# Summary of: African swine fever virus survival in buried wild boar carcasses

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Key Points:

- Carcasses of ASF infected wild boars have long been thought to play a major role as ASFV reservoirs that can contribute to virus spread.
- No infectious ASFV was isolated in samples from known ASFV infected boar carcasses that had been buried and re-excavated.
- These results do not rule out the possibility of infection if boars come into contact with an excavated infected carcass, but does support the viability of burial as an ASF mitigation strategy in areas where other disposal options are unavailable.

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Since the first introduction of African swine fever (ASF) into the European wild boar population in 1957, the question of virus survival in carcasses of animals that succumbed to the disease has been discussed. The causative African swine fever virus (ASFV) is known to be very stable in the environment. Thus, carcasses of infected wild boars could play a major role as an ASFV reservoir and thereby help to locally maintain and spread the virus to other wild boar populations. To minimize this risk, removal of wild boar carcasses in ASF affected areas is regarded to be crucial for effective disease control. If removal is not feasible, carcasses are usually disposed by burial on the spot to avoid direct contact of wild boar with contaminated carcasses.

In this study, carcasses of ASFV infected wild boars buried in Lithuania at different time points and locations were excavated and retested for the presence of ASFV. Bone marrow and, if available, residual organ matrix were collected from each excavated carcass. In addition, soil was sampled on each excavation site by pooling three samples randomly collected next to the decomposing bodies. For better comparison, the decomposition status of each sampled carcass was scored. Score “1” denotes that the cadaver was generally intact with closed skin and identifiable organs. Carcasses that were dismembered or open but had residual tissue obtained score “2”, and if only bones were left, the carcasses received score “3”. After excavating and sampling, the carcasses were buried again on the spot. Local soil surfaces and all involved instruments were treated using a broad-spectrum disinfectant containing peroxides, surfactants, organic acids and an inorganic buffer system. To avoid unintentional virus inactivation of carcass samples, disinfectants were strictly applied after completion of the sampling process. The obtained specimens were subsequently tested by qPCR and virus isolation.

Sampled carcasses were buried between 18 and 440 days before excavation. From a total of 45 bone marrow samples and 18 tissues samples collected from 20 sites, ASFV genome was detected by qPCR in all except three carcass samples. The mean ct value of the initial testing was 29.8 with a SD of 3.94. The mean ct value of excavated carcass samples was 31.14 with a SD of 3.32. Soil samples from seven out of 20 sites yielded qPCR ASFV positive results with a mean ct value of 34.12 (SD 1.29). Traces of swine cytochrome B gene were found in all but six excavation sites with a mean ct value of 31.18 (SD:3.84). Only at one site was ASFV genome detected without the detectable presence of swine DNA. All soil samples tested positive for internal control (beta-actin gene). Infectious ASFV could not be obtained from any of the bone marrow and tissue samples. Also, the direct immunofluorescence test did not reveal any indication for the presence of infectious ASFV.

Due to high stability of the virus and the fact that pigs dying during the acute phase of the disease usually show high titres of ASFV in blood it has been assumed that carcasses of infected wild boar contain considerable amounts of viable virus. The detection of very stable quantities of ASFV genome in the obtained samples shows that passive surveillance by sampling dead wild boar is highly reasonable even if the carcass is in advanced stage of decomposition and only bone marrow is available.

Regardless of carcass burial time and condition, infectious ASFV could not be isolated from any samples which was unexpected. One factor affecting virus stability might be the unknown conditions during the decomposing process of the carcasses. The typical summer climate in Lithuanian forests, the higher average soil temperature at this time, and the additional temperature increase during decomposition make it likely that the virus had been inactivated effectively during the decomposition process. This result could be also influenced by factors like the difficulty of analyzing the sample material. It has been shown that pigs were successfully orally infected with ASFV even if the sample previously tested negative in virus isolation. Animal infection was not included in this study and the possibility of carcasses causing infection with negatively tested virus cannot be ruled out.

The study could not demonstrate the presence of infectious ASFV in buried wild boar carcasses at different decomposition status. This result was unexpected but should interpreted with caution considering a risk of infection for swine coming into contact with dead wild boar which succumbed to ASF. However, it might indicate that there are additional sources of infection that might have been underestimated such as direct and indirect contact between wild boar. The results of this study support that proper burial as an alternative tool for wild boar carcass disposal can be regarded as a safe way to mitigate ASFV spread within the habitat if safe collection and transport to rendering plants is not feasible.

The complete paper can be found at: https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.13554