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Short Communication

Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar

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ABSTRACT

In view of the fact that African swine fever (ASF) was recently introduced into the wild boar population of the European Union and that classical swine fever (CSF) keeps reoccurring, targeted surveillance is of utmost importance for early detection. Introduction of both diseases is usually accompanied by an increased occurrence of animals found dead. Thus, fallen wild boar are the main target for passive surveillance. However, encouraging reporting by hunters and sampling of these animals is difficult. Partly, these problems could be solved by providing a pragmatic sampling approach. For this reason, we assessed the applicability of three different dry/semi-dry blood swabs, namely a cotton swab, a flocked swab, and a forensic livestock swab, for molecular swine fever diagnosis. After nucleic acid extraction using manual and automated systems, routine quantitative real-time polymerase chain reactions (qPCR) were carried out. Results obtained from swabs or their fragments were compared to results generated from EDTA blood.

It was shown that reliable detection of both pathogens was possible by qPCR. Shifts in genome copy numbers were observed, but they did not change the qualitative results. In general, all swabs were suitable, but the forensic swab showed slight advantages, especially in terms of cutting and further storage. Robustness of the method was confirmed by the fact that different extraction methods and protocols as well as storage at room temperature did not have an influence on the final outcome. Taken together, swab samples could be recommended as a pragmatic approach to sample fallen wild boar.

1. Introduction

Both African and classical swine fever are among the most important and devastating viral diseases of domestic pigs and wild boar (Edwards et al., 2000; Sanchez-Vizcaino et al., 2013) and are notifiable to the World Organization for Animal Health (OIE). Recently, African swine fever (ASF) was introduced into the wild boar population of the European Union (EU), but also classical swine fever (CSF) keeps reoccurring (WAHID interface, visited May 10th 2014). As was observed with classical swine fever (CSF), disease occurrence in the wild boar population is often accompanied by spill over into the domestic pig population (Fritzemeier et al., 2000), with severe socio-economic consequences. Similar pattern were recently seen with African swine fever (ASF) that was also transmitted from wild boar to domestic pigs and back (Gogin et al., 2013). Only timely detection and intervention can lower the impact on both pig industry and wildlife and therefore, appropriate surveillance and warning systems are needed for countries at risk (De la Torre et al., 2013).

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As introduction of both diseases into a naïve wild boar population is usually accompanied by high morbidity and mortality (Artois et al., 2002; Costard et al., 2013), and thus an increased occurrence of animals found dead, passive surveillance is crucial. However, the number of sample submissions from fallen wild boar is usually very low, even in times of increased risk. This could be partly due to the fact that sampling and/or transport of wild boar carcasses in various stages of decay is difficult and in some cases even nauseating. Thus, encouraging hunters to report and sample fallen wild boar could be facilitated by provision of an easy to handle and pragmatic sampling and transport approach.

In the presented pilot study, dry blood swabs were investigated. To this means, different swabs were immersed in EDTA blood samples from experimentally infected wild boar and domestic pigs and subsequently subjected to molecular swine fever diagnosis using different nucleic acid extraction methods and specific quantitative real-time polymerase chain reaction (qPCR) techniques. Preparatory methods were chosen to allow detection of both diseases at the same time. Additional samples were tested to assess field applicability and transferability to other sample matrices including organ swabs.

2. Materials and methods

2.1. Swabs

Three different swabs, namely a routine cotton swab (COPAN), a flocked swab (FLOQSwabs, COPAN), and a forensic livestock swab (Genotube, Prionics) were used.

2.2. Processing and testing of samples

As a first proof of concept experiment, the above mentioned cotton swabs were immersed in EDTA blood samples from experimentally infected animals ($n = 7$ for ASF, collected at 4 dpi; $n = 11$ for CSF, collected at 4, 5, 7, 10 dpi). Samples were chosen to represent animals in the clinical phase of infection and had been stored at −70 °C until further use. The resulting blood swabs were stored three days (ASF) or over night (CSF) at room temperature to mimic sample transport without cooling. For nucleic acid extraction, swabs were dipped into the AVL buffer of the QIAamp Viral RNA Mini Kit (Qiagen) and used to stir it. After removal of the swab, all subsequent extraction steps were carried out according to the manufacturers instructions. A slight modification concerned the addition of an internal control DNA/RNA (5 μl per reaction with 2×10^5 copies per μl). Subsequently, qPCR or reverse transcription qPCR (RT-qPCR) was performed according to the protocols published by King et al. (2003) for ASF, and Hoffmann et al. (2005) for CSF. The PCR reactions were carried out using a Bio-Rad CFX Cycler (Bio-Rad Laboratories) and its accompanying software. Results were presented in a semi-quantitative way as quantification cycle (cq) values.

In a second pilot experiment, three different swabs (cotton, flocked and forensic) were used along with one manual and one automated nucleic acid extraction system. This time, swab fragments were subjected to nucleic acid extraction to ensure a retesting option.

For ASF, 10 samples from wild boar experimentally infected with ASF virus "Armenia08" (including samples from 6, 8 and 9 dpi) were used to soak the above mentioned swabs in parallel. After storage over night at

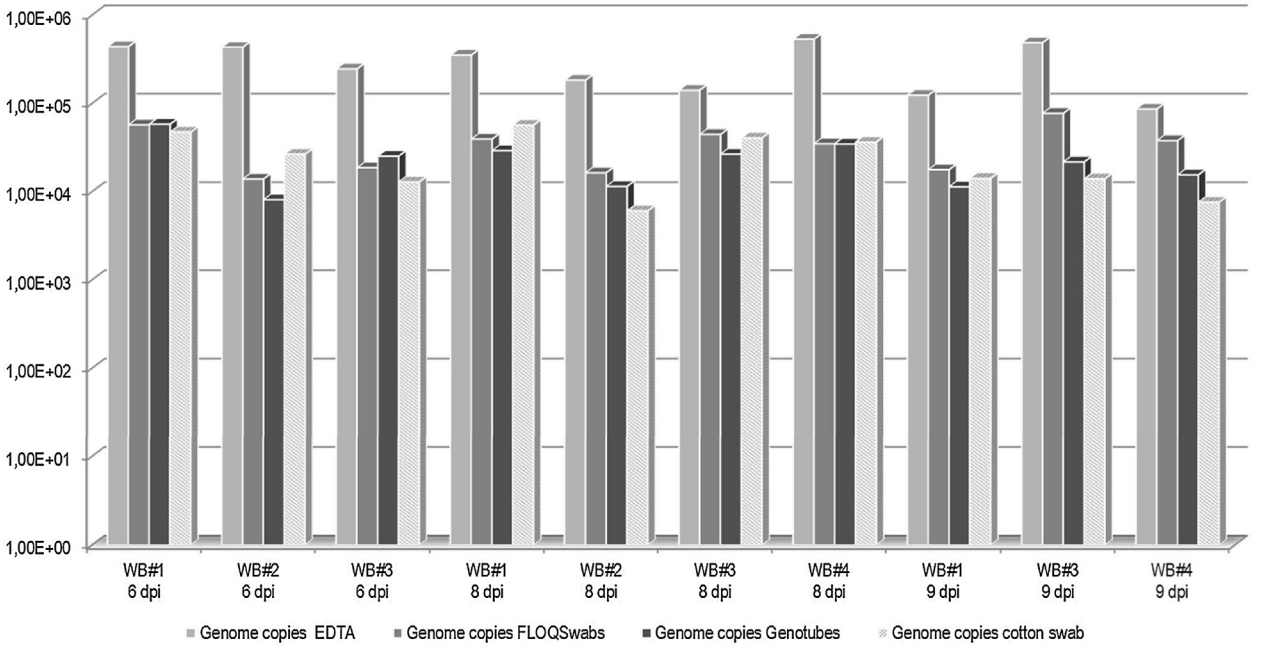

Fig. 1. Comparison of blood and different blood swab samples after manual nucleic acid extraction using the QIAamp Viral RNA Mini Kit (Qiagen) and ASFV specific qPCR. Results are presented as genome copy numbers per μl based on a synthetic standard. Samples were taken from experimentally infected wild boar between days 6 and 9 post infection (dpi) with ASFV "Armenia08".