Received: 28 January 2020 | Revised: 1 March 2020 | Accepted: 18 March 2020

DOI: 10.1111/tbed.13554

ORIGINAL ARTICLE

Transboundary and Emerging Diseases WILEY

African swine fever virus survival in buried wild boar carcasses

Laura Zani[1] | Marius Masiulis[2,3] | Paulius Bušauskas[2] | Klaas Dietze[1] | Gediminas Pridotkas[4] | Anja Globig[1] | Sandra Blome[1] | Thomas Mettenleiter[1] | Klaus Depner[1] | Birutė Karvelienė[3]

[1]Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

[2]State Food and Veterinary Service, Vilnius, Lithuania

[3]Lithuanian University of Health Sciences, Kaunas, Lithuania

[4]National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania

Correspondence
Laura Zani, Friedrich-Loeffler-Institut, International Animal Health Team, Suedufer 10, 17493 Greifswald-Insel Riems, Germany.
Email: laura.zani@fli.de

Funding information
Parts of this work have been funded by the FLI internal African swine fever virus research network.

Abstract

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 boar could play a major role as ASFV reservoir and thereby help to locally maintain and spread the disease in 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 to the infection source. In this study, carcasses of ASFV infected wild boar buried in Lithuania at different time points and locations have been excavated and retested for the presence of infectious ASFV by in vitro assays and for viral genome by qPCR. Soil samples potentially contaminated by body fluids have been additionally tested for viral genome. In seventeen out of twenty burial sites, samples of excavated carcasses were positive for ASFV genome. However, in none of the carcass samples ASFV could be isolated. On seven sites soil samples contained ASF viral DNA. These results unexpectedly negate the long-term persistence of infectious ASFV in wild boar carcasses independent from the burial time. In this context, sensitivity of ASFV isolation from carcass samples versus susceptibility of animals and doses needed for oral inoculation has to be further investigated. Furthermore, research is required to consider alternative ASF infection sources and drivers in the infection cycle among wild boar.

KEYWORDS

African swine fever, tenacity, virus survival, wild boar carcasses

1 | INTRODUCTION

African swine fever (ASF) affects all members of the *Suidae* family. While it is asymptomatic in African indigenous wild pigs, for example warthogs (Thomson, Gainaru, & Van Dellen, 1980), it causes a fatal disease in domestic pigs and Eurasian wild boar (*Sus scrofa*). Due to its extensive spread within the last decade, ASF became a tremendous threat to the global pig industry (Sánchez-Cordón, Montoya, Reis, & Dixon, 2018). Since there is no vaccine and no treatment available, biosecurity and culling of animals are the only tools to fight the disease in pig holdings. As long as ASF affected pig holdings are detected early and all measures are accompanied by proper cleaning

and disinfection, the risk for further spread of the disease can be minimized (Zani et al., 2019). If ASF occurs in wild boar, the situation is much more complex. The infected area has to be defined, and ASF control strategies like ban of hunting and/or fencing to limit the wild boar movement must be adapted to the local situation (EFSA Panel on Animal Health & Welfare, 2018). Since carcasses of ASF infected wild boar could act as a source of infection, they have to be detected and removed as soon as possible. Leak-proof collection and transport of infected carcasses to incineration plants are regarded to be the safest way of carcass disposal (Depner et al., 2017). However, carcass removal and transport to rendering facilities can be problematic or even impossible if the terrain is difficult to access or covered by dense vegetation. In most of the countries, carcass disposal by burning on spot is strictly prohibited due to environmental purposes. In the case of Lithuania, where a rendering plant was not available for the disposal of wild boar carcasses, burying of carcasses as suggested by the Food and Agricultural Organization of the United Nations (Miller & Flory, 2018) was applied to reduce the risk for disease spread. Consequently, in Lithuania, with its large and remote forests, but with soil suitable for digging, many wild boar carcasses have been buried since the start of the epidemic in this country in 2014 (Pautienius et al., 2018). In this study, wild boar carcasses buried at different time points and locations in Lithuania in 2017–2018 were excavated and tested for the presence of infectious ASFV and viral DNA to check for virus survival in those carcasses. Virus survival has been analysed in several studies (Table 1) that investigated meat products (Mebus et al., 1997; Petrini et al., 2019), pig excretions (Davies et al., 2017) and different environmental samples (EFSA Panel on Animal Health & Welfare, 2014). However, mainly samples from experimentally infected animals or spiked samples have been tested. To evaluate if the data holds true for samples under field conditions, we investigated specimens taken from wild boar that succumbed to ASF and were buried in Lithuania if proper carcass removal was impossible. Bone marrow, surrounding soil and, if available, residual organ matrix were sampled and tested.

2 | MATERIALS AND METHODS

2.1 | Study design

Wild boar carcass excavation including decontamination measures of the excavation place and surrounding areas has been approved by the Lithuanian Ministry of Environment and the State Food and Veterinary Service of the Republic of Lithuania. Wild boar carcasses, initially found and tested positive for ASFV infection by quantitative polymerase chain reaction (qPCR) as part of disease control measures, have been excavated during 8–11 October 2018 on 20 different locations across Lithuania (see Figure S1). Bone marrow and, if available, residual organ matrix were taken from each excavated carcass. In addition, soil was sampled on each excavation site by pooling three samples randomly taken 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

TABLE 1 Summary of studies conducted on African swine fever virus tenacity

Material	Duration and conditions	Method	Reference
Blood	140 days in the dark	Bioassay	Eustace Montgomery (1921)
Blood	>6 years at 4–6°C	Bioassay (injection)	Kovalenko, Sidorov, and Burba (1972)
Blood	>90 days	Virus isolation (high titres)	Blome and Dietze, 2011 (unpublished data)
Putrefied blood	15 weeks	Unknown	USDA, 1997 cited by EFSA Panel on Animal Health and Welfare (2010)
Spleen	240 days (6–8°C)	Bioassay (injection)	Kovalenko et al. (1972)
Spleen	>90 days	Virus isolation (high titres)	Blome and Dietze, 2011 (unpublished data)
Muscle	155 days (6–8°C)	Bioassay (injection)	Kovalenko et al. (1972)
Muscle	183 days	Unknown	McKercher et al. (1987)
Muscle	90 days	Virus isolation (low titre)	Blome and Dietze, 2011 (unpublished data)
Fat	123 days	Virus isolation	McKercher et al. (1987)
Cured pork belly	60 days	Virus isolation + bioassay (oral)	Petrini et al. (2019)
Cured loin	83 days	Virus isolation + bioassay (oral)	Petrini et al. (2019)

bones were left, the carcasses received score ③ (Figure 1). 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 (Virkon® S, Lanxess). 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 at the National Food and Veterinary Risk Assessment Institute, harbouring the National Reference Laboratory (NRL) for ASF in Lithuania and at the Friedrich-Loeffler-Institut (FLI), Germany.

2.2 | Laboratory investigations

2.2.1 | Sample processing

After collection, samples were stored at −80°C until further use. For qPCR and virus isolation, bone marrow samples were homogenized with a 5 mm stainless steel bead in 1 ml phosphate-buffered saline (PBS) using TissueLyser II (Qiagen). Tissue samples were homogenized by grinding in sterile sand with pestle and mortar.

2.2.2 | Genome detection

At the Lithuanian NRL, ASF viral nucleic acid was extracted using the QIAamp® RNA Viral Mini Kit (Qiagen). All samples were screened for ASFV genome by qPCR according the protocol of King et al. (2003). At FLI, nucleic acids of soil and decay matrix samples have been extracted by using the DNeasy® PowerSoil® Kit (Qiagen). The obtained eluates were screened for ASFV genome by qPCR (Tignon et al., 2011) with beta-actin gene serving as internal control. In addition, soil samples were screened for swine cytochrome B gene (Forth, 2015) to check for the presence of wild boar DNA.

2.2.3 | Virus isolation

To detect infectious ASFV, homogenized bone marrow and tissue samples were tested for hemadsorption on peripheral blood mononuclear (PBMC)-derived macrophages by directly adding the homogenate but also by running three blind passages first.

Blood for the preparation of PBMC-derived macrophages was collected from healthy domestic donor pigs. In brief, PBMCs were obtained from EDTA-anti-coagulated blood using Pancoll animal density gradient medium (PAN Biotech). PBMCs were grown in RPMI-1,640 cell culture medium with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 10% foetal calf serum (FCS) at 37°C in a humidified atmosphere containing 5% CO_2. The medium was supplied with amphotericin B, streptomycin and penicillin to avoid bacterial and fungal growth. To facilitate maturation of macrophages, GM-CSF (granulocyte-macrophage colony-stimulating factor; Biomol) was added to the cell culture medium at 2 ng/ml. For blind passaging, 1 ml of the homogenate was added to PBMC-derived macrophages (1×10^7 cells/ml) in a T 12.5 cell culture flask (Corning™). After an incubation time of 48–72 hr, the cell culture flasks were freeze-thawed before further passaging and testing.

The hemadsorption test (HAT) was carried out according to slightly modified standard procedures (Carrascosa, Bustos, & Leon, 2011). In brief, 100 µl of isolated PBMCs per well was seeded into a 96-well microplate (Corning™ Primaria™) at a density of 5×10^6 cells/ml. After 16–24 hr, non-adherent cells were removed and cell culture medium containing GM-CSF was replenished as described above. The culture was then incubated for 24 hr to allow initial maturation of macrophages. Subsequently, 30 µl of the supernatant of the homogenized sample was added to each well. From blind passaged samples, 100 µl of the cell culture supernatant was added to each well. Samples were tested in eight replicates. After 24 hr of incubation, 20 µl of homologous 1% erythrocyte suspension was added to each well. For readout, cultures were checked for hemadsorption over a period of 3 days. When using bone marrow or tissue homogenates directly, cells were washed after 2 hr of adsorption time at 37°C using lukewarm PBS, whereas cell culture supernatant was left on the cells until the final evaluation of the test. ASFV Armenia 2007 virus stocks were used as positive control. Doubtful results were confirmed by an additional passage and qPCR testing (King et al., 2003) of cell supernatant.

To exclude the presence of non-hemadsorbing ASFV strains, samples of each carcass were additionally screened by indirect immunofluorescence staining according to the protocol published by Carrascosa et al. (2011). In brief, the formalin-fixated macrophages were incubated with monoclonal antibodies against the viral protein p30 (kindly provided by Linda Dixon, Pirbright Institute, UK) and then stained with commercially available fluorescent antibodies Goat anti-mouse IgG (H + L), Alexa Fluor® 488 (Thermo Fisher). Blood donation from pigs kept for laboratory investigation at FLI, Germany was approved by the competent

FIGURE 1 Exemplary pictures of applied carcass condition score. Score ① skin closed, organs intact; Score ② residual tissue; Score ③ only bones

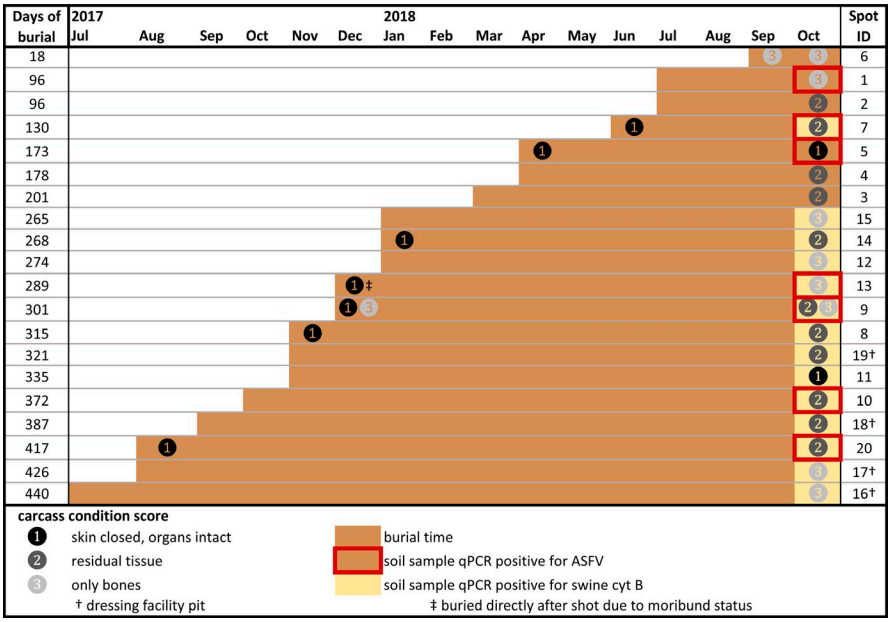

FIGURE 2 Overview of burial time and condition of sampled carcasses. Carcass condition score after excavation has been assessed for each location (right side). If more than one carcass was buried and carcasses at the same location showed different level of decay, separate scoring was applied. If the information was available, carcass condition score at time of burial has been added (left side)

authority (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei (LALLF) Mecklenburg-Vorpommern) under reference number LALLF 7221.3-2-041/17. No further animal experiments have been conducted within the present study. Data analysis was performed using Microsoft Excel 2007 (Microsoft) and SigmaPlot for Windows version 11.0 (Systat software, Inc.).

3 | RESULTS

3.1 | Sample amount and decay conditions of excavated wild boar carcasses

In total, 45 bone marrow samples and 18 tissue samples from carcasses excavated at 20 sites have been obtained (Table S1). The sampled carcasses were buried between 18 and 440 days before excavation and were in different stages of decay. The decomposition status varied between the sites and burial times. On two sites, carcasses with score ① were sampled. Eleven carcasses were excavated in condition score ② and from eight carcasses only bones (score ③) could be found and sampled (Figure 2). Only on excavation site #9, two carcasses with different decomposition score could be clearly identified and separate sample panels were obtained. On all other sites, either only one carcass was buried or, if the identification of corresponding body pieces was not feasible, a general carcass scoring was applied. For seven sites, additional information on the carcass decay condition at the time point of burial was available (Figure 2).

3.2 | Genome detection in all but three excavation sites and in soil samples of seven sites

All sites selected for this study contained wild boar carcasses that were initially tested positive for ASFV genome by qPCR during routine diagnostics. When retested after excavation, in all except three carcass samples, ASFV genome could be detected again by qPCR. 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 (Figure 3). On seven sites (Figure 2), soil samples yielded ASFV positive results in qPCR 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 on one site (#5) ASFV genome was detected without the detectable presence of swine DNA. All soil samples tested positive for the internal control (beta-actin gene).

3.3 | Virus isolation

From none of the bone marrow and tissue samples obtained from excavated carcasses, infectious ASFV could be isolated. Neither in samples passaged three times on macrophages nor in those that have been applied without previous passaging, was any hemadsorption observed. Also, the direct immunofluorescence test did not reveal any indication for the presence of infectious ASFV.

4 | DISCUSSION

Several studies investigating ASFV tenacity in pork products have been conducted. Under favourable conditions, meaning cool and associated with tissue or blood the virus has shown to be rather stable in uncooked pork (Table 1).

Nonetheless, there is so far no data available for the survival of ASFV in wild boar carcasses decomposing under field conditions. Due to the 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 (Zhao et al., 2019) it has been assumed that carcasses

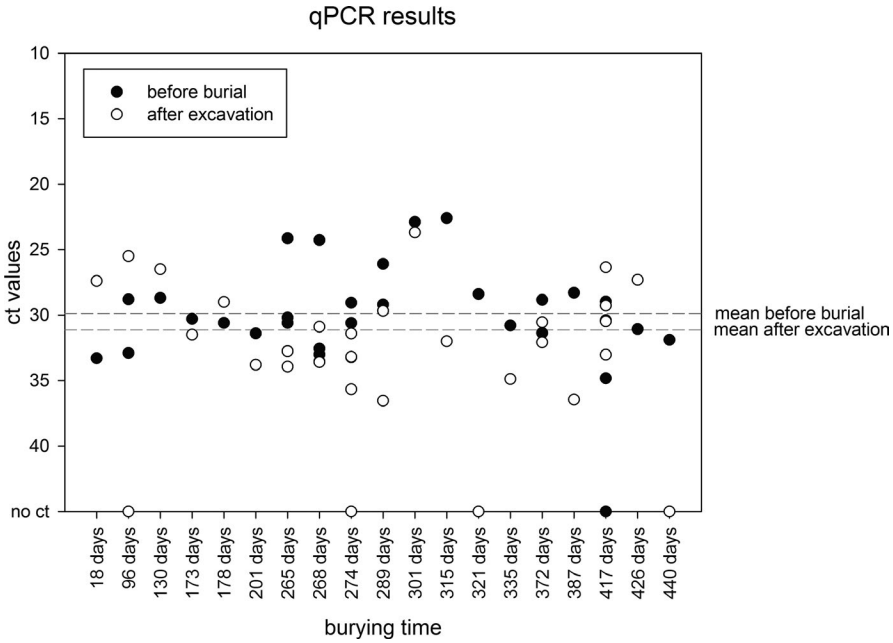

FIGURE 3 qPCR results of wild boar carcasses. Black dots indicate ct values of qPCR testing shortly before the carcass was buried, and white dots indicate ct values after carcass excavation. If more than one dot for each category is displayed for a time point, several samples of the same carcasses have been tested or carcasses on different spots have been sampled after the same burial time

of infected wild boar contain considerable amounts of viable virus (Chenais, Stahl, Guberti, & Depner, 2018).

The detection of very stable quantities of ASFV genome (Figure 3) 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. In our study, only four bone marrow samples (from carcasses after 96, 274, 321 and 440 days of burial) were negative for ASFV genome. However, only on three sites (96, 321 and 440 days of burial) all obtained carcass samples tested negative. The fluctuation of ct values within samples obtained from the same carcass before and after burial shows that viral DNA is rather stable but not homogeneously distributed in body tissues as shown in other studies (Petrov, Forth, Zani, Beer, & Blome, 2018). However, mean ct values after carcass excavation were comparable to previous testing (Figure 3).

In soil samples, ASF viral DNA was found at seven out of twenty sites while swine-specific cytochrome B DNA was detected in fourteen samples. This indicates a rather low virus contamination of soil surrounding buried carcasses although host DNA is present. This might be different in case of carcasses decomposing on the surface where the body is exposed to outdoor conditions and insects or vertebrate species have access. The virus load in soil samples could also have been reduced by the natural presence of humic acids that have been shown to reduce infectivity of other pathogens (Kuznetsova, Cullingham, McKenzie, & Aiken, 2018). Wild boar have shown to root in ground soaked with decay liquids (Probst, Globig, Knoll, Conraths, & Depner, 2017). Therefore, the low contamination rate shown in the presented study would decrease the chance of disease spread by this behaviour. However, the number of obtained soil samples is limited and further studies are under way to evaluate the transmission potential of soil. As mentioned before, soil samples were tested by using the protocol of Tignon et al. (2011) while carcass samples have been tested by using the protocol of King et al. (2003). Both protocols are listed in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Sánchez-Vizcaíno, Dixon, & Heath, 2019) and show similar sensitivity and specificity but in our hands the (Tignon et al., 2011) protocol led to less inhibition artefacts with problematic soil samples. Therefore, ct values of carcass and soil samples cannot be directly compared with the ct values of carcass samples in this study but the contamination rate of the obtained soil samples could be reliably assessed.

In none of the carcasses sampled during this study, infectious ASFV could be isolated independent from burial time and carcass condition. This result was rather unexpected and might have been influenced by different factors.

The obtained sample material is challenging for hemadsorption testing due to advanced decomposition status of sampled tissues and high fat content of white bone marrow. To mitigate the influence of sample quality, blind passaging was performed and antibiotics were added to the cell culture medium. In case of direct testing, the sample was removed after 2 hr from the cell layer to avoid possible damage due to toxic effects. However, a negative influence of sample material on test sensitivity cannot be ruled out. Bioassays, that is testing of infectivity by administration of the sample to live pigs, are known to be more sensitive (Petrini et al. 2019; Table 1). It has been shown that pigs were successfully orally infected with ASFV even if the sample previously tested negative in virus isolation (Niederwerder et al., 2019; Petrini et al., 2019). However, for animal welfare reasons and the low amount of sample material, this was not attempted in our study.

Another factor affecting virus stability might be the unknown conditions during the decomposing process of the sampled carcasses. In published tenacity studies, samples are usually stored at a standardized setting which is different from field conditions. Lithuania has a humid continental climate with average air temperatures not above −3°C in the coolest month and up to 22°C in the

warmest months. At least for 4 months, the average air temperature is above 10°C (Bukantis, 2001). Compared to air temperature, soil surface is on average 3–6°C warmer during summer and several tenths of a degree colder in winter depending on vegetation and humidity (Juknevičiūtė & Laurinavičius, 2008). All sampled carcasses were buried for at least one summer period, and in case of advanced decomposition status at time of burial (Figure 2) it can be assumed that the exposure to outdoor conditions was even longer. ASFV is known to be heat sensitive (Plowright & Parker, 1967) and inactivated at 37°C after around 20 days (Mazur-Panasiuk, Zmudzki, & Wozniakowski, 2019). Therefore, taking into consideration the additional temperature increase of the carcass during decomposition (above 32°C at an outer temperature of 23°C) it does not seem unlikely that the virus has been inactivated effectively during the decomposition process (Johnson, Mikac, & Wallman, 2013). Nonetheless, as the presence and titres of infectious virus at the time of death of the wild boar included in this study are not known, inactivation is difficult to prove and further research is required.

5 | CONCLUSIONS

Due to the high stability of ASFV, carcasses of infected wild boar are considered as major driver for local spread and the maintenance of ASF in the habitat. However, the presented study could not demonstrate the presence of infectious ASFV in buried wild boar carcasses at different decomposition status. This result was unexpected but should be taken with care 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. Furthermore, 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 are not feasible.

ACKNOWLEDGEMENTS

The authors would like to thank MSc Felix Närmann, University of Greifswald for preparing the map of carcass excavation spots.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available in the supplementary material of this article or are available from the corresponding author upon request.

ORCID

Laura Zani https://orcid.org/0000-0001-5263-8389
Klaas Dietze https://orcid.org/0000-0002-6138-6707
Sandra Blome https://orcid.org/0000-0001-5465-5609

REFERENCES

Bukantis, A. (2001). Climatic fluctuations in Lithuania against a background of global warming. *Acta Zoologica Lituanica*, *11*(2), 113–120. https://doi.org/10.1080/13921657.2001.10512366

Carrascosa, A. L., Bustos, M. J., & de Leon, P. (2011). Methods for growing and titrating African swine fever virus: Field and laboratory samples. *Current Protocols in Cell Biology*, *53*(1). https://doi.org/10.1002/0471143030.cb2614s53

Chenais, E., Stahl, K., Guberti, V., & Depner, K. (2018). Identification of wild boar-habitat epidemiologic cycle in African swine fever epizootic. *Emerging Infectious Diseases*, *24*(4), 810–812. https://doi.org/10.3201/eid2404.172127

Davies, K., Goatley, L. C., Guinat, C., Netherton, C. L., Gubbins, S., Dixon, L. K., & Reis, A. L. (2017). Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 isolate. *Transboundary and Emerging Diseases*, *64*(2), 425–431. https://doi.org/10.1111/tbed.12381

Depner, K., Gortazar, C., Guberti, V., Masiulis, M., More, S., Oļševskis, E., … Dhollander, S. (2017). Epidemiological analyses of African swine fever in the Baltic States and Poland. *EFSA Journal*, *15*(11), e05068. https://doi.org/10.2903/j.efsa.2017.5068

EFSA Panel on Animal Health and Welfare. (2010). Scientific Opinion on African swine fever. *EFSA Journal*, *8*(3), 1556. https://doi.org/10.2903/j.efsa.2010.1556

EFSA Panel on Animal Health and Welfare. (2014). Scientific Opinion on African swine fever. *EFSA Journal*, *12*(4), 3628. https://doi.org/10.2903/j.efsa.2014.3628

EFSA Panel on Animal Health and Welfare. (2018). African swine fever in wild boar. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2018.5344

Eustace Montgomery, R. (1921). On a form of swine fever occurring in British East Africa (Kenya Colony). *Journal of Comparative Pathology and Therapeutics*, *34*, 159–191. https://doi.org/10.1016/S0368-1742(21)80031-4

Forth, J. H. (2015). *Standardisierung eines nicht-invasiven Beprobungssystems zur Infektionsüberwachung bei Wildschweinen Sus scrofa*. Retrieved from https://www.openagrar.de/receive/openagrar_mods_00012495

Johnson, A. P., Mikac, K. M., & Wallman, J. F. (2013). Thermogenesis in decomposing carcasses. *Forensic Science International*, *231*(1), 271–277. https://doi.org/10.1016/j.forsciint.2013.05.031

Juknevičiūtė, L., & Laurinavičius, A. (2008). *Analysis of the change in the depth of frozen ground in different soils under Lithuanian conditions*. Paper presented at the 7th International Conference "Environmental engineering", Vilnius, Lithuania. http://dspace.vgtu.lt/handle/1/660

King, D. P., Reid, S. M., Hutchings, G. H., Grierson, S. S., Wilkinson, P. J., Dixon, L. K., … Drew, T. W. (2003). Development of a TaqMan® PCR assay with internal amplification control for the detection of African swine fever virus. *Journal of Virological Methods*, *107*(1), 53–61. https://doi.org/10.1016/S0166-0934(02)00189-1

Kovalenko, J. R., Sidorov, M. A., & Burba, L. G. (1972). *Afrikanskaia chuma svinei*. Thesis translated from Russian. Moskau.

Kuznetsova, A., Cullingham, C., McKenzie, D., & Aiken, J. M. (2018). Soil humic acids degrade CWD prions and reduce infectivity. *PLOS Pathogens*, *14*(11), e1007414. https://doi.org/10.1371/journal.ppat.1007414

Mazur-Panasiuk, N., Zmudzki, J., & Wozniakowski, G. (2019). African swine fever virus – Persistence in different environmental conditions and the possibility of its indirect transmission. *Journal of Veterinary Research*, *63*(3), 303–310. https://doi.org/10.2478/jvetres-2019-0058

McKercher, P. D., Yedloutschnig, R. J., Callis, J. J., Murphy, R., Panina, G. F., Civardi, A., … Scatozza, F. (1987). Survival of Viruses in "Prosciutto di Parma" (Parma Ham). *Canadian Institute of Food Science and Technology Journal*, *20*(4), 267–272. https://doi.org/10.1016/S0315-5463(87)71198-5

Mebus, C., Arias, M., Pineda, J. M., Tapiador, J., House, C., & Sánchez-Vizcaíno, J. M. (1997). Survival of several porcine viruses in different Spanish dry-cured meat products. *Food Chemistry*, *59*(4), 555–559. https://doi.org/10.1016/S0308-8146(97)00006-X

Miller, L., & Flory, G. (2018). Carcass management for small- and medium-scale livestock farms – Practical considerations. *Food and Agriculture Organization of the United Nations*, FOCUS ON FAO, No. 13, *13*, 1–10.

Niederwerder, M. C., Stoian, A. M. M., Rowland, R. R. R., Dritz, S. S., Petrovan, V., Constance, L. A., … Hefley, T. J. (2019). Infectious dose of African swine fever virus when consumed naturally in liquid or feed. *Emerging Infectious Diseases*, *25*(5), 891–897. https://doi.org/10.3201/eid2505.181495

Pautienius, A., Grigas, J., Pileviciene, S., Zagrabskaite, R., Buitkuviene, J., Pridotkas, G., … Stankevicius, A. (2018). Prevalence and spatiotemporal distribution of African swine fever in Lithuania, 2014–2017. *Virology Journal*, *15*(1), 177. https://doi.org/10.1186/s12985-018-1090-8

Petrini, S., Feliziani, F., Casciari, C., Giammarioli, M., Torresi, C., & De Mia, G. M. (2019). Survival of African swine fever virus (ASFV) in various traditional Italian dry-cured meat products. *Preventive Veterinary Medicine*, *162*, 126–130. https://doi.org/10.1016/j.prevetmed.2018.11.013

Petrov, A., Forth, J. H., Zani, L., Beer, M., & Blome, S. (2018). No evidence for long-term carrier status of pigs after African swine fever virus infection. *Transboundary and Emerging Diseases*, *65*(5), 1318–1328. https://doi.org/10.1111/tbed.12881

Plowright, W., & Parker, J. (1967). The stability of African swine fever virus with particular reference to heat and pH inactivation. *Arch Gesamte Virusforsch*, *21*(3), 383–402. https://doi.org/10.1007/BF01241738

Probst, C., Globig, A., Knoll, B., Conraths, F. J., & Depner, K. (2017). Behaviour of free ranging wild boar towards their dead fellows: Potential implications for the transmission of African swine fever. *Royal Society Open Science*, *4*(5), 170054. https://doi.org/10.1098/rsos.170054

Sánchez-Cordón, P. J., Montoya, M., Reis, A. L., & Dixon, L. K. (2018). African swine fever: A re-emerging viral disease threatening the global pig industry. *The Veterinary Journal*, *233*, 41–48. https://doi.org/10.1016/j.tvjl.2017.12.025

Sánchez-Vizcaíno, J. M., Dixon, L., & Heath, L. (2019). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (Section 3.8., Chapter 3.8.1). Paris, France: World Organization for Animal Health.

Thomson, G. R., Gainaru, M. D., & Van Dellen, A. F. (1980). Experimental infection of warthogs (*Phacochoerus aethiopicus*) with African swine fever virus. *Onderstepoort Journal of Veterinary Research*, *47*(1), 19–22.

Tignon, M., Gallardo, C., Iscaro, C., Hutet, E., Van der Stede, Y., Kolbasov, D., … Koenen, F. (2011). Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. *Journal of Virological Methods*, *178*(1–2), 161–170. https://doi.org/10.1016/j.jviromet.2011.09.007

Zani, L., Dietze, K., Dimova, Z., Forth, J. H., Denev, D., Depner, K., & Alexandrov, T. (2019). African swine fever in a Bulgarian Backyard farm—A case report. *Veterinary Sciences*, *6*(4), 94. https://doi.org/10.3390/vetsci6040094

Zhao, D., Liu, R., Zhang, X., Li, F., Wang, J., Zhang, J., … Bu, Z. (2019). Replication and virulence in pigs of the first African swine fever virus isolated in China. *Emerging Microbes & Infections*, *8*(1), 438–447. https://doi.org/10.1080/22221751.2019.1590128

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Zani L, Masiulis M, Bušauskas P, et al. African swine fever virus survival in buried wild boar carcasses. *Transbound Emerg Dis*. 2020;67:2086–2092. https://doi.org/10.1111/tbed.13554