28 days. Regarding immune parameters, Cd-exposure lowered the lysozyme activity and difference was significant after 28 days of exposure, compared to control. Alternative complement pathway activities were slightly higher in Cd-exposed group upto 14 days and thereafter it reduced; however, immunoglobulin M levels were slightly lower in Cd-exposed group at any point of time. Peroxidase activity was always higher in Cd exposed group but the increment was not significant at any time point, and Cd exposure had not significant effect on phagocytic activity. The serum glutamic –pyruvic transaminase and serum alkaline phosphatase activities were significantly higher in fish exposed to Cd for 14 to 28 days. Further, Cd had an immunosuppressive effect and leading to down- regulation ($P < 0.05$) of IL-10 and IFN-γ mRNA. However, Cd-exposure led to the up- regulation of HSP47, HSP60, HSP70, HSP78, and HSP90 mRNA indicating Cd-induced cellular stress.

Conclusions: Overall, Cd-exposure in *L. rohita* affects the immune system and this make the fish immunocompromised and could increase the susceptibility of fish to pathogen infection.

References

[1] Pretto A, Loro VL, Morsch VM, Moraes BS, Menezes C, Clasen B, Hoehne L, Dressler V, 2010. Acetylcholinesterase activity, lipid peroxidation, and bioaccumulation in silver catfish (Rhamdia quelen) exposed to cadmium. Arch Environ Contam Toxicol 58: 1008–1014.

[2] Barwick M, Maher W, 2003. Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW. Australia. Mar Environ Res 56: 471–502.

[3] Jindal R, Verma S, 2015. In vivo genotoxicity and cytotoxicity assessment of cadmium chloride in peripheral erythrocytes of Labeo rohita (Hamilton). Ecotoxicol Environ Safety 118: 1-10.

O-003

Molecular detection and phylogenetic analysis of *Borrelia afzelii* in ticks infested on Korean water deer

Dorene Van Bik[1], Seung-Hun Lee[1], Kyoo-Tae Kim[2], Dongmi Kwak[*1]

[1]College of Veterinary Medicine, Kyungpook National University, Daegu, Korea; [2]Animal Health Center, Zoo Land, Daejeon O-World Theme Park, Daejeon, Korea

Introduction: Lyme borreliosisis one of the most prevalent infectious vector-borne diseases in Europe, America and Asia. It is a multi-organ systemic disease which is caused by spirochetes of the *Borrelia burgdorferi* sensu lato group with its species (Lee et al. 2000). Among them, *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* are the major etiological agents of human Lyme disease (Chu et al. 2008). In Korea, *B. afzelii* has been detected from environmental ticks (*Ixodes persulcatus*, *I. nipponesis*, *I. granulatus*), wild rodents (*Apodemus agarius*) and sera of febrile disease patients (Kee et al. 1994). However, there is no report on the detection of *B. afzelii* from infested ticks yet. Thus, this study assessed the presence of *B. afzelii* in the ticks infested on Korean water deer.

Materials and Methods: A total of 48 ticks attached to Korean water deer were collected from Gyeongbuk province in Korea from 2013 to 2015. Identification of tick species was done by microscopy. Nested PCR was performed with the primers designed to amplify the *Borrelia* specific 5S–23S intergenic spacer region (*rrf-rrl*), with the expected sizes of 226-266 bp depending on the *Borrelia* strain (Chu et al. 2008). For the positive sample, DNA sequencing and phylogeny was assessed by comparing the sequences from NCBI GenBank database.

Results: From the microscopic examination, all the collected ticks were identified as *Haemaphysalis longicornis*. By nested PCR, the *Borrelia* specific DNA fragment was amplified in 1 out of 48 ticks (2.1%) with the amplicons at 246 bp of *B. afzelii rff-rrl* gene. The amplicon revealed a high sequence homology (96-100% similarity) to *B. afzelii* spirochetes deposited in the GenBank database.

Conclusions: The result indicates that *B. afzelii* was detected from *H. longicornis* in Korea. To the extent of our knowledge, this report is the first molecular detection of *B. afzelii* in ticks infested on Korean water deer. Since ticks can act as vectors for a number of pathogens, knowledge on their characteristics is important to humans and also in veterinary practices. Moreover, changes of environmental temperature may also cause changes in the proliferation of wild animals and the arthropod vectors as well as the pathogens they transmitted. Thus, further screening ticks for disease-causing pathogens using molecular epidemiological tools would be necessary for the data about the distribution and prevalence of tick-borne pathogens.

References

[1] Chu CY, Jiang BG, Liu W, Zhao QM, Wu XM, Zhang PH, Zhan L, Yang H, Cao WC. Presence of pathogenic Borrelia burgdorferi sensu lato in ticks and rodents in Zhejiang, south-east China. J Med Microbiol. 2008 57:980-985.

[2] Kee S, Hwang KJ, Oh HB, Park KS. Identification of Borrelia burgdorferi isolated in Korea using outer surface protein A (OspA) serotyping system. Microbiol Immunol. 1994 38:989-993.

[3] Lee SH, Kim BJ, Kim JH, Park KH, Yeo SU, Kim SJ, Kook YH. Characterization of Borrelia burgdorferi strains isolated from Korea by 16S rDNA sequence analysis and PCR-RFLP analysis of rrf (5S)-rrl (23S) intergenic spacer amplicons. Int J Syst Evol Microbiol. 2000 50:857-863.

O-004

Effect of polymorphisms of GBP1, Mx1 and CD163 genes in pigs infected with PRRSV

Nadeem Shabir[1], Pengxia Niu[2], Amina Khatun[1], Byoung-Joo Seo[1], Suna Gu[3], Sang-Myoung Lee[3], Kwan-Suk Kim[2], and Won-Il Kim[1]

[1]College of Veterinary Medicine,Chonbuk National University;

[2]College of Agriculture, Life & Environment Sciences, Chungbuk National University; [3]College of Environmental & Biosource Science, Chonbuk National University

Introduction: Porcine reproductiveand respiratory syndrome (PRRS) is characterized by reproductive failure ofsows and respiratory disease in pigs. Commercially available Modified-livevirus (MLV) vaccines offer good homologous protection but lack proper heterologous protection as a result of which the disease still remains a bigger threat to the swine industry worldwide. Previous studies on PRRS have focussed on the virulence, pathogenecity and immune response elicited by PRRSV alone, however, to provide a holistic view of the host-pathogen interaction in PRRS, host genetic factors playing a role in host-pathogen interaction need to be explored. Guanylate-binding protein 1 (GBP1) and myxovirus resistance protein 1(Mx1) are two important proteins belonging to the GTPase superfamily that havebeen previously described to show antiviral effects. CD163 is considered the most important receptor for PRRSV attachment and internalization. Therefore, the aim of the present study was to evaluate the effects of these genes on host resistance against PRRSV infection in conjunction with the host immune response following PRRSV challenge.

Materials and Methods: To determine the diversity of the polymorphisms in the GBP1, CD163 and Mx1 genes in swine herds in Korea, their genotyping was conducted in 689 pigs from 22 farms as a pre-screening procedure. Blood samples were collected from the pigs and genotyped for polymorphisms in the GBP1(exon2), CD163 and Mx1 genes using the polymerasechain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Thereafter, a total of forty, PRRSV-negative, three-week old pigs with relatively high genotypic heterogeneity were purchased from a farm. Thirty-eight pigs were housed in the same room and intramuscularly challenged with the JA142 strain of North American PRRSV at a titre of 10^3 $TCID_{50}$/mL and twon on-challenged pigs were maintained as negative controls and housed in a separate room. All of the pigs were bled for serum collection at 0, 4, 11, 18,and 25 days post-challenge (dpc), and whole blood was collected at 0, 4, and 18 dpc for PBMC isolation. PBMCs were evaluated for the surface marker expressionof activated T cells ($CD4^+CD25^+$/$CD8^+CD25^+$) and γδ T cells ($CD8\alpha^+TcR1N4^+$). Viremia levels in all pigs were evaluated weekly, and all pigs were weighed at -6, 11 and 25 dpc. All of the pigs were euthanized at 25 dpc and subjected to pathological evaluation.

Results: The results demonstrated that pigs with AG genotype for the GBP1 exon2 and with CC genotype for theCD163 gene exhibited a significantly higher average daily weight gain (ADWG)and lower average viremia. Also, pigs harbouring the AG genotype for the GBP1gene presented higher $CD4^+CD^+25$ and $CD8^+CD25^+$T cell populations at 4 and 18 days post challenge (dpc), respectively, whereas pigs with the CC genotype for the CD163 gene displayed significantly higher nucleocapsid-specific antibody titers at 11 dpc. However, pigs with a single11-bp deletion or insertion in the Mx1 gene did not show significant differences in either weight gain or viremia.

Conclusions: Collectively, these results suggest that the GBP1 gene might be the most effective genetic marker associated with resistance of pigs to PRRSV infection among the three candidate genes evaluated in the present study while as CD163 might also represent an additional candidate for PRRSV resistance. The increased resistance to PRRSV observed in animals with the AG genotype for the GBP1 gene might be a consequence of the enhanced activation of $CD4^+$ and $CD8^+$ T cells in response to infection.

References

[1] Benfield, D.A., Nelson, E., Collins, J.E., Harris, L., Goyal, S.M.,Robison, D., Christianson, W.T., Morrison, R.B., Gorcyca, D., Chladek, D.,1992, Characterization of swine infertility and respiratory syndrome (SIRS) virus (isolate ATCC VR-2332). J. Vet. Diagn. Invest. 4, 127-133.

[2] Boddicker, N., Waide, E.H., Rowland, R.R., Lunney, J.K., Garrick, D.J.,Reecy, J.M., Dekkers, J.C., 2012, Evidence for a major QTL associated with host response to porcine reproductive and respiratory syndrome virus challenge. J. Anim.Sci. 90, 1733-1746.

O-005

High genetic and biological stability of a PRRSV strain resistant to lethal dose of ribavirin during sequential passages in pigs

Amina Khatun, Nadeem Shabir, Byoung-Joo Seo, Bum-Seok Kim, Won-Il Kim*

College of Veterinary Medicine, Chonbuk National University

Introduction: Porcine reproductive and respiratory syndrome (PRRS) is the most economically important infectious disease in pigs caused by PRRS virus (PRRSV). MLV vaccines are widely used to control PRRS; however, there has been serious concerns regarding the safety issue as MLV vaccine virus quickly reverted to virulence during replication in pigs. Therefore,it is important to develop a novel strategy to stabilize the PRRSV genome during replication for the purpose of vaccine safety. Ribavirin, a RNA virus mutagen, has been successfully used as an antiviral drug against different viruses maintained through lethal mutagenesis. However, ribavirin-resistant mutants reemerged by escaping lethal mutagenesis when the treatment concentration is sub-lethal, and those mutants were found to be genetically more stable than their parental viruses as reported in many studies. In a previous study, this strategy was applied and two ribavirin-resistant PRRSV mutants (RVRp13 and RVRp22) was selected, which are genetically and phenotypically more stable than their parental virus through acquired resistance to random mutation was shown in cell culture systems. Consequently,in the present study, both ribavirin-resistant mutant viruses were evaluated in terms of their genetic and phenotypic stability during replication in 3 pig-to-pig passages compared with a commercial MLV vaccine (Ingelvac®PRRS MLV).

Materials and Methods: Six3-week-old PRRSV-negative pigs