

froze in less than 80% in hard water. With these concentrations, the mixtures of citric acid with ethylene glycol or windshield washer fluid could reduce the infectivity of the viruses by more than 4 log₁₀ in 5 minutes as well as 30 minutes at -20°C. But sodium carbonate mixed with ethylene glycol or windshield washer fluid in the same manner did not reduce the infectivity of the viruses in 30 minutes at -20°C.

Conclusions: The efficacy of the citric acid in inactivating FMDV was not decreased with the addition of the anti-freezer compound or the windshield washer fluid even at the freezing temperature (-20°C). Propylene glycol would be used more environment friendly than ethylene glycol in terms of the toxicity to people and other animals. To apply these anti-freezers or windshield washer fluid in the field, the limitations need to be considered and more tests need to be done in the field.

References

- [1] Pereira, H.G., Foot-and-mouth disease. New York, N.Y: Academic Press, 1981, 333-363
- [2] Bachrach, H.L., Foot-and-mouth disease. Annu Rev Microbiol, 1968. 22, 201-44
- [3] Domingo, E, et al., Evolution of foot-and -mouth disease virus. Virus Res, 2003. 91(1), 47-63.

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Enhanced Inhibition of Foot-and-Mouth disease Virus by Combination of Chemical Reagents and Recombination Adenovirus Expressing Small Interference RNAs

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Introduction: Foot-and-mouth disease (FMD) is highly contagious disease in cloven-hooked animals such as pigs, sheep, goats and cattle causing reduction productivity of milk, growth rate of meat animals. Foot-and-mouth disease virus (FMDV) is the type species of the *Aphthovirus* genus of the *Picornaviridae*. The current vaccines for FMD is not complete prevention methods because it induced immune response 7 days after vaccinated. It reduced protection effect of FMD. Therefore, we need to find complementary strategy for FMDV vaccine. Small interfering RNA (siRNA), known as silencing RNA, takes part in RNA interference (RNAi) pathway. RNAi interferes with specific gene with complementary nucleotide sequence. Recent study reported that the recombinant adenovirus expressing three siRNAs (Ad-siRNA) significantly inhibits FMDV replication (1). In this study, we tested the combination effect of chemical agents such as Ribavirin or 6-azauridine and Ad-siRNA.

Materials and Methods: Recombinant human adenovirus carrying small interference RNA (siRNA) was produced following the manufacturer's instructions (1). IBRS-2 cells were plated to 96-well with 90% confluence. Cells were inoculated 50 TCID₅₀ and 5 TCID₅₀ of Adenovirus. After 6h inoculation, various concentrations of Ribavirin or 6-azauridine which known as

antiviral reagents (2) were treated followed by removing the Ad-siRNA supernatant and washed twice with D-MEM. 24hr after infection of adenovirus, O/SKR/2002 serotype of FMDV was inoculated with 1000 TCID₅₀. After 1hr absorption at 37°C, cells were washed twice with D-MEM after removed supernatant and filled with D-MEM containing 2% FBS. 24 h and 48 h post infection (P.I.), supernatant were collected and viral RNAs were extracted. Real-time RT-PCR were performed for FMDV quantification.

Results: IBRS-2 cells treated only antiviral reagent, ribavirin and 6-azauridine showed higher viral copy number value compared with cells treated only Ad-siRNA. In addition, the cells treated combination mixture of Ad-siRNA and each antiviral reagent exhibited the lowest viral copy number value. The sequential treatment of Ad-siRNA and Ribavirin or 6-azauridine was not improved antiviral effect comparing with simultaneous treatment of Ad-siRNA and chemical reagents.

Conclusions: It is necessary to find alternative methods for increasing protection effect for FMDV. The mixture of Ad-siRNA and Ribavirin or 6-azauridine exhibited significantly inhibition of FMDV replication. These results demonstrated that combination of siRNA and antiviral reagent could be one of alternative methods for fill up the immunity gap. Animal experiment of combination effect is needed for further study.

References

- [1] Kim SM, Lee KN, Lee SJ, Ko YJ, Lee HS et al. (2010) Multiple shRNAs driven by U6 and CMV promoter enhances efficiency of antiviral effects against foot-and-mouth disease virus. Antiviral Research 87: 307-317.
- [2] Kim SM et al. (2012), Enhanced inhibition of foot-and-mouth disease virus by combination of antiviral reagents and recombinant adenovirus expressing porcine IFN- α , Antiviral Research, accepted and in press.

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Antimicrobial Resistant Enterococci Isolated from Companion Animals, Owners, Veterinary Staffs and People Who Have No Companion Animals

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Introduction: Population living with companion animals has rapidly increased. Thus, there has been concern that companion animals can transfer antimicrobial resistant bacteria to humans. However, there are a few studies about distribution of antimicrobial resistant bacteria among companion animals and humans. Enterococci are one of the major normal flora that colonize the skin, mucosal membranes, and the gastrointestinal tract of humans and animals. Furthermore, these bacteria cause a wide range of nosocomial infections in humans and some of them possess serious antimicrobial resistance such as vancomycin resistant enterococci. In this study, we investigated antimicrobial resistance of enterococcal isolates from companion animals, owners,

veterinary staffs and people who do not have companion animals. **Materials and Methods:** Enterococci were isolated in 2012. Samples were collected from external auditory meatus, nasal cavity, medial part of arm, and anus of humans and medial canthus, external auditory meatus, interdigital cleft, anus, and skin infection of companion animals. The disk diffusion tests were carried out for determining antimicrobial susceptibility for 11 antimicrobials including tetracycline, chloramphenicol, high-level gentamicin, high-level streptomycin, erythromycin, ciprofloxacin, ampicillin, quinupristin-dalfopristin (Q/D), vancomycin, teicoplanin, and linezolid according to the Clinical and Laboratory Standards Institute guideline. Statistical analysis was performed by the method of chi-square test on SPSS program (SPSS Institute, IL, US).

Results: A total of 91 enterococci were isolated. Vancomycin, teicoplanin, and linezolid resistances were not detected. Except Q/D resistance, all the resistance rates were the highest in the group of veterinary staffs. There was no significant difference of antimicrobial resistance rates between owners of companion animals and people who do not have them. The rates of multidrug resistance (MDR) were significantly higher among veterinary staffs (100%) than in companion animals (44.2%), owners (35.7%) and people who don't have them (29.6%) ($P < 0.05$).

Conclusions: Antimicrobial resistance and MDR rates were exceptionally high in the group of veterinary staffs but there was no significant difference between companion animal owners and other people. Accordingly, a guideline about antimicrobial use for animals is needed and an awareness of the concept of hospital acquired infection control should be improved in the veterinary hospital.

References

- [1] Kwon KH, Moon BY, Hwang SY, Park YH. Detection of CC17 *Enterococcus faecium* in Dogs and a Comparison with Human Isolates. *Zoonoses Public Health*. 2012 Sep;59(6): 375-8.
- [2] Moon BY, Youn JH, Shin S, Hwang SY, Park YH. Genetic and phenotypic characterization of methicillin-resistant staphylococci isolated from veterinary hospitals in South Korea. *J Vet Diagn Invest*. 2012 May;24(3):489-98.

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The Infectivity of Foot-and-Mouth Disease Virus in the Leachate of Burial Site

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Introduction: During the last two decades, in the Republic of Korea, five discontinuous foot-and-mouth disease (FMD) outbreaks occurred in 2000, 2002 and 2010-2011. The causes of the outbreaks were serotype A in January 2010, but in the other outbreaks they were serotype O. Since November 2010, about 3.5 million of cattle, pig, goat or deer were killed and buried at more than 4,000 sites nationwide. However, the study on the infectivity and detectability of the viruses contaminated

in the leachate from the burial site with the dead infected animals is rare. So, we studied the inactivation rate of the infectivity of FMDV in the leachate, used as a matrix, and the range of viral detection with rRT-PCR at different temperature conditions.

Materials and Methods: Two different strains of serotype O and one strain of serotype A (O/Andong/ROK/2010 (Accession no. JQ070321, $10^{6.17}$ TCID₅₀/ml), O/Paju/ROK/2010 (Accession no. not register, $10^{6.38}$ TCID₅₀/ml) and A/Pocheon/ROK/2010 (Accession no. JQ070331, $10^{5.19}$ TCID₅₀/ml)) were propagated and titrated in LF-BK cells. Three different FMDV-negative sources of leachates (no. 1, 5, and 9) from the real burial sites and DMEM (negative control) were spiked with these three kinds of viruses and put on the two different temperature conditions (4 and 37°C) in the darkness. After 0 h, 1 day, 4 days, 7 days, 14days, 29 days and 35 days, the infectivity and the copies of viral genome contained in the leachates were quantified.

Results: At 4°C the viruses in the leachates no. 5 and 9 did not lose the infectivity much by two weeks (1-2 log₁₀ reduction), but in leachate no. 1, there were more than 4log₁₀ reduction in the infectivity of the viruses. By contrast, at 37°C, within 1-4 days the viral samples in the leachates (no.1, 5, and 9) lost their infectivity completely. The interaction effect between the sources of the leachate and temperature was shown in leachate no. 1. For rRT-PC, regardless of the sources of leachate and virus isolates, at 4°C, Ct values were not changed upto 35 days, but, at 37°C, Ct values were delayed in proportion to the lapse of time.

Conclusions: If we suppose that the temperature in the depth of dead bodies of the infected animals buried is around 15-20°C and the pH of the leachate from them reaches neutral zone shortly after the burial, we can guess that the infectivity in the leachate is likely to be lost in a few months from the date of burial. However, the detection of viral genomes with rRT-PCR would be feasible for a longer period without infectivity. So this discrepancy needs to be considered for the proper handling and risk assessment of the burial sites.

References

- [1] Muhammad T.G. et al. Physical Factors Affecting *in Vitro* Replication of Foot and Mouth Disease Virus (Serotype "O"). *Pakistan veterinary journal*. 2011, ISSN: 0253-8318.
- [2] Anette B., Graham J.B. Virus survival in slurry: Analysis of the stability of foot-and-mouth disease, classical swine fever, bovine viral diarrhoea and swine influenza viruses. *Veterinary Microbiology*. 2012, Vol. 157, Issues 1-2: 41-49.

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Plasmid-Mediated Quinolone Resistance and Extended-Spectrum Cephalosporin Resistance in Enterobacteriaceae Strains Isolated from Breeder Flocks

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