

GM1 receptors was determined by means of GM1ELISA. Efficacy of the *Salmonella* Typhimurium-LTBghost as a vaccine candidate was evaluated in a chicken model using 56 five-week-old chickens, which were divided into four groups (n=14); Group A was designated as the non-vaccinated control group, whereas the birds in groups B, C, and D were intramuscularly immunized with 10^9 , 10^8 , and 10^7 ghost cells, respectively. Compared with the non-immunized chickens (group A), immunized chickens (groups B, C and D) exhibited increased titers of plasma IgG and intestinal secretory IgA antibodies.

Conclusions: After oral challenge with 10^9 CFUs of a virulent *Salmonella* Typhimurium strain, the vaccinated group B birds showed a decrease in internal organ colonization with the challenge strain

P-04

Prevalence and phylogenetic analysis of equine hepaciviruses in Korea

Ho-Seong Kim¹, Hyuk-Moo Kwon^{*1}

¹Laboratory of Veterinary Microbiology, College of Veterinary Medicine, Kangwon National University, Chuncheon, Republic of Korea.

Introduction: Non-primate hepacivirus (NPHV) was discovered 5 years ago in respiratory samples of domestic dogs in USA for the first time. In phylogenetic analysis, NPHV is classified to the genus *Hepacivirus* of the family Flaviviridae, which contains hepatitis C virus (HCV). HCV was reported over 20 years ago. The equine NPHV has been isolated in the United States, Scotland, Hungary, Japan, Brazil, and Germany. Some studies reported no relationship between NPHV infection and disease. However, recent study showed that the RNAs of NPHV were mainly isolated from the liver of horses in both chronic and acute stages of its infection. Furthermore, two other studies demonstrated that NPHV in horses had infection kinetics similar to that of HCV, and that NPHV infection was related with acute and chronic liver disease. Also, in one clinical case about horse with hepatitis, the high level of viremia with NPHV was detected. In this study, we detected and sequenced NPHV for the first time from horses in Korea.

Materials and Methods: A total of 74 equine serum samples were collected from horses in Chuncheon-si, Gwacheon-si, Icheon-si and Jeju-si regions in Korea between August and December 2015. We performed nested PCR for NPHV NS3 protein to detect NPHV. Eluted PCR products were cloned into the PCR2.1-TOPO vector using a TOPO TA Cloning Kit (Invitrogen, USA) and transformed into One-shot TOP 10 competent cells (Invitrogen). Plasmid DNAs of positive clones were extracted by DNA-spin Plasmid DNA Purification Kit (IntRON). Plasmid DNAs were sequenced in both directions by fluorescence-based sequencing. The nucleotide sequence data were compiled and analyzed by means of the Lasergene computer program (DNASTAR, Inc).

Results: The proportions of positive rates for NPHV RNA were compared based on horse gender, age, breed and area.

With regard to gender of horses, there were higher positive rates in male and gelding horses (7/24, 29.2%) than female horses (7/50, 14.0%) in this study. With regard to age of horses, there were high positive rates for NPHV RNA in horses of 1-2 years (4/10, 40%) than horses of 3-10 years (10/51, 19.6%). However, horses of 11 or more years were all negative for NPHV RNA (n=13). With regard to breed of horses, Thoroughbred horses had high positive rates for NPHV RNA (14/56, 25.0%). The other breeds including Korean pony (11), Warm blood (1), Shetland pony (3), Appaloosa (1), Australian Pony (1), and Connemara Pony (1) were all negative for NPHV RNA (n=18). With regard to area, there were significant differences in positive rates for NPHV RNA. The positive rates of 4 cities were 16.7% (2/12) in Chuncheon-si, 0% (n=12) in Gwacheon-si, 21.6% (8/37) in Icheon-si and 30.8% (4/13) in Jeju-si. We could not find positive horses in Gwacheon-si, but the other cities had relatively high positive rates compared to other studies.

A total of 14 horses were positive among 74 horses (18.9%). In the NS3 gene, the 14 Korean NPHVs shared approximately 85.3-99.6% nucleotide sequence identities with each other. The nucleotide sequences of equine NPHV NS3 genes detected in Korea were significantly different from those of HCV (55.8-63.4%), bat NPHV (56.2-59.6%), rodent NPHV (56.2-58.9%), and bovine NPHV (53.8-56.1%). However, they showed relatively high similarity with those of canine NPHV (85.3-96.6%). We found various sequence divergences of NS3 genes between horses in our study and horses from other countries (83.4-100%). Phylogenetic analyses based on the NS3 gene of Korean NPHVs showed that Korean NPHVs were clustered into 2 clusters which were reported previously. Ten isolates were classified into one cluster which contained NPHVs from Hungary and Germany. Four isolates were classified into the other cluster which contained canine NPHV. We found that the sequences of equine HCV detected in same horse farm showed high sequence divergences.

Conclusions: This study is the first report of NPHV in Korea. Prevalence (14/74, 18.9%) of equine NPHV infection in Korea was higher than foreign countries. According to the phylogenetic analysis, 2 or more lineages of equine NPHV were existed worldwide.

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P-05

Prevalence of *Salmonella* spp. in pet turtles and their environment in Korea

Gang-Joon Heo^{*1}, Du-San Back¹, Gee-Wook Shin², Mitchell Wendt¹, Sabrina Hossain¹, Sudu Hakuruge Madusha Pramud Wimalasena¹

¹College of Veterinary Medicine, Chungbuk National University,

²Bio-Safety Research Institute and College of Veterinary Medicine,

Introduction: Pet turtles are known as a source of *Salmonella* infection to humans when handled in captivity. Human cases of salmonellosis are often associated with food, but frequently people become infected by handling infected material such as infected turtles, water and soil.

Materials and Methods: Thirty four turtles purchased from pet shops in Korea were examined to determine whether the turtles and their environment were contaminated with *Salmonella* spp. We studied the isolation rate of *Salmonella* spp. from fecal samples of pet turtles purchased in pet shops as well as water and soil samples in their environment over time.

Results: *Salmonella* spp. were isolated from 17 (50.0%) of 34 turtle fecal samples; positive samples were from 8 of 10 Chinese stripe-necked turtles, 3 of 8 yellow belly sliders, 3 of 6 river cooters, 3 of 4 northern Chinese softshell turtles and no *Salmonella* spp. was isolated from the western painted turtles and common musk turtles. The isolation rate of *Salmonella* spp. increased over time. In other words, on day 2 *Salmonella* spp. was isolated from five water samples and 8 soil samples; however, *Salmonella* spp. was isolated from 14 water samples and 15 soil samples on day 10. This demonstrates that most of the cage environments were contaminated with *Salmonella* spp. over time.

Conclusions: We concluded that a high percentage of turtles being sold in pet shops were infected with *Salmonella* spp., and their environments tend to become contaminated over time unless they are maintained properly. These results indicate that pet turtles could be a potential risk of salmonellosis in Korea.

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P-06

Cigarettesmoke exposure impaired maturation of ovarian follicle and normal growth of uterus inner wall of female rats

Hae-Miru Lee¹, Kyung-Chul Choi*¹

¹Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea

Introduction: Cigarette smoke (CS) is well known to be very harmful to human body functions such as fertility, reproduction, and development. To elucidate the effect of CS on women's fertility more definitely, we examined the histopathological characteristics of the uterus and ovary

Materials and Methods: We received ovary and uterus sample from Korean Conformity Laboratories (KCL), which were obtained from the female rats exposed to the different amounts (low, medium, and high concentrations) of smoke of the standard cigarette (3R4F) for 2h/day and 5 days/week for 28 days according to the OECD guidelines. The animals used for the present study were the spontaneously hypertensive female Wistar Kyoto (WK) rats. We manufactured tissue slides from uterus and ovary samples and evaluated maturation of follicle of ovary and uterus development through H&E and immunohistochemistry (IHC).

Results: It was shown that CS decreased maturation of follicle and abnormal uterus development by CS exposure. In IHC and H&E analysis on ovary tissues, we confirmed that the number of stages of follicles was decrease. (As compared to the non-exposed rats). For uterus, the thickness of inner wall of uterus was decreased by the exposure to CS at low and medium concentrations. In accordance with this result, the expression of PCNA was decreased, but the expression of Bax and CHOP was increased by exposure to CS at low and medium concentrations. However, acute exposure to CS at high level induced the abnormal over-growth of uterus wall.

Conclusions: These results may help elucidate the action of common CS on female rats reproductive organs. The exposure of CS may have a harmful effect on women's fertility and pregnancy by inducing decreased maturation of ovarian follicle and abnormal growth of uterus inner wall.

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P-07

Identification and characterization of outer membrane vesicles (OMVs) derived from antimicrobial resistant and sensitive *Escherichia coli*

Si Won Kim¹, Seong Bin Park², Se Pyeong Im¹, Jung Seok Lee¹, Jae Wook Jung¹, Tae Sung Jung¹

¹Laboratory of Aquatic animal diseases, College of Veterinary