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Suitability of faeces and tissue samples as a basis for non-invasive sampling for African swine fever in wild boar

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ABSTRACT

A challenging aspect of ASFV control in wild boar populations is the design and implementation of effective surveillance and monitoring programmes, both for early warning, and to determine the ongoing epidemiological situation in an infected population. Testing blood samples requires invasive sampling strategies like hunting or capture of wild boar. Besides being biased towards healthy animals, such strategies are also linked to further spread of the virus. Non-invasive sampling strategies would increase the reliability of surveillance of ASFV in wild boar populations, without the negative side effects. This study evaluates the potential of faeces and tissue samples as a basis for non-invasive sampling strategies for ASFV in wild boar. In the acute phase (0–21 days after infection), in comparison with virus detection in blood, virus can be detected in faeces 50–80% of the time. This percentage decreases to below 10% for the subacute/chronic phase. ASFV DNA is quite stable in faeces. Half-lives range from more than 2 years at temperature up to 12 °C, to roughly 15 days at temperatures of 30 °C. In tissue samples, stored at 20 °C, half-lives mostly range from 1.7 to 7.4 days. The sample of preference is the spleen, where the highest titres and highest half-life of ASFV DNA are observed. The level and duration of excretion of ASFV in the faeces, combined with the stability of the DNA, suggest that sampling of faeces could be the basis for a non-invasive sampling strategy to monitor ASFV in wild boar.

1. Introduction

African swine fever virus (ASFV), an enveloped double-stranded DNA virus, is the causing agent of African swine fever (ASF), a highly lethal, haemorrhagic disease of swine. ASF is present in several African countries and Sardinia (OIE, 2014). In 2007, after first being reported in Georgia, it spread to several neighbouring countries, including Armenia, Azerbaijan and the Russian Federation, where it is still circulating (Gogin et al., 2013). Recently, ASFV has also been reported in Ukraine, Belarus, Lithuania and Poland (OIE, 2014). The endemic presence of the virus in Russia, coupled with ongoing spread to neighbouring countries is being seen as a serious threat for pig populations in Europe and Asia. The reported presence of ASFV in wild boar populations has been of particular concern, due to their possible role as disease reservoirs (Blome et al., 2013; Jori and Bastos, 2009). In relation to their free-roaming behaviour, this may further complicate control efforts across national borders. One particularly

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challenging aspect of ASFV control in wild boar populations is the design and implementation of effective surveillance programmes (Sanchez-Vizcaino, 2006).

To obtain the necessary epidemiological information from a potentially infected population, samples can usually be obtained from two sources: from hunted/captured animals, or from animals found dead/animal debris. Testing hunted and/or captured wild boar is heavily biased towards healthy animals (Artois et al., 2002), which will not result in correct information on current spread of ASFV in the population. Additionally, hunting and capturing are quite invasive practices, which may be linked to further disease spread (Choisy and Rohani, 2006; Laddomada, 2000). Among the non-invasive, alternative methods to hunting and capture methods in wildlife ecology, is sampling of faeces (Engeman et al., 2013). The design of a surveillance strategy employing faeces found in the field would require information on the duration and titres of ASFV shed in faeces, and stability of ASFV DNA in faeces. Especially on the latter, there is very little quantitative information available.

For an early detection of ASFV, after its first introduction into the population, wild boar found dead constitute the best sampling target. Although decomposed samples may not be suitable for virus isolation and immunofluorescence tests, testing by polymerase chain reaction (PCR) is considered valid (OIE, 2012). However, there is no information on decay rates of ASFV DNA in badly preserved tissues from infected pigs.

This study evaluates the diagnostic potential of faeces and tissue samples, as a basis for non-invasive sampling strategies for ASFV in wild boar, using domestic pigs as a model. The potential of faeces is evaluated by taking into consideration ASFV titres in faeces and duration of shedding, as well as the effect of time and temperature on the detection of ASFV DNA in faeces. Stability of ASFV DNA in tissues over time was investigated to determine the suitability of the PCR on testing wild boar in various stages of decomposition.

2. Materials and methods

2.1. Animal experiment design

Animal experiments were performed with three different ASFV isolates: Brazil'78, Malta'78 and Netherlands'86 (de Carvalho Ferreira et al., 2012). Brazil'78 behaved as highly virulent in these experiments, while Malta'78 and Netherlands'86 behaved as moderately virulent. Further details regarding animal experiment design (e.g. number of inoculated and contact pigs, age, inoculation dose) can be found in de Carvalho Ferreira et al. (2012). Although the experiments were carried out in domestic pigs, wild boars are expected to have comparable pathogenesis and excretion patterns (Blome et al., 2013; Jori and Bastos, 2009). In these experiments, all pigs became infected with ASFV either by inoculation or contact with infected pigs. The experiments were approved by the Ethical Committee for Animal Experiments of the Animal Sciences Group.

2.2. Collection and processing of faeces

To estimate the amounts of excreted virus and duration of virus excretion, 20 pigs infected with Malta'78 and 10 pigs with Netherlands'86 were sampled daily from dpi 0 to 13, three times per week from dpi 14 to 27, and twice per week from dpi 28 until dpi 70. Ten pigs infected with Brazil'78 were sampled three times per week, up until the end of the experiment, on 9 dpi (de Carvalho Ferreira et al., 2012). Pigs infected with Malta'78 and Netherlands'86 were sampled for a total of 33 times and the pigs infected with Brazil'78 for a total of 5 times.

To estimate the stability of ASFV DNA, 24 additional faeces samples were collected in larger amounts (on average 27 g, ranging from 1.5 to 78 g) from pigs showing acute clinical signs: 8 originating from pigs infected with the Brazil'78 isolate, 10 from pigs infected with Malta'78 and 6 from pigs infected with Netherlands'86. Samples were taken 8–9 dpi in the Brazil'78 group, and between 7 and 15 dpi in the Malta'78 and Netherlands'86 groups. Each faecal sample was homogenized and divided in 4 equal amounts, placed in 50 ml tubes and kept at different temperatures: 5 °C, 12 °C, 20 °C, or 30 °C, for up to 35 days. Depending on the amount of faeces, 4–10 time points were sampled from each tube.

All faecal suspensions were made by adding 1 g of faeces to 9 ml Eagle's minimum essential medium (EMEM [Gibco, Invitrogen, Breda, The Netherlands]) with 10% heat-inactivated pig serum and 10% antibiotics solution ABII (1000 U/ml penicillin, 1 mg/ml streptomycin, 20 μg/ml fungizone, 500 μg/ml polymixin-B and 10 mg/ml kanamycin), and vortexed with glass beads. After centrifugation ($2500 \times g$ for 15 min) the supernatants were stored at −70 °C until they were analysed by quantitative real-time polymerase chain reaction (qPCR).

2.3. Collection and processing of tissue samples

In total, tissue samples from 22 pigs were collected, of which 10 had been infected with the Brazil'78 isolate, 6 with the Malta'78 isolate and 6 with the Netherlands'86 isolate. Tissue samples were collected from pigs that died as a result of the ASFV infection, with necropsies taking place between 7 and 9 days post-infection (dpi) in the Brazil'78 group, between 9 and 18 dpi in the Malta'78 group, and between 18 and 25 dpi in the Netherlands'86 group.

During the necropsy, 5 g of spleen and liver, one retropharyngeal lymph node, one parotid lymph node and the entire tonsil were collected. These tissues were placed at room temperature (approximately 20 °C). The tissues were sampled after 0, 1, 2, 3, 5, 7, 14, 21 days. On every sampling day, 0.1 g of tissue was excised, and placed in a "MagNA Lyser Green Beads" tube (Roche Applied Science, Mannheim, Germany) filled with 600 μl of EMEM with 5% heat-inactivated pig serum and 10% antibiotics solution ABII. The sample was then homogenized in a MagNa Lyser instrument (Roche Applied Science, Mannheim, Germany) for 30 s at $3500 \times g$, and then centrifuged for 1 min at $9500 \times g$. A volume of 100 μl of tissue suspension was suspended in 200 μl of medium. Samples were stored at −70 °C until analysis by qPCR.