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P-016

Genetic Analysis of the Spike Gene of a Porcine Epidemic Diarrhea Virus (PEDV) Strain Isolated from Gyeonggi Province in 2014

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Introduction: Porcine epidemic diarrhea virus (PEDV) is an etiological agent causing acute diarrhea, dehydration and high mortality in sucking piglets. PED has been reported sporadically since explosive outbreaks in Korea in the 1990's, but recently recurs as more severe cases despite of vaccination in national wide. Thus, there is a strong controversy on the protective efficacy of commercial vaccines available on the market, speculating that newly emerging virus strains might be evolved over a period of time. In this light, new vaccine candidates capable of dealing with virus strains prevalent in swine farms are actively being searched. Here we report that a field strain was newly isolated in 2014 and its spike gene was genetically analyzed.

Materials and Methods: Virus isolation was performed on Vero cells with the small intestine of a piglet affected with severe diarrhea. When cytopathic effect obviously appears, the virus was purified by a serial plaque assay and then subjected to immunofluorescence assay with PEDV- specific monoclonal antibody. After amplification of the purified virus, viral RNA was extracted from culture supernatant using the RNeasy Mini kit (Qiagen). PEDV-specific spike gene was amplified by PCR using the One-Step RT-PCR kit (Invitrogen). The sequence of amplified DNA products was confirmed in a both-direction sequencing method. The full-length spike gene of the isolate was analyzed using a DNAMAN program (Lynnon Biosoft) and neighborhood-joining tree was generated using a Clustral X program.

Results: A field strain, named KBNP-PPC410, was isolated from a diseased piglet submitted from a swine farm located on the province of Gyeonggi in 2014. It was confirmed by PEDV-specific PCR, and immunofluorescence assay. Homology analysis with the full-length spike gene sequence revealed that KBNP-PPC410 showed a similarity of 94.0%, 96.5% to SM98, CV777, respectively, at the nucleotide. Also, the similarity of 95.5%, 96.5% to SM98, CV777, respectively, was found at amino acid. However, three distinct insertion sequences, including a neutralizing epitope on the spike gene were detected compared to that of a vaccine strain SM98.

Conclusions: KBNP-PPC410 was isolated as a field strain currently circulating in swine farms in Korea and classified into a similar group of a prototype CV777. Nevertheless, it would be worth of investigating the biological significance of such insertions leading to genetic differences between KBNP-PPC410 and SM99 strains.

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P-017

Evaluation of Nine Germicides against the Pathogen of Avian Botulism

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Introduction: Recently, avian botulism has emerged as a problem in Korea. The pathogens of all avian botulism cases in Korea since 2012 have been *Clostridium botulinum* toxin type C/D. But, there is no article on effective germicide of this pathogen. So weconducted the experiment to find effective germicide killing spores of *C. botulinum* toxin type C/D.

Materials and Methods: Nine kinds of germicides (3 oxidants, 1 acidulant, 4 aldehydes, 1 extra germicide)distributed in Korea were selected for this study. To evaluate the sporicidal activity of these nine kinds of germicides, dilution-neutralization method was used. 100㎕ of germ suspension was added in 400㎕ of each germicides. Neutralizing broth has been applied after 30m each. And each sample is spread on McClung Toabe Agar after 10-fold serial dilution. Colony Forming Unit (CFU) has been checked after anaerobic incubation for 24 hours in 37℃. Spore-killing ability of germicides had been expressed as Inactivation Factor (IF), calculated by $\log_{10}$ (CFU decrease). And germicides which were represented more than 4 IF have been judged as 'effective'.

Results: Only 'Willow vet HALASOL (Sodiumhypochlorite, Yuhan Corporation)' represented more than 4 IF.

Conclusions: Based on this result, we would recommend HALASOL to farm affected by avian botulism.