E-COST scientific report

Short-term scientific mission (STSM):

Application of fast molecular techniques for African swine fever virus detection

COST-STSM-ECOST-STSM-CA15116-020117-083188

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Period of research stay: 20.03.2017-14.04-2017
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1. Purpose of the STSM

The purpose of the STSM was to compare different fast molecular techniques, including real-time PCR- and isothermal amplification assays, for the detection of African swine fever virus (ASFV) DNA in field samples obtained in Poland. A fast and reliable diagnosis of such samples is one of the most important measures in the control of ASFV transmission in affected regions, as the application of the fastest and most sensitive diagnostic methods could enable earlier detection of the virus and thus earlier intervention by the competent authorities.

Currently, real-time PCR methods are applied in numerous laboratories for the detection of ASFV, and several methods have shown a high diagnostic sensitivity and specificity, including the OIE recommended real-time PCR using Universal Probe Library (UPL-probe) [1]. New real-time PCR methods and kits for the detection of ASFV are continuously marketed, some applying ready to use reaction mixtures and fast reaction conditions. Hence, these kits may provide a faster diagnosis of the infection within the laboratories. Still, as the real-time PCR methods rely on the use of sophisticated laboratory equipment, diagnosis could be delayed due to shipping time to the laboratories. Identification of ASFV cases under field conditions would allow an even faster diagnosis of suspected cases. Such prelaboratory identification could be obtained by the use of different isothermal methods, which are not dependent on the use of a thermal cycler [2].

2. Description of the work carried out during the STSM

During the STSM, different real-time PCR and isothermal methods for the identification ASFV DNA in Polish field samples were compared. Hence, the OIE recommended real-time PCR with UPL-probe was compared to two new commercial ready-to-use duplex real-time PCR kits (kits A and B – not on the market yet), both containing an internal swine β-actin control. For kit A, samples were analyzed using standard reaction conditions and fast reaction conditions, respectively. For kit B, samples were analyzed using standard reactions conditions, only. In addition, some samples were analyzed using an isothermal amplification assay, cross-priming amplification (CPA) [2]. Amplified product from the CPA reaction were visualized both by using SYBR Green I dye yielding a greenish fluorescence under UV illumination and by using a commercial lateral flow device.

In addition to the evaluation of different methods for the detection of viral DNA in field samples, methods for the isolation and quantification of infectious virus applied at PIWet were introduced. This included virus isolation methods for field isolates in both primary cells and continuous cell lines, and the detection of ASFV infected primary cells by hemadsorption. Finally, the STSM also included an introduction to the diagnostic ASFV laboratory at the Department of Swine Diseases, where hands on experience with handling and pre-processing of blood, tissue and bone marrow samples, DNA extraction methods and serological analysis (ELISA and IPT) have been obtained.

3. Description of the main results obtained

3.1. Evaluation of new real-time PCR kit A

The test panel consisted of 46 samples, representing Polish cases. Forty-five samples tested positive when using the real-time PCR with UPL-probe, while one sample was found ASFV-negative. This sample was previously identified as negative by PCR and antibody positive by ELISA and a confirmatory IPT. When using kit A, a FAM fluorescent amplification signal was produced in 44 samples previously identified as ASFV positive by the UPL-method. The positive samples yielded similar C_q-values when using the UPL-based method and kit A. In two samples ASFV remained undetected, one representing the seropositive ASFV-negative sample and one representing a sample in which Cq-values were high (around 38), when it was analyzed with the UPL-based real-time PCR. All porcine samples yielded a VIC fluorescent amplification signal, i.e. the internal swine β-actin control was successfully detected in these samples. The amplification profile of the negative control samples remained below the background fluorescence level for both FAM and VIC as expected.

Similar results were obtained when using the standard - and fast reaction protocols, respectively.

3.2. Evaluation of new real-time PCR kit B

When testing the panel of 46 ASFV samples a FAM fluorescent amplification signal was only produced in the samples, in which medium to low Cq-values (below 30) had been detected by using the real-time PCR with UPL-probe. In several samples, ASFV remained undetected or the C_q-values were above a predetermined cut-of at 40, and in correctly identified samples C_q-values were higher when using kit B compared to the real-time PCR with UPL-probe. None of the porcine samples yielded a VIC fluorescent amplification signal, i.e. the internal swine β-actin control was not detected. As for kit A, the amplification profile of the negative control samples remained below the background fluorescence level for both FAM and VIC.

3.3. Evaluation of isothermal methods

Different isothermal methods has previously been compared to each other and the real-time PCR methods by a PhD student at the host institute, and the results obtained during these studies were presented and discussed during the research stay. In order to gain hands-on experience with the isothermal methods and further insight into the advantages and drawback when using these methods, CPA was performed on selected samples. Thus, a panel of 10 ASFV positive samples, selected from the 46 previously tested samples (see 3.1. and 3.2) were used for this analysis. All samples were identified as ASFV-positive by visual examination after the addition of SYBR Green I, where a greenish fluorescence under UV illumination was observed. ASFV-positive results for all samples were also obtained when using a commercial lateral flow device, where two lines appeared on the flow device, indicating a control line plus a test line. The negative control sample, however, also yielded a slight positive (inconclusive) result when using the lateral flow device. Based on these results the problems with false positive reactions due to the interaction of multiple primer pairs in the CPA were discussed. Hence, it was discussed how the use of fewer primer pairs in the polymerase cross-linking spiral reaction (PCLSR), could offer a higher diagnostic specificity [3].

3.4. Other activities

Finally, spending time in the diagnostic laboratory, has given me the opportunity to gain more insight into the current disease situation in Poland. During my PhD, I have investigated infection stages and transmission of a Polish ASFV in Danish pigs under experimental settings. Hence, it has been interesting to learn which infection stages are currently being diagnosed (PCR/serology) by the laboratory and which transmission patterns they experience in the Polish restricted zones.

4. Future collaboration with host institution

It is anticipated, that the two departments will collaborate further in the field of molecular diagnosis and characterization of ASFV strains. Currently, next-generation-sequencing methods for field- and experimental ASFV samples are being established at PIWet and DTU-Vet, respectively. Hence, it would

be possible to exchange knowledge obtained using different pre-processing- and extraction methods as well as sequencing platforms. Finally, the introduction to other diagnostic - and research methods than the ones applied at the home institute, DTU-Vet, have fostered new ideas for the application of such methods during my PhD, including for the detection of infectious virus and viral DNA. Hence, it is anticipated, that the knowledge obtained from the comparisons of real-time PCR methods can be used at the home institute, DTU-Vet, guiding the choice of method for identification of ASFV DNA in experimental samples where viral loads are low (e.g. air samples, swab samples and fecal samples).

5. Confirmation of the host institution of the successful execution of the STSM

The confirmation letter from the host is attached below.

6. References

1. Fernández-Pinero, J., Gallardo, C., Elizalde, M., Robles, A., Gómez, C., Bishop, R., Heath, L., Couacy-Hymann, E., Fasina, F.O., Pelayo, V., Soler, A., Arias, M., 2012. Molecular Diagnosis of African Swine Fever by a New Real-Time PCR Using Universal Probe Library. Transbound Emerg Dis, 2012, 1-11. doi: 10.1111/j.1865-1682.2012.01317.x
2. Frazczyk, M., Woźniakowski, G., Kowalczyk, A., Niemczuk, K., Pejsak, Z., 2016. Development of cross-priming amplification for direct detection of the African Swine Fever Virus, in pig and wild boar blood and sera samples. Lett Appl Microbiol, 62, 386-391. doi:10.1111/lam.12569
3. Woźniakowski, G., Frazczyk, M., Kowalczyk, A., Pomorska-Mól, M., Niemczuk, K., Pejsak, Z., 2017. Polymerase cross-linking spiral reaction (PCLSR) for detection of African swine fever virus (ASFV) in pigs and wild boars. NATURE Scientific Reports, 7:42903, doi: 10.1038/srep42903