POPULAR SCIENCE SUMMARY

Development and evaluation of loop mediated isothermal amplification assays for the detection of Classical swine fever virus and African swine fever virus

Classical swine fever (CSF) and African swine fever (ASF) are highly contagious viral disease caused by classical swine fever virus (CSFV) and African swine fever virus (ASFV) respectively. They both top the list of the Office International des Epizooties (OIE) List A diseases. Both wild and domestic pigs can be infected. CSFV is present in Eastern Europe, Southeast Asia, Central America and South America. At present CSFV is eradicated from domestic pigs in Western Europe but still remains common in some populations of wild boar and therefore the farms around these areas are at a greater risk of re-infection. Presently ASFV has affected quite a number of animals in African countries, Mediterranean island and Sardinia (Italy).

The ASFV infection causes a wide range of clinical signs and that are quite similar to those of CSFV infection so there is a need of convenient tool or an assay for simultaneous detection of CSFV/ASFV. The assay used in this project was "LAMP" (Loop-mediated isothermal amplification) assay which is a simple, rapid, specific and cost-effective nucleic acid amplification technique developed by Eiken Chemicak Co., Ltd. The chemical pathway for nucleic acid amplification in LAMP is quite complex. DNA amplification is accomplished through the use of Bst DNA polymerase which exhibits strand displacing activity and therefore a thermal denaturation step is not required. This strand displacing activity allows the LAMP reaction to proceed under single temperature (isothermal) unlike PCR which requires a thermal denaturation step to produce single stranded DNA. This technique can also be made use for amplification for RNA templates by just adding reverse transcriptase enzyme (which converts RNA to DNA) to the reaction mixture.

In this project, single-plex and multi-plex LAMP assays were developed for the detection of CSFV and ASFV. The assays provide a simple, rapid and reliable tool for the detection of CSFV and ASFV with acceptable sensitivity. The assays can also be used as a simple-to-use field diagnostic tool which can help in field detection of CSFV and ASFV. A duplex LAMP assay is required to investigate simultaneously if a sample is positive for CSFV or ASFV. To differentiate CSFV and

ASFV, restriction enzyme digestion was performed. Latter, the performance of the LAMP assays was compared with real-time PCR for the detection of CSFV and ASFV. We found that LAMP assay was 10 to 100 fold less sensitive when compared to real-time PCR. We observed that the duplex LAMP assay was about 10 fold less sensitive than single-plex LAMP assay. This could be due to the presence of additional primers, which might interfere with specific amplification of targets.

Although LAMP assay has high analytical sensitivity but a small contamination can readily cause false positive results. LAMP assay is useful for less equipped laboratories due to its isothermal nature. It could also be used as a simple-to-use field diagnostic tool which can help in field detection of CSFV and ASFV and can also compliment for the detection of other swine diseases.