LinkTADs

Rapid detection and differentiation of ASFV and CSFV under field conditions. LinkTADs evaluation of a portable PCR assay

Montpellier 2015-09-02

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No: 613804

Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

- In a collaboration between Harbin Veterinary Institute a duplex LAMP assay for porcine hemorrhagic diseases (ASFV/CSFV) was planned

- This should be very useful in China, EU and Africa for rapid testing of suspicious cases and carcasses in the field or in basic laboratories

However!

Duplexing an ASFV LAMP assay with a newly developed CSFV RT-LAMP-LFD assay was unsuccessful!

Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

- Therefore a portable real-time PCR assay, using Tetracore T4 thermocycler, was developed and evaluated in China, to detect and differentiate ASFV from CSFV in basic laboratories or under field conditions

Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

- Central laboratory settings:
  - Laminar Flow Hood
  - Centrifuge
  - Fully automated sample prep using spin-column kits

- **Under field condition**:

  Minimal requirement for equipment

  Not feasible using spin-column kits

  Easy samples, e.g., blood or serum

➢ Magnetic beads-based extraction of DNA/RNA from blood/sera using MagMAX kit

Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

Published primers and probes were used

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PLOS | ONE

Development and Validation of a Multiplex, Real-Time RT PCR Assay for the Simultaneous Detection of Classical and African Swine Fever Viruses

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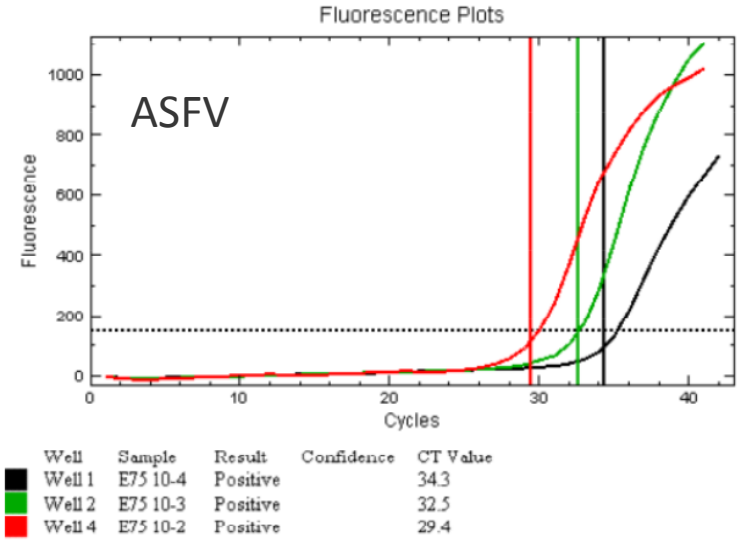
Abstract

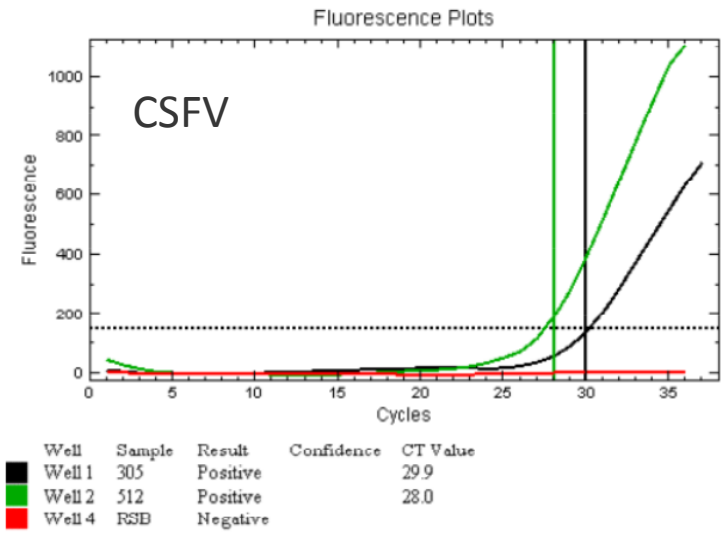
A single-step, multiplex, real-time polymerase chain reaction (RT-PCR) was developed for the simultaneous and differential laboratory diagnosis of Classical swine fever virus (CSFV) and African swine fever virus (ASFV) alongside an exogenous internal control RNA (IC-RNA). Combining a single extraction methodology and primer and probe sets for detection of the three target nucleic acids CSFV, ASFV and IC-RNA, had no effect on the analytical sensitivity of the assay and the new triplex RT-PCR was comparable to standard PCR techniques for CSFV and ASFV diagnosis. After optimisation the assay had a detection limit of 5 CSFV genome copies and 22 ASFV genome copies. Analytical specificity of the triplex assay was validated using a panel of viruses representing 9 of the 11 CSFV subgenotypes, at least 8 of the 22 ASFV genotypes as well as non-CSFV pestiviruses. Positive and negative clinical samples from animals infected experimentally, due to field exposure or collected from the UK which is free from both swine diseases, were used to evaluate the diagnostic sensitivity and specificity for detection of both viruses. The diagnostic sensitivity was 100% for both viruses whilst diagnostic specificity estimates were 100% for CSFV detection and 97.3% for ASFV detection. The inclusion of a heterologous internal control allowed identification of false negative results, which occurred at a higher level than expected. The triplex assay described here offers a valuable new tool for the differential detection of the causative viruses of two clinically indistinguishable porcine diseases, whose geographical occurrence is increasingly overlapping.

Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

Adaption to a portable T-COR4

- The same set of primers and probes
- Ag Path-ID™ One-Step RT-PCR Kit
- Run on T-COR4

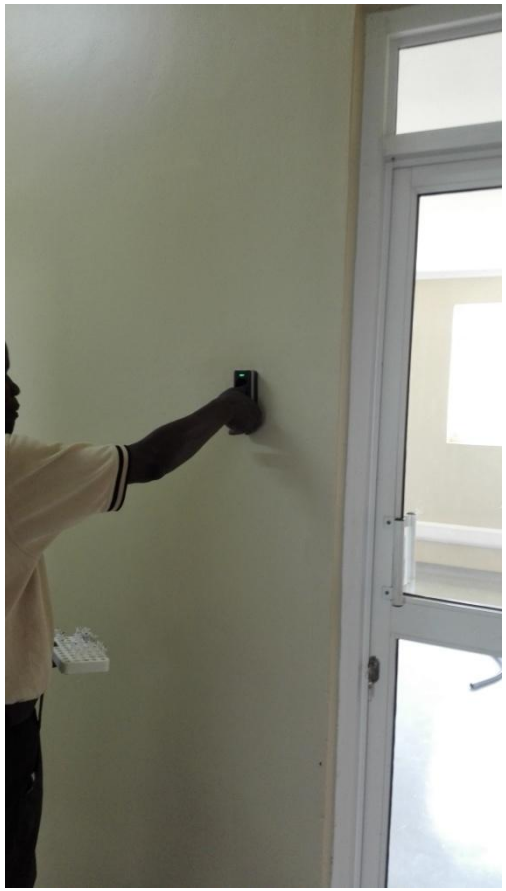




Evaluation at Harbin Veterinary Research Institute (HVRI), China

- 56 samples of 4 types (whole blood, serum and already homogenized tissue and sera from experimental infection)
- Tested by gel-based and /or real-time RT-PCR

Evaluation at NADDEC, Uganda



Evaluation within LinkTAD of a portable PCR assay for the rapid detection and differentiation of ASFV and CSFV under field conditions

A collaboration between;

The National Veterinary Institute (SVA), Sweden and Harbin Veterinary Resarch Centre, China (HVRI), CReSA, Spain and NADDEC, Uganda

Hua Ji Qiu / Yuzi Luo / Frederik Widén /
Lihong Liu

Conclusions

- In a collaboration between Sweden and China a duplex real-time RT-PCR assay was adapted to the portable PCR instrument T-COR4 and tested in a collaboration between SVA, HVRI, CReSA and NADDEC.
- The portable PCR assay performed well with chinese samples and others, thus providing a useful tool for detection and differentiation of ASFV and CSFV in the field or basic laboratories in EU, Africa and China

Conclusions

- This one example of the very useful work that can be performed in cooperation with China.

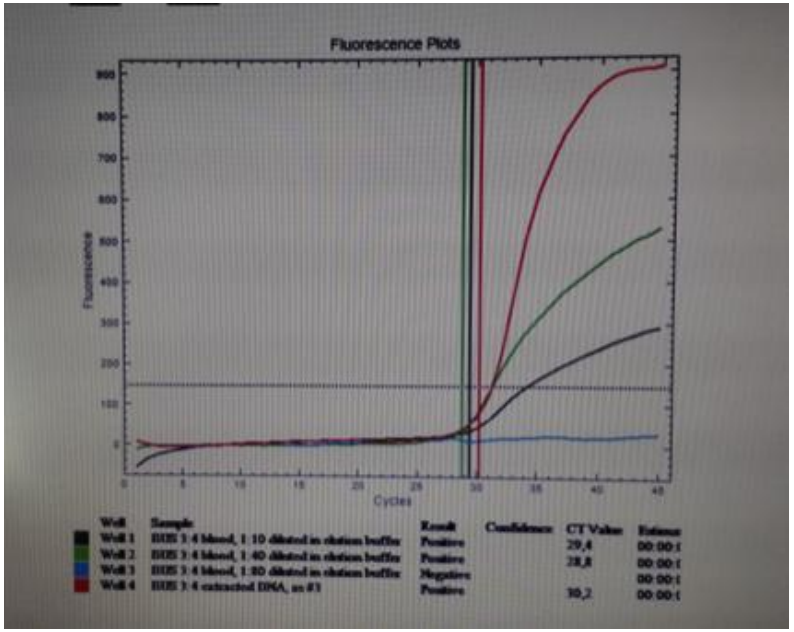
Acknowledgements

- Supported by LinkTAD, RAPIDIA-FIELD project and OIE twinning program
- HVRI: Yuzi Luo, Huaji Qiu
- CReSA: Francesca Accensi, Llilianne Ganges, Fernando Rodríguez
- NADDEC: Stella Atim, Chrisostom Ayebazibwe
- Markerere University, Uganda: Charles Masembe
- Tetracore Inc., USA: David Almassian
- SVA: Sándor Belák, Karl Ståhl, Frederik Widén, Lihong Liu

Thank you for your attention!

Evaluation at NADDEC, Uganda

64 samples from the field
10 positive



I. **About LinkTADs**

II. Project objectives

III. Partnership

Tables

Lorem Ipsum is simply dummy text of the printing and typesetting industry. Lorem Ipsum has been the industry's standard dummy text ever since the 1500s, when an unknown printer took a galley of type and scrambled it to make a type specimen book.

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Partnership

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