Literature Review: Non-animal Origin Feed Ingredients and the Transmission of Viral Pathogens of Swine

United States Department of Agriculture
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1 Executive Summary

The United State Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) has conducted a literature review on the scientific evidence regarding whether non-animal origin ingredients of commercial swine feed could introduce or transmit viral pathogens of swine into or within the United States. The purpose of this literature review was to identify, evaluate, and summarize the current scientific knowledge published through March 2018 regarding this topic and to identify information gaps, thereby, making the available evidence more accessible to decision makers, other stakeholders, and the scientific community. The results may support future scientific research and/or risk quantifying models for evaluating the risk (or likelihood) of entry of exotic viral pathogens via specific feed ingredients from source countries and subsequent exposure to United States (U.S.) swine populations.

The methodology of this literature review follows the basic framework of a qualitative systematic review and has four main components: 1) identifying and selecting research evidence, 2) data extraction and quality assessment, 3) data synthesis, and 4) report writing [1]. Twenty-six published articles were included in the literature review. The major findings and information gaps are highlighted below:

- A subset of the studies reviewed provided experimental evidence that swine viruses can survive in non-animal origin feed ingredients under various experimental conditions. Virus survival times were variable (range: 7 days to > 180 days) and dependent on the simulated environmental conditions applied (e.g., temperature and relative humidity) and the virus-ingredient combination. Additional research is needed to verify virus survival times (and infectivity) in complete feed and feed ingredients, with various virus-ingredient combinations under various environmental conditions, including actual field conditions.
- Several experimental studies provided evidence that feed contaminated with virus can transmit disease to naive piglets. However, the experimental methods used in these studies such as spiking feed ingredients with a predetermined virus load or inoculating piglets via methods other than natural feeding behaviors do not necessarily reflect the field setting and results may not be generalizable to field conditions, particularly the large, commercial swine production setting. Additional laboratory and field-based studies are needed to determine the extent of reproducibility and applicability of these experimental findings to field settings.
- Several studies have investigated whether some individual feed ingredients are more likely than others to support virus survivability. Viable virus (meaning positive by virus isolation test and/or bioassay) was detected in the following non-animal origin ingredients that were experimentally spiked with virus: organic and conventional soybean meal, dried distillers grain with solubles (DDGS), lysine HCL, D/L methionine, choline chloride, and vitamin D. Two experimental studies, using different experimental conditions, observed porcine epidemic diarrhea virus (PEDV) viability in three PEDV-spiked ingredients: conventional soybean meal, lysine, and choline chloride. Virus viability (and infectivity) as determined in separate swine bioassays was observed

with choline chloride and two swine viruses: PEDV and Seneca virus A. The implications of these findings for field settings are unclear.

- A major knowledge gap exists with respect to potential source(s) of contamination and where feed or feed ingredients may be contaminated, particularly for non-animal origin ingredients sourced outside the United States. Current studies have produced little scientific evidence of how, or if, non-animal origin feed ingredients could become contaminated with swine viruses in regions outside the United States. The critical point(s) of susceptibility to contamination along the feed production, processing, and distribution continuum, from harvesting the plant-derived feed ingredients in the field to on-farm delivery of feed to swine premises, have not been identified. Neither the contamination route of exotic swine pathogens into non-animal origin feed (ingredients) nor the virus entry route into the United States has been decisively proven.
- Under laboratory-simulated model conditions, both the formaldehyde-based liquid antimicrobial SalCURB® and a medium chain fatty acid blend were concluded to be effective chemical mitigants against PEDV. The real-world application of these mitigants for eliminating swine viruses or decreasing their level of infectivity under field conditions has yet to be determined. Additional mitigation strategies should continue to be explored, including other chemical treatments, the application of heat or pressure (pelleting) to feed and of various holding times to feed or feed ingredients.
- When considering non-animal origin feed ingredients as potential fomites for swine virus transmission, a major knowledge gap exist with how the primary transmission pathways (e.g., exposure to infected live pigs, contaminated transport vehicles, personnel, etc.) interface with one another, particularly how the production and distribution of feed interact with other potential sources of virus contamination (e.g., infected live pigs, contaminated transport vehicles, personnel, etc.) to contribute, if at all, to disease transmission.
- Reliable and validated assays and sampling techniques capable of detecting infective virus (i.e., to determine the level of contamination is sufficient to transmit disease) in large quantities of (bulk) ingredients are not available.
- The entry of PEDV (and new or emerging swine viruses) onto presumably biosecure commercial premises suggests that current biosecurity standards may be insufficient to prevent virus incursion. Virus characteristics and the characteristics of the commercial swine industry (globalization of trade, intensification and vertical integration of production, and extensive movement of pigs and related production components) could contribute to biosecurity breaches. Robust biosecurity measures may be the only tool, in the absence of effective vaccines or treatments, to prevent the entry and spread of some diseases. Thus, biosecurity strategies, particularly the extensive movement of production inputs, need to be re-evaluated and adjusted to meet today's swine industry paradigm.

While investigators have addressed some critical experimental questions pertaining to transmission of swine viruses via feed and feed ingredients, the current body of scientific knowledge has yet to provide conclusive evidence for the source(s) of contamination of non-animal origin feed ingredients with swine viruses and the epidemiology of virus transmission to swine under field conditions. If the primary

concern of the swine industry and associated stakeholders lies in the importation of contaminated feed and feed ingredients, then additional research and investigative studies of how ingredients are sourced, processed, and transported prior to importation into the United States are needed. However, the lack of feed and feed ingredient diagnostic assays capable of detecting virus in large volumes of material limits our ability to determine if and at what point non-animal origin feed or feed ingredients may become contaminated with viruses and limits our ability to establish critical control points in feed production, distribution, and storage to mitigate risk(s). Until these data are available, it is difficult to evaluate the biosecurity risk posed by non-animal origin feed and feed ingredients. Moving forward, studies designed to examine the likely source(s) of contamination and virus mitigation steps in processing and post-processing may be the most fruitful focus of research.

2 Acronyms

APHIS	Animal and Plant Health Inspection Service
ASF(V)	African swine fever (virus)
CSFV	classical swine fever virus
Ct	cycle threshold
DDGS	dried distillers grain with solubles
DPI	day(s) post-inoculation
EFSA	European Food Safety Authority
FMDV	foot and mouth disease virus
HP-PRRS	highly pathogenic porcine reproductive and respiratory syndrome
IAV-S	influenza A virus of swine
LA	liquid antimicrobial
MBM	meat and bone meal
MCFA	medium chain fatty acid blend
OIE	World Organization for Animal Health
PCR	polymerase chain reaction
PCV2	porcine circovirus type 2
PDCoV	porcine delta coronavirus
PED(V)	porcine epidemic diarrhea (virus)
PHFD	porcine high fever disease
PRRS(V)	porcine reproductive and respiratory syndrome (virus)
PRV	pseudorabies virus
RBC	red blood cells
RNA	ribonucleic acid
RT-PCR	reverse transcription-polymerase chain reaction
SDPP	spray-dried porcine plasma
SVDV	swine vesicular disease virus
$TCID_{50}$	tissue culture infectious dose 50
TGEV	transmissible gastroenteritis virus
U.S.	United States
VESV	vesicular exanthema of swine virus
VI	virus isolation
VSV	vesicular stomatitis virus
VTM	vitamin/trace mineral

3 Introduction

Over the past three decades, the swine industry in the United States has experienced several significant disease outbreak events with highly pathogenic viral pathogens, including porcine reproductive and respiratory syndrome virus (PPRSV), porcine circovirus type 2 (PCV2), and, most recently, the swine enteric coronaviruses, including porcine delta coronavirus (PDCoV) and porcine epidemic diarrhea virus (PEDV) [2-5]. These disease events have resulted in significant clinical consequences with increased morbidity and mortality, in some cases reaching 100%, as well as economic devastation to the swine industry with financial losses estimated in the hundreds of millions to billions of dollars [4, 6]. Among other shared characteristics, all three causative agents had been previously known to cause mild or non-pathogenic disease in swine prior to the re-emergence event. Additionally, novel agents, such as PEDV, may be present yet remain undetected for some time, contributing to (wide) spread transmission amongst the industry and hindering local containment. Thus, once identified, the unforeseen emergence of swine diseases with high morbidity and mortality and rapid, transboundary spread brings about fundamental questions (how, what, why, when, and from where) as well as an immediate need to find solutions for both short- and long-term response activities and mitigation strategies to control the outbreak and prevent future events [5].

During the 2013 - 2014 outbreak of porcine epidemic diarrhea (PED) in North America, contaminated feed and feed ingredients were suspected as a potential introduction and/or transmission route for spread as early cases of PED in Canada were linked to a common feed source containing spray-dried porcine plasma (SDPP) [7]. Additionally, genetic and phylogenetic analyses revealed that United States (U.S.) strains were closely related to Chinese PEDV strains, particularly the 2012 strain from the Anhui Province in China [8], fueling concerns that imported (contaminated) commodities from China may have been the route of introduction into the United States. Growing anecdotal evidence and early investigative studies [9] have further implicated feed and feed ingredients as the possible transmission vehicle for PEDV although a definitive introductory cause remains unknown. Compounded by the recent outbreaks of African swine fever (ASF) in China and the European Union [10], there is rising concern that contaminated imported commodities, particularly non-animal origin feed ingredients of commercial swine feed, could introduce and transmit viral pathogens of significant concern to the United States (U.S.) swine industry.

The Animal and Plant Health Inspection Service (APHIS) has conducted two analyses concerning the introduction of exotic swine diseases into the United States. The scope of each assessment included feed and feed ingredients. In 2014, APHIS completed the *Pathways Assessment: Entry Assessment for Exotic Viral Pathogens of Swine.* This assessment estimated the likelihood of entry of exotic swine viruses via several import pathways based on the quantity of the commodity imported, the likelihood that a hazard would be associated with the pathway, and the likelihood that a hazard would persist under current import mitigation procedures. Several pathways were estimated to pose a non-negligible risk of introducing exotic viral pathogens of swine into the United States, including animal

feed ingredients derived from unprocessed plants or plant products; however, significant gaps in knowledge and available data prohibited a conclusive assessment. In 2015, APHIS completed the *Swine Enteric Coronavirus Introduction to the United States: Root Cause Investigation Report.* This report investigated several plausible import pathway scenarios including flexible intermediate bulk containers (FIBC or tote bags) used to transport feed ingredients from China as a possible pathway for the introduction of PEDV into the United States [11].

Despite extensive investigative work in the field and the laboratory, the specific mode of introduction of exotic viral pathogens such as PEDV, into the United States and, subsequently, into domestic swine premises remains unknown. In order for feed or feed ingredients to be a route of disease introduction into the United States, it must become contaminated with the causative agent; avoid inactivation through (trans-ocean) travel, feed manufacturing, processing, and distribution processes; and be ingested at a dose sufficient to cause infection in a susceptible pig (see Figure below).

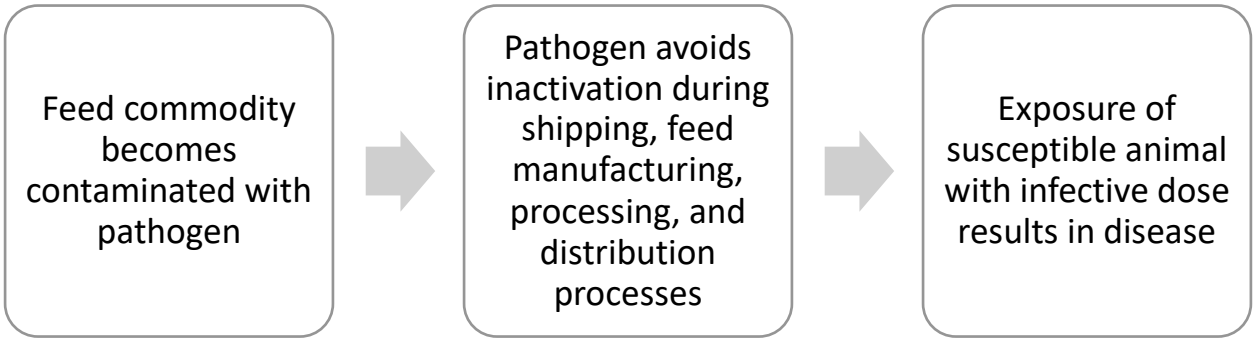

Figure 1: Simple generic pathway for transmission of pathogens in feed

The risk of disease transmission via contaminated feed and feed ingredients is non-zero as pathogens such as *Salmonella* are known to be transmitted via swine feed [12, 13]. However, questions regarding the true risk level that contaminated feed and feed ingredients pose in the introduction and subsequent transmission of PEDV and other exotic swine viruses remain unanswered, particularly in comparison to other recognized risk factors such as movement of infected pigs, transport vehicles, personnel, and waste feeding of unprocessed or improperly processed animal products. Thus, making sound decisions regarding risk mitigation measures in the face of an uncertain risk level is challenging. Additionally, the economic costs and downstream impacts of broadly banning certain feed ingredients or applying other mitigation measures to feed and feed ingredients without objective data may outweigh the costs of direct production losses from a disease event [5].

The emergence of PEDV in North America and growing concerns of introduction of ASF from China, the European Union, or other affected regions has put a spotlight on the possible role of contaminated feed and feed ingredients in the introduction and transmission of viral swine pathogens. The characteristics of modern swine production – globalization of trade (including significant increases in the volume of

imported bulk feed ingredients), intensification and vertical integration of production, and extensive movement of pigs and related production components (e.g., transport vehicles, feed, personnel) – and the trend of emerging swine pathogens in new geographic ranges (e.g., ASF) and/or with increased pathogenicity (e.g., PEDV) suggests that the critical production inputs along with existing biosecurity and mitigating measures that have historically delivered an acceptable level of protection may need to be re-evaluated.

In order to better inform policy-makers, the U.S. swine industry, and associated stakeholders, APHIS has conducted a literature review on the scientific evidence regarding whether non-animal origin ingredients of commercial swine feed could introduce or transmit viral pathogens of swine into or within the United States. This literature review was prepared by APHIS Veterinary Services at the request of the swine industry and other associated stakeholders. The goal of the literature review is to understand the current scientific knowledge and to identify information gaps. The results may support future scientific research and/or risk quantifying models for evaluating the risk (or likelihood) of entry of exotic viral pathogens via specific feed ingredients from source countries and subsequent exposure to U.S. swine populations.

4 Methods of the Literature Review

4.1 Overview

The methodology of this literature review follows the basic framework of a qualitative systematic review [1]. The literature review aims to identify, evaluate, and summarize the findings of relevant research studies, thereby making the available evidence more accessible to decision makers, other stakeholders, and the scientific community. When appropriate, combining the results of several studies gives a more reliable and precise estimate of the available knowledge, intervention, or control measure's effectiveness than one study alone. This literature review aims to answer the following research question:

> *What evidence is available in published scientific literature regarding whether non-animal origin ingredients of commercial swine feed could transmit viral pathogens of swine into or within the United States?*

The methodology of the literature review has four main components: 1) identifying and selecting research evidence, 2) data extraction and quality assessment, 3) data synthesis, and 4) report writing [1].

4.2 Identification of studies and study selection

A systematic search of the National Library of Medicine/PubMed, National Agricultural Library/PubAg, National Agricultural Library/Navigator (including major databases: AGRICOLA, AGRIS, BIOSIS, CABI, EBSCO Environment Complete, GEOBASE, GeoRef, MEDLINE, Scopus, Web of Science, and Zoological Record) and Google Scholar was conducted to identify published scientific literature pertaining to evidence regarding whether non-animal origin ingredients of commercial swine feed could transmit viral pathogens of swine into or within the United States. Studies published any time through March 2018 were identified.

The study selection process was performed in two stages. In the first stage, an initial screening of search results was performed based on title and abstract. In the second stage, the full text of the preliminary list of studies was evaluated. Additional articles were obtained through manual review of reference citations in the relevant literature. Figure 2 provides an overview of the study selection process. Studies were excluded that did not meet the purpose statement of the literature review. Reasons for exclusion include:

- Focus on non-viral pathogens (e.g., Salmonella, toxoplasmosis, etc.)
- Focus on disease transmission routes other than feed and feed ingredients (e.g., airborne)
- Animal origin feed ingredients (e.g., swill, SDPP, etc.)
- Focus on mitigations/treatment/disinfection of equipment or sanitizing feed
- No English translation available
- Full text not available (e.g., abstract or conference proceeding only)

- Duplicate publication
- Subject matter outside the scope of the review.

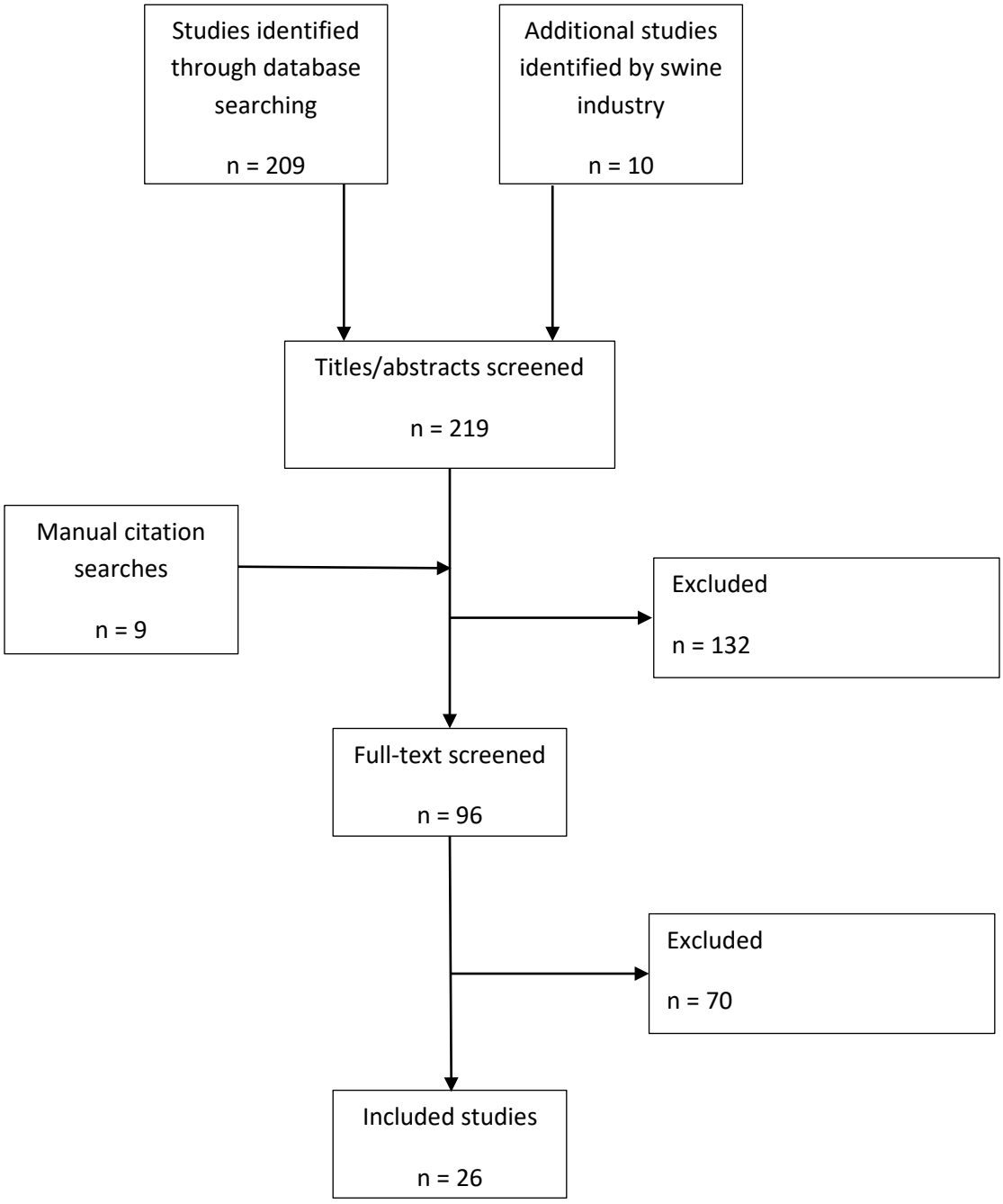

Figure 2: Overview flow chart of study selection process

The two-stage study selection resulted in retention of 26 included studies. The most common reasons for exclusion (in descending order) included: focus on disease transmission routes other than feed or feed ingredients; focus on mitigations/treatment/disinfection of equipment or sanitizing feed; subject matter outside the scope of the review; and focus on animal origin feed ingredients.

4.3 Data extraction and quality assessment

The data extraction component is the process by which research reviewers obtain the necessary information about study characteristics, methods, and findings from the included studies. The quality assessment component aims to identify internal and external validity of the selected studies. Standardized data extraction provides consistency in a literature review, thereby reducing potential bias and improving validity and reliability.

A fillable, pdf data extraction form was created for the purpose of this literature review. Twenty-six published articles were included in the data extraction and quality assessment process. Six reviewers worked in pairs to perform the data extraction and quality assessment. Each article was reviewed for general information, study characteristics, and outcome results. For the quality assessment portion, the reviewers reported on potential sources of bias, shortfalls in the statistical and analyses methodology, the quality of reporting, and the generalizability of the study to the commercial swine industry in the United States. An example of the form is provided in Appendix I.

4.4 Data synthesis and report writing

A qualitative, narrative approach was used for the data synthesis and report writing. The information extracted in the data extraction process was summarized into data synthesis tables. These tables are provided in Appendix II. From the data synthesis tables, relevant information from the individual studies was collated and summarized in the Literature Review Results section below.

5 Animal-origin Feed Ingredients

Although outside of the scope of this literature review, the exploration of animal-origin feed ingredients as possible vehicles for pathogen transmission, in particular swine-origin ingredients such as SDPP, has been on-going. Recent studies have associated SDPP with the transmission of PEDV. Many of the studies reviewed in this literature review included swine-origin feed ingredients as part of the experimental data and/or the overall study discussion. Some studies more broadly or generically used the term "feed" without distinguishing whether animal origin ingredients were excluded. Thus, several recent studies on the transmission of PEDV via feed are summarized below to provide context for the discussion of the epidemiological role, if any, of contaminated animal-origin feed and feed ingredients in pathogen transmission.

5.1 Spray-dried porcine plasma

Several experimental studies and epidemiological investigations have been conducted on the role of PEDV-contaminated SDPP in the transmission of PED with inconsistent conclusions [7, 14-20]. In particular, the first outbreak of PED in Canada in January 2014 focused on imported SDPP from the United States as the possible transmission vehicle. Using complete feed and SDPP samples associated with the positive case herds in Canada, Pasick et al. (2014) conducted a swine bioassay with PEDV polymerase chain reaction (PCR)-positive SDPP and feed mixed with PEDV PCR-positive SDPP. The results provided evidence that the PEDV PCR-positive SDPP [cycle threshold (Ct)[1] value range 36.35 – 36.69] was infectious as clinical signs of diarrhea were observed and significant amounts of PEDV were shed in the feces of piglets inoculated with the PEDV PCR-positive SDPP. In contrast, the bioassay results from the piglets challenged with the feed matrix[2] containing PEDV PCR-positive SDPP (Ct value range 37.22 – 42.88) were inconclusive and, thus, infectivity could not be definitely demonstrated [7]. In a retrospective case-control study, Perri et al. (2018) investigated the role of feed and other factors in the Canadian outbreak. The authors found that the odds of PED occurrence in herds receiving feed from a specific feed company that provided potentially contaminated feed was 38.1 times greater than for herds that did not receive that feed. The number of live pig movements, semen deliveries, and the frequency of dead stock pickups were not identified as risk factors for PED [20]. Similarly, Aubry et al. (2017) determined the attack rate for farms with confirmed consumption of feed containing SDPP was 28.1% while the attack rate of unexposed farms was estimated at 0.17%. The strength of association increased with increasing concentration of SDPP in feed [18]. In a separate study, spatiotemporal findings included that cases were more likely to neighbor cases than controls, and the pattern of spread indicated point source introduction with secondary transmission [17].

[1] A cycle threshold (Ct) of less than 38 is considered PEDV positive (Dee et al. 2014).

[2] Food matrix (plural matrices) as defined by the USDA National Agricultural Library is the nutrient and non-nutrient components of foods and their molecular relationships, i.e. chemical bonds, to each other. The glossary can be accessed at https://agclass.nal.usda.gov/glossary.shtml.

Collectively, these studies of the epidemiological investigation of the Canadian PED outbreak concluded that a single lot of SDPP imported from the United States was the vehicle of infection and found that the proportion of cases exposed to creep and nursery feeds contaminated with this product was significantly higher than expected. The investigation of the initial cases found no association with other exposures, such as feed providers, transporters, a rendering company or livestock haulers [7, 17, 18]. Despite previous evidence that the spray-drying process is effective at inactivating viruses, including PEDV [16, 21-23], the investigations into the Canadian PED cases suggest that a breach of good manufacturing practices and/or biosecurity practices led to contamination of the SDPP during processing, or post-processing contamination occurred during packaging, storage, and transportation of the SDPP and subsequent mixing into the complete swine feed [18].

However, independent studies on the same single lot of SDPP (provided directly from the plasma manufacturer not affected farms) by the Food and Drug Administration and the North American Spray Dried Blood and Plasma Producers Association determined the PEDV PCR-positive SDPP was not infectious; pigs remained negative as determined by PCR and serology testing. To account for low sensitivity of the bioassay and to mimic commercial feeding practices, two additional bioassay studies were performed with longer feeding times (14 and 28 days, respectively); both determined that pigs fed a diet with 5% SDPP that was PCR-positive for PEDV (Ct values of 30.1 and 30.0, respectively) did not contain infectious PEDV and did not transmit PEDV to pigs [16]. Similarly, Opriessnig et al. (2014) found that a commercial feed containing 5% commercial SDPP confirmed positive for PEDV ribonucleic acid (RNA) (SDPP diet contained 3.3±0.3 $\log_{10}$ PEDV RNA copies/g) was not infective and not capable of transmitting PEDV to pigs [14]. Dee et al. (2015) were also not able to reproduce or support the Pasick et al. (2014) findings despite repeat bioassays with high viral load (Ct value of 16.34) [7, 15]. Given the varying results, the role, if any, of SDPP and other swine origin feed ingredients in the general epidemiology of PEDV requires further investigation, particularly in identifying the critical points in the production and distribution processes where contamination may occur and the likely sources for the viral contamination.

6 Literature Review Results

The core studies included in this literature review have been collated into three categories: background information on risk factors for transmission and fomite survivability, epidemiology and outbreak investigations, and experimental studies with swine bioassays. Studies were grouped into the best-fit category. Information extracted from the core studies may overlap into one or more categories and, thus, may be discussed in more than one section. Additionally, the study summaries provided in this section are not meant to be detailed and fully comprehensive but rather are focused on the information pertinent to this literature review, namely, non-animal origin feed ingredients. Studies conducted on feed and feed ingredients containing animal-origin ingredients, such as SDPP, are summarized in Section 5. For additional study details, please see Appendix II or consult the full-text paper (see References).

6.1 Risk factors for transmission and fomite survivability

Numerous investigators have reviewed the epidemiology and impact of swine viral pathogens and analyzed industry expert opinion and outbreak information to identify risk factors for transmission of swine pathogens. Additionally, several studies have looked at the survivability of viruses on various fomites related to swine production and husbandry, including feed and feed ingredients. Methods used by these investigators include expert elicitation, questionnaire-based post-hoc outbreak investigations, and experimental studies of the survival of viruses in contact with fomites. Here, we summarize such investigations and their findings, focusing on non-animal origin feed and feed ingredients. The studies are grouped into two categories: risk factors for virus transmission and virus survival in contact with fomites. The virus scope of these studies includes African swine fever virus (ASFV), porcine high fever disease, (highly pathogenic) porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PRV), Aujeszky's disease virus, and blue eye disease virus.

6.1.1 Risk factors for virus transmission

Three studies examined the epidemiology of specific swine pathogen(s) or outbreak events in various regions of the world [24-26]. The pathogens covered include porcine high fever disease virus in Vietnam and ASFV in Nigeria and Eastern Europe. One study used expert elicitation methods to identify risk factors for transmission of highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) into and within Australia [27].

- A study by Le et al. (2012) examined risk factors that may have contributed to the spread of porcine high fever disease (PHFD) from China to Vietnam in 2008. PHFD is considered a PRRS-related syndrome. A survey was administered to individual households in a southern province of Vietnam which included questions regarding observed clinical signs in the owner's swine herd, suggestive of PHFD, as well as various risk factors that could contribute to the spread of this virus. Probability incidence of PHFD was estimated based on description of clinical signs but presence of the virus was not confirmed with diagnostics. Investigators found that, among other things, the presence of ducks and the feeding of water green crops, together, were positively

associated with clinical signs consistent with PHFD in swine. Water green crops are fed directly to pigs after harvest, without processing. Authors hypothesize that ducks potentially amplify the virus, similar to data shown for PRRSV, and contaminate the water green crops fed to pigs. It is also believed that PHFD virus can persist in water further contaminating the greens before harvest. The findings of this study suggest that unprocessed non-animal origin feed ingredients could be contaminated with virus by other animals and transmitted to swine [25].

- The study by Fasina et al. (2012) sought to identify risk factors associated with ASF outbreaks in Nigerian swine herds. Investigators conducted a matched case control investigation of farms that experienced ASF outbreaks between 2006 and 2009. Case farms met OIE (World Organization for Animal Health) guidelines for confirmation of ASF including clinical signs, pathological signs, and laboratory diagnostic confirmation of ASF. Control farms were selected based on farm characteristics, similarity in operations and biosecurity measures, and absence of ASFV infection. An epidemiological questionnaire was administered to all participating farm owners and a number of factors associated with increased risk of ASF were identified. These included but were not limited to purchase of untested pigs from neighboring farms, presence of an abattoir in the community, wild bird access to pig pens, sharing of equipment between farms, and unprotected feed sources (rodent access). The use of commercial feed on farm, instead of swill feeding, was negatively associated (protective) with ASF risk. The final logistical regression model showed that protecting food and water sources, separation of sick and healthy pigs, and washing/disinfecting equipment were negatively associated (protective) with ASF infection. These findings indicate that preventing rodent access to feed sources and the use of commercial feeds, as opposed to swill feeding, were potentially protective measures in the farms studied. The investigators of this study conducted extensive statistical analysis on a wide range of ASF risk factors found on small and large swine farms. This study was conducted in a developing country; thus, direct application of findings to large scale, industrial swine farms in the United States may be limited [24].

- The review by Guinat et al. (2016) summarizes findings of ASFV transmission studies performed in Eastern European and Baltic countries. In respect to feed-to-pig transmission pathways, the authors referenced a European Commission (2014) epidemiological report in Latvia and Lithuania suggesting that fresh grass and seeds may have been contaminated by wild boar feces containing ASFV and transmitted the virus to domestic backyard pigs. A similar study focused on ASF in Latvia also suggested that feeding potentially contaminated (via wild boar) fresh grass or crops was a risk factor for ASF disease in backyard holdings; however, swill feeding could not be excluded as a source [28]. A study conducted in Kenya in 1921 demonstrated that ASFV could be transmitted to pigs when they consumed infected feces and urine but failed to transmit when contaminated sweet potatoes or bananas were consumed [29]. Several references were provided for documented transmission of ASFV to domestic pigs through feeding of swine-origin

feed ingredients such as preserved or uncooked meat products; some primary references from the review were not accessible to the reviewers, making it difficult to gauge the robustness of the referenced studies. However, the studies referenced demonstrated that there is very little field data exploring the relationship of ASFV transmission and non-animal origin feed ingredients [26].

- Brookes et al. (2015) elicited industry expert opinions to identify entry and exposure routes with the highest probability of occurrence for introduction of HP-PRRS from south-east Asia to Australia. Pig industry experts attending the Australian Pig Veterinarians' Annual Conference in Melbourne, Australia in June 2013 were given a questionnaire and asked to indicate the probability of occurrence of 28 entry routes and 36 exposure routes within fixed probability ranges over one year. Agreement between participants was assessed using a chi-squared statistical test by comparing the frequency distribution of probability ranges for each route with a random distribution of probability ranges. There was statistically significant agreement on 29 exposure routes; the routes with the highest estimated probability of occurrence all involved disposal of waste to feral or backyard pigs. The highest probability exposure route for commercial pigs was thought to be contact with a human acting as a fomite or access to animal feed/additives from south-east Asia. (Note: the definition of "animal feed/additives" was not provided and it is unclear if this term refers to non-animal origin feed, individual ingredients, or complete feed.) There was significant agreement on 16 entry routes; however, there were fewer consistent agreements or patterns of responses regarding a high proportion of entry routes in comparison to the exposure routes. This may be due to the fact that participant knowledge was of the pig industry and associated biosecurity practices, not border security or trade policy. The entry routes with the highest probability of occurrence *and* statistically significant agreement among participants included entry of HP-PRRS by humans or animal feed acting as fomites (and traveling or being shipped by air) and raw pork entering through the postal service or by a private individual. There was not agreement on the probability of entry of these fomites traveling or being shipped by sea. [27].

6.1.2 Virus survival in contact with fomites

Three studies examined virus survivability on various swine-related fomites, including feed and feed ingredients [30-32]. The viruses covered by these studies were PRV, PRRSV, Aujeszky's disease virus, and blue eye disease virus.

- Schoenbaum et al. (1991) investigated the survival duration of PRV in contact with various solid and liquid fomites commonly found in hog-raising environments. Feed or feed ingredients included in the study were green grass, whole corn, pelleted feed, and alfalfa. The authors did not report whether the feed included any animal-origin ingredients. The authors mixed stock PRV with various diluents, namely saline, swine saliva, or swine nasal washings, and inoculated fomites with those mixtures. After incubating the inoculated fomites at 25 °C for up to 14 days,

the authors tested for virus activity through use of a cell-culture-based assay. They found that, in general, the quantity of infectious virus decreased over time. Of the combinations of PRV and diluent with feed or non-animal origin feed ingredients, the combination of PRV/saline/whole corn remained infectious the longest at 7 days with an estimated half-life of 36.3 hours. The durations of infectiousness of the other combinations of PRV/diluent/feed or non-animal origin feed ingredients were shorter, ranging from 1 to 4 days with an estimated half-life of 1.0 hour to 5.1 hours. The conditions of this study do not reliably reproduce field conditions. The fomites were inoculated with stock virus at doses that might not reflect contamination levels under field conditions. The authors used liquid diluents in all experiments and deliberately avoided field conditions (drying and ultraviolet light exposure) deleterious to virus activity. Similarly, the authors acknowledged that virus survival time is impacted by temperature, and speculated that virus survival times would be longer at lower temperatures and shorter at higher temperatures [30].

- Pirtle and Beran (1996) investigated the survival time of PRRSV in contact with various liquid and solid fomites. Feed and non-animal origin feed ingredients included in the study were ground corn, pelleted swine starter feed mix, and alfalfa. The authors did not report whether the feed included any animal-origin ingredients. The authors mixed stock PRRSV with saline, and inoculated fomites with those mixtures. After incubating the inoculated fomites at 25 to 27 °C for up to 11 days, the authors tested for virus activity through use of a cell culture based assay. In the PRRSV-spiked alfalfa sample, the authors detected PRRSV only on day 0. They did not detect any virus in the PRRSV-spiked starter feed mix and ground corn samples. The authors speculated that the pH (<7) of the samples tested and/or unknown substances present in the samples could have contributed to virus inactivation. The conditions of this study do not reliably reproduce field conditions. The addition of saline to each of the samples increased the moisture content of the samples and might have impacted the virus survival kinetics. In addition, the feed and feed ingredient samples were inoculated with stock virus at doses that might not reflect contamination levels under field conditions [31].

- Martínez-Gamba et al. (2001) examined the persistence of bacterial and viral pathogens in feces fermented for use in animal feed (silage). Feces from 30 pigs was collected, inoculated with Aujeszky's disease virus and blue eye disease virus, and mixed with molasses and sorghum for fermentation. Flasks of ensilage were incubated at room temperature for 0, 7, 14, 28, and 56 days. Presence of virus was detected by cytopathic effects on cellular monolayers and indirect immunofluorescence. Samples were positive for virus on day 0 but not at any subsequent time point. The findings indicate that fermentation is sufficient for the inactivation of the viruses tested and could be an acceptable means for eliminating harmful pathogens from feces used for animal feed. These results may not be applicable to viruses of other families [32].

6.1.3 Summary of findings

In summary, most of the reports and studies summarized above were not specifically designed to focus on non-animal origin feed and feed ingredients as a potential vehicle for virus transmission; however, feed and feed ingredients were included in the study design or discussion. In some cases, the author did not clearly state or define whether the feed includes animal-origin ingredients. Similarly, some findings in this group of studies may not be generalizable to other virus families or the commercial swine industry in the United States. Given these limitations, several key points are highlighted below.

Le et al. (2012) identified owning ducks and using water green crop as pig feed (together) as being associated with an increased risk of PHFD, speculating that unprocessed non-animal origin feed ingredients could be contaminated with virus by other animals and transmitted to swine [25]. Fasina et al. (2012) identified food and water control to be significantly associated with decreased risk of ASF infection in Nigeria, noting that feed and water biosecurity practices can prevent virus contamination by rodents and wild birds [24]. Consistent with these findings, expert opinion conducted in Australia found industry representatives believed commercial pigs were most likely to be exposed to HP-PRRS through access to contaminated animal feed and feed additives imported from southeast Asia [27]. These studies suggest that protecting unprocessed non-animal origin feed ingredients from contact with animals may decrease the likelihood of pathogen contamination. This may be particularly important for ingredients that are not processed before being fed to animals.

Several investigators have examined the persistence of viral activity in the presence of various fomites, including non-animal origin feed ingredients. In experiments in which they spiked fomites with stock virus, they found, in general, that viral activity did not persist for long periods of time under the experimental conditions used. Schoenbaum et al. (1991) found that the combination of PRV/saline/whole corn remained infectious the longest at 7 days with an estimated half-life of 36.3 hours [30]. Similarly, Pirtle and Beran (1996) detected PRRSV activity only on day 0 in the PRRSV-spiked alfalfa sample [31]. Interestingly, Martínez-Gamba et al. (2001) found that fermentation is sufficient for the inactivation of Aujeszky's disease virus and blue eye disease virus [32]. Collectively, these studies suggest that, under certain conditions, swine viruses can survive in non-animal origin feed ingredients.

Among the knowledge gaps identified in these studies are the need for a comprehensive evaluation of transmission pathways involving non-animal origin feed ingredients and swine viruses. The scientific literature regarding the survival times for various non-animal origin feed ingredient/pathogen combinations as well as determination of infectivity is incomplete and warrants further studies along with documented replication of studies. Furthermore, the likelihood of non-animal origin feed ingredients incurring contamination and documented scenarios in which cross-contamination occurs under field conditions is unknown.

6.2 Epidemiology and outbreak investigations – PEDV and PDCoV

In 2013, swine enteric coronaviruses, such as PEDV and PDCoV, emerged as pathogens of significance for the swine industry in the United States and several other countries. Efforts were made to describe the epidemiology of the outbreaks which included a focus on the source of virus introduction and transmission. Among other things, feed and non-animal origin feed ingredients were suspected as one possible route of virus introduction and spread. The following section summarizes reports pertaining primarily to PEDV and PDCoV outbreak investigations, findings related to feed and feed ingredients, and experimental studies that sought to determine the survivability of swine viruses in feed and feed ingredients.

Studies reviewed in this section have been separated into two major categories: 1) epidemiological reports, reviews and surveys and 2) feed and feed ingredient studies. These studies are primarily focused on PEDV and PDCoV; however, other swine pathogens are included such as transmissible gastroenteritis virus (TGEV), PRRSV, and PCV2. The epidemiological reports collated information from literature and descriptive reviews, disease outbreak analyses, as well as information from retrospective surveys [5, 11, 33-38].

Studies focused on virus-contaminated feed and non-animal origin feed ingredients evaluated the ability of these materials to serve as fomites for virus introduction and spread. The studies are grouped into descriptive and experimental reports. The descriptive studies obtained samples from feed, feed facilities and feed transport vehicles linked to disease outbreaks and analyzed them for presence of viral RNA [39, 40]. Experimental studies included in this section investigated survivability of swine viruses on feed fomites by adding virus to the feed or non-animal origin feed ingredient and examining virus titers over time [41, 42].

6.2.1 Epidemiological reports, reviews and surveys

Eight epidemiological reports examining the global outbreaks of PED and PDCoV have been reviewed. The geographic scope of these reports include Europe, Asia, and North America [5, 11, 33-38]. The authors sought to describe the outbreaks and describe associations between risk factors for transmission and disease status.

- In 2014 and 2016, the European Food Safety Authority (EFSA) published two reports pertaining to PED and PDCoV [33, 34]. In 2014, EFSA published a scientific opinion on PEDV and PDCoV based on a review of the scientific literature published in the preceding 10 years [34]. In regards to the global epidemiologic picture of PEDV and PDCoV, the authors found that only limited active monitoring is conducted for PED in Europe, Asia, and the Americas. Few outbreaks of PED have been reported in Europe (Germany and Italy 2014); in Asia, multiple outbreaks have been reported in several countries. In the Americas, the first outbreak of PED in the United States was reported in May 2013 with rapid spread within the country and reports in North, Central and

South America. The authors found no clear evidence that PDCoV is causing significant impacts on animal health [34].

When examining the possible geographic differences in PEDV strains and the potential for immunologic cross-protection, the authors found a high level of sequence identity between PEDV in Germany and Italy and the PEDV in the Americas. Findings of retrospective studies suggest that at least two PEDV strains were introduced into the United States at similar times. Differences in the nucleotide sequence of PEDV have been identified but their effects on virulence, if any, are unknown. Serological cross-reactivity between PEDV in Europe and the Americas is reported; no data regarding cross-protection are available. For reasons not well understood, outbreaks of PEDV in Asia and the United States seem to be more severe than those in Europe. However, it's difficult to compare impacts in different regions because of differences in age group of affected pigs, production systems, biosecurity, farm management, herd size, and immune status and sanitary status of the herd population [34].

In a literature review covering the preceding 10 years (2004 – June 2014), EFSA concluded that the scientific literature supports the following statements [34]:

- Infected live animals and feces are reported to transmit PEDV.
- PEDV can survive in slurry but the epidemiologic role of this matrix is unknown.
- High levels of infectious PEDV are shed in feces, contributing to contamination of various fomites, including vehicles, humans, and feed.
- The transmission of PEDV via feed has been shown but more data are required to assess its epidemiologic importance.
- PEDV RNA has been detected at low levels in serum but the role of this epidemiologic matrix is unknown.
- Fecal cross-contamination of blood during collection at the slaughterhouse cannot be excluded.
- Manufacturing techniques for SDPP can inactivate PEDV; however, different spray-drying techniques exist and variations in SDPP manufacturing procedures or breach in quality control (e.g., inadequate time and temperature holding times) may result in incomplete inactivation of PEDV. For example, infectious SDPP was detected in one study, but the origin of the PEDV in the SDPP is unknown (post-processing cross-contamination versus inadequate SDPP processing).
- Infectious PEDV has been detected in air under experimental conditions and virus may be transmitted via air over short distances.

- Swill, particularly untreated pig intestines, can contain PEDV but the role of this epidemiologic matrix is unknown.
- There is no data on the presence of PEDV in embryos, pork meat, or other swine-derived feed components such as red blood cells (RBC), hydrolyzed proteins, fat, gelatin and collagen.

In 2016, EFSA published a scientific report of PEDV epidemiology and impact as reported in scientific literature in 2014 and 2015, together with an analysis of PED cases in the European Union [33]. The goals of the report were to provide guidance on PEDV data to be collected by European Union Member States in order to optimize response coordination, and an analysis of the epidemiologic data to quantify PED impact on pig production in the European Union. Also, an updated literature search was conducted to obtain data from October 2014 to October 2015 to update information in the EFSA scientific opinion report from 2014. Transmission of PEDV via feed or feed ingredients was not directly addressed in this report; thus, a summary of the report is not provided. However, the major recommendation(s) relevant to feed and non-animal origin feed ingredients included the importance of strict biosecurity, in particular with vehicles, to prevent introduction of PEDV onto the farm [33].

- Although PEDV was first identified in Japan in the 1990s, Japan experienced renewed outbreaks of the disease in 2013. Scientists determined that the strain of PEDV in Japan was highly similar to the viral strain affecting the United States. In a retrospective questionnaire-based case-control study, Sasaki et al. (2016) focused on risk factors associated with these new outbreaks of PEDV in Japan. The investigators tested the hypothesis that epidemiologic factors associated with a high risk of PEDV infection at swine farms located within 5 km of at least one other infected farm (locally exposed farms) differ from factors associated with high risk of infection at farms more distant from other infected farms (non-locally exposed farms). Using logistic regression analyses, they found that on locally exposed farms, PED was associated with a larger total number of swine, shorter distance to the nearest PEDV-positive farm, and a disinfectant contact time of less than 20 min. On non-locally exposed farms, PED was associated with increased feed truck visits to the farm, no visit by a veterinarian, and a disinfectant contact time of less than 20 min. Feed or non-animal origin feed ingredients were not explicitly included in the scope of this study. Feed-related items included on the questionnaire included feed truck visits and artificial milk. The authors found neither to be significantly associated with PED on either type of farm [36].

- In the descriptive report by Davies (2015), the first half of the article discusses the similarities of 3 major disease epidemics in the swine industry caused by PRRSV, PCV2, and PEDV. All three share the following features: highly host specific, rapid rates of mutation, and appear to be associated with swine as non-pathogenic (or associated with mild disease) for years before

becoming highly pathogenic. The author suggests that the proximate source of the highly pathogenic variants of the 3 viruses was the extant swine virome. The emergence of these 3 highly pathogenic viruses over 25 years may be considered extremely rare events but the author argues that intensive single species food production systems along with globalization, intensification, and extensive movements of pigs have contributed to the geographic expansion of these viral agents and points to the likelihood that future "new" viruses in swine are likely to emerge from already recognized (non-pathogenic or ignored) swine viruses.

The second half of the article discusses the risk of feeding animal products (SDPP) to swine and whether or not the true risk warrants excluding animal origin products (or other ingredients) from swine diets. The author concludes that the risk of PEDV survival in SDPP is extremely low, but non-zero. The challenge lies in how to manage a non-zero risk. The likelihood of transmitting PEDV via feed in comparison to transmitting PEDV via infected animals is likely extremely low. However, the consequences of a disease outbreak are significant and the trend toward increasing herd sizes (and assuming fixed biosecurity practices) in developed countries such as the United States along with greater flux of inputs (animals, semen, feed, water, personnel, vehicles, etc.) leads to a higher temporal frequency of adverse events. The author argues that a blanket ban on certain ingredients in swine feed (of swine origin) may not be the solution. A comprehensive evaluation of transmission pathways as well as cost-benefit analyses of managing feed-related risks and balancing risks with nutritional value are needed [5].

- In the review by Lowe (2014), the author summarized aspects of the U.S. outbreak of PEDV in 2013, including factors that were found to be associated with greater risk of PEDV transmission. The author concluded that current research supports transmission of PEDV through livestock transportation, movement of people, vehicles, and other contaminated fomites as well as shared resources and equipment among farms [35]. The author cited reports by the Canadian Food Inspection Agency and others that demonstrated the level of PEDV found in SDPP added to feed around the time of the outbreak was not sufficient to transmit virus to naïve pigs [7, 14]. It is stated that feed was not likely a primary transmission route of PEDV in 2013 [35]; however, Lowe cites other clinical studies and epidemiological investigations that provide evidence that feed can serve as a fomite for PEDV if contaminated during the manufacturing, storage, and transport processes [35].

- In 2014, the United States experienced the emergence of a second novel swine enteric coronavirus, PDCoV. McCluskey et al. (2016) administered a retrospective survey to 42 U.S. swine breeding herd operations that experienced a confirmed outbreak of PDCoV in order to identify factors that may have contributed to the introduction and spread of the virus. Among other things, the source and timing of feed delivered to the affected farms in the 10 days prior to the outbreak of PDCoV was examined. All farms surveyed had a delivery of complete feed or

feed ingredients in the 10 days prior to the outbreak; 60% of those had a delivery of SDPP while 40% received blood products or pork fat. One third of the farms received feed components from outside of the United States. There was no common source of feed or SDPP for the farms surveyed [37].

- In April/May of 2013, PEDV was first identified in the United States. Scott et al. (2016) addressed the question of how PEDV entered the United States, through various methods including scenario development, post hoc investigations, epidemiologic surveys, a case control study, brainstorming, and speculation. They used previously collected epidemiologic data to develop scenarios and identify hypothetical routes of PEDV introduction into the United States and conducted follow-up studies to gather evidence for the most plausible scenarios. The follow-up studies included testing organic soybeans and pet jerky treats imported from China and archived serum samples opportunistically collected from feral swine; all results were negative. The authors did not identify a proven source or route of PEDV introduction into the United States. However, it was suggested that incomplete farm feed/ration records negatively impacted investigators' ability to thoroughly evaluate the potential epidemiologic role of feed or feed ingredients. The authors identified totes used to transport bulk feed as providing "the simplest explanation" for the investigation findings [11].

- A retrospective survey was administered to U.S. swine veterinarians and producers in 2017 to collect opinions regarding, among other things, the suspected source of PEDV and PDCoV introduction into the United States in 2013-2014. The majority of respondents believed trucks coming onto the farm (26%), feed (29%), and variable biosecurity protocols (18%) were responsible for virus introduction. Survey participants were also asked about control measures implemented in response to outbreaks. None of the participants noted a change in feed practices although 56% implemented enhanced biosecurity protocols. At the time of the survey the majority of respondents had either never experienced an outbreak of PEDV or PDCoV (28.9%) or reported that the virus had been eliminated from their farms (56.6%) [38].

6.2.2 Feed and feed ingredients

Feed and feed ingredients have been hypothesized to serve as fomites for virus introduction and spread, leading several investigators to examine environmental samples (feed, fomites etc.) for the presence of virus and/or to conduct assays testing the ability of feed to serve as a fomite for virus. The following summarizes the reported findings.

Descriptive

- Associated with the U.S. outbreak of PED in 2013, an epidemiological investigation was conducted at an affected Ohio swine operation to determine the source of virus introduction. The timing of the outbreak coincided with a switch to a new out-sourced feed pellet.

Environmental samples were obtained and analyzed by real-time RT-PCR. The investigators report that PEDV RNA was detected in newly opened bags of pellets on-farm; and in pellets and individual ingredients sampled at the source (supplier) facility. No virus isolation (VI) assays were performed on these samples. In the bioassay conducted, pigs were observed to be healthy and no clinical signs of disease were observed [39].

- A retrospective case study by Greiner (2016) investigating PEDV and PDCoV outbreaks in the United States found a positive association between feed truck visits to farms and presence of PEDV. To investigate the role of feed and feed mills in these outbreaks, environmental sampling was conducted at 24 feed mills, some of which served farms known to be positive for PEDV or PDCoV. The investigators swabbed office floors, bulk ingredient pit grates (exterior surfaces), mixer/pellet coolers, incoming bagged-ingredient truck trailers, the interior of feed compartments on trucks servicing farms, and feed truck foot pedals. None of the samples obtained were positive for PEDV RNA, 5% of truck foot pedals and 1% of bulk ingredient pit grates were suspect for PDCoV RNA, and 3.4% and 2.2% of truck foot pedals and office floors, respectively, were positive for PDCoV. All other samples were negative for PDCoV. There were no significant associations between viral RNA at feed mills and the disease status of farms served. Further, with the exception of the 3.4% of suspect samples, none of the incoming ingredient trucks, bulk ingredient pits, or outgoing feed compartments were positive for PEDV or PDCoV RNA [40].

Experimental

- Trudeau et al. (2017a and 2017b) conducted experimental assays to investigate inactivation kinetics of PEDV, PDCoV, and TGEV in feed and feed ingredient matrices and on solid surfaces. Feed and non-animal origin feed ingredients used in these studies included complete feed, corn, soybean meal, corn dried distillers grains with solubles, and vitamin and trace mineral premix. The authors mixed stock virus with liquid medium, and then spiked samples of feed and feed ingredients with the mixture and incubated the combinations at various temperatures. In one study, they incubated the spiked samples at room temperature (approximately 25 °C) for 0 to 56 days [42]. In another study, they incubated the spiked samples at 60 to 90 °C for up to 30 min [41]. The authors used a cell culture based assay and model fitting to estimate a delta value, calculated as an indicator of the time necessary to reduce virus concentration by 1 log. For the spiked samples incubated at room temperature, the largest delta values were obtained for PDCoV and TGEV in soybean meal, at approximately 42 days each. Soybean meal at room temperature also provided the highest delta value for PEDV, at 7.5 days. Other findings indicated that at room temperature, moisture and ether content were important determinants of virus survival. The authors found no difference in virus survival in feed or non-animal origin feed ingredients incubated at temperatures higher than 70 °C. The maximum level of virus inactivation occurred upon heating the spiked samples at 90 °C for 30 minutes [41, 42].

6.2.3 Summary of findings

Of the retrospective studies, epidemiological investigations and reviews conducted in the aftermath of the PEDV and PDCoV outbreaks in 2013-2014, none found definitive evidence linking the transmission of PEDV and PDCoV to non-animal origin feed ingredients [11, 34-37]. Scott et al. (2016) proposed imported virus-contaminated feed totes as a possible scenario for the source of PEDV introduction into the United States in 2013; however, no conclusive data were presented to support this hypothesis [11]. The perception by the swine industry, however, that feed could have been the source of virus introduction is reflected in the results of the 2017 survey administered to veterinarians and swine producers, in which 29 percent of respondents believed feed was linked to virus entry and spread. Trucks (26%) and variable biosecurity protocols (18%) were also believed to be linked with PED spread [38]. The EFSA 2014 scientific opinion report notes that while transmission of PEDV via feed has been experimentally demonstrated [9], more data are needed for reliable assessment of the epidemiologic importance of this route in the field [34].

Lowe (2014) concluded that feed was not a primary transmission route in the spread of PEDV in the United States [35]. However, on further investigation, Lowe et al. (2014) found evidence that transport vehicles moving in and out of collection points, such as harvest facilities and livestock auction markets, served as a source of contamination of other transport vehicles, noting that the cost-saving industry practice of consolidating resources such as sharing building maintenance, manure handling equipment, personnel, and livestock transportation equipment likely contributed to the spread of the PED outbreak across multiple geographic regions in the United States [43]. Similarly, McCluskey et al. (2016) reported on findings that movement of feed trucks onto farms was significantly associated with PED status. Collectively, these studies suggest that biosecurity measures on the farm and at the feed manufacturing plant as well as limiting the movement of animals, people, vehicles, and other shared inputs could serve as critical control points to stop the transmission of PEDV between herds [35, 37, 43].

Two studies conducted environmental sampling of feed, feed facilities, and feed transport vehicles to determine whether these items contributed to the cause of the PEDV and PDCoV outbreaks in the United States [39, 40]. Collectively, the findings indicate that none of the source ingredients nor the outgoing feed at the feed mills sampled were positive for viral RNA, suggesting the non-animal origin ingredients and feed were not contaminated with PEDV or PDCoV. However, experimental studies by Trudeau et al. (2017a and 2017b) did show that feed ingredients spiked with PEDV, PDCoV, and TGEV in a laboratory setting could maintain live virus for a period of time at room temperature. The authors also found that different ingredients produced different virus survival periods which they believe may be due to moisture and ether content or pH of each ingredient. These data indicate that, under certain conditions, swine enteric coronaviruses are able to survive in feed ingredients [41, 42].

In summary, the epidemiologic investigations and outbreak studies did not definitively link (or exclude) transmission of PEDV or PDCoV with non-animal origin feed or feed ingredients. Inconsistent findings impart uncertainty towards feed or feed ingredients as a transmission pathway [35, 37, 39, 40, 43]. The

most common mechanical fomite implicated in this group of studies was transport vehicles, including a positive association between feed truck movements onto farms and disease status [34-38, 40, 43]. Two experimental studies reviewed did demonstrate that virus added to feed can survive for a period of time under certain conditions [41, 42]. However, these studies did not investigate nor were able to demonstrate that virus present in feed could be transmitted to naïve animals through normal feeding behavior.

Among the knowledge gaps identified in these studies are the identification of critical control points for pathogen contamination of feed and/or non-animal origin feed ingredients and consensus on the feed-based transmission pathways for PEDV and PDCoV. The point source(s) for virus contamination of feed or non-animal origin feed ingredients are not clearly defined.

6.3 Experimental studies on feed transmission with swine bioassays

The rapid spread of PED in commercial swine in North America in 2013-2014 prompted several experimental (non- randomized) studies with *in vivo* biological assays (bioassays) to be conducted. The bioassays were used to assess the biological activity or potency of the pathogen(s) of interest by measuring the magnitude of response such as observed clinical signs consistent with the study disease(s) and/or positive findings to diagnostic testing and necropsy examination. Most studies included in this section of the literature review used swine bioassays to determine the infectivity of the pathogen(s) of interest in feed or feed ingredients subsequent to detection by PCR of virus nucleic acid in the matrix (feed or feed ingredients) under examination.

For the purposes of discussion, the experimental studies with swine bioassays have been collated into two categories: field-based experimental studies with bioassays [9, 39, 44], and laboratory-controlled experimental studies with bioassays [15, 45-48]. In general, the bioassays involved naïve piglets of various ages, ranging from four days old to 21 days old [9, 48]; in some studies, pigs were re-used after a negative bioassay and the subsequent ages at re-introduction to the bioassay were not easily discernable [15, 47]. Piglets were sourced from healthy herds and tested by PCR and serological assays to confirm negative status to the respective pathogen(s) prior to the initiation of the bioassay. Exposure routes of the challenge matrix to the piglets varied, including ad libitum (natural) feeding [9, 39], oral administration via syringe [15, 47, 48], orogastric gavage [44-46], intramuscular injection, and intranasal administration [48]. In most studies, the bioassays consisted of daily diagnostic monitoring, lasting 6-7 days post-inoculation, culminating with euthanasia and complete necropsy examinations of piglets.

6.3.1 Experimental studies with swine bioassays using field-sourced challenge virus

Three experimental studies with swine bioassays exposed piglets to challenge feed samples inoculated with field-sourced virus from an index farm or contaminated feed facility. Prior to the bioassay, the virus

material was further processed or prepared in the laboratory before inoculating the challenge matrix. The bioassay results yielded mixed findings [9, 39, 44].

- Pillatzki et al. (2015) used a swine bioassay to investigate whether PEDV PCR-positive samples of complete feed, feed pre-mix, and SDPP (Ct values of 33.8, 34.2, and 30.0, respectively) that had been retained by feed manufacturers could serve as a source of PEDV transmission to neonatal swine. The piglets inoculated with the PEDV-positive feed samples along with the negative-control piglets remained negative for PEDV by PCR and clinically healthy throughout the study period. In contrast, only the positive-control piglets (Ct value = 25.5) developed clinical signs of PED; PEDV RNA was detected in fecal swabs; villous atrophy was observed in the ileum; and PEDV was detected in the ileum by immunohistochemistry [44].

- Bowman et al. (2015) conducted a swine bioassay on a newly started pelleted diet that was implicated as the transmission vehicle of PEDV into an Ohio swine operation. PEDV was detected by RT-PCR in the interior of the unopened bags of the new supplier's pellets, suggesting contamination occurred prior to delivery of the feed to the farm. Additionally, the source facility (supplier) tested positive for PEDV as well as individual ingredients at the source facility. Piglets were provided ad libitum access to the RT-PCR positive mash[3] (mean Ct = 36.5) along with dry pellets from the same lot for 7 days and observed for clinical signs of PEDV. During the bioassay, none of the pigs developed clinical signs of disease. Environmental and rectal swabs collected daily during the study were negative for PEDV as determined by RT-PCR. Microscopic examination of intestinal tissues collected from the piglets at the end of the study revealed no significant morphologic lesions [39].

- Following an outbreak of PED on three breeding herd premises in the United States, Dee et al. (2014) used a novel on-farm sampling method to collect remnants of feed samples from empty feed bins that previously contained feed consumed by the index populations. Analysis of feed material across the 3 affected sites by real-time RT-PCR indicated the presence of PEDV RNA with Ct values ranging from 19.50-22.20. For the swine bioassay, piglets were divided into three groups: the treatment group was fed 454 g of the challenge feed (natural feeding method) (pooled Ct value = 20.65); the positive-control group was challenged with feed spiked with stock PEDV (Ct value = 18.23); and the negative-control group was fed a placebo feed. The challenge feed for the treatment group was prepared using 30 g of feed material from the PCR-positive bin samples from the 3 affected sites and diluted in 30 mL of sterile phosphate buffered saline. The solution was vortexed and centrifuged. The supernatant was mixed with 454 grams of PEDV-free feed. Clinical signs consistent with PED were observed in piglets in the treatment group and the positive-control group. At necropsy, rectal swabs and intestinal tract samples from the treatment group and the positive-control group were positive for PEDV by PCR and

[3] Mash refers to dry pellets, grain or meal mixed with (hot) water to form a moist, pulpy feed.

immunohistochemistry with evidence of microscopic lesions. In the negative group, clinical signs, viral shedding, or PEDV-positive intestinal tract samples were not observed. Molecular sequencing of viral RNA obtained from treatment and positive control groups confirmed consumption of feed and not cross-contamination as the source of infection [9].

6.3.2 Experimental studies with swine bioassays using laboratory-sourced challenge virus

Five experimental studies with swine bioassays involved laboratory-controlled experimental studies in which piglets were exposed to the challenge matrix spiked with a predetermined pathogen dose sourced from laboratory stock viruses [15, 45-48]. Goyal (2014) and Schumacher et al. (2016) conducted experimental studies with a swine bioassay aimed at determining PEDV survivability in various organic materials and minimum infectious dose, respectively.

- Goyal (2014) investigated the survival of PEDV and TGEV in fresh feces, manure slurry, animal feed, and water. Stock PEDV prepared from pigs infected experimentally with a field strain of PEDV and a laboratory strain of TGEV were inoculated into samples of fresh feces, slurry, dry and wet swine feed, and drinking water and the mixtures were incubated at various humidity percentages and temperatures and for up to 14 days. PEDV and TGEV could be detected by PCR in fresh feces for 1-7 or 14 days, respectively, depending on temperature and humidity. Both viruses could be detected in slurry, non-chlorinated water, and dry and wet feed samples for ≥ 28 days at room temperature [45].

  For the bioassay, a subset of pigs were inoculated via orogastric gavage with virus aliquots obtained from 7 day old fresh feces, ≥ 28 day old room temperature slurry samples and wet feed samples, 7 day old dry feed samples, and two week old water samples. Bioassay results showed that PEDV survives in fresh feces for 1-7 days. Infective virus was recovered from inoculated slurry samples stored for ≥ 14 days at various temperatures, suggesting that spreading of manure could disseminate virus onto fields. The findings of this study also suggest that PEDV inoculated into wet and dry swine feed can remain infective for up to 28 days and 7 days, respectively [45].

- Schumacher et al. (2016) used a swine bioassay to estimate the minimum infectious dose of PEDV in virus-inoculated feed. The authors mixed serial dilutions of stock PEDV with feed, and administered the mixtures to 10-day-old piglets by orogastric gavage. The feed used in the study was corn- and soybean meal-based and included vitamin and trace mineral premixes and a source of phytase. The lowest concentration of virus in feed to cause infection in the piglets was 5.6×10^1 tissue culture infectious dose 50 ($TCID_{50}$)/g which corresponds to a Ct value of 37. Based on this infective dose, the authors estimated that 1 g of fecal matter could contaminate up to 450,000 kg of feed. The authors noted that there was a 10 unit difference in PCR Ct value for PEDV mixed with feed (Ct value of 37) in comparison to an equivalent dose of PEDV mixed with tissue culture medium (Ct value of 27). The authors proposed a variety of mechanisms that

might account for this difference, such as differences in the methods of dilution and virus extraction, differential RNA degradation, or enhanced viral persistence through binding of virus particles or RNA to feed particles [46].

Three experimental studies with swine bioassays reported by the same primary author examined the infectivity of PEDV in common swine feed ingredients in the presence or absence of a formaldehyde-based liquid antimicrobial SalCURB® (LA) [15]; the infectivity of PEDV in common swine feed ingredients with or without LA and 2% custom medium chain fatty acid blend (MCFA) following a simulated trans-Pacific shipment from China to the United States [47]; and the infectivity of select viral pathogens in common swine feed ingredients following two simulated transportation conditions across two different regions of the world [48].

- Dee et al. (2015) examined PEDV viability in various feed ingredients common in swine diets in the presence or absence of LA. Eighteen common swine feed ingredients were selected: corn, conventional soybean meal (SBM), dried distillers grain with solubles (DDGS), SDPP, purified plasma, intestinal mucosa, meat and bone meal (MBM), RBC, 3 vitamin/trace mineral (VTM) mixes, choice white grease, soy oil, lysine HCL, D/L methionine, threonine, limestone, and dry choline chloride. Sixteen 30 g samples of each ingredient were divided into two groups in replicate. One group was treated with 0.1 mL of LA (treated group) and the other group was treated with saline (non-treated group). The samples were spiked with 2 mL PEDV to reach a Ct mean of 25 (range 19-30) following mixing. There were 32 control samples of complete feed inoculated with PEDV (16 positive controls) or saline (16 negative controls). Also, 8-10 mL samples of stock PEDV served as stock virus controls. The samples were stored outside in winter conditions in plastic totes. At 1, 7, 14, and 30 days post-inoculation (DPI), samples were removed for diagnostic testing. The samples were tested for PEDV, PDCoV, and TGEV by RT-PCR. The presence of viable virus was determined by VI. Viable PEDV (positive VI) on 30 DPI was detected from non-treated SBM, DDGS, RBCs, MBM, lysine HCL, and D/L methionine. Non-treated choice white grease, threonine, and limestone were positive on VI at varying sampling days. Only SBM and MBM remained PCR positive at day 30; all LA-treated ingredients were VI negative [15].

  The swine bioassay was used for PCR positive but VI negative feed samples. This included the non-treated ingredients of corn, 3 VTM mixes, intestinal mucosa, soy oil, choline chloride, SDPP, purified plasma, as well as the LA-treated ingredients. Choice white grease, limestone, and threonine were tested as well. Piglets (n = 24) of 5-7 days old were divided into groups of four. Each pig received 1 mL of the designated inoculum orally via syringe. Pigs were observed for 7 days for clinical signs and rectal swabs were collected daily. If clinical signs were observed, swabs were collected from diarrhea and vomit samples and tested by PCR. Following completion of the bioassay, viable PEDV was detected only in piglets challenged with non-treated choline and choice white grease [15].

- Dee et al. (2016) designed a model to evaluate the transboundary risk of PEDV-contaminated swine feed ingredients during a simulated shipment from China to the United States and tested the effect of two mitigation strategies on the reduction of PEDV in feed using LA and 2% custom MCFA. Fourteen swine feed ingredients commonly imported from China to the United States were selected: organic and conventional soybeans and soybean meal (SBM), lysine HCL, D/L methionine, tryptophan, vitamins A, D, & E, choline chloride, rice hulls, corn cobs, and feed-grade tetracycline. The samples were organized into four batches, each representing a specific segment of the 37 day shipping journey. Each ingredient was allocated into each of the four batches in two replicates. Each batch of ingredients had a positive control group (non-treated), a negative control group (PEDV-negative ingredients), a LA-treated group (LA and PEDV spiked), and a MCFA-treated group (MCFA and PEDV spiked). The samples were housed in a programmed environmental chamber based on the temperature and percent relative humidity for each segment of the shipping journey. For exposure to ambient air, two holes were drilled into each plastic container. At designated DPI, samples were removed and submitted for diagnostic testing by RT-PCR and VI. Viable PEDV (positive VI) in batch 4 (representing shipment to and storage in Iowa) was found in non-treated organic and conventional SBM, lysine, and vitamin D [47].

  The swine bioassay was used to test ingredients that were PCR-positive for PEDV but negative by VI. This included non-treated ingredients vitamins A and E, tryptophan, D/L methionine, soybeans (organic and conventional) and choline chloride. Ingredients treated with LA or MCFA included soybean meal (conventional and organic), lysine, vitamin D, and choline chloride. Piglets (n = 24) of 5-7 days old were divided into groups of four. All four piglets in each unit received the same ingredient. Samples from batch 4 (to represent ingredients that would travel the entire 37-day journey to Iowa) were prepared for the bioassay. Piglets received 1 mL of the designated inoculum orally via syringe. Pigs were observed for 7 days for clinical signs and daily rectal swabs were taken and, if clinical, swabs were collected from diarrhea and vomit samples and tested by PCR. If diagnostic samples were PEDV-positive by PCR, all animals swabbed were euthanized and piglets re-stocked. If pigs remained PEDV-negative, the pigs were added to a different experimental group and inoculated with a different ingredient (re-used). Positive bioassay findings were observed in piglets that were administered non-treated choline chloride. None of the piglets fed LA- or MCFA-treated ingredients spiked with PEDV were positive on the swine bioassay [47].

- Dee et al. (2018) evaluated the survival of select viral pathogens in feed ingredients using models designed to simulate transportation conditions across two different regions of the world. Eleven viruses were selected: foot and mouth disease virus (FMDV), classical swine fever virus (CSFV), ASFV, influenza A virus of swine (IAV-S), PRV, Nipah virus, PRRSV, swine vesicular disease virus (SVDV), vesicular stomatitis virus (VSV), PCV2, and vesicular exanthema of swine

virus (VESV). Surrogate viruses were used for FMDV, CSFV, PRV, VESV, Nipah virus, and SVDV. Eleven feed ingredients were selected: organic and conventional SBM, soy oil cake, DDGS, lysine HCL, vitamin D, choline chloride, moist cat food, moist dog food, dry dog food, and pork sausage casings. Two transboundary shipping journeys were modeled: trans-Pacific (per Dee et al., 2016) to simulate travel between China and the United States and trans-Atlantic to simulate travel between Europe (Poland) and the United States (for ASFV only). The samples were organized into four batches, each representing a specific segment of each trans-ocean shipping journey. Duplicate samples of each ingredient were organized into the batches. Five gram samples of gamma-irradiated ingredients were spiked with 100 µL of minimum essential media containing 1 X 10^5 $TCID_{50}$/g of each virus. All samples were incubated in a programmed environmental chamber regulated for temperature and percent relative humidity. Samples of each ingredient/virus combination were removed from each batch at the predetermined DPI and tested by RT-PCR and VI. A wide variation in viability was observed across the virus-ingredient combinations from batch 4 [48].

The swine bioassay was used to determine infectivity of feed ingredients that tested positive by PCR but negative on VI in cell culture. The bioassay was performed with Seneca virus A (FMDV surrogate), PRRSV, porcine sapelovirus (SVDV surrogate), PCV2, ASFV, and IAV-S in selected virus-ingredient combinations. Pigs were inoculated by various methods, including orally via syringe, intramuscularly, and intranasally. A positive bioassay was observed for the following virus-ingredient combinations: PRRSV and conventional SBM; PRRSV and DDGS; Seneca virus A and choline; and PCV2 and lysine, choline, and vitamin D. The finding of Senecavirus A in choline was not detailed in the discussion section [48].

The investigators reported that from the virus-ingredient combinations subjected to various simulated environmental conditions, 7 viruses remained in a viable form in 2 or more ingredients: Seneca virus A, ASFV, PRRSV, porcine sapelovirus, PCV2, feline calicivirus (surrogate to VESV), bovine herpesvirus-1 (surrogate to PRV), and PEDV. The highest degree of stability was for Seneca virus A as viable virus was recovered from 10 of 11 ingredients. ASFV stock virus was the only virus to survive the simulated 30-day shipping journey in the absence of feed matrix. Half-life was calculated for Seneca virus A, porcine sapelovirus, feline calicivirus, bovine herpesvirus-1, and ASFV for each virus-ingredient combination. Feline calicivirus and Seneca virus A had extended half-lives in conventional SBM, 26.6 days and 9.7 day, respectively, as compared to other virus-ingredients combinations. Feline calicivirus had the longest half-life in conventional SBM at 26.6 days but much shorter half-life in other ingredients. In contrast, Seneca virus A had the least variability of half-life in feed, ranging from 1.7 to 9.7 days across 10 ingredients. The non-animal origin feed ingredients that supported survival of multiple viruses (n) included conventional SBM (n = 7), lysine (n = 5), vitamin D (n = 4), choline (n = 4), organic

SBM (n = 3), and DDGS (n = 2). The findings suggest that viruses can survive in feed but survival duration is variable and dependent on virus properties and feed matrix [48].

6.3.3 Summary of findings

For the experimental studies using field-sourced challenge virus, the swine bioassays in the Pillatzki et al. (2015) and Bowman et al. (2015) studies failed to demonstrate the infectivity of PEDV PCR-positive feed. Pillatzki et al. (2015) provides several plausible explanations, including the nucleic acid detected in the feed samples did not represent infectious virus; the feed samples had relatively high Ct values (range: 30.0 – 36.5); and an extended storage time between collection of the sample and the bioassay may have reduced or eliminated the infectivity of the PEDV. Similar explanations were provided by Bowman et al. (2015), adding that the small number of piglets used in the bioassay and the short feeding trial period lowered the sensitivity of the bioassay and did not realistically reflect the field setting. Despite the negative findings, Bowman et al. (2015) maintains that the contaminated feed pellets were the source of the outbreak in the Ohio swine operation. Pillatzki et al. (2015) also concluded that feed contaminated with infectious PEDV can serve as a vehicle for disease transmission, citing as evidence that the positive-control piglets that were administered spiked feed did develop clinical signs of PED and PEDV fecal shedding. The findings in Pillatzki et al. (2015) are consistent with the findings of Dee et al. (2014). Both studies produced challenge feeds with a low Ct value (range: 18.23 – 25.5) and the bioassay conducted with the PEDV-contaminated feeds produced disease.

For the experimental studies using laboratory-sourced challenge virus, Goyal (2014) produced preliminary evidence that under laboratory conditions, PEDV can survive in PEDV-spiked wet and dry feed at room temperature and remain infectious for ≥ 28 days and 7 days, respectively [45]. Other studies on PEDV survival in individual swine feed ingredients support these findings [15, 41, 42, 47]. Schumacher et al. (2016) produced preliminary evidence on the minimum infectious dose of PEDV-inoculated feed as 5.6×10^1 $TCID_{50}$/g with Ct values ranging from 27 – 37 [46].

The findings in three studies, under different experiment conditions, suggest that virus survival is ingredient-dependent. Varying physical and chemical characteristics of feed ingredients may enhance or protect virus survival [15, 47, 48]. Dee et al. (2015) noted several interesting or novel findings, including an extended survival time observed in conventional SBM; recovery of viable PEDV from (all non-treated) DDGS, 3 synthetic amino acids, and dry choline chloride; and the inability to recover viable PEDV from SDPP [in contrast to Pasick et al. (2014)] or the 3 VTM mixes. Dee et al. (2016) study also found viable PEDV in PEDV-spiked conventional SBM, lysine, and choline. Additionally, PEDV did survive and remain infectious in vitamin D [47]. Both formaldehyde-based LA and MCFA treatment rendered virus inactive, regardless of ingredient type, suggesting these mitigants might be useful as part of a risk management strategy for reducing viral load in feed ingredients [15, 47].

Building upon the shipping model methods developed in the Dee et al. (2016) study, the Dee et al. (2018) study expanded the selection of viral pathogens as well as added an additional trans-oceanic

shipping route from Europe (Poland) to the United States for ASFV. Like the two preceding studies, Dee et al. (2018) found that certain feed ingredient matrices enhance or protect viral survival. Of interest, the authors noted that virus viability in organic SBM could not be demonstrated. This finding could discount previous speculation that the rise in organic swine farming may have contributed to PEDV introduction in to the United States. Additionally, the authors noted that ASFV demonstrated strong survivability characteristics, remaining viable under laboratory-simulated conditions with or without the feed matrix [48].

In summary, the infective dose of PEDV is low and, experimentally, infectivity of the feed material is dependent on viral load (e.g., Ct value). Additionally, experimental evidence indicates that the duration of virus survival in swine feed ingredients is dependent on the ingredient matrix and the virus-ingredient combination. The feed ingredients that have shown to support virus survivability and viability include conventional soybean meal, lysine, choline chloride, and vitamin D. ASFV demonstrated strong survivability characteristics.

Among the knowledge gaps identified in these studies are the identification of vulnerable (risky) non-animal origin feed ingredients for viral contamination, ingredient (matrix) characteristics that support or hinder virus survival, and identification of the critical point(s) in the transboundary feed production and distribution continuum where (fecal) contamination of non-animal origin ingredients could occur. There is a paucity of field data demonstrating if, how, and when non-animal origin feed ingredients may become contaminated with swine viruses. Further, although some field epidemiological investigations have associated contaminated feed (PCR positive) with the source of virus introduction on affected farms, to date, experimental studies designed to prove causation of virus transmission via feed and feed ingredients have yielded inconsistent data. It is also unknown what characteristics of non-animal origin feed ingredients may contribute to virus survival, if present. Additionally, the development of diagnostic assays and sampling techniques, capable of testing large volumes of non-animal origin feed ingredients to determine whether the level of contamination is sufficient to transmit disease is required to better assess the frequency with which these materials are contaminated with virus, which viruses are present, and at what concentration. Subsequent assays to determine contamination of the final, processed feed product would be useful to determine the likelihood that contaminated non-animal origin feed ingredients would transmit virus to swine and to what degree feed should be prioritized as a biosecurity risk.

7 Discussion

The objective of this literature review was to gather and analyze the evidence in published scientific literature regarding whether non-animal origin ingredients of commercial swine feed could introduce and transmit viral pathogens of swine into or within the United States. The goal was to understand the current scientific knowledge and to identify information gaps to better inform decision makers, other stakeholders, and the scientific community. To achieve this, the basic steps of a systematic review methodology were followed [1], resulting in a qualitative summary report of findings.

The results of the literature review demonstrate that a limited number of studies currently address swine viral pathogen transmission through non-animal origin feed and feed ingredients. Of the studies available, several suffered from limitations that hinder generalizing the findings to real-world commercial swine scenarios. For example, studies were limited by small feed sample volumes (as small as 5 grams) which does not directly equate to the quantities (tonnage) in actual swine production and feed scenarios; small sample sizes (2 replicates) which reduced statistical strength and confidence of findings; low sensitivity in swine bioassays; experimental methods which do not mimic natural feeding behaviors of swine or large-scale commercial swine production; and environmental scenarios that cannot be easily extrapolated to other seasons or geographical regions. Bioassays, including those reviewed in this document, often suffer from low sensitivity due to low number of subject animals (n = 4 in the studies reviewed); health status of subject animals often does not reflect the immune status variability observed in the field setting; and subject animals are typically exposed or inoculated once (single-hit concept) whereas multiple or on-going exposures occur in the field setting. Thus, results of the bioassay may not equate to real-life scenarios. Other studies, particularly those with retrospective questionnaire or survey components, were limited by inherent sources of internal bias such as selection and recall bias. Furthermore, robust replication of studies in independent laboratories and field settings to validate or corroborate findings has not occurred. Thus, conclusions drawn from these studies should be interpreted with caution until repeatability of the findings can be demonstrated, particularly under conditions that mimic the field setting.

Despite the small number of published studies, several key themes have emerged, many of which warrant additional exploration and research:

- A subset of the studies reviewed provided experimental evidence that swine viruses can survive in non-animal origin feed ingredients under various experimental conditions [15, 30, 45, 47, 48]. Virus survival times were variable (ranging from 7 days to > 180 days) and dependent on the simulated environmental conditions applied (e.g., temperature and relative humidity) and the virus-ingredient combination. Others concluded that duration of virus survival (and infectivity) is ingredient-dependent and certain feed ingredients provide a more favorable matrix than others for extended survival [15, 47, 48]. Additional research is needed to verify virus survival times (and infectivity) in complete feed and feed ingredients, with various virus-ingredient combinations under various environmental conditions, including actual field conditions.

- Several authors speculated that characteristics of the ingredient such as the physical supportive matrix and/or the chemical (bromatological) composition contributed to virus survival [15, 30, 31, 48]. However, the specific characteristic(s) of the ingredient that contribute to viral persistence have not been identified or proven.
- A subset of experimental studies provided evidence that feed contaminated with virus can transmit disease to naive piglets [9, 15, 45-48]. However, the experimental methods used in these studies such as spiking ingredients with predetermined virus load or inoculating piglets via methods other than natural feeding behaviors, do not reflect the field setting and results may not be generalizable to field conditions, particularly large-scale commercial swine facilities. Additional laboratory and field-based studies are needed to determine the extent of finding reproducibility and applicability to field settings.
- A subset of studies have attempted to identify individual feed ingredients that may be more likely than others to support virus survivability [15, 47, 48]. Viability and infectivity of each virus were assessed through VI and/or swine bioassay. The presence of a viable form (meaning a positive VI and/or bioassay) of virus (at ≥ 30 days) was confirmed in the following non-animal origin ingredients that had been experimentally spiked with virus inoculate: organic and conventional SBM, DDGS, lysine HCL, D/L methionine, choline chloride, and vitamin D [15, 47, 48]. Extended survival was observed in virus-spiked conventional SBM [15]. Two experimental studies, using different experimental conditions, observed PEDV viability in three PEDV-spiked ingredients: conventional SBM, lysine, and choline chloride. [15, 47]. Virus viability (and infectivity) as determined in separate swine bioassays was observed with PEDV-contaminated choline chloride [15, 47] and Seneca virus A-contaminated choline chloride [48].
- A major knowledge gap exists in sources of potential contamination and where feed or feed ingredients may be contaminated. These experimental studies conducted with inoculated non-animal origin feed ingredients do not address the question of whether these ingredients are contaminated with swine viruses under field conditions. The critical point(s) of susceptibility to contamination along the feed production, processing, and distribution continuum, from harvesting the plant-derived feed ingredients in the field to on-farm delivery of feed to swine premises, have not been identified.
- Neither the contamination route of exotic swine pathogens into non-animal origin feed (ingredients) nor the virus entry route into the United States has been decisively proven.
- Under the laboratory-simulated model conditions, both LA and MCFA were concluded to be effective chemical mitigants against PEDV in individual feed ingredients stored under simulated shipping conditions, suggesting they might be useful for reducing viral load in feed ingredients [15, 47]. The real-world application of LA and MCFA for eliminating swine viruses or decreasing their level of infectivity under field conditions has yet to be determined. If field-contamination of non-animal origin ingredients is determined to be a concern for pathogen transmission, additional mitigation strategies should continue to be explored, including other chemical treatments, the application of heat or pressure (pelleting) to feed, and holding times for feed or feed ingredients.

- Some of the outbreak epidemiological investigations provided evidence that the transmission route(s) for swine viruses onto the index farm may differ from the transmission route(s) among housing units within the index farm and between secondary farms. Similarly, the entry route (e.g., for PEDV) into the United States may differ from the transmission route(s) among domestic swine farms. Thus, as with other infectious diseases, multi-modal transmission mechanisms are likely occurring. When considering non-animal origin feed ingredients as a potential fomite for swine virus transmission, it's important to understand how the primary transmission pathways (e.g., infected live pigs, contaminated transport vehicles, personnel, etc.) interface with one another, particularly how the production and distribution of feed interacts with other sources of virus contamination (e.g., infected live pigs, contaminated transport vehicles, personnel, etc.) to contribute, if at all, to disease transmission.
- Similarly, it's important to understand the relative risks of various transmission pathways and where feed ingredients fit in among broadly accepted risk pathways such as movement of infected pigs and fecal contamination of fomites (e.g., transport vehicles). By understanding the magnitude of the risk of feed ingredients, one can better balance the costs of sourcing "safe" feed ingredients and the nutritional needs of pigs with the costs of applying various mitigation strategies to potentially contaminated feed (e.g., heat or chemical treatment or feed holding times).
- Reliable and validated assays and sampling techniques capable of detecting infective virus (i.e., to determine the level of contamination is sufficient to transmit disease) in large quantities of (bulk) ingredients are not available.
- As mentioned above, the critical point(s) of contamination along the feed production, processing, and distribution continuum, from harvesting the plant-derived feed ingredients in the field to on-farm delivery of feed to swine premises, have not been identified. Because of this some of the experimental studies reviewed were performed under the assumption that ingredients are contaminated in the post-processing stage of feed production. Very little information is available on how non-animal origin feed ingredients are produced and sourced outside the United States and current studies have produced little scientific evidence of how, or if, non-animal origin feed ingredients could become contaminated with swine viruses in regions outside the United States. Taking a systematic approach to the entire (transboundary) feed production system, similar to the hazard analysis and critical control points process used in food safety, may help to identify vulnerabilities in the production process, better inform the development and application of mitigation measures to reduce viral contamination risks, and help stakeholders allocate resources towards mitigation measures based on the likelihood of virus contamination.
- Over the past several decades, the U.S. commercial swine industry has improved biosecurity measures on commercial premises to prevent transmission of economically significant viruses such as PCV2, PRRSV, and PEDV. Many commercial swine operations use the absence of PRRS on the farm as an indicator of thorough implementation and enforcement of biosecurity measures. In the studies reviewed, many favorably described their biosecurity measures and echoed sentiments expressed by Bowman et al. (2015) that the "effectiveness of the biosecurity

measures in place was evidenced by the absence of PRRS cases". However, the entry of PEDV (and new or emerging swine viruses) onto presumably biosecure commercial premises suggests that current biosecurity standards are insufficient to prevent virus incursion. Virus characteristics and the characteristics of the commercial swine industry (globalization of trade, intensification and vertical integration of production, and extensive movement of pigs and related production components) could contribute to biosecurity breaches. Robust biosecurity measures may be the only tool, in the absence of effective vaccines or treatments, to prevent the entry and spread of some diseases. Thus, biosecurity strategies, particularly the extensive movement of production inputs, need to be re-evaluated and adjusted to meet today's swine industry paradigm.

While investigators have addressed some critical experimental questions pertaining to transmission of swine viruses via feed and feed ingredients, the current body of scientific knowledge has yet to provide conclusive evidence for the source(s) of contamination of non-animal origin feed ingredients with swine viruses and the epidemiology of virus transmission to swine under field conditions. If the primary concern of the swine industry and associated stakeholders lies in the importation of contaminated feed and feed ingredients, then additional research and investigative studies of how ingredients are sourced, processed, and transported prior to importation into the United States are needed. However, the lack of feed and feed ingredient diagnostic assays and sampling techniques capable of detecting virus in large volumes of material limits our ability to determine if and at what point non-animal origin feed or feed ingredients may become contaminated with viruses and limits our ability to establish critical control points in feed production, distribution, and storage to mitigate risk(s). Until these data are available, it is difficult to evaluate the biosecurity risk posed by non-animal origin feed and feed ingredients. Moving forward, studies designed to examine the likely source(s) of contamination and virus mitigation steps in processing and post-processing may be the most fruitful focus of research.

8 Appendix I: Sample data extraction form

Data Extraction Form for Non-animal Origin Feed Ingredient (NOFI) Literature Review

General Information

1. Reviewer(s):
2. Date of data extraction:
3. Author(s):
4. Article Title:
5. Type of publication:

☒ No Selection ☐ Journal article ☐ Government publication
☐ Conference abstract ☐ Other (specify)

6. Publication name/year:
7. Country of origin:
8. Source of funding/author affiliation:

Study Characteristics

1. Study objectives:
2. Study design:
3. Study inclusion and exclusion criteria:
4. Unit of allocation:
5. Study description (brief)

Outcome data/results

1. Unit of assessment/analysis

2. Statistical techniques used:

3. Results of study:

Quality Assessment

1. Appropriateness of study design to the research objective:

2. Sources of potential bias:

3. Statistical/analysis issues:

4. Quality of reporting:

5. Generalizability:

6. Overall quality:

☒ No selection ☐ High ☐ Medium ☐ Low

7. Comments:

9 Appendix II: Data synthesis table

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
Bowman et al., 2015 [39]	Epidemiological case report with bioassay Funding provided by National Pork Checkoff, PIC North America, and USDA.	PEDV	Feed, starter feed pellets	Through epidemiological investigation of a swine operation in Ohio, contaminated feed (starter feed pellets) was identified as the likely source of PEDV introduction. The feed and feed source was RT-PCR-positive for PEDV. A bioassay was performed with samples of cryopreserved feed.	Authors determined the starter pellet to be the source of PEDV introduction. PEDV RNA was detected inside unopened bags of new pellets, at the source facility (supplier), and in individual ingredients at the source facility. For the bioassay, naive pigs fed PCR-positive feed from the supplier remained negative for PEDV.	The results of the bioassay were negative but experimental conditions may not reflect field conditions. The authors made several hefty assumptions regarding biosecurity at the affected farm and ruled out other possible transmission routes without providing the supporting data.
Brookes et al., 2015 [27]	Expert elicitation Funding provided by Australian Pork Limited.	PRRSV	Multiple	Through expert elicitation, authors examine the most probable route of introduction of highly pathogenic PRRSV from southeast Asia into Australia. Participant answers were analyzed for commonalities and	Overall, significant agreement of respondents' opinions for exposure routes involved disposal of waste to feral and backyard pigs. For commercial pigs, the highest probability exposure route was	The study design has several sources of potential bias, including participant selection, response/cognitive bias, framing bias, and recency bias. Authors examine multiple entry and exposure routes to analyze perceived risks by

[4] A fomite is defined as an inanimate object or material that is likely to carry infection such as animal feed, feed ingredients, organic substrates, transport vehicles, boots, etc.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
				agreements were grouped and ordered.	human fomite or access to animal feed/additives from SE Asia (very low). Less agreement on entry routes; animal feed/additives were given moderate probability.	industry experts' opinions. Thus, specific pathways (e.g. animal feed/additives) are not adequately defined or examined to the level of detail needed for in-depth analysis.
Davies, 2015 [5]	Review article Funding source not listed.	PRRSV, PCV2 and PEDV	Feed, SDPP	The article describes 3 major swine diseases, including PRRSV, PCV2 and PEDV. Similarities among the viruses are discussed. The virus transmission likelihood resulting from feeding animal products (SDPP) to swine and whether or not the true risk warrants excluding animal origin products from swine diets is examined.	The author suggests that "new" viruses in swine are likely to emerge from already recognized (non-pathogenic or ignored) swine viruses. The likelihood of PEDV survival in SDPP is extremely low, but not zero. A blanket ban on certain ingredients in swine feed may not be the solution. A comprehensive evaluation of transmission pathways as well as cost-benefit analyses of managing feed-related risks and	The discussion focused on SDPP; however, key points can be generalized to non-animal origin ingredients. Increasing herd sizes (and assuming fixed biosecurity practices) along with greater flux of inputs translates to a higher temporal frequency of adverse events. The features of modern swine production (global trade, intense production, extensive movements) have contributed to the emergence of these pathogenic strains and we should expect this trend to continue.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
					nutritional value is needed.	
Dee et al., 2014 [9]	Experimental with bioassay Funding provided by Pipestone Applied Research.	PEDV	Complete feed	At-risk feed bins were sampled on 3 index farms. Feed bins at 4 PEDV-negative farms were also sampled. All samples were tested for PEDV by RT-PCR. For the bioassay, 11 pigs were divided into 3 groups – a treatment group fed PCR-positive feed bin samples, a positive control group fed PEDV- spiked feed, and a negative control group fed a placebo. Groups were fed PCR-positive feed or placebo feed (ad libitum) on day 0 and PCR-negative feed throughout the remainder of the study. Pigs were necropsied on day 7 post- challenge.	Assessment of feed material in the at-risk bins across the 3 sites were PCR-positive for PEDV. All samples from control bins and PEDV-negative sites were PCR-negative. For the bioassay, treatment and positive control groups exhibited clinical signs of PED and were PCR-positive for virus. The negative control group displayed no clinical signs of disease and were PCR-negative.	The bioassay sought to mimic on-farm transmission conditions by using PCR-positive feed material from confirmed PED affected farms and using a natural feeding method (ad libitum). An acknowledged limitation was that the *in vivo* study was not designed to estimate the frequency of feed-related PEDV infections. Results were based on a very small populations of pigs and cannot be extrapolated to today's commercial farm and field conditions.
Dee et al., 2015 [15]	Experimental with bioassay Kemin Industries and Dr. Mark Bienhoff were	PEDV	Feed ingredients: corn, SBM, DDGS, SDPP, purified plasma, intestinal	Common swine feed ingredients (18) were divided into two groups in replicate – LA treatment group and a non-treated group. Controls included complete feed spiked with	Only LA-treated samples of SBM and MBM remained PEDV-positive at 30 DPI. Supplementary testing of SBM (non-LA treated) was	The study assumes post-processing contamination of ingredients; the index step or point of contamination in feed manufacturing/feed delivery is relatively

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
	acknowledged for providing technical expertise, funding and in-kind resources.		mucosa, MBM, RBC, 3 VTM mixes, white grease, soy oil, lysine HCL, D/L methionine, threonine, limestone, dry choline chloride	PEDV (positive) or saline (negative). The samples were stored outside in winter conditions in plastic totes. At 1, 7, 14 and 30 DPI, feed samples were removed and were tested for PEDV, PDCoV, and TGE by RT-PCR. The presence of viable virus was tested by VI. A swine bioassay was conducted for PCR positive/VI negative feed samples. Piglets 5-7 days old were divided into groups of 4. Pigs received the designated inoculum orally via syringe and were observed for 7 days. The negative control group was given saline PO. If clinical signs observed, swabs were taken of diarrhea and vomit. Swabs were tested by PCR. If PEDV positive, all animals swabbed were euthanized; units were cleaned and piglets re-stocked as needed.	negative by PCR and VI for up to 210 days. PEDV was not detected in other treated ingredients. The following ingredients were used in the bioassay: (non-LA treated) corn, 3 VTM mixes, intestinal mucosa, soy oil, choline chloride, SDPP, purified plasma; (LA-treated) white grease, limestone, and threonine. Viable PEDV was detected only in piglets given choline and choice white grease. PEDV viability may be influenced by ingredient type. Extended virus survival observed in SBM. LA is effective in rendering PEDV inactive, independent of ingredient type.	unknown. Only 2 replicates per ingredient were used; confidence intervals and confidence levels were not provided. Small ingredient samples (30 g) may not equate to the quantities (tonnage) in actual swine production and feed scenarios. Outdoor winter weather conditions in this study may not be extrapolated to other climates or time periods. For the bioassay, pooled sampling was used across days 7, 14, and 30 DPI (not daily testing of samples) and piglets were re-used following negative bioassays. Bioassay inoculums were given orally via syringe which does not mimic normal feeding habits and may not correlate to field conditions.
Dee et al., 2016 [47]	Experimental with bioassay	PEDV	Feed ingredients:	The shipping journey from Beijing, China to Des	First proof of concept study indicating PEDV	The shipping timetable was based on one

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
	Kemin Industries, APC, Inc. and National Pork Board were acknowledged for providing technical expertise, funding and in-kind resources.		organic and conventional soybeans, SBM, Lysine HCL, D/L methionine, tryptophan, Vitamin A, D, & E, choline chloride, rice hulls, corn cobs, and feed-grade tetracycline	Moines, Iowa was divided into 4 travel segments, represented by 4 sample batches. Two replicates of the 14 ingredients were allocated into the 4 travel segments. Each batch had a positive control group (PEDV with no LA treatment), LA-treated group and MCFA-treated group. Also negative controls (PEDV-negative feed with saline) and stock PEDV samples. Samples were housed in a programmed environmental chamber to mimic weather between China and Iowa in Dec 2012/Jan 2013. At designated DPI, samples were submitted for diagnostic testing with RT-PCR and VI. A bioassay was performed for PCR-positive, VI-negative samples. Piglets (5 days old) received inoculum (from batch 4) orally via syringe and were observed for 7 days. If	survival in specific feed ingredients under modeled shipping conditions (China to US). After 37-day period, viable PEDV found (via VI) in Vitamin D, lysine hydrochloride, organic and conventional SBM. For the bioassay, piglets became PEDV-positive when administered non LA-treated choline chloride. Both LA and MCFA were concluded to be effective chemical mitigations as a means to reduce the risk of PEDV in feed ingredients.	website, not (multiple) data from actual shipping times and may not reflect real-life scenarios. Small ingredient samples (30 g) may not equate to the quantities (tonnage) in actual swine production and feed scenarios. Simulated environmental conditions may not be extrapolated to other environmental conditions. Bioassay inoculums were given orally via syringe which does not mimic normal feeding habits. In the discussion, authors may have overstated (or did not adequately provide justifications for) the "risk" of organic farming and imported soybean products. Similar inferences were not stated in the discussion for ingredients commonly used in commercial swine despite similar study results.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
				clinical, swabs were taken of diarrhea and vomit and tested by PCR. If PCR-positive, animals swabbed were euthanized; units cleans and piglets re-stocked as needed.		
Dee et al., 2018 [48]	Experimental with bioassay Funding provided by Swine Health Information Center (SHIC); American Association of Swine Veterinarians Foundation; State of Kansas National Bio and Agro-defense Facility Fund; SDSU Animal Disease Research and Diagnostic Laboratory. Salaries of four authors paid by	SVA (FMDV)[5], bovine viral diarrhea virus (CSFV), BHV-1 (pseudorabies virus), canine distemper virus (Nipah virus), PSV (SVDV), FCV (vesicular exanthema of swine virus), ASFV, IAV-S, PRRSV, vesicular stomatitis virus, and PCV2.	Feed ingredients: organic & conventional SBM, soy oil cake, DDGS, lysine HCL, Vitamin D, choline chloride, moist cat food, moist dog food, dry dog food and pork sausage casings	Similar shipping model to Dee et al. 2016 employed. Two shipping routes: Trans-Atlantic (Poland to US) for ASFV and Tran-Pacific (China to US) for all other viruses. Eleven ingredient/11 virus combinations were assembled for trip segments. Five gram, gamma-irradiated samples were spiked with virus; stored in environmentally-controlled chambers. Each ingredient/virus combo was tested by RT-PCR and VI on the appropriate day (based on simulated travel). A bioassay was performed for PCR-positive,	Seven of 11 viruses remained viable in 2 or more ingredients (SVA, ASFV, PRRSV, PSV, PCV2, FCV and BHV-1). SVA was recovered from 10 of 11 ingredients. ASFV samples survived the simulated 30-day shipping in the absence of feed matrix. FCV and SVA had extended half-lives in conventional SBM; SVA had the most stable half-life range (1.7 to 9.7 days across 10 ingredients). FCV had the longest survival in	Small ingredient samples (5 g) may not equate to the quantities (tonnage) in actual swine production and feed scenarios. Confidence intervals were not calculated due to too few replications. Samples were spiked with the same amount of virus which may not reflect proposed field contamination. Bioassay inoculums were given IM, IN or orally via syringe which does not mimic normal feeding habits. Results seem to negate previous reports (Dee et al. 2016) that organic

[5] Surrogate viruses were used for viruses listed in parentheses. For other viruses listed, actual virus was used.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
	Pipestone Applied Research.			VI-negative samples, which included SVA, PRRSV, PSV, PCV2, ASFV, and IAV-S.	conventional SBM (26.6 days). Findings indicate viruses can survive in feed; survival duration is variable and dependent on virus properties and feed matrix. Data indicates non-enveloped viruses are more resistant in the environment than enveloped.	soybean products pose an increased "risk" of virus transmission compared to non-organic ingredients.
EFSA AHAW Panel, 2014 [34]	Descriptive; qualitative literature review 2004-2014 Funding source not listed.	PEDV, PDCoV	N/A	At the request of the European Commission, EFSA AHAW Panel was tasked to deliver a scientific opinion on the current scientific evidence, epidemiological situation, and knowledge/data gaps regarding PEDV and PDCoV.	Transmission of these viruses in feed or feed ingredients was not directly addressed in this report. Overall, the major recommendation(s) relevant to NOFI included the importance of strict biosecurity, in particular with vehicles, to prevent introduction of PEDV onto the farm.	Comprehensive summation of the current knowledge of PEDV from 2004 to September 2014.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
EFSA, 2016 [33]	Descriptive; qualitative literature review Oct 2014 - Oct 2015 Funding source not listed.	PEDV	N/A	The European Commission requested EFSA to 1) provide guidance on PEDV data to be collected by EU Member States in order to optimize coordination of response, and 2) analyze the epidemiological data from EU Member States and in the scientific literature. The review focused on occurrence of infection with different PEDV strains, morbidity/mortality rates and severity of clinical disease.	Conclusions and recommendations starts pg. 20 & many annexes collate the findings and data from the updated literature review.	EU-centric report; however, recommendations pg. 20-21 are generalizable to US. For the impact of PEDV to EU farms, the authors noted in Table 3 that data were missing and analysis is difficult for non-reportable diseases. Due to the missing data, results in Table 3 must be interpreted with caution.
Fasina et al., 2012 [24]	Retrospective case-control study Funding source not listed.	ASFV	Feed (swill), water, rodents, equipment, people	A survey of farm characteristics, farm operations, and self-reported biosecurity measures was administered to case and control farm owners. Statistical analysis was performed on responses and risk factors using univariable and multivariable conditional logistic regression models.	Protection of feed and water (from rodents) and purchasing of commercial feed (vs swill feeding) was negatively associated (protective) with acquiring ASFV. Presence of abattoir in the community and infected neighboring farms was positively associated with risk for ASFV.	The study examined associative relationships between farm practices and risk of ASFV. Swill feeding was not defined but presumably swill would contain both animal and non-animal origin feed components. Self-reporting and recall bias is possible given study design.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
Goyal, S.M., 2014 [45]	Experimental; quasi-experimental Funding provided by Pork Checkoff.	PEDV; TGE virus	Feces, slurry, wet and dry feed, water	PEDV and TGE virus were inoculated into animal feed (dry and wet) and water. Feces and slurry from infected animals were tested for virus. Spiked feed was incubated and instilled into the esophagus of piglets. Animals were scored for signs of PED/TGE and sacrificed.	PEDV and TGE virus were detected by RT-PCR in dry and wet feed, feces samples, slurry and water. All piglets inoculated with spiked feed became infected with PEDV at viral dilutions up to 10^{-9}.	The study used a small sample set for the bioassay (12 piglets). No statistical analyses were performed for the experiments. Bioassay inoculation performed by gavage instead of free feed. Author demonstrates that virus can survive in various organic materials and that pooled samples of spiked feed can infect piglets with PEDV. Authors attempt to extrapolate findings to infections that occur in the field but this is unsubstantiated.
Greiner, L.L., 2016 [40]	Descriptive Funding provided by the National Pork Board.	PEDV; PDCoV	Feed mill fomites: office floors, bulk ingredient pit grates, trucks carrying bagged ingredients, mixer/pellet cooler, inside feed	Fomites at 24 US feed mills were swabbed for 5 consecutive days. Samples were analyzed by PCR at 1 of 4 laboratories. Eighteen of the 24 feed mills serviced farms known to be positive for PEDV; 5 delivered to PDCoV-positive farms.	No samples tested positive for PEDV RNA; 5% of truck foot pedals and 1% of bulk ingredient pits were suspect for PDCoV RNA; 3.4% of truck foot pedals and 2.2% of office floors were positive for PDCoV RNA. No bulk	The study examined various control/entry points for virus at feed mills. Authors did not find a positive correlation between virus presence at the feed mill and probability of PEDV infection on farms serviced.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
			compartment on feed truck, foot pedals of feed delivery truck		ingredient pits or mixer/coolers were positive for either virus.	
Guinat et al., 2016 [26]	Review Article Funding provided by the European Seventh Framework Programme.	ASFV	Review examining ASFV transmission (pig-to-pig, fomites, ticks, feed)	Research summarized describes transmission of ASFV via feeding of contaminated animal meat. ASFV was not transmitted by contaminated sweet potatoes or bananas (1921 study). It was reported that a study in East Africa showed that ASFV was not transmitted by consuming non-animal origin feed (review 1969).	Authors conclude current research supports transmission of ASFV in feed containing contaminated swine products. Transmission through non-animal origin feed is less conclusive. Additional studies are required to determine if transmission occurs in feed not containing swine products.	In the section on feed, authors conclude viral strain may impact transmission via this route. In this respect, extrapolation of findings to other viruses/different feeds may not be appropriate.
Le et al., 2012 [25]	Retrospective survey Funding source not listed.	PHFDV	N/A	Retrospective survey classified cases of PHFD in southern Vietnam. Statistical analysis was conducted to identify potential risk factors associated with disease status at household level.	The study found PHFDV prevalence was 33.4% and risk factors included: higher numbers of sows and finishing pigs, receiving pigs from an external source and the interaction between using 'water green	The smaller study area limits extrapolation of results to other areas. Case identification was based only on clinical signs and no diagnostic assays were used. Farm size is a confounding factor.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
					crop' as pig feed and owning ducks with or without direct contact with pigs.	
Lowe, J. F. 2014 [35]	Review article Funding source not listed.	PEDV	Feed, vehicles, people, and other fomites	The article reviews the US PED outbreak in 2013. The author summarizes clinical signs, virus shedding, immune responses, epidemiology, origin of the outbreak and risk factors contributing to transmission such as herd/farm management, transportation and fomites.	In this outbreak, PEDV was transmitted by livestock transport, movement of people, vehicles, and other contaminated fomites, and shared resources/equipment. The author suggests feed contaminated with infectious fecal material could transmit virus and that enhanced control procedures may provide protection against outbreaks of PED or other novel diseases in the future.	None
Martinez-Gamba et al., 2001 [32]	Experimental Funding provided by PAPIIT-UNAM Project # INI210997.	Aujeszky's Disease virus; Blue Eye Disease virus	Ensilages (solid fraction of pig feces)	Swine feces was obtained from 30 pigs to prepare ensilage. A serological survey of the animals was performed to see if they were free of both pathogens. ADV and BEDV were inoculated into micro-	No animals had antibodies against either the ADV or the BEDV and all samples obtained from micro-silos at different times of ensilage were negative for both	Due to the small sample set (1 farm, 30 animals, and 5-15 samples from each area) the study is not easily extrapolated to other farms/conditions. Ensilage appears to

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
				silos and tested by viral identification methods.	viruses. Immunofluorescence and electron microscopy were positive only at 60 min after inoculation.	inactivate both viruses examined.
McCluskey et al., 2016 [37]	Retrospective testing; case series study Funding source not listed.	PDCoV	Feed, feed components, trucks, equipment and drivers, farm employees, and visitors	Banked samples (feces, fecal swabs, intestines, or oral fluids) from commercial swine farms in 27 states were tested by PCR to estimate initial time point of PDCoV introduction. A survey was conducted to examine biosecurity practices and disease status over time.	Only 4 samples out of 2286 were PCR-positive for PDCoV. Nearly 29% of sites with ill gestating sows and gilts that purchased feed delivered in the 10 days prior to onset of PDCoV sourced feed ingredients from outside the US. The authors conclude that the earliest detections in August and October 2013 may have had limited spread due to warm summer and fall temperatures.	The study examined a small number of operations (42 breeding farms). Authors did not discuss potential sources of bias – (e.g. survey and recall biases).
Niederwerder and Hesse, 2018 [38]	Review article	PEDV, PDCoV	Feed, trucks	Review examined SECV detection, epidemiology, and control efforts in the U.S. and Canada. Transmission and risk	The authors conclude that fecal–oral is the primary transmission route for SECV. Those surveyed (73.6%)	Survey results cannot be extrapolated due to the low number of respondents (40) and

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
	Funding provided by the National Pork Board.			factors for introduction were also discussed. A survey of U.S. swine veterinarians and managers was conducted to compile information related to SECV including suspected sources of introduction.	believed truck movements onto farms, feed and biosecurity issues were the most likely routes of SECV introduction.	small number of herds (83).
Pillatzki et al., 2015 [44]	Experimental with bioassay Funding provided by the American Association of Swine Veterinarians.	PEDV	Complete feed, feed pre-mix, and dried porcine plasma retained by feed manufacturers from April and May 2013	Investigators obtained 3 PEDV feed samples - complete feed, feed pre-mix and SPDD. After confirming the feed samples were PCR-positive, they performed a swine bioassay. Piglets were separated into 5 groups and inoculated with untreated feed (negative control), one of the PEDV-contaminated feeds (3 experimental groups), or feed spiked with PEDV stock virus (positive control). Feed samples were mixed with saline and supernatants were instilled into animals by gavage. Rectal swabs were collected daily. Pigs were euthanized on day 7,	No clinical signs were observed in piglets from the negative controls or treatment groups inoculated with PCR-positive pre-mix feed, SDPP, or complete feed. Also, fecal swabs collected from these groups were PEDV-negative, no histologic lesions were, and PEDV was not detected by IHC. The positive control group developed clinical signs at 3 DPI, and feces was PCR-positive. No histology or IHC results were presented for positive controls.	The extended storage of the feed samples might have impacted virus viability. Authors suggest that these contaminated feed samples might not have been representative of the overall concentration of PEDV in the entire batch of feed. The method of inoculation for the bioassay (gastric gavage) does not reflect natural field transmission conditions.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
				necropsied, and small intestine and colon samples were collected for analysis.		
Pirtle and Beran, 1996 [31]	Experimental Funding provided by Iowa Pork Producers.	PRRSV	Solid- stainless steel, plastic, boot rubber Porous- ground corn, pelleted swine starter feed mix, wood shavings, alfalfa, straw, denim cloth Liquid- PBSS, saline G, well water, city water, and swine urine, saliva, and fecal slurry	Three solid fomites, 6 porous fomites, and 7 liquids (25-27 °C) were contaminated with PRRSV. Samples were obtained on day 0 through day 11 for VI, cell culture assay, and fluorescent antibody staining.	Only the day 0 samples of the 3 solid fomites contained PRRSV. PRRSV was isolated only at day 0 for 3 porous fomites (alfalfa, wood shavings, and straw) and not detected in any samples for 3 porous fomites (corn, swine starter feed and denim cloth). PRRSV was isolated only at day 0 from all swine secretions (urine, saliva, fecal slurry). PRRSV was detected in two buffer solutions through days 4 and 6; in well water through day 9; and in city water through day 11.	Fomites were spiked with stock virus at doses that may not reflect contamination levels under field conditions, therefore, extrapolation to field conditions is limited.
Sasaki et al., 2016 [36]	Retrospective case-control study	PEDV	People, trucks, equipment, feed, artificial	Japanese swine producers were surveyed for information regarding herd management practices for	For locally-exposed farms, 8 of 20 variables were associated with PED	Authors report that participants selected for this study may not be representative of the

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
	Funding provided by a KAKENHI Grants-in-Aid for Scientific Research) from the Japan Society for the Promotion of Science.		milk, manure, pests	a two-week time period relevant to PEDV exposure. The goal of the study was to test the hypothesis that factors associated with high risk of PEDV infection were different for locally exposed farms (within 5 km of another PEDV-infected premises) than for non-locally exposed farms (greater than 5 km of another PEDV-infected farm). Investigators sought to quantify the dynamics of PEDV spread and support the design and implementation of PED prevention and control measures in Japan.	status such as, increased farm size, shorter distances to the closest PEDV-positive farm, and a disinfectant contact time of less than 20 minutes. In non-locally exposed farms, PED status was associated with increased feed truck visits to the farm, visits by a veterinarian, and disinfectant contact time of less than 20 minutes.	overall Japanese swine industry. Results should be interpreted cautiously.
Schoenbaum et al., 1991 [30]	Experimental study Funding provided by a grant from USDA APHIS.	PRV	Swine nasal washings, saliva, & urine, swine lagoon water and pit effluent, swine bile, chlorinated water, well water, heat-sterilized	Fomites were spiked with stock virus, and the combinations were incubated at 25 °C. Samples were collected on days 0, 1, 2, 3, 4, 7, and 10, or until a PRV titer of < 10 PFU/ml was obtained. Swine bile was also sampled at 1 h, and swine urine on day 14. Virus titers were	Of the combinations of PRV and diluent with feed or non-animal origin feed ingredients, the combination of PRV/saline/whole corn remained infectious longest, at 7 days with an estimated half-life of	Generalizability of study findings to field conditions is poor. Non-animal origin feed and feed ingredients were mixed with diluents prior to spiking with virus. Field conditions detrimental to virus activity (drying and UV light exposure) were deliberately not used.

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			chlorinated water, heat-sterilized PBSS, steel, concrete, polypropylene plastic, vinyl rubber, denim cloth, loam soil, green grass, whole corn, pelleted feed (starter and finisher), MBM, alfalfa, straw, wood, swine feces	determined by counting plaques in cell culture.	36.3 hours. The durations of infectiousness of the other combinations of PRV/diluent/feed or non-animal origin feed ingredients ranged from 1 to 4 days with an estimated half-life of 1.0 h to 5.1 h. Authors report that the "quantity of infectious virus decreased logarithmically" over time. The rate of decrease varied among fomites.	
Schumacher et al., 2016 [46]	Experimental with bioassay Funding provided by the National Pork Board.	PEDV	Feed containing corn, SBM, VTM, and a source of phytase	Feed was mixed with stock PEDV at various doses, and the mixtures were administered to pigs by orogastric gavage. Fecal swab specimens were collected. Pigs were euthanized at 7 days after exposure. Fecal swab samples, tissue samples, and cecal contents were analyzed by PCR, histology, and/or	The lowest concentration of virus in feed to cause infection in pigs was 5.6×10^1 $TCID_{50}$/g. The PCR cycle threshold value was 10 units lower for PEDV mixed with feed than for an equivalent dose of PEDV mixed with tissue culture medium.	Generalizability of study findings to field conditions is poor; virus-spiked feed was administered to pigs by orogastric gavage.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
				immunohistochemistry. Virus titers were determined by RT-(quantitative) PCR.		
Scott et al., 2016 [11]	Scenario development, post hoc investigation, epidemiologic survey, case/control, brainstorming and speculation Funding source not listed.	PEDV, SECV	Products or equipment identified as having the potential to carry PEDV or other SECVs: feed totes, organic soybeans, pet treats, SDPP, biologicals, plant materials, amino acid supplements, and VTM	The authors used previously collected epidemiologic data to develop scenarios and identify possible routes of PEDV introduction into the United States, and initiated follow-up studies "to gather more evidence for the most plausible scenarios".	No PEDV was detected in imported organic soybeans, pet jerky treats, or feral swine samples. Source of epidemic was not identified. Authors identified totes used to transport bulk feed as providing the simplest explanation for the investigation findings.	Unclear objective(s) and reporting. Appears to be an emphasis or assumption towards identifying Asia or China as the location of origin and towards imported organic soybeans as the point source feed ingredient.
Trudeau et al., 2017a [42]	Experimental study Funding provided by the National Pork Board.	PEDV, PDCoV, TGEV	Complete feed, SDPP, meat meal, MBM, blood meal, corn, SBM, and DDGS.	Fomites were spiked with stock virus, and the combinations were incubated at room temperature for 0 to 56 days. Virus titers were determined through use of a cell-culture-based assay.	The first log decrease in PDCoV and TGEV activity took longest in SBM. Moisture and ether content were indicated as being important determinants of virus survival in feed ingredients.	Generalizability to field conditions is poor. Fomites were spiked with stock virus. Increased moisture content due to virus inoculation likely altered virus survival kinetics.

Study	Study Design and Funding Source(s)	Viral Pathogen(s)	Fomite[4]	Study Description	Core Outcomes	Methodological Comments
Trudeau et al., 2017b [41]	Experimental study Funding provided by the National Pork Board.	PEDV	SBM, swine growing-finishing VTM, SDPP, meat meal, MBM, blood meal, corn, and DDGS, complete feed, galvanized steel, stainless steel, aluminum, plastic	Feed and feed ingredients were spiked with stock virus, and the combinations were incubated at various temperatures for 0-30 min. Four fomite surfaces were spiked with stock virus and held at various temperatures for 0-10 days. Virus titers were determined through use of a cell-culture-based assay.	The authors found no difference in virus survival in feed or feed ingredients at temperatures higher than 70 °C. Maximum virus decrease occurred upon heating at 90 °C for 30 min. Inactivation kinetics did not differ among the surfaces tested.	Generalizability to field conditions is poor. Fomites were spiked with stock virus. Increased moisture content due to virus inoculation likely altered virus survival kinetics.

Acronyms - Data synthesis table

ADV	Aujeszky's disease virus
AHAW	Animal Health and Welfare
BEDV	blue eye disease virus
Ct	cycle threshold
DDGS	distillers dried grains with solubles
DPI	day(s) post-inoculation
EFSA	European Food Safety Authority
EU	European Union
FCV	feline calicivirus
FMDV	foot and mouth disease virus
IAV-S	influenza A virus of swine
LA	liquid antimicrobial
MBM	meat and bone meal
MCFA	medium chain fatty acid
PBSS	phosphate buffered saline solution
PCV2	porcine circovirus 2
PDCoV	porcine deltacoronavirus
PED(V)	porcine epidemic diarrhea (virus)
PHFDV	porcine high fever disease virus
PRV	pseudorabies virus
PSV	porcine sapelovirus
RBC	red blood cells
(RT)-PCR	(real time)-polymerase chain reaction
RNA	ribonucleic acid
SBM	soybean meal
SDPP	spray dried porcine plasma
SECV	swine enteric coronavirus
SVA	Seneca virus A
SVDV	Swine vesicular disease virus
$TCID_{50}$	tissue culture infectious dose 50
TGE(V)	transmissible gastroenteritis (virus)
VI	virus isolation
VTM	vitamin/trace mineral

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