SCIENTIFIC REPORTS

OPEN

Deletion at the 5′-end of Estonian ASFV strains associated with an attenuated phenotype

Received: 22 January 2018
Accepted: 29 March 2018
Published online: 25 April 2018

Laura Zani[1], Jan Hendrik Forth[1], Leonie Forth[1], Imbi Nurmoja[2,3], Simone Leidenberger[1], Julia Henke[1], Jolene Carlson[1], Christiane Breidenstein[1], Arvo Viltrop[3], Dirk Höper[1], Carola Sauter-Louis[1], Martin Beer[1] & Sandra Blome[1]

African swine fever (ASF) was introduced into the Eastern European Union in 2014 and led to considerable mortality among wild boar. In contrast, unexpected high antibody prevalence was reported in hunted wild boar in north-eastern Estonia. One of the causative virus strains was recently characterized. While it still showed rather high virulence in the majority of experimentally infected animals, one animal survived and recovered completely. Here, we report on the follow-up characterization of the isolate obtained from the survivor in the acute phase of infection. As a first step, three *in vivo* experiments were performed with different types of pigs: twelve minipigs (trial A), five domestic pigs (trial B), and five wild boar (trial C) were inoculated. 75% of the minipigs and all domestic pigs recovered after an acute course of disease. However, all wild boar succumbed to infection within 17 days. Representative samples were sequenced using NGS-technologies, and whole-genomes were compared to ASFV "Georgia 2007/1". The alignments indicated a deletion of 14560 base pairs at the 5′ end, and genome reorganization by duplication. The characteristic deletion was confirmed in all trial samples and local field samples. In conclusion, an ASFV variant was found in Estonia that showed reduced virulence.

In 2014, African swine fever virus (ASFV) was introduced into Poland and the Baltic European Union (EU) member states Latvia, Lithuania and Estonia. Since then, slow but constant spread of this notifiable disease has been observed[1]. With regard to outbreak characteristics, detection of fallen animals and virus prevails. However, in some regions, a different pattern in cause of the epidemic has been observed[1]. In the follow-up of those observations, we recently reported an animal experiment that aimed at the biological characterization of an ASFV strain from north-eastern Estonia, where an unexpectedly high ASFV-antibody prevalence was found in hunted healthy animals[2]. In this previous animal trial, ten wild boar were inoculated with the above mentioned ASFV strain to evaluate if the clinical course of the disease differed from infections with the so far known highly virulent Caucasian strains[3–6]. In brief, nine out of ten animals succumbed to the infection showing typical lesions. The surviving wild boar recovered completely and was slaughtered in good health status 96 days post infection (dpi). Comingling of the survivor with three sentinel wild boar from 50 dpi did not lead to disease transmission. Taken together, the virus showed still considerable virulence and lethality, but one animal recovered and could represent one of the antibody positive wild boar found in the hunting bags of north-eastern Estonia. These results left us with several unanswered questions, including: Is the survival of one animal within the normal range of clinical courses of a highly virulent ASFV strain or is it an indication for true attenuation? Could a further animal passage lead to a more attenuated phenotype? If there is attenuation, what is the genetic basis?

To address these questions and to further characterize the virus isolated from the surviving boar, three additional animal trials were performed to characterize the virus with different pig types. Since the survival rates and clinical courses were rather variable in the different trials, representative samples from each trial were full-genome sequenced using next-generation sequencing technologies and the resulting sequences were compared to ASFV "Georgia 2007/1" (FR682468.1). In order to confirm the circulation of the variant strain, Estonian field samples were screened for the mentioned mutation by *real-time* quantitative polymerase chain reaction (qPCR).

[1]Friedrich-Loeffler-Institut, Suedufer 10, 17493, Greifswald – Insel Riems, Germany. [2]Estonian Veterinary and Food Laboratory, Kreutzwaldi 30, 51006, Tartu, Estonia. [3]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51014, Tartu, Estonia. Correspondence and requests for materials should be addressed to S.B. (email: sandra.blome@fli.de)