empirical evidence showing that susceptible individuals not only are more prone to viral infection but are also supporting more replication of the pathogen in their tissues, shedding more viral particles to the environment and infecting more than resistant individuals do.

Developing Cas9 expressing MDBK cell lines for GeCKO screening against bovine diseases

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Recent developments in CRISPR/Cas9 technology have opened several possibilities for gene editing in various model organisms. However, seldom research has been done in applying this research in developing suitable tools for gene editing in large production animals against viral diseases. The objective of this project was to develop Cas9 integrated Madin-Darby Bovine Kidney (MDBK) cell lines for a genome wide CRISPR/Cas9 knockout screening against Bovine Viral Diarrhea Virus (BVDV) and Bovine Coronavirus (BCoV). Two-step vector was used for the screening. LentiCas9-Blast was transduced into MDBK cells for generating Cas9 integrated cell lines. Stable cell lines were then selected using blasticidin scelection, followed by expanding homogenous cell lines from single cell clones obtained through serial dilutions. The lines were then grouped based on Cas9 gene expression (using RT-qPCR) and protein levels (using Western blotting). ADAM 10 gene was then cloned into lentiGuide-Puro and was then transduced into the grouped cell lines and selected using Puromycin. The lines were then sequenced to confirm the genome editing. The major result involved the successful development of multiple Cas9 integrated cell lines with varied Cas9 expression, ready for the subsequent genome wide screening. Additionally, CRISPR editing efficiencies of cell lines were also correlated with their Cas9 gene expression to obtain a few optimal cell lines ready to perform GeCKO screening.

Keywords: BVDV, BCoV, CRISPR/Cas9, MDBK cell lines, Transduction, GeCKO screening

Effects of *TLR* gene diversity on reproductive traits

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To date, a series of observations of the effects of TLR gene variants on different health traits of cattle have been published. They comprise mostly the effects on mastitis incidence, somatic cell count, and paratuberculosis resistance. Consequently, diversity in this group of genes was documeneted in the population of the Czech Red Pied cattle (Czech Simmental) and the association study with a set of production, health, and reproductive traits has been performed. The genotyping workflow included design of the targeted amplification, hybrid resequencing in the population with two independent technologies, discovery of the polymorphism and validation with primer extension reactions, genotyping of a set of bulls representing the population and calculation of the association indicators. Eight polymorphisms in *TLR1*, *-2* and *-6* were significantly associated with reproductive traits, like calving ease. Calf vitality index

seems to be correlated as well. On the other hand, unaffected traits included early traits like cyst formation in ovaries and the index of early reproduction disorders. Shared pattern of associated traits in this group of *TLR* genes might be due to the interaction of their products in the heterodimers formed by TLR2 on one side with TLR1 and TLR6 on the other. A similar effect on reproductive traits was observed in *TLR4* and *TLR5*. No effects on udder health traits were observed, in contrast to the expectation. The association with the milk production traits was present only in the *TLR5* polymorphisms. This finding is consistent with the *TLR* variation effects known in model species, mouse and in humans. Non-immune functions of *TLR*s, e.g., participation in Rho/ROCK signalling, can be responsible for the observed effect on reproductive traits. However, the absence of correlation between the predicted effects and detected associations for individual alleles points at the localization of causative variants in the non-coding regulatory regions. Consequently, the knowledge of the long-range variability in the regulatory regions of *TLR*s would reduce the possibility of misinterpretation of the association studies, otherwise difficult to avoid.

Balancing selection acting on haplotypes of bovine *TLR*s

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The *TLR* genes coding for Toll-like receptors of anti-bacterial series, namely *TLR1*, *-2*, *-4*, *-5* and *-6*, were re-sequenced in Czech Simmental (Czech Red). PacBio sequencing allowed to determine haplotypes within the range of the designed amplicons. A more general statistical reconstruction of haplotypes from individual reads was carried out in parallel. The PacBio read results demonstrated randomly distributed frequencies of haplotypes in the amplicons 2 – 5, 15 haplotypes revealed in amplicon 1 in the proximal part of the transcript formed two distinct groups. Similar results were found in the haplotypes obtained by statistical reconstruction in *TLR2* and *TLR5*. Similarly to direct reconstruction in *TLR2*, the trend for bimodal distribution was expressed stronger in proximal regions of the transcripts. The bimodal clustering of *TLR* haplotypes has been reported earlier in cattle and other animal models, however, a final interpretation of this disequilibrium is still missing. Alternating infectious agents might be a factor causing balancing selection. Two different functions performed by the *TLR2* gene or its product are another possible mechanism. An example of a dual function might be formation of two kinds of heterodimers, TLR2/TLR1 and TLR2/TLR6. Nevertheless, the association of the groups of haplotypes with the transcript proximal region suggests the selection target in the 5'- regulatory regions of the *TLR* genes, although functional interactions in the proximal part of the transcript cannot be excluded.

Characterization of ovalbumin coding sequence revealed altered antigenic index in predicted ovalbumin protein structure in two strains of WLH chicken

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Ovalbumin is the most abundant protein in egg white which contributes up to 54%. It is a storage protein and major source of amino acids for the developing embryo. Sequencing of ovalbumin CDS from IWI and IWK lines of white leghorn chicken revealed that both the products were 1161bp in length. Comparison of obtained nucleotide sequence and deduced amino acid sequence with that of available sequences of reference genome confirmed that the sequence was of ovalbumin gene encoding a peptide of 386 amino acid sequence in both the cases. At 15th position T>C transition and at 237th position C>T transitional mutations were observed in both the lines. At 291th position, T>C transition and 562nd position G>A transitional mutations were observed in IWI line. 562nd G>A transition had changed the amino acid alanine to threonine at 188th position in amino acid sequence, while other mutations did not change the any of the amino acids. Based on the predicted structure of protein using coding sequence it is found that molecular weight of ovalbumin protein from IWI and IWK lines were 42.91 KDa and 42.55 KDa respectively. The antigenic index was found out for predicted protein structure for both IWI and IWK lines. It was observed that the index was similar in both the lines till amino acid number 300 from N-terminal and from 300 to 386 the index graph shown multiple changes. Based on our results, we hypothesize that there can be a potential antigenic determinant candidates present in this region and may have association with consumer egg protein allergy and these candidates.