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AUSTRALIAN Pork®

Review of research on epidemiological aspects of transportation-related biosecurity for African swine fever - Risk factors for environmental persistence of the virus

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Executive Summary

Background

African swine fever (ASF) is a high priority for the Australian pork industry. Though the industry has been proactive in responding to the threat of ASF and has worked in conjunction with the states and territories on numerous ASF preparedness actions, it is critical that the industry continues to progress key gaps, opportunities and outputs related to ASF preparedness. To continue the strong progress made in response to the threat of ASF to date, the industry has identified a need to strengthen biosecurity across the supply chain particularly with respect to transportation.

The potential risks to pigs from disease due to pathogen contamination brought into the piggery by people, vehicles, and/or animal movements are minimised by good on-farm biosecurity practices. Understanding how the consistency and effectiveness of truck washing practices being used at each pork export abattoir can support on-farm biosecurity is necessary.

As part of a larger project funded by APL to improve biosecurity around transportation of pigs from farms to abattoir, a review of published literature related to transportation-related epidemiological risk factors for spread of ASF virus, the kinetics of virus shedding in faeces and other pig fluids, survival of the virus in the environment, and the efficacy of various cleaning and disinfection (C&D) protocols in inactivating the virus was commissioned. This report describes the key findings from that review.

Key findings

1) No peer reviewed reports relative to truck washing or cleaning and disinfection, for either full-sized or scale-model trucks contaminated with ASF virus, were found despite extensive literature searches.
2) Transportation of infected pigs (and return of potentially contaminated trucks from infected regions/farms to uninfected regions/farms) is a recognized risk factor for spread of ASF virus. However, much of the concern is based on this being a plausible risk rather than being supported by any substantial amount of experimental data or case report findings. Only a tiny fraction of ASF cases reported to OIE include information about the suspected or confirmed route of exposure; most are simply listed as 'unknown'. There is case report data in the scientific and grey literature that implicates contaminated transport vehicles being the route of virus introduction into farms; these reports have most often come from China and other countries in SE Asia. Trader-networks that rely on commingling pigs for collection and delivery to slaughter have been a significant concern related to the frequency and rate of spread of ASF in China.
3) While differences exist amongst regions, feral pigs are believed to act as a persistent, long-term reservoir of ASF virus for infected regions of Europe and Asia; they are likely responsible for a small number of new outbreaks in domestic (usually small holder) pigs through direct or indirect (contaminated environment, faeces, forages, or carcasses) contact. There is strong anecdotal evidence that most new infections in domestic holdings are related to feeding of ASF virus contaminated swill; unfortunately this evidence is more often simply based on 'the farm fed untreated swill' rather than evidence that in fact, the

swill was contaminated with ASF virus. The source of infection in commercial-sized domestic pig holdings to our knowledge has almost always been 'unknown'.

4) ASF virus is shed in all body fluids and faeces, though at varying concentrations based on number of days post-infection. Virus strain also impacts shedding levels and duration. The ASF virus involved in the Eurasian outbreak is highly virulent and can be considered virtually 100% fatal, with death occurring five to 30 days post-infection. Other strains are less virulent and less lethal. In either case, virus can be assumed to be shed continuously, albeit at decreasing concentrations, for the life of the pig. There are likely outliers (pigs or virus strains) to these assumptions, but for planning purposes one should accept these assumptions.
5) The is little evidence that dose or route of infection (inoculated or naturally infected) substantially influences virus excretion kinetics or concentration.
6) The literature presents some conflicting evidence on the likelihood pigs will become infected after coming into contact with an ASF virus contaminated environment (e.g. a pig pen or truck compartment). However, as first principles:
    a) ASF virus is shed in faeces, and
    b) ASF virus is infectious through oral exposure
7) Therefore, one should assume contaminated environments will remain contaminated for an extended period (weeks to months) in the absence of cleaning and disinfecting. Under warm and dry conditions typical for much of Australia where commercial pigs are farmed, virus may only remain infectious for days to weeks. However, cool temperatures and moisture such as might be found in some outdoor settings (farmed or feral) during some of the year will help the virus persist for longer periods. A number of factors relate to the conflicting literature, namely: Stage of infection that the 'seeder' pigs were in when vacating the environment, the interval between seeder pigs vacating the space and naïve pigs coming into contact, the level of contamination, the surface of the environment (concrete, solid floor, perforated floor, etc.), virus strain, and others. Bedding materials may help the virus persist longer as they can be expected to protect the virus from sunlight and drying. However, the relative 'protective' effect of one bedding material versus another has not been reported in detail.
8) Many reports exist that suggest a wide range of disinfectants are active against ASF virus. ASF virus resists inactivation by disinfectants or desiccation when in the presence of proteinaceous fluids such as blood or meat juice, or in faeces. Some disinfectants are formulated to include surfactants which can improve their performance, particularly when the surface has been imperfectly cleaned.
    a) Citric acid is not usually the best choice for ASF virus but can be effective when used at high concentration (>= 3%) and when given at least 30 minutes of contact time. Acids at this concentration are particularly corrosive to aluminium and therefore may present a problem for trailer disinfection.
    b) Alkalis are generally more effective than acids (concentration varies depending on which chemical is used). Many alkalis at effective concentrations are corrosive to materials and can present a particular hazard to human health.
    c) Aldehydes (including formalin and formaldehyde gas) are effective against ASF virus.
    d) Alone, drying is unlikely to provide sufficient inactivation of ASF virus under the time constraints related to truck and trailer cleaning.

e) Virkon S (1% for 30 minutes, 2% for 10 minutes) is very effective at inactivating ASF virus.
f) Other commercial products, often formulated as a combination of chemicals, are available and some of these have label claims against ASF virus.
g) Wood and unsealed concrete are challenging to clean and to disinfect.
h) There has been limited study of the interaction between temperature and disinfectant efficacy though general recommendations by disinfectant manufacturers and some scientific literature suggest that disinfectants (including the water in which they are dissolved) should be at room temperature when applied or that contact times be extended when temperatures are below this level.

9) There is no evidence in the literature that supports a 'minimum downtime' is required after depopulation and C&D (in a farm or for a truck). Essentially, an environment can be considered either 'disinfected, or not'. Downtime serves only to provide some extra security around not being able to reliably ascertain if a surface is in fact disinfected. The EU requires a minimum of 40 days downtime (plus sentinels for 45 days OR on-going monitoring in the new population for 45 days) as part of their OIE recognised ASF control strategy. If there is evidence that tick vectors were involved in the original outbreak, repopulation is prohibited for six years.
10) Following any decision to implement compulsory truck washing at abattoirs detailed SOPs should be drawn up and independent auditing of the procedures implemented to ensure compliance.
11) In the event that ASF (or another EAD) reaches Australian pig herds, it is likely that abattoirs will inadvertently slaughter infected pigs. Given the uncertainty surrounding virus inactivation in pondage systems and the common Australian abattoir practice of disposing of the pondage effluent by irrigation onto pasture, consideration should be given to Implementation of a process that minimizes the opportunity for exotic pathogens of pigs to remain viable in abattoir effluent before land application. This may include chemical or heat treatments, and be combined with management processes such as drying, dilution, subsoil application, and bio-exclusion (fencing).

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1. Methods

A search of the scientific peer-reviewed literature was conducted to identify published work related to African swine fever (ASF) and risk factors for spread of the disease related to transportation. Three separate searches were conducted using the following search strings:

Search 1: 993 hits (PubMed)
((ASF OR 'african swine fever') AND
(epidem* OR risk OR 'risk factor' OR biosecur* OR transpor* OR truc*))

Search 2: 23 hits (PubMed)
((ASF OR 'african swine fever') AND
(manure* OR faeces OR feces OR effluent) AND
(pig OR swine))

Search 3: 10 hits (PubMed), 321 hits (Google Scholar), and 23 hits (Web of Science)
(("African swine fever" OR ASF) AND
(disinfectant* OR decontam* OR clean* OR wash*) AND
(truck* OR transport* OR trail*))

The search results (n = 1,370) were combined, duplicates removed, and the abstract of each paper was reviewed for relevance. One-hundred and fifteen papers were identified and then reviewed in full. An additional 14 publications were subsequently identified as part of ad hoc searches during review of the initial 115 papers resulting in a total of 129 papers being reviewed in full.

2. Introduction

African swine fever (ASF) was first described in Africa (Kenya) in 1921 (Montgomery 1921) but probably had been diagnosed earlier in June 1910. The author noted that in all cases, warthogs were known to be present in the vicinity of the outbreak. For 15 outbreaks that occurred between September 1909 and September 1912, the mortality rate was 98.9% (Montgomery 1921) though in other outbreaks, the mortality was as low as 2-3%.

Since this first description of ASF, the disease has spread to many countries in Europe, Africa, Asia and of geographical importance to Australia; Indonesia, East Timor and Papua New Guinea. The disease is currently not present in Australia, North, Central or South America, or New Zealand.

Once ASF virus enters a country, the disease spreads via direct contact, contaminated vehicles, pork products and carrier pigs. These routes of transmission need to be recognized when considering measures to control the spread of ASF virus. Virus replication is essentially limited to cells in the mononuclear phagocytic system and the virus does not appear to replicate in epithelial tissue. The amount of virus shed from body fluids and faeces is low with the exception of blood where virus concentration may be very high (Mebus 1988). Clinical signs of the disease may present across a spectrum of severity from peracute to subclinical with an incubation period ranging between five and seven days.

3. Epidemiology of ASF

There are many recent and comprehensive reviews of the epidemiology of ASF and the agent itself and only aspects of ASF epidemiology related to transportation are summarized in this report. For readers that require information about other aspects of ASF or the virus, several open-source, recent reviews are recommended below:

Dixon, L. K., Stahl, K., Jori, F., Vial, L., & Pfeiffer, D. U. (2020). African Swine Fever Epidemiology and Control. Annu Rev Anim Biosci, 8, 221-246. doi:10.1146/annurev-animal-021419-083741

Link: https://www.annualreviews.org/doi/pdf/10.1146/annurev-animal-021419-083741

Schulz, K., Conraths, F. J., Blome, S., Staubach, C., & Sauter-Louis, C. (2019). African Swine Fever: Fast and Furious or Slow and Steady? Viruses, 11(9). doi:10.3390/v11090866

Link: https://www.mdpi.com/1999-4915/11/9/866/pdf

Mazur-Panasiuk, N., Żmudzki, J., & Woźniakowski, G. (2019). African Swine Fever Virus - Persistence in Different Environmental Conditions and the Possibility of its Indirect Transmission. J Vet Res, 63(3), 303-310. doi:10.2478/jvetres-2019-0058

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6749736/pdf/jvetres-63-303.pdf

4. ASF virus shedding and persistence

4.1 Physical properties of the virus

ASF virus is the sole member of the family *Asfarviridae*, genus *Asfivirus.* The genome is a double strand of DNA of approximately 170-190 kilobase pairs which encode for between 151 and 167 open reading frames (ORFs). The average diameter of this enveloped virus is about 172 to 191 nm and consists of concentric layers surrounded by an external hexagonal membrane (Carrascosa *et al.* 1984). A total of 22 genotypes have been identified, no serotypes have been identified as the virus does not induce neutralizing antibodies (Boshoff *et al.* 2007).

4.2 Virus shedding

4.2.1 Faeces and other secretions or excretions

Important to understanding within and between-farm spread of ASF is recognizing the role of contaminated fomites in spread of the disease. Contaminated fomites arise through physical objects coming into contact with virus contaminated fluids from infected pigs and therefore some understanding of virus shedding patterns is important in mitigating the risk of ASF virus being spread amongst farms.

Bellini *et al.* (2016) reviewed published work on ASF virus shedding and concluded that infected pigs are usually contagious (i.e. shedding virus) during the incubation period of the disease when clinical signs are not yet apparent. During incubation, pigs may shed virus for up to 48 h before showing clinical signs. Large amounts of the virus are then shed from the time the disease produces clinical signs of infection (the acute stage). Pigs that survive the acute phase of the disease and progress into more chronic stages, continue to shed virus into the environment until they succumb albeit at reduced levels and frequency. During the acute phase, large quantities of virus are present in blood, secretions, and excretions including oral and nasal fluids, urine, faeces, and blood (when present); the likelihood of virus being shed in semen is contentious but is at least plausible given the very high and persistent viremia that develops with the disease.

The earliest excretion of ASF virus usually occurs by the nasopharyngeal route, as early as one or two days before the onset of fever (Greig and Plowright 1970) though the exact time and concentration of virus can vary depending on strain of the virus. The titres of pharyngeal and nasal swabs rise rapidly to reach mean levels of about 10^4-10^5 haemadsorbing doses (HAD_{50}, or the amount of virus present to cause a positive HA reaction in 50% of test wells) in the two to three days following the onset of pyrexia. Virus in the secretions of the conjunctiva or lower urogenital tract appear somewhat later and tend not to attain as high of levels. The amount of virus present in faecal and urinary excretions, and therefore the extent of environmental contamination, appears to be related to virus strain which helps to explain the failure of infected pigs to transmit the disease to stall mates during the first 12 to 24 hours of pyrexia (and later) in some studies (Greig and Plowright 1970). Though blood reliably has the highest peak concentration of any tissues (often exceeding 10^8 HAD_{50} per ml), mean faecal titres can still be high with concentrations reaching as much as 10^2 HAD_{50} per gram – even higher levels (10^4 to 10^5 genome copies per mL of rectal fluid) are reported when using the more sensitive PCR to detect the virus (Guinat *et al.* 2014).

In a separate study, ASF virus was present in substantial amounts in secretions and excretions of acutely infected pigs for only seven to 10 days after the onset of fever and was present in the greatest concentration in the faeces (McVicar 1984). In this study, the authors used a moderately virulent strain (Dominican Republic 1979) as compared to other work done with highly virulent strains such as Georgia 2007/1 or Lisbon 1960. However, studies with this lower virulence strain showed the virus to persist in blood of recovered and clinically normal pigs for at least eight weeks and in the lymphoid tissues for at least 12 weeks. Concentrations of virus in various secretions were as follows (HAD_{50} per mL or gram of secretions): Nasal mucus $10^{4.3}$, saliva $10^{3.3}$, conjunctival fluid $10^{4.8}$, tonsil swab $10^{6.8}$, rectal swab $10^{6.3}$ (contained blood), prepuce or vaginal swab and urine $10^{6.1}$ (contained blood). Blood from these pigs had ASF virus titres of $10^{5.3}$-$10^{9.2}$ HAD per mL (McVicar 1984).

Using a strain of ASF virus (Pol18_28298_O111) isolated from an outbreak isolated in Poland and given intranasally, a minimum dose of 5 HA units was required for the infection of naïve pigs (Walczak *et al.* 2020) indicating the infectious dose by this route is very low.

Susceptible pigs exposed to pigs that had been infected with the virulent Georgia 2007/1 ASF virus were used to measure within- and between-pen transmission scenarios (Guinat *et al.* 2014). ASF virus was first isolated in blood among the inoculated pigs by day 3, and then chronologically among the direct and indirect contact pigs, by day 10 and 13, respectively. Close to the onset of clinical signs, higher virus titres were found in blood compared with nasal and rectal fluid samples among all pigs. Pig rectal fluid titres were 10^4 to 10^5 genome equivalents per mL (or around 10^2 HAD_{50} per mL). Consistent with other studies, blood titres were highest amongst the tissues tested at 10^7 to 10^8 per mL when measured either by HAD_{50} or genome equivalents.

In contrast to previous work done studying virus shedding during the acute stages of infection, excretion dynamics in persistently infected animals was reported in 2012. In the study, virus excretion through different routes was quantified for up to 70 days after infection using three ASF virus isolates of moderate virulence (Brazil 1978, Malta 1978, and Netherlands 1986) (de Carvalho Ferreira *et al.* 2012). For each isolate and dose level, 10 animals were used, some of which were inoculated directly, while others were left to become infected through contact with the inoculated pigs. It was shown that neither dose nor route of infection (inoculated or naturally infected) influenced virus excretion kinetics or concentration. Nasal, ocular, and vaginal excretions showed the lowest titres and virus was consistently present in the oropharyngeal swabs for up to 70 days post-infection (dpi). Virus was occasionally present in the faeces, sometimes with very high titres. Results presented in this study show that a high proportion of persistently infected animals shed virus into the environment for at least 70 days. In faeces, mean virus concentration reached nearly 10^4 $TCID_{50}$ genome equivalents per gram depending on strain. Shedding persisted routinely through 45 dpi and occasionally over 60 dpi.

The shedding pattern and stability of ASF virus in faeces, urine, and oral fluid from pigs infected with the highly virulent Georgia 2007/1 virus isolate has been reported (Davies *et al.* 2017). In this study, When measured by virus isolation, the half-life was estimated at 8.48 and 15.33 days at 4°C and 3.71 and 2.88 days at 37°C, for faeces and urine respectively. When measured using with PCR, the half-life of ASF virus DNA was 8 to 9 days in faeces and 2 to 3 days in oral fluids, at all temperatures that were tested. In urine, the half-life of ASF virus DNA was found to be 32.54 days at 4°C, decreasing to 19.48 days at 37°C.

Olesen *et al.* (2018) conducted a study that examined the likelihood that exposure of healthy pigs to the pen environment of pigs that had died from ASF, would result in infection. Following euthanasia of pigs that had been infected with a virulent isolate of ASF virus from Poland (POL/2015/Podlaskie/Lindholm), healthy pigs were introduced into the contaminated pens either 1, 3, 5, or 7 days later. Importantly, the infected pigs used to 'seed' the pen environment with ASF virus were euthanized within 1 to 4 days following the detection of clinical signs (i.e. the peracute to acute stage of the disease). Pigs that were introduced into the contaminated environment within one day of the infected pigs being removed developed clinical disease within one week (and were virus positive in blood). However, pigs introduced into the contaminated pens after 3, 5 or 7 days did not develop any signs of ASF infection, and no viral DNA was detected in blood samples obtained from these pigs within the following three weeks. Thus, it was shown that exposure of pigs to an environment contaminated with ASF virus can result in infection but the time window for transmissibility may be shorter than expected.

Virus shedding in faeces presents a potential for using faeces as an ASF diagnostic or surveillance tool. During the acute phase of the infection (0 to 21 dpi), virus could be detected in faeces (by PCR) only around 50 to 80% of the time, far less sensitive than applying the same diagnostic procedure to a blood or serum sample de Carvalho Ferreira *et al.* (2014). This percentage decreased to below 10% after 21 dpi. The authors reported that ASF virus DNA was quite stable in faeces with the half-life ranging from more than two 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 preferred sample in this study was spleen, which had both the highest titres and highest half-life of any tissue that was assessed.

4.2.2 Meat and blood

Bellini *et al.* (2016) provides a convenient review of the key routes of ASF virus transmission between pigs and farms and offers preventive measures to minimise the risk of transmission. The authors' note that ASF virus can survive for long periods in a protein rich environment, remaining stable across a wide range of (pH 4 to 10). The virus' resistance to changes in pH and affinity for survival in protein rich environments explain why the virus is not substantially affected by meat maturation processes. Meat from pigs slaughtered in the infective stages of ASF or that die spontaneously of the disease therefore provide a ready source of infective virus to naïve pigs via the practice of feeding uncooked waste food. Pork products fed to pigs as garbage or swill that has not been cooked to comply with the OIE Terrestrial Code poses a significant risk to naïve animals.

ASF virus can be inactivated by heating for 30 minutes at 60˚C (Plowright and Parker 1967) or 70 minutes at 56˚C (Mebus 1988) but the virus is much more hardy when held in a moist and proteinaceous environment, surviving in blood heated to 50˚C for 3 hours (Montgomery 1921). In defibrinated blood kept in the dark at room temperature, the virus can survive for 140 days, in filtered serum for 404 days, and in putrefying blood for at least 16 days (Montgomery 1921). Survival of the virus in pig blood kept at 4˚C can be as long as 18 months (Plowright and Parker 1967). The virus will survive across a wide range of pH conditions with inactivation occurring below pH 3.9 or

above pH 11.5; the virus will survive at pH 13.4 for 20-22 hours in medium containing 25% serum (Mebus 1988).

ASF virus will survive in Parma hams for at least 300 days but not 400 days (McKercher *et al.* 1987). Infectivity of ASF virus is lost by 110 days in chilled deboned meat, bone-in meat, or ground pork, and after 30 days in smoked deboned meat, as cited by (Adkin *et al.* 2004).

Recently, EFSA has been requested to review its previous evaluation (Anonymous 2014) of the ability of different matrices, such as meat and meat products from ASF virus-infected pigs and ASF virus-contaminated materials, including vegetables, arable crops, hay, straw as well as sawdust, wood chips and similar materials, to transmit ASF virus to domestic pigs, and to rank the different matrices on the basis of their level of risk of transmitting the virus (Anonymous 2020). Virus was reported to remain viable in frozen organ tissues for ≥60 (liver, kidney, and heart), and ≥735 (spleen) days. EFSA previously (Anonymous 2014) cited (Adkin *et al.* 2004) suggesting virus could remain viable in frozen pork meat for at least 1000 days.

The World Organisation for Animal Health (OIE) publishes guidelines for conditions that would be expected to inactivate ASF virus in various commodities (Anonymous 2019a).

Article 15.1.22: Procedures for the inactivation of ASFV in swill
For the inactivation of ASFV in swill, one of the following procedures should be used:

1. the swill is maintained at a temperature of at least 90°C for at least 60 minutes, with continuous stirring; or
2. the swill is maintained at a temperature of at least 121°C for at least 10 minutes at an absolute pressure of 3 bar; or
3. the swill is subjected to an equivalent treatment that has been demonstrated to inactivate ASFV.

Article 15.1.2: Safe commodities
When authorising import or transit of the following commodities, Veterinary Authorities should not require any ASF-related conditions, regardless of the ASF status of the exporting country or zone:

1. meat in a hermetically sealed container with a F_0[1] value of 3 or above;
2. gelatine.

[1] The F- value for a process is the number of minutes required to kill a known population of microorganisms in a given food under specified conditions. This F-value is usually set at 12 D-values to give a theoretical 12 log reduction of the most heat-resistant species of mesophilic spores in a can of food. The D-value is the time required to reduce the spore survivors by 90%, or 1 log10. When F is used without a subscript (F0), 250°F (121°C) is assumed and so F3 defines the 'equivalent killing conditions to what would occur over 3 minutes at 121°C). F3 is a typical value for most commercially retorted foods in international trade.

Article 15.1.23: Procedures for the inactivation of ASFV in meat
For the inactivation of ASFV in meat, one of the following procedures should be used:

1. Heat treatment

Meat should be subjected to:

a. heat treatment for at least 30 minutes at a minimum temperature of 70°C, which should be reached throughout the meat; or
b. any equivalent heat treatment which has been demonstrated to inactivate ASFV in meat.

2. Dry cured pig meat

Meat should be cured with salt and dried for a minimum of six months.

Article 15.1.24: Procedures for the inactivation of ASFV in casings of pigs
For the inactivation of ASFV in casings of pigs, the following procedures should be used: treating for at least 30 days either with dry salt (NaCl) or with saturated brine (Aw < 0.80), or with phosphate supplemented dry salt containing 86.5% NaCl, 10.7% Na2HPO4 and 2.8% Na3PO4 (weight/weight/weight) at a temperature of 12°C or above.

Article 15.1.27: Procedures for the inactivation of ASFV in litter and manure from pigs
For the inactivation of ASFV in litter and manure of pigs, one of the following procedures should be used:

1. moist heat treatment for at least one hour at a minimum temperature of 55°C;
2. moist heat treatment for at least 30 minutes at a minimum temperature of 70°C.

5. ASF virus inactivation in faeces

Contact with faeces is a key means by which fomites can become contaminated with ASF virus and therefore, having a good knowledge of the concentration and inactivation kinetics of ASF virus in faeces is important in developing control strategies focussed on pig transportation. For the purpose of this review, pig waste (faeces and urine) that is held in open storage external to a building or under the building or is the waste water (regardless of whether it is treated or untreated water) used to wash down trucks that have carried pigs or other animals will be regarded as slurry. Unlike Northern hemisphere pig production which tends to occur in areas with more severe winters, the need to store large quantities of slurry is not a critical issue in Australia as the material can be applied to land throughout most of the year.

A possible risk associated with slurries generated at commercial or on-farm truck washes is that slurry may bypass municipal sewage treatment and be applied directly to a crop as fertilizer, thereby creating a substantial risk of spreading any pathogens present in the slurry.

Fischer *et al.* (2020) studied the survival of ASF virus on plant material that had been brought into contact with ASF contaminated slurry. The issue under investigation in the study was related to concerns that virus shed by ASF infected wild boars (in urine or faeces), or contaminated by the carcass of a pig that had died of ASF, could lead to contamination of animal feeds that were derived from the plant materials and hence create a risk of infection in unrelated susceptible pigs. Part of the rationale for this study was related to observations reported from a study of the early part of the outbreak of ASF in Latvia (Oļševskis *et al.* 2016). Fischer *et al.* (2020) found that after being contaminated with ASF virus contaminated blood, six different types of field crops (wheat, barley, rye, triticale, corn, and peas) were positive for ASF viral genome by PCR even after being dried at room temperature for two hours, or after being dried and then exposed for one hour to moderate heat (40°C and 75°C). However, no infectious virus could be detected after two hours drying using virus isolation in porcine macrophages in combination with the detection of ASF virus by the haemadsorption (HA) test.

A study was undertaken to determine the survival time of ASF virus on selected fomites including water, wet soil, and wet leaf litter (Mazur-Panasiuk and Woźniakowski 2020). The samples were tested at -20°C, 4°C, 23°C, and 37°C either 0, 3, 7, or 14 days later. Five grams of each matrix was spiked with 500 µL of culture medium containing $10^{6.52}$ HAD_{50} per mL of ASF virus (Pol16/20540/Out10 isolate). Infectious virus was isolated from all water samples at all sampling times. For the other fomites, virus infectivity was lost after 3 days, regardless of temperature. The same study also investigated the survival of ASF virus in putrescent spleen tissue when the tissue was held in these same fomites. Contaminated spleen samples were put into water, soil, leaf litter, straw (type not specified), hay (type not specified) and grain (type not specified) and incubated at 4 and 23°C for 56 days. Virus titres were determined at 7, 14, 28 and 56 days. A temperature of 4°C was sufficient to preserve virus viability for at least 56 days in water, straw, and hay. Soil and grain samples were inactivated after 28 days, whereas leaf litter resulted in the fastest inactivation of the virus, with its titre decreased to less than or equal to $10^{1.31}$ HAD_{50} per mL between day 7 and 14. At 23°C, no samples were positive beyond 7 days of incubation (calculated half-life 0.44 days).

Out of concern around the potential for forage and feeds to act as ASF virus fomites, the European Commission (EC) has developed recommendations for management of these materials

(SANTE/7113/2015 - Rev 12 2020). Though generally the risk of commercially traded crops, vegetables, hay and straw to contain and maintain infectious ASF virus is considered to be low by the EC, if the use of locally harvested grass and straw is considered to represent a risk under local prevailing conditions, then the EC recommends that feeding of fresh grass or grains to pigs should be banned unless the materials have been treated to inactivate ASF virus, or be stored out of reach of wild boar for at least 30 days before feeding. Further, use of straw for bedding of pigs should also be banned unless it has been treated to inactivate ASF virus or stored out of reach of wild boar for at least 90 days before use.

Turner and Williams (1999) examined the effectiveness of alkali treatment (NaOH or $Ca(OH)_2$) or heating (4°C or 22°C) for inactivating ASF and swine vesicular disease (SVD) viruses in pig slurry, then went on to design a pilot plant for heat inactivation of slurry that could be used in a field setting (Turner *et al.* 1998). The desired level of inactivation was to achieve a 10^4-fold reduction of infectious virus titre (in alignment with the UK standard for disinfectant performance at the time). In their initial work, the authors used slurry from two different farms that had been spiked with ASF virus. The virus was inactivated in less than 1 minute at 65˚C for a sample from only one of the farms while no infectious virus was detected after 15 minutes at 60˚C from both farms. Addition of 1% (w/v) of NaOH or $Ca(OH)_2$ caused the inactivation of ASF virus within 150 seconds at 4°C, while 0.5% (w/v) of NaOH or $Ca(OH)_2$ required 30 minutes for inactivation. While extending their work to develop a pilot plant, the authors produced slightly different estimates of the chemical or thermal conditions necessary to inactivate ASF virus (Turner *et al.* 1998). After making a detailed study of the kinetics of inactivation from 4°C to 60°C for time periods of up to 24 hours, they concluded that ASF virus was inactivated within a few seconds at 60°C and within 90 seconds at 56°C. The authors also determined that ASF virus could be inactivated with 1% of either NaOH or $Ca(OH)_2$ equally well at either 4°C or 22°C for 150 seconds.

In the authors' further work with their pilot treatment plant, a transportable device was constructed that could continuously treat pig slurry at a rate of up to 100 litres per hour (Turner *et al.* 1999). Based on findings from their laboratory-based work, the heating mechanism was designed in a manner that allowed for at least 99.99% of the slurry to be maintained at the required temperature for a minimum period of five minutes. ASF virus was inactivated by operating the plant at a temperature of 53°C at a pH of 8. For the very large volumes of slurry found on modern commercial farms, heat treatment or chemical treatment with either NaOH or $Ca(OH)_2$ may still be impractical, but the published work suggests it is not impossible.

No published literature on the survival of ASF virus in straw or other bedding materials could be located (Anonymous 2020).

In an older review, Haas *et al.* (1995) summarised the inactivation kinetics of various transboundary pathogens in faeces or slurry. The authors suggested that Aujeszky's disease virus may survive for 3-15 weeks, Borna disease virus for 22 days, Marek's disease virus for 7 days, Teschen disease virus for 3-25 days, ASF virus for 60-100 days, and foot and mouth disease virus for 21-103 days. However, they note that under practical field conditions, survival time is strongly dependent on many variables such as temperature, pH value, and the initial concentration of the pathogen which are out of the control of a farmer or disease control officials. Tables reflecting the authors' best estimates of pathogen survival time under various conditions are reproduced below (Table 1,Table 2, and Table 3).

Table 1. Inactivation times for animal viruses in slurry at various temperatures (reproduction of Table 1 from Haas et al. (1995)).

Virus	Origin of slurry	Initial concentration ($TCID_{50}$/50 µl)	Inactivation time at various temperatures (detection limit = 0.7 $\log_{10}$ $TCID_{50}$/50 l)						
			5°C	20°C	35°C	40°C	45°C	50°C	55°C
Swine influenza virus	Pigs	$10^{5.8}$	9 weeks	2 weeks	> 24 h *	> 24 h *	ND	> 2 h 30 min	1 h
Porcine parvovirus	Pigs	$10^{6.0}$	> 40 weeks *	> 40 weeks *	21 weeks	9 weeks	> 19 days *	5 days	8 days
Bovine virus diarrhoea virus	Cattle	$10^{5.2}$	3 weeks	3 days	3 h	50 min	20 min	5 min	5 min
Infectious bovine rhinotracheitis virus	Cattle	$10^{5.1}$	> 4 weeks *	2 days	24 h	3 h	1 h 30 min **	40 min	10 min
Aujeszky's disease virus	Pigs	$10^{5.2}$	15 weeks	2 weeks	5 h	2 h	45 min	20 min	10 min
Foot and mouth disease virus	Pigs	$10^{4.8}$	> 14 weeks	2 weeks	24 h	10 h	5 h	1 h	1 h
	Cattle	$10^{4.8}$	ND	5 weeks	> 24 h *	ND	ND	ND	> 60 min *
Classical swine fever virus	Pigs	$10^{4.2}$	> 6 weeks *	2 weeks	4 h	> 3 h *	> 3 h *	***	***
Transmissible gastroenteritis of pigs virus	Pigs	$10^{5.2}$	> 8 weeks *	2 weeks	24 h	> 5 h *	2 h 30 min	1 h	30 min

$TCID_{50}$: 50% tissue culture infective dose
ND: no data
* time of complete inactivation not reached
** infectious virus verified through inoculation tests on calves; after 2 h 30 min at 45°C, similar inoculation tests on calves yielded negative results
*** instantaneous

Table 2. Survival of classical swine fever virus in pig slurry at various temperatures (reproduction of Table 2 from Haas et al. (1995)).

Day	Titre at 4°C *			Titre at 17°C *		
	1st test	2nd test	Control **	1st test	2nd test	Control **
0	7.0	6.75	6.75	6.5	6.5	6.75
14	5.5	5.25	5.25	5.0	4.75	5.25
28	4.5	4.75	4.0	4.25	4.25	3.75
42	4.25	4.25	3.25	4.0	3.75	2.25
56	3.75	3.75	2.5	3.5	3.25	2.0
70	2.75	3.0	2.0	2.75	≤ 2.5	≤ 1.5
84	≤ 2.5	2.75	≤ 1.5	≤ 2.5	≤ 2.5	≤ 1.5
98	≤ 2.5	≤ 2.5	≤ 1.5	≤ 2.5	≤ 2.5	≤ 1.5
112	≤ 2.5	≤ 2.5	≤ 1.5	≤ 2.5	≤ 2.5	≤ 1.5
126	≤ 2.5	≤ 2.5	≤ 1.5	≤ 2.5	≤ 2.5	≤ 1.5

* virus titres in $\log_{10}$ $TCID_{50}$/carrier
** control carriers were closed so that virus was not exposed to slurry

Table 3. Survival of African swine fever virus in pig slurry at various temperatures (reproduction of Table 3 from Haas et al. (1995).)

Day	Titre at 4°C *			Titre at 17°C *		
	1st test	2nd test	Control **	1st test	2nd test	Control **
0	6.25	6.5	6.5	6.25	6.0	6.25
14	5.5	5.5	4.5	4.75	5.5	4.75
28	4.5	4.75	4.25	4.25	4.0	3.5
42	4.5	4.25	4.0	3.75	3.25	2.5
56	4.25	4.0	3.5	3.5	3.5	1.75
70	3.5	3.75	2.0	3.0	2.75	≤1.5
84	3.75	3.25	1.75	2.75	≤2.5	≤1.5
98	3.0	2.75	≤1.5	≤2.5	≤2.5	≤1.5
112	2.75	≤2.5	≤1.5	≤2.5	≤2.5	≤1.5
126	≤2.5	≤2.5	≤1.5	≤2.5	≤2.5	≤1.5

* virus titres in $\log_{10}$ $TCID_{50}$/carrier .
** control carriers were closed so that virus was not exposed to slurry

Further, the same authors offered some practical recommendations for the treatment of liquid manure:

- Wherever possible, slurry should be utilised on tillage crops (excluding those for fresh consumption)
- Slurry should be stored for at least 60 days in summer or 90 days in winter, before being spread on pasture
- After application of slurry, a period of 30 days should be allowed before grazing, preferably with adult or non-susceptible animals.
- If possible, storage tanks should not be filled to the limit, to enable the addition of a sufficient amount of chemicals.
- Chemical disinfectants must be thoroughly dissolved and evenly distributed in the slurry. Vigorous stirring is necessary before, during and after the addition of chemicals.
- Chemicals should be added to the storage tank at several points simultaneously.
- As powdery or granular substances are difficult to dissolve in liquid manure, the application of aqueous suspensions is strongly recommended, unless high performance (e.g. 100 hp per 500m^3) stirring equipment is available.
- The efficacy of treatment with aqueous solutions depends on the intensity of stirring. Usually, the equipment available on farms will not achieve sufficiently vigorous stirring of sediments. Therefore, mobile high-performance stirring equipment should be made available by veterinary authorities, farmers' associations or other institutions.
- Liquid manure must be disinfected by chemical means if no heat disinfection is applied.
- Before and during the addition of disinfectants, and for six hours afterwards, the liquid manure must be thoroughly stirred.
- Stirring, for at least two hours, must be repeated daily until the manure is considered safe.

- After treatment, the manure should be ploughed into arable land.
- The following chemicals are recommended for disinfection of liquid manure:
- Ca(OH)2 (slaked lime, lime hydrate): 40% solution at a rate of 40-60 litre/m^3; exposure time >4 days; also suitable for use at temperatures between 0 and -10°C.
- NaOH (sodium hydroxide): 50%, 16-30 litre/m^3, exposure time >4 days; pH >12; also suitable for use at temperatures between 0 and +10°C.
- Formalin: 35-37% solution of formaldehyde in water, 25-40 litre/m^3, exposure time >4 days. The efficacy of formalin is reduced when temperature is below +20°C and it is not suitable for use when temperatures are below +10°C.
- Peracetic acid: 25-40 litre/m^3, exposure time > 1h; only suitable in special situations, due to strong formation of foam; also suitable for use between 0 and +10°C.
- The incubation time of 4 days requested in the regulations should be considered the absolute minimum, and exposure for 7 days would be more advisable.

There appears to be little published evidence available assessing inactivation of ASF virus during composting of manure or animal composting (Costa and Akdeniz 2019).

6. Transportation-related risk factors

Most country reports to OIE of ASF outbreaks in domestic pigs since the Eurasian pandemic began indicate that the source of the infection for the outbreak is 'unknown'. Though contaminated transport vehicles are a plausible and recognized risk factor for spread of ASF and other diseases amongst farms (Beltran-Alcrudo *et al.* 2019), documented cases of such occurrences are rare (Guinat *et al.* 2016). It is highly likely that trucks were a significant factor in the spread of ASF in China (Li *et al.* 2020) and trucks have also been considered to be a potentially important risk for spread of ASF into and around Europe (Mur *et al.* 2012) though much of the evidence is anecdotal or assumed. However, a number of ASF outbreaks that occurred in large commercial farms in Russia and Lithuania were thought to be result of poor compliance with biosecurity rules, such as improper disinfection of clothing and boots, or contaminated food brought onto the premises therefore highlighting the potential for fomite-related spread (Gogin *et al.* 2013; Oganesyan *et al.* 2013). In these cases, authors suggested generally poor biosecurity and inadequate implementation of centralized disease control measures were key anthropogenic factors related to ASF introduction and spread in the region.

The current Eurasian epidemic was initiated when ASF entered Georgia in 2007 followed by spread into the EU in 2014. In the EU, the virus primarily spread through wild boar (*Sus scrofa*) in the period from 2014 to 2018. However, from the summer 2018, an increasing number of domestic pig farms became infected, in Romania in particular. During the period from May to September 2019, 655 Romanian pig farms were included in a matched case-control study investigating possible risk factors for ASF incursion in commercial and backyard pig farms (Boklund *et al.* 2020). The results showed that close proximity to outbreaks in domestic farms was a risk factor in commercial as well as backyard farms. Furthermore, in backyard farms, herd size, wild boar abundance around the farm, number of domestic outbreaks within 2 km around farms, short distance to wild boar cases and visits of professionals working on farms were statistically significant risk factors. Additionally, growing crops around the farm, which could potentially attract wild boar, and feeding forage from ASF affected areas to the pigs were risk factors for ASF incursion in backyard farms.

Identifying the route of introduction of ASF virus onto infected farms, even at the early stages of an outbreak can be difficult. During 2015 to 2017, 26 cases of ASF were identified on backyard and commercial pig farms in Estonia (Nurmoja *et al.* 2018). Detailed investigations of each herd by government and international specialist teams were undertaken however, the specific route of introduction could not be determined on any of the herds, though the belief was that some indirect pathway was likely responsible. None of the outbreaks could be linked to the direct introduction of infected pigs.

Further assessments of risk factors related to infection as spread have been done and support the importance of contaminated fomites in the disease epidemiology and this body of work has been recently reviewed (Bellini *et al.* 2016).Simply the presence of an infected pig farm or abattoir in the area, and visits by veterinarians and para-veterinarians have been identified as important risk factors – factor probably related to fomite spread as the agent is not known to spread by aerosol over any more than a few metres (Wilkinson *et al.* 1977; Olesen *et al.* 2017). A spatial regression analysis found density of the road network, of water bodies and of the domestic swine population to be associated with outbreaks in Russia and another spatial spread model found the movement of infected animals to be the most important factor in the spread of ASFV; vehicles collecting dead

animals have also been suggested as a risk factor though there appears to be little data to support this.

Given the lack of experimental and high-quality case study data on between-farm spread, researchers in the Netherlands assembled a group of 45 people considered experts in 'livestock disease control' to participate in a workshop to elicit quantitative estimates of the relative risks of various activities that contributed to introduction of exotic transboundary diseases into countries of Europe. Amongst the activities discussed, livestock trucks returning from infected to uninfected countries was assessed to be an activity with the second highest level of risk. The group noted that this was an important finding as the risk was effectively controllable at borders through inspection of trucks for sufficient cleaning and disinfection (Horst *et al.* 1998).

By contrast to the above, the scientific literature includes numerous efforts by authors to quantify the risk of ASF introduction or spread (into a farm, country, or region) through 'transportation' but typically these papers are considering the risk associated with movement of infected pigs, as distinct from a contaminated vehicle itself acting as a fomite (Vergne *et al.* 2017; Ferdousi *et al.* 2019; Taylor *et al.* 2019; Gao *et al.* 2020).

A majority of ASF outbreaks reported to the World Organisation for Animal Health (OIE) in the current Eurasian ASF pandemic have been in feral pigs and domestic smallholder farms. Despite their arguably important role in many transboundary diseases of pigs (ASF, Aujeszky's disease, classical swine fever, and others), good quality information from these sub-sectors is notoriously difficult to come by as neither tends to receive much institutional support from government or industry. This means that the links between veterinary services and backyard smallholders are often missing or very weak. This, coupled with other issues such as a lack of trust or cultural barriers between farmers and the veterinary services, an absence of farm or animal identification and traceability systems, and insufficient funding for ongoing disease surveillance activities make implementation of disease prevention and control activities difficult (Beltrán-Alcrudo *et al.* 2018).

Concern about spread of ASF from eastern Europe into countries of the EU with significant commercial pork industries prompted an effort to estimate the risk of ASF virus introduction into the EU through three types of transport routes: returning trucks, waste from international ships, and waste from international planes – the authors' collectively referred to these as transport-associated routes (TAR) (Mur *et al.* 2012). A semi-quantitative model based on the weighted combination of risk factors was developed to estimate the risk of ASF virus introduction by TAR with relative risks of each estimated by expert opinion elicitation. The researchers concluded that the relative risk for ASF virus introduction through TAR in most of the EU countries was low, although some countries, specifically Poland and Lithuania had higher levels of risk mostly due to their proximity to other infected countries. 'Livestock trucks returning to non-infected countries from infected countries was thought to pose the highest risk for ASF virus introduction into the EU. The risk for ASF introduction associated with returning trucks accounted for 65% of the total TAR risk.

Retrospective information from outbreaks of ASF in the Russian Federation was used to assess the most likely source of ASF virus introduction onto farms (Khomenko *et al.* 2013).The route of introduction into new pig populations (primary outbreaks, as opposed to secondary onward spread) was unidentified in 28.3 percent of cases (45 out of 159). For those situations where there was some certainty around the source of introduction, 97% were through feeding contaminated swill (n = 109),

2% were through contact with wild boar, and 1% were through fomites such as contaminated vehicles. The route of secondary spread was unidentified in 58.1 percent of cases (25 out of 43) but when the route of introduction was identified, spread occurred through contaminated vehicles (62.1%), direct contact with pigs or people from holdings nearby (33.3%), or through the introduction of new pigs in the herd during the incubation period (5.6%).

A case report of an ASF outbreak that occurred in a large-scale Chinese commercial pig farm was recently published (Li *et al.* 2020). The outbreak started in 2018 and the spatial and temporal spread of infection into and throughout an intensive pig farm was described. Despite a number of standard operating procedures being in place to manage worker and transportation biosecurity, minor infractions related to movement of slaughter pigs off the farm were identified as the most likely route ASF virus was introduced. It is believed that ASF virus was introduced onto the farm during the process of loading slaughter pigs onto a truck (owned by the farm) that had likely been cross contaminated during a prior trip to a commercial abattoir.

Several authors have suggested that the emergency sale of pigs during ASF outbreaks contributes to the spread of ASF with particular examples in Russia and countries in Africa; similar occurrences have been hypothesized in China and in countries of southeast Asia (Costard *et al.* 2015).

One author reviewed published literature in an effort to identify critical features of ASF control strategies that assisted in successful eradication of ASF from historically infected countries (Danzetta *et al.* 2020). Unfortunately, data for this effort was quite limited with suitable evidence to include in the review only available for Belgium, Brazil, Cuba, the Dominican Republic and Haiti, France, mainland Italy, Malta, Portugal, and Spain. Across these countries, movement of ASF contaminated pork and infected live pigs were critically related to introduction of the virus and ongoing spread in all the countries. Insufficient details were available to understand the specific role (if any) of contaminated transport vehicles in these outbreaks. Further, the issue of movement of infected live pigs is inherently confounded by the use (potentially) of contaminated vehicles making the issue difficult to study in the absence of data collected on cleaning and disinfection behaviours in the transport industry.

Published information related to transportation risk is also available for other pathogens from which perhaps, some inferences can be made about ASF virus. Similar to work cited above for ASF, researchers assessed the likelihood of naïve pigs becoming infected with classical swine fever (CSF) after coming into contact with the environment of pigs previously inoculated with virus, but removed before the naïve pigs were introduced into the pen (Dewulf *et al.* 2002). Eight days after experimental infection (when all pigs were viraemic for at least 3 days), the pens were depopulated and 20 h later, restocked with susceptible pigs which stayed in these pens for 35 days. During the first three weeks of the experiment, the pens were neither cleaned nor disinfected. During the observation period, none of the susceptible pigs became infected. This result indicates that CSF virus spread via excretions is of minor importance in the early stages of infection. At the moment of restocking, the floor of each pen was almost fully covered with excretions. The experiment was designed to correspond as much as possible to a field situation where susceptible pigs are transported with a vehicle that previously transported infectious pigs. Therefore, the incubation period was deliberately limited to eight days to allow all pigs to become viraemic but to avoid the pigs to become undeniably clinically diseased as visibly diseased animals are unlikely to be transported during a CSF epidemic. The time interval between depopulation and restocking was set

to be 20 h mimicking a vehicle transporting infectious pigs on one day and susceptible pigs the next day. The fact that the pens were neither cleaned nor disinfected between depopulation and restocking mimics a worst-case scenario where even the most basic hygiene procedures were omitted.

In another study of transport risk related to CSF, the rate at which CSF was transmitted by several different types of inter-herd contact during the 1997–98 epidemic in The Netherlands was quantified (Stegeman *et al.* 2002). During that epidemic, 428 CSF virus-infected pig herds were detected, 403 of which provided data to this study. The estimated rates of transmission were 0.065 per shipment of live pigs, 0.011 per contact by a pig transportation lorry, 0.0068 per person contact, 0.0007 per dose of semen, 0.0065 per contact with a potentially contaminated pig assembly point, 0.027 per week per infected herd within a radius of 500 metres, and 0.0078 per week per infected herd at a distance between 500 and 1000 metres. Quite extensive reporting has done around this CSF outbreak. In a separate study, researchers studied possible origins of the initial introduction of the virus into the Netherlands and other countries involved in the outbreak (Elbers *et al.* 1999). It appeared as though the virus was introduced into The Netherlands by a transport lorry that had been in contact with infected pigs or infectious material in the Paderborn area in Germany which then returned to The Netherlands and came into contact with the index herd there. Further, CSF was diagnosed in a mixed sow-finishing herd in Bocholt in Belgium (near the border area with The Netherlands) and seemed to be associated with use of a transport lorry that had been returning from The Netherlands (which was infected at the time).

7. Cleaning and disinfection

Disinfection is a critical step in controlling the spread of ASF virus by fomites. However, disinfection must be preceded by a thorough mechanical cleaning of the space in order for the disinfectant to be effective. Normal 'cleaning and disinfection' includes first, removal of bedding, straw, feed and manure; second, washing using detergents; and third, application of an effective disinfectant. Attempted disinfection of faecal and bedding materials in the absence of prior cleaning will often fail to inactivate viruses and bacteria. ASF virus is an enveloped virus and therefore tends to be more susceptible to a wider range of disinfectants than nonenveloped viruses, for example Enteroviruses (Juszkiewicz *et al.* 2019a).

Juszkiewicz *et al.* (2019b) reported the results of *in vitro* testing of four commercial disinfectants against ASF virus. Only two products in this study were found to be effective when tested at 10°C for 30 min: Disinfectants containing sodium hypochlorite (1%, 0.5% in low level soiling) and potassium peroxymonosulfate (1% in high level soiling).

There are multiple choices available for use in disinfecting premises that have been contaminated with ASF virus. However, their exact efficacy in a field setting is uncertain given important variables such as the presence of organic matter, temperature, physical characteristics of the surface being disinfected, etc. are not identical across situations. De Lorenzi *et al.* (2020) provides a recent review of the topic. Lipidic solvents, which destroy the envelope of the virus and commercial disinfectants based on iodine and phenolic compounds appear to be amongst the most effective chemicals in inactivating the ASF virus though disease control officials in countries and regions often maintain their own list of 'approved' disinfectant compounds.

The main piece of legislation providing the guidance for the control of ASF in the EU is Council Directive 2002/60/EC which establishes the minimum measures to apply for the control of ASF, including the principles for cleaning and disinfection (Appendix 1). De Lorenzi *et al.* (2020) attempts to use this guidance in constructing an overview of how to establish an effective cleaning and disinfection (C&D) programme that should be effective for ASF virus and the full paper is attached to this report as Appendix 2. Additionally, OIE dedicated an entire issue of Revue Scientifique et Technique (International Office of Epizootics) to cleaning and disinfection of livestock facilities entitled 'Disinfectants: actions and applications' which is attached to this report as Appendix 5.

General knowledge and experience in the use of disinfectants against enveloped viruses have shown that the chemical compounds effective in inactivation of ASFV are:

- Formaldehyde 1%
- Sodium hypochlorite (0.03% to 0.0075%)
- Caustic soda solution 2%
- Glutaraldehyde
- Sodium or calcium hydroxide 1% (effective at virus inactivation in slurry at 4°C)
- Phenols – Lysol, lysephoform, and creolin
- Chemical compounds based on lipid solvents
- Multi-constituent compounds – Sodium chloride, potassium peroxymonosulfate, ysoformin, Desoform, Octyldodeceth-20 (OD-20) surfactants, active substances, organic acids, glycosal, etc.

The USDA has published a list of approved disinfectants for ASF virus (Table 4).

Table 4. Disinfectants effective against ASFV (Table reproduced from (De Lorenzi et al. 2020)).

Active ingredient(s)	**Contact time**	**Application(s)**
Sodium chloride Potassium peroxymonosulfate (Virkon S)	10 min	In/on animal feeding and watering equipment, livestock barns/pens/stalls/stables, livestock equipment, hog farrowing pen premises, hog barns/houses/pens, animal quarters, animal transportation vehicles, agricultural premises/ equipment, human footwear
Sodium dichloro-s-triazinetrione (Clearon Bleach Tablets, Klor-Kleen, Klorsept, Klorkleen2)	30 min	In/on animal living quarters, animal feeding/watering equipment, animal equipment, transportation vehicles, farm premises, shoe baths.
Sodium hypochlorite	15 min nonporous 30 min porous	Indoor or outdoor use sites such as agricultural, transportation, quarantine, and laboratory equipment and facilities; footwear/personal protective equipment.
Citric acid	15 min nonporous 30 min porous	Indoor or outdoor use sites such as agricultural and non-agricultural equipment and facilities; laboratory equipment and facilities; footwear/personal protective equipment, personnel decontamination.

FAO has produced a table of disinfectants appropriate for use against a number of important animal pathogens (Table 5); in the context of this table, ASF virus is considered a 'Category A' pathogen.

Table 5. FAO list of recommended disinfectants and concentrations for inactivation of viruses (Reproduced from (Geering et al. 2001), page 87 and similar to Table 3.1 in AUSVETPLAN Operational Procedures Manual for Decontamination, Version 3.2 (Anonymous 2008)).

Disinfectant group	**Form**[1]	**Strength**[2]		**Contact time**[4]	**Applications and virus category**
		Usual dilution	Final[3]		
Soaps and detergents:					
Miscellaneous	Solids or liquids	As appropriate	10 min		Thorough cleaning is an integral part of effective decontamination. Use for category A viruses.
Oxidizing agents:					
Sodium hypochlorite (NaOCl)	Concentrated liquid (10-12% available chlorine)	1:5	2-3% available chlorine (20,000-30,000 ppm)	10-30 min	Use for virus categories A, B and C. Effective for most applications except when in the presence of organic material. Less stable in warm, sunny conditions above 15C.
Calcium hypochlorite ($Ca(OCl)_2$	Solid	30 g/litre		10-30 min	
Virkon®	Powder	20 g/litre	2-3% available chlorine (20,000-30,000 ppm) at 2% w/v		Excellent disinfectant active against all virus families.
Alkalis:					
Sodium hydroxide	Pellets	20 g/litre	2% (w/v)	10 min	Very effective against virus categories A, B and C. Do not use in the presence of aluminium and derived alloys.
Sodium carbonate anhydrous (Na2CO3)	Powder	40 g/litre	4% (w/v)	10 min	Recommended for use in the presence of high concentrations of organic material.
Sodium carbonate decahydrate ($Na_2CO_3 \cdot 10H_2O$)	Crystals	100 g/litre	10% (w/v)	30 min	
Acids:					
Hydrochloric acid	Concentrated acid (10 Molar)	1:50	2% (w/v)	10 min	Used only when better disinfectants not available. Corrosive for many metals and concrete.
Citric acid	Powder	2 g/litre	0.2% (w/v)	30 min	Safe for clothes and body decontamination. Especially useful for FMD virus decontamination.
Aldehydes:					
Glutaraldehyde	Concentrated solution	as appropriate	2% (w/v)	10-30 min	Excellent disinfectant effective against virus categories A, B and C.
Formalin	40% formaldehyde	1:12	8% (w/v)	10-30 min	Disinfectant; releases irritating, toxic gas.
Formaldehyde gas	Special generation required			15-24 hr	Toxic gas, recommended only If other methods of decontamination cannot be used.

[1] Usual form supplied.
[2] Recommended working strength.
[3] Final concentration.
[4] Required contact time for inactivation of disease agents.

Notes:

- Commonly used general disinfectants such as phenolics and quaternary ammonium compounds are very effective antibacterials but have limited effectiveness against category B and C viruses; they are not included in Table 4.
- Products effective for decontamination of viruses on the hands and the skin arc limited. Virkon® is reported to have low toxicity and to be effective against members of all 17 virus families but it has not been approved for use on skin. Alternatively, citric acid or sodium carbonate may be added to washing water to induce antiviral conditions by lowering or raising the pH as appropriate for the agent to be inactivated.
- w/v = weight/volume (e.g. 2g/100ml)

Unsealed concrete is a porous material widely used in livestock facilities. Quantitative efficacy testing of chemical disinfectants applied to porous unsealed concrete is often hindered by insufficient recovery of viral loads from concrete control samples – this control information is necessary to assess the effectiveness of disinfectants on the surface – i.e. disinfectant properties of the surface itself confounds assessment of the efficacy of the disinfectant chemical placed upon it. Success can only be measured if there is sufficient recovery of microorganisms from untreated, positive control surfaces. Insufficient recovery (<4-log10) of viable virus from control surfaces precludes demonstrating the minimum 4-log10 reduction in infectious titre required for viricidal efficacy determination and subsequent product registration with the U.S. Environmental Protection Agency.

The pH of freshly prepared concrete is highly alkaline, measuring approximately pH 13. ASF virus is inactivated at pH levels ≥10. Prolonged exposure to natural atmospheric and environmental conditions results in a gradual decrease in the pH of concrete over time. This process, termed carbonation, occurs due to the interaction of atmospheric carbon dioxide (CO2) with the hydration products of cement. However, the chemical reaction is largely dependent on relative temperature and humidity, and thus may take many years to complete under natural conditions.

A study was conducted to determine the influence of concrete pH on the recovery of infectious ASF virus (Gabbert *et al.* 2020). Virus recovery from untreated, high-pH concrete was compared to that from concrete in which the pH had been lowered through accelerated gaseous carbonation in a laboratory environment. Following demonstration of sufficient virus recovery from carbonated, pH-adjusted concrete, quantitative efficacy tests with Virkon S were conducted. For pH-adjusted carbonated concrete (without Virkon treatment), viable virus was recovered at levels comparable to, and sometimes better than, recovery from stainless steel controls. Subsequent experiments showed that a 10-minute contact time for ASF virus was required because the 5-min contact time was insufficient to completely inactivate the virus.

An alternative to using disinfectants to kill viral and bacterial pathogens using ozonized water has been reported (Zhang *et al.* 2020). A two log10 reduction (99%) was observed within one minute when $10^{5.0}$ $TCID_{50}$ per mL wild-type or reporter ASF virus was exposed to 5 mg per L of ozonized water, and a three log10 (99.9%) reduction in virus was observed within one to three minutes when exposed to either 10 or 20 mg per L of ozonized water. Inactivation kinetics were also similar at higher virus concentrations. In the study, ozonised water was shown to be relatively stable for one to two days (Table 6).

Table 6. Stability of the ozonized water over time. Reproduced from (Zhang et al. 2020).

	Time (h)				
Starting concentration (mg/L)	24	48	72	96	120
5	3	1.16	0.56	0.27	0
10	4.9	1.66	0.52	0.34	0

8. Efficacy of truck-washing protocols in managing infectious disease risk

8.1 ASF

No reports were found for which real life trials described the attempted disinfection/decontamination of trucks or trailers contaminated with ASF virus. The sensitivity of ASF virus to various disinfectants has been described previously in this report.

Several reports by the same authors have assessed the efficacy of several disinfectants against ASF virus on surfaces found in abattoirs, porous material (likely to be used as bedding in trucks) and hard surfaces (likely to be material used to build trucks) (Krug *et al.* 2011; Krug *et al.* 2012; Krug *et al.* 2018).

Wood shavings, sawdust or chips may be used as bedding when transporting pigs but little work has been reported to understand their particular risk with regards to ASF virus (Anonymous 2020). As there is no standardized method for porous surface disinfection; commercial disinfectants are only certified for use on hard, nonporous surfaces (Krug *et al.* 2012). To model porous surface disinfection in the laboratory, FMD and ASF virus stocks were dried on wood coupons and exposed to citric acid or sodium hypochlorite. It was found that 2% citric acid was effective at inactivating both viruses dried on a wood surface by 30 min at 22°C. While 2000 ppm sodium hypochlorite was capable of inactivating ASF virus on wood under these conditions, this chemical did not meet the 4-log disinfection threshold for FMD virus. The data supports the use of chemical disinfectants containing at least 2% citric acid for porous surface disinfection of FMD and ASF viruses.

To model the inactivation of transboundary animal disease (TAD) viruses on fomites, the authors' tested selected chemicals to inactivate foot-and-mouth disease (FMD) virus, ASF virus, and CSF virus dried on steel and plastic surfaces (Krug *et al.* 2011). For each of these viruses, a 2 to 3 log reduction of infectivity due to drying alone was observed. A modified surface disinfection method was applied to determine the efficacy of the selected disinfectants to inactivate surface-dried high-titre stocks of these three structurally different TAD viruses. ASF and FMD viruses were susceptible to sodium hypochlorite (500 and 1000 ppm, respectively) and citric acid (1%) resulting in complete disinfection. Sodium carbonate (4%), while able to reduce FMD virus infectivity by greater than 4-log units, only reduced ASF virus by 3 logs. Citric acid (2%) did not totally inactivate dried CSF virus, suggesting it may not be completely effective for disinfection in the field. Based on these data, the authors (Krug *et al.* 2011) recommended disinfectants be formulated with a minimum of 1000 ppm sodium hypochlorite for ASF or CSF virus disinfection, and a minimum of 1% citric acid for FMD virus disinfection.

To assess the situation within abattoirs, commercial disinfectants used by the food industry were tested and assessed against ASF virus when dried on steel, plastic, and sealed concrete surfaces (all commonly found in abattoirs), in the presence of swine faeces, meat juice, or blood (Krug *et al.* 2018) The commercial disinfectants used in this study included quaternary ammonia with surfactant (800 ppm, pH 1.8), stabilized sodium hypochlorite (600 ppm, pH 10.8), potassium peroxymonosulfate with surfactant (2% w/v, pH 2.2, presumed to be Virkon S), and citric acid (2%). However, disinfectant activity was greatly inhibited in the presence of dried blood and meat juices. As compared to virus dried in PBS, the efficacy of citric acid and sodium hypochlorite was strongly inhibited in the presence of blood. In swine faeces that were dried on stainless steel, citric acid was effective in inactivating ASF virus, but sodium hypochlorite was not. Commercial disinfectants used

by the food industry were generally effective against ASF virus when dried in the absence of swine products on various surfaces. Conversely, when the virus is dried in swine blood and meat juices on steel, disinfection was strongly inhibited, and the disinfectants were unable to completely inactivate ASF virus dried in swine faeces. Taken together, these data reinforce the need to physically remove contaminated swine excretions from surfaces prior to disinfection and to choose effective chemicals to ensure complete virus inactivation.

8.2 Porcine reproductive and respiratory syndrome (PRRS)

The need for effective truck washing to reduce the risk of transfer of PRRS virus has been demonstrated during cold weather conditions (below 0°C). A field strain of PRRS virus was inoculated into mechanical fomites comprised of snow and water, which were then adhered to the undercarriage of a vehicle. The vehicle was driven approximately 50 km to a commercial truck washing facility where the driver's boots contacted the carriers after they were washed off the vehicle, introducing the virus to the vehicle cab. The vehicle was then driven 50 km to a simulated farm site where the driver then entered the 'farm office'; the driver's boots were found to have readily spread the virus into the farm premises (Dee *et al.* 2002). This study demonstrated the risks posed by truck washing and truck washes where care was not taken to prevent contamination of other parts of a truck even though the animal compartment may have been adequately cleaned. By contrast, using the same experimental model in conditions above 0°C using virus contaminated compacted soil attached to the wheel wells of the truck it was found that transfer of PRRS virus was an infrequent event (Dee *et al.* 2003).

To evaluate the effectiveness of various trailer cleaning regimes, four cleaning/disinfecting methods were designed then evaluated using truck scale models that had been artificially contaminated with PRRS virus (Dee *et al.* 2004). Treatment 1 consisted of manual scraping of the interior to remove soiled bedding (wood chips). Treatment 2 consisted of bedding removal, washing (80°C, 20,500 kPa), and disinfection (1:256 phenol; 10-min contact time). Treatment 3 consisted of treatment 2, followed by a freezing and thawing cycle. Treatment 4 consisted of bedding removal, washing, disinfecting, and air drying overnight. Ten replicates were conducted per treatment. Pre-treatment swabs from all trailers tested positive by polymerase chain reaction (PCR) for the presence of PRRS virus. Post-treatment swabs were PCR-positive for all trailers except those that were washed, disinfected, and dried (Treatment 4). Thus, drying appears to be an important component of the truck washing under the prescribed treatment conditions. To further evaluate the efficacy of drying on the inactivation of PRRS virus, the use of forced heating to dry trucks versus overnight drying at environmental temperature was trialled (Dee *et al.* 2005). Scale model trailer interiors were artificially contaminated with 5×10^5 $TCID_{50}$ of PRRS virus strain MN 30-100, then treated with 1 of 4 treatments: 1) Thermo-assisted drying and decontamination (TADD); 2) Air only (no supplemental heat); 3) Overnight (8 h) drying; and 4) Washing only. Following treatment, swabs were collected from the trailer interiors at 0, 10, 20, and 30 min post-treatment and from the overnight group after 8 h. TADD treated trailers were raised to 71°C for 30 min to promote drying and degradation of PRRS virus. All tests for the presence of infectious PRRS virus were negative for trailers treated with TADD and overnight drying.

TADD may be an option to assist in truck decontamination of ASF along with washing and will reduce overall drying times though no experimental work has been done to assess the TADD

conditions that would be required to inactivate ASF virus. Under Australian conditions, washed trucks may not require long periods to dry out but whether simply drying out thoroughly washed trucks will inactivate any ASF virus that has not been killed by an approved disinfectant is unknown.

8.3 Porcine epidemic diarrhoea (PED)

Porcine epidemic diarrhoea virus (PED) virus causes watery diarrhoea, dehydration, and a high mortality rate among suckling pigs and is present in many parts of the world including North America, Europe and China. The role of trucks in the spread of PED in Italy has been reported by (Boniotti *et al.* 2018). In this study in total, 14.1% (154/1091) of the environmental swabs collected from trucks at slaughterhouses after animals were unloaded tested positive for PED virus before the trucks were cleaned and disinfected, and 46% (71/154) remained positive after cleaning and disinfection processes were performed. Moreover, environmental swabs indicated that 17.3% (14/81) of the empty trucks arriving at the farms to load animals were PED virus positive. Not only does this study indicate the risk that trucks pose for non-infected farms but that this risk may arise from the failure to adequately clean and disinfect trucks after delivering pigs to abattoirs and before arriving at farms to load pigs.

A study by (Lowe *et al.* 2014) investigated the role of trucks in the spread of PED in the USA during June 2013. Samples were collected from 575 trailers unloading pigs at six abattoirs in the mid-west of the USA. Sample collection consisted of rubbing a phosphate-buffered saline–moistened pad (Swiffer, Procter & Gamble, Cincinnati, OH, USA) over an ≈900 cm2 area of the trailer floor, 15 cm from the rear door. Before unloading 6.6% (38/575) trailers were contaminated. Of those trailers not contaminated at unloading, 5.2% (28/537) became contaminated during the unloading process. The authors concluded 'This study suggests that collection points, such as harvest facilities and livestock auction markets, can be an efficient source of contamination of transport vehicles that return to pig farms and likely played a role in rapidly disseminating PED virus across vast geographic regions shortly after PEDV was first identified in the United States. These data also suggest that the contamination of transport vehicles leaving the harvest facilities increased as the prevalence of PED virus positive transport vehicles and virus load coming into the facility increased' (Lowe *et al.* 2014).

8.4 Salmonella

As an alternative to virus detection, the use of faecal bacteria surrogates such as Enterobacteriaceae (e.g. Salmonella, E. coli), may be useful to assess the effectiveness of truck washing procedures. Several studies have reported on the effectiveness of truck washing as part of studies into *Salmonella*. For example, (Door *et al.* 2005) reported an increase in the percentage of positive isolations of *Salmonella* and *Campylobacter* from trucks using one particular truck wash in North Carolina which used recycled water with a phenol disinfectant and had a post wash *Salmonella* and *Campylobacter* prevalence of 100% and 100%, respectively. Three other truck washes that were part of the study reduced the level of contamination, but none reduced the prevalence to zero. A similar report by (Mannion *et al.* 2007) from the Republic of Ireland where truck washing was mandatory before leaving an abattoir indicated not all trucks were free of *Salmonella* post washing.

9. Truck-washing regulations and methods

9.1 European Union

In accordance with the European Regulation (EU) No 853/2004, abattoirs must have "a separate place with suitable facilities for the cleaning, washing and disinfection of transport equipment for animals". Even though facilities must be provided as per this regulation, a study by (Weber and Meemken 2018) found that at two of five abattoirs in Germany individual vehicles left without any washing, and in 31 to 97% of all 750 vehicles examined only cleaning of the vehicle was carried out, and a subsequent disinfection did not take place. A cleaning followed by disinfecting took place in only 3 to 59% of all vehicles. Numerous excuses were offered by the vehicle owners as to why a full wash and disinfection was not carried out.

For movement of pigs within the EU truck washing must be carried out in accordance with Article 12, Section 1, sub-section (a), ii) Council Directive 64/432/EEC. The vehicles must be constructed in a way to prevent spillage or leakage of feed, litter or faeces. Trucks must be cleaned and disinfected immediately after every transport of animals or of any product which could affect animal health, and if necessary, before any new loading of animals, using disinfectants officially authorized by the competent authority.

According to pork industry information,[2] Denmark has since 2002 required that all trucks entering Denmark to pick up pigs or cattle has to go through one of three industry-approved truck wash facilities, where they will be inspected on the inside, washed on the outside, and disinfected inside and outside. Trucks that do not pass the inspection, are sent back across the border. Until 2010, this was a Danish government regulation but since 2010, the process has been managed as an industry requirement.

At arrival to the truck wash facility, GPS-data from the truck are analysed, and if the truck has been in an ASF-affected area, the truck is quarantined for 7 days after leaving the truck wash, and is only allowed to pick up pigs from an officially approved collection point in that period. If the truck has been in an area close to an affected area, there is a 48 hours quarantine.

Based on the certificate, a physical and electronic certificate is issued. On arrival to a farm, the pig producer can obtain the certificate electronically from an app or ask the driver for the physical certificate.

In Denmark approved disinfectants include Vanodox® (peracetic acid-based disinfectant) and Virkon. Washdown of vehicles must use an alkaline detergent and potable water. Water with added disinfectant must be stored at 20-30°C.

[2] Dahl, J. (2020) Danish Agriculture & Food Council (Landbrug & Fodevarer F.m.b.A.), personal communication.

9.2 Australia

AUSVETPLAN

Australia's response to exotic animal disease incursions are outlined in AUSVETPLAN[3] and procedures for cleaning and disinfection of livestock vehicles (and other items) are described in the Operational Procedures Manual for Decontamination (Anonymous 2008). The section of the document related to livestock vehicles is included below:

4.4.2 Livestock vehicles

In addition to trucks and semitrailers used to haul production stock, livestock vehicles include horse boxes, vehicles used to carry stud and show stock, and racing pigeon carriers. For any vehicle known to have carried stock susceptible to the EAD agent, the principles of vehicle and trailer decontamination are the same. All solid debris, faecal matter and bedding must be removed. All water, feedstuff and litter carried in the vehicle must be disinfected and burned or buried. The vehicle should then be soaked in disinfectant using a detergent and scrubbed down to bare metal or wood. All fixtures and fittings must be dismantled to ensure that infected material has been removed. All surfaces must be cleaned down to metal and then disinfected. Wooden surfaces must be cleaned and disinfected, where appropriate, or valued before removal and destruction. The wheels, wheel arches, bodywork and undercarriage must be cleaned of detritus and disinfected. The driver's cabin and sleeping compartment, if fitted, also need to be cleaned and disinfected. When the crate structure of a trailer has been decontaminated, the stock crate should be lifted free from the body. The underside of the stock crate and the parts of the trailer on which it rests should be decontaminated. The vehicle must be closely inspected to determine if there is a double layer. If this is so, the top layer of metal tread plate or wood must be removed to reach areas where contaminated material could be trapped. Any metal flooring that appears solid must be weight tested to ensure that welds are not cracked and that there is no rubbish under the flooring. Some trailers may carry extra equipment under the body; if so, this must be treated.

The outside dual wheels and spare wheels must be removed to ensure adequate decontamination of the wheel hubs and to allow inspection of the spare wheel hangers, which can be hollow and therefore could hold contaminated material. The driver should be asked to identify the clothing and boots they were wearing when in contact with suspect stock. Those articles must be decontaminated, and arrangements made for dry cleaning, where applicable (see Section 4.2.2). It is common practice for specialised vehicles to be self-contained with water, food and litter supplies for the animals. If the vehicle is known to have carried diseased or suspect stock, and such materials were removed before departmental officers identified the vehicle as being contaminated, every effort should be made to locate the discarded material. Once identified, the material must be disinfected and disposed of by burial or burning.

[3] AUSVETPLAN Manuals and Documents. Available from https://www.animalhealthaustralia.com.au/our-publications/ausvetplan-manuals-and-documents/, accessed July 29, 2020.

Australian Pork Industry Quality Assurance Program (APIQ)

The pork industry in Australia also provides guidance to livestock haulers and farmers that help to support compliance with the Australian Pork Industry Quality Assurance Program (APIQ). The APIQ Transport Standards and Performance Indicators describes driver behaviour and the requirement for vehicle cleanliness as follows (Anonymous 2019b).

Section 7.2 Drivers, Vehicles, & Facilities Standard

Drivers and vehicles used to carry pigs follow the farm's Biosecurity Standards (as per the on-farm Biosecurity Plan). Facilities promote effective and safe handling of pigs when loading or unloading.

Performance Indicators:

1) *Drivers and other transport personnel do not enter designated "clean areas".*
2) *Vehicles are cleaned between consignments.* (Note no SOPs provided)
3) *Handling, assembly, loading and/or unloading of pigs is conducted with care and in a manner that minimises stress to pigs.*
4) *Loading facilities, unloading facilities and farm roads are designed and maintained to facilitate safe loading and delivery of pigs and safety for operators.*

While there is a requirement for vehicles to be cleaned between consignments within the APIQ standard, there is no guidance on how this should be carried out.

All producers supplying export abattoirs are required to be APIQ certified, thus in theory all trucks will have been washed between consignments. However, no washing standards appear to have been mandated in APIQ.

Information about the availability and quality of livestock truck washes in Australia is not readily available. However, two limited reviews have been recently conducted. First in 2016, consultants working for the Tasmanian government undertook a strategic review of truck wash facilities which relied primarily on interviews with haulers, government officials, farmers, and allied industries such as abattoirs (Murphy *et al.* 2016). Though the review was limited to Tasmania (which has relatively few commercial pig farms), the authors reported key findings which they believed were also likely to apply to other parts of the country: Stakeholders believed that clean trucks were an industry responsibility and that transporters themselves (not just their clients) have an overall obligation to assist in controlling the spread of disease through livestock transport; that management and containment of in-transport effluent was a consistent problem; that there was unmet demand for suitable, publicly-accessible livestock truck washdown infrastructure; and that improved truck washdown infrastructure would be likely to deliver additional benefits (aside from biosecurity) including improved workplace health and safety. The authors also noted the existence of the National Truckwash System which was established in 1993 to provide users with visibility around the location of commercial truck wash facilities in Australia, including indicative user costs for accessing

the truck washes. As of August 21, 2020, there were 125 truck washes listed on the website;[4] the completeness of the data on this system in unknown.

A second review of truck washing capacity was completed in 2019 focussing on facilities available at four major pork abattoirs and one saleyard, all in South Australia (LLoyd and Dunstan 2019). The authors noted several challenges found at most of the facilities that had the potential to compromise biosecurity namely, an absence of high-pressure washing equipment, uncoordinated foot and vehicle traffic patterns that contributed to cross-contamination between trucks, no equipment to clean the undercarriage of trucks or trailers, and limited attention given to drainage and effluent capture on the sites. The authors felt a combination of driver and abattoir staff training as well as increased capital investment in the truck washing facilities themselves were required to bring the truck washing capacity at these facilities to an acceptable level of biosecurity.

A list of disinfectants approved for use with ASF virus in Australia is included as (Appendix 6). Some States such as New South Wales have published additional guidance for ASF virus decontamination.[5,6]

[4] AVDATA National Truckwash System. https://avdata.com.au/truckwashes/#Truckwashes-using-our-system

[5] Primefact 1710 'African swine fever (ASF) investigation (March 2020)'. Available at https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/1193250/asf-investigation.pdf, accessed August 19, 2020.

[6] Guide to decontamination of vehicles and equipment v2 (August 27, 2018). Available at https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0010/545554/procedure-decontamination-vehicles-and-equipment.pdf, accessed August 19, 2020.

10. Cost and benefits of implementing a biosecurity programme for ASF

Little information is available to estimate the financial benefit of implementing a biosecurity programme for ASF. However, in endemically infected Nigeria, investigators used a 122-sow piggery unit small-holder herd to develop a financial model for estimating the economic benefits of effective biosecurity against African swine fever (Fasina *et al.* 2012). Though the 122-sow model was substantially different than a typical Australian herd, per pig costs and revenues approximated Australian commercial conditions; the model farm generated a profit of approximately US$109,637.40 per annum (or a profit of $38.75 per weaned pig). The implementation of a biosecurity plan was calculated to give a benefit: cost ratio of 29. In this model, full implementation of a biosecurity plan would result in a 9.70% reduction in total annual profit but was thought to be justified in view of the substantial costs incurred in the event of an ASF outbreak. Biosecurity efforts were focussed on exclusion (buildings and fencing), clothing and boot changes, restricted entry of people and vehicles, effective cleaning and disinfection, etc.

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Appendix 1. European Union regulations on control of ASF

Council Directive 2002/60/EC of 27 June 2002 laying down specific provisions for the control of African swine fever and amending Directive 92/119/EEC as regards Teschen disease and African swine fever

Link: http://data.europa.eu/eli/dir/2002/60/2008-09-03

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ANNEX II

Principles and procedures for cleansing, disinfection and treatment with insecticides

1. General principles and procedures:

   (a) the cleansing and disinfection operations and, where necessary, the measures to destroy rodents and insects using officially authorised products must be carried out under official supervision and in accordance with the instructions given by the official veterinarian;

   (b) the disinfectants to be used and their concentrations must be officially approved by the competent authority to ensure destruction of African swine fever virus;

   (c) the efficacy of disinfectants must be regularly checked before use, as the efficacity of certain disinfectants is diminished by prolonged storage;

   (d) the choice of disinfectants, insecticides and of procedures for disinfection and disinsectisation must be made taking into account the nature of the premises, vehicles and objects which are to be treated;

   (e) the conditions under which degreasing agents, disinfectants and insecticides are used must ensure that their efficacy is not impaired. In particular technical parameters indicated by the manufacturer, such as pressure, minimum temperature and required contact time must be observed;

   (f) irrespective of the disinfectant used, the following general rules should be applied:

      — thorough soaking of bedding and litter as well as faecal matter with the disinfectant,

      — washing and cleansing by careful brushing and scrubbing of the ground, floor, ramps and walls, if possible after the removal or dismantling of equipment or installations so as not to impair the effective cleansing and disinfection procedures,

      — then, further application of disinfectant for a minimum contact time as stipulated in the manufacturer's recommendations,

      — the water used for cleaning operations must be disposed of in such a way as to avoid any risk of spreading the virus, in accordance with the instructions of the official veterinarian;

   (g) where washing is carried out with liquids applied under pressure, re-contamination of the previously cleansed parts must be avoided;

   (h) washing, disinfecting or destroying of equipment, installations, articles or compartments likely to be contaminated must be included;

   (i) following the disinfection procedures, re-contamination must be avoided;

   (j) cleansing, disinfection and disinsectisation required in the framework of this Directive must be documented in the holding or vehicle register and where official approval is required, be certified by the supervising official veterinarian.

2. Special provisions on the cleansing and disinfection of infected holdings:

   (a) preliminary cleansing and disinfection:

      — during the killing of the animals, all necessary measures must be taken to avoid or minimise the spread of African swine fever virus. Those measures include, *inter alia*, the installation of temporary disinfection equipment, supply of protective clothing, showers, decontamination of used equipment, instruments and facilities and the interruption of power supply to the ventilation,

      — carcases of killed animals must be sprayed with disinfectant,

      — if the carcases have to be removed from the holding for processing, covered and leak proof containers must be used,

      — as soon as the carcases of the pigs have been removed for processing, those parts of the holding in which the animals were housed and any parts of other buildings, yards etc., contaminated during killing, or post-mortem examination must be sprayed with disinfectants approved in accordance with Article 12,

▼B

— any tissue or blood spilled during slaughter or post-mortem or gross contamination of buildings, yards, utensils etc. must be carefully collected and processed with the carcases,

— the disinfectant must remain on the surface for at least 24 hours;

(b) final cleansing and disinfection:

— manure and used bedding must be removed and treated as provided in point 3(a),

— grease and dirt must be removed from all surfaces by the application of a degreasing agent and the surfaces washed with water,

— after washing with cold water, further spraying with disinfectant must be applied,

— after seven days the premises must be treated with a degreasing agent, rinsed with water, sprayed with disinfectant and rinsed again with water.

3. Disinfection of contaminated bedding, manure and slurry:

(a) manure and used bedding must be stacked to heat, sprayed with disinfectant and left for at least 42 days or destroyed by burning or burying;

(b) slurry must be stored for at least 60 days after the last addition of infective material, unless the competent authorities authorise a reduced storage period for slurry which has been effectively treated in accordance with the instructions given by the official veterinarian so as to ensure the destruction of the virus.

4. However, by way of derogation from points 1 and 2, in the case of open-air holdings the competent authority may establish specific procedures for cleansing and disinfection, taking into account the type of holding and the climatic conditions.

Appendix 2. Framework for development of a cleaning and disinfection programme for ASF virus

De Lorenzi, G., Borella, L., Alborali, G. L., Prodanov-Radulović, J., Štukelj, M., & Bellini, S. (2020). African swine fever: A review of cleaning and disinfection procedures in commercial pig holdings. Research in Veterinary Science, 132, 262-267. doi:10.1016/j.rvsc.2020.06.00

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African swine fever: A review of cleaning and disinfection procedures in commercial pig holdings

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ABSTRACT

African swine fever (ASF) is one of the most important diseases in pigs. Since there are no effective vaccines against the virus, farm biosecurity and good farming practices are the only effective tools to prevent the spread of the ASF virus (ASFV) in pig holdings. Hence, an important component of farm biosecurity is the Cleaning and Disinfection (C&D) procedure.

Precise indications regarding the ideal disinfectant against ASFV are lacking, but every country has approved and/or authorized a list of biocides effective against ASFV. Lipidic solvents, which destroy the envelope of the virus and commercial disinfectants based on iodine and phenolic compounds are effective in inactivating the ASFV. This review describes the C&D protocol to apply in pig holdings with particular reference to ASFV.

1. Introduction

The recent expansion of African swine fever (ASF) epidemic throughout several regions of the world has placed the majority of the world's swine population under threat (Sánchez-Vizcaíno et al., 2019).

ASFV is a complex large enveloped virus with icosahedral morphology that contains a linear double-stranded DNA genome. It is currently classified as the only member of the *Asfaviridae* family, genus *Asfivirus* (Dixon et al., 2004).

ASFV is able to survive for long periods in a protein rich environment and remains stable at pH 4–10 (Geering et al., 2001). The extreme environmental resistance of the ASFV can play a significant role in local persistence and geographical spread of the virus (Juszkiewicz et al., 2019).

Susceptible suids can be infected by direct or indirect contact with infectious animals or their fluids, ingestion of contaminated animal feed, pork or pig products, or contact with contaminated surfaces or fomites acting as mechanical vectors of the disease (Guinat et al., 2016). Fomites, such as contaminated clothing and footwear, farming tools, equipment and vehicles has been widely reported in the spread of ASFV (Kleiboeker, 2008). In the southern and eastern parts of Africa and in the Iberian Peninsula, bites of infected soft ticks belonging to *Ornithodoros* genus were another source of ASF infection (Sánchez-Vizcaíno et al., 2019).

One of the major challenges for ASF control is the absence of available vaccines. Therefore, farm biosecurity and good farming practices are the only effective tools for preventing ASF introduction to pig holdings (Guinat et al., 2016).

Upon confirmation of ASF outbreak its eradication depends on the application of a combination of measures aimed at eliminating the source of pathogen, through: 1) the killing or slaughter of animals infected or suspected of being infected and safe disposal of dead animals and potentially contaminated products; 2) the cleaning, disinfection and, if relevant, disinfestation of premises, vehicles and equipment. The main piece of legislation providing the indications for the control of ASF in the EU is the Council Directive 2002/60/EC (EC, 2002), which establishes the minimum measures to apply for the control of ASF, including the principles for cleaning and disinfection.

Considering ASFV's characteristics of resistance, the current epidemiological situation and the relevance of implementing effective C&D to control the spread of ASFV, a database research on C&D was performed with the aim of identifying the effective procedures to be applied in commercial pig holdings. A database research was carried out between the 9th and the 19th of September 2019 using PubMed database and was supplemented with further search between the 26th of March and 6th of April 2020 using CAB Abstracts database. All the scientific papers reviewed were written in English, in the period from 1973 to 2019. The key words used were "Disinfection", "Virus", "Pig

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Farms". Based on these test searches, the search terms applied were: "Disinfection" AND "Virus" AND "Pig Farms".

A two-step process was applied to select the references for the aim of this review. The first exclusion criteria was carried out reading the title and the abstract (when available) of the papers found. In the first step of the process, duplicates and papers which abstracts and full text were not available were removed, as well as the references not related to the themes of this review. Then, the full text of each selected piece of literature was screened. As a second exclusion criteria, articles that were not concerning viral diseases, pig diseases and papers in which the information provided about the themes "Disinfection", "Virus" and "Pig Farms" were not sufficient, were discarded.

The search performed on PubMed generated 46 articles, while the search performed on CAB Abstracts generated 27 papers, obtaining 73 papers in total. After applying the primary exclusion criteria, 60 papers were identified. After applying the secondary exclusion criteria, 12 articles were identified for the review, among which 5 included the search terms "African swine fever" in the title and/or in the abstract. To make sure that other relevant documents such as technical guidelines, scientific opinions and conference proceedings were included, the literature search was performed following the same query on the internet using a common browser.

This search provided several papers about biosecurity in veterinary medicine, with particular reference to cleaning and disinfection of farms and vehicles. In particular, 7 technical guidelines, 5 books and one conference proceedings published in English were consulted for review. Moreover, one thesis and 4 technical guidelines published in Italian have been included as a source of literature. At the end of the reviewing process, 37 papers were identified for this review.

2. Cleaning and disinfection (C&D) protocol

C&D procedures are fundamental for pathogen inactivation, to prevent the spread of the disease and to facilitate the repopulation after an outbreak (Ford, 1995). Cleaning represents one of the most important steps in the C&D process. It removes over 90% of microorganisms when properly performed and improves the disinfection efficacy (FAD PReP, 2014). The C&D protocol described below follows a general scheme to apply in a systematic manner (FAD PReP, 2018) also within the framework of an ASF control program. EU Council Directive 2002/60/EC (EC, 2002) establishes that in case of ASF, the destruction of carcasses shall be followed by the thorough C&D of all premises, vehicles and equipment. These operations shall be conducted under the supervision of the Veterinary Authority and C&D must be documented in the holding or vehicle register and where official approval is required, be certified by the supervising official veterinarian. Adequate safety measures must be taken during the implementation of the disinfection processes and all team members equipped with appropriate protective clothing, boots, hats, visors, gloves and respirators (FAD PreP, 2014).

2.1. Cleaning

Cleaning protocol comprises the following phases:

2.1.1. Dry cleaning

The removal of all the residual organic material (food, feces, litter, dust) from production areas or equipment is an essential first step in the C&D process (CERVES, 2004). Mobile equipment (such as feeders and water troughs) must be disassembled, transferred outside the premises, cleaned and washed separately. Permanently attached equipment (e.g., fan blades, light fixtures, louvers, electrical panels) must be cleaned on site (Alborali, 2009; OIE, 1995). Pressure aerators are not recommended during dry cleaning, as they increase the risk of spreading microorganisms in the environment (FAD PReP, 2014).

2.1.2. Pre-soaking

Pre-soaking for at least two hours is suggested to facilitate the subsequent cleaning process. Pre-soaking prevents the raising of dust from the surfaces during washing, limiting the environmental spread of pathogens (FAD PReP, 2014). For this reason, pressure washers with a high water flow but low pressures are recommended (OIE, 1995).

2.1.3. Wet cleaning

Following the removal of gross contamination, areas or items shall be washed with detergent by foaming, if possible, and leave the detergent for the recommended period. The washing process and detergents helps to further reduce the number of microorganisms as well as removing any oil, grease, or exudates that may inhibit the action of disinfection (CERVES, 2004).

Washing requires warm water (32–54 °C) and high volumes of water and adequate working pressures according to the type of surface and the amount of dirt to remove. Usually high-pressure washers are used with a flow rate of at least 20 l per minute and pressures between 130 and 200 bar (Dal Capello, 2010–2011). The complete removal of the dirt residues can be obtained by rubbing the irregular surfaces with metal brushes (FAD PReP, 2014). Special attention should be given to dislodge accumulated grime in deep cracks, crevices, pits, pores, or other surface irregularities (CERVES, 2004; FAD PReP, 2014).

Detergents remove soil and organic materials reducing surface tension and increasing the penetrating ability of water. They improve disinfectant's ability to reach and destroy microorganisms within or beneath the organic material. Some disinfectants (i.e., quaternary ammonium compounds) have also detergent properties (OIE, 1995).

The detergent can be previously mixed with water or it can be directly distributed on surfaces and equipment, according to the instructions on the label. The application of foam is generally more effective. It is necessary to allow sufficient contact time for the detergent to be effective (20–60 min) and during this time surfaces should be monitored to make sure they do not dry off. If surfaces are drying, they should be sprayed with detergents again (Alborali, 2009).

The cleaning solution can contain different components as water, alkaline or acids detergents. The main alkaline compounds used for their cleaning properties are sodium hydroxide (caustic soda), potassium hydroxide and sodium carbonate (washing soda) (OIE, 1995). They are particularly effective against organic protein material, but have the disadvantage of precipitating hard water ions, forming foam with soaps; they are corrosive and difficult to rinse (Holah, 1995; OIE, 1995; Verghese, 1998).

Some detergents are effective against ASFV, among these sodium hydroxide (caustic soda) which is corrosive and must only be used on resistant materials (e. g. walls); users should be aware that it will remove paint (CEREP, 2004; OIE, 1995). Aluminum surfaces should be protected from contact with caustic soda (OIE, 1995). Caustic soda can be sprinkled on carcasses of dead animals and used for the treatment of slurry (CEREP, 2004). Potassium hydroxide is effective against ASFV but is very corrosive (CEREP, 2004; OIE, 1995). Sodium carbonate is characterized by a low microbicide activity, so it is used in formulations with other detergents to increase its grease-removing properties, enhance penetration capacity and raise the pH (OIE, 1995). In case of ASF outbreak, it can be used on farm equipment and stables (CEREP, 2004).

Acids detergents have minor detergency properties than alkaline detergents, but they are very useful in solubilizing carbonate, mineral scales (including hard water salts) and proteinaceous deposits (Holah, 1995).

The choice of the most suitable detergent agent should take into account several factors as the type of surface (e.g material composition, porosity) (Table 1), the characteristics of the cleaning solution (water concentration, water hardness and temperature), nature and solubility of the organic material to be removed, the efficacy and practicability under farm conditions and compatibility with the disinfectant selected (Alborali, 2009; Holah, 1995; Marriott et al., 2018; Verghese, 1998).

Table 1
Characteristics of the materials and detergents recommended (Marriott et al., 2018 modified).

Material	Characteristics	Recommended detergents
Ferrous metal	Easily damaged by rust due to the use of acid and alkaline chlorine detergents	Neutral detergents
Aluminum	Damaged by acid and strongly alkaline detergents	Neutral or weakly alkaline detergents
Concrete	Damaged by acid detergents	Alkaline detergents
Glass	Damaged by strongly alkaline detergents	Neutral or weakly alkaline detergents
Paints and resins	Damaged by alkaline detergents	Acid detergents
Rubber	Damaged by strong acids. It must not be porous or spongy	Alkaline detergents
Stainless steel	Smooth, non-porous, easy to clean, resistant to corrosion, high temperature oxidation	Indifferently acidic or alkaline detergents

Table 2
Detergent/disinfectants not to be used in combination (Holah, 1992; Turner et al., 1999).

Disinfectant	Detergent	Cause
Quaternary ammonium compounds (QACs)	Alkalis	Alkaline detergents may react chemically with QACs and destroy their antimicrobial properties
Hypochlorite	Acids detergents	If these compounds are mixed, the resultant reaction releases toxic chlorine gas
Phenols	Soaps based on tallow, tall oil or oleic acids	These detergents are able to markedly decrease the activity of phenol compounds
Chlorexidine	Alkalis	Alkaline detergents may interfere with the disinfectant action of chlorexidine

2.1.4. Rinsing and drying

After washing, all surfaces should be rinsed in order to remove detergents and all traces of material used in the cleaning process because residues can neutralize or inactivate some chemical disinfectants (Table 2). In this procedure high volume of cold water at low pressure should be used. Surfaces should dry completely before the application of the disinfectant. The excess of humidity in fact, especially on porous surfaces, can dilute and decrease disinfectant efficacy (OIE, 1995).

2.2. Disinfection

In the areas affected or at risk of introducing ASF the disinfectants to choose for the control of the disease must be effective against ASFV and approved by the official veterinarian. In case of ASF outbreak the procedures to follow are described in CD 2002/60/EC.

2.2.1. Application

According to Van Immerseel et al. (2018) two main ways of disinfectant application can be distinguished: wet (surface) and dry disinfection. Surface disinfection is often carried out with high pressure. In the case of dry disinfection (thermal fogging), a highly concentrated disinfectant is heated and subsequently converted to fog by a fogger. Disinfection applied in a fumigation stage may ensure the elimination of the pathogens in difficult-to-reach zones and can be performed where it is possible to seal the building completely. Calculating the right amount of solution needed to disinfect the animal premises is fundamental (Van Immerseel et al., 2018). The amount of disinfectant needed to disinfect a polished and non-porous floor is 100 ml/m^3 (FAO, 2001).

2.2.2. Contact time

Adequate contact time must be allowed for the process to be effective and it can vary according to the surface to be treated (FAD PReP, 2014; FAD PReP, 2018). In some cases, the disinfectant may need to be applied again to keep the surface wet for the required contact time and it must remain on the surfaces for at least the time indicated in the instructions (OIE, 1995). In case of ASF, disinfectants must remain on the surfaces for at least 24 h (EC, 2002).

2.2.3. Drying

Cleaned and disinfected premises should also have a period of down time following the disinfection procedures. Premises should remain empty after drying of the disinfectant in order to avoid the accidental absorption of residues by the animals (FAD PReP, 2014). In case of ASF outbreak this period is 40 days (EC, 2002).

3. Disinfectants effective against ASFV

The choice of the disinfectant must take into consideration different aspects as the type of surfaces, the spectrum of activity, the efficacy and practicability under farm conditions (e.g. ease of handling, risk of corrosion of equipment, temperature stability), safety for operative staff, animals and the environment, costs, risk to store, etc. (FAO, 2010; Missouri Department of Agriculture, 2008). The preparation of disinfectant solutions must be performed by qualified operators strictly following the manufacturer's instructions (concentration, contact time, pH, temperature) (FAO, 2010). It is important to keep solutions clean and freshly made. Mixing disinfectants is inadvisable, as the potency of each may be nullified or a dangerous reaction may be caused, releasing heat or dangerous gases (CEREP, 2004).

There are no indications in literature regarding the ideal disinfectant against ASFV, but every country has approved and/or authorized a list of biocides effective against ASFV and thus only authorized biocides should be used and applied according to the producer's instructions (Juszkiewicz et al., 2019). However, on the OIE website effective disinfectants against ASFV are reported (OIE, 2019). General knowledge and experience in the use of disinfectants against enveloped viruses (CEREP, 2019; Gallina and Scagliarini, 2010; Krug et al., 2012; Krug et al., 2011; OIE, 2019; Shirai et al., 2000; Stone and Hess, 1973; Turner and Burton, 1997) have shown that the chemical compounds effective in inactivation of ASFV are:

- Formaldehyde 1%,
- Sodium hypochlorite (0.03% to 0.0075%),
- Caustic soda solution 2%,
- Glutaraldehyde, formic,
- Sodium or Calcium hydroxide 1% (effective at virus inactivation in slurry at 4 °C),
- Phenols – lysol, lysephoform, and creolin,
- Chemical compounds based on lipid solvents,
- Multi-constituent compounds – Sodium chloride, Potassium peroxymonosulfate, ysoformin, Desoform, Octyldodeceth-20 (OD-20) surfactants, active substances, organic acids, glycosal, etc.

The use of some effective disinfectants against ASFV is limited due to their toxicity or safety (e.g. formaldehyde). In practice, only some of the chemical compounds mentioned above are contained in commercial

Table 3
Disinfectants effective against ASFV (Aphis-Usda, 2011 modified; Geering et al., 2001).

Active ingredient(s)	Contact time	Application(s)
Sodium chloride Potassium peroxymonosulfate	10 min	In/on animal feeding and watering equipment, livestock barns/pens/stalls/stables, livestock equipment, hog farrowing pen premises, hog barns/houses/pens, animal quarters, animal transportation vehicles, agricultural premises/equipment, human footwear
Sodium dichloro-*s*-triazinetrione	30 min	In/on animal living quarters, animal feeding/watering equipment, animal equipment, transportation vehicles, farm premises, shoe baths.
Sodium hypochlorite	15 min nonporous 30 min porous	Indoor or outdoor use sites such as agricultural, transportation, quarantine, and laboratory equipment and facilities; footwear/personal protective equipment.
Citric acid	15 min nonporous 30 min porous	Indoor or outdoor use sites such as agricultural and non-agricultural equipment and facilities; laboratory equipment and facilities; footwear/personal protective equipment, personnel decontamination.

disinfectants. The disinfectants recommended in the USA by The Environmental Protection Agency (EPA) are shown in Table 3. Currently there are commercial disinfectants based on phenolic and iodine compounds which are effective against the virus and can inactivate the ASFV at pH < 4 and > 11 (Gallardo et al., 2015; Geering et al., 2001).

4. Procedures for C&D

4.1. Buildings

C&D of premises must be carried out using the "all in/all out" (AI / AO) system between each batch, followed by ten-day resting period between batches to maintain low infection rates (Alborali, 2009; OIE, 1995). The AI / AO principle is essential for an adequate cleaning and disinfection procedure between consecutive cycles (Dewulf et al., 2018).

It is important to include the whole stable during the C&D process, including the ceiling, walls, floor, pipelines, feeding troughs, drinking nipples and other equipment, to minimise the potential contamination of previously cleaned areas (Van Immerseel et al., 2018). After C&D measures in farrowing pens, it is necessary to clean and disinfect without leaving residual chemicals cause the building will house parturient and neonatal animals. Phenolic disinfectants should be avoided as they can be toxic to swine (FAD PReP, 2014). According to Aphis-Usda (2011) disinfectants effective against ASFV may include single active ingredient (sodium dichloro-*s*-triazinetrione) and multi-constituent compounds like combination of sodium chloride and potassium peroxymonosulfate (Table 3). According to CEREP (2004) sodium carbonate can be used for stables disinfection.

4.2. Vehicles

All vehicles (e.g., cars, livestock carriers, feed trucks, milk trucks, and carcass transporters) and heavy machinery (e.g., excavators, backhoes and bulldozers) that have been used on infected premises must undergo proper C&D processes because they can potentially transport pathogens from one site to another (Missouri Department of Agriculture, 2008). The cargo space of animal transport vehicles specifically need to undergo comprehensive C&D between animal loads (FAD PReP, 2018).

A C&D station should be established and vehicle should follow the C &D protocol described previously. During dry cleaning procedures, soiled bedding and refuse have to be removed and placed in a proper area to avoid the re-contamination of the cleaned vehicles (FAO, 2010).

Shovels, manure forks, brushes, low-pressure sprayers, or mechanical scrapers can be used to remove all visible organic material from the exterior of the vehicle. During the washing procedures, detergent and warm water (32–54 °C) have to be used to wash the vehicle. Wheels and wheel wells can be a particular fomite that requires detailed attention to ensure proper C&D. Any deposits of mud and straw should be removed from the exterior of the vehicle (FAD PReP, 2018).

Items with debris that is difficult to remove should be pre-soaked. Vehicles should be rinsed with cold water, but if it is not possible, the vehicle should sit for 5–10 min to allow the residual rinse water to drip off (FAD PReP, 2018). Cold weather is a significant constraint to vehicle disinfection, cold temperatures in fact can preserve pathogens, freeze the water and make drying difficult. Thus, in cold climates, an indoor washing facility is essential. Additionally, forced air fans and heaters can facilitate drying process (FAO, 2010). If ambient temperature is below freezing, either heat the surfaces to prevent freezing, heat blankets around liquid containers should be used, or 40% propylene glycol in water could be added when mixing solutions (FAD PReP, 2018).

The disinfection procedure must be performed only on dried surfaces and following a precise order: the application of the disinfectant starts at the top of the vehicle and moves downward from the outside to the inside, paying particular attention to the wheels and the underlying parts of the vehicle (CERVES, 2004).

All external areas should be sprayed and the chassis of the vehicle should be disinfected with a non-corrosive disinfectant (Table 4). Then, after a proper contact time, vehicles should be rinsed and dried thoroughly. Interior C&D of the vehicle is necessary if the driver leaves the cab (FAD PReP, 2018). All non-fixed items should be removed from the vehicle to be cleaned and disinfected (FAD PReP, 2014).

4.3. Equipment

All the equipment that will come in contact with pigs must be purchased new and without any previous contact with other holdings (Carr et al., 2018). In case of ASF, items in contact with infected animals that can be difficult to clean and disinfect should be discarded. If surfaces and ambient temperature are below freezing, the same techniques used with vehicles to prevent freezing should be used for the equipment (FAD PReP, 2018).

A C&D station for small equipment should be established possibly in proximity to a water supply and drainage. The most practical method of decontaminating electronic equipment (e.g., generators, motors) involves placing the equipment inside an airtight enclosure (e.g., plastic sheeting) for fumigation. When possible, equipment should be dismantled so all parts can be fumigated (FAD PReP, 2018). Some electrical items may be inherently airtight, in which case they can be safely disinfected by wiping down with disinfectant. Exposure to ultraviolet

Table 4
List of disinfectants incompatible with metal surfaces (FAD PReP/NAHEMS, 2014).

Chemical Disinfectant	Effect on metal surfaces
Sodium hydroxide	Corrosive to aluminum and derived alloys and galvanized metal
Sodium carbonate	Corrosive to aluminum and derived alloys
Acids	Highly corrosive to metals
Glutaraldehyde, Virkon® S	Mildly corrosive to metals
Iodophors, hypochlorites, formaldehyde	Corrosive to some metals
Phenolics	Relatively non-corrosive

light can be another option for disinfecting complex equipment (FAD PReP, 2014; OIE, 1995). Equipment used to euthanize livestock (e.g., captive bolt guns and firearms) should be considered grossly contaminated. After use, they need to be scrubbed with disinfectant at the location where they were used and again at the disinfection station. C&D equipment (e.g., rakes, shovels, brushes, sprayers) must be cleaned and disinfected after use and stored in a secure location. Special care should be used when cleaning and disinfecting rubber equipment because many disinfectants are corrosive to rubber. For this reason these tools should be destroyed (CERVES, 2004; FAD PReP, 2014). The USA EPA-approved list of disinfectans for ASF includes several active ingredient(s) for farm equipment like sodium chloride and potassium peroxymonosulfate, sodium dichloro-striazinetrione and sodium hypochlorite (Aphis-Usda, 2011).

4.4. Personnel

Personnel engaged in C&D procedures should wear at the least a minimum coveralls, boots and gloves (Ford, 1995). Face protection and a mask should be worn based on the product or application method used and when mixing disinfectant solutions. Personal protective equipment, such as chemical-resistant suits (including both pants and jackets with hoods) or respirators may be necessary for some situations (e.g., formaldehyde or acidic disinfectants) (FAD PReP, 2014). The portable electronic equipment (e.g., hand-held radios, cameras) should be used while protected inside plastic bags. Personnel must practice proper personal decontamination and doffing procedures, before leaving an infected premise or any quarantined area, to prevent the spread of pathogens (FAD PReP, 2018). For personnel body decontamination, citric acid 0.2% (*w/v*) as safe and active ingredient effective for ASFV is recommended (Aphis-Usda, 2011; Geering et al., 2001).

4.5. Manure management

ASFV may be transmitted via faecal material therefore, issues involving manure collection systems (e.g. slurry pits, lagoons) must be considered (Davies et al., 2015). In case of ASF occurrence, manure and used bedding must be stacked to heat, sprayed with disinfectant and left for at least 42 days or destroyed by burning or burying. Whereas, slurry must be stored for at least 60 days after the last addition of infective material, the veterinary authority may authorise a reduced storage period for slurry if it has been effectively treated to ensure the destruction of the virus (2002/60/EC). The most practicable method to inactivate the manure is the disinfection with chemicals (FAD PReP, 2018). In case of ASF, the chemical considered effective for manure is sodium hydroxide [(lye, caustic soda) 2%, 15 lt/m3)] (CEREP, 2004).

The disinfectant should be added to the storage tank at several points simultaneously to ensure that the disinfectant is properly distributed throughout the pit. . During exposure of slurry to disinfectants, no fresh slurry must be introduced into the tank (OIE, 1995; FAD PReP, 2018).

5. Conclusions

C&D procedures are fundamental for pathogen inactivation, to prevent the spread of the disease and to facilitate the repopulation after an outbreak. The completion of C&D procedure is also one of the requirements foreseen by the OIE for the recovery of the free status after the occurrence of ASF (OIE, 2019). In the areas affected or at risk of introduction of ASF, the disinfectants to choose for the control of the disease must be effective against the ASFV and approved by the official veterinarian. The choice of disinfectants and of procedures for disinfection should always take into account the type of premises, vehicles and objects to be treated. However, disinfectants should be officially authorized by the veterinary service and the conditions for their use strictly respected. In fact, a list of effective chemicals and disinfectants is reported in literature but the use of some of them is limited by their toxicity. Many ASF outbreak investigations have reported biosecurity shortcomings as a critical element for virus introduction and spread. In this perspective, C&D procedures described in this article provide the elements to prepare an adequate hygiene protocol aimed at minimizing the risk of spread.

Declarations of Competing Interest

The authors have no conflict of interest to declare.

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Appendix 3. OECD standards for assessing efficacy of disinfectants

Organisation for Economic Co-operation and Development (OECD) (2013) Quantitative method for evaluating viricidal activity of microbicides used on hard nonporous surfaces. OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 187 and Series on Biocides No. 6. http://www.oecd.org/chemicalsafety/testing/evaluating-the-activity-of-microbicides-used-on-hard-non-porous-surfaces.htm

Link: http://www.oecd.org/chemicalsafety/testing/evaluating-the-activity-of-microbicides-used-on-hard-non-porous-surfaces.htm

Appendix 4. ASTM standards for assessing efficacy of disinfectants

ASTM International. E1053-97: Standard Test Method for Efficacy of Viricidal Agents Intended for Inanimate Environmental Surfaces. West Conshohocken, PA. .

Link: https://www.astm.org/Standards/E1053.htm

Appendix 5. Revue Scientifique et Technique special issue entitled 'Disinfectants: actions and applications', published by OIE (1995)

Anonymous. 'Disinfectants: actions and applications', Revue Scientifique et Technique (International Office of Epizootics); Vol. 14, N° 1, Mar. 1995.

Link: http://www.epi-insight.com/docs/ALL_TOC_OIE_Rev%20Sci%20Tech_14-1_1995_Disinfectants-actions-and-applications.pdf

Appendix 6: APVMA permit for disinfectants for ASF virus (PER88135)

Australian Government
Australian Pesticides and Veterinary Medicines Authority

PERMIT TO ALLOW MINOR USE OF REGISTERED AND UNREGISTERED AGVET CHEMICAL PRODUCTS

FOR USE AS DISINFECTANTS FOR TREATMENT OF EQUIPMENT, FABRIC AND SURFACES IN CASE OF AN OUTBREAK OF AFRICAN SWINE FEVER OR CLASSICAL SWINE FEVER.

PERMIT NUMBER – PER88135

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows any person to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 19 DECEMBER 2019 to 31 DECEMBER 2024.

Permit Holder:
DEPARTMENT OF AGRICULTURE AND WATER RESOURCES
18 Marcus Clarke Street
CANBERRA ACT 2601.

Persons who can use the product under this permit:
Owners and employees of affected properties; owners or persons in charge of vehicles or equipment operating in declared areas; employees of the Australian Government and state and territory government Departments of Agriculture, Primary Industries or equivalent. Other personnel as directed by the Chief Veterinary Officer of the jurisdiction in which the disinfectant is to be used.

CONDITIONS OF USE

Products to be used

Registered products:

VIRKON S THE BROAD SPECTRUM VIRUCIDAL BACTERICIDAL FUNGICIDAL DISINFECTANT [APVMA No. 48185]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 494 g/kg POTASSIUM PEROXOMONOSULFATE TRIPLE SALT, 132 g/kg SODIUM DODECYL BENZENE SULFONATE and 15 g/kg SODIUM CHLORIDE as the only active constituents.

VIRKON AQUATIC BROAD SPECTRUM VIRUCIDAL BACTERICIDAL FUNGICIDAL DISINFECTANT [APVMA No. 68503]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 497 g/kg POTASSIUM PEROXOMONOSULFATE and 15 g/kg SODIUM CHLORIDE as the only active constituents.

GEA AG CHLOR DAIRY SANITISER [APVMA No. 84678]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 125 g/L available CHLORINE (Cl) present as SODIUM HYPOCHLORITE as the only active constituent.

RUA-KLENZA RKL 400 LOW FOAM DAIRY DETERGENT [APVMA No. 54229]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 400g/L SODIUM HYDROXIDE as the only active constituents.

TERMINATOR BROAD SPECTRUM DISINFECTANT [APVMA No. 51744]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 150 g/L GLUTARALDEHYDE and 100 g/L QUATERNARY AMMONIUM as the only active constituents.

HY-CLOR AQUATIC PREMIUM CALCIUM HYPOCHLORITE [APVMA No. 84936]
PLUS OTHER SIMILAR REGISTERED PRODUCTS
Containing: 700g/kg available CHLORINE (Cl) present as CALCIUM HYPOCHLORITE as the only active constituent.

Unregistered products:
CITRIC ACID, Anhydrous powder.

SODIUM CARBONATE, Anhydrous powder.

SODIUM CARBONATE, Washing soda crystals.

Directions for Use

For use as disinfectants for the treatment of equipment, fabric and surfaces in case of an outbreak of african swine fever or classical swine fever.

- For all situations you **MUST** clean with soap or detergent first and then rinse with water to remove organic matter.
- Ensure all wastewater remains on site.
- Ensure best management practices are followed to prevent or mimismise odour and water pollution.
- All users must comply with their relevant state and territory Environmental legislation.

Disinfectant	Rate	Application	Caution
494g/kg of potassium peroxomonosulfate triple salt, 132g/kg of Sodium Dodecyl Benzene Sulfonate, and 15g/kg of sodium chloride (Virkon S) 497g/kg Potassium peroxymonosulfate, 49g/kg sulfamic acid and 15g/kg Sodium chloride (Virkon Aquatic)	20g/L	Final dose: 2-3% solution (equivalent to 20g/L). Soak clothes/small items and equipment for at least 10 minutes For surface cleaning, apply at rate of 1-1.5L/m2. Do not use high pressure sprays. Decontaminate removed organic matter before disposal.	Mildly corrosive for many metals.
Sodium hypochlorite 125g/L	40ml/L	Final dose: 0.5% solution (equivalent to 40ml/L). Soak clothes, footwear and small equipment for 15-30 minutes. For surfaces apply at a rate of 1-1.5L/m2 and soak for 15 minutes on non-porous surfaces and 30 minutes on porous surfaces. Do not use high pressure sprays.	Toxic for eyes and skin – wear protective clothing, mask and gloves. Corrosive for many metals. Corrosive liquid. Harmful if swallowed. Will damage the eyes. Product will irritate the eyes, nose, throat and skin. Avoid contact with eyes, skin and clothing. DO NOT inhale vapour. Ensure adequate ventilation when using. When opening the containing and using the product, wear rubber gloves. If product on skin, immediately wash area with soap and water. If product in eyes, immediately wash out with water. Wash hands after use. Use clean containers for dispensing. DO NOT mix with

			other chemicals. DO NOT mix with different types of chlorinating chemicals. Mix with water only
Calcium hypochlorite 700g/kg	7.2ml/L	0.5% solution concentration (equivalent to 7.2ml/L) for 10-30 mins. Less stable in warm, sunny conditions above 15◦C.	Toxic for eyes and skin – wear protective clothing, mask and gloves. Corrosive for many metals. Corrosive liquid. Harmful is swallowed. Will damage the eyes. Product will irritate the eyes, nose, throat and skin. Avoid contact with eyes, skin and clothing. DO NOT inhale vapour. Ensure adequate ventilation when using. When opening the containing and using the product, wear rubber gloves. If product on skin, immediately wash area with soap and water. If product in eyes, immediately wash out with water. Wash hands after use. Use clean containers for dispensing. DO NOT mix with other chemicals. DO NOT mix with different types of chlorinating chemicals. Mix with water only.
Sodium hydroxide 400g/L	50ml/L	Final dose: 2% solution (equivalent 50ml/L). Always add the product to the water. Soak clothes, footwear and small equipment for at least 10 minutes. For surfaces apply at a rate of 1-1.5L/m2 and soak for at least 10 minutes. Do not use high pressure sprays.	Do not use in the presence of aluminium or derived alloys. Wear protective (water resistant) clothing, gloves and safety glasses. Corrosive. Product is poisonous if swallowed. The product is alkaline. Avoid contact with eyes, skin and clothing. When preparing and using the wash solution wear elbow-length neoprene gloves and face-shield or goggles. If product or wash solution on skin, immediately wash area with soap and water. Wash hands after use. After each day's use, wash gloves, face-shield or goggles and contaminated clothing.

Sodium Carbonate Anhydrous	40g/L	4% solution (equivalent to 40g/L) for 20 mins.	Mildly caustic for eyes and skin. Avoid use on aluminium and similar alloys.
Sodium Carbonate Washing Soda	100g/L	10% solution (equivalent to 100g/L) for 30 mins	
Glutaraldehyde (only for AI and ND) with Quaternary Ammonium Compounds (QACs) (Available as 150g/L of glutaraldehyde. Therefore 1 part 15% glutaraldehyde to 7.5 parts water = 2% final conc. 1000/7.5=133ml/L.)	133ml/L	2% solution (equivalent to 133ml/L) Clean equipment with soap or detergent first and rinse with water. Immerse for minimum of 10 mins at 35ºC and 20 mins at 25ºC. Maintain solution at pH>7. Efficacy may be increased by raising the solution temperature e.g. to 60ºC.	Avoid eye and skin contact.
Citric Acid	30g product/L	3% solution (equivalent to 30g/L) Non-porous surfaces apply for 15 minutes Porous surfaces apply for 30 minutes	Corrosive. Avoid contact with eyes and skin. Wear protective eye wear.

NOTE: Glutaraldehyde and Quaternary Ammonium Compounds are only available as a combined product. The final concentration is based off the Glutaraldehyde % or ppm

NOTE: EFFICACY of some of the products and proposed uses under this permit has not been thoroughly determined however efficacy is reasonably expected due to the broad spectrum nature of the products.

Safety Directions and Personal Protective Equipment

- Follow label First aid instructions as listed on the product label for registered products. In some cases, the safety directions listed on the label are not sufficiently protective, due to the higher concentrations to be used. Additional Safety Directions are specified in the 'caution' column above.
- For industrial grade chemicals (e.g. citric acid and sodium carbonate) follow *Safety Directions* and First aid instructions as listed on the label and the *Safety Data Sheet* issued by the supplier/manufacturer of the product.
- As a minimum, when opening product containers, preparing spray solutions and during use wear elbow length butyl rubber or PVC gloves, cotton overalls buttoned to the neck and wrist, washable hat, chemical resistant boots and goggles/face mask.
- If products or prepared solutions come into contact with skin, immediately wash the area with soap & water.
- If products or prepared solutions come into contact with eyes, immediately wash it out with water.

Jurisdiction:
ALL States and Territories.

Additional Conditions:

This PERMIT provides for the use of a product in a manner other than specified on the approved label of the product. Unless otherwise stated in this permit, the use of the product must be in accordance with instructions on its label.

PERSONS who wish to prepare for use and/or use products for the purposes specified in this permit must read, or have read to them, the DETAILS and CONDITIONS of this permit.

Issued by the Australian Pesticides and Veterinary Medicines Authority.