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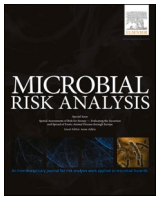

A qualitative risk assessment of imports of animal feed as a potential pathway for Aujeszky's disease virus incursion

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ABSTRACT

Aujeszky's disease (AD) is a highly contagious disease of pigs that primarily transmits by respiratory and oral routes. Evidence from recent outbreaks suggests that some swine viruses can survive in contaminated animal feed, thus posing a risk of entry via imports from other countries. To this end, a qualitative risk assessment was undertaken to determine the risk of introduction of AD virus (ADV) and infection of pigs via this route to determine if contaminated animal feed is a viable pathway for the spread of ADV. The feed categories investigated were soya bean/meal/oilcake, pet food, choline/lysine and spray dried porcine plasma. These were chosen based on their use in animal feed and the available data on viral contamination. The overall probability of an animal becoming infected from the importation of feed contaminated with ADV was estimated as Negligible or Very Low for all feed categories. The uncertainty associated with the estimates was assessed as Medium, due to the lack of data around the mechanisms that ADV could contaminate feedstuffs and for infection of susceptible animals from ADV infected feed.

1. Introduction

Aujeszky's Disease (AD), also known as Pseudorabies, is caused by the virus Suid herpesvirus 1 (SuHV-1) (Mettenleiter et al., 2019). Pigs and wild boars are the natural hosts and the only animals to become latent carriers. However, almost all mammals can be infected, and clinical cases have been reported in domesticated mammals including cats; farmed species including cattle, sheep, goats, mink; various captive wild animals and uncommonly in wildlife (Spickler 2017). Some species such as horses and birds require high viral doses to become infected (WOAH 2018). SuHV-1 does not appear to infect the tailless apes, although some other primates are susceptible (Spickler 2017).

AD is economically important and highly contagious, and poses a threat to both pigs and wild boar with mortality as high as 100% (Wittmann, 1986). AD is a World Organisation of Animal Health (WOAH) reportable disease that can cause significant economic losses (Spickler 2017). Recently, in Europe, there have been increasing reports of isolated AD virus (ADV) detections in countries that have been free of the disease previously, with the disease moving from South and East Europe into the West and Central countries. However these detections remain sporadic and not sufficient in number to cause countries to lose disease freedom (Bacigalupo et al., 2023; Gale, Perrin, Freath, and Bacigalupo, 2023).

The virus spreads primarily through venereal, respiratory, and oral transmission (Spickler 2017). In acute infections the virus can be present for more than 2 weeks in tonsillar epithelium, milk, urine, and vaginal and preputial secretions, posing a risk for other routes of transmission. Transmission between farms has also been attributed to airborne spread (Christensen et al. 1990) and spread to other species through close contact with infected pigs, or the consumption of infected meat from pigs or other animals (Spickler 2017). Published evidence on the survival of ADV, and other swine viruses, in different feed matrices suggests that potentially contaminated animal feed, including unprocessed feed compounds, could pose a risk of viral entry from imports (Dee et al., 2018; Gordon et al., 2019; Jones et al., 2019; Patterson et al., 2019; Stoain et al., 2020). Commercial swine feed was previously not deemed a significant concern as a fomite for transmission of viral pathogens until soon after porcine epidemic diarrhoea virus (PEDV) was diagnosed in the United States of America (USA) in 2013. It was reported that contaminated feedstuffs or their packaging arriving from Asia may have been involved in the introduction and transmission of PEDV in this case (Elijah et al., 2021). Another contributing factor to the quick spread of

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PEDV in the US was the feed mill. Once introduced into the feed mill, PEDV became widely distributed, as the feed mill served as a continuous source of contamination of workers and feed delivery vehicles (Elijah et al., 2021). More recently contamination of feed or a feed transport vehicle with PEDV was considered the most likely cause of a PEDV outbreak in Mexico and highlighted the need for more consideration of mitigation of the spread of disease via feed and feed transport (Garrido-Mantilla et al., 2022). Cases of PEDV via feed and subsequent mitigations have also been reported in China (Wu et al., 2022).

Senecavirus A virus has also been associated with feed imports from endemically infected countries (Dee et al., 2022). Foot and Mouth disease (FMD) virus has also been proven to be viable for 37 days in soybean meal, although there are currently no reported cases of FMD virus transmission via a feed route (Stenfeldt et al., 2022). Historically, introductions and spread of African swine fever virus (ASFV) into new countries or regions have also had epidemiological associations with contaminated feed. In Romanian backyard farms, one of the risk factors for ASFV incursion was feeding plant material that had originated from ASFV-positive regions to pigs (Niederwerder 2021). Another example occurred in Latvia, where contaminated grass and crops fed to pigs were implicated in the 2014 outbreaks of ASFV on backyard farms (Niederwerder 2021). Contamination of cereal grains and grasses fed to commercial pigs was also a likely route of ASFV introduction on farms in Estonia between 2015 and 2017. Further, dried blood products contaminated with ASFV and used as feed additives were suggested as contributors to disease spread in China (Niederwerder 2021). However, there are no published reports confirming detection of ASFV in feed ingredients of complete feed, which is thought to be primarily due to the difficulty in detecting ASFV in feed (Shurson et al., 2022). Although inoculation studies show that ASFV survives in matrices, the reasons behind survival time variation across different matrices are not well understood. These challenges, in addition to lack of data for ASFV contamination of feed and feed supply chains, means few risk assessments have been conducted (Shurson et al., 2022).

There are no published reports indicating that ADV has been detected in feed ingredients or complete feed. Several in vitro studies have been conducted to evaluate ADV viability and inactivation when ADV or a suitable proxy (a proxy in this case being a different but similar virus that can be representative of ADV, such as Bovine Herpesvirus-1 (BHV-1) as used in (Dee et al., 2018) was added to various feed ingredients and complete feed. These inoculation studies have shown that some feed matrices support virus survival longer than others although the reasons for this are unknown. Current analytical methodologies on swine viruses have significant limitations in terms of sensitivity, repeatability, ability to detect viable virus particles and association with infectivity, meaning interpretations of findings can lead to misleading conclusions (Shurson et al., 2022). Scientific data on the persistence of swine viruses on crops under field conditions and on representative matrices are scarce in comparison with the frequency with which such feed stuffs are used as feed for pigs and the potential efficiency of these routes as viral transmission pathways (Patterson et al., 2019; Stoian et al., 2020).

Given the increased understanding that animal feed could be an influential route of import transmission, this risk assessment was therefore undertaken to assess whether or not imported contaminated animal feed is a viable risk pathway for transboundary spread of ADV. There are currently several published risk assessments for spread of ADV via imports, but the routes assessed were legal trade of live animals and animal products, import of pigs for slaughter, and import of pigs for breeding or fattening (de Vos et al., 2022; Martínez-López et al., 2009; Morley, 1993). With this focus on risks from live animals, there currently appear to be no risk assessments focussing on the import of ADV via contaminated feed.

The amount and provenance of feed imports is an important consideration when assessing risk as the worldwide prevalence of ADV will affect the prevalence within the imported feed (Patterson 2021; Blomme 2021). However, given the complicated trade network of feed, we decided to perform this risk assessment for a generic importing country. Therefore, the results are applicable across the spectrum of importing countries. Similarly, other important factors such as virus survival and probability of raw commodity contamination need to be included. Given the uncertainties in these latter factors, and a lack of quantitative information specific to swine feed and ADV, this risk assessment was therefore conducted qualitatively.

2. Methods

The aim of this risk assessment was to assess the probability of transboundary spread of ADV through the import of contaminated feed. This risk assessment is an entry assessment that considers the entry of a pathogen into an importing country, up to and including the point of first infection in an animal in the importing country. The specific risk question assessed was:

What is the risk of Aujeszky's disease virus introduction into an importing country via the import of virus contaminated swine feed?

The risk pathway highlighting the individual steps that would need to occur for an ADV incursion to occur via this route is shown in Fig. 1. Fig. 1 also shows several factors taken into account to estimate the probability that exported feed is contaminated. These factors are not separately estimated probabilities.

We use standard qualitative risk assessment methodology as set out by the World Organisation for Animal Health (WOAH) to estimate the probability of ADV being introduced via imported animal feed and lead to an infection (WOAH, 2004). The risk pathway is developed by taking the start point: "Feed for export is contaminated", and the end point: "Animal in importing country is infected", and then populating all the steps that would need to occur to complete the pathway. For each stage of the pathway (Fig. 1) we provide qualitative estimates of probability, along with an associated uncertainty level. Systematic literature reviews were undertaken for each step, using SCOPUS (scopus.com), PubMed (www.ncbi.nlm.nih.gov/), Google Scholar (scholar.google.com/) and relevant keywords to identify papers and other sources with relevant information. This information was then used to determine these probability and uncertainty estimates.

The definitions of the probability of an event occurring were taken from EFSA (2006) namely, Negligible, so rare that it does not merit to be considered; Very Low, very rare but cannot be excluded; Low, event is rare but does occur; Medium, event occurs regularly; High, event occurs very often; and Very High, event occurs almost certainly. The overall probability was obtained by qualitatively combining these individual probabilities. As these estimates were essentially conditional probabilities (the probability of each step occurring is estimated given that the previous step has already occurred), these were combined using a risk matrix approach as described in Gale et al., 2010.

For each stage of the pathway an uncertainty score was also given according to the definitions given in Table 1. This uncertainty reflects both the data availability and the confidence in the probability score given. For a high uncertainty, we could expect the actual probability to range higher or lower than estimated, but are unable to clearly ascertain an exact probability due to missing or absent data.

The feed matrices considered in P1 are soya beans/meal, soy oilcake, choline/lysine, spray dried porcine plasma, and pet food.

2.1. Parameterisation of P1

The information required to determine a probability for exported feed being contaminated (P1) is related to a larger number of discrete factors than in other steps, and as such, the information for each factor is presented here separately. The final probability for P1 takes all of them into account when determining the probability and uncertainty.

2.1.1. ADV prevalence in exporting country

For feed ingredients to serve as vectors of ADV, feeds or ingredients

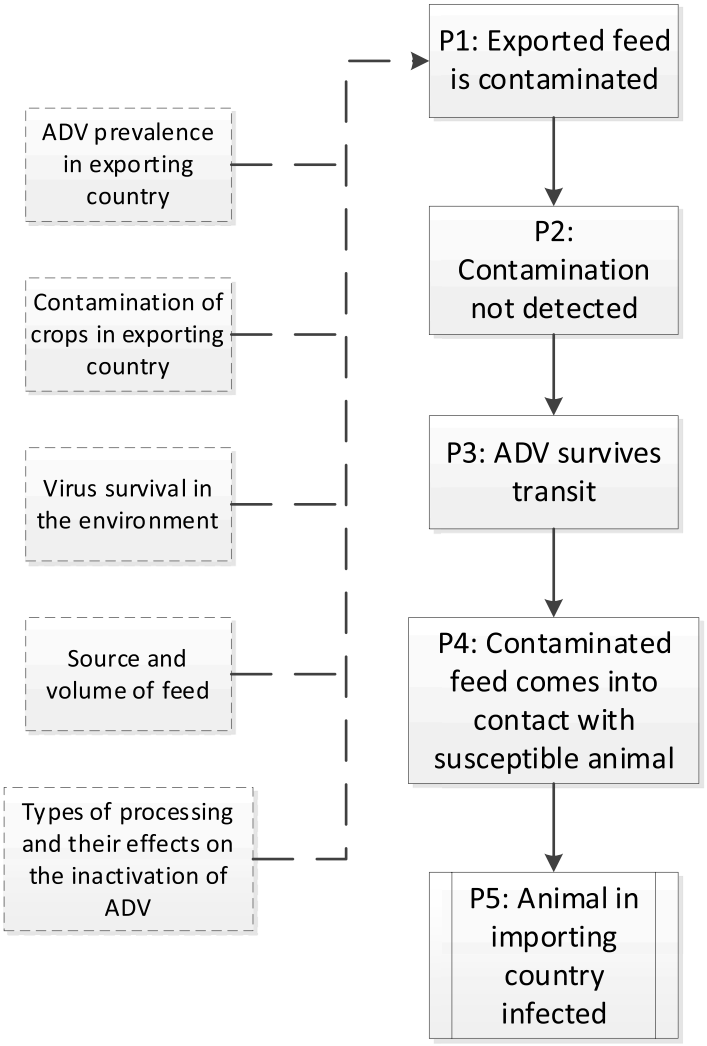

Fig. 1. Pathway for entry of ADV and onward transmission to a native Suid population via imported feedstuffs.

must first have a source of virus contamination and must therefore be sourced from a country with known ADV in the wild boar/domestic swine population (see Table 2). Table 2 suggests there may be some element of seasonality to ADV infections, but no further evidence was found during literature review to support this. It may be due to the 6-month reporting schedule of WOAH WAHIS, or possibly related to with wild boar hunting seasons.

Prevalence of ADV in each country can vary greatly, reported as ranging from 5 to 26% within countries in which the disease is endemic (DAFF, 2004). In Argentina, a prevalence in farmed pigs of 33% has been reported. In Brazil the prevalence in wild boar is as high as 47.3%, with Brazil also reporting its first case in farmed pigs in 20 years in December 2022 (Aznar et al., 2022; Paes et al., 2013; Swine Health 2023).

Management of ADV can be effective in reducing prevalence with studies in China indicating that the high prevalence in the 1980s was brought to as low as 10% across the country by the 1990s after widespread vaccine usage (Tong and Chen, 1999; Kong, 2000). However, as the vaccination campaign was not compulsory, and combined with a lack of a Differentiating Infected from Vaccinated Animals (DIVA) strategy, insufficient regulations and poor biosecurity measures, the campaign was not fully effective and ADV was endemic or sporadic again in all regions of China by the mid-2000s, highlighting the importance of completing management strategies (Zhang and Chen, 2008).

2.1.2. *Contamination of crops in exporting country*

The mechanics by which ADV could contaminate various feed matrices are not well understood. Contamination risks are present at several critical control points during feed manufacture. Specific examples of contamination risks at different control points include exposure of pre-harvest field crops to infected wild boar, exposure of post-harvest grains drying on roadways to vehicles transporting infected pigs, exposure of feed-ingredient processing facilities to infectious fomites such as personnel shoes and exposure of ingredients post-processing to infectious fomites such as multi-use containers (Niederwerder 2021). For example, genetic sequencing after a FMD outbreak in Japan suggested that use of forage (wheat straw) from countries in East Asia was the source of infection. Some of the wheat straw from China was found to be stained with faecal-like substances and was imported in winter when FMD virus reportedly survives for longer periods (Sugiura et al., 2001).

Agricultural commodities, such as straw, forage/roughage and cereals, may all be contaminated with virus from remains of wild boar carcasses or wild boar excretions. Straw may be left to dry on the field for several days before being baled, which also constitutes an opportunity for contamination by wild boar. Longer storage of cereal grains and drying at ambient and high temperatures is expected to result in lower probabilities of virus survival to the point of usage (Nielsen et al., 2021). Conversely, amino acids and vitamins such as choline and lysine are produced in laboratory-type settings, where there is little risk for

Table 1
Qualitative categories for expressing uncertainty given the available evidence; based on definitions within the literature [Spiegelhalter et al., 2011].

Uncertainty category and definition	Type of information/evidence to support uncertainty category
Low Further research is very unlikely to change our confidence in the assessed risk	• Solid and complete data available (e.g. long term monitoring results) • Peer reviewed published studies where design and analysis reduce bias (e.g. • systematic reviews, randomised control trials, outbreak reports using analytical epidemiology) • Complementary evidence provided in multiple references • Expert group risk assessments, specialised expert knowledge, consensus • opinion of experts • Established surveillance systems by recognised authoritative institutions • Authors report similar conclusions
Medium Further research is likely to have an important impact on our confidence in the risk estimate	• Some but no complete data available • Non peer-reviewed published studies/ reports • Observational studies/surveillance reports/outbreak reports • Individual (expert) opinion • Evidence provided in a small number of references • Authors report conclusions that vary from one another
High Further research is very likely to have an important impact on our confidence in the risk estimate	• Scarce or no data available • No published scientific studies available • Evidence is provided in grey literature (unpublished reports, observations, • personal communication) • Individual (non-expert) opinion • Authors report conclusions that vary considerably between them

Table 2
Most recent ADV outbreaks in the last 5 years (WOAH WAHIS, 2023). Countries that do not feature in this table have no recent outbreak data reported in the World Animal Health Information System (WAHIS).

Country	Time period	Domestic or Wild
Argentina	Jul-Dec 2012	Both
Belgium	Jul-Dec 2012	Wild
Bosnia and Herzegovina	Jan-Jun 2021	Domestic
Brazil	Jul-Dec 2019	Both
China	Jul-Dec 2019	Domestic
Croatia	Jan-Jun 2021	Domestic
Cuba	Jan-Jun 2019	Domestic
Estonia	Jul-Dec 2022	Wild
Finland	Jul-Dec 20	Wild
France	Jan-Jun 2023	Both
Germany	Jan-Jun 2018	Wild
Hungary	Jul-Dec 2021	Domestic
Italy	Jul-Dec 2022	Domestic
Mexico	Jul-Dec 2019	Domestic
Panama	Jul-Dec 2020	Domestic
Papua New Guinea	Jul-Dec 2023	Domestic
Poland	Jul-Dec 2020	Domestic
Portugal	Jul-Dec 2022	Both
Serbia	Jan-Jun 2020	Domestic
Spain	Jul-Dec 2022	Both
Timor-Leste	Jul-Dec 2018	Domestic
United States of America	Jul-Dec 2020	Both
Venezuela	Jul-Dec 2018	Domestic

contamination except if mixed with contaminated carriers or placed in contaminated packaging or vehicles during distribution (Korosh et al., 2017). Minerals from animal origin undergo a heat treatment and drastic pH changes that would eliminate any virus present in the original material. Minerals originating from mining have no contact with infected animals.

Contamination in the field will depend on infection in wild boar (i.e., excretions or carcass decomposition) and the persistence of the virus in the environment. The main excretion of the virus from infected pigs is likely to be in the nasal and oral secretions, and urine, faeces and milk likely play, at the most, only a minor part in the dissemination of the disease. Although Nikitin (1961) claimed that recovered pigs could excrete virus in their urine for periods of up to 186 days, other researchers have not been able to repeat this work. McFerran and Dow (1965) did not find detectable virus in either urine or faeces and Beran et al. (1976) did not find it in the kidney or the intestinal wall. Other investigators have not found ADV in the intestines, colon or ileum of infected pigs (Sabo et al. 1968; Zhang et al. 2019). Infected sows have been shown to secrete virus in their milk and pigs suckling them contracted the disease (Kojnok, 1957). The virus has been detected in rectal swabs but not in faeces, and occurs irregularly in urine (Wittmann, 1986).

While in piglets ADV has a multi-organ distribution, the disease is limited to the respiratory and reproductive tract in older animals (Sehl et al. 2020). Field evidence has demonstrated that the virus may remain viable in the carcasses of dead pigs, since these have been shown to infect a pack of hounds after a period of time (Gore et al., 1977). Ustenko (1958) also noted that the virus could live in the muscle of carcasses for 11–18 days at summer temperatures and from 21 to 36 at spring temperatures. The detection of ADV DNA across sample types (blood, nasal, oral, and genital [vaginal] swabs) suggests that viral shedding via direct (oronasal or venereal), and potentially indirect (through carcass consumption) methods, may occur (Hernandez 2018). In the field, it is likely that contamination will not be homogeneous and will involve a lower viral load than experimental investigations (Dee et al., 2018). Contamination of crops may also occur from birthing products. For instance, the finding that 1/24 pooled foetuses from seropositive and viral shedding wild boar sows tested positive by polymerase chain reaction (PCR) suggests this as an additional transmission route (Pacini et al. 2020).

2.1.3. *Virus survival in the environment*

The survival of ADV is a few days in contaminated bedding, soil, feed, manure, grass and water, with some reports of environmental survival for up to 2 weeks, under certain conditions, at 20–24 °C but ADV may remain viable longer when temperatures are very cold (Spickler 2017). The survival of ADV outside the living host has also been found to be dependent on the diluents and fomite combinations into which the virus is suspended. The maximal survival times expressed in days for ADV at 25 °C under moist conditions were found to be as follows: Swine nasal washings, < 2 days; swine saliva, < 4 days; steel, < 4 days; loam soil, < 6 days; green grass, < 2 days; whole corn, < 4 days; pelleted feed, < 1 day; meat and bone meal, < 2 days; alfalfa, < 1 day; straw, < 4 days. The maximum survival time for ADV at 25 °C, under moist conditions, when suspended in samples of fresh swine faeces, or effluent from swine pits or lagoons, was less than 2 days, 1 day, or 2 days, respectively. Sow urine, uncontaminated with faecal products, maintained titres infectious to 2-week-old pigs for up to 14 days, at 25 °C (Freund 1981).

As detailed in Section 3.1.2, the virus remains viable in carcasses of dead pigs, which would allow carcasses to remain infectious in the environment (Gore et al., 1977; Ustenko 1958).

Under direct sunlight exposure with a lowest temperature of 38 °C, a highest temperature of 39 °C and an average temperature of 38.16 °C, ADV lost the ability to infect cells after approximately 10 min. Under the same temperature conditions, the virus samples blocked by cardboard survived for more than 30 min. Therefore, at the same temperature, sunlight exposure will accelerate virus inactivation, and sunlight can kill ADV (Gong 2020).

2.1.4. Types of processing and their effects on the inactivation of ADV

Some raw products may undergo a thermal, enzymatic, or pH-driven step during processing that reduces viral viability. Due to the different processing methods for different matrices, we consider these separately.

2.1.5. Spray dried porcine plasma (SDPP)

Spray dried porcine plasma is a feed ingredient commonly used in diets of young animals. Typically, SDPP is manufactured from blood collected from pigs or at inspected abattoirs. Experimental investigations have found that PEDV RNA and porcine circovirus type 2 DNA present in commercial SDPP is not infectious to naïve pigs (Opriessnig et al. 2014; Pujols et al., 2008). This confirms that while SDPP may be PCR positive, PCR-positive test results do not imply infectivity (Russell et al., 2020). The WOAH Scientific Commission on Animal Diseases determined that SDPP is not a likely source of infectious virus if good manufacturing procedures are followed. According to the WHO guidelines, viral safety is derived from three complementary approaches during manufacturing: donor selection, testing of donation and plasma pools and viral inactivation by spray drying at 80 °C. Experimentally, bovine plasma was inoculated with ADV and spray dried in the same manner and conditions of industrial SDPP production. Before spray-drying, all samples contained $10^{5.3}$ tissue culture infectious dose ($TCID_{50}$)/mL of ADV, but no viable virus was detected in samples of spray dried bovine plasma (Polo et al., 2005).

2.1.6. Soya meal

During harvesting, field crops might be protected by a spike, cop, legume or shell, and weather conditions may wash off any contaminants. In addition, the raw material is likely to be transported several times (harvest, rail, truck, etc.) before it reaches a feed mill for processing into animal feed. All these factors may reduce the amount of contamination or infectious virus on a product. However, this also increases opportunities for cross-contamination to occur during the transport process.

Soybean meal is the by-product of the extraction of soybean oil. Several processing methods exist, but in modern soybean processing plants, soybeans are processed using a solvent extraction process which removes most of the oil. The oil-extracted residue is then heated to drive off the solvent and carefully toasted to a prescribed temperature (Cromwell, 2012). Toasting of the product at temperatures of 60 °C upwards is common and also converts the vegetable protein into a readily digestible form. The following processes can also be applied to the production of full fat soybean meal: boiling/autoclaving, roasting/toasting and extruding (wet or dry). Extrusion is a continuous process and dry extruders generate heat and pressure mechanically as a result of the frictional and shear forces produced within the extruder barrel. The heat and pressure generated typically raises the temperature to 150–160 °C (Riaz 2008).

Although there are no data for ADV, the effect of drying and heat treatment on the inactivation of ASFV on six different types of field crops, namely wheat, barley, rye, triticale, corn, and peas, contaminated with infectious blood, has been investigated. Samples were analysed for the presence of viral DNA and infectious virus after drying for 2 hr at room temperature or after drying and 1 hr exposure to moderate heat at a specific temperature between 40 °C and 75 °C. The ASFV genome was detected in all samples by real time PCR, including samples that had been dried for 2 hr and incubated for 1 hr at 75 °C. However, no infectious virus could be detected after 2 hr drying using virus isolation in porcine macrophages (Fischer et al. 2020).

Contamination of feed processing mills has also been investigated by introducing PEDV and ASFV contaminated feed. Data was not found specific to ADV, but ASFV and PEDV are porcine diseases known for causing infections via contaminated feed routes (Elijah et al., 2021). ASFV has a similar persistence in the environment when compared to ADV, with similar increases in survival time under colder conditions, although the maximum time ASFV can survive in the environment exceed that of ADV by a number of months (Spickler, 2017). Flushing animal food-contact surfaces with low-risk feed is commonly used to reduce cross-contamination in animal feed manufacturing but even after 4 subsequent flushes after PEDV had been introduced, animal food-contact surfaces retained PEDV RNA (28/33 positive samples/total samples), with the conveying system being more contaminated than the mixer. A bioassay to test infectivity of dust from animal food-contact surfaces, however, failed to produce infectivity. This study demonstrates the potential widespread viral contamination of surfaces in an animal food manufacturing facility (Schumacher et al., 2017). A later study with PEDV found that pigs that were fed flushed feed batches after PEDV introduction were positive by real-time reverse transcriptase semiquantitative polymerase chain reaction (rRT-PCR) on faecal swabs by the end of the study and that sequential batches had reduced quantities of PEDV RNA, although flushed feed without detectible PEDV RNA by rRt-PCR could also be infectious (Schumacher et al., 2018). For ASFV, a greater amount of viral genome was detected on transient surfaces compared to other surfaces ($P < 0.05$). This study illustrated that once ASFV enters the feed mill environment it can become widespread and movement of people can significantly contribute to the spread of ASFV in a feed mill environment (Elijah et al., 2021; Gebhardt et al., 2022). A significant limitation of these studies is the lack of infectivity data associated with the feed containing quantitative PCR detectable ASFV-specific DNA. It is important to note the difference between PCR and virus/infectivity (Fischer et al. 2020).

2.1.7. Pet food

Species other than pigs can become infected by close contact with infected pigs, or by ingesting contaminated offal from pigs or other animals (Spickler 2017). Pet food could become contaminated if animals infected with ADV were slaughtered and their offal used for pet foods. Animals with AD may not exhibit clinical signs (Underwood, Blauwiekel et al. 2015). However, testing detects prevalence ranging from 5% (in holdings with eradication programmes) to 10% (in holdings in AD-free areas) annually {Allepuz, 2009 #48}. Hence, in regions with eradication programs, it is likely that infected animals will be detected by clinical signs or testing before being sent to slaughter. Farms in AD-free regions will be less likely to detect cases before sending animals to slaughter, given their lower rates of testing. Neither designation has sufficient controls in place to negate the probability of infected animals going to slaughter. There is no legal requirement to test slaughtered animals for ADV, and all statutory surveillance is done ante-mortem on animals to be processed into meat for human consumption. Inspection at slaughter, without suspicion of disease, is therefore unlikely to isolate and remove infected carcasses.

Using pet food produced in the European Union (EU) as an example, pet food is produced according to rules set by FEDIAF, the European Pet Food Industry. Canned pet food and other hermetically sealed heat-treated containers must be subject to heat treatment to a minimum Fc value of– 3, a processing standard that specifies that the core temperature of the product has reached 121 °C for 3 min, or an equivalent time-temperature parameter. Processed pet food other than canned pet food or other hermetically sealed heat-treated containers must be subject to a heat treatment of at least 90 °C throughout its substance. Dog chews must be subject to a heat treatment during processing sufficient to destroy pathogenic organisms. After treatment, every precaution must be taken to ensure that the product is not exposed to contamination. After heat treatment all products must then be repacked in new packaging (FEDIAF, 2010). ADV is susceptible to inactivation at temperatures of 60–62 °C (Turner et al., 2000), so this treatment would be sufficient to inactivate any virus at this stage. This risk would need to be re-assessed if pet food were to be imported from non-EU countries, as per their own pet food production and export guidelines.

2.1.8. Source and amount of feed

The amount of feedstuff imports that could be potentially contaminated with ADV is an important factor in determining the overall

probability of introduction. Data on the weight of feedstuffs a country imports will be specific to that nation. To give an indication of the scale involved in feed import/export, the largest global exporters of soybeans for 2022 were Brazil with 50% of the world soybean exports at $46 billion, the USA was 37% ($34 billion) and next highest was Argentina with 3.3% ($3.08 billion) (TrendEconomy, 2023). In 2022 the average price for a metric ton of soybeans was 675 nominal U.S. dollars, meaning Brazil exported approximately 68,148,148 tonnes of soybeans (World Bank, 2023).This matches the order of the top three global producers of soybeans (Agriculture, 2023). The largest global importers of soybeans were China at 70%, Mexico at 3.02% and Japan at 2.98% (TrendEconomy, 2023).

A country with a small proportion of contamination could export large amounts of potentially harmful feed if they are a large exporter. Additionally, as modern supply chains are complex, it is difficult to determine the true origin of feed. Export goods may pass through multiple countries and import data can often only report the most recent country passed through. For example, the Netherlands is the largest EU exporter of soybeans, but produces very little of its own and primarily re-exports from Brazil (EFECA 2018).

This problem, multiplied over the number of potentially contaminated feed products that are imported, raises the uncertainty of the probability exported feed is contaminated.

2.2. Parameterisation of P2

Literature review did not provide any evidence of testing systems for ADV on import of feedstuffs. However, there are studies showing detections of PEDV and ASFV are detectable in bulk feed, although there is varying effectiveness of detection depending on the testing methods used but also the initial dosage used to spike the feed (Jones et al., 2020; Diel, 2021; Gebhardt et al., 2022).

As ADV contamination of soybeans is a viral contamination rather than live animal infection or pathogenic growth on the material, visual inspection of the load would not be sufficient to identify contamination. PCR, cell culture, and virus isolation testing methods are able to detect ADV contamination in a variety of feed matrices (Dee et al. 2018; Stoian et al. 2020), however there do not appear to be any legal requirements for testing of feed not of animal origin for viruses in, for example in the EU.

2.3. Parameterisation of P3

If an ingredient is contaminated with a virus, the pathogen must survive transport to be able to cause onward transmission. For imported feed, this would involve shipment in varying temperatures and humidity, as well as significant variation in time spent in transport or storage. Given the stability of the virus in feed and the high variation in the chain of transport it is impossible to estimate the length of time a given contaminated export would take through manufacture, transport, import checks, import processing, and potential storage for an unknown amount of time at potentially multiple points in the journey. Although using a proxy virus for ASFV, and therefore limited in usefulness when compared to ADV, one study followed spiked feedstuff matrices across a 23-day commercial transport model. The study found the proxy virus in all matrices without degradation in viability after the full 23 days. The paper concluded that sampling sensitivity rather than virus inactivation was the main reason for variation in virus quantity detected after transport (Palowski et al., 2022).

As ADV is a virus, growth does not occur outside the host cell so viral loads on any transported feed can only remain stable or be reduced, with the reduction increasing as time increases. Schoenbaum et al. (1991) conducted an experimental study to investigate the survival duration of ADV in contact with various solid and liquid fomites commonly found in swine production environments. Feed or feed ingredients included in the study were green grass, whole corn, pelleted feed, and alfalfa. The authors inoculated various solid and liquid fomites with mixed stock ADV and incubated the samples at room temperature for up to 14 days. Virus activity was assessed through a cell culture-based assay. They found that, in general, the quantity of infectious virus decreased over time. Of the feed or feed ingredients included in the study, the combination of ADV/saline/whole corn remained infectious the longest at 7 days with an estimated half-life of 36.3 h (Schoenbaum et al. 1991). There is evidence of environmental survival of ADV, but not data to show the effect of environmental factors on the survival of ADV in feed matrices. The general survival of ADV is a few days in contaminated bedding, soil, feed, manure, grass and water, with some reports of environmental survival for up to 2 weeks, under certain conditions, at 20–24 °C but ADV may remain viable longer when temperatures are very cold (Spickler 2017).

The potential for entry of swine viruses through import of ingredients has been determined directly for ASFV, and indirectly via surrogates for FMDV and classical swine fever virus (CSFV) (Dee et al., 2018). The survival of ADV in feed matrices is less well studied although two papers suggest the survival of ADV in various matrices is between 4.4 to 37 days (Dee et al. 2018; Stoian et al. 2020). The large difference in these survival times could be due to the study by Dee et al. using Bovine Herpesvirus-1 (BHV-1) as a proxy virus for the survival of ADV, whereas the paper by Stoian et al. used ADV directly. The BHV-1 proxy had a halflife of 4.4 days in conventional soybean meal and soybean oilcake, whereas the ADV survived for 37 days in a variety of matrices: conventional and organic soybean meal, vitamin D, moist dog food, stock virus control, lysine, choline, moist cat food and dry dog food. Neither Dee et al. nor Stoian et al. tested whole corn hindering a comparison to Schoenbaum's earlier work.

In addition, Stoian et al. tested some of the matrices for ADV via cell culture and bioassay PCR after 37 days of exposure to modelled transport conditions. With an initial inoculation of 10^5 $TCID_{50}$, after 37 days cell cultures produced mean titres of $10^{3.6}$ to $10^{4.2}TCID_{50}$ for soybean meal, $10^{3.3}TCID_{50}$ for moist dog food and $10^{4.3}TCID_{50}$ for Vitamin D; compared to $10^{3.0}TCID_{50}$ for virus grown in media. Cell culture could not detect virus in oil cake, lysine, choline, and moist cat food. The bioassay PCR found detectable virus in lysine, choline, moist cat food and dry dog food, with higher concentrations in the tonsil samples compared to serum or cerebrum. PCR did not detect any virus in oil cake either. The chosen matrices provide at least as suitable an environment for environmental survival as virus in media, showing that ADV could survive transport, albeit with a reduction in dosage.

While these data are important, they are also limited. First, it is proof-of-concept research with high levels of viral inoculum and limited sample size and quantity. Furthermore, the research utilised one combination of time × temperature scenarios but in real life, as temperature fluctuates, virus degrades at varying rates. For example, viral degradation would be faster in a hot warehouse during summers versus a cold warehouse in the winter. Additionally, surrogates were necessary due to the limited number of facilities where this research could be conducted. Even with these gaps, these data are important in that it establishes the theoretical potential for import of ingredients to be a transboundary vector of virus entry into the domestic feed supply (Jones et al., 2019).

There do not appear to be any rules or laws prescribing certain transport methods or temperatures for transit of other animal feeds. However, information from the German Transport Information Service (2021) suggests an ideal transport temperature of between 5 and 25 °C and that transport can be done via train, truck and boat.

2.4. Parameterisation of P4

A recent European Food Safety Authority (EFSA) report used expert knowledge elicitation (EKE) to assess how imported feed would potentially be distributed in an importing country. For cereals, including soy beans, it was considered that a large proportion of grains harvested will be used as animal feed and will go directly to a farm. The rest will be

used in the production of compound feed. Cereal grains would be transported mostly by ship and in large amounts and could be produced both in Eurasia and in the EU. Many small farms were considered to produce their own cereal grains or to use compound feed rather than commercial grains. Large shipping containers were more likely to be delivered to feed merchants and then distributed. The EKE experts estimated that the average number of farms receiving a delivery from a single consignment was significantly higher for small farms than for large farms. The discussion around the estimation indicated that not only will large-scale farms import multiple consignments of a single commodity, but also that the small-scale farms may not use a whole consignment but may in fact share it with other local farms through a feed distributor (Nielsen et al., 2021).

The probability of contaminated feed coming into contact with pigs will therefore depend on the size of farm, whether the farm buys in feed and, whether the feed is single ingredient or compound. Further transport, storage, processing or mixing may also occur to imported feed once it has entered an importing country. Processing or manufacture of different formulations of feed for breeding, rearing, or finishing post-import, will also impact the probability of any contaminant coming into contact with a susceptible animal. Any contamination will be considerably diluted as the feed undergoes further post-import processing such as mixing and bagging before distribution to different farms. This could, however, mean that a greater number of animals may come into contact with virus, albeit at a lower dose.

2.5. Parameterisation of P5

ADV spreads primarily through venereal, respiratory, and oral transmission (Spickler, 2017). It can also be transported through airborne transmission between farms (Christensen et al., 1990) and can spread through the consumption of meat from infected pigs by wild animals. Almost all mammals can be infected but some species such as horses and birds require high viral doses to become infected (WOAH, 2018).

To cause infection within an animal, there must be sufficient quantities of virus within a feed or ingredient to cause infectivity. Due to the large variation in travel time possible with the transport of feed, there is a possibility that virus levels could diminish and no longer be sufficient to cause disease when in contact with susceptible animals, but this is difficult to quantify as data on travel times and ADV degradation in relevant matrices are not well studied.

One of the reasons that PEDV is so easily spread through the feed supply chain is its low infectious dose – just 5.6×10^1 TCID50/g in feed has been experimentally demonstrated to be infectious via bioassay (Schumacher et al., 2017). Importantly, the probability of infection is based on both dose and number of exposures. For example, if pigs with a single exposure to feed containing 10^4 $TCID_{50}$ virus have a probability of infection of 25%, the probability of infection would increase as the number of exposures increases. Conversely, the process of feed manufacturing would likely homogenise viral contamination throughout a batch of feed, reducing its dose, but increasing the potential number of exposures (Jones et al., 2019).

The dose response for ADV through natural feeding behaviour consuming contaminated feed has yet to be established, nor has the dose response in suidae via other infection methods been well studied. Pigs inoculated in the nostril with $10^1$$TCID_{50}$ of NIA-2 strain of ADV still developed pyrexia and nervous signs albeit much delayed – nervous signs were not apparent in all 6 pigs until 12 days post-infection (dpi). In addition, pigs infected with such a low dose did not show any other typical signs such as sneezing or respiratory distress (Baskerville 1972). The oral infectious dose of ADV infection for pigs has been estimated to be 10^1 to 10^3 $TCID_{50}$ for piglets, 10^4 $TCID_{50}$ for young pigs and 10^4 to 10^5 $TCID_{50}$ for adult pigs (Wittmann 1991).

3. Results

3.1. Probability exported feed is contaminated (P1)

The probability of the commodity being contaminated (P1) taking into account the sub-steps (ADV prevalence in exporting country, contamination of crops in exporting country, processing, import of feed, virus survival in the environment), was considered to be **Negligible** for choline/lysine, SDPP and pet food due to either the lack of contact with infected animals (choline/lysine), the sourcing from pathogen free herds (SDPP) or the processing steps which are known to inactivate ADV (pet food) (Table 3) (Korosh et al., 2017; Polo et al., 2005; FEDIAF 2010). Therefore, commodities other than soya beans, meal and oilcake were not considered further.

Although China and South American countries are major exporters of soya beans/meal crops and have high prevalence of ADV in both domestic and wild animals (Table 2), the probability that contaminated soya beans/meal crops would be exported is considered to be **Very Low** due to the effects of processing, with a **Medium** uncertainty surrounding the initial contamination on the crop and the range of processes which it may have undergone before export (Riaz, 2008).

However, there are data gaps when estimating these probabilities. Tracking the full trade path of an import can be difficult or impossible and does not accurately reflect the probability of contamination if the source country is misrepresented. Prevalence data is likewise readily available from WOAH WAHIS but limited in that it lacks granularity and detail. It is also unknown how, or if, the crop would initially become contaminated. This is reflected by the **Medium** uncertainty associated with this step.

3.2. Probability contamination is not detected (P2)

The probability that contamination is not detected is considered to be **Very High**, with a **Medium** uncertainty as no evidence for compulsory testing schemes were found.

3.3. Probability ADV survives transit (P3)

The probability ADV survives transport is considered to be **Medium** with **High** uncertainty, due to the high range of survival time of ADV depending on conditions (Dee et al. 2018; Stoian et al. 2020; Schoenbaum et al. 1991). However, there is currently a lack of data for survival time specific to feedstuffs in the field and there are large differences in results from the experimental data.

3.4. Probability contaminated feed comes into contact with a susceptible animal (P4)

It is assumed that imported animal feed is intended for animal consumption and that the vast majority of imported feed will come into direct contact with animals. Whilst soya meal can be used as feed for other livestock sectors, the swine industry is the second largest consumer of soy products (EFECA 2018). The probability for this step was therefore considered to be **Very High** with a **Low** uncertainty.

Table 3
Probability estimates of commodities being contaminated before and after processing steps.

Commodity	Prior to processing	After processing	Overall probability
Soya beans/meal	Low	Very low	Very low
Soy oilcake	Low	Very low	Very low
Choline/lysine	Very low	Negligible	Negligible
Spray dried porcine plasma	Negligible	Negligible	Negligible
Pet food	Very low	Negligible	Negligible

3.5. Probability an animal in importing country is infected (P5)

Any contamination of animal feed that does occur is expected to be in very low amounts. It is unclear by what mechanism ADV contaminated feed would infect pigs as no confirmed cases of natural ADV infection from contaminated feed have ever been reported. It is possible that in addition to direct consumption of contaminated feed, ADV contained within feed could become airborne as feed is churned around either by the replenishing of the feed or the movement of feed during consumption, but viral titres are expected to be very low. The probability of infection is considered to be **Very Low** with a **Medium** uncertainty, due to the absence of information regarding transmission from contaminated feed by ADV in swine.

3.6. Risk summary

The final probability and uncertainty estimates are presented in Table 4. The overall probability of an animal becoming infected from the importation of feed contaminated with ADV was assessed to be **Very Low**. This was primarily due to the probability that exported feed is contaminated at the beginning of the pathway and the probability that an animal becomes infected at the end of the pathway. Probabilities were multiplied together using a risk matrix approach as described in Gale et al., 2010.

The uncertainty for these key steps is considered to be **Medium** for both, due to the lack of data around the mechanisms that ADV could contaminate feedstuffs and for infection of susceptible animals from ADV infected feed.

4. Discussion

This risk assessment was undertaken to assess the risk of ADV contaminated imported feed as a route of ADV introduction and determined that the overall probability was **Very Low** with **Medium** uncertainty. Other published risk assessments for the spread of ADV via imports have concluded that the risk from live animals is less important than the risk from animal products (de Vos et al., 2022; Martínez-López et al., 2009; Morley, 1993). One paper found there was a 99% probability of less than one swine herd ADV infection in 50 years as a result of the importation of pigs from USA to abattoirs in Canada (Morley, 1993), whereas another concluded that the mean probability of introducing ADV-infected animals, when breeding pigs were quarantined but not tested prior to shipment, is likely up to 21% (Martínez-López et al., 2009). The risks involved between ADV and the import of live animals are complex and depend on testing protocols as well as the destination and use of the animals. This suggests that a route of introduction via feed is not a pathway of particular concern, although this could change in the future depending on the prevalence of ADV in feed exporting countries and also if any new evidence to suggest mechanisms for ADV to infect naïve swine from contaminated feed.

However, whilst experimental studies have demonstrated that ADV survives in certain feed matrices including those used for pig feed, there are still significant data gaps throughout the risk pathway (Dee et al. 2018; Stoian et al. 2020; Schoenbaum et al. 1991). The data suggest that viral loads do reduce during transport, but no consistent rate of degradation is available, and, in addition, there is a lack of data for ADV dose response. Studies into contaminated feed matrices, particularly pertaining to pig viruses, appear to have arisen as a response to outbreaks of PEDV from contaminated feed tote bags containing feed pellets (Scott et al. 2016; Schumacher et al. 2017; Bowman et al. 2015). PEDV is transmitted to pigs via the ingestion of virus-contaminated faeces (WOAH, 2014); faecal contamination on clothes and boots is able to transfer to tote bags and the feed within (Scott et al. 2016). This is contrary to ADV, which has never been detected in faeces (Spickler 2017). Whether ADV could infect pigs via ingestion of contaminated feed has not been confirmed and requires further study.

This assessment agreed with a recent review which found that whilst the scientific literature has addressed some critical experimental questions pertaining to transmission of swine viruses via feed and feed ingredients, the current body of scientific knowledge lacks conclusive evidence of virus contamination of non-animal origin feed ingredients of commercial swine feed, particularly for imported commodities. Further investigation into the mechanics of virus transmission via feed to swine under field conditions through natural feeding behaviour is needed. Additional studies of how imported ingredients of commercial swine feed are sourced, processed, transported and, thus, contaminated prior to importation are also needed (Gordon et al., 2019). There are no published reports indicating that porcine viruses other than PEDV have been detected in feed ingredients or complete feed. Because of this lack of viral contamination data in feed supply chains, quantitative risk assessments have not been conducted for other viruses such as ASFV (Shurson et al., 2022).

Several key uncertainties were identified whilst carrying out this risk assessment. Several parameters of the virus were lacking confirmed data, such as how ADV could contaminate feed in order to present an initial risk, in addition to uncertainty on the prevalence of ADV, even in countries where it is considered endemic. There is also high variance in the survival of ADV under different environmental conditions, and few studies on the dose response for ADV. There is also uncertainty due to a lack of information on the feed matrices, primarily data on their production and traceability, but also on inspection practices. Whilst data on the number of imports by product are available, much uncertainty remains around the other steps of the pathway e.g., probability of commodity contamination and virus survival throughout transit.

This risk assessment concluded that the risk of transboundary spread of ADV via import of contaminated feed was **Very Low**, with the caveat of **Medium** uncertainty. This represents the first comprehensive risk assessment of ADV in feed, and has identified key data gaps and uncertainties that, should they be addressed in the future, would give a clearer picture of the risks of this pathway. The most key of these data gaps is the lack of research into a method of transmission by which pigs could become infected from ADV contaminated feed, which could warrant further study.

Table 4
Combination of risk scores determined using the risk matrix from Gale et al., 2010.

Pathway Step	Probability estimate	Uncertainty estimate
P1: Exported feed is contaminated	Very Low	Medium
P2: Contamination not detected	Very High	Medium
P3: ADV survives transit	Medium	High
P4: Contact with susceptible host	Very High	Low
P5: Animal in importing country becomes infected	Very Low	Medium
Overall Probability	**Very Low**	**Medium**

Author statement

All authors have agreed with the amendments for the manuscript as submitted.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a risk assessment article with no original research data.

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Daniel Evans: Writing – original draft, Investigation. **Verity Horigan:** Writing – review & editing, Writing – original draft, Investigation. **Rachel A. Taylor:** Writing – review & editing. **Louise Kelly:** Writing – review & editing, Conceptualization.

Declaration of competing interest

None.

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