

Article

Stability of African swine fever virus in soil and options to mitigate the potential transmission risk

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Abstract: Understanding African swine fever virus (ASFV) transmission is essential for strategies to minimize virus spread during an outbreak. ASFV can survive for extended time periods in animal products, carcasses, and the environment. While ASFV genome was found in the environment around infected farms, data on the virus survival in soil are scarce. We investigated different soil matrices spiked with ASFV-positive blood from infected wild boar to see if ASFV can remain infectious in the soil beneath infected carcasses. As expected, ASFV genome detection was possible over the entire sampling period. Soil pH, structure, and ambient temperature played a role for the stability of infectious ASFV. Infectious ASFV was demonstrated in specimens originating from sterile sand for at least three weeks, from beach sand for up to two weeks, from yard soil for one week, and from swamp soil for three days. Virus was not recovered from two acidic forest soils. All risk mitigation experiments with citric acid or calcium hydroxide resulted in complete inactivation. In conclusion, stability of infectious ASFV is very low in acidic forest soils but rather high in sandy soils. However, given the high variability, treatment of carcass collection points with disinfectants should be considered.

Keywords: African swine fever virus; stability; soil; disinfection; risk mitigation;

1. Introduction

In the last decade, African swine fever (ASF) has reached an unprecedented geographical spread affecting wild boar and domestic swine in large parts of Europe and Asia, as well as several areas in Africa [1]. The notifiable disease of suids can be accompanied by signs of a viral hemorrhagic fever in domestic pigs and Eurasian wild boar [2]. The virus is transmitted directly between infected swine and wild boar by the oro-nasal route, as well as indirectly by ingestion of contaminated meat, by fomites or by a contaminated environment. It can also be transmitted by competent vectors, i.e. soft ticks of the genus *Ornithodoros*. They play an important role in Africa, but only in a few areas outside this continent [3]. Despite its limited host range and non-existent zoonotic potential, the socio-economic impact is high and many stakeholders are involved [4].

During the first years of the current epidemic that started in Georgia in 2007, infections were mainly seen among pig farms with generally low biosecurity, and with incidental spill over to the wild boar population. In the EU, however, the infection survived in the wild boar population independently from outbreaks in domestic pigs [5]. For the transmission among wild boar, carcasses and the contaminated habitat seem to play a crucial role, together with humans as long-distance spreaders [5].

So far, there are only a few cases in the current European outbreak where the disease was completely eradicated from a country's wild boar population. One example is the Czech Republic, where the control measures were successfully applied and can be used as guide for ongoing efforts elsewhere [1]. Another case is Belgium which is very close to becoming free of ASFV as no new wild

boar cases have been detected [6]. Both countries followed EU policy to keep the virus concentrated in one zone as much as possible.

An integral part of the control strategy is to search for and remove carcasses as a potential virus source. In this context, the question was raised whether the soil under a removed wild boar carcass should be also removed or treated to prevent virus transmission to other wild boar rooting in the contaminated soil. It was shown that viral genome can be detected in contaminated soil [7] and in order to minimize the transmission risk, simple physical measures such as tilling the soil, but also the application of disinfectants were intensively debated. Commercial disinfectants as well as lime products, i.e. quicklime and limewater (an aqueous solution of calcium hydroxide), were considered as possible options.

Consequently, our experiments began simply to establish a protocol to isolate ASFV from soil samples but evolved over time as we obtained more data. We set out to assess the stability of ASFV in soil matrices and to determine how infectivity could be reduced.

2. Results

2.1. Recovery of ASFV from yard soil on macrophages (Experiment 1)

In this pilot experiment yard soil was spiked with blood from ASFV infected wild boar and stored for four weeks at 25°C or 4°C. A blood-only control was conducted under the same conditions.

Regarding the blood-only control stored at 4°C, a high variability of the determined virus titers was observed during the first 48 hours (Figure 1). Titers of the three biological replicates ranged between 3.75 log₁₀ 50% hemadsorbing doses (HAD₅₀) per mL and 7.00 log₁₀ HAD₅₀/mL after an incubation time of six hours at 4°C. Such a variation did not reoccur at later time points or in the blood-only control stored at 25°C. In general, virus titers in pure blood decreased clearly after two weeks storage at either temperature. However, the blood-only controls (4°C and 25°C) remained infectious over the entire observation period.

Virus titers in yard soil spiked with infectious blood and stored at 4°C or 25°C generally decreased within the first 72 hours (Figure 1). In contaminated yard soil stored at 25°C no infectious virus was detectable after 72 hours. After one week, however, a high variability between the biological replicates was observed in yard soil at both storage temperatures, with virus titers up to 5.50 log₁₀ HAD₅₀/mL at 25°C. Hence, we found that ASFV remained infectious in yard soil (pH 6.7) for up to 7 days at both temperatures. After two weeks, contaminated yard soil was clearly negative for infectious virus until the end of the study.

Irrespective of the storage temperature, ASFV genome copy numbers were constant over time in the blood-only control and in yard soil samples.

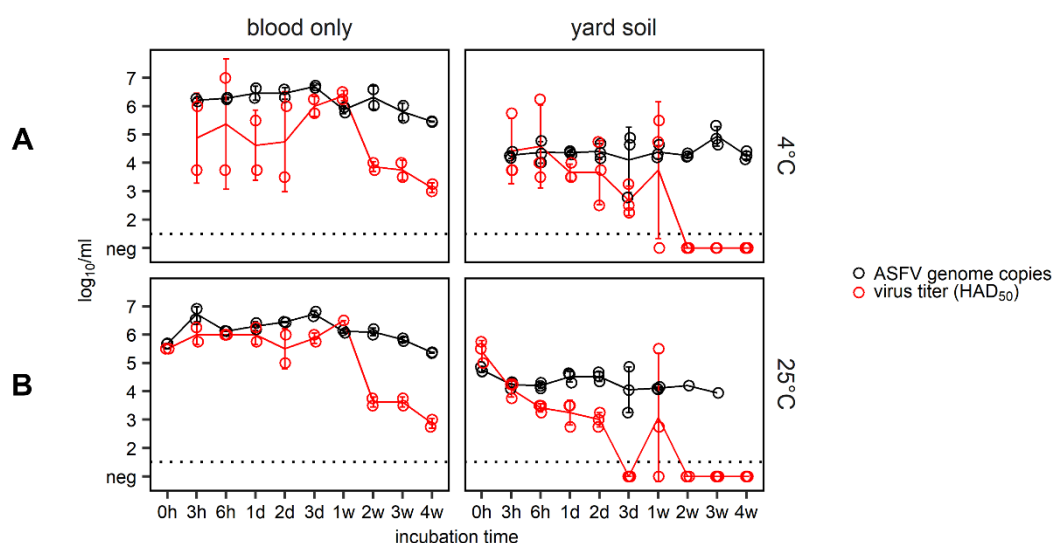


Figure 1: Infectious wild boar blood ($7.25 \log_{10} \text{HAD}_{50}/\text{mL}$ of ASFV “Armenia08”) and yard soil spiked with $400 \mu\text{L}$ of it was stored at 4°C (A) or 25°C (B). ASFV genome copies per mL are depicted in black and virus titer (as $\log_{10} \text{HAD}_{50}/\text{mL}$) is shown in red. Experiments were completed in triplicates and each open circle represents an individual replicate, solid lines and error bars represent the mean and standard deviation. The dotted line is the limit of detection of the virus titration.

2.2. Virus recovery from sterile sand, beach sand, swamp mud, and forest soil on macrophages (Experiments 2 and 3)

Three different soil types (beach sand, swamp mud, forest soil) were inoculated with blood from an ASFV infected wild boar and stored at room temperature for up to three weeks. A blood-only control and sterile sand mixed with infectious blood were used as process controls under the same conditions. In these experiments, virus titers in pure blood remained stable over the three-week storage period at room temperature and no decline in virus titers was observed after two weeks (Figure 2). Virus titers in the sterile sand control, however, decreased constantly over time. Nevertheless, both process controls (blood-only and sterile sand) contained infectious virus over the entire observation period.

In beach sand, high virus titers between $5.50 \log_{10} \text{HAD}_{50}/\text{mL}$ and $6.50 \log_{10} \text{HAD}_{50}/\text{mL}$ were observed directly after application of infectious blood (0h), but no infectious virus could be detected from three days until the end of the experiment (Figure 2). In contrast, no infectious virus could be recovered from either forest soil specimen (pH 4.1 and 3.2), even immediately after the application of infectious blood. In swamp mud (pH 5.1), however, low residual titers were found directly after the addition of infectious blood. From day three until the end of the observation period, no infectious virus was recovered from swamp mud.

ASFV genome, however, was detectable in all investigated soil types/matrices and no distinct decline in copy numbers was recorded over the entire observation period.

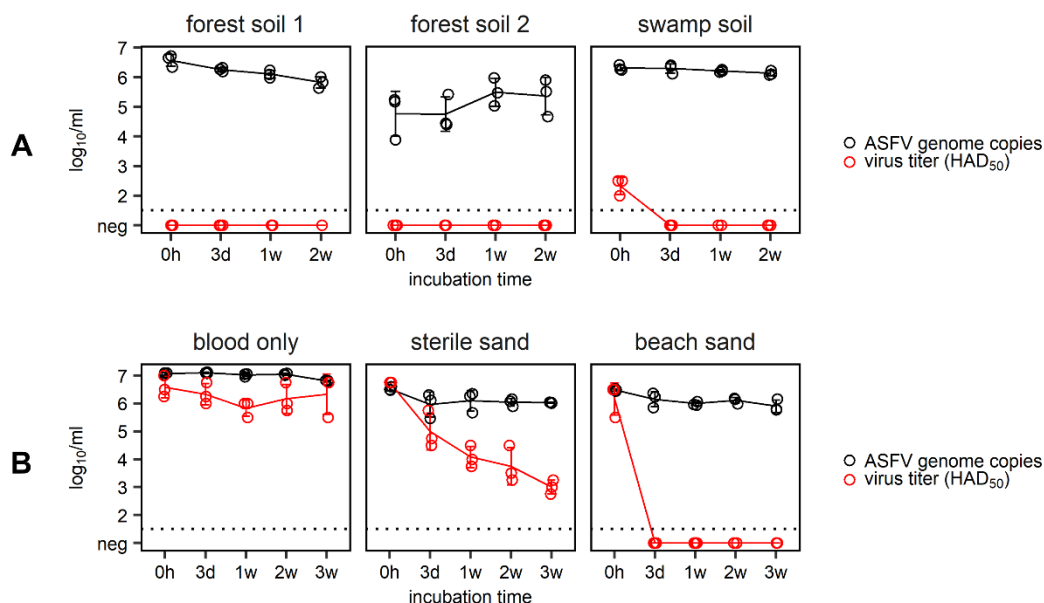


Figure 2: Different soil types spiked with 1.2 mL infectious blood (6.0 \log_{10} $\text{HAD}_{50}/\text{ml}$ of ASFV “Armenia08”) and stored at room temperature (A). Beach sand and sterile sand were inoculated with 2 mL of infectious blood and also incubated at room temperature (B). Blood only samples served as controls in both experiments. ASFV genome copies are depicted in black and virus titers are shown in red. Experiments were completed in triplicates and each open circle represents an individual replicate, solid lines and error bars represent the mean and standard deviation. The dotted line is the limit of detection of the virus titration.

2.3. Recovery of infectious WSL-adapted CD2v-deleted ASFV Kenya and disinfection treatments (Experiment 4)

To evaluate the improved cell culture technique using WSL cells (Figure 3), beach sand or yard soil were inoculated with blood spiked with WSL-adapted CD2v-deleted ASFV Kenya and stored at room temperature for three weeks. Furthermore, the different matrices were treated with two different disinfectants for one or three hours. A blood-only control and sterile sand mixed with infectious ASFV-blood were used as process controls under the same conditions. Cytotoxicity was not observed in cultures after matrix treatment with the respective disinfectants.

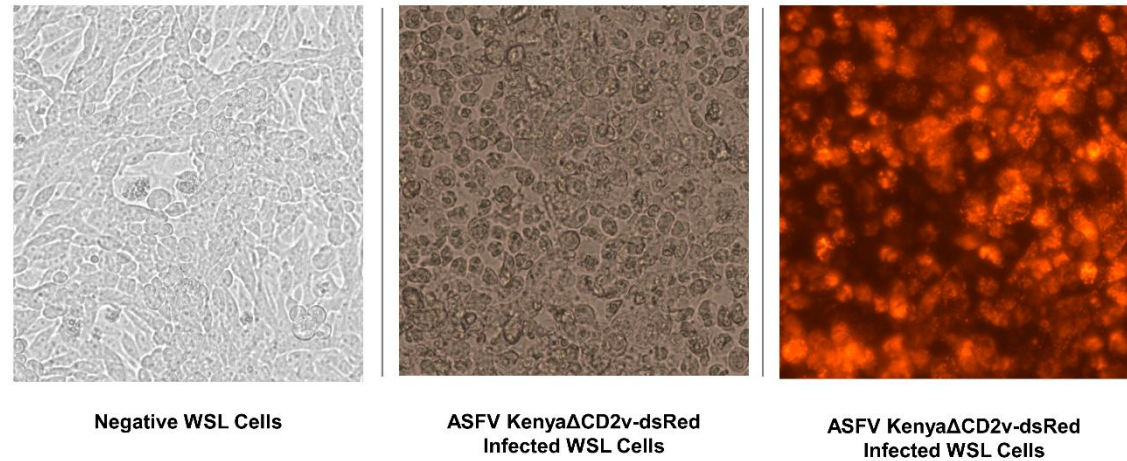


Figure 3: Cytopathic effect and simple ASFV titration readout of WSL cells infected with ASFV Kenya Δ CD2v-dsRed.

Virus titers in the blood-only and sterile sand controls remained constant over the entire observation period, no decrease in virus titer was observed (Figure 4). Thus, untreated blood or sterile sand were infectious for the entire test interval of three weeks. Inoculated beach sand and yard soil however, displayed a steady decline in virus titer over time (during the first week). After one week a high variability between the biological replicates was observed in the beach sand. Moreover, in both soil types, no infectious virus could be detected after two weeks storage at room temperature.

Regardless of the matrix (sterile sand, beach sand, potting soil), no infectious virus could be recovered after a one-hour disinfectant treatment (calcium hydroxide or citric acid) at either concentration (Figure 4). ASFV in pure blood was also fully inactivated after treatment with either disinfectant for one hour at room temperature. In all tested matrices, ASFV genome copy numbers were relatively constant over time. In disinfectant-treated samples, slightly fewer genome copies were detected (Figure 4).

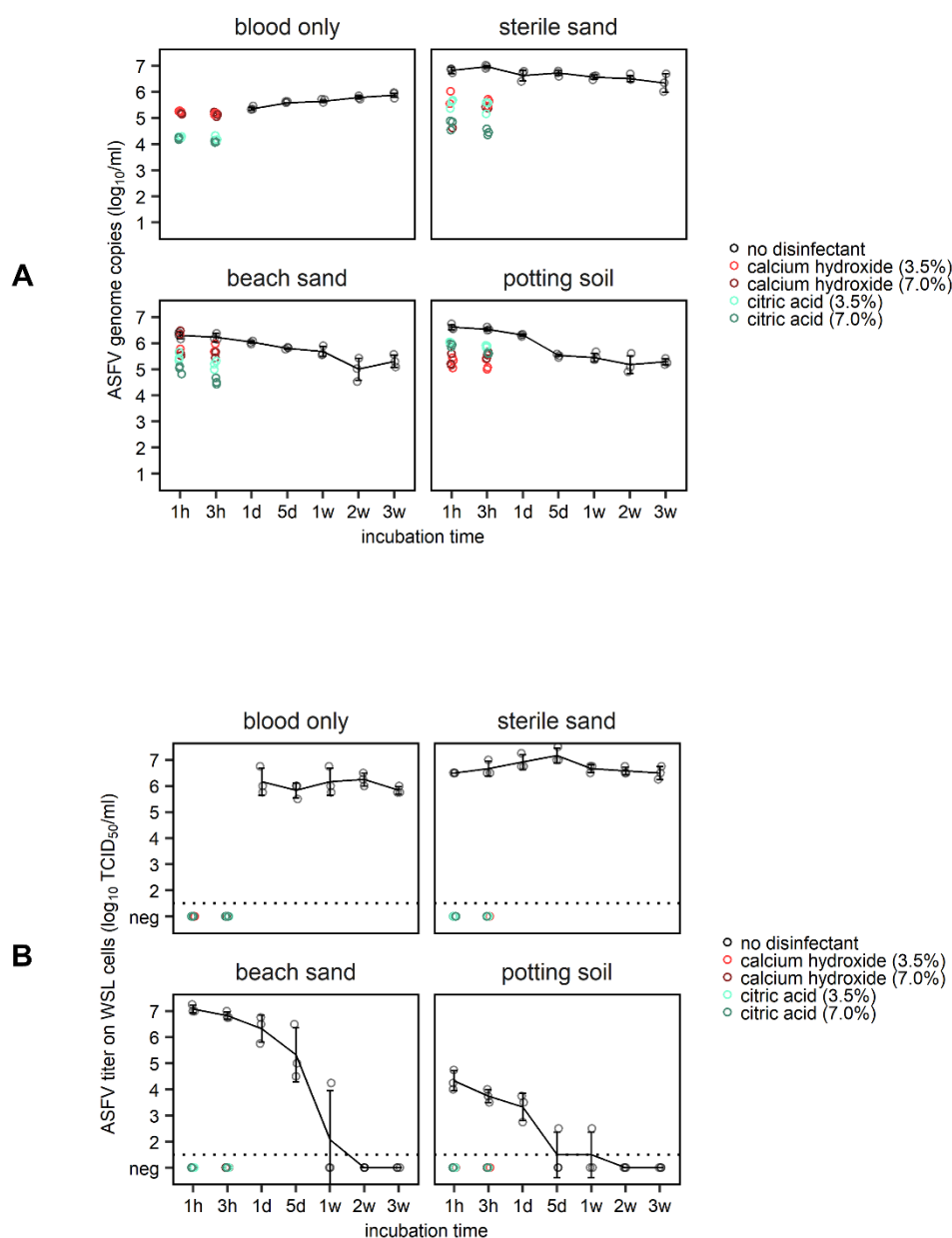


Figure 4: Different soil types were spiked with 2 mL of spiked blood ($6.0 \log_{10} \text{HAD}_{50}/\text{mL}$ of ASFV Kenya $\Delta\text{CD}2\text{v-dsRed}$) and stored at room temperature for indicated times. ASFV genome copies (A) and

virus titers (B) on WSL cells in untreated or disinfectant-treated matrices are shown. Experiments were completed in triplicates and each open circle represents an individual replicate, solid lines and error bars represent the mean and standard deviation. The dotted line is the limit of detection of the virus titration.

3. Discussion

African swine fever is no longer an exotic disease and has established self-sustaining, complicated transmission cycles in European wild boar populations. Slow but constant local spread is observed (data from the Animal Disease Notification System [8]). This was rather unexpected as historical experience did not indicate that wild boar could sustain an endemic infection cycle [9]. Field observations and experimental studies indicate a high lethality [10,11] and low contagiousity, especially in the initial phase of an ASF outbreak among wild boar. The low level of contagiousity requires a rethinking and an adapted approach to control ASF in the wild boar population [12,13]. Evidence suggests that ASF in a wild boar population tends to behave more like a long-term (rather stationary) habitat-bound disease (persisting in the ecological niche comprising wild boar, their carcasses, contaminated fomites, and other biotic and abiotic factors) with no tendency to spread rapidly. It is mainly infectious cadavers, combined with the high tenacity of the ASF virus and the low contagiousity, that can contain the disease within a region [13]. ASFV-contaminated soil rooted by wild boar is one of the habitat factors that could play a role in transmission. Probst et al. [14] reported that wild boar show interest in the soil where carcasses have been found previously, with wildlife cameras documenting animals rooting in soil even when only bones remained. Furthermore, Estonian colleagues and others have demonstrated viral genome in these soils [7,15].

In our study we tried to create a data set for a risk assessment of the role of contaminated soil in ASFV transmission and possible mitigation measures.

We demonstrate that virus stability depends on the soil type, pH, organic material percentage, and to a lesser extent, on ambient temperature. While contaminated sand retains infectivity for weeks, virus stability is very low in acidic forest soils from different locations. This kind of soil is commonly found in northern Germany [16]. Soils are very complex in nature; the interaction of trees, vegetation, animals, microbes, temperatures, location can alter the biology and chemistry of soil ecosystems. In this respect, the existence of different soil types and horizons in forest ecosystems would need further attention. Intermediate times of stability were found in swamp mud and yard soil. Within the limits of our experimental setup, and assuming that the animal is an even more sensitive detection system, we cannot rule out a persistence of low-level infectivity. The HAT detection limit was within the range that was shown to be infectious when orally applied to susceptible animals [17,18]. These results contradict to a certain extent previously published studies [19], where water, soil and leaf litter inactivated ASFV quickly. In this study Mazur-Panasiuk and Wozniakowski [19] were able to re-isolate ASFV from soil and leaf litter immediately after adding culture supernatant to the matrix, but even a short 3-day incubation caused complete loss of virus infectivity independent of temperature conditions. This is in line with our results from swamp mud but not from yard soil or sand, where much longer periods of infectivity were observed. Sand and yard soil mirror the situation in backyard farm settings and other urban habitats. In contrast, re-isolation immediately after adding the contaminant to forest soil was impossible in our hands. Thus, virus inactivation seems to occur after short contact with the matrix e.g. due to the acidic conditions in both investigated forest soil specimens (pH 4.1 and 3.2).

Risk mitigation could involve the use of disinfectants despite the obvious limitation that decontamination of soils in fields and forests, which are very different in structure, consistency and composition, is generally difficult and the organic matter in body fluids impairs disinfection [20]. We used citric acid and calcium hydroxide in our study, which both have proven efficiency against ASFV [21,22], the former with known inhibition by blood [23]. It must be noted that in the past, lime products were used in the control of classical swine fever in wild boar, e.g. in Germany. It is assumed that they not only have a disinfectant effect but also repel wild boar. Furthermore, these products were well accepted by the hunters. The application of lime was therefore included in the official recommendation of the German government for the use of disinfectants in an epizootic [24]. Despite

the above information, it can be questioned whether the application of a basic chemical to acidic soils in the wild boar habitat is appropriate. ASFV is quite reliably inactivated at a pH of below 4 [25]. Therefore, acidic disinfectants could be more useful and here, citric acid was our candidate.

In our study, ASFV was inactivated after 1 h of disinfectant treatment. In spiked beach sand and commercial potting soil not treated with disinfectant, ASFV was fully inactivated after 2 weeks. However, untreated blood or sterile sand were infectious for the entire test interval of 3 weeks with consistent results from virus isolation (macrophages) and titration on WSL cells.

ASFV stability is very low in acidic forest soils but rather high in sandy soils. Given the high variability of wild boar habitats and unpredictable effects of the decay matrix, treatment of carcass locations with disinfectants should be considered when setting up control measures. Not all forest soils are the same globally nor are they homogenous within a single forest. The powder format of the used chemicals could be beneficial and practical. Nevertheless, regulations on the use of biocides and occupational safety have to be considered. Off-label use of commercial products could be an alternative. In this context, disinfectants based on potassium peroxymonosulfate (Trifectant, Virkon S) were recently shown to inactivate ASFV on porous surfaces [26] but had problems with blood under certain circumstances [23]. Removal of ASFV-positive carcasses is of utmost importance and remains a critical control measure as live virus may remain infectious in certain soil matrices for weeks. These studies establish useful protocols to isolate ASFV from soil matrices, while providing insight to potential management options useful in the field to mitigate transmission.

4. Materials and Methods

4.1. Collection and analysis of soil

Half a kilogram of soil was collected from each of five locations in Mecklenburg-Western Pomerania, Germany (Figure 5). The soil types chosen (yard soil, two kinds of forest soil, swamp mud and beach sand) were based on locations where wild boar are commonly found. In addition, a bag of commercial potting soil was purchased to have a more controlled matrix with neutral pH (Table S1), compared to the acidic forest soil samples collected, and sterile sea sand was obtained from a lab supplier (Carl Roth, Karlsruhe, Germany). The parameters of collected soils were commercially analysed by an agricultural laboratory in Rostock, Germany (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, LUFA) (Table S2).

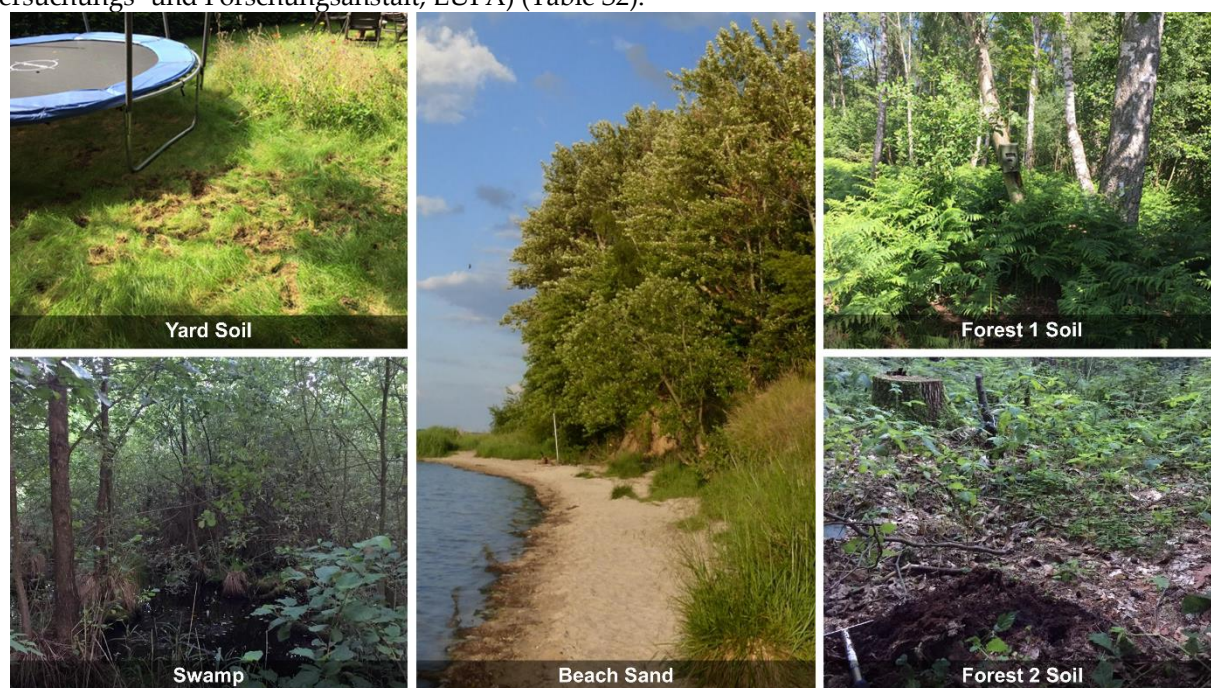


Figure 5: Areas where soil was collected in northern Germany. Sources of yard soil, swamp mud, beach sand, and two forest soils are shown.

4.2. Description of inoculums prepared for soil spiking.

In experiments 1-3, whole blood was collected from wild boar experimentally infected with ASFV “Armenia08”. These animal trials were previously conducted for pathogenesis studies [11]. The blood was mixed for 15-20 minutes with glass beads to remove fibrin. The blood was then stored at -80°C until use. Since experiments were completed at different time points, stocks for spiking the soil matrices had different titers, but differed no more than one log in considered volumes. Infected blood used in experiment 1 had a titer of 7.25 log₁₀ 50% hemadsorbing doses (HAD₅₀) per mL, blood used for experiment 2 had a titer of 6.00 log₁₀ HAD₅₀/mL and blood for experiment 3 had a titer of 7.00 log₁₀ HAD₅₀/mL.

ASFV-Kenya1033ΔCD2vdsRed was derived from ASFV-Kenya1033 as described by Hübner et al. [27] by substitution of the CD2v ORF (EP402R) from codon 77 to the translational stop codon (386) by a dsRed expression cassette.

4.3. Experiment 1: Recovery of ASFV from yard soil on macrophages, a first pilot experiment

In a pilot experiment, 5 g of yard soil were spiked with 400 µL of infectious blood at a titer of 7.25 log₁₀ HAD₅₀/mL and stored at 4°C or 25°C. In this study, blood and soil were tested at time points 0, 3, 6, 24, 48, and 72 h. At the respective time points, 5 ml of RPMI-1640 cell culture medium (Thermo Fisher Scientific, Schwerte, Germany) with 10% fetal bovine serum (FBS) and 2% antibiotics (Gibco Penicillin-Streptomycin mix, 10000 U/ml; Thermo Fisher Scientific) was added to the inoculated soil. Then the soil was agitated in the media by vortexing for 45 seconds (see Figure 6 for all steps). Next, soil and media were sonicated for 45 seconds at 4°C with the settings: duty cycle 40%, output 3.5 with a Branson Sonifier 450 (Heinemann Ultraschall- und Labortechnik; Schwäbisch Gmünd, Germany). After sonication, the soil suspension was centrifuged for 30 minutes at 2,500 x g at 4°C. The supernatant was poured over a coffee filter, pushed through a 0.45 µm syringe filter (Millex Filter Units; Merck Millipore Ltd., Tullagreen, Ireland) and the filtrate was stored at -80°C prior to real-time PCR, virus isolation and titration (see 4.7.).

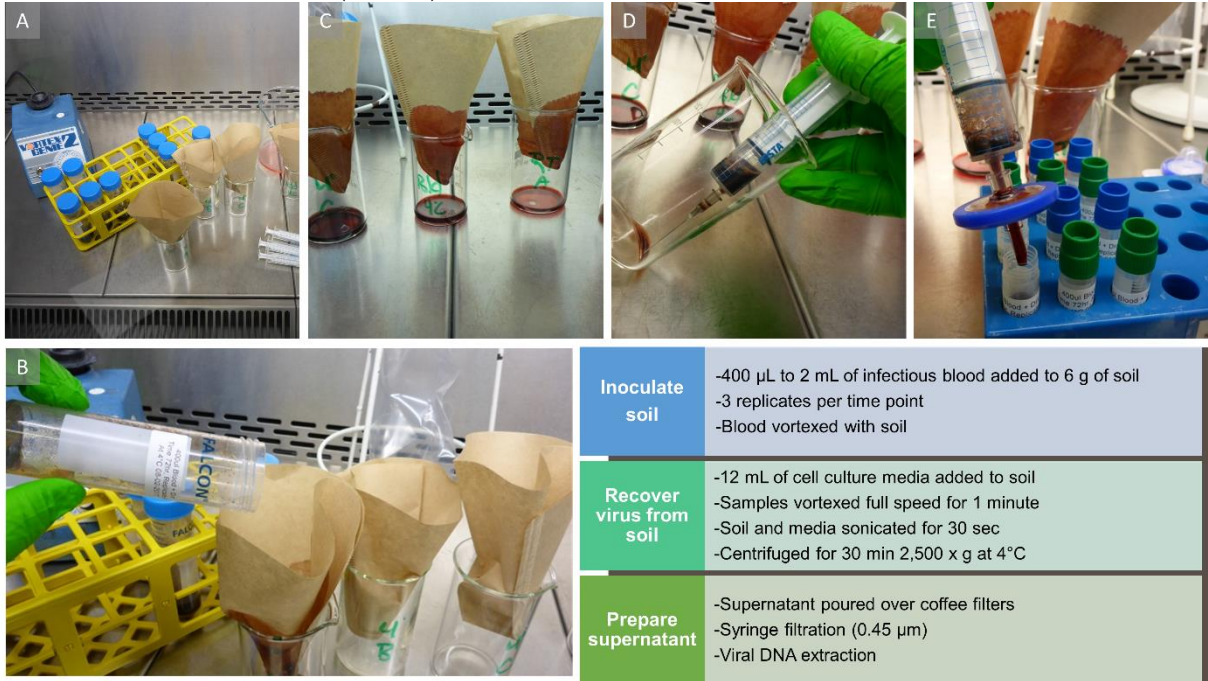


Figure 6: Downstream protocol for processing of soil samples for virus isolation and qPCR. Panel A depicts samples after media and soil matrix have been sonicated and centrifuged for 30 minutes at 2,500 x g at 4°C. Afterwards supernatant was poured over coffee filters (B). The filtration step is shown in panel C. The filtrate was drawn up with a syringe (D) and subsequently passed through a 0.45 µm syringe filter (E).

4.4. Experiment 2: Recovery from sterile sand, beach sand, swamp mud, and forest soil on macrophages

The collected soils were tested together with two controls: blood-only and blood mixed with 6 grams of sterile sea sand (Carl Roth, Karlsruhe, Germany). All soils and controls were spiked with 1.2 mL of ASFV-positive blood with a titer of $6.00 \log_{10} \text{HAD}_{50}/\text{mL}$ and in three replicates per condition. The forest soils were extremely dry, therefore 12 mL of media were added to all samples for virus isolation. We continued experiment 2 and subsequent experiments at room temperature (25°C), since we had not seen significant differences between soil stored at 4°C or 25°C in experiment 1. We limited our testing to 14 days in experiment 2 as it seemed unlikely that live virus would be detected beyond one week. Sample processing was performed analog to experiment 1. Resulting filtrates were stored at -80°C prior to real-time PCR, virus isolation and titration (see below). In addition to the protocol described in experiment 1, we also used a dedicated kit for DNA extraction from soil (DNeasy PowerSoil; Qiagen, Hilden, Germany) from 0.25 grams of all matrices and time points.

4.5. Experiment 3: Recovery of live virus from beach sand on macrophages.

Experiment 2 was repeated with beach sand and 2 mL of ASFV-positive blood with a titer of $7.25 \log_{10} \text{HAD}_{50}/\text{mL}$ at room temperature, due to inconclusive virus isolation results (data not shown). A larger volume of blood was used to increase the chances for virus detection in this matrix that previously gave mixed results. In the repeated experiment, blood-only and sterile sand were included as controls. Every experimental condition was completed with three replicates.

4.6. Experiment 4: Recovery of live virus with WSL-adapted CD2v-deleted ASFV Kenya and disinfection treatments

A virus stock was prepared by mixing 160 mL of supernatant from WSL cells infected with WSL-adapted CD2v-deleted ASFV Kenya virus with 500 mL of defibrinated whole blood from a domestic swine resulting in a final ASFV titer of $6.00 \log_{10} \text{TCID}_{50}/\text{mL}$.

A 2 mL volume of the spiked blood was used to inoculate 6 g beach sand and commercial potting soil at room temperature. A blood-only tube and sterile sand were again included as controls. The protocol was followed as described above, with the omission of sonication as the samples were too numerous to sonicate in the 3-hour intervals between the first three collections. All sample conditions were completed with 3 replicates. Samples were treated with 3.5% and 7.5% calcium hydroxide or 3.5% and 7.5% citric acid (by weight of soil). After each soil matrix was inoculated, the powdered disinfectants were added, vortexed and incubated for 1 or 3 hours at room temperature. To exclude cytotoxicity, cells were monitored under a light microscope daily.

4.7. Virus isolation on porcine macrophages and on WSL cells

Virus isolation and titration were completed with macrophages derived from peripheral blood mononuclear cells (PBMCs). Blood was collected from healthy domestic pigs in heparin tubes. The whole blood was diluted 1:1 in phosphate-buffered saline (PBS), 35 mL of diluted blood was overlaid on 12 mL of Pancoll (PAN-Biotech, Aidenbach, Germany) and spun at $730 \times g$ for 40 minutes at 20°C with slow acceleration and no brake. The PBMCs were collected and washed twice in PBS and passed over 70 μm nylon strainers to remove any fatty debris. For blind passages, 5×10^6 PBMCs were seeded into each well of 24-well Corning Primaria plates (Corning, Durham, USA). Titrations were completed with 7.5×10^6 cells per mL in 96-well Corning Primaria plates (100 μL per well; Corning, Durham, USA).

PBMCs were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2% antibiotics, 75 μL mercaptoethanol (Merck, Darmstadt, Germany) and 2.5 ng/mL granulocyte macrophage colony-stimulating factor (GM-CSF; Biomol, Hamburg, Germany) for the first day, then with 5 ng/mL GM-CSF from the second day after a media change.

A volume of 300 μL soil supernatant was inoculated per well on a 24-well plate with 700 μL of media. The following day, the cells were washed with media once and the supernatant in each well was replaced with 1 mL of fresh media. Cells were examined daily for cytotoxicity with a light microscope. The cells were cultivated for 5 days prior to freezing at -80°C for subsequent virus titration. Titrations were performed as detailed below.

Wild boar lung cells (WSL) were cultivated in Iscove's Modified Dulbecco's Medium with Ham's F-12 Nutrient Mix (Thermo Fisher Scientific), 10% FBS and 2% antibiotics. For virus isolation, WSL cells were seeded the day before with 10^6 cells per well in a 24-well tissue culture plate (Corning, Durham, USA). A volume of 300 μ L soil supernatant was inoculated on a 24-well plate with 700 μ L of media, and subsequently changed the next day and replaced with 1 mL of fresh media. During daily observations, cell proliferation and confluent monolayers were observed in wells negative for virus. The cells were cultivated for 5 days prior to freezing at -80°C for subsequent virus titration. Titrations were completed with 4×10^5 WSL cells per mL using 100 μ L per well in a 96-well tissue culture plate (Corning, Durham, USA) seeded one day prior to inoculation. Cells were inoculated with 100 μ L of virus isolation supernatant from one round of virus amplification in the 24-well plate described above. Titrations were completed in ten-fold dilutions starting with 10^{-1} to 10^{-8} and calculated by the Spearman-Kärber method \log_{10} 50% end point dilution with a limit of detection of 1.75 TCID₅₀/mL in 96-well plates.

4.8. Viral genome detection via real-time PCR

Prior to real-time PCR analysis, nucleic acids from soil samples were extracted using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Subsequently, nucleic acids were analyzed using a published real-time PCR assay targeting the ASFV p72 gene [28] in combination with an internal control based on beta actin [29] on a CFX96 real-time cyclor (Bio-Rad Laboratories, Hercules, USA). PCR was performed with the QuantiTect Multiplex PCR Kit (Qiagen, Hilden, Germany) in a total volume of 25 μ L. Using a dilution series of an ASFV DNA standard, the genome copies in the respective samples were determined. For generation of the ASFV standard, DNA from an ASFV "Armenia08" PBMC culture supernatant was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Subsequently, the DNA concentration was determined by spectrophotometry using a Nanodrop 2000c (Thermo Fisher Scientific) and the exact number of DNA molecules was calculated with an online tool [30].

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Parameters of purchased potting soil, Table S2: Soil analysis results.

Author Contributions: J.C., M.F., and S.B. conceived the idea of the study design and methodology. J.C. and S.B. collected the different soils used in the experiment. J.C., M.F., L.Z., W.F. conducted the experiments, processed samples, and collected the data. J.C. and M.E. provided visuals and charts. J.C., M.F., S.B., M.B., T.M., W.F., M.E. all wrote and edited the manuscript. S.B., M.B., and T.M. funding acquisition.

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Conflicts of Interest: The authors declare no conflict of interest.

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