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Short Communication

Novel rope-based sampling of classical swine fever shedding in a group of wild boar showing low contagiosity upon experimental infection with a classical swine fever field strain of genotype 2.3

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ABSTRACT

Several classical swine fever (CSF) epidemics in wild boar and domestic pigs in Europe during the last decades have been caused by CSF virus (CSFV) strains of genotype 2.3. This genotype is known to be virulent leading to high morbidity and mortality. We experimentally infected two eight months old wild boar with $10^{5,5}$ $TCID_{50}$ of CSFV genotype 2.3 and kept the animals together with five noninoculated wild boar of the same age. Our original purpose was to evaluate a non-invasive sampling method based on saliva collection using "rope-in-a-bait" sampling baits. While expecting high morbidity, high level of virus shedding and some mortality, we actually observed a subclinical course of infection with an unexpected low contagiosity. The two inoculated animals infected only three contact animals while two contact animals remained uninfected. These findings substantially add to our epidemiological understanding of CSFV circulation in wild boar populations. CSFV infected animals older than six months and in good condition may not shed sufficient virus to transmit infection to all seronegative in-contact animals. The contagiosity in relation to the animal's age is discussed. This supports the hypothesis of silent perpetuation of CSFV in wild boar populations for several months if the wild boar density is sufficiently high. The feasibility of the "rope-in-a-bait" sampling method could be proven during the short viraemic phase of infected animals during the second week of infection.

1. Introduction

Classical swine fever (CSF) is caused by an RNA virus of the genus Pestivirus of the *Flaviviridae* family. The disease is often fatal, affecting pigs and wild boar (*Sus scrofa*) alike, and causes major economic losses especially in countries with an intensive pig production system (Horst et al., 1999). The role of wild boar in CSF is primarily of epidemiological interest since they are regarded as a reservoir for CSF virus (CSFV) and a possible source of infection for domestic pigs (Artois et al., 2002; Staubach et al., 2013). Therefore, the presence of CSFV in wild boar populations represents a high risk for domestic pigs. Under these conditions adequate surveillance which enables an early detection of CSF in the wild boar population is crucial. However, collecting a statistically significant number of samples from wild boar for early detection of infection, e.g. for demonstration of the presence of virus, viral RNA or antigen, is logistically difficult and up to now linked exclusively to hunting or trapping activities (Alexandrov et al., 2013). Furthermore, sampling

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methods for antibody detection provide a retrospective analysis of the disease situation, but are not useful for early warning.

Our primary aim in this study was to explore the efficiency of CSF viral RNA detection in saliva samples collected by a non-invasive method using specific sampling baits consisting of ropes imbedded in a bait matrix (“rope-in-a-bait”). For validating the method we conducted an experimental infection of wild boar to probe animals which shed CSFV and which can be sampled regularly. In particular, we wanted to assess the sensitivity of the novel “rope-in-a-bait” sampling technique by comparison with blood tests and oronasal swabs.

2. Materials and methods

Seven wild boar were used in the experiment. The animals were eight months old, weighing around 45 kg each. They were all in good condition and kept in the same stable. Two randomly selected wild boar (A1 and A2) were inoculated oronasally with 4 ml of cell culture medium containing $10^{5.5}$ $TCID_{50}$ (tissue culture infectious dose 50%) of CSFV isolate 2.3 “Rösrath”. The CSFV strain “Rösrath” (CSF1045, GenBank accession number GU233734) was originally isolated from a wild boar piglet in Germany in 2009 and has been previously described by Leifer et al. (2010) as a strain of moderate virulence causing varying clinical pictures of different severities ranging from unspecific symptoms to haemorrhagic lesions. The challenge virus was obtained from the German National Reference Laboratory for CSF and passaged twice on PK15 cells prior to use. The inoculum was from the same virus stock used in earlier experiments showing a higher virulence.

Clinical signs were recorded daily. Blood samples (Monovette® EDTA KE/9 ml resp. Serum/9 ml Sarstedt, Numbrecht, Germany) for virological and serological investigations as well as oronasal swabs (Copan Rayon Regular Tip cat. no. 155C, Hain Lifescience GmbH, Nehren, Germany) were taken twice a week during the first four weeks, and once a week afterwards. The last samples were taken 81 days post infection. For collecting oronasal swabs and blood samples the animals had to be sedated using Tiletamin and Zolezepam (0.5–1 ml Zoletil® 100 per animal). While the animals were under sedation the body temperature was also measured. Increased body temperatures between 40 °C and 41 °C were regarded as an effect caused by the handling and agitation of the animals while body temperatures above 41 °C where regarded as fever.

For non-invasive sampling we used rope-in-a-bait sampling baits which were manufactured in analogy to the CSF oral vaccine baits (Faust et al., 2007) by embedding a raw cotton rope with a length of 10 cm and a diameter of 0.8 cm (Kanirope GmbH, Dortmund, Germany) in a cereal-based bait matrix. At least seven sampling baits were distributed every morning on the floor of the pen. The chewed cotton ropes were collected either the same day or next morning.

RNA was extracted from all samples using the MagAttract Virus Mini M48 Kit for automated extraction (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s recommendations. For CSF viral RNA detection real time reverse transcription PCR (RTqPCR) according to the protocol of Hoffmann et al. (2005) was used. Samples with C_q values below 40 were considered as positive.

For antibody detection a commercially available CSF antibody ELISA (IDEXX CSFV Ab, Idexx Laboratories, Inc., Westbrook Maine, USA) was used according to the protocol of the producer. Additionally, selected serum samples were tested in the virus neutralization assay (NT) according to the OIE manual of diagnostic tests and vaccines for terrestrial animals (OIE, 2008).

After completion of the trial necropsy was performed.

3. Results

Liveliness and appetite of all animals remained normal during the whole observation period and no clinical signs of CSFV infection were seen (for example haemorrhages in the skin, central nervous symptoms). The only sign which could be attributed to CSF was fever in some of the animals during the second week of infection (Fig. 1). However, fever did not affect their lively roaming and eating behaviour.

The virological and serological results are shown in Table 1. The first RTqPCR positive results were detected on day 3 post inoculation (3 dpi) in the blood sample of one inoculated animal (A1) and on 6 dpi of the second inoculated wild boar (A2). Three contact animals (C3, C4, C5) reacted positive one week later (10 dpi), while two contact animals (C6 and C7) remained negative during the entire observation period. Seroconversion started in the inoculated animals two weeks post infection (10 dpi), followed by the three positive RTqPCR contact animals (13 and 17 dpi). CSF specific antibodies could not be detected in serum samples from the two wild boar with negative RTqPCR results. These two animals were repeatedly tested negative until 81 dpi.

In the “rope-in-a-bait” samples, positive RTqPCR reactions were measured on 12 dpi (C_q 36) and 13 dpi (C_q 35) while only one oronasal swab from 10 dpi gave a positive RTqPCR signal (C_q 37).

At necropsy no gross pathological signs of a CSFV infection were seen. Viral RNA was detected in tonsils (C_q 33–34), salivary gland (C_q 37), mandibular lymphnotes (C_q 33–36) but not in spleen.

4. Discussion

The experimental trial presented in this paper was aimed to validate a non-invasive sampling method for the detection of CSF viral RNA in saliva of wild boar. The unexpected subclinical course in the five infected animals and the absence of infection in two sentinel animals did not allow a thorough evaluation of the non-invasive sampling method. Virus excretion through saliva could only be demonstrated with two sampling baits and one oronasal swab during the second week of infection. The low virus dose in the saliva is reflected by the high C_q values (>30) measured. Presumably, insufficient amounts of virus had been excreted during the viraemic phase which lasted less than one week. Since measurements of temperature and blood sampling could not be performed