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DIFFERENTIATING CLASSICAL SWINE FEVER VIRUS INFECTED FROM VACCINATED WILD BOAR USING A RECENTLY DEVELOPED MULTIPLEX REAL-TIME RT-PCR ASSAY

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Introduction

Classical swine fever (CSF) is one of the most important diseases of swine and can affect both domestic pigs and wild boar. While prophylactic vaccination is banned within the European Union, oral vaccination of affected wild boar populations is carried out in several countries. Following detection of CSF in the German wild boar population in 2009, oral vaccination using the live modified vaccine "Riemser Schweinepestoralvakzine" was implemented in the affected regions. Within the restriction zones, all wild boar shot or found dead were subjected to reverse transcription polymerase chain reaction (RT-PCR) testing. This also meant, that animals were shot in the short period after baiting where the vaccine virus could be detectable by molecular methods. In order to differentiate between field virus infected from only vaccinated animals, a fast discriminatory assay was needed.

Materials & methods

As a discriminatory approach for genetic differentiation of infected from vaccinated animals (DIVA), a set of real-time RT-PCR assays was developed and validated. Specific primers and probes were designed for detection of the C-strain "Riems" vaccine virus, and a heterologous internal positive control was included. The assays were then multiplexed to detect simultaneously CSF field virus, C-strain "Riems, and the internal control (1).

For validation, a RNA panel representing different Pestiviruses and their types was used in addition to experimental samples.

After initial validation, this protocol was used for all samples sent to the German National Reference Laboratory for CSF for confirmation from regional laboratories with a positive PCR result. The animals came mainly from the 2009 CSF restriction zones in Federal States North-Rhine Westphalia and Rhineland Palatinate.

Subsequent to observation of sensitivity problems caused by quasispecies variations (2), the assay was adjusted and validated again for the use in wild boar monitoring. The adjusted assay lost its specificity for C-strain "Riems" but is still specific for 1.1 CSFV strains.

Results

Analytical sensitivity was determined using a 10-fold dilution series of C-strain "Riems" RNA taken from the above mentioned RNA panel. It was shown that the detection limit was six dilution steps of the used standard, and the detection limit of the control RNA goes linearly down to 10^1 copies/well.
From the RNA panel, only C-strain "Riems" was detected showing high specificity.

In order to allow simultaneous detection, the vaccine virus specific RT-PCRs were combined with a RT-PCR protocol for detecting field virus and an internal control. Comparison of single and multiplex assays based on the RNA panel showed no noticeable influence on specificity and sensitivity.

In the initial phase of routine use, all samples positive for C-strain "Riems" gave a positive result in the RT-PCR assay. Later on, discrepancies were found that resulted from quasispecies variations in the vaccine itself.

To further use the assay, the protocol was amended. Validation and routine use showed that the new assay again detects C-strain "Riems" with high sensitivity. The specificity is now genotype 1.1.

Discussion & conclusions

It was shown that differentiating real-time RT-PCR assays can be a useful tool in genetic differentiation of infected from vaccinated animals in areas with vaccination against CSF.

In routine use, the assay also revealed that "Riemser Schweinepestoralvakzine" contains more than one quasispecies. Adjusting the assay to this fact rendered it highly sensitive again but C-strain "Riems" specificity was lost.

As recent CSF outbreaks in Europe were caused by genotype 2 strains, this limitation is of lesser importance but has to be taken into account in other settings.

The diagnostic value of this approach also shows that genetic DIVA can be considered.

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