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INVITED REVIEW

Is transportation a risk factor for African swine fever transmission in Australia: a review

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African swine fever (ASF) is a viral disease of the pigs that was first described in Africa during the early part of the twentieth century. The disease has periodically occurred outside of Africa, including an ongoing epidemic in Europe and Asia that started in 2007; the disease has never occurred in Australia or New Zealand. Once introduced into a country, spread can occur through direct and indirect routes of transmission. Infected feral pig populations have the potential to act as a long-term reservoir for the virus, making eradication difficult.

Just before and throughout the period of clinical signs, ASF virus is shed in oronasal fluids, urine, faeces and blood. This results in contamination of the pig's environment, including flooring, equipment and vehicles. Transportation-related risk factors therefore are likely to play an important role in ASF spread, though evidence thus far has been largely anecdotal.

In addition to the existing AUSVETPLAN ASF plan, efforts should be made to improve transportation biosecurity, from the time a pig leaves the farm to its destination. Collection of data that could quantify the capabilities and capacity of Australia to clean and disinfect livestock trucks would help to determine if private and/or public sector investment should be made in this area of biosecurity. No peer-reviewed research was identified that described a specific process for cleaning and disinfecting a livestock truck known to be contaminated with ASF virus, though literature suggests that transportation is an important route of transmission for moving the virus between farms and countries.

Keywords African swine fever; biosecurity; epidemiology; pig; risk factors; transportation

Abbreviations APIQ, Australian Pork Industry Quality Assurance Program; ASF, African swine fever; CSF, classical swine fever; EU, European Union; FMD, foot-and-mouth disease; OIE, World Organisation for Animal Health; PED, porcine epidemic diarrhoea; PRRS, porcine reproductive and respiratory syndrome; TAR, transport-associated routes

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African swine fever (ASF) was first described in Kenya in 1921[1] and has spread to many countries in Europe, Africa and Asia. Of importance to Australia, Indonesia, East Timor and Papua New Guinea have recently become infected as part of the current Eurasian pandemic of the disease that first started in Georgia in 2007. The disease is currently not present in North or South America, Australia or New Zealand.

Once ASF virus enters a country, the disease spreads via direct contact between infected and non-infected pigs, and through indirect contact via contaminated vehicles, equipment, personnel and pork products. In the current Eurasian outbreak, infected feral pigs act as a long-term reservoir for the disease. They may transmit ASF to domestic pigs through direct contact and also indirectly by contaminating the environment, feed stuffs (grains and forages) and bedding (straw) through contact with excrement, body fluids and carcass remnants (from ASF-induced mortality).[2,3]

In an uninfected country, the risk of introducing ASF virus onto a farm can be minimised by good on-farm biosecurity practices.[4] In particular, transportation-related risk factors deserve special attention as they can be both a source of infection for a country (via return of potentially contaminated trucks from infected regions/countries to uninfected regions/countries) and as means of propagating an epidemic once a country becomes infected. Furthermore, the widespread use of contract livestock haulers (rather than farmer-owned transport) is common in the pig industries of many countries including Australia, and biosecurity-relevant behaviours of contracted transport companies and drivers cannot be completely controlled by the farmer.

The proximity of Australia to ASF-infected countries has made the disease a high priority for the Australian pork industry. In preparing for a response to an ASF incursion, the industry has identified a need to strengthen biosecurity across the supply chain,[5] specifically with respect to transportation.

A review of published literature in PubMed and Web of Science on transportation-related risk factors for spread of ASF virus was conducted using the following Boolean strategy:

((ASF OR 'african swine fever') AND (epidem* OR risk OR 'risk factor' OR biosecur* OR transpor* OR truc* OR disinfectant* OR decontam* OR clean* OR wash* OR manure* OR faeces OR feces OR effluent))

Ad hoc searches were also conducted to find additional relevant material when discovered through study of the sources identified in the peer-reviewed literature. The review aimed to identify key features of this risk that should be considered in the context of Australian pork production systems and transportation methods. 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 protocols in inactivating the virus were therefore included in the review.

Epidemiology of ASF

General reviews of ASF epidemiology and virology have been recently published and therefore only aspects of ASF epidemiology related to transportation risk are summarised in this review. For readers that require information about other aspects of ASF epidemiology or virology, several open-source, recent reviews are recommended.[6–8]

ASF virus shedding, persistence and transmission

Faeces and urine. The role of contaminated fomites in the spread of ASF within and between farms is particularly important, and therefore some understanding of virus-shedding patterns is important in mitigating the risk of ASF virus being spread among farms.

ASF virus infected pigs are contagious (i.e. shedding virus) during the incubation period of the disease and 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) until the pig succumbs or recovers (a rare outcome of the disease in the current Eurasian outbreak), albeit at reduced levels and frequency.[9]

During the acute phase, large quantities of virus are present in oral and nasal fluids, blood, urine and faeces. The high levels found in faeces may be related to the presence of occult blood.[10] The likelihood of virus being shed in semen is contentious but is at least plausible given the remarkably high and persistent viremia that develops with the disease. The World Organisation for Animal Health (OIE) in its Terrestrial Animal Health Code chapter on ASF acknowledges this risk by recommending that semen from ASF infected countries be sourced only from donor males that have been kept since birth or for at least 3 months prior to collection in an establishment in which ASF surveillance demonstrates that no case of ASF has occurred in the past 3 years and that show no clinical sign of ASF on the day of collection of the semen.[11] The European Union recently reviewed the adequacy of their existing regulations related to movement of semen potentially contaminated by ASF virus, agreeing with the OIE that the risk was quantifiable and that explicit measures were required to manage the risk.[12] There are few peer-reviewed publications that provide data that help quantify the risk of ASF virus in semen and the potential for it to be transmitted venereally. One author notes that although sexual transmission of ASF virus in pigs has not been documented, the virus is shed in genital secretions,[13] information which is consistent with a study published in 1970 that detected infectious ASF virus in vaginal swabs of experimentally infected pigs.[14] Evidence of the occurrence of transmission of ASF virus through semen is often attributed to a 1984 paper by Thacker et al but this reference notes the finding only as a personal communication with another scientist, and not as original data.[15]

The earliest excretion of ASF virus usually occurs by the nasopharyngeal route, as early as 1 or 2 days before the onset of fever[14] though the exact time and concentration of virus can vary depending on the strain of the virus. Virus in the secretions of the conjunctiva or lower urogenital tract appears somewhat later and tends not to attain as high of levels. The amount of virus in faeces, secretions and excretions during various stages of infection appears to be related to virus strain.[10]

It has been shown that neither dose nor route of infection (inoculated or naturally infected) has a dramatic influence on virus excretion kinetics or concentration and that a high proportion of persistently infected animals shed virus into the environment for at least 70 days.[16]

A study done in 2017 estimated the half-life of the highly virulent Georgia 2007/1 isolate in faeces and urine.[17] 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 PCR, the half-life of ASF virus DNA was 8–9 days in faeces and 2–3 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.

A study examined the likelihood that exposure of healthy pigs to the pen environment of pigs that had died from ASF would result in infection.[18] Following euthanasia of pigs that had been infected with a virulent isolate of ASF virus from Poland, healthy pigs were introduced into the pens either 1, 3, 5 or 7 days later. Pigs that were introduced into the contaminated environment within 1 day of the infected pigs being removed developed clinical disease; 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 could be detected in blood samples.

Detection of ASF virus shedding in faeces has the potential for use as an ASF diagnostic or surveillance tool. However, in one experiment virus was detected in faeces (by PCR) only around 50%–80% of the time from 0 to 21 days post-infection (dpi), far less sensitive than applying the same diagnostic procedure to a blood or serum sample.[19] 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 2 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 ranged from 1.7 to 7.4 days.

Meat and blood. Meat from pigs slaughtered in the infective stages of ASF provide a ready source of virus to naïve pigs via the practice of feeding uncooked waste food. Pork products fed to pigs as swill that has not been cooked to comply with the World Organisation for Animal Health (OIE) Terrestrial Code (90°C for at least 60 min; or at least 121°C for at least 10 min)[11] poses a significant risk to naïve animals.

ASF virus can be inactivated by heating for 30 min at 60°C[20] or 70 min at 56°C[21] in culture media, but the virus is much more hardy

when held in a moist and proteinaceous environment, surviving in blood heated to 50°C for 3 h.[1] Survival of the virus in pig blood kept at 4°C can be as long as 18 months.[20] 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 to 22 h in medium containing 25% serum.[21]

ASF virus will survive in Parma hams for at least 300 days but not 400 days.[22] Data suggest that 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.[23] The literature on survival of ASF virus in many pork products has been recently reviewed by at least two authors documenting that a number of tissues (especially blood) are able to provide an environment that enables the virus to persist for hundreds of days, especially when tissue has been chilled to 4°C or less.[24,25]

Transportation-related risk factors

Although contaminated transport vehicles are a plausible and recognised risk factor for the spread of ASF and other diseases among farms,[26] documented cases of such occurrences are rare.[27] It is highly likely that trucks were a significant factor in the spread of ASF in China[28] and trucks have also been considered to be a potentially important risk for spread of ASF into and around Europe[29] though much of the evidence is anecdotal or assumed.

A number of ASF outbreaks that occurred in large commercial farms in Russia and Lithuania were thought to be the result of contact with contaminated fomites related to improper disinfection of clothing and boots or by bringing contaminated pork onto the premises.[30,31] In these cases, authors suggested generally poor biosecurity and inadequate implementation of centralised 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 Europe in 2014, then China and other countries of East Asia in 2018. 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 into commercial and backyard pig farms.[32] Results of the study showed that 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, growing crops around the farm (which could potentially attract wild boar), feeding forage from ASF affected areas to the pigs and visits by professionals working on farms were significant risk factors.

Identifying the route of introduction of ASF virus onto infected farms, even at the early stages of an outbreak, can be difficult. During 2015–2017, 26 cases of ASF were identified on backyard and commercial pig farms in Estonia.[33] Detailed investigations of each herd were undertaken, but 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.

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. Among the activities discussed, livestock trucks returning from infected to uninfected countries was assessed to be the activity with the second highest level of risk. The group noted that this was an important finding as the risk was largely able to be controlled at country borders through inspection of trucks for sufficient cleaning and disinfection.[34]

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 consider the risk associated with movement of infected pigs, as distinct from a contaminated vehicle itself acting as a fomite.[35–38]

Concern about the spread of ASF from Eastern Europe into countries of the European Union (EU) 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 – these were collectively referred to as transport-associated routes (TAR).[29] A semi-quantitative model based on the weighted combination of risk factors was developed to estimate the risk of ASF virus introduction by TAR. The researchers concluded that the relative risk for ASF virus introduction through TAR in most of the EU countries was low. The risk for ASF introduction associated with returning trucks accounted for 65% of the total TAR risk. Similar modelling work in France reached a similar conclusion with virus transmission from commercial herds almost (99%) exclusively related to pig movements.[39]

Retrospective information from outbreaks of ASF in the Russian Federation was used to assess the most likely source of ASF virus introduction onto farms.[40] The route of introduction into new pig populations (i.e. primary outbreaks) was unidentified in 28.3% of cases. For those situations where there was some certainty around the source of introduction, 97% were through feeding contaminated swill, 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% of cases 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 (5.6%).

A case report of an ASF outbreak that occurred in a large Chinese pig farm was recently published.[28] Despite standard operating procedures being in place to manage biosecurity, 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 contaminated during a prior trip to an abattoir.

Several authors have suggested that emergency sale of pigs during ASF outbreaks contributes to the spread of ASF with particular

examples available from Russia, countries in Africa, China and other countries in southeast Asia.[41]

Published information related to transportation risk is available for other pathogens, which may provide some insight about ASF spread. Researchers assessed the likelihood of pigs becoming infected with classical swine fever (CSF) after coming into contact with the pen environment of pigs experimentally infected with CSF virus, but removed before the naïve pigs were introduced into the same dirty pen.[42] Eight days after experimental infection (when all pigs had been viraemic for at least 3 days), the pens were depopulated and restocked (20 h later) with susceptible pigs, which stayed in these pens for 35 days. During the first 3 weeks of the observation period (during which time the pens were neither cleaned nor disinfected), none of the susceptible pigs became infected. This result indicates that CSF virus spread via excretions may be of minor importance in the early stages of infection. 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 8 days to allow all pigs to become viraemic but to avoid the pigs becoming overtly '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 1 day and susceptible pigs the next.

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.[43] During that epidemic, 428 CSF virus-infected pig herds were detected, 403 of which provided data to the 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 m and 0.0078 per week per infected herd at a distance of between 500 and 1000 m. In a separate study of the same European CSF outbreak, researchers studied possible origins of the initial introduction of the virus into the Netherlands and other countries involved in the outbreak.[44] 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 Germany, which then returned to The Netherlands and came into contact with the index herd there. Furthermore, CSF was diagnosed in a mixed sow-finishing herd in Belgium (near the border with The Netherlands), which seemed to be associated with the use of a transport lorry that had been returning from The Netherlands.

Inactivation of ASF virus

Of relevance to countries with significant pork exporting activity such as Australia, a major concern is the contamination of abattoirs that would occur as a result of processing pigs infected with an exotic animal disease. Although only healthy animals should be presented for slaughter, it is possible that some pigs acutely infected with ASF would be presented to slaughter during the 'silent spread phase' that occurs between the time of disease incursion and the time when a diagnosis is established. ASF virus and other high-consequence animal pathogens are found in pig products such as blood and faeces and are therefore likely to be present in the tissues of infected animals that arrive to an abattoir, thus jeopardising the export-eligible status of that abattoir.

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 contained in external storage or under the pig building, and the waste water used to wash down trucks that have carried pigs will be referred to as slurry.

A possible risk associated with slurries that the material may bypass municipal sewage treatment and be applied directly to a crop as fertiliser, thereby creating a risk of spreading any pathogens present by aerosol or through pig contact with the crop.

The survival of ASF virus on plant material contaminated with slurry containing ASF virus has been studied,[45] prompted by 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; observations reported from ASF outbreaks in Latvia further support this potential risk.[46] After contaminating six different types of field crops (wheat, barley, rye, triticale, corn and peas) with ASF virus contaminated blood, all remained positive for ASF viral genome even after being dried at room temperature for 2 h, and after being dried and then exposed for 1 h to moderate heat (40°C and 75°C).[45] However, no infectious virus could be detected by virus isolation after 2 h drying.

A study was undertaken to determine the survival time of ASF virus on selected fomites including water, wet soil and wet leaf litter.[47] The samples were tested at −20°C, 4°C, 23°C and 37°C either 0, 3, 7 or 14 days after exposure to culture medium containing ASF virus. 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 plus straw, hay 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 decreasing to less than or equal to $10^{1.31}$ haemadsorption in 50% of infected cultures per mL between days 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 EU has developed recommendations for management of these materials.[48] Though generally the risk of commercially traded crops, vegetables, hay and straw to contain and maintain infectious ASF virus is considered to be low, if the use of locally harvested

grass and straw is considered to represent a risk under local prevailing conditions then the EU 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. Furthermore, the 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.

The effectiveness of alkali treatment (NaOH or $Ca[OH]_2$) or heating (4°C or 22°C) for inactivating ASF and swine vesicular disease viruses in pig slurry was investigated in 1999,[49] and based on the findings, the researchers then went on to design a pilot plant for heat inactivation of slurry that could be used in a field setting.[50] In the authors' work with their pilot treatment plant, ASF virus was inactivated by operating the plant at a temperature of 53°C for approximately 5 min 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 be impractical, but not impossible.[51]

In an older review, the inactivation kinetics of various transboundary pathogens in faeces or slurry were summarised and authors suggested ASF virus may survive in the material for 60–100 days.[52] However, they noted that under practical field conditions, survival time was 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.

There appears to be little evidence available assessing inactivation of ASF virus during composting of manure or animal composting.[53]

Cleaning and disinfection efficacy

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 for the disinfectant to be effective. Normal cleaning and disinfection include the following: First, removal of bedding, straw, feed and manure; second, washing using detergents; and third, application of an effective disinfectant. 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.[54,55]

The results of in vitro testing of commercial disinfectants against ASF virus using a method similar to that described in the European Standard EN 14675:2015 for quantitative assessment of veterinary chemical disinfectants have been reported.[56] Disinfectants were assessed with or without the presence of organic contaminants (bovine serum albumin = 'low-level' contamination, bovine serum albumin plus yeast extract = 'high-level' contamination). Sodium hypochlorite (3.0, 1.5 and 1.0%), caustic soda (2%), phenol (1%), potassium peroxymonosulfate (3.0, 1.0 and 0.5%) and glutaraldehyde (0.5%–0.1%) were found to reduce virus concentrations by at least fourfold even in the presence of high-level contamination. Other products or concentrations were less effective at inactivating ASF virus. The authors emphasised the substantial effect of organic matter in reducing the effectiveness of all compounds at all concentrations.

An alternative to using disinfectants to kill viral and bacterial pathogens using ozonised water has been reported.[57] A 2-log_{10} reduction (99%) was observed within 1 min when $10^{5.0}$ $TCID_{50}$/mL wild-type or reporter ASF virus was exposed to 5 mg/L of ozonised water and a 3-log_{10} (99.9%) reduction in virus was observed within 1–3 min when exposed to either 10 or 20 mg/L of ozonised water. Inactivation kinetics were also similar at higher virus concentrations. In the study, ozonised water was shown to be relatively stable for 1–2 days.

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, and physical characteristics of the surface being disinfected are not identical across situations.[58] Lipidic solvents, which destroy the envelope of the virus and commercial disinfectants based on iodine and phenolic compounds, appear to be among 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.

Several international agencies have published principle of cleaning and disinfection as they relate to transboundary diseases, including ASF. The main 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[59] and attempts to use this guidance in establishing an effective cleaning and disinfection programme for ASF virus are available.[58] The OIE,[60] the Food and Agriculture Organisation of the United Nations[61] and the United States Department of Agriculture have published guidance for appropriate use of disinfectants against important animal pathogens including ASF virus all of which are broadly in agreement with Australia's approved list of disinfectants effective against ASF virus (Table 1).[62]

Efficacy of truck-washing protocols in managing infectious disease risks

No reports were identified in the literature of trials that attempted to directly assess methods to decontaminate trucks or trailers contaminated with ASF virus, though 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) has been reported.[63–65]

Wood shavings, sawdust or chips may be used as bedding when transporting pigs. As there is no standardised method for porous surface disinfection; commercial disinfectants are only certified for use on hard, nonporous surfaces.[64] To model porous surface disinfection in the laboratory, foot-and-mouth disease (FMD) virus and ASF virus stocks were dried on wood surfaces 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. Although 2000 ppm sodium hypochlorite was capable of inactivating ASF virus on wood under these conditions, this chemical did not meet the 4-log effective disinfection threshold for FMD virus. The data support the use of chemical disinfectants containing at least 2% citric acid for porous surface disinfection of FMD and ASF viruses. The same authors extended their work to assess the efficacy of drying and disinfectants on steel and plastic

surfaces.[65] For ASF, CSF, and FMD viruses, a 2- to 3-log reduction of infectivity due to drying alone was observed. 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 that it may not be completely effective for disinfection in the field. Based on these data, the authors 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 an abattoir environment, the authors mentioned earlier evaluated common disinfectants used by the food industry 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.[63] The commercial disinfectants used in this study included quaternary ammonia with surfactant (800 ppm, pH 1.8), stabilised sodium hypochlorite (600 ppm, pH 10.8), potassium peroxymonosulfate with surfactant (2% w/v, pH 2.2) and citric acid (2%). Disinfectant activity was greatly inhibited in the presence of dried blood and meat juices. As compared to virus dried in phosphate buffered saline, 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.

Though experimental work with ASF virus and truck decontamination appears to be limited, work has been done with porcine reproductive and respiratory syndrome (PRRS) virus and porcine epidemic diarrhoea (PED) virus, both of which are high-consequence pig diseases exotic to Australia. Mechanical fomites consisting of snow and water were contaminated with a field strain of PRRS virus and 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 fomites after they were washed off the vehicle, which resulted in introduction of the virus into 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.[66] By contrast, using the same experimental model in warmer conditions using compacted soil as the fomite found that transfer of PRRS virus was an infrequent event.[67] To evaluate the effectiveness of various trailer cleaning regimes, four cleaning/disinfecting methods were designed and then evaluated using truck-scale models that had been artificially contaminated with PRRS virus, including manual scraping only to remove soiled bedding; a combination of bedding removal, washing, and disinfection; the combined treatments followed by a freezing and thawing cycle; and the combined treatments followed by air drying overnight.[68] Post-treatment swabs were PCR-positive for all treatments except the combination protocol accompanied by drying. 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.[69] Scale-model trailer interiors were artificially contaminated with PRRS virus and then treated with one of four treatments: Thermo-assisted drying and decontamination (TADD; or raising the interior surface of the trailer to 71°C for 30 min); air drying only without supplemental heat; overnight (8 h) air drying without supplemental heat; and 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. All tests for the presence of infectious PRRS virus were negative for trailers treated with TADD and overnight drying, with TADD having the advantage of requiring much less time to implement.

PED virus causes watery diarrhoea, dehydration and a high mortality rate among suckling pigs and is present in many parts of the world since a major global pandemic occurred during 2013–2017. In a study of the role of transportation in spread of PED in Italy, a study reported that 14.1% environmental swabs collected at slaughterhouses from trucks after animals were unloaded tested positive for PED virus before the cleaning and disinfection operations were performed.[70] In addition, 7.4% of environmental swabs of the same trucks, collected after routine cleaning and disinfection operations, still tested positive for the virus thus the cleaning and disinfection procedures succeeded in eliminating the virus in only 54% of the trucks that initially tested PED virus positive. More concerning was that 17.3% of the empty trucks that were tested before arriving at farms to load animals were PED virus positive.

The role of trucks in the spread of PED in the United States during the 2013 epidemic was studied by collecting environmental samples from near the rear door of 575 trailers unloading pigs at six different abattoirs.[71] Before unloading, 6.6% trailers were found to be contaminated and of those trailers not found to be contaminated at the time of unloading, 5.2% 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'.[71]

The situation in Australia

In 2019, there were an estimated 3700 pig producers in Australia producing around 420,000 tonnes of pork per year of which around 10% was exported.[72] However, Australian Pork Limited has estimated that only around 1500 of these producers raise pigs at a scale from which the owner can claim income from the

Table 1. Australian Pesticides and Veterinary Medicines Authority (APVMA) list of approved disinfectants and concentrations for treatment of ASF virus

Disinfectant	Rate	Application[a,b]
494 g/kg of potassium peroxymonosulfate triple salt, 132 g/kg of sodium dodecylbenzene sulfonate, 44 g/kg sulfamic acid and 15 g/kg of sodium chloride (Virkon S) 497 g/kg potassium peroxymonosulfate, 49 g/kg sulfamic acid and 15 g/kg sodium chloride (Virkon Aquatic)	20 g/L	Final dose: 2–3% solution (equivalent to 20 g/L). Soak clothes/small items and equipment for at least 10 min. For surface cleaning, apply at the rate of 1–1.5 L/m^2. Do not use high-pressure sprays. Decontaminate removed organic matter before disposal.
Sodium hypochlorite 125 g/L	40 mL/L	Final dose: 0.5% solution (equivalent to 40 mL/L). Soak clothes, footwear and small equipment for 15–30 min. For surfaces, apply at a rate of 1–1.5 L/m^2 and soak for 15 min on nonporous surfaces and 30 min on porous surfaces.
Calcium hypochlorite 700 g/kg	7.2 mL/L	Final dose: 0.5% solution concentration (equivalent to 7.2 mL/L) for 10–30 min.
Sodium hydroxide 400 g/L	50 mL/L	Final dose: 2% solution (equivalent 50 mL/L). Soak clothes, footwear and small equipment for at least 10 min. For surfaces, apply at a rate of 1–1.5 L/m^2 and soak for at least 10 min.
Sodium carbonate anhydrous	40 g/L	Final dose: 4% solution (equivalent to 40 g/L) for 20 min.
Sodium carbonate washing soda	100 g/L	Final dose: 10% solution (equivalent to 100 g/L) for 30 min.
Glutaraldehyde with quaternary ammonium compounds[c] Available as 150 g/L of glutaraldehyde. One part of 15% glutaraldehyde to 7.5 parts water = 2% final concentration = 133 mL/L.	133 mL/L	Final dose: 2% solution (equivalent to 133 mL/L). Clean equipment with soap or detergent first and then rinse with water. Immerse for minimum of 10 min at 35°C and 20 min at 25°C. Maintain solution at pH > 7. Efficacy may be increased by raising the solution temperature to 60°C.
Citric acid	30 g product/L	Final dose: 3% solution (equivalent to 30 g/L). Nonporous surfaces apply for 15 min; porous surfaces apply for 30 min.

[a] 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 product.
[b] For all situations, APVMA requires that users clean with soap or detergent first and then rinse with water to remove organic matter before applying disinfectant and that users must comply with their relevant state and territory environmental legislation.
[c] Glutaraldehyde and quaternary ammonium compounds are only available as a combined product. The final concentration is based on the glutaraldehyde% or its ppm.

enterprise.[73] According to the Australian Bureau of Statistics,[1] there were around 2.4 million domestic pigs in the country during 2017, including 273,000 breeding sows. The industry raises approximately 5.3 million pigs for slaughter annually. Although there are in excess of 70 abattoirs that are licensed to slaughter pigs in Australia, only seven are qualified to process pigs for the export market. These seven abattoirs are responsible for around 85% of the total annual pork slaughter.

PigPass is the national tracking system designed to provide real-time information on the movements of all pigs in Australia. The objective of the system is primarily to enable authorities to quickly determine the source of a disease outbreak and extent of potential spread by pig movement. A PigPass National Vendor Declaration form must be completed (electronically or on paper) any time pigs leave a property regardless if ownership of the pigs' changes or not.[74] Since the programme became compulsory in 2018, PigPass has not been systematically reviewed for accuracy or compliance but limited reporting against the available data in 2018 found that approximately 80% of movements recorded in PigPass were to abattoirs, as opposed to movements to other farms or saleyards.[75] Farrow to finish farms were the dominant users of PigPass at the time, accounting for 54% of all recorded movements (47% of which were to abattoirs and 7% to other farms).

Australia's response to exotic animal disease incursions are outlined in AUSVETPLAN[2] and procedures for cleaning and disinfection of livestock vehicles are described in the Operational Procedures Manual for Decontamination.[61] The section of the document related to

[1] Australian Bureau of Agricultural and Resource Economics and Sciences (2018). Agricultural commodity statistics 2018, Table 14.2 Australian pig numbers, by state and territory. Available at: https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/abares/data/acs2018-meat-pigs.xlsx. Accessed 21 August 2020.

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

livestock vehicles is very detailed and lists requirements for interior and exterior areas of the truck, cab and trailer that need to be inspected, cleaned and disinfected including areas that need dismantling before these steps are undertaken. Importantly, these standards only apply to livestock transport undertaken as part of an exotic animal disease response and do not necessarily apply at other times.

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).[76] The APIQ Transport Standards and Performance Indicators describes driver behaviour and the requirement for vehicle cleanliness and mandate that: Drivers and vehicles used to carry pigs follow the farm's Biosecurity Standards, facilities promote effective and safe handling of pigs when loading or unloading; that drivers do not enter designated clean areas; that vehicles are cleaned between consignments; that handling, assembly, and loading or unloading of pigs are conducted with care and in a manner that minimises stress to pigs; and that loading facilities and farm roads are designed and maintained to facilitate safe loading and delivery of pigs. Although there is a requirement for vehicles to be cleaned between consignments, there is no guidance on how this should be carried out. All producers supplying pigs to export abattoirs are required to be APIQ certified, thus in theory all trucks will have been washed between consignments. Producers supplying 'non-export' abattoirs are not required to meet APIQ standards.

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.[77] Although 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 21 August 2020, there were 125 truck washes listed on the website;[3] the completeness of the data on this system is 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.[78] 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 was required to bring the truck washing capacity at these facilities to an acceptable level of biosecurity.

Conclusions

Transportation-related risk factors likely play an important but as yet unquantified role in the introduction and spread of ASF. In addition to the existing AUSVETPLAN guidance that focuses primarily on truck washing, efforts should be made by the industry to improve the biosecurity around all aspects of pig transport from the time a pig leaves the farm of origin through to its destination.

There does not appear to be objective data that describe the frequency or quality of cleaning and disinfection procedures of pig transport vehicles on-farm or at abattoirs in Australia. Similarly, data that describe the behaviours (and their effectiveness) that are routinely undertaken by farmers to minimise possible contamination at the time of loading pigs on-farm or unloading at an abattoir by truck drivers or farm/abattoir staff is not currently available. Transport biosecurity has been embraced by pig industries in other countries and examples such as the Danish Specific Pathogen Free Program (https://spfsus.dk/en), established in 1971, demonstrate that systemic control of pig transport biosecurity could be adopted by the Australian pork industry. Producers and abattoirs should understand that ASF virus-contaminated trucks represent a significant threat to the Australian industry and that this risk is controllable.

Initiatives that help to educate and improve farmer and transporter behaviours such as improving the use of electronic real-time submission of movement data into PigPass, minimising cross-contamination events during loading/unloading, better containment and treatment of effluent generated during truck washing, increasing the quality of cleaning and disinfection procedures at load-out facilities, loading ramps, and lairage areas, and perhaps considering segregation of trucks used for farm-to-farm pig movements from those used for farm-to-abattoir movements would improve exotic disease preparedness and minimise spread of endemic diseases in the country. Collection of data that could quantify the capabilities and capacity of Australia to clean and disinfect livestock trucks would help to understand if further private and/or public sector investment should be made in this important area of biosecurity.

Conflicts of interest and sources of funding

The authors Neumann, Hall and Hamilton have previously applied for and received funding for research from Australian Pork Limited. None of the authors have any other potential conflicts of interest to declare that are relevant to this publication.

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

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