LinkTADs

D4.7 New Diagnostic Technologies

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National Veterinary Institute

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Harbin Veterinary Research Institute

China Animal Health and Epidemiology Center

China Animal Disease Control Center

Sociedade Portuguesa de Inovação

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01.

Summary

Summary

Within the LinkTADs project, the Joint Laboratory of Veterinary Microbiology between HVRI and SVA has been established in 2014, and relevant collaboration research on the new diagnostic technologies in infectious animal diseases has been strengthened and good progress has been made.

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02.

Development of an accompanying ELISA test for marker vaccines against classical swine fever

Development of an accompanying ELISA test for marker vaccines against classical swine fever

Dr. Hua-Ji Qiu's research team at HVRI has established an accompanying E^{rns}-based ELISA for marker vaccines against CSFV. The assay has been co-evaluated in SVA, and EU and OIE Reference Laboratory for CSF, at the University of Veterinary Medicine Hannover (TiHo), by testing diverse swine serum samples. The assay was able to detect the CSFV-infected pigs during the early infection, and discriminate the CSFV-specific antibodies from CSFV-infected pigs from those vaccinated with E2-based marker vaccine rAdV-SFV-E2. The iELISA showed comparable sensitivity and specificity with the virus neutralization test and higher sensitivity (90.4%) compared with PrioCHECK CSFV E^{rns} (59.6%). The E^{rns}-based ELISA is specific and sensitive, representing a promising DIVA test for E2-based marker vaccines against CSF. A jointly authored paper has been published in Journal of Virological Method with LinkTADs acknowledged (Luo Y et al., 2015).

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03.

Real-time PCR and other research achievements between SVA and HVRI

Real-time PCR and other research achievements between SVA and HVRI

Research activities on the techniques on the early diagnosis and rapid diagnosis of ASFV and CSFV.

Drs. Frederik Widén and Lihong Liu from SVA, Prof. Paul Becher and Dr. Alexander Postel from TiHo, and Fernando Rodríguez from Centre de Recerca en Sanitat Animal (CReSA/IRTA) participated in the Seminar on Classical/African Swine Fever organized by Dr. Hua-Ji Qiu's team at HVRI on July 17, 2015.
During the seminar the researchers exchanged opinions and discussed the basic and applied research activities and progress on ASFV and CSFV. Dr. Lihong Liu introduced the techniques on the early diagnosis of the animal diseases and presented the recent research progress on the rapid diagnosis of ASFV and CSFV. He demonstrated the great potential of the real-time PCR assay performed on a portable thermocycler T-COR4 for effective disease surveillance by its application data in the disease epidemic areas. Dr. Alexander Postel from TiHo presented the progress on the epidemiology and vaccine development of pestiviruses. Dr. Yuzi Luo from HVRI introduced a promising CSFV E^{rns}-based indirect ELISA for antibody differentiation of infected from vaccinated animals. Dr. Yimin Wang from HVRI introduced the generation and efficacy evaluation of a recombinant pseudorabies virus variant expressing the E2 protein of CSFV in pigs. They also discussed further strengthening of relevant collaboration research within the Joint Laboratory between HVRI and SVA. During the meeting HVRI and SVA agreed to hold The 1st Workshop on the Joint Laboratory in the end of this year.

Real-time PCR development

Pen-side tests in veterinary virology are agent-oriented assays, aiming at obtaining results rapidly to support clinical diagnosis and differentiation of infectious diseases, such as African swine fever and Classical swine fever. Such assays are very useful in the field to do quick screening of carcasses and investigate suspect cases on farms.
Within the LinkTADs project, SVA and HVRI organized and participated in an evaluation of a real-time PCR assays performed on a portable battery powered thermocycler as a field diagnostic tool. Other institutes that took part in this evaluation were CReSA in Spain and NADDEC in Uganda. The PCR instrument was supplied by Tetracore free of charge. Our data suggested the new assay could be used in a simple laboratory or under field conditions for rapid diagnosis and differentiation of the two diseases.
Previously two LAMP assays were combined in a duplex assay. However, this was unsuccessful. Therefore a portable real-time PCR assay, using Tetracore T4 thermocycler, was developed based on a published duplex assay. For this Ag Path-ID One Step RT-PCR kit and published primers and probes were used. Extraction of RNA from sera was performed using the Mag Max kit and a magnetic comb. No centrifugation was required. For evaluation CSFV samples from the EURL for CSFV in Hannover and from HVRI in Harbin were used. To test the capability to detect ASFV samples from CReSA were used. Finally the assay was tested by NADDECC in Uganda under field conditions.

The portable PCR assay performed well, thus providing a useful tool for detection and differentiation of ASFV and CSFV in the field or basic laboratories in EU, Africa and China. The big advantage of this assay is the

minimal requirement for equipment which makes it useful in the field or in basic laboratories. Furthermore, it can be used without connection to the electrical grid. The portable assay should be useful for screening of wild animal carcasses and domestic animals.

Jointly authored manuscripts have been published or drafted.

Luo Y, Li L, Austermann-Busch S, Dong M, Xu J, Shao L, Lei J, Li N, He WR, Zhao B, Li S, Li Y, Liu L, Becher P, Sun Y, Qiu HJ. Enhanced expression of the E^{rns} protein of classical swine fever virus in yeast and its application in an indirect enzyme-linked immunosorbent assay for antibody differentiation of infected from vaccinated animals. J Virol Methods. 2015 Sep 15;222:22-7.

Lihong Liu, Stella A Atim, Yuzi Luo, Chrisostom Ayebazibwe, Hua-Ji Qiu, Francesc Accensi, Llilianne Ganges, Fernando Rodríguez, Hu Shan, Charles Masebe, Karl Ståhl, Widén F, Sándor Belák. Feasibility and reliability of portable real-time PCR as a field diagnostic toll: Experiences from testing clinical samples for African and classical swine fever viruses. (in preparation)

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04.

Conclusions

Conclusions

The objective of WP4 is to upgrade and implement the diagnostic technologies for early and rapid diagnosis of animal infectious diseases including zoonosis via collaboration between EU and China. Within the LinkTADs project, the Joint Laboratory of Veterinary Microbiology between HVRI and SVA has been established, and relevant collaboration research has been made and strengthened. HVRI has established an accompanying E^{rns}-based ELISA for marker vaccines against CSFV, the assay has been co-validated in SVA and TiHO. SVA has also co-evaluated with HVRI and other partners on the potential of the real-time PCR assay performed on a portable thermocycler T-COR4 for disease surveillance. The next steps will be to strengthen the collaborations between SVA and HVRI, and organize more exchange activities and collaborations within the joint laboratory.