

with Arithmetic Mean (UPGMA) analysis, and the horses in each cluster were differed by frequency of feeding, amount of water consumption, and type of bedding.

Conclusions: To our knowledge, this is the first study to investigate the gastric microbiome of Thoroughbred racehorses and to evaluate the microbial diversity in relation to the severity of the gastric ulcer and management factors. This study is important for further exploration of the core gastric microbiome in racehorses and ultimately applicable to improving animal health.

References

- [1] The equine gastric ulcer council 1999. Recommendations for the diagnosis and treatment of equine gastric ulcer syndrome (EGUS): The Equine Gastric Ulcer Council. Equine veterinary Education 115: 262-272.
- [2] Moyaert, H., F. Haesebrouck, M. Baele, et al. 2007. Prevalence of *Helicobacter equorum* in faecal samples from horses and humans. Veterinary microbiology 1213: 378-383.
- [3] Perkins, G. A., H. C. den Bakker, A. J. Burton, et al. 2012. Equine stomachs harbor an abundant and diverse mucosal microbiota. Appl Environ Microbiol 788: 2522-2532.

P-065

Selection of Potential Canine Probiotics Enhancing the *in vitro* Innate Immune System of Dogs

Yeong-Im Kang, Sang-Won Lee, In-Soo Choi, Chang-Seon Song, Joong-Bok Lee, Seung-Yong Park*

College of Veterinary Medicine, Konkuk University, Seoul, Korea

Introduction: A myriad of studies have shown that probiotics augment the health of animals, and it is generally believed that the effects of probiotics are species-specific. To develop probiotics for dogs, we have screened bacteria from feces of healthy dogs, and selected two strains with potential capacities in enhancing the innate immune system of dogs.

Materials and Methods: Strains: Two lactic-acid bacteria, *Lactobacillus reuteri* 42-3 and *L. plantarum* 51-2, were chosen based on the *in vitro* effects on the innate immune system. *L. lactics* which was isolated from commercial probiotic product was used as a reference strain. Phagocytosis: Canine heparinized blood was diluted by RPMI 1640 media and was incubated with samples for 30 min 37°C in the presence of Fluorospheres (1.0 µm diameter). After removing the RBC, the cells were analyzed by a flow cytometer to detect the phagocytosed cells. Oxidative burst: Canine heparinized blood was incubated with samples for 20 min at room temperature, which was followed by addition of dihydrorhodamine 123 (DHR). The formation of reactive oxidants during oxidative burst was measured by the oxidation of DHR with a flow cytometer.

Results: Without stimulation 5% of granulocytes were involved in phagocytosis. *L. reuteri* 42-3 and *L. plantarum*

51-2 increased the phagocytosed cells to 21% and 32% respectively, which were 2 or 3 times higher than those of reference strain. The mean fluorescence intensities, which reflect the level of oxidative burst, of *L. reuteri* and *L. plantarum*, and *L. lactics* were 2815, 3333, and 1851 respectively.

Conclusions: Phagocytosis and generation of reactive oxidants are pivotal arms in the innate immune system. We have demonstrated that *L. reuteri* 42-3 and *L. plantarum* 51-2 enhanced these functions of granulocytes, which indicate that these strains have potentials as probiotics for dogs.

P-066

Protectivity by Guanosine Analog and Ad-siRNA against Foot-and-Mouth Disease Virus in Mice

Joo-Hyung Choi¹, Gun do Park¹, Kwiwan Jeong², Kwang-Nyeong Lee¹, Jin-Mo Ku², Myoung-Heon Lee¹, Su-Mi Kim¹, Jong-Hyeon Park^{*1}

¹Foot and Mouth Disease Division, Animal and Plant Quarantine Agency, Anyang, Republic of Korea; ²GyeongGi Bio-Center, Gyeonggi Institute of Science and Technology Promotion, Suwon, Republic of Korea

Introduction: Foot-and-mouth disease (FMD) causes severe economical problems in livestock industry because of rapid spread and inducing low productivity. Effective antiviral agent is required for FMD outbreak control because the current vaccine of FMD maybe provides no protection until 7 days post vaccination. For these reasons, we tested that FMDV replication would be inhibited by agent Guanosine analog-1 (GA-1). The Guanosine analog is taken immunomodulation by making interferon-gamma and tumor necrosis factor (TNF)-alpha or being mutated RNA of virus. These days, the GA-1 has been researched for curing FMD in cell. We tested whether GA-1 as a treatment could maintain mice's survival after FMD challenge. Also, we tested effect of si-RNA which is known for stimulating interferon pathways, especially interferon gamma and helps to protect from FMD. So, we examined interaction with Ad-siRNA, 2B and 3C target, and GA-1 against FMDV.

Materials and Methods: The virus was used 50% lethal dose (LD₅₀) of Asia 1/Shamir FMDV which is known for virulent pathogenic virus in C57BL/6mice. C57BL/6 mice were injected 10mg of GA-1 from day 0 to day 3 by intraperitoneal (IP) or intramuscular (IM) route to find proper routes of injection. Survival rates of the mice were monitored for 10 days. Moreover, we tested 5 × 10⁸ of Ad-siRNA or 10mg of GA-1 and the combination. One group was injected Ad-siRNA on one day before challenge, another group was fed a pellet feed containing GA-1 from day 0 to day 6, all mice were challenged with 50 LD₅₀ FMDV Asia 1/Shamir on day 0. The mice were monitored for 10 days.

Results: All mice were survived for 10 days by IP or IM

injection against FMDV. Body weights of the mice were reduced up to 15% by IP and 25% by IM, and the body condition of the mice injected by IP route were recovered the faster than that by IM route. Also, only injecting Ad-siRNA and feeding GA-1 helped to cure mice against FMD infection. However, each of single trials was shown 80% of survival rate and weight loss of the groups was reduced up to 30% of their onset weight. The mice treated with Ad-siRNA and GA-1 were completely survived for 10 days, and they showed approximately 10% weight loss. It means that combination of Ad-siRNA and GA-1 was the best antiviral measure in this experiment in C57BL/6 mice.

Conclusions: GA-1 and Ad-siRNA as an inhibitor of RNA virus replication, especially in FMDV Asia 1/Shamir challenge model with C57BL/6 mice were confirmed an efficient antiviral agents. We suggest that si-RNA and GA-1 are antiviral agents against FMDV replication in animal model.

References

- [1] Su-Mi Kim, Jong-Hyeon Park, Kwang-Nyeong Lee, Se-Kyung Kim, Young-Joon Ko, Hyang-Sim Lee, In-Soo Cho: Enhanced inhibition of foot-and-mouth disease virus by combinations of porcine interferon- α and antiviral agents. *Antiviral Research* 2012, 96:213-220.
- [2] Su-Mi Kim, Kwang-Nyeong Lee, Su-Jung Lee, Young-Joon Ko, Hyang-Sim Lee, Chan-Hee Kweon, Hyun-Soo Kim, Jong-Hyeon Park: Multiple shRNAs driven by U6 and CMV promoter enhances efficiency of antiviral effects against foot-and-mouth disease virus. *Antiviral Research* 2010, 87:307-317.

P-067

Comparative Evaluation of a *Brucella abortus* Ghost Strain Constructed using GI24 for Brucellosis with *B. abortus* strain RB51 in Murine Models

Won Kyong Kim, Ja Young Moon, Jin Hur*

College of Veterinary Medicine, Chonbuk National University

Introduction: Bovine brucellosis, caused by a gram negative facultative intracellular bacterium *Brucella abortus*, is one of the most important zoonotic diseases in the world (Olsen 2013). Brucellosis in cattle causes abortion, infertility and decreased milk production thus resulting in major economic losses. It also affects humans and cause headache, fever, arthritis and chronic fatigue; however it does not spread among them (Olsen 2013). A number of strategies, including spreading awareness, improving hygiene standards and using vaccines, have been implemented to prevent the spreading of bovine brucellosis, due to its high socioeconomic impact (Olsen 2013). In past few years, bacterial ghost have emerged as effective inactivated non living vaccine strategy against wide variety of gram negative bacteria. Bacterial ghosts are empty, non-living bacterial envelopes of gram negative bacteria devoid of cytoplasmic content but with intact cellular morphology including cell

surface structures (Delvecchio et al., 2006). The objective of this study is to compare the efficacy of *Brucella abortus* ghost strain constructed by lysis of *Brucella abortus* biotype 1 isolate from Korean cattle using GI24 with *Brucella abortus* strain RB51 vaccine using the mouse model.

Materials and Methods: Forty BALB/c mice were divided equally into 4 groups (n=10); group A mice were intraperitoneally (ip) inoculated with sterile phosphate buffered saline as a control. Groups B and C mice were ip immunized with live *B. abortus* strain RB51 and the *Brucella* ghost vaccine and group D mice were orally inoculated with the ghost cells. The *B. abortus* LPS-specific IgG and IgA titers were determined in serum and vaginal washing samples, respectively, by enzyme-linked immunosorbent assay (ELISA). The cytokines such as IL-4, IL-10, IFN- γ and TNF- α were estimated from splenocytes stimulated with heat-inactivated *B. abortus* cells. All mice were challenged with a wild type *B. abortus* strain 544 at 6 WPI.

Results: The *B. abortus* LPS-specific serum IgG titers were significantly higher in groups C and D mice than in control group from 4 weeks post immunization (WPI) until 6 WPI. In addition, the LPS-specific IgA titers were significantly increased in groups B, C and D compared to those of control group from 4 WPI until 6 WPI. The levels of IL-4, TNF- α and IFN- γ in groups B, C and D were significantly higher than those of control group. The significant levels of IL-10 were increased in groups C and D. All immunized group mice showed the significant level of resistance against the challenge strain colonization in spleen compared to control group mice. Especially, the highest levels of resistance against the challenge strain colonization were shown in groups C and D mice.

Conclusions: Taken together, these results show that feasibility of *B. abortus* ghost vaccine lysed by PMAP-36 (GI24) can be used as an effective and safe vaccine to induce protection against systemic infections with virulent *Brucella abortus*.

References

- [1] DelVecchio V.G., Alefantis T., Ugalde R.A., Comerci D., Marchesini M.I., Khan A., Lubitz W., Mujer C.V., 2006. *Methods of Biochemical Analysis* 49: 363-377.
- [2] Olsen S.C., 2013. *Revue Scientifique et Technique* 32: 207-217.

P-068

Detection of *Lawsonia intracellularis* in Horses using Serology and PCR in the Republic of Korea

Md Mukter Hossain¹, Sung-Hyun Moon¹, Byoung-Joo Seo¹, Won-Il Kim¹, Byeong Yeal Jung², Se-Ji Cho², Eu-Tteum Song², Jae-Won Byun², Ho-Seong Cho^{*1}

¹College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Iksan 570-752, Korea; ²Animal and Plant Quarantine Agency, Anyang 430-757, Korea

Introduction: Over the past decade, *Lawsonia intracellularis*,