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DEEP-SEQUENCING CHARACTERIZATION OF TWO ROTAVIRUS A OUTBREAKS IN SUCKLING PIGLETS IN CATALONIA REVEALS A HIGH FREQUENCY OF RECOMBINATION EVENTS

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Introduction

Group A rotaviruses are one of the main causes of diarrhoea in pigs worldwide. In the present study, two outbreaks of diarrhoea in suckling pigs were investigated. Four rotavirus A isolates were deep sequenced and analysed to characterize the genetic diversity and identify recombination events.

Materials and methods

Two outbreaks of diarrhoea in suckling piglets were investigated. In each farm faeces from 10 diseased and 5 healthy new-borns were sampled. Faeces were initially examined for Rotavirus A by means of a qRT-PCR. Four positive samples were selected based on their low Ct (<22) in the RT-qPCR and used for further sequencing. RNA was extracted by using a TRIzol-based protocol and RNA was deep sequenced in an Illumina platform. The output for each sample was filtered and a viral quasi-species and a consensus sequence for all Rotavirus A genes (namely, VP1, VP2, VP3, VP4, VP5, VP6, VP7, NSP1, NSP2, NSP3, NSP4, NSP5) was constructed.

Results

The deep sequencing analysis obtained high quality reads (mean QC scores >30), and a deep coverage for each nucleotide along the Rotavirus A genome (>1,000 reads per position). Four recombinant events were detected in three different genes (NSP1, VP4 and VP7), leading to 3 genome types. In the first outbreak, both samples shared the same pattern, being recombinant for the VP4 and VP7 genes, while in the second outbreak, only one sample was recombinant, specifically for the NSP1 and VP7 genes.

Discussion and conclusion

The sequence analysis showed a strong case component, since sequences from the same farm were always closely related compared to the sequences of the other farm. Moreover, the results pointed to a high frequency of recombination events within different genes along the genome. The analysis clearly indicated that the circulating field strains had different ancestors, suggesting several introduction events.