by sequencing 326 bp RNA dependent RNA polymerase region. Amongst the 932 diarrheic calves, 17 (1.8%) were found to be positive for norovirus genogroup III. The age of these calves was between 3 days and 1 year. Among the BNoVs examined, one Korean BNoV strain had the lowest nucleotide (60.8%) and amino acid (71.7%) identity, while the remaining 16 strains had nucleotide (88.6~94.4%) identity and amino acid (86.9~96.9%) identity with the genotype 2 BNoV (GIII-2) strains.

Conclusions: The alignment indicated that the seventeen BNoVs clustered with the GIII-2 prototype. Further investigations are needed to determine the clinical symptoms and epidemiology of norovirus infections in calves in Korea.

References

[1] H.Yilmaz., N.Turan., E.Altan., K.Bostan., A.Yilmaz., C.R.Helps., K.O.Cho., 2011. First report on the phylogeny of bovine norovirus in Turkey. Arch Virol. Springer. 156:143-147.

[2] S.I.Park., C.Jeong., H.H.Kim., S.H.Park., S.J.Park., B.H.Hyun., D.K.Yang., S.K.Kim., M.I.Kang., K.O.Cho. 2007. Molecular epidemiology of bovine noroviruses in South Korea. Vet. Microbiology. 124:125-133.

P-095

Multiplex reverse-transcription loop-mediated isothermal amplification (mRT-LAMP) assay for differential detection of porcine reproductive and respiratory syndrome viruses

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Introduction: Porcine reproductive and respiratory syndrome virus(PRRSV) is an important swine pathogen that causes tremendous economic losses in swine production worldwide. This virus divided into two genetically and antigenically distinct genotypes: European (EU) and North American (NA).Recently, co-infection with both genotypes has been increasing in Europe, North America, and Asia, resulting in a significant impact on PRRSV diagnostics and management. In this study, to simultaneous detection of EU and NA PRRSVs more simple, rapid and cost-effective, Reverse transcription loop-mediated isothermal amplification assay was developed by applying previously reported multiplex LAMP strategy (Nyan et al., 2015)[2].

Materials and Methods: The Web-based primer design software PrimerExplorer V4 was used to design the six primers targeting the conserved regionsof the nucleocapsid (N) gene of EU and NA PRRSVs and bi-labeled backward loopprimer (LBp) was used for multiplex LAMP assay. bi-labeled backward loop primer(LBp) covalently linked with 6-ROX as reporter on the 5' end and BHQ2 as quencher on the 3' end for EU-PRRSV and HEX as reporter on the 5' end and BHQ1as quencher on the 3 end for NA-PRRSV. For detecting EU or NA PRRSV, RNA template extracted from primary porcine alveolar macrophages and MARC-145 cell cultures infected with the Lelystad virus and VR2332 strain at the initial viral titer of 104 50% tissue culture infection dose ($TCID_{50}$))/0.1mL. A single RT-LAMP assay for each EU and NA PRRSV was optimized and later combined and optimized as a multiplex reaction by adjusting concentration of each set of primers and reaction time. Multiplex LAMP amplification was carried out at 58 ℃ for 60min and then the mixture was heat inactivated at 80 °C for 5min to terminate the reaction. Results of the mRT-LAMP assay were detected by fluorescence signal.

Results: The mRT-LAMP assay successfully detected EU and NA PRRSVs in a single reaction but not any other porcine pathogens. The limit of detection of the mRT-LAMP was 0.1 $TCID_{50}$/1 mL for EU and NA PRRSV, respectively, and was comparable to that of previously reported singular types of RT-LAMP assays and real-time RT-PCR assays[1]. Clinical evaluation of the mRT-LAMP assay with various porcine samples showed that the assay sensitivity was comparable to that of the previously reported real time RT-PCR and more sensitive than that of the conventional PCR.

Conclusions: The highly specific andsensitive mRT-LAMP assay developed in this study can serve as a rapid, cost-effective, and user-friendly diagnostic tool for EU and NA PRRSVs,even in under-equipped laboratories.

References

[1] Park, J.Y., Park, S.J., Park, Y.R., Kang, D.Y., Kim,E.M., Jeon, H.S., Kim, J.J., Kim, W.I., Lee, K.T., Kim, S.H., Lee, K.K., Park,C.K., 2016. Reverse-transcription loop-mediated isothermal amplification(RT-LAMP) assay for the visual detection of European and North American porcine reproductive and respiratory syndrome viruses. J. Virol. Methods 237, 10-13.

[2] Nyan, D.C., Swinson, K.L. 2015. A novel multiplex isothermal amplification method for rapid detection and identification of viruses. Sci. Rep. 5, 17925.

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Multiplex reverse transcription loop-mediated isothermal amplification assay for the rapid detection of M and H5 gene of avian influenza viruses

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Introduction: The H5 subtype of the highly pathogenic avian influenza virus (HPAIV) has caused significant economic losses in the poultry industry and poses a potential threat to animal and human health worldwide. Rapid and accurate diagnostic methods are essential for monitoring, managing disease and controlling early infection of new HPAIV. Molecular diagnostic methods including reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time RT-PCR (RRT-PCR)analysis have been successfully applied to rapid and reliable AIV detection. However, these technologies require sophisticated and expensive equipment and specialists, limiting their effectiveness in small, streamlined laboratories in developed or developing countries. Since the development of loop-mediated isothermal amplification (LAMP) in 2000, this method has been regarded as a useful tool for the detection of various pathogens with high sensitivity, specificity, rapidity and simplicity. However, considering that various subtypes of AIV and H5 HPAIV coexist between wild birds and poultry birds in the highly pathogenic avian influenza epidemic, the RT-LAMP method, which can simultaneously detect the different subtypes of H5HPAIV and AIV, It is necessary for monitoring. In this study, we developed a multiplex RT-LAMP of influenza virus by applying previously reported multiplex LAMP strategy (Nyan et al., 2015).

Materials and Methods: The M gene and H5 gene- specific primer sets for detecting all subtypes of AIV and H5 subtypes of HPAIV were taken from our previous research, with some modifications (Park et al., 2017). For simultaneous detection of M and H5 gene, Fluorescence dye was attached to loop backward primer(LB) as previously described (Nyan et al.,2015). 6-Carboxyfluorescein(FAM) dye was attached to M gene specific loop backward primer, and ROX dye was attached to H5 gene specific loop backward primer. To improve sensitivity, mRT-LAMP was performed by mixing LB and LB labelled with fluorescent dye. The mRT-LAMP reaction was carried out as previously described (Park et al., 2017). The reaction was performed in a single step at 58°C for 40 min, followed by heating at 80°C for 2min to terminate the reaction. Results of the mRT-LAMP assay were detected by fluorescence signal.

Results: The mRT-LAMP assay successfully detected all tested AIVs and H5 subtypes of AIV simultaneously. The detection limit of the assay was approximately $10^{2.0}$ copies for the M and H5 genes, respectively, and was comparable to that of previously reported single RT-LAMP and real-time RT-PCR assays. These results suggest that the present mRT-LAMP assay, with its high specificity, sensitivity, and simplicity, will be a useful diagnostic tool for surveillance of currently prevalent H5 HPAIVs and other subtypes of AIV, even in under-equipped laboratories.

Conclusions: The highly specific and sensitive mRT-LAMP assay developed in the study can serve as a rapid, cost-effective, and user-friendly diagnostic tool for currently prevalent H5HPAIVs and other subtypes of AIV in avian populations, even in under-equipped laboratories.

References

[1] Park, Y.R., Kim, E.M., Han,D.H., Kang, D.Y., Yeo, S.G., Park, C.K., 2017. Reverse transcriptionloop-mediated isothermal amplification assay for the rapid and simultaneousdetection of H5 and other subtypes of avian influenza viruses. J.Vet. Serv. 40, 15–20.

[2] Nyan, D.C., Swinson, K.L.2015. A novel multiplex isothermal amplification method for rapid detection andidentification of viruses. Sci. Rep. 5, 17925.

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Multiplex realtime polymerase chain reaction for simultaneous detection of beak and feather disease virus (BFDV) and avian polyomavirus (APV)

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Introduction: Recently, the number of parrot lovers have been increasing in Korea followed by consistently increasing parrot importation from foreign country. However, parrots have not been taken disease screening in quarantine process when they are imported. Thus, it is presumed that various parrot diseases were already flowed into Korea and have been infecting captive parrot groups.[2] Avian polyomavirus (APV), also known as Budgerigarfledgling disease (BFD), and psittacine beak and feather disease virus (PBFDV)are the common viral diseases of psittacine birds. APV is a nonenveloped, icosahedral virus with a double stranded, circular 4981-bp genome. The molecular properties of APV isolates from various avian species have been characterized. The genomic organization of BFDV-1 from Budgerigar, BFDV-2 from chicken, and BFDV-3 from parakeet has been shown to be similar to that of other polyomaviruses such as SV40.[2] Among these viruses, infection case of BFDV and APV are more frequently detected and because of similar feather disorder, laboratory diagnostic tool is needed for differential diagnosis of the disease pathogens. However diagnostic tool for differential detection of BFDV and APV have not been developed yet in Korea. In this study, to simultaneous detection of BFDV and APV, rapid and sensitive multiplex quantitative real time PCR (mqPCR) was developed.

Materials and Methods: BFDV primers and probes were designed on the V1 gene and APV primers and probes were designed on the T/t antigen gene using a clone manager (SEcentral, USA) based on the nucleotide sequence registered in NCBI. Standard samples of Both viral target genes were prepared as plasmids by cloning. Each