PO-21

Development of DNA Chip System for Differential Diagnosis of Porcine Enteric Pathogens

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Introduction

Intestinal infections are common in growing pigs and can be caused by multiple pathogens, environmental and management factors [1]. Among the most important viruses in swine enteritis are porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine enteric calicivirus (PECV), porcine group A rotavirus (PRV gp A) and bacteria are Escherichia coli and Salmonella spp. and protozoa is Isospora suis [1].

The DNA chip system can serve as a powerful tool that can be utilized for simultaneous detection of specific pathogenic bacteria strains and viruses [2,3]. The combination of PCR and DNA chip technology will provide a novel method for the detection of porcine enteric pathogens thus revolutionize the diagnosis and management of the disease. The aim of this study is to develop DNA chip system for the rapid and reliable detection of five major porcine enteric pathogens based on oligonucleotide DNA chip hybridization.

Materials and Methods

Tissue culture fluid containing TGEV (Miller), PECV (Korean isolate), PEDV (KPEDV-9) and PRV gp A (Gottfried) and PRV gp C (Cowden); *E. coli, Salmonella spp.* and *Isospora suis* obtained from Choong Ang Vaccine Lab (Daejeon, Korea) were used as reference strains. 40 fecal samples from cases of diarrhea in post-weaning pigs were collected from several pig farms. Viral RNA, bacterial and protozoa DNA were extracted using standard extraction method according to the manufacturer's protocol. The oligonucleotide primers and probes used in the DNA chip and RT-PCR were designed from published sequence of the nucleocapsid (N) gene of TGEV (GenBank accession No. AJ271965), the spike (S) gene of TGEV (GenBank accession No. AJ271965), the matrix (M) gene of PEDV (GenBank accession No. AF015888), the RNA-dependent RNA polymerase (RDRP) gene of PECV (GenBank accession No. AF182760), VP 7 gene (GenBank accession No. X06759) of PRV group A and C. For E. coli, the 16s rRNA (GenBank accession No. J01859), F4 gene, EAST1 gene, STa gene, STb gene and LT gene obtained from GenBank were used as oligonucleotide primers and probes; whereas fimbrial genes were used for *Salmonella spp.* The sequence for *Isospora suis* was also obtained from GenBank.

Results

Positive signals were detected for TGEV, PECV, PEDV, PRV gp A and C, E. *coli, Salmonella spp* and *Isospora suis* using DNA chip system. Overall, the agreement of RT-PCR and DNA chip was higher when compared with PCR. The sensitivity and specificity of DNA chip was also better when compared with PCR technique. The result showed that DNA chip system is a rapid and reliable method with high specificity and sensitivity for the detection of major causes of porcine diarrheal disease.

Discussion

The application of DNA chip system for major porcine enteric viruses, bacteria and protozoa from fecal samples has been described. The ability of DNA chip system to simultaneously detect various causative agents for porcine diarrhea is valuable method for the differential diagnosis of porcine enteric diseases. DNA chip system provides powerful tools which is rapid and reliable in one single reaction. This will enable the veterinarian to respond with proper treatment and management for porcine enteric disease promptly, thus assisting the livestock industry.

References

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