RESEARCH REPORT

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Risk assessment of feed ingredients of porcine origin as vehicles for transmission of Porcine Epidemic Diarrhea Virus (PEDV)

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Abbreviations

ASFV: African Swine Fever virus
AIV: Avian Influenza Virus
APPI: American Protein Producers Industry
a_w : Water Activity
BVDV: Bovine Diarrhea Virus
Ct: Cycle threshold in quantitative PCR tests (inversely proportional to nucleic acid content)
CSFV: Classical Swine Fever Virus
DMED: Dulbecco's Modified Eagles Medium
D value: Decimal reduction time or time needed at a given temperature to inactivate 1 log of virus
FDA: Food and Drug Administration
FPRF: Fats and Proteins Research Foundation
GMPs: Good Manufacturing Practices
GRAS: Generally Recognized as Safe
HACCP: Hazard Analysis Critical Control Point
HEV: Hemagglutinating Encephalomyelitis Virus
HAV: Hepatitis A Virus
kGy: kilo Gray
NASS: National Agricultural Statistics Service
NRA: National Renderers Association
OIE: World Organization for Animal Health
PCR: Polymerase Chain Reaction
PDCoV: Porcine Deltacoronavirus
PEDV: Porcine Epidemic Diarrhea Virus
PEV: Porcine Enterovirus
PPV: Porcine Parvovirus
PRCV: Porcine Respiratory Coronavirus
PRV: Pseudorabies Virus
PRRSV: Porcine Reproductive and Respiratory Syndrome Virus
RT-PCR: Real Time - Polymerase Chain Reaction
SDPP: Spray-dried Plasma Protein
SVDV: Swine Vesicular Disease virus
$TCID_{50}$: the amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated
TGEV: Transmissible Gastroenteritis virus
UMN: University of Minnesota
USDA: United States Department of Agriculture
WRO: World Rendering Organization

Executive Summary

The objective of this project was to assess the likelihood that feed ingredients of porcine origin may function as vehicles of Porcine Epidemic Diarrhea virus (PEDV) transmission via feed. The scope of the assessment included rendered ingredients, ingredients derived through spray drying porcine blood, and ingredients derived by hydrolyzing porcine tissues. The project was coordinated by a multidisciplinary group at the University of Minnesota (UMN) which included expertise in swine health and epidemiology, swine nutrition, food safety risk analysis, and food engineering. The UMN team convened a stakeholder working group that included a range of technical experts from the animal feed industry and swine industry. The stakeholder group was integrally involved in collection of information relevant to the processes being assessed, and participated in regular conference calls, occasional face to face meetings, and email or phone communications as necessary. The stakeholder group also enabled visits of the UMN team to relevant processing sites to observe facilities and operations. An iterative process of information gathering, synthesis and review was used to ensure the details gathered on industry processes were representative of existing operations. The stakeholder group also reviewed the final document.

Recent North American field studies have indicated that both feed ingredients and cross-contamination of feed are potential routes for PEDV transmission. For any feed ingredient, the risk of the release of infective PEDV is a function of: (1) the concentration of PEDV in the raw materials; (2) the virus survival after ingredient processing (3) the survival of virus during post-processing storage and distribution; and 4) the likelihood of post-processing contamination incorporating PEDV into the finished ingredient. As anticipated, data limitations were a significant constraint in this assessment. The approach taken was to acquire data from diverse sources (industry, scientific literature, experimental studies and industry reports), to document likely caveats for the data sources, and to identify priorities for future data acquisition that would enable more robust conclusions. It is noted that most of the data used in the analysis were unavailable at the commencement of the project, and are derived from very recent studies that have yet to be independently replicated.

No data on PEDV contamination of raw materials were available for the rendering and hydrolyzed protein sources. Estimates of PEDV contamination of liquid plasma were available from industry, based on PCR testing of ingredients over time, and were used in quantitative modeling. Flow charts were designed to illustrate the respective processes used to manufacture the ingredients, and to identify points of likely pathogen inactivation. Data on the thermal and other treatments used to inactivate pathogens during processing were obtained from industry sources. Where available, ranges of key variables (e.g., temperature and time) were documented to indicate likely variability in industry practices.

Previously published studies did not enable adequate portrayal of the thermal inactivation kinetics of the virus. To assess the likelihood of PEDV survival in all three ingredient types, we used recent (and as yet unpublished) experimental data on the thermal inactivation kinetics of PEDV characterized by D values [time at a given temperature needed to inactivate 90% (1 log reduction) of virus]. The estimated

D values ranged from 2.71-7.94 min at 120-145°C in complete feed and 2.0-17.7 min at 60-90°C in different matrices (spray-dried plasma, meat meal, bone meal, meat and bone meal, grow finish premix). In general, D values obtained in damp plasma were higher (more time needed to inactivate 1 log) than in other matrices, suggesting a relatively favorable milieu for virus (or RNA) survival. Based on these D-values, the combinations of temperature and time used in the rendering process (115 to 145°C for 30 to 90 min) were estimated to result in 3.7 to 21.9 log reduction in virus. The combinations of temperature and time during the hydrolyzed process (60 to 90°C for 380 to 440 min plus drying for 1 to 25 min at 115°C) were estimated to result in a cumulative inactivation reduction of 50 logs. Consequently, the likelihood of PEDV survival of either the rendering and hydrolyzed protein processes was deemed to be negligible. However, more extensive data on thermal inactivation of PEDV under conditions similar to the processes evaluated is desirable to verify these conclusions.

Unlike for the rendering and hydrolysis processes, additional information on inactivation of PEDV by spray drying became available during the project. Two sources of information were used to assess the likelihood of PEDV survival after spray-drying. For both scenarios, the initial concentration of virus in liquid plasma (3.8 to 7.8 log RNA copies PEDV/mL) was derived from data provided by the industry. Following the same approach used for the rendering and hydrolyzing assessments, for Scenario 1 the experimental D values at 60-80°C in damp plasma (9.6 to 12.1 min) were used to estimate virus thermal inactivation. Exposure of damp plasma to temperatures between 80-84°C for 20-90 s resulted in an estimated viral inactivation between 0.07-0.26 log. For Scenario 2, we used estimates from 2 published studies estimating PEDV inactivation of 4.2 logs when liquid plasma was dried in a laboratory scale spray dryer.

Simulations in Scenario 1 indicated some likelihood of PEDV survival if at least 0.1% of viral RNA in liquid plasma represented viable virus. An important limitation for Scenario 1 is that it models only thermal inactivation in damp spray-dried plasma, but it is known that other inactivation mechanisms occur during spray-drying. Consequently, the simulation presents a very conservative estimate of PEDV inactivation. Simulations in Scenario 2 indicated negligible risk or PEDV survival across all assumptions of virus viability. An important limitation for Scenario 2 is the uncertainty of extrapolating results from a laboratory scale spray dryer to an industrial scale dryer. Further research is advised to explore the relationship between PEDV survival in laboratory scale and commercial scale spray dryers, and to understand which components of the process contribute most to virus inactivation. Better theoretical understanding of the mechanisms of viral inactivation would be helpful to addressing risk management of viruses generally. In line with practices recently adopted in industry, the effect of storage of spray-dried plasma at room temperature (20-22°C) for two weeks was also estimated using published and experimental studies to achieve an additional 3-5 log inactivation. Taking in consideration the post-processing storage step, the risk of PEDV survival after this storage period was estimated to be negligible to low (PEDV survival predicted only if 100% of the virus in raw plasma were viable) for Scenario 1 and negligible for Scenario 2.

To assess the likelihood for post-processing contamination of the finished ingredient with PEDV, site visits were performed at the processing plants for each of the ingredients. Information gathered from

the visits were compared with general Good Manufacturing Procedures (GMP) for food and feed industry. The potential pathways for PEDV cross-contamination identified were related to: 1) personnel movement from raw material areas to finished ingredient areas where virus carriage under footage, clothing and hands could potentially transfer the virus to the finished ingredient; 2) vehicle movement (i.e. skid-steer loaders to outside areas) where contaminated manure carried out by raw material delivery trucks could be picked up by loader vehicles and contaminate the finished ingredient; 3) airflow patterns within the plants where viral particles may be entrapped in the air and move with it, thus there is potential for cross-contamination if the air reaches finished ingredient areas. In general, most of these pathways were categorized as negligible to low due to the low occurrence of these events and the measures taken by the industry. However, rendering plants categorized as 'open facilities' where vehicles (skid-steer loaders) are used to move the finished ingredient, the likelihood for post-processing contamination was low to moderate due to possible cross-contamination that may occur outside the plants and longer virus persistence during winter in contaminated materials.

In summary, the assessments made in this project are constrained by a paucity of specific data on several aspects that are germane to the risk of PEDV transmission in feed ingredients of porcine origin. Available data on thermal inactivation of PEDV indicate that risk of virus surviving the processes of rendering and hydrolysis (peptone production) are negligible. The time and temperature profiles used in spray-drying are much less severe, and therefore, the possibility of virus survival is inherently greater if non-thermal mechanisms are ignored. Overall, currently available data indicate that probability of PEDV surviving the spray-drying process and current commercial storage periods is extremely small. In the course of the project, several data gaps were identified that contributed to the uncertainty. Risk assessment is an iterative process and the findings of this report may be revised in the future if new knowledge becomes available.

Background

Emergence of Porcine Epidemic Diarrhea virus

Porcine epidemic diarrhea virus (PEDV) is a single-stranded, positive-sense, enveloped RNA virus that belongs to the *Alphacoronavirus* genus of the *Coronaviridae* family. PEDV was first associated with diarrheal disease in feeder pigs and fattening swine in England in 1978 (Chasey et al., 1978). PEDV is not closely related to other pathogenic coronaviruses of swine [i.e., transmissible gastroenteritis virus (TGEV), Porcine Respiratory Coronavirus (PRCV), Hemagglutinating Encephalomyelitis Virus (HEV)], but shows some immunological cross reactivity with feline infectious peritonitis virus, a pathogen of cats (Zhou et al., 1988). Epidemic outbreaks of PEDV have been reported from different countries in Europe as well as Asia, including Japan, China, South Korea and Thailand (Takahashi et al., 1983; Xuan et al., 1984; Kweon et al., 1993; Puranaveja et al., 2009). A marked increase in PEDV outbreaks in China since 2010 was associated with substantial economic losses and attributed to the emergence of new strains (Chen et al., 2014). Until 2013, PEDV was not known to exist in the Western Hemisphere. However, in less than one year, the virus was identified for the first time in several countries in the Western Hemisphere including the USA and Canada (Huang et al, 2013; Pasick et al., 2014). Despite similarities between the strain affecting herds in USA and Canada with a strain in China, definitive sources of these outbreaks have not been established. Subsequently, PEDV has continued to spread domestically within the USA and, as of November 2014, had been confirmed in 32 states of the USA.

The role for contaminated feed ingredients in the transboundary spread of PEDV, and its subsequent propagation within countries, has been speculated upon since the original cases were diagnosed within the USA (Alumbaugh, 2014). The initial cases of PEDV in Canada were linked to a common feed manufacturer and led to the voluntary recall of swine feed suspected to contain PEDV contaminated spray-dried plasma protein (SDPP) (Byrne, 2014; Pasick et al., 2014). In many cases, recommendations to exclude ingredients of porcine origin from swine feed were also implemented in the USA. These precautionary decisions were driven by the high economic consequences of introducing PEDV to swine farms. However, they were made in a virtual vacuum of objective data on the risk of PEDV transmission via feed. Such changes in feeding strategies add cost to production and disrupt markets, augmenting the impact of PEDV beyond direct production losses. On May 6, 2014, the European Commission announced precautionary measures that pig blood products imported for use in pig feed be treated at 80°C and stored for 6 weeks at room temperature due to perceived risks of PEDV (Anonymous, 2014). Therefore, the emergence of PEDV in the Western hemisphere has significantly impacted swine production and the animal feed industry on both a national and international level.

Great uncertainty surrounds all facets of PEDV transmission via feed and the mechanisms by which these risks may be mitigated. Given the complexity of the feed supply chain and diversity in feed handling practices, a comprehensive and systematic approach is required. Established risk analysis methods for foodborne hazards provide an effective framework to do so (Anonymous, 1995). Although there are, theoretically, a vast number of potential pathways by which animal feed could become

contaminated with any pathogen prevalent in the environment, the scope of this assessment is focused upon products of porcine origin that can be included in swine diets. The objective of this risk assessment is to analyze currently available scientific knowledge relevant to the risk of PEDV contamination posed by inclusion of ingredients of porcine origin in swine diets. The purpose of the assessment is to provide objective information to guide decision makers in both the feed industry and swine production to optimize animal feeding practices while minimizing the risk of PEDV transmission via this route of transmission.

Utilization of ingredients of porcine origin in swine diets

Utilization of rendered ingredients in swine diets

Most rendered ingredients of porcine origin can be utilized in swine diets. The most commonly used rendered ingredients include meat and bone meal, meat meal, and choice white grease (Cromwell, 2006). Rendered products are good sources of standardized ileal digestible amino acids, standardized total tract digestible phosphorus, energy, and vitamins of the B complex (NRC, 2012). There are no specific reasons to limit the inclusion of rendered ingredients in swine diets beyond the constraints of balancing nutrients (e.g. lysine, tryptophan, and phosphorus) and economic considerations.

Utilization of spray-dried ingredients in swine diets

Spray-dried porcine plasma (SDPP) is commonly included in diets for recently weaned nursery pigs and less commonly for lactating sows (Torrallardona, 2010). A range of inclusion rates of spray-dried porcine or bovine plasma have been reported in the literature, with early reports of up to 10-12% (van Dijk et al., 2001). Further refinement work suggests that the inclusion rates of SDPP in nursery pig diets range from 0.0 to 7%. The first diet that young pigs consume after weaning (i.e., phase 1 diets) usually contains higher levels (e.g., 2.5 to 7%) of SDPP and these levels are typically reduced by approximately half (e.g.,0 to 3.5%) as pigs mature (i.e., phase 2 diets). Inclusion rates vary depending on weaning age, health status, local availability, and cost of ingredients (Van Dijk et al., 2001; Torrallardona, 2010). Plasma constitutes approximately 60% of whole blood and contains 6 to 8% proteins composed of a complex blend of proteins (i.e., albumin, globulins, and fibrinogen), growth factors, bioactive peptides, hormones, etc. Spray-dried plasma (porcine or bovine) has a nutritional value that is beyond that indicated by the energy and amino acid content. Replacement of spray-dried plasma with dried whey, casein, egg proteins, or other sources of high quality protein (e.g. soy protein isolate) does not seem to result in the same growth benefit (Torrallardona, 2010). Therefore, it is hypothesized that spray-dried plasma contains beneficial components other than amino acids such as immunoglobulins (specifically IgG) or other blood derived factors (e.g. epithelial growth factor, insulin growth factors). Inclusion of spray-dried plasma augments the growth of pigs between 10% and 40% compared with other non-plasma protein sources (i.e., whey, casein). Inclusion of spray-dried plasma has also been suggested in diets for lactating and gestating sows, but is a less common practice (Crenshaw et al. 2007; Frugé et al., 2008). Spray-dried red blood cells are utilized in nursery pig diets as a source of amino acids, but have an unusual amino acid profile with a relatively low concentration of the branched chain amino acid isoleucine and the amino acid methionine (Wagespack et al., 2011). Therefore, utilization of spray-dried

red blood cells in diets for young pigs is limited due to the need to balance diets for these 2 amino acids. Inclusion rates of blood cell ingredients are usually between 2 and 8% of the diet.

Utilization of hydrolyzed ingredients in swine diets

In recent years, research has confirmed the value of porcine specific peptones as a protein source in diets for nursery pigs (Cho et al., 2010; Myers et al., 2014) and lactating sows (Johnston et al., 2003). The diets that were used in these studies were a defined blend of hydrolyzed porcine proteins. Hydrolyzed porcine proteins are added to diets at an inclusion rate of 3 to 7% and are an alternative to other high protein sources such as fishmeal, whey protein concentrate, and spray-dried plasma (Jones et al., 2010; Solà-Oriol et al., 2009).

Hazard Identification

Hazard identification is a key preliminary step in the risk assessment process and typically involves identification of biological, physical, and chemical hazards that could potentially produce adverse consequences associated with the use of ingredients or processes of interest. This agent-oriented risk assessment is focused upon PEDV as a biological hazard PEDV and evaluates some putative pathways by which this agent might be transmitted via the swine feed supply chain.

As described above, PEDV is a coronavirus. Coronaviruses are enveloped, single stranded positive sense RNA viruses classified in the order *Nidovirales*, family *Coronaviridae* and subfamily *Coronavirinae*. Coronaviruses are further divided into 4 genera. Alpha- and Betacoronaviruses are predominantly associated with mammalian hosts. Gamma- and Deltacoronaviruses are predominantly associated with avian hosts. It is proposed that bats (Alpha-, Beta-) and birds (Gamma-, Delta-) may be the ancestral source hosts of coronaviruses (Woo et al., 2012). Coronaviruses have been found in a wide range of hosts including primates, ungulates (including pigs), lagomorphs, carnivores, and rodents (Drexler et al., 2014). Some coronaviruses appear to be highly host specific, while others have shown considerable potential for interspecies transmission, notably the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) viruses (Drexler et al., 2014).

There are now 5 distinct coronaviruses that have been associated with disease in swine: Transmissible Gastroenteritis Virus (TGEV) and the closely related Porcine Respiratory Coronavirus (PRCV); the Hemagglutinating Encephalomyelitis Virus (HEV); Porcine Deltacoronavirus (PDCoV); and PEDV (Saif, 2012; Huang et al., 2013; Wang et al., 2014). PDCoV's were first described in Asia in 2012, (Woo et al., 2012) and recently have been associated with enteric disease in pigs in the USA after the initial recognition of PEDV in this country (Wang et al., 2014). PEDV appears to be a highly host specific virus and, although also an alphacoronavirus, is not closely related to TGEV. The known coronaviruses most closely related to PEDV appear to be of bat origin (Drexler et al., 2014).

Coronaviruses are genomically the largest known RNA viruses, ranging from 27 to 32 kilobases in length. The genome of the prototype European PEDV strain (CV777) is 28,033 bases, and the genome includes 5' and 3' untranslated regions, and at least 7 open reading frames (ORFs) encoding 4 structural proteins and 3 non-structural proteins. Structural proteins include a glycosylated peplomer 'spike' (S) protein, a glycosylated membrane protein (M), an envelope (E) protein, and an unglycosylated RNA-binding nucleocapsid (N) protein (Song and Park, 2012). The virus envelope is derived from the host cells, and includes glycolipids and phospholipids of host origin (Saif, 2012). Most PEDV sequences documented recently in China and the USA contain 2 insertions and a deletion in the spike gene compared with the prototype European PEDV strain (CV777) (Tian et al., 2014).

The mutation rate of RNA viruses is generally considered to be greater than for DNA viruses, and rapid genetic change and diversity are common features of RNA viruses. The diameter of PEDV virions range from 95 to 190 nm (mean diameter: 130 nm), including the projections of the spike proteins (Song and

Park, 2012). Phylogenetic analyses of 3 USA PEDV strains indicated a close relationship to a PEDV isolate from Anhui Province in China, suggesting a likely Chinese origin (Huang et al., 2013). It is suggested that USA PEDV strains have diverged genetically and can be classified into two sub-lineages (Huang et al., 2013). A recent analysis of sequences of genomes of 74 North Americas PEDV strains found they clustered into 2 distinct clades (Vlasova et al., 2014). Seven (9.7%) contained insertions and deletions in the spike gene (S-INDEL strains), which shared 96.2%–96.7% nucleotide identity with the virulent PEDV strains detected initially in the USA. The INDEL strains formed a distinct cluster within North American clade II and the authors concluded that the S INDEL and original PEDV strains are co-circulating and could have been introduced simultaneously into the USA (Vlasova et al., 2014).

The predominant site of PEDV replication in pigs is the villus epithelial cells of the small intestine (Saif, 2012), although there is one recent report of PEDV replication in alveolar macrophages (Park and Shin, 2014). The clinical signs and gross pathological lesions are indistinguishable from TGEV infections of pigs. The incubation period for onset of clinical signs is approximately 24-48 hours in young pigs (Jung et al., 2014). Lesions are confined to the small intestine. Grossly, the intestinal wall becomes thin and translucent and there are watery yellow intestinal contents. The clinical syndrome is acute gastroenteritis and dehydration with variable mortality. Similar to TGEV infection, the severity of disease is highly age-dependent and, in naïve herds, 100% mortality is expected in suckling piglets up to 2 weeks of age. Typical clinical signs include diarrhea, vomiting, and anorexia. Morbidity is high in pigs of all ages, but high mortality is uncommon in pigs after three weeks of age. Although viremia is not considered to be a feature of PEDV pathogenesis (Pensaert and Yeo, 2006; Saif, 2012; Gerber et al., 2014), it was detected in pigs infected experimentally or naturally with USA strains of PEDV, including 55% of naturally infected from pigs aged 13 to 20 weeks (Jung et al., 2014).

The predominant route of PEDV transmission fecal-oral and may be direct or indirect (Saif, 2012). PEDV spread rapidly and widely across the USA swine industry within 12 months of being identified and, at an industry level, it is the routes of transmission between herds practicing high levels of biosecurity that are of most concern. There is field and experimental evidence indicating that contaminated vehicles (Lowe et al., 2013), aerosol transmission (Alonso et al., 2014), contaminated feed ingredients (Pasick et al., 2014; Aubry et al., 2014) and contaminated feed (Dee et al., 2014) are all plausible routes of transmission between farms.

Evidence for feedborne transmission of PEDV

The study by Dee et al (2014) was motivated by outbreaks of PEDV in 3 breeding farms that were PRRSV negative and reportedly had relatively high standards of biosecurity. In each of the outbreaks, clinical signs of PEDV started in production areas supplied with feed from feed bins that had been recently emptied prior to being refilled. Notably, the feed being fed through these bins did not contain ingredients of porcine origin. Residual material was aseptically collected from the internal surfaces of the suspected contaminated feed bins from the each of the 3 PEDV affected herds. As well, bins from 3 PEDV negative herds were sampled as negative controls. PCR results from the suspected contaminated feed bins ranged between Ct = 19.50 and Ct = 22.20. These concentrations of viral RNA were much

higher than the highest concentration that has been observed in SDPP (Ct = 25.1, Table 14). A bioassay was completed to evaluate infectivity of the isolated PEDV using an inoculum of pooled materials collected from the inside of the contaminated feed bins on the affected farms. For the bioassay, piglets were divided into 3 groups: 1) treatment group that received feed that was PEDV PCR positive from the affected herds, 2) positive control group that was fed feed spiked with PEDV, and 3) a negative control group that was fed a placebo. In both the treatment and positive control groups, bioassay piglets showed diarrhea and tested PCR positive for PEDV in feces and intestines post-mortem. No clinical signs or positive tests were seen in the negative control group.

Similarly, epidemiological investigations of the first cases of PEDV identified in Canada implicated contaminated feed ingredients and feed as a vehicle of transmission (Pasick et al., 2014; Aubry et al., 2014). SDPP imported from the USA and associated with the outbreak tested positive (Ct = 36) for PEDV RNA, whereas only one feed sample (containing 6% SDPP) tested weakly positive. Bioassays were performed using both SDPP and feed containing 6% SDPP by administering 5g of material in 50ml of suspension per pig. No infection occurred in the pigs receiving feed, but PEDV infection confirmed by immunohistochemistry and serology occurred in the pigs receiving SDPP and also in pigs placed in contact with them 11 days after exposure (Pasick et al., 2014). These bioassays were performed approximately 4 (SDPP) and 5 (feed) weeks after the onset of clinical disease on affected farms (Soren Alexandersen, personal communication)

Although these 2 reports confirm the possibility of PEDV transmission by contaminated feed or feed ingredients, they do not provide insight into the likelihood of these events or the relative importance of feedborne transmission in the epidemiology of PEDV.

Environmental stability of PEDV

For feed to be a vehicle of pathogen transmission, it must become contaminated with an infectious agent that can maintain its infectivity from the point of contamination until it can be ingested in sufficient dose to cause infection. Contamination may occur anytime during the feed manufacturing and distribution stage or earlier in the feed supply chain. Understanding the ability of a virus to maintain infectivity throughout the feed manufacturing chain is critical to evaluate the potential for feed to be a vehicle for the spread of infectious agents. PEDV infections, like TGEV and bovine coronavirus infections, tend to be seasonal and more prevalent in winter (Song and Park, 2012). Overall, PEDV survives much better under colder and high moisture conditions than in warmer and drier conditions.

Early studies indicated that PEDV is chloroform and ether sensitive, and loses infectivity when heated to >60°C for 30 minutes, but it is 'moderately stable' at 50°C (Pensaert and Yeo, 2006). Other studies have tested the environmental persistence of PEDV under different conditions. PEDV was shown to be stable between pH 5.0 and 9.0 at 4°C, and between pH 6.5 and 7.5 at 37°C (Hoffman, 1989).

A study conducted by Pujols and Segalés, (2014) tested the survivability of the prototype European strain (CV777) of PEDV under different storage conditions in spray-dried bovine plasma. The virus (9 mL

of PEDV cell culture) was inoculated on spray-dried bovine plasma (60 g) to a concentration of $10^{2.8}$ $TCID_{50}$/g and maintained at 4, 12 and 22°C for 7-21 days. After the storage, samples were assayed in VERO cells. Results indicated that the virus lost 2 log of infectivity (final concentration of $10^{0.8}$ $TCID_{50}$/g) after storage at 4°C for 7 days. The virus was completely inactivated after 21 days at 4°C (i.e. 2.8 log inactivation). At 12°C the virus was completely inactivated after 14 days while at 22°C the virus was completely inactivated after 7 days.

Verma and Goyal (2013) studied the survivability of PEDV in different matrices (i.e. pig feces, pig slurry, water and feed). In this study, the virus was spiked on the matrices, and stored at different temperatures (-20, 4, 20, 40, 50 and 60°C), storage times (up to 28 days), and relative humidity (RH) levels [30, 50 and 70%; Appendix A: Environmental resistance of PEDV (Verma and Goyal, 2014)]. In order to assess the survivability of PEDV, a bioassay with 10-day old piglets was performed and the clinical signs evaluated. The piglets were then euthanized and the Ct values estimated from samples obtained from the piglets' jejuni (i.e. region of the small intestine) by RT-PCR. In summary, the study showed that PEDV was able to survive in fresh feces up to 7 days at 40, 50 and 60°C at RH levels less than 70%. PEDV was detected at 7 days in the 40 and 50°C samples, but not in 60°C samples (Appendix A: Environmental resistance of PEDV (Verma and Goyal, 2014)). In pig slurry, PEDV survived up to 14 days at 25°C at all RH levels. At 4°C and -20°C, PEDV was able to survive more than 28 days.

For the purpose of this risk assessment, the results from the feed matrix portion of the study are reviewed in more depth here. The observations were made on a feed slurry (i.e. 10 mL of PBS to 5 g of dry feed) and in damp feed (i.e., 1 mL of PBS to 5 g of dry feed) samples. The initial Ct value at day 0 of the experiments was 19.1 for the feed slurry, or 9.7 log copies RNA PEDV/g. For the damp feed, the Ct was 16.0 or 10.6 log copies RNA PEDV/g. The results in feed slurry indicated that PEDV was still infectious after 28 days at 20°C (there were no diarrhea symptoms found in piglets but positive Ct values from intestinal samples were reported). In damp feed, the virus lost infectivity between 1 and 2 weeks (there were no symptoms of diarrhea and negative Ct values from intestinal samples after two weeks). One limitation of this study is that both of matrices contained greater moisture concentration and water activity (a_w) than would be present in commercial SDPP or complete feed offered to pigs. This limitation also applies to the study of Pujols and Segalés (2014) cited above, and could lead to overestimation of PEDV survival in the feed matrix due to less dehydration inactivation than would occur in commercial materials. This would imply that inactivation of PEDV during storage of drier materials could be greater.

In a recent study conducted by Thomas et al., (2014), the authors aimed to mimic the environmental conditions of livestock trailers and investigated the combinations of time and temperature required to inactivate PEDV on metal surfaces. The authors spread PEDV positive feces, collected from experimentally infected pigs 2 to 3 days post-infection, on the bottom surface of a metallic tray. Ct values of feces ranged from 12 to 16 with the majority around 13. Trays were maintained under different time/temperature combinations and then the recovered feces were diluted and fed to 4-week old pigs. Their results indicated that the optimal time/temperature combination to inactivate PEDV was 71°C for 10 minutes, or maintaining the trailer at 20°C for 7 days. A limitation of this study is that the amount of infectious viral particles was unknown, so the reduction in virus infectivity survival could not

be estimated. However, as indicated by some authors, the concentration of virus in fresh feces from PEDV infected pigs at the peak of diarrhea can be very high ranging from 10^8–10^9 $TCID_{50}$/mL (Goyal, 2013; Oglesbee, 2014). In addition, another limitation of the study was the lack of control of the external humidity level where the samples were placed. Humidity is likely to affect the rate of moisture loss of the samples and thus the rate of inactivation of the virus.

In most of the studies reporting the effect of environmental variables on PEDV survivability, virus infectivity was difficult to measure by cell culture and most of the diagnostics were largely based on RT-PCR testing, with results expressed as cycle threshold (Ct). In this report, a calibration curve obtained at UMN and published in Alonso et al., (2014) was used to estimate the amount of RNA contained in a sample and expressed as copies of RNA PEDV/g or mL. The diagnostic test had a limit of detection of Ct=40 (50 copies of RNA/reaction or 7,000 copies of RNA/g or mL). We acknowledge that other veterinary diagnostic laboratories may use a different calibration curves, and some variability in genomic copies estimated from Ct values would be expected across laboratories.

For reference, Ct values observed in a range of field samples submitted to the UMN veterinary diagnostic laboratory are listed in Table 1.

Table 1: RT-PCR values for PEDV virus in various tissues, secretions, and excreta of pigs confirmed or suspected to be infected with PEDV.

Sample[1]	Average Ct values[2]	Max. Ct values	Min. Ct values
Feces (n=178)	21.01	39.31	12.19
Fecal swab (n=33)	24.59	33.88	15.22
Intestines (n=170)	23.32	39.18	13.82
Saliva (n=15)	23.63	30.63	18.03

[1]Data was provided by Dr. Albert Rovira, UMN-VDL.
[2]Cycle threshold - samples with Ct less than 35 were considered as positive.

Environmental persistence of other swine viruses

Given the scarcity of data on survival of PEDV, survivability data for some other swine viruses were also reviewed. This review was conducted simply for comparative purposes, recognizing that survival data cannot be extrapolated between viruses. Botner (1991) showed that pseudorabies virus, an enveloped virus from the family Herpesviridae, was infectious for periods of hours to weeks at ambient temperatures. Lund et al., (1983) using pig slurry and cattle manure, showed that at 5°C it took 27 and 300 days to obtain 1 log reduction of Talfan virus (initial concentration of 10^4 $TCID_{50}$ /mL) under aerobic and anaerobic conditions respectively. At 20°C, it took 4 and 13 days to obtain 1 log reduction under

aerobic and anaerobic conditions respectively. Weesendorp et al., (2008) showed that classical swine fever virus (CSFv) became undetectable between 42 and 64 days in feces from infected pigs whereas in urine no virus was detectable after 18 days at temperatures ranging from 5 to 30°C. Comparing these data to the experimental studies with PEDV, it seems that PEDV may be relatively sensitive to dry conditions during storage and thus the survivability may be shorter than some other swine viruses studied.

Thermal resistance of viruses

It is well recognized that inactivation processes vary widely among viruses, and also among strains of the same virus (Farcet et al., 2012; Nims and Plavsic, 2013). Virus specific data are therefore indispensable for risk assessment, but are generally lacking for PEDV. For context, a brief overview of thermal resistance of viruses is included. Studies on several non-enveloped non-swine viruses (i.e. poliovirus, bovine parvovirus, poliovirus Sabin, adenovirus type 5, parechovirus 1, murine norovirus, human norovirus, murine hepatitis virus) have shown that the classification of viruses according to their morphological characteristics (presence or absence of a viral envelope or nucleic acid type), lipophilicity or hydrophilicity is useful to characterize sensitivity to chemical biocides, but is less helpful for the characterization of thermal resistance (Daniel et al., 1987; Sauerbrei et al., 2009; Tudlahar et al., 2012). Several studies have estimated the survival of enveloped and non-enveloped viruses exposed to different thermal processes (Appendix D). In general, non-enveloped viruses tend to be more resistant to heat than enveloped viruses (Nims and Plavsic, 2013). However, there are many exceptions to that generality, therefore the presence of an envelope is insufficient to predict thermal resistance of individual viruses (Daniel et al., 1987; Sauerbrei et al., 2009; Tudlahar et al., 2012; Nims and Plavsic, 2013). (Daniel et al., 1987; Sauerbrei et al., 2009; Tudlahar et al., 2012; Nims and Plavsic, 2013).

Temperatures above 80°C have been reported to inactivate some enveloped viruses even after short periods of time (<1 s). Porcine reproductive and respiratory syndrome (PRRS) virus and pseudorabies virus (PRV) were inactivated by 4 and 5.3 log respectively after spray-drying at 90°C (outlet temperature) for 0.41 s (Polo et al., 2005; Hermann et al., 2007). It is again important to note that inactivation by spray drying is not solely a result of thermal inactivation and other non-thermal effects may take place increasing the inactivation level. For temperatures below 80°C, there was variation among the thermal resistance of swine-related viruses. Classical swine fever virus (CSFV) was inactivated by 5 log after 1 hour at 60°C (Torrey et al., 1964) whereas African swine fever virus (ASFV) was not completely inactivated (1.5 log) at 52°C after 5 min (Turner et al., 2000).

For other enveloped viruses (non-swine) there was also variation in their thermal resistance (Appendix D-2). Canine Coronavirus, a PEDV-like coronavirus, was inactivated by 5.75 log at 75°C after 5 minutes (Prateli et al., 2008). For Severe Acute Respiratory Syndrome (SARS) coronavirus, thermal resistance varied depending on the matrices tested (immunoglobulin preparation, anti-thrombin III preparation, haptoglobin preparation and 25% human serum albumin preparation) with reductions ranging from 3.5 to 5.5 log at 60°C for processing times from 30 min to 2 hours (Yunoki et al., 2004). Sindbi virus was not

completely inactivated in a blood product (lyophilized Factor VIII of coagulation) after 20 hours at 60°C (Espíndola et al., 2006).

Porcine circovirus type 2, which was very resistant to dry heat but more sensitive to wet heat, saw a reduction of 3.6 log when heated at 80°C for 15 minutes (O'Dea et al., 2008). Porcine parvovirus was reduced by 5 log after 72 hours at 80°C, but was not completely inactivated (Blümel, 2008). Pujols et al., (2007) showed an inactivation of 5 to 6 log of swine vesicular disease virus (SVDV) in plasma after a spray-drying process that used a lab-scale unit with exit temperatures of 80°C and 90°C.

Thermal resistance of PEDV

There are few studies describing the thermal inactivation kinetics of PEDV (Table 2). Hoffmann et al., (1989) found that the virus (German isolate V215/78 of PEDV) lost its infectivity when heated to 60°C for 30 min (5.5 log reduction), and it was moderately stable at 50°C (virus infectivity decreased as a rate of 1.1 log per hour) (Hoffmann et al., 1989). In a recent study, Pujols and Segalés (2014) studied the inactivation of PEDV in bovine plasma. The study showed a 4.2 log inactivation by a combination of spray-drying at 80°C (outlet temperature) in a laboratory scale spray dryer for <1 s, then maintaining the samples at 90°C in a water bath for 30 s (time to reach an inner temperature of 70°C) or 60 s (time to reach an inner temperature of 80°C) to simulate processing times of an industrial scale spray dryer. The final moisture of the samples after spray-drying was not published. Similarly, Gerber et al. (2014) demonstrated a 4.2 log inactivation in liquid plasma spiked with PEDV (the plasma was harvested from a naïve pig then spiked with PEDV) and spray-dried at 80°C (outlet temperature) in a laboratory scale spray dryer and stored at 4°C for 7 days.

Verma and Goyal (2014) studied the thermal inactivation kinetics of PEDV in complete feed and animal-based feed ingredient samples. The complete feed was held in an oven at elevated temperatures (120 to 145°C for 0 to 120 min). As well, complete feed and select animal-based feed ingredient matrices, (including SDPP, meat meal, meat and bone meal, and blood meal), were similarly held in an oven at 60 to 90°C for 0 to 30 min at RH of 30, 50 and 70%. Appendix B outlines the protocols and the resulting virus concentrations following the different combinations of temperature and time for each matrix.

In general, the protocol in the Verma and Goyal (2014) study called for the addition of 1 mL of PBS containing the virus to 5 g of each matrix. This increased the moisture content of samples to approximately 18 to 25%, which is not representative of the moisture content of commercial feed ingredients. This limitation is an important consideration when evaluating these findings.

Table 2: Thermal inactivation of PEDV

Type of sample	Process conditions	Detection method	Inactivation (log TCID50 reduction)	Referen ce
Spray- dried porcine plasma	Lab bench scale spray dryer (Yamato) Inlet air temp: 166°C Outlet temp: 80°C Drying time: <1 s	RT-PCR Sequencing Bioassay	Initial Concentration 4.2 log TCID50/mL Inactivation after spray-drying and storage for 7 d at 4°C: 4.2 log TCID50/mL	Gerber et al., 2014
Spray- dried bovine plasma	Lab spray-dryer (Buchi): Inlet air temp: 200°C Outlet temp: 80°C Drying time: <1 s Post-Drying (water bath): 90°C, 30 s (time to reach an inner T 70°C) 90°C, 60 s (time to reach an inner T 80°C)	Microtiter assay procedure in VERO cell monolayers	Initial concentration: $10^{4.2}$ $TCID_{50}$/mL $10^{5.1}$ $TCID_{50}$/g Inactivation after spray-drying and post-processing in a water bath: 4.2 log	Pujols and Segalés, 2014
Eagle's Minimum Essential Media	Water bath: 50°C for 5 to 180 min, 50-80°C for 30 min	Plaque test	Initial inoculum: $10^{5.5}$pfu/mL Inactivation: 60°C, 30min: 5.5 log 50°C, 30min: 0.4 log	Hofman n et al., 1989

Differences in moisture content are known to affect virus inactivation kinetics in general. For example, Savage et al. (1998) studied the effect of moisture content on hepatitis A virus (HAV) and porcine parvovirus (PPV) at a temperature of 80°C during 72 hours. For both viruses, the achieved level of inactivation was lower when the matrix (i.e. Eagle minimal essential medium) had a moisture content lower than 0.8% (much drier than commercial feed or ingredients). When the moisture content was greater than 0.8%, the inactivation level achieved was greater than 4 and 3.2 log for HAV and PPV respectively. When the moisture content was less than 0.8%, the inactivation level achieved was only

0.5 and 2.5 log for HAV and PPV respectively. In another study, Zimmer et al (2012) studied vesicular stomatitis virus (VSV) inactivation on dry surfaces and with the virus in suspension at different temperature and time combinations. From their findings, they concluded that the survival time of VSV on dry surfaces was shorter than that of VSV in suspension, probably because humidity was important for the virus to maintain infectivity. Other components such as proteins may also affect the virus survivability. Bozkurt et al., (2014) found that when murine norovirus and feline calicivirus were inoculated in a buffer solution with 10% fetal bovine serum and spinach the inactivation kinetics were much higher in spinach than in the buffer. In this case, the authors argued that the presence of proteins in the buffer may offer protection for the virus against heat inactivation.

Figure 1 shows an example of PEDV inactivation curve in damp spray-dried plasma at 60°C and RH of 70% and the predicted values using a log-linear model using the data of Verma and Goyal (2014).

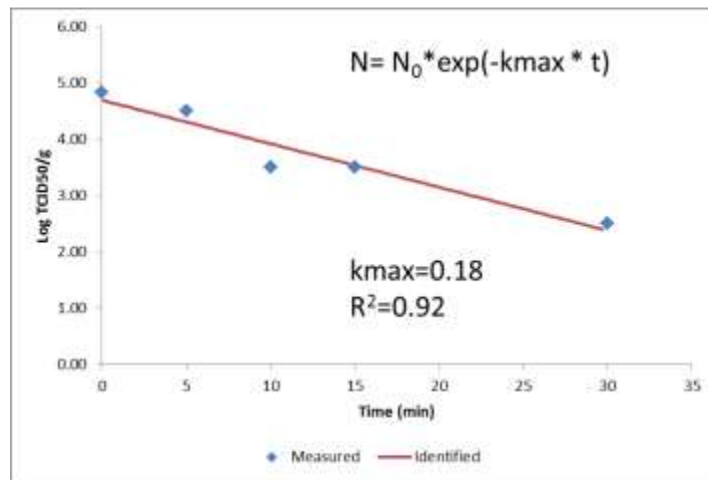


Figure 1: Inactivation of PEDV in damp spray-dried plasma at 60 °C and RH of 70%. Blue dots correspond to experimental data and red line to the values predicted by the log- linear model.

Appendix C shows the estimated D values of PEDV in different animal-based ingredients. D values ranged from 7.9 to 2.7 min in feed as temperature changed from 120 to 145°C. As temperature was adjusted from 60 to 90°C for RH 30 to 70%, D values ranged between 13.5 to 2.1 min in feed, 12.8 to 9.6 min in SDPP, 14.4 to 2.4 min in meat meal, 14.4 to 2.2 in meat and bone meal, 14.3 to 2.1 in blood meal, and 16.4 to 3.1 in grow finish premix. In general, the D values tend to trended lower as temperatures rose. This tendency is typical for microbial inactivation kinetics. No significant differences were observed between 30% and 50% RH levels. However, at 70% RH greater inactivation was achieved and this was reflected in lower D values. For example at 90°C, D values changed from 11.5 min at 50% RH to 2.0 min at 70% RH in feed, from 6.7 to 2.4 in meat meal, 10.9 to 2.2 in meat and bone meal, and 10.4 to 3.1 in grow finish premix. Interestingly, this effect was not observed in SDPP, where the D values did not change with temperature or RH. The D values for SDPP at 90°C were the highest observed. This may be due to a protective effect of the matrix against virus inactivation, but it is important that this observation be replicated and refined.

Scope of the risk assessment

This risk assessment addresses the likelihood that feed ingredients of porcine origin may function as vehicles of PEDV transmission. Therefore, the scope of the assessment includes ingredients of porcine origin that may be included in feed provided to pigs in the USA, and therefore present potential pathways for the transmission of PEDV in this country. The assessment includes ingredients derived from rendering process (e.g., meat & bone meal, choice white grease), ingredients derived by spray drying porcine blood (e.g., spray dried porcine plasma), and ingredients derived by hydrolyzing porcine tissues linked to the extraction of heparin (e.g., dried porcine solubles).

This risk assessment evaluates the following likelihoods:

- Likelihood of PEDV survival through the rendering, spray-drying and hydrolyzing processes used to produce ingredients of porcine origin.
- Likelihood of PEDV cross-contamination after processing of rendered, spray-dried and hydrolyzed ingredients.

Other potential pathways by which materials of porcine origin could enter the swine feed chain (e.g., feeding of waste from pet food production) are not addressed in the assessment. Evaluation of spray drying focused on spray-dried plasma rather than spray-dried red cells owing to the wider use of spray-dried plasma of porcine origin in swine nutrition. Rendering facilities that complete blending of proteins on site were within scope, but geographically detached facilities for blending of fats and/or proteins were out of the scope of this risk assessment. The distribution phase (e.g.; transport of materials from the rendering facilities) was not within the scope.

Assumptions

- All unprocessed feed ingredients or environmental samples that are PCR positive for PEDV may contain infectious PEDV. This is a conservative assumption that represents a 'worst-case scenario' with respect to the risk assessment. It is recognized that PCR testing of processed samples cannot be used to evaluate infectivity, as PCR testing detects nucleic acids that may or may not be associated with infective viral particles. It is acknowledged that virus contaminated materials that have been processed by methods that inactivate PEDV can still test positive by PCR for viral nucleic acids.
- It is assumed that the manufacturers of ingredients of porcine origin will be following their respective industry promoted GMPs when available.
- The reduction in virus load is described by the first-order reaction model: $N=N_o \times e^{-kt}$ where the time for one log reduction is expressed by $D_T= 2.3/k$ and N is the titer value for time t after heating at a given temperature T for t minutes.
- A calibration curve obtained at UMN and published in Alonso et al., (2014) was used to estimate the amount of RNA contained in a sample from a Ct value obtained in RT-PCR. The values were expressed as copies of RNA PEDV/g or ml. The diagnostic test had a limit of detection of Ct = 40 (50 copies of RNA/reaction or 7,000 copies of RNA/g or mL).

Overview of data analysis

Risk Assessment Overview

This risk assessment is based on the World Organization for Animal Health (OIE) guidelines and methodology for import risk assessment with some modifications (OIE, 2014). The OIE model is comprised of hazard identification and three steps within a risk assessment:
1) entry assessment (release of virus to the environment through the commodity);
2) exposure assessment (exposure of susceptible animals);
3) consequence assessment (consequences of the exposure to the hazard)
4) risk estimation (considers the entry, exposure and consequence assessments to provide the overall risk estimation).

The exposure and consequence assessment steps of the OIE framework were out of scope of the risk assessment. The emphasis of this risk assessment is the release of PEDV associated with the processing and movement of ingredients of porcine origin to the feed mill. If the release assessment demonstrates a negligible likelihood of the ingredient being contaminated with PEDV, then the risk assessment may be concluded. However, if the risk is estimated to be greater than negligible, the next step in the risk assessment process would be to complete an exposure assessment, which would assess the likelihood that susceptible animals will be infected by PEDV through the commodity in question.

As recommended by the OIE, the entry assessment process is described as the determination of the likelihood of a commodity (e.g., ingredient of porcine origin) being infected or contaminated with a hazard (e.g., PEDV) and describes the biological pathways necessary for that hazard to be introduced into a particular environment with susceptible livestock. It includes an estimation of the likelihood (i.e., qualitative or quantitative) of each of the pathways. Risk estimation consists of integrating the results from the entry and exposure assessments to produce summary measures of the risk associated with the identified hazard.

Likelihood and Risk Evaluation

The likelihood for each pathway was assessed and categorized using the descriptive scale in Table 3. The risk estimation is based on the combination of the likelihoods for each of the pathways in the entry assessment.

Table 3: Descriptive scale to estimate the likelihood for an event to occur.

Likelihood	Descriptive Definition
Extremely high	This event would almost certainly occur
High	The event would likely occur
Moderate	This event would be nearly as unlikely to occur as likely to occur
Low	The event would be unlikely to occur
Negligible	This event would almost certainly never occur

Uncertainty Estimation

The uncertainty of the likelihood estimation is indicated by using a range within the descriptive definitions listed in Table 3. When uncertainty about the estimation is low (i.e., the estimation was somewhat certain), a single descriptive definition is used to state the likelihood (e.g., low). When the uncertainty surrounding the estimation is moderate or high, the likelihood is stated within a range (e.g., moderate to high). The broader the range, the higher is the uncertainty surrounding the risk estimation.

Modeling Overview

Experimental data on thermal inactivation kinetics of PEDV was fitted to the traditional log-linear model to estimate the kinetic parameter k (min^{-1}) by using the GinaFit software (Geeraerd et al., 2005):

$$N=N_0*\exp(-k*t) \quad \text{Eq. 1}$$

where N is the surviving viral particles after the treatment expressed as $TCID_{50}$/mL, N_o is the initial virus concentration in $TCID_{50}$/mL, t is the processing time in min and k is the kinetic constant (min^{-1}).

D values (min) were calculated from k values by using the following equation:

$$D=2.3/k \quad \text{Eq. 2}$$

The D value is defined as the time at a certain temperature to inactivate 90% or 1 log of the initial virus concentration.

A probabilistic model was developed to estimate the amount of PEDV in the spray-dried blood plasma after processing and storage. Input parameters were obtained from industry, scientific literature and experimental studies. Each input parameter was characterized by probability distributions using @Risk 6.2 for Excel (Palisade Corporation, NY). Once the input parameters were characterized, a one-dimensional Monte Carlo simulation was carried out using a Microsoft Excel 2003 (Microsoft Corporation) spreadsheet software and @Risk 6.2 for Excel (Palisade Corporation, NY). The analysis was

performed using 10,000 iterations with the Latin-hypercube method. A detailed explanation of the model can be found in the spray-drying section of the entry assessment.

Overview of industry practices in the USA

Rendering

Overview of the rendering industry in the US

The rendering industry is a critical component of the global food animal production cycle. The rendering process transforms raw materials from the food animal supply chain into a range of products used across many industries, including animal feed. Rendering processes prevent serious challenges to public, animal, and environmental health that could result from the mismanagement of the potentially infective raw materials.

The rendering industry in the USA and Canada has evolved from having more than 900 independent rendering facilities in 1927 (Meeker et al., 2006) to approximately 200 facilities operated by 36 firms in 2014 (National Rendering Association, Personal Communication, 2014). Of these facilities, approximately 180 are located within the USA and 20 facilities are located within Canada (National Rendering Association, Personal Communication, 2014). These operations consist of both integrated and independent renderers and the approximate breakdown of these facilities into renderers, protein blenders, fat blenders, and fat recyclers is outlined in Table 4.

Table 4: Breakdown of rendering industry enterprises

Enterprise Description	Approximate Number of Operations
Independent Renderers	79
Integrated Cattle/Swine Processors with Rendering	50
Integrated Poultry Processors with Rendering	30
Integrated and Independent Protein Blenders	26
Integrated and Independent Renderers that also Blend On Site	8
Total	193

Source: American Protein Producers Industry Records (National Rendering Association, Personal Communication, 2014)

In 2013, approximately 50 billion pounds (i.e.; 22.7 million tonnes) of raw material were processed into rendered products (National Rendering Association, Personal Communication, 2014). Approximately 85% of rendered products are used as animal feed ingredients (e.g., fats, meat & bone meal, meat meal, blood meal, specialized protein & fat blends) (Meeker et al., 2006). In addition to animal feeding, other main applications for rendered products include human food products (e.g. edible tallow, lard, and gelatin), soaps and personal care items, industrial lipids and lipid derivatives, and biodiesel (NRA, 2003)

(Tables 5 and 6). In 2010, the total volume of rendered animal protein and fat based products was approximately 8.88 and 9.40 billion pounds respectively (i.e.; 4,027 and 4,265 thousand tonnes) (Informa Economics, 2011).

Table 5: Estimated Share of Rendered Protein Sales to Main Markets (2010)

Market	Percent
Poultry	39
Pets	31
Swine	9
Cattle	6
Fish	3
Export	8
Other	4

Source: Informa Economics, 2011

Table 6: Estimated Volume Share of Rendered Animal Fat Sales to Main Markets (2010)

Market	Percent
Livestock	35
Export	22
Pet Food	15
Biofuel	10
Oleo/Chemical	9
Human Food	8
Other	1

Source: Informa Economics, 2011

Raw materials of animal origin (e.g. mortality, offal, skin, hides, bones, horns, etc.) are expected to be contaminated with many microorganisms (e.g., bacteria and viruses), some of which can be pathogenic to animals or humans (Meeker, 2009). While one of the most significant preventive roles of rendering is the large scale elimination of these microorganisms, the potential for some pathogen survival or cross contamination during rendering processes poses an ongoing challenge for industry to verify its ability to

provide hygienic ingredients for animal feed consumption that will not contribute to the risk of disease transmission.

Rendering industry organizations have responded to this challenge in a systematic and science-based manner. A major responsibility of organizations such as the World Renderers Organization (WRO), the National Renderers Association (NRA), the Fats and Proteins Research Foundation (FPRF), and the Animal Protein Producers Industry (APPI) has been to address safety concerns. In North America, over 95% of the industry is represented by the NRA (Meeker et al., 2006; NRA, 2010) which has promoted good hazard management practices (i.e., chemical, physical, and biological hazards) and feed ingredient safety amongst its membership by facilitating ongoing education and promoting the implementation of Codes of Practice, recommended HACCP plans, GMPs, and Third Party Certification.

The three essential goals of rendering processes are to: 1) Remove moisture, 2) Separate fats from protein materials, and 3) Inactivate microorganisms (Leaphart et al., 2012). Figure 1 shows the general flow chart of the rendering process. These goals are accomplished using mechanical processes (e.g. grinding, pressing, and centrifugation), thermal processes (e.g. cooking, evaporating, and drying), and occasionally chemical processes (e.g. solvent extraction) (Auvermann et al., 2004). In North America, the cooking process is typically carried out at atmospheric pressure as opposed to the European rendering industry, which commonly performs cooking at elevated pressures (Annel Greene, Personal Communication, 2014).

Raw Material Sourcing

The USDA NASS survey reported that 73.4 billion lbs. (i.e.; 33.3 billion kg) of cattle and swine (USDA: NASS, 2014) and 58.6 billion lbs. (i.e.; 6.6 billion kg) of poultry (i.e., chickens, turkeys, ducks, etc.) (USDA: NASS, 2014) were harvested in the USA in 2013. On average, 50-66% of each harvested animal is used for meat, eggs, hides, and fiber for human consumption (Meeker, 2009). The raw materials for rendering consist largely of the portions of harvested livestock that do not directly enter the human food supply chain.

In the case of integrated renderers (i.e. renderers that process raw materials from their own processing facilities), raw materials (e.g. fat, offal, bones, skin, hair, blood, condemnations, etc.) are sourced solely from the animal or poultry processing facilities with which they are associated. It is estimated that integrated renderers process 48% of the total raw material in the USA (Informa Economics, 2011 National Rendering Association, Personal Communication, 2014).

Independent renderers process the remaining 52% of raw materials that are not processed within integrated systems. Raw materials may be sourced from various stages of the food animal supply chain. While their raw materials originate primarily from animal and poultry processing (~80%), they also process restaurant grease (~10%), trim from retail outlets such as grocery stores and butcher shops

(~7%; e.g. trimmings: bones, cartilage, meat, fat), and pre-harvest animal mortalities (~4%; National Rendering Association, Personal Communication, 2014).

The disposal of pre-harvest mortalities (e.g., live animal losses on farms or in transport, and ante-mortem condemnations) is a critical function of the rendering industry. The industry's ability to process normal and catastrophic animal losses in an efficient and hygienic manner has proven to be an effective way to mitigate potentially negative impacts on public safety and the environment (Auvermann et al., 2004). Across all species of livestock and poultry, it was estimated that 2.1 billion lbs (953 million kg) of animals that had died prior to slaughter were rendered in 2010, which comprised 3.75% of all raw materials rendered in that year (National Rendering Association, Personal Communication, 2014)

Table 7: Estimated Amounts of Species Specific By-products Processed by Renderers in 2010

Species	Amount in Billions of Pounds
Cattle	18.7
Poultry	16.0
Swine	7.8

Source: Informa Economics, 2011

Raw Material Receiving

Depending on the locations of rendering facilities in relation to raw material sources, raw materials are often collected and transported from source locations (e.g. offal from harvest facilities, mortality from livestock production facilities, trimmings from grocery store meat departments, or waste restaurant grease) to rendering locations. When transport is necessary, raw materials are handled following established State and federal regulations.

In most cases, the raw materials are delivered to fully enclosed receiving areas that can accommodate complete truck and trailer units, and have overhead allowances that enable full extension dumping capabilities. The transport trailers normally deposit their contents into a receiving pit. In some cases, raw materials (e.g. animal mortalities) may be deposited onto the floor of the receiving area and are then transferred mechanically (e.g., with payloaders) to the receiving pits.

Internal biosecurity measures are implemented to prevent cross-contamination between raw materials reception areas and the final rendered products. (e.g. proper labeling of shovels, front end loaders, and containers as 'Raw Only' or 'Finished Product Only'; guidelines for personnel movements between production areas; employee training to properly reprocess or dispose of spilled product in an appropriate container to prevent recontamination, etc.). The GMPs of each facility will typically outline these measures and third party audits will certify their implementation.

The receiving pits are normally emptied by large screw conveyors that transfer the raw material to the sizing stage of the rendering process. The material is discharged across magnets to remove any metal material that may be present.

The initial step of raw material processing is the sizing process. Sizing refers to the cutting and grinding or raw materials into consistently sized pieces and is completed before the thermal processing stage (Auvermann et al., 2004). The target size is 40 mm, with a maximum size of 70 mm (Auvermann et al., 2004). Consistent sizing is considered critical for efficient heat transfer during cooking (Meeker et al., 2006).

After the raw materials are transformed to a consistent particle size, they undergo thermal processing or cooking. The rendering industry has employed various systems for thermal processing throughout its evolution. The advancement of these systems has been driven by the advent of technologies that have facilitated production efficiencies (e.g. batch systems versus continuous flow systems, re-use of waste heat, grinding of raw material) and quality control (e.g. temperature monitoring for pathogen control, magnets for metal contaminants, centrifuging to purify fats, and pressing to separate fat from solids, etc.). Original systems added water and raw materials and were cooked in an open kettle or under pressure in batch format (Meeker et al., 2006). Although batch systems are still used, the predominant systems used currently in USA rendering operations are continuous flow systems that use the dry rendering process (Meeker et al., 2006). As opposed to wet rendering, dry rendering does not add water. Instead, the raw materials are thermally processed in their own fats. These systems may employ a heat recovery system that recirculates the waste heat from the vapor of the continuous cookers (Dupps, 2005).

The most pertinent parameters of thermal processing are the application temperature, residence time, and pressure. As temperature and pressure increase, the time to complete the rendering process decreases (USDA-Carcass Disposal, 2004). The exit temperature of the rendered material when it exits the thermal processor ranges from 240 to 290°F (i.e.; 115 to 143 °C) (Meeker, 2009) and is normally considered the critical control point for biological hazards of the rendering process under most rendering HACCP plans. Although the minimum time spent by the materials in the cooker stage (i.e.; 'residence time') is 30 minutes (Leaphart et al., 2012), the residence time typically ranges from 40 to 90 minutes (Meeker, 2009) and varies depending on raw material characteristics. Within North America, thermal processing is typically performed at atmospheric pressure (Leaphart et al., 2012).

The slurry that is discharged from the thermal processor enters handling systems that are designed to separate the liquid fats from the solids. Initially, the free liquid fat is drained as it is conveyed from the thermal processor. The remaining solids and retained fats enter a screw press system that applies pressure to express additional fat from the mixture. This process reduces the fat content of the solids to approximately 10 to12% (Meeker et al., 2006). The application of pressure at this stage can cause the temperature to rise above the exit temperature from thermal processing (Annel Greene, Personal Communication, 2014).

The liquid fat output is centrifuged to clarify the fat by removing any residual solid impurities which are recycled back into the thermal processing unit. The clarified liquid fat is transferred to storage through a closed loop liquid handling system for further processing into final commercial products, or stored as a finished fat product (Meeker et al., 2006). The nutrient content of the rendered fats varies among batches. In order to produce final fat products with consistent nutrient composition, samples from each batch are analyzed, and batches may be blended to achieve the desired nutrient profile. Fats from other sources (e.g. animal or vegetable origin) may also be blended to achieve the desired end product.

The solid output from the screw press system (i.e.; ''pressed cake', 'cracklings' or 'crax' (Meeker et al., 2006) is generally referred to as the protein output of the rendering process. However, in addition to protein, it also includes minerals, fiber, and residual fats (NRA, 2003). The solids enter a pressed cake conveyor following the screw press system and are further ground before being stored as a protein meal prior to final processing (i.e.; blending).

The type of meal produced by the rendering processes is dependent upon the raw materials entering the process. Common meal products that include materials of porcine origin are 'Meat and Bone Meal', 'Meat Meal', and 'Blood Meal'. As with fats, the nutrient content of protein meals will vary between batches and the blending of batches is performed to yield ingredients with consistent nutrient composition. Final meal products may be species specific or of mixed species origin. Recently there is an increased interest in single species animal proteins, such as chicken meal, beef meal, pork meal and lamb meal.

Inventory Management and Distribution of Final Rendered products

The distribution and storage of liquid fat products occurs at temperatures sufficient to ensure the liquidity and ease of movement and use. Upon entry into the storage tank the liquid fat will retain the post processing temperature for several hours. Fats entering storage will cool approximately 68°F (i.e.; 20°C) in the first 5 hours. Thus, temperatures can be expected to be above 212°F (i.e.; 100°C) for a minimum of 5 hours. Typically, final holding temperatures of 129 to 144.5°F (i.e.; 54 to 62.5°C) are maintained for liquid fat handling.

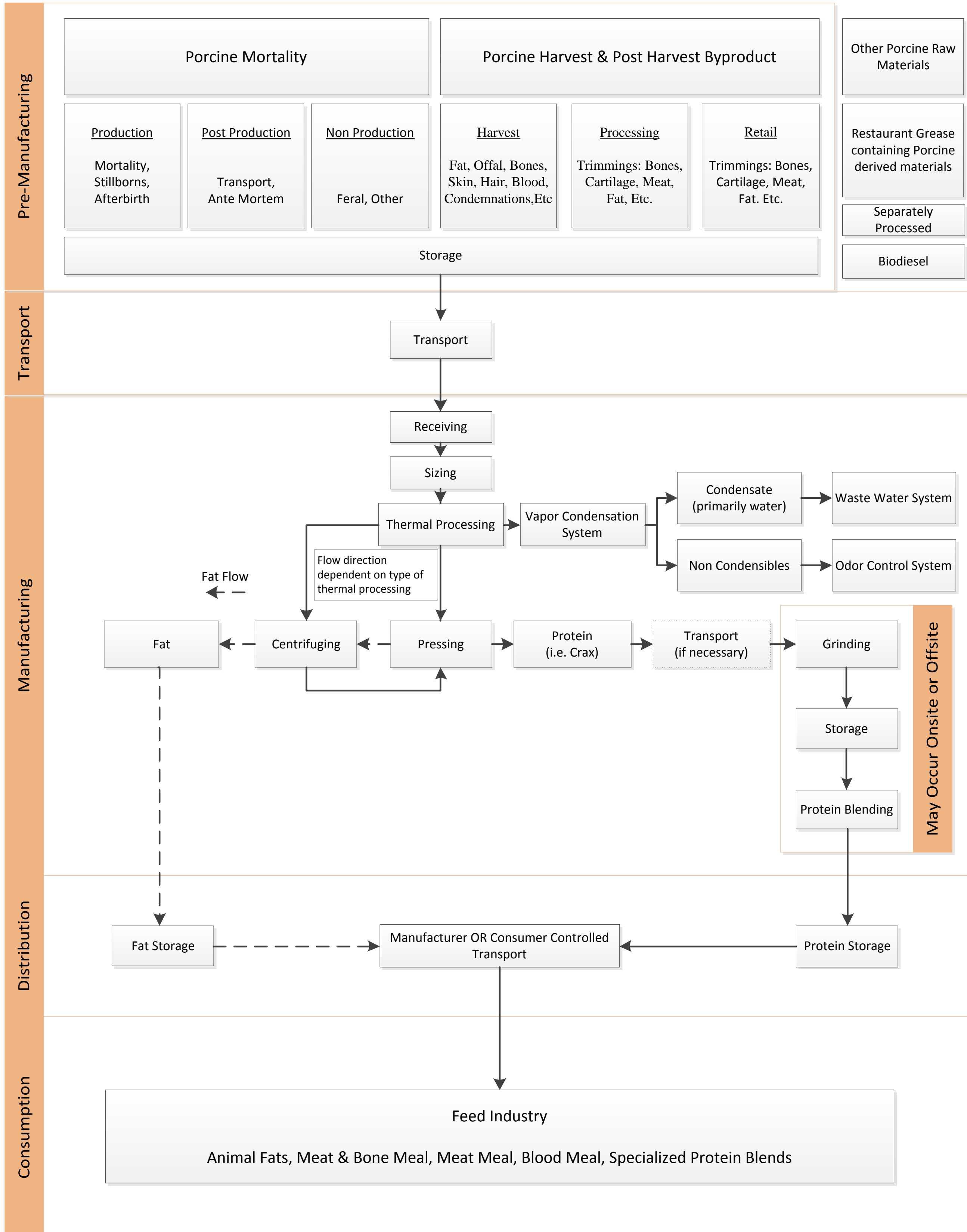
Distribution of the solid output from the rendering process may occur as pressed cake, unblended meal, or blended meal. Individual rendering facilities may undertake all stages of processing from reception of raw materials to meal blending into final protein products. However, in many facilities, pressed cake or unblended meals are transported to separate locations for grinding and blending to meet a final mix composition for specific buyer required specifications (e.g.; % protein).

If the pressed cake or unblended meals are to be transferred between locations for analysis and blending, the method for loading transport vehicles from bulk storage will depend on the type of the storage containers. Handling systems may employ: 1) either a closed loop bulk bin and auger system; or 2) open bin systems and front end loader vehicles. As mentioned above, GMPs that focus upon the flow of people and transport vehicles are often implemented to prevent cross-contamination from likely

contaminated areas (e.g.; raw material receiving) to final product inventory and distribution areas. GMPs also insure that trailers utilized for final product are not used for the transport of raw materials. New biosecurity procedures in dead stock and offal collections from swine operations have been implemented by most of the rendering industries in the US and Canada “” available here: have been adopted by most of the rendering companies in the US and Canada

Inventory holding times of final products will vary from facility to facility due to variation in demand. However, the usual retention time of final liquid fat and solid protein outputs are typically at least 1-2 days.

Figure 2: Rendering Process Flow Chart



Spray Drying

Background

Spray-dried porcine plasma is an ingredient derived from blood collected at pork harvesting plants. Spray-dried porcine plasma enhances growth of weaned pigs to a degree that cannot be explained by the nutrient concentration and which may be due to the presence of immunoglobulin or other growth factors including some short chain protein peptides which exhibit biological functionality (Torrallardona et al., 2010). Therefore, preserving the structure and function of these proteins is essential to the quality of the ingredients. Common methods for removing moisture from raw materials and feed ingredients involve heat. However, heat causes changes to the structure and function of proteins in raw plasma that can reduce the quality. Figure 3 shows a general flow chart outlining the spray-drying process.

Collection of raw materials

The collection of whole blood from swine as a source of spray-dried plasma and spray-dried red blood cells occurs in federally inspected slaughtering plants in the USA. Animals destined to slaughter are subject to official USDA ante-mortem and post-mortem inspections to determine their suitability for human consumption. Whole blood is collected immediately after stunning by using an open trough system that is specifically designed to minimize environmental contamination through the use of rapid collection, controlled flow, and rapid removal from the collection area. Blood drips from hanging carcasses by gravity during the exsanguination process. An anticoagulant (e.g., sodium citrate, sodium phosphate) is usually added to the whole blood as it flows in the collection trough to prevent clotting and facilitate flow. After the blood collection stage, further processing is conducted within a closed system that precludes any contact with the external environment. The system is maintained closed and batches are separated by 'clean in place' protocols. Collected raw blood is initially filtered to remove clots and particulate matter. Raw blood is centrifuged to separate the plasma fraction from the cellular fraction. Depending on the facility, plasma is concentrated by reverse osmosis, nanofiltration, or ultrafiltration membranes to obtain approximately 20% solids. Plasma is then chilled in steel storage tanks at approx. 39 to 50°F (i.e.; 4 to 10°C) and transported from the pork harvesting plant to the plasma processing plant. In some facilities, plasma is refrigerated and transported unconcentrated to the processing plant. Alternatively, in some systems, blood is refrigerated and transported to a spray drying facility where plasma separation is conducted. In these cases, the concentration at the manufacturing plant is usually greater and achieves 27-30% solids before spray-drying.

The total amount of blood collected in each plant varies depending on the system utilized and speed of the pork harvesting plant. Blood represents approximately 4% of the whole body weight of pigs (Bah et al., 2013). Therefore, each harvested pig will render approximately 0.92 US gallons (i.e.; 3.5 L) of whole blood. Large plants in North America can harvest approx. 19,000 to 22,000 pigs/ day and collect 17,570 to 20,300 US gallons (i.e.; 66,500 to 77,000 L) of whole blood (North American Spray-dried Blood and Plasma Producers, Personal Communication, 2014). There are various designs, but the majority of plants in the USA will hold plasma for 1 tanker truck load (approx. 5,000 to 6000 US gallons (18,900 to 22,700

L)). Plasma constitutes approximately 60% of whole blood. Therefore, some pork harvesting plants may collect as much as 12,150 US gallons (i.e.; 46,000 L) of liquid plasma per day.

Transportation

Chilled whole blood or plasma is transported at 39 to 50°F (i.e.; 4 to 10°C) in closed stainless steel tanks to the spray-drying plant. Large pork harvesting plants generate sufficient liquid plasma for collection once per day. These transportation tanks use tamper-resistant seals labeled with a corresponding and unique shipment identification number. Upon arrival at the spray-drying plant, each tank is verified for seal integrity, matching identification, and security of space documentation. Shipping documentation includes seal number, lot number, truck and driver's name, and plant of origin. The receiving spray-drying plant has specific areas for reception of incoming tankers loaded with raw plasma. Receiving of raw materials and dispatching of final product occur in separate areas within the plasma processing plant.

Plasma processing

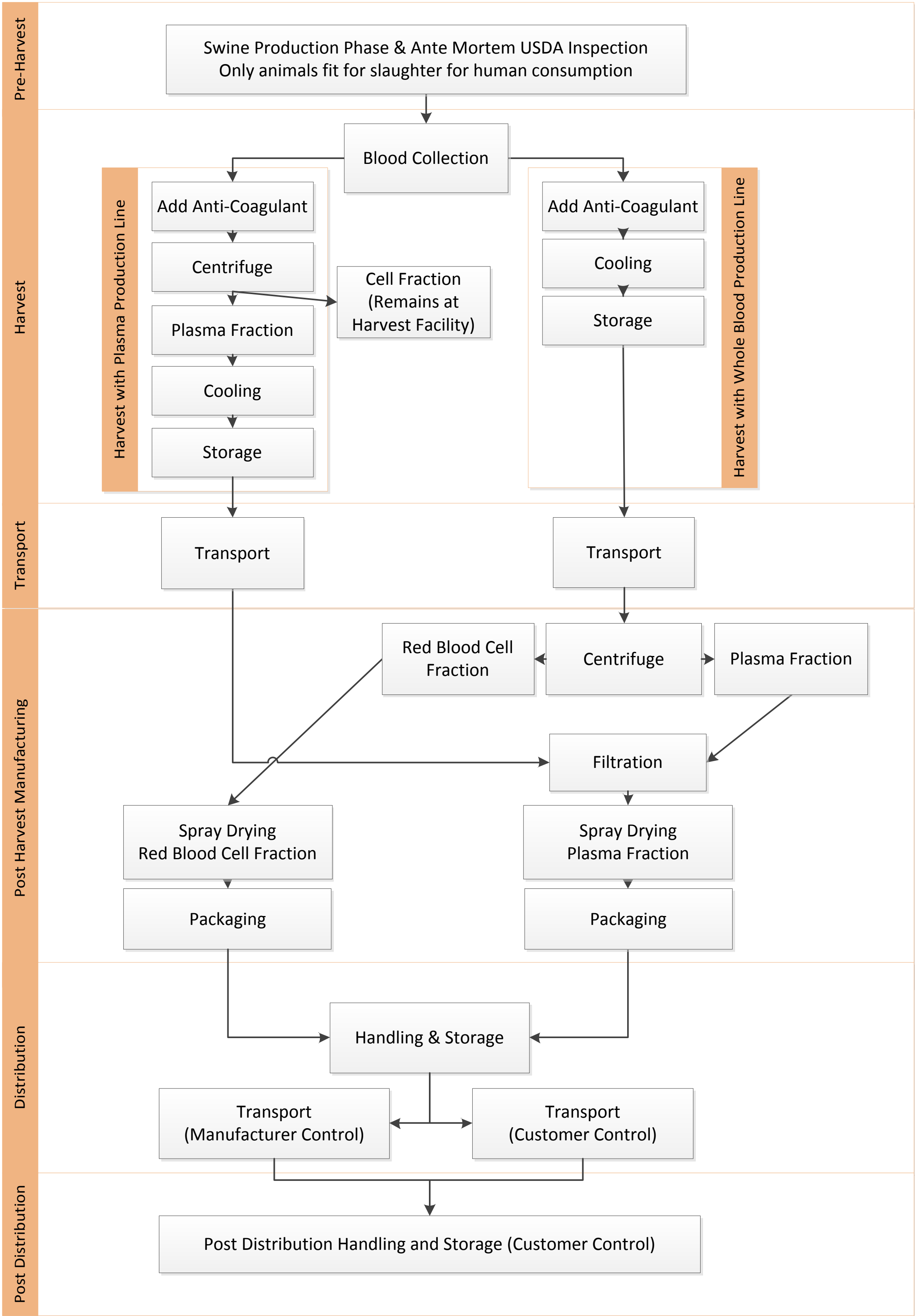
Spray-drying plants may process plasma or whole blood sourced from multiple harvest facilities. Plasma spray-drying plants are specialized in drying either porcine or bovine plasma. Therefore, the majority of SDPP is source specific and never mixed with bovine plasma at the drying plant.

Spray-drying consists of the evaporation of water as a function of the difference in the water activity (a_w) of the wet particle and the humidity of the inlet air, and thermal differences (Δt) between the inlet air (approx. 338 to 590°F or 170-310°C) and the changing particle temperature as it enters and passes through the dryer and approximates (in 2 to 5 seconds) the dryer outlet temperature (approximately 176 to 183°F or 80 to 84°C). Total transit times (i.e.; nozzle to cyclone collector) are usually between 20 to 90 seconds. The final spray-dried plasma has water activity (a_w) values of less than 0.6 which limits bacterial growth that may degrade product, and preferably 0.4 which avoids stickiness of the particles. Commercial dryers generally create particle sizes in the range of 45-150 microns. Processes are generally optimized for product quality and standardized regarding the exit temperatures and post-drying storage. However, there are other processing parameters that vary widely among installed spray-dryers and may influence virus inactivation kinetics (flow rates of inputs, temperature of materials entering the spray-drying units, retention time of the particles in spray dryer, and the distribution of thermohydric histories of particles during the process).

Product packing and storage

Both spray-dried plasma and spray-dried red blood cells are packaged into one of the following: 1) 55 lb (25 kg) plastic lined paper bags, 2) 2,200 lb (1,000 kg) super-sacks, or 3) 50,000 lb (~22,800 kg) bulk loads. There is limited data on time and temperature of storage for spray-dried plasma products. However, due to perceived risks associated with PEDV, during 2014 plasma manufacturers in the USA implemented additional inventory time of at least 2 weeks at room temperature (~20-22°C) before delivery to customers (North American Spray Dried Blood and Plasma Producers, 2014).

Figure 3: Spray-Drying Flow Chart



Hydrolysis

Background

Hydrolyzed porcine proteins are a specific subset of the industrial component hydrolysates (peptones). Peptones are a protein derivative formed by the partial hydrolysis of either animal or plant based proteins (Bridson et al., 1970; Pasupuleti et al., 2010). The sources of the raw material for the hydrolysate process can be placed into two groups: defined and complex. These grouping labels are based on the desired end product. Defined media are of limited product/tissue origin, whereas complex media can be any amalgamation of animal and/or plant protein sources (Biosciences, 2006). Currently, the most common worldwide use of peptones is for biotechnological fermentation media (Pasupuleti et al., 2010).

Hydrolyzed porcine proteins are considered a feed additive and are regulated by the Food and Drug Administration (FDA) which categorizes them as Generally Recognized As Safe (GRAS) (21 CFR 184.1553). A consequence of the GRAS designation is that there is no additional import restrictions placed on the materials other than general import laws (FDA, 2014). Although peptones are manufactured in many countries, including India and China, there is no known large importation market for the USA animal feed industry. New requirements under the FDA Food Safety Modernization Act (FSMA), which was signed into law in 2011, will require foreign manufacturers to: 1) have preventive GMPs and HACCP procedures that meet US domestic standards; 2) be registered as an approved processor; and 3) have mandatory audits by certified auditors. Once implemented, these requirements may inhibit the importation of these ingredients to the USA.

Currently, the majority of hydrolyzed porcine proteins used for feed ingredients are co-products of the enzymatic hydrolysis of porcine intestinal mucosa for the purpose of heparin extraction. The heparin production is exclusively for the human pharmaceutical use while the peptone co-products are used as feed ingredients. Currently, peptones are produced by their manufacturers as either a pure porcine origin hydrolysate powder that is soluble in water, or as a porcine origin hydrolysate dried onto a substrate (carrier) mixture.

Hydrolysate process: Heparin production

The raw materials consist of a combination of washed intact porcine small intestines and porcine small intestinal mucosa after the serosa is stripped for the production of sausage casings. Both materials are chopped or ground at the harvest facilities and a preservative such as bisulfite is added (Lindhart et al., 1999). This amalgamation of raw materials and preservative is known as “hash gut.” The hash gut is then shipped via tanker trucks to the heparin extraction plant, and is received at the plant at 32 to 38°C.

Once the hash gut arrives at the heparin extraction plant, it is heated to 60 to 65.5°C for a minimum of 3 hours in large hydrolysis vats that are continuously stirred. During this time the pH is adjusted to 7.8 by the addition of a buffer such as sodium hydroxide. Enzymes are also added at this time to hydrolyze the proteins. After the mixture reaches a desired consistency, the pH is neutralized and the fat is separated

by maintaining the mixture at 62 to 65.5°C for additional 1 to 2 hours. The liquid fat is then heated to 76 to 82.5°C for purification, and maintained at that temperature for a minimum of 2 hours. The fat is then held in a storage vat after decanting and maintained at 76°C until shipment (which usually occurs a minimum of 24 hours after decanting) (Aspen API Inc, Personal Communication, 2014). This liquid fat is then sold to a third party where it may or may not reenter the food supply chain. The remainder of the original material is pumped through ion exchange resin beads and heated to 79 to 82°C for a minimum of 30 minutes. During this process, the highly negatively charged heparin is bound to the positively charged resin beads (Aspen, Personal Communication, 2014).

The mixture with the added resin beads is then passed through a filter screen to remove the beads from the liquid hydrolysate. At this point, the resin beads and the liquid hydrolysate are separated and the beads placed in a separate vat for heparin removal and purification. The hydrolysates are then sent to a storage vessel that is maintained at ≥73.5°C for a minimum of 30 minutes prior to shipping to the drying facility (Aspen API Inc, Personal Communication, 2014). A fleet of dedicated tanker trucks ships the peptones to the drying facility where it is placed in a storage vat. Depending on the manufacturer, an evaporation process is implemented either before or after shipping to the drying facility. In either situation, the hydrolysate goes through an evaporator and it is heated to 76.5 to 93°C for a minimum of 20 minutes to remove moisture and yield a mixture that is 40 to 55% solids (TechMix, Personal Communication, 2014/ NutraFlo, Personal Communication, 2014).

Drying

Drying of the hydrolysate is performed by using either a drum dryer or a double drum dryer system. Each of the two drying processes is used by one of two manufacturers (system A and system B), who also use different bagging and distribution processes .The two systems are outlined separately in the next sections.

System A

Once the hydrolysate is delivered, it is kept in a closed system until the bagging stage. Only employee maintenance access points are available for product access during the drying process. Upon delivery, the condensed hydrolysate material is placed in a storage vat and maintained at 70°C until it is ready for drying. The hydrolysate liquid is moved through a closed loop system to the dryer and is then run over two heated rollers (double drum drying system). The product exits the unit as a sheet of highly hydrophilic, dried hydrolyzed porcine protein (NutraFlo, Personal Communication, 2014). The rollers have a surface temperature of 177°C and the material takes no longer than 1 minute to pass from the start to the end of the drying process. The product is then cooled and sized via a grinder (NutraFlo, Personal Communication, 2014).

System B

In system B, the drying process is performed within a drum dryer. After delivery, the liquid hydrolysate is stored in bulk tanks and then is run through a closed system until the bagging and distribution stage. During the transfer to the dryer, a non-animal protein substrate is added. The dryer has an inlet air

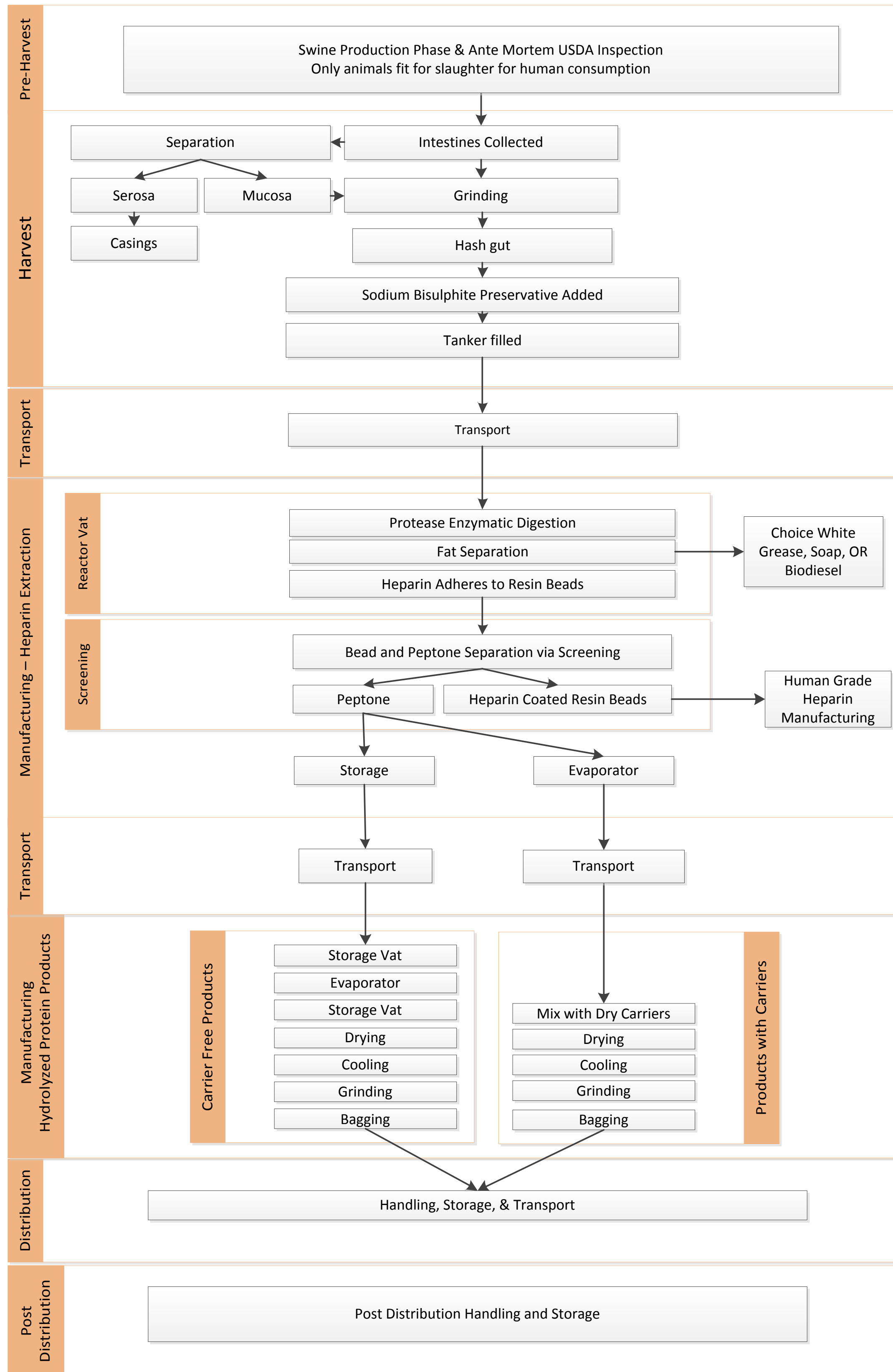
temperature of ~280.5°C and the drying process takes about 20-25 minutes (Dee et al., 2014/ TechMix, Personal Communication, 2014), and the dried product exits the dryer at ≥115°C. The product then takes a minimum of 1 min to be moved along a mechanical feeder to be air blown into a cooling system. After the product is cooled, it is ground and air blown to storage tanks where it is held until bagging and distribution. The end product water content is 5% (TechMix, Personal Communication, 2014).

Bagging and distribution

In system A, after the product is ground to the desired size, it is immediately sent for bagging. It is bagged into either 25 kg bags or loaded into 1,000 kg totes. The bags used within this system are heat sealed triple-walled bags with an internal moisture barrier (commonly used in the feed industry). They are filled in a completely mechanical system, including pallet stacking. The pallets are shrink-wrapped and held until shipment. Due to the hydrophilic nature of the end product, the distribution is via enclosed trailers (NutraFlo, Personal Communication, 2014).

In system B, the product is stored in closed hoppers until it is needed for distribution. It is then packaged in one of three ways: bagged into 25 kg bags, filled into totes, or loaded into a bulk product trailer. The bags used for smaller use are a heat sealed triple-walled bag with an internal moisture barrier. These smaller bags are shrink-wrapped and held in a warehouse until shipment. (TechMix, Personal Communication, 2014). Neither system has a standardized product hold time and the products are shipped based upon market demand.

Figure 4: Hydrolyzed Porcine Protein Flow Chart



Irradiation

Background

Irradiation is a process in which food or other items are exposed to ionizing radiation. The main uses of this technology in the food industry are to extend the shelf-life of products (mainly ground meat, chicken fruits and vegetables and spices), to control insect infestation in stored products, and to kill (or reduce the number of) pathogens that might be present in food products (Moseley, 1990). There are different types of irradiation applied to food products, including gamma rays emitted from radioisotopes, and high energy electrons or X-rays produced by equipment sources (Farkas, 2006). An absorbed dose of irradiation energy is measured in a unit called kilo Grays (kGy), for which 1 KGy equals a 100,000 rads (Tauxe, 2001).

Electron-Beam (e-beam) technology uses a stream of high energy electrons that are emitted from an electron gun. Electrons can only penetrate several centimeters of food, and that is why food is usually treated in thin layers, approximately 2 inches thick. The penetration in food is usually limited.

In the US, irradiation is currently used to treat some packaged spray-dried plasma products as a further killing step post drying. Electron-beam irradiation is applied to both sides of a large flat package at a dose of 15 kGy per side (betaGRO, 2014).

Effect of irradiation on pathogens

The effect of electron-beam irradiation on biological organisms has been studied by many authors. Tauxe (2001) reported on the doses needed to inactivate a range of organisms. For instance, a low dose of 0.1 kGy would kill insects and parasites; a medium dose (between 1.5-4.5 kGy) would kill most bacteria other than spores, and a higher dose (10 to 45 kGy) was required to inactivate spores and some viruses.

With respect to viral inactivation, there are a few studies that mostly address human pathogens. Bidawid et al., (2000) observed a linear decrease in Hepatitis A virus (HAV) titer as irradiation doses increased for lettuce and strawberries; at 10 kGy >3 log inactivation occurred for both products. Brahmakshatriya et al., (2009) used electron-beam irradiation to evaluate its effect on Avian Influenza Virus (AIV) in poultry products. They found that the dose required to achieve 90% reduction (1 log) of viable AIV was 2.3 kGy in PBS, 1.6 kGy in egg-white, and 2.6 kGy in ground turkey meat samples. Also, more recently, Skowron et al., (2013) found e-beam processing to be very effective in inactivating bacteria (*Salmonella* spp., *Escherichia coli*, and *Enterococcus* spp.) and parasite (*Ascaris suum*) eggs in cattle and swine slurry of varying dry matter contents. They showed that doses of less than 1 kGy achieved 90% inactivation (1 log) of the tested microorganisms.

For viruses of animals, Preuss et al., (1997) compared e-beam irradiation and chemical inactivation with ethylenimine applied to 3 viruses: Porcine Parvovirus (PPV), Porcine Enterovirus (PEV), and Bovine Viral Diarrhea Virus (BVDV). With e-beam inactivation, they found that, in general, the rate of inactivation in

liquid samples was almost twice as fast as in frozen samples. In order to obtain a 1 log reduction of PPV, 11.8 (frozen) and 7.7 (liquid) kGy was needed; for BVDV the required dose was 4.9 and 2.5 kGy respectively; and for PEV it was 6.4 and 4.4 kGy. Schmidt et al., (2012) studied the inactivation kinetics for several viruses (HIV-2, HAV, Pseudorabies Virus (PRV) and PPV) after application of e-beam processing to a tendon transplant model. To achieve a virus titer reduction of 4 log, a dose of 34 kGy of the fractionated e-beam irradiation was necessary in case of HIV-2, which was the most resistant virus investigated in this study; PPV was the second most resistant with a range of inactivation between 32-36.8 kGy. Both HAV and PRV were inactivated with less than 34 kGy.

In contrast, Sanglay et al., (2011) reported that e-beam irradiation was not as effective on the inactivation of Murine Norovirus 1, a surrogate of human noroviruses. They measured the inactivation of the virus by e-beam (at doses of 0, 2, 4, 6, 8, 10, and 12 kGy) in PBS, Dulbecco's Modified Eagle's Medium (DMEM), and in fresh foods. They found that in PBS and DMEM, e-beam at 0 and 2 kGy provided less than 1 log reduction of the virus. At the higher doses, viral inactivation in PBS ranged from 2.37 to 6.40 log, while in DMEM the inactivation ranged from 1.40 to 3.59 log. In the fresh food, inoculated cabbage showed a reduction of 1 log at 4 kGy, and less than 3 log reduction at 12 kGy; strawberries showed less than 1 log reduction with doses up to 6 kGy.

Entry Assessment

The entry assessment addresses the risk associated with the processing of PEDV infected materials of porcine origin into ingredients that may be fed to pigs. For estimating the risk, the study evaluated the likelihood that raw materials are contaminated with PEDV, the effect of the processes on virus survival, and the likelihood for post-processing contamination. Each of these events was characterized within defined pathways, and for each of the pathways the likelihood of occurrence was evaluated based on available scientific information, stated assumptions, input from experts and simulation models. The pathways considered were:

- Likelihood of PEDV survival through the rendering process in ingredients of porcine origin
- Likelihood of PEDV survival through the spray-drying process in ingredients of porcine origin
- Likelihood of PEDV survival through the hydrolysis process in ingredients of porcine origin
- Likelihood of PEDV cross-contamination after processing of rendered ingredients
- Likelihood of PEDV cross-contamination after processing of spray-dried ingredients
- Likelihood of PEDV cross-contamination after processing of hydrolyzed porcine protein ingredients

Likelihood of PEDV survival through the rendering process in ingredients of porcine origin

Summary

The thermal inactivation kinetics of PEDV in feed at high temperatures suggests D values between 2.71-7.94 min at 120-145°C. The current temperature-time combination during the rendering process (115-145°C for 30-90 min) will achieve a predicted inactivation level of 3.7-21.9 log cycles. Liquid fats derived from rendering are held at temperatures sufficient to ensure the liquidity and ease of liquid flow during handling (above 100°C for a minimum of 5 hours and at 54-62.5°C for the rest of the process). *Likelihood estimation: The likelihood that PEDV would survive the rendering process is negligible.*

Thermal inactivation kinetics of PEDV in feed at high temperatures

The thermal inactivation kinetics of PEDV in complete feed at 120-140°C from the study of Verma and Goyal (2014) (see Appendix B) were fitted into the log-linear model (Equation 1) to obtain the D values to characterize the thermal resistance of PEDV (see Appendix F). Table 8 shows the kinetic parameters estimated.

Table 8: Kinetic parameters and correlation coefficient of PEDV in complete feed

Temperature (°C)	k value (min^{-1})	D value (min)*	Adj. R^2
120	0.29±0.06	7.94	0.79
130	0.54±0.11	4.26	0.88
140	0.56±0.07	4.11	0.95
145	0.85±0.17	2.71	0.92

*: These values may change depending on the moisture content of the sample and the matrix type. Values shown in this table correspond to complete feed.

Values in Table 8 were used to estimate the overall degree of inactivation achieved during the cooking step in the production of feed ingredients by the rendering industry.

Table 9 shows the inactivation in log scale predicted for the rendering process.

Table 9: Inactivation (log scale) predicted during the cooking process

Temperature (°C)	Retention time (min)	Inactivation (log)*
120	30-90	3.77-11.33
130	30-90	7.04-21.12
140	30-90	7.29-21.89

*: These values may change depending on the moisture content of the sample and the matrix type. Values shown in this table correspond to complete feed.

Likelihood of PEDV survival through the spray-drying process in ingredients of porcine origin

Summary

The Ct values for PEDV in commercial plasma samples indicate that during 2013-2014 pooled blood collected at harvest of pigs from multiple farms was highly likely to contain variable concentrations of PEDV RNA. The most likely sources are the contaminated external surface of the pig carcass and/or the oral cavity (saliva) at the slaughter plant. To model virus survival during spray-drying, two scenarios were evaluated based on independent sources of data:

1) Experimental data on thermal inactivation of PEDV in damp plasma at 60-90°C and RH levels of 30-70% (D values between 9.6 to 12.8 min); and
2) Experimental data on inactivation of PEDV after spray-drying in a lab-scale dryer. Simulations were also conducted across a range of assumptions about the viability of viruses in raw plasma in relation to viral RNA content.

Simulations in scenario 1 indicated likelihood of PEDV survival if at least 0.1% of viral RNA in liquid plasma represented viable virus. It is noted that the processes for estimating the D values used in scenario 1 were not equivalent to the processes occurring during spray drying (e.g., atomization, rates of moisture loss and heat exchange, etc.) and the moisture conditions used in the experiment are not equivalent to the moisture content of commercial spray-dried plasma. Therefore the inferences derived from them need to be interpreted with reservation.

Simulations in scenario 2 indicated negligible risk or PEDV survival across all assumptions of virus viability. Likelihood estimation: The likelihood that PEDV would survive spray-drying process itself was negligible to moderate for scenario 1 and negligible for scenario 2.

Warm storage (20-22°C) for 2 weeks was predicted to yield an additional inactivation level between 3 to 5 log cycles. The likelihood that PEDV would survive the warm storage before releasing the product was estimated to be negligible. Overall, the robustness of the assessments can be questioned due to the paucity of specific data regarding the thermal inactivation of PEDV, limited understanding of the mechanisms of inactivation of viruses during spray-drying, and the representativeness of laboratory scale spray-drying with respect to the range of commercial spray-drying facilities.

PEDV contamination of blood at the harvesting facility

The majority of raw plasma is collected from market age pigs. Therefore, concentration and prevalence of PEDV in raw plasma is likely a function of the prevalence of infection at finisher sites. The prevalence of infected pigs at harvest is unknown, and likely has varied greatly temporally and spatially during the course of the PEDV epidemic in the USA. Data from the UMN VDL have shown (see section on PEDV environmental persistence) low Ct values for saliva and fecal samples (i.e. average Ct values of 21 and 23 for fecal and oral fluid samples, respectively). This corresponds with a concentration of ~10^7 copies of

RNA/ml. It is noted that the number of genomic copies in any medium should not be interpreted as equivalent to the number of intact or infective virions (Weidmann et al., 2011). The most likely sources of PEDV in raw plasma include fecal material originating directly from the gastrointestinal tract, or indirectly from the contaminated external surface of the carcass or oral cavity (saliva) which may drip into the blood collection trough when animals are being exsanguinated. Viremia was reported by Jung et al., (2014) in experimentally infected gnotobiotic pigs and in 11 of 20 naturally infected pigs during the acute phase of disease. However, another experimental study did not detect viremia in PEDV infected pigs (Gerber et al., 2014), and more research is needed to confirm whether viremia may contribute substantially to the contamination risk of raw blood and plasma.

Effect of plasma concentration on PEDV load

Filtration/concentration processes (e.g., reverse osmosis, ultrafiltration, nanofiltration) are used to remove water and small molecules, thereby concentrating the plasma. Any filtration system is based on a separation medium that allows molecules of less than a given molecular weight range to pass through the membrane. As shown in Figure 5, viral particles will be retained by nanofiltration (NF), ultrafiltration (UF) and reverse osmosis (RO) processes. As stated by Hofmann et al., (1989) a pore size of 100 nm, typical of reverse osmosis, will retain PEDV. Thus, with the pore size filters used by the industry, PEDV virions would remain in the concentrated plasma.

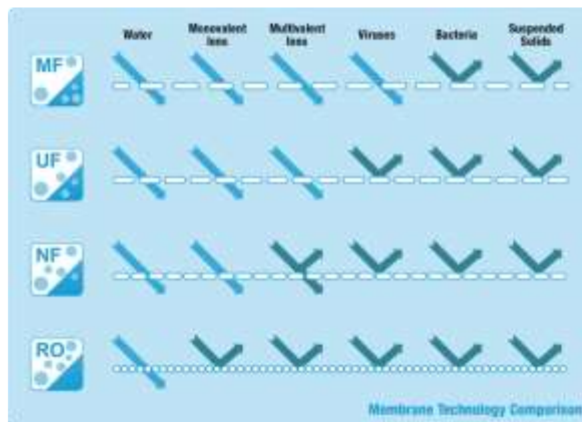


Figure 5: Substances removed from water by membrane filtration processes: ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) processes (http://www.kochmembrane.com/Learning-Center/Technologies.aspx)

Spray-drying characteristics

Spray-drying is the process of transforming a solution or suspension from a fluid into a dried particulate form by spraying the feed into a hot drying medium. Spray-drying involves four stages of operation: (1) atomization of liquid source into a spray chamber; (2) contact between the spray and the drying medium (very hot air at a high gas mass to liquid mass flow volume ratio to ensure that the exit air condition is at a low enough %RH such that the particles will not be sticky); (3) moisture evaporation resulting in particle formation; and (4) separation of dried products from the air stream (Kuriakose et al.,

2007). Atomization is the process where the bulk-liquid breaks up into a large number of small droplets of sizes that may vary over a wide range (e.g. 10 to 150 µm of diameter). During spray-air contact, droplets meet hot air in the spraying chamber. At the beginning, the particle is maintained at the adiabatic wet bulb temperature as moisture is lost, the droplet temperature increases to reach a value close to the outlet air temperature. The time needed for the particle to reach the final temperature may vary depending on the air volume rate to liquid flow rate, initial particle temperature, hot air temperature, size of the droplet, configuration, size of the dryer, and thermal properties of the matrix (Woo and Bandari, 2013). In a typical commercial dryer this may be between 3 to 5 seconds and takes place within the first 5 feet of space from the nozzle. It is generally accepted that for most practical purposes, the temperature gradient inside the particle is minimal so that the droplet temperature is uniform (Woo and Bandari, 2013). The dried powder is then collected either at the base of the dryer or more generally the air stream is directed into a cycle collector where the particles are separated from the air stream. The air then flows out of the cyclone collector through a pipe to the roof. In some cases there may be a filter in the line to collect the fines that pass through the cyclone collector. This prevents contamination of the environment. Screw conveyors or pneumatic systems remove dried particles contained in either the dryer or cyclone collector (Kuriakose et al., 2010).

Figure 6 depicts the three main temperature and moisture transitions that occur in particles during spray-drying. At the start of the spray-drying process, the water activity (a_w) values for the particle and inlet air are around 0.95 and 0.001 respectively, thus drying is rapid (i.e. 90-95% of the water in the particle will be lost in 2 to 5 seconds). As the moisture is being lost from the particle, it is incorporated to the air, thus the air cools down quickly close to the final exit temperature (e.g. from 280°C to 80°C) (Green et al., 2007). This stage, during which the surface of the particle is still wet and the drying occurs from inside, is considered the adiabatic heating stage and the temperature of the particle is at the wet-bulb temperature, which is much below the inlet air temperature, for about 3 to 5 seconds. During this time the evaporating water takes the heat from the air to supply the energy of evaporation (~ 45 Kj/gram water) and the air quickly cools down to close to the exit temperature. For the remainder of the retention time inside the dryer, the particle will approach the outlet air temperature and will lose most of the remaining moisture content (i.e., final moisture of ~ 5-10%). This stage is termed the dry heating stage due to the low moisture content of the particle. As shown in a study conducted by Anandharamakrishnan et al., (2007) by spray-drying a whey protein isolate (20-40% w/v) in a tall form co-current spray dryer (11 ft high,3 ft diameter), the wet-bulb temperature of the particle was at 46-48°C and the final temperature of the particle was around 75°C (lower bound) when outlet air temperature was set at 80°C. However, other authors have indicated that the dry particle may achieve the outlet air temperature during the spray-drying process under specific conditions (Perdana et al., 2013, 2014; Straatsma et al., 2007).

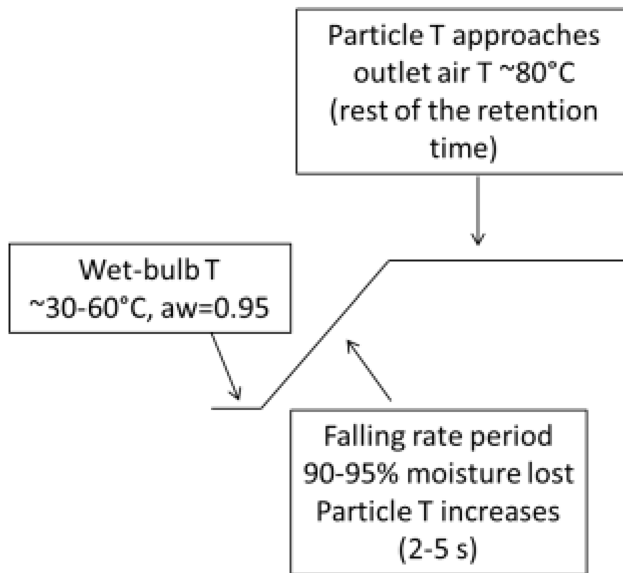


Figure 6: Typical drying history of a particle (adapted from Woo and Bandari, 2013)

Figure 7 shows a psychrometric chart which is used to estimate the moisture and temperature of the particle and inlet air as an example of a spray-drying process.

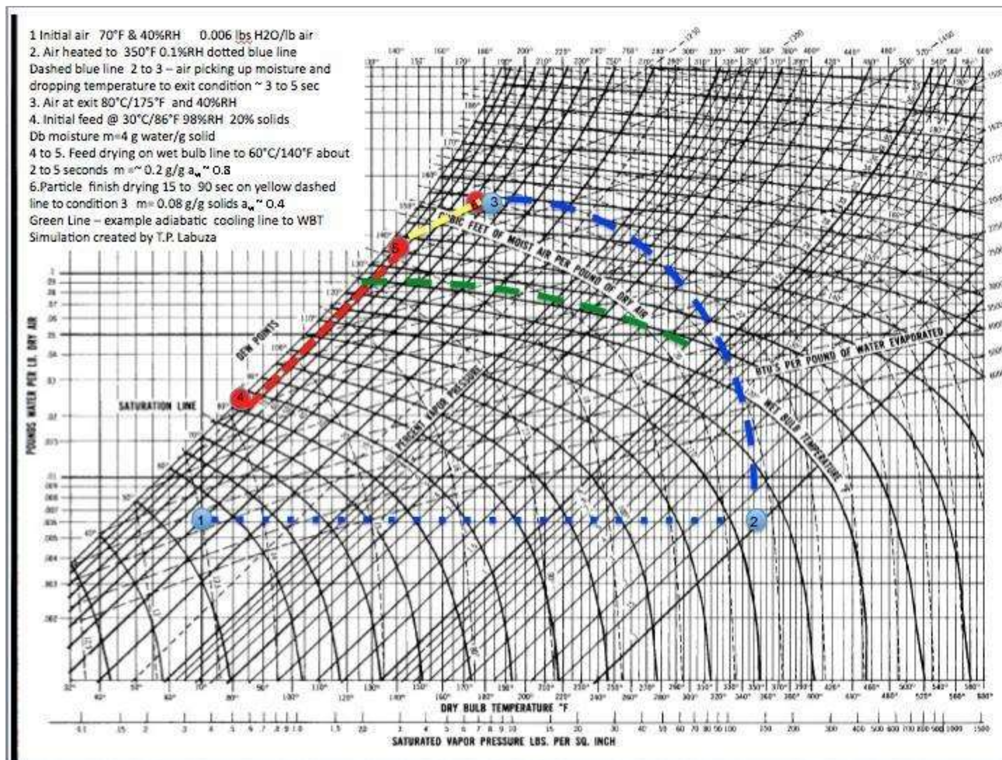


Figure 7: Temperature and moisture content profile of a particle and air during spray-drying

A course-scale simulation model was applied to the experimental data related to the dryer process parameters provided by industry (Table 10). The model predicted the temperature and humidity profile of the plasma particles.

Table 10: Input parameters for the simulation model of the moisture and temperature of particles during the spray drying process.

Inlet	Outlet
Air temperature: 304 – 329 ºC Air humidity: 40% RH (6 g water/kg air) Air flow rate: 10,501 CFM[1]	Air temperature: 80°C Air humidity: Not supplied
Plasma temperature: 4 to 10°C for 20% solid or 25-30ºC for 30% dry mater Plasma flow rate: 1472 L/h @ 3000 PSI Plasma composition (solids %): 23 º - 33º Brix (assume 20% - 30% dry matter)	Particle flow rate: 317 kg plasma powder/h Particle temperature (after cooling during transport to packaging): 42.2°C (7 % moisture)

[1]Cubic feet per minute

Effect of spray-drying on the inactivation of microorganisms

Understanding the contribution of spray-drying to the inactivation of PEDV entails more than just determining the temperature and humidity profile of the plasma in the spray dryer itself. Just as in the case of other spray-dried protein products (non-fat milk, whey proteins, etc.), each step in the process, from pre-drying processing to pre-packaging conveying to post packaging storage, contributes to the overall effectiveness of the integrated system for pathogen control. The effect of spray-drying on the inactivation of microorganisms will depend on the physicochemical characteristics of the organism, the composition of the matrix (e.g. fat, moisture and solids content), and the process conditions (inlet and outlet temperatures, flow volumes, residence time, particle size).

The mechanisms of pathogen inactivation during spray drying are incompletely understood, and have been investigated more for bacteria and yeasts than viruses (Fu and Chen, 2011; Ghandi et al., 2012; Gong et al., 2014). Conventionally, concurrent processes of dehydration inactivation and thermal inactivation are thought to play a major role in pathogen inactivation (To and Etzel, 1997a,b; Gong et al., 2014), with the rates of temperature changes and moisture loss having an important impact on cell viability (Gong et al., 2014). Fu and Chen (2011) explain that cell survival during thermal drying is influenced by the combined effects of heat and dehydration damage, which are determined by multiple factors including the intensity of each stress (e.g., temperature and water potential), the times that of cells are exposed to the respective stresses, and the changing rate of the stresses (e.g., drying rate and the rate of temperature change). However, other factors including oxidative stress, osmotic stress, and

shearing forces have been shown to contribute significantly to loss of cell viability (Fu and Chen, 2011; Ghandi et al., 2012; Gong et al., 2014; Perdana et al., 2013). Furthermore, wide variability in survival of spray drying has been seen between strains of the same bacteria, and is affected by the composition of the matrix. Ghandi et al. (2012) stated that the atomization process of the feed liquid (e.g., plasma), which determines the initial droplet size distribution, affects organism survival due to extensional and shear stresses. In turn, particle size influences bacterial survival by altering the droplet and particle temperature and moisture trajectories, and altering oxygen exposure by changing the surface:volume ratio. In general, finer droplets experience more rapid drying and subsequently reach a higher temperature than coarser ones (Ghandhi et al., 2012). Other authors (Perdana et al., 2014; Straatsma et al., 2007) have also indicated that larger particles require more time for drying (related to the moisture diffusion through the dried particle) and doubling the particle size leads to a fourfold longer drying time to reach the same moisture content. It is important to note that understanding of mechanisms of pathogen inactivation during spray drying is overwhelmingly based on studies of bacteria. Although the basic mechanisms of inactivation (e.g., protein denaturation, oxidative damage to membranes, etc.) are likely similar for viruses, the relative importance of different mechanisms is likely to be very different due to the different structural characteristics of virus particles compared with bacteria, and can also be expected to vary greatly among viruses.

Effect of spray-drying on the inactivation of PEDV

Table 11 shows available data regarding PEDV inactivation by spray-drying. In the study by Pujols and Segalés (2014), the prototype European PEDV strain (CV777) was inoculated into bovine plasma and spray-dried in a lab bench-dryer for 0.41 seconds (80°C outlet temperature). The samples were then processed in a water bath for an additional 30-60 s to achieve 70 and 80°C temperature in the materials in order to simulate commercial exposure time conditions (estimated between 20 to 90 seconds). The virus inactivation (4.2 log reduction in TCID $_{50}$), was estimated after the post-processing step thus the extent of inactivation attributable to the spray drying step is unknown. These data need to be considered with some caveats. Firstly, there are no studies comparing the thermal resistance of the European strain versus U.S. strains, and variability in survival of different isolates of the same virus species is documented (Platt et al., 1979; Saif et al., 2012) In addition, results obtained from laboratory scale dryers may not reliably predict the performance of industrial scale dryers due to the different processing parameters used. According to Thybo et al., (2008), in order to scale-up from laboratory setting to pilot plant or industrial scale, process parameters need to match for atomization, mixing of droplets and drying gas, drying kinetics and separation. Typically, industrial spray drying employs longer drying times (~30 s) compared with laboratory scale spray dryers (approximately 1.3 s), due to the size of the drying chamber and the volume of material being processed. In addition, particle size is usually smaller in laboratory dryers compared to particle size in industrial dryers (Perdana et al., 2013).

Table 11: Effect of spray-drying on the inactivation of viruses

Virus	Dryer type	Matrix	Process conditions	Inactivation (log)	Source
Pseudorabies virus, Porcine Respiratory and Reproductive Syndrome virus	Pilot-plant spray-dryer (Anhydro)	Bovine Plasma	Inlet air temp: 240°C Outlet temp: 90°C Flux: 10 L/h Processing time: 0.41 s	Initial inoculum: 5-6.3 log TCID50/mL Inactivation: 5.3 log (PRV) 3.5-4 log (PRRS)	Polo et al., 2005
Swine Vesicular Disease virus	Lab scale (Buchi)	Porcine plasma from SPF animal	Inlet air temp: 200°C Outlet temp: 80ºC or 90°C Flux: 10 L/h Processing time: 0.41 s	Initial inoculum: 5.64 log TCID50/mL Inactivation: 5.64 log	Pujols et al., 2007
PEDV (European strain CV777)	Lab-scale (Buchi)	Bovine plasma	Inlet air temp: 200°C Outlet temp: 80°C Processing time: 0.41 s Post-processing (water bath): 90°C for 20 s (inner T 70°C) 90°C for 30 s (inner T 80°C)	Initial inoculum: 4.2 log TCID50/mL Inactivation after spray-drying and post-treatment: 4.2 log	Pujols et al., 2014
PEDV (U.S. strain)	Lab-scale (Yamato)	Porcine plasma	Inlet air temp: 166°C Outlet temp: 80°C	Initial inoculum: 4.25 log TCID50/mL Inactivation after spray-drying and storage for 7 d at 4°C: 4.25	Gerber et al., 2014

The longer drying time in industrial spray dryers may lead to greater microbial inactivation. For example, Perdana et al. (2013) compared bacterial (*L. plantarum*) inactivation in laboratory and commercial spray dryers and suggested that there are differences between small scale-laboratory spray dryers and single droplet drying (individual droplets are dispensed and dried on a flat surface). Single droplet dryers inactivated the bacterial suspension by a slow dehydration process, while small scale-laboratory spray dryers inactivated the bacterial suspension by thermal inactivation. Therefore, greater residual bacterial viability was observed in small scale spray dryers at a 50C outlet temperature compared with large scale single droplet drying. These data demonstrate significant differences between types of dryers and mechanisms of bacterial inactivation (dehydration vs. thermal shock). These data also highlights the difficulties of extrapolating data from small scale spray dryers to large scale conditions.

The data provided by Verma and Goyal (2014) suggest that PEDV appears to be very resistant to heat when the moisture content of samples was 18 to 25%. The D values for PEDV in damp plasma at 80°C were in the range of 9.6 to 12.8 min, which greatly exceeds the time encountered during spray-drying (20-90 s). A caveat to these data is that they are derived from a single study with experimentally inoculated virus, and that thermal inactivation alone does not represent the entire scope of inactivation processes that occur during spray drying (Fu and Chen, 2011; Ghandi et al., 2012; Gong et al., 2014). In addition, the plasma product used in the experiment contained more moisture than commercially produced plasma. More extensive research of the mechanisms and kinetics of inactivation kinetics of PEDV under conditions simulating spray-drying processes is required to more confidently predict virus inactivation during spray drying.

Recently, Gerber et al., (2014) reported inactivation of PEDV (culture adapted USA strain) after spray-drying in a lab dryer followed by storing the sample for 7 days at 4°C before inoculating it into pigs (bioassay). The caveat stated earlier in extrapolating results from laboratory scale experiments to industrial settings remains. Although the storage period at 4°C could also contribute to inactivation, this is likely more favorable to survival than the usual lag from production to feeding of SDPP under swine industry conditions (approximately 30 days). As shown by Pujols and Segalés, (2014), PEDV can lose up to 2 log cycles when stored at 4°C for 7 days.

Simulation of the final concentration of PEDV after spray-drying and during storage

A probabilistic model was used to estimate the final concentration of PEDV after spray-drying and subsequent warm storage before releasing the product. Two different scenarios were used to predict the survival of PEDV: 1) Experimental thermal inactivation data for PEDV in damp plasma at 60-80°C and RH levels of 30, 50 and 70% and the corresponding D values (Verma and Goyal 2014;) (Appendix B and C); 2) Experimental data from Pujols and Segalés (2014) and Gerber et al., (2014) where the virus was inactivated at around 4.2 log after spray-drying followed by post-heating treatment to 70 or 80°C during 30 to 60s or spray-drying and further storage for 7 days at 4°C, respectively.

For both scenarios, the concentration of PEDV in positive batches of liquid plasma (copies of RNA PEDV/mL) was estimated from Ct values provided by industry. The number of genomic copies in any medium should not be interpreted as equivalent to the number of intact or infective virions (Weidmann et al., 2011). Distinction needs to be made between genomic copies, complete virions, viable virions (i.e., capable in productive infection of the most permissive cell types in the host species) and how these measures may relate to experimental methods to assess infectivity such as plaque formation, $TCID_{50}$, and bioassay. In cell culture systems, the particle to genome ratio can be of the order of several logs (Weidmann et al., 2011), and has been estimated to be approximately 3 logs (1000 fold) for foot and mouth disease virus (Callahan et al., 2002). No data are available on the particle to genome ratio of PEDV therefore we performed a sensitivity analysis with a range of assumptions for the ratio of infective virus to genome copies (Table 12).

Table 12: Ct values and the corresponding copies of RNA/mL of PEDV in positive batches of raw plasma

Ct values	Copies of RNA/mL	Log RNA/mL	Possible amount of infective particles (Log)
25.9 (min.)	$6.5x10^7$ (max)	7.8	7.8 (100% of the virus is infective) 6.8 (10%, 90% is not viable) 5.8 (1%, 99% is not viable) 4.8 (0.1%, 99.9% is not viable) 3.8 (0.01%, 99.99% is not viable)
31.4 (most likely)	$1.8x10^6$(most likely)	6.3	6.3 (100% of the virus is infective) 5.3 (10%, 90% is not viable) 4.3 (1%, 99% is not viable) 3.3 (0.1%, 99.9% is not viable) 2.3 (0.01%, 99.99% is not viable)
34.2 (max.)	$3.0x10^5$(min.)	5.5	5.5 (100% of the virus is infective) 4.5 (10%, 90% is not viable) 3.5 (1%, 99% is not viable) 2.5 (0.1%, 99.9% is not viable) 1.5 (0.01%, 99.99% is not viable)

For scenario 1, a simulation model was developed to predict the total inactivation achieved based on the thermal profiles expected during spray-drying and the thermal inactivation indicated experimentally (Appendix G). For that, a conservative estimate of thermal profile during spray drying was developed as follows: 1) Adiabatic heating around 3 seconds where the particle was at 60°C and 100% RH. To predict the inactivation in this step, the estimated D value of PEDV at 60°C and 70% RH in plasma was used; 2) Dry heating for the rest of the retention time up to 90 s. To predict the inactivation, the estimated D value at 80°C and 70% RH was used.

The effect of storage on PEDV concentration was modeled using the data from Pujols and Segalés (2014) and Goyal and Verma (2013). Pujols and Segalés (2014) reported inactivation of 2.8 log cycles after storing the spray-dried bovine plasma for 7 days at 22°C. The spray-dried bovine plasma was spiked with the European prototype strain of PEDV at an initial concentration of $10^{2.8}$ $TCID_{50}$/g (this concentration was limited by the maximum concentration in the stock solution). Table 13 shows the values obtained

by Goyal and Verma (2013) who spiked a sample of complete feed (5 g) with 1mL of PBS containing the virus and stored the sample at 22°C for 5 weeks. Each week, they exposed 10-day old piglets to stored materials and observe clinical symptoms and determine Ct values from intestinal samples. Assuming different levels of infectivity, the table shows that PEDV is very sensitive to storage conditions at room temperature when placed in complete feed. These data are consistent with other published studies (Appendix H) demonstrating that bacterial and viral agents can be significantly inactivated during storage, especially at elevated temperatures.

Table 13: Ct values and the corresponding copies of RNA/mL of PEDV in complete feed samples stored at 22°C

Ct values	Copies of RNA/g	Log RNA/g	Infective in pigs (clinical symptoms)	Possible amount of infective particles (Log)
Day 0: 16.0	4.0×10^{10}	10.6		10.6 (100% of the virus is infective) 9.6 (10%, 90% is not viable) 8.6 (1%, 99% is not viable) 7.6 (0.1%, 99.9% is not viable) 6.6 (0.01%, 99.99% is not viable)
One week: 30.39	3.5×10^{6}	6.5	Yes (diarrhea) Yes (positive in intestine, Ct=16.5)	6.5 (100% of the virus is infective) 5.5 (10%, 90% is not viable) 4.5 (1%, 99% is not viable) 3.5 (0.1%, 99.9% is not viable) 2.5 (0.01%, 99.99% is not viable)
Two weeks: 33.41	5.0×10^{5}	5.7	No symptoms No positive intestine samples	5.7 (100% of the virus is infective) 4.7 (10%, 90% is not viable) 3.7 (1%, 99% is not viable) 2.7 (0.1%, 99.9% is not viable) 1.7 (0.01%, 99.99% is not viable)
Three weeks: 33.63	4.3×10^{5}	5.6	No symptoms No positive intestine samples	5.6 (100% of the virus is infective) 4.6 (10%, 90% is not viable) 3.6 (1%, 99% is not viable) 2.6 (0.1%, 99.9% is not viable) 1.6 (0.01%, 99.99% is not viable)

The remaining input data for the simulation model were obtained from industry, scientific literature and experimental studies as shown in Table 14.

Table 14: Input data of the probabilistic model for PEDV during plasma processing

Variable	Value	Distribution function	Source
Percentage of plasma lots contaminated with PEDV	20-70%	=binomial(1,beta(289+1,486-289+1))	Industry
Concentration of PEDV in raw plasma	Ct values: 25.9-34.2 5.5-7.8 log RNA PEDV/mL (100% infective) 4.5-6.8 log RNA PEDV/mL (10% infective) 3.5-5.8 log RNA PEDV/mL (1% infective)	=IF(prevalence=1,pert(5.5,6.3,8.0),0) =IF(prevalence=1,pert(4.5,5.3,6.8),0) =IF(prevalence=1,pert(3.5,4.3,5.8),0)	Industry
Process parameters in the dryer	Tinlet air: 304-329°C Toutlet air: 80°C 20-90 s	=uniform (20,90)	Industry
Thermal inactivation or Spray Drying	SCENARIO 1: $D_{60°C}$ RH70%=12.79 min $D_{80°C}$ RH70%=9.60 min 0.07-0.26 log inact. SCENARIO 2: Lab-dryer: 4.2 log inact.	=uniform(0.07-0.26) Point estimate	Verma and Goyal 2014 Gerber et al., 2014 Pujols and Segalés, 2014
Effect of storage temperature on PEDV concentration	20°C for 2 weeks 3-5 log inactivation	=uniform (3,5)	Pujols and Segalés, 2014 Goyal and Verma, 2013
Effect of irradiation on PEDV concentration (optional scenario)	Two passes of 15 KGy D values 4.4-6.4 (kGy) for porcine enteric virus 4.6-6.8 log inactivation	=uniform (4.6-6.8)	betaGRO, 2014 Preuss et al., 1997

The assumptions made in the model were:

- Raw materials of porcine origin (blood, carcasses, intestines) may be contaminated with PEDV at various concentrations
- The cutoff to distinguish positive and negative samples of PEDV by PCR is set at values of 40 Ct (Ct<40 are considered positive, and Ct>40 are negative, non-detectable).
- The formula used to transform the Ct values into estimated copies of PEDV RNA/mL was: $y = 9E * 12e^{-0.648x}$ (Alonso et al., 2014) for copies of RNA/reaction (5 μL) and then multiplied by 140 to estimate copies of RNA/mL.
- Cross-contamination risks at the transportation, feed mill and distribution steps were not considered
- All the plasma producers will have implemented a warm storage (20-22°C) period of spray-dried product for 2 weeks
- Centrifugation and solid concentration steps will not affect the virus concentration
- Culturable virus isolate will have the same characteristics as the wild strain
- 100% of virus is viable before the spray-drying process and virus surviving the spray-drying process is infectious

A sensitivity analysis was done to account for the variability in the viability of the virus. The scenarios of virus viability modeled ranged from 1) ‘Worst-case’ scenario assuming 100% of the genomic copies are viable virions; 5) 0.01% of genomic copies are viable virions (Table 15). Figures 8 and 9 represent the final virus concentration after spray-drying and storage in the ‘worst-case’ scenario of having 100% of the viral RNA viable. Figures 10 and 11 represent the final virus concentration after spray-drying and storage where only 10% of the RNA is viable.

Table 15: Outputs from the probabilistic model and sensitivity analysis (SCENARIO 1)

Scenario 1	**PEDV concentration after spray-drying (copies RNA PEDV/g)**	**PEDV concentration after storage (copies RNA PEDV/g)**
100% viable virus	60% of positive batches ($4.4x10^5$-$6.2x10^7$)*	1.1% of positive batches ($7.0x10^3$-$4.1x10^4$)*
10% viable virus	60% of positive batches ($4.4x10^4$-$4.2x10^6$)*	0% of positive batches (non-detectable by PCR)**
1% viable virus	50% of positive batches ($7.9x10^3$-$3.8x10^5$)*	0% of positive batches (non-detectable by PCR)**
0.01% viable virus	0% of positive batches (non-detectable by PCR)**	0% of positive batches (non-detectable by PCR)**
0.1% viable virus	5.5% of positive batches ($7.5x10^3$-$3.4x10^5$)*	0% of positive batches (non-detectable by PCR)**

Irradiation (100% viable virus)	0% of positive batches (non-detectable by PCR)***	0% of positive batches (non-detectable by PCR)***

**: Minimum and maximum concentration within the positive samples.*

***: Non-detectable by PCR means Ct values > 40 (50 copies of RNA/reaction or 3.8 log copies RNA PEDV/mL)*

****: Concentration after spray-drying and irradiation*

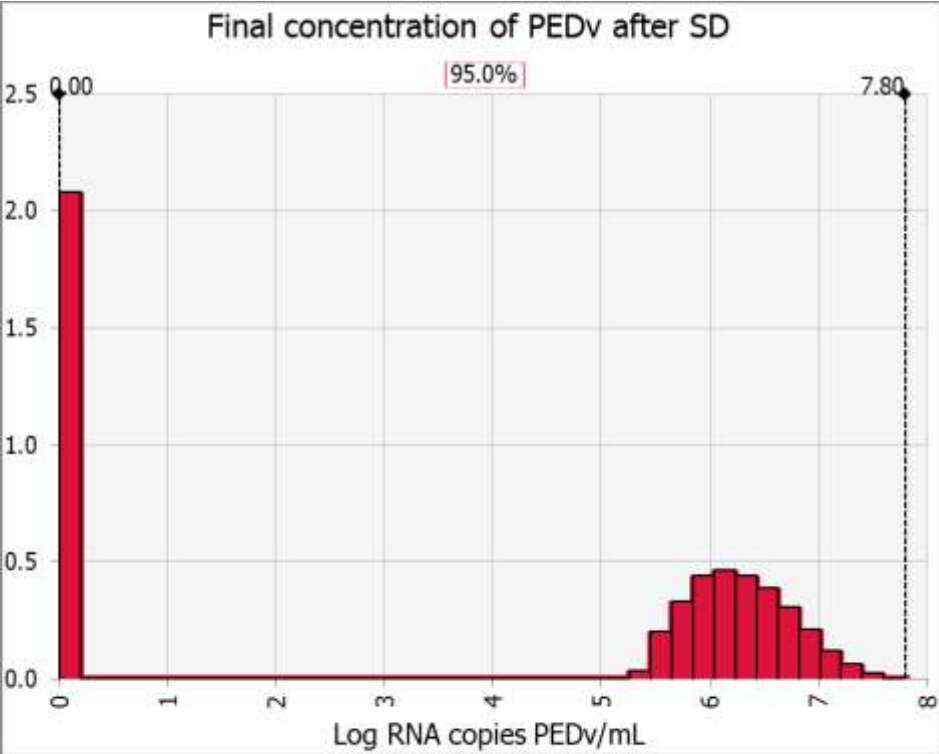


Figure 8: PEDV concentration after spray-drying (Scenario 1, 100% infective).

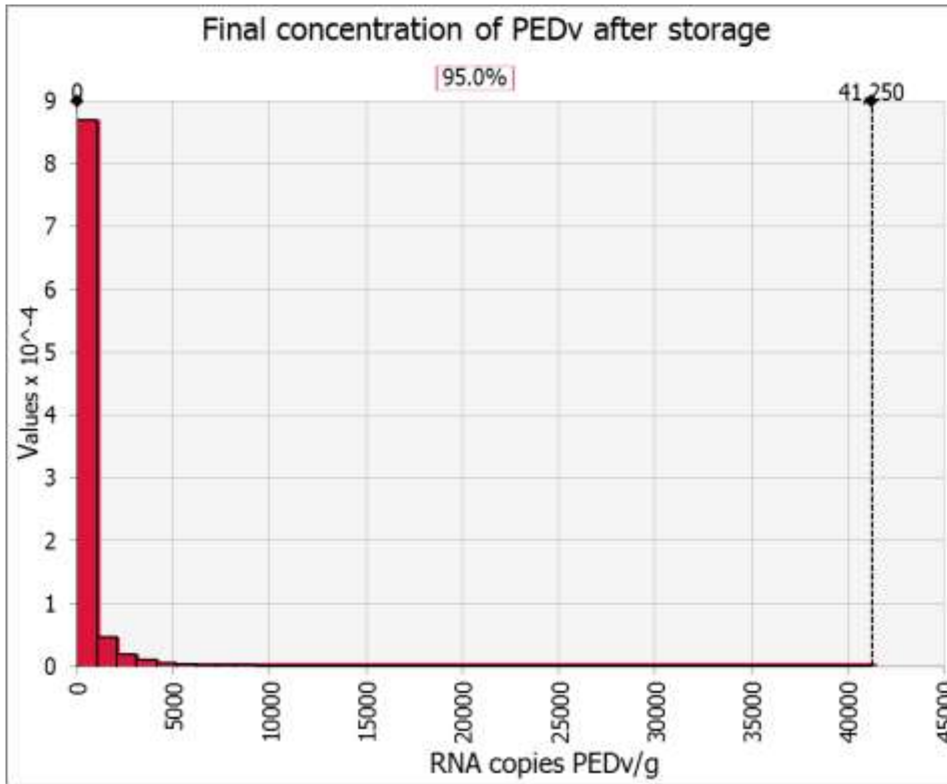


Figure 9: PEDV concentration after storage (Scenario 1, 100% infective).

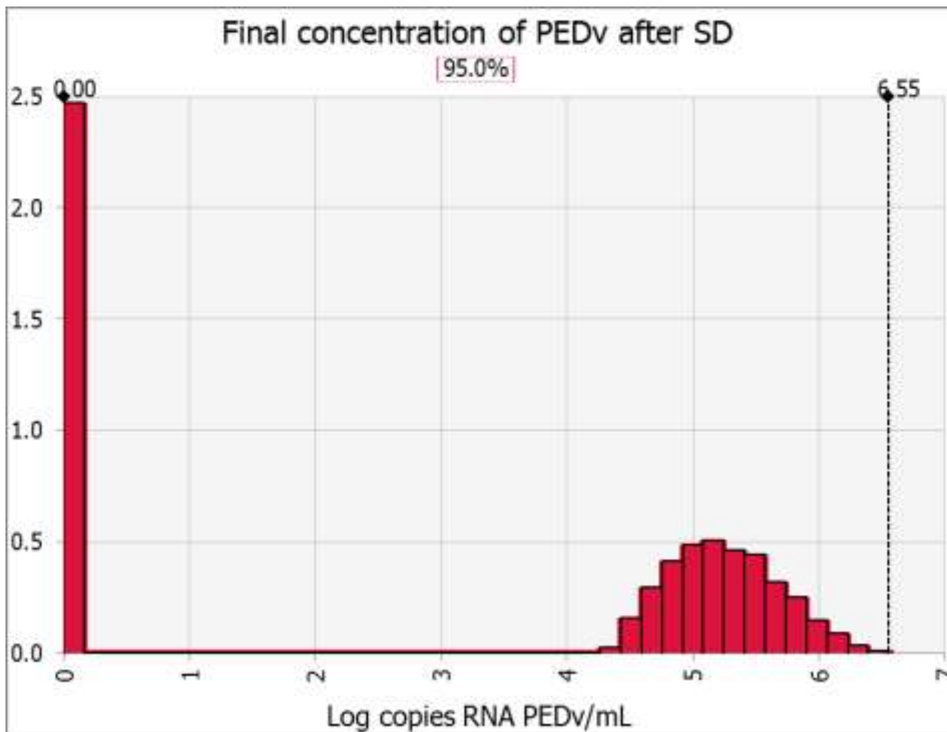


Figure 10: PEDV concentration after spray-drying (Scenario 1, 10% infective)

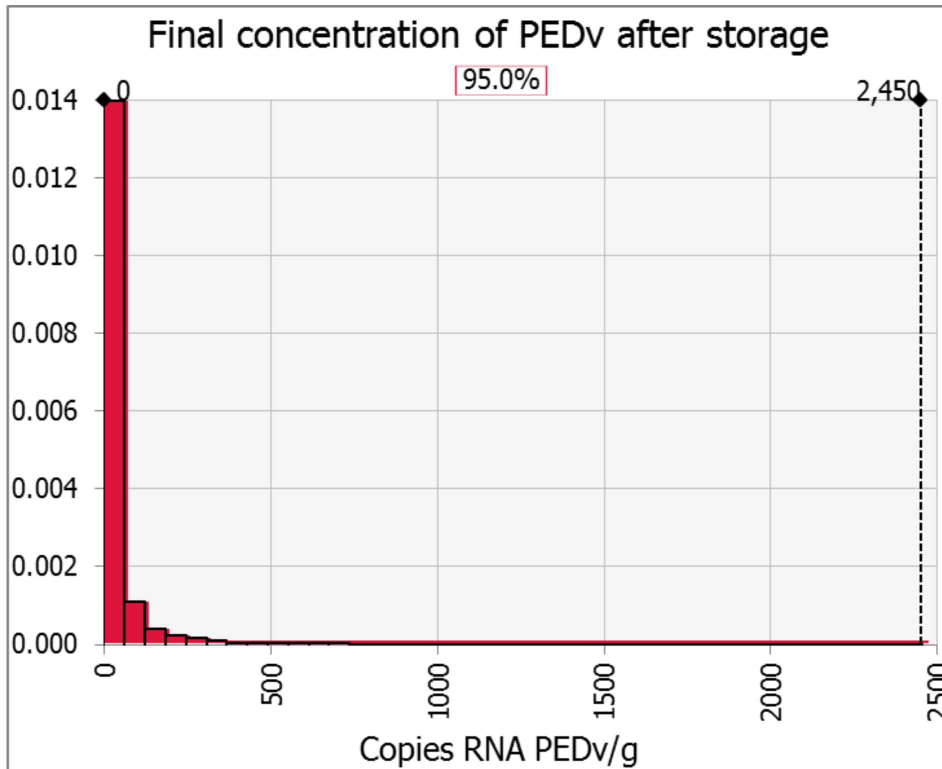


Figure 11: PEDV concentration after storage (Scenario 1, 10% infective)

Based on experimental data of thermal inactivation alone, the model predicted that residual viable virus could occur provided at least 0.1% of genomic copies in raw material represented viable virus. Note that 0.1% represents a ratio of 1 in 1000, which corresponds with the ratio reported for FMD virus in cell culture (Callahan et al., 2002), but comparable estimates for PEDV are not available. After storage, the model predicted no viable virus would remain other than in the 'worst-case' scenario where all of the genomic material represented viable virions, which is considered unlikely.

For scenario 2 (data from laboratory scale spray-drying), the model predicted that no viable virus would remain following spray-drying (Table 16).

Table 16: Outputs from the probabilistic model and sensitivity analysis (SCENARIO 2)

Scenario 2	**PEDV concentration after spray-drying (copies RNA PEDV/g)**	**PEDV concentration after storage (copies RNA PEDV/g)**
100% viable virus	0% of positive batches (Non-detectable by PCR)*	0% of positive batches (Non-detectable by PCR)*
50% viable virus	0% of positive batches (Non-detectable by PCR)*	0% of positive batches (Non-detectable by PCR)*

10% viable virus	0% of positive batches (Non-detectable by PCR)*	0% of positive batches (Non-detectable by PCR)*
Irradiation (100% viable virus)	0% of positive batches (Non-detectable by PCR)**	0% of positive batches (Non-detectable by PCR)**

**: Non-detectable by PCR means that values lower than Ct=40 (50 copies of RNA/reaction or 3.8 log copies RNA PEDV/mL) will be considered non-detectable.*

***: Concentration after spray-drying and irradiation*

Likelihood of PEDV survival through the hydrolysis process in ingredients of porcine origin

Summary

The series of processes used to obtain hydrolyzed proteins employ different temperature-time combinations. For most of the steps, the D values obtained for PEDV in bone meal, blood meal and meat and bone meal at 70% RH were used to estimate virus inactivation before drying as an approximation to the raw material used for the process. For the drying process, the D values obtained in complete feed at high temperatures at RH 30% were used to predict the virus inactivation. The total log reduction that would likely occur at the end of this process will exceed in 1.6-26x times the reduction required to inactivate the virus that may be found initially in the raw materials (intestines). *Likelihood estimation: The likelihood that PEDV would survive the protein hydrolyzing process is negligible.*

Time-temperature combination for processing steps

Table 17 indicates the inactivation (log scale) of PEDV after the different processing steps. The log inactivation ranged from 15.2-50.0 after all processing steps. The UMN-VDL has reported Ct values 13 to 39 for PEDV contamination of intestines (Table 1). This corresponds to 1.9 to 9.0 log RNA copies/mL. Assuming 100% of the virus is viable as the 'worst-case' scenario, the combinations of temperature and time for the production of hydrolyzed proteins will greatly exceed the requirements to inactivate PEDV in the raw materials used by the industry.

Table 17: Inactivation of PEDV during the hydrolyzed porcine protein product process

Step	**Time (min)-Temp (ºC)**	**D value (min)***	**Log inactivation**
Protease Enzymatic Digestion	180 min/60-65.5ºC	10.99	~ 16 log
Fat separation	60-120 min/62-65.5ºC	10.99	5.4 log-10.9 log
Heparin adhered to resin beads	30 min/79-82º C (~80ºC)	8.93	3.36 log
Bead/Peptone separation (Screening)	N/A*	N/A	N/A
Peptone storage	30 min/≥73.5º C (~70ºC)	9.45	3.17
Transport	N/A*	N/A	N/A
Storage vat	N/A*	N/A	N/A
Evaporator	20 min/76.5-93º C (~70-90ºC)	6.87	2.91 log
Storage vat	60 min/70ºC	9.45	6.35 log
Drying**	A:1 min/177ºC (~145ºC) B: 20-25 min/≥115ºC (~120ºC)	A: 2.71 B: 7.94	A: 2.71 B: 3.55-6.80
Cooling	N/A**	N/A	N/A
Grinding	N/A**	N/A	N/A
Bagging	N/A**	N/A	N/A

*: These values may change depending on the moisture content of the sample and the matrix type. Values shown in this table correspond to a dry sample.

**: N/A: Not Applicable

***: For this step, complete feed was used, given that this matrix (complete feed) was the one tested at highest temperatures, which approximate more to the temperatures from this process.

Likelihood for PEDV cross-contamination of ingredients of swine-origin

Introduction

The likelihood statements presented in this section are based upon information gathered during visits performed by the UMN risk assessment group to processing facilities for rendered, spray-dried and hydrolyzed porcine protein products, and provided by industry partners, review of Good Manufacturing Practices (GMP) plans, expert opinion, and scientific literature. These activities fostered a better understanding of the potential risk pathways for PEDV cross-contamination via sources and/or fomites. A source is defined as a reservoir (e.g.; contaminated transport equipment) or an infected host that may shed the organism and a fomite is an inanimate object (e.g.; footwear) that can facilitate the indirect contamination of the final product.

Industry stakeholder groups recognize that production sites and facilities should include design considerations that will prevent the cross-contamination of final products with micro-organisms from direct and indirect source and vector contamination. As well, these industry groups have created recommended GMPs that promote the biosecure production of their products. As noted in the assumptions section of this risk assessment, the implementation of commonly accepted GMPs is assumed when assigning likelihood statements. Several of the commonly observed GMP principles and recommendation include the following:

- Segregation and dedication of equipment and facilities for delivery, reception, and processing raw materials
- Segregation and dedication of equipment and facilities for storage, shipping, and delivery of the processed product
- Closed loop systems for the handling of raw materials and processed product
- Air intake and exhausted air systems that prevent aerosol contamination of processed products
- Equipment, vehicle, and facility cleaning and disinfection protocols
- Production management systems (i.e. batch production) that facilitate adequate cleaning and disinfection (both internal and external) of equipment, vehicles, and facilities
- Defined traffic patterns for personnel, equipment, and vehicles
- Defined Personal Protective Equipment (PPE) usage
- Defined Clothing and footwear management protocols

Finally, the potential for cross contamination with the PEDV is dependent upon the presence of the organism in raw materials and upon the equipment and facilities (i.e.; fomites) involved in the management of those raw materials and the processed product. Likelihood statements assume that the PEDV is present and, therefore, cross contamination is possible.

Likelihood of PEDV cross-contamination after processing of rendered ingredients

Summary

This risk assessment evaluated the likelihood for the cross-contamination of rendered products at rendering facilities between processing and the point of distribution. As described in the background section, rendering facilities may distribute unblended fat or protein products to external blending facilities or complete the blending into a final product within the rendering facility itself. The goal of this risk assessment was to identify and evaluate the potential pathways for PEDV contamination of unblended or final blended products between processing and the point of distribution from the rendering facility. Cross-contamination risks beyond the rendering facility were not considered.
Likelihood estimation: The likelihood that rendered products would be contaminated with PEDV after processing will be low to moderate for protein-based products processed in open handling systems, negligible to low for protein-based products processed in closed handling systems, and negligible for fat-based liquid products.

PEDV cross-contamination pathways after processing

In the rendering industry, the potential for the cross contamination after the rendering process primarily exists as a result of: 1) Contamination of the equipment and facilities used to deliver, receive, and process the raw materials; 2) Contamination of the equipment and facilities used to blend, store, and deliver the processed product; and 3) Effectiveness of the implementation of GMPs targeting the prevention of cross contamination through the proper management of these equipment and facilities.

The rendering industry's recommended biosecurity GMPs are primarily focused upon ensuring the segregation of the equipment used for raw materials and the equipment used for rendered products. As well, GMPs may also include steps that minimize the transfer of organisms from raw material management areas to areas where rendered products are managed by outlining protocols that address personnel movement restrictions, footwear disinfection practices, and equipment and facility cleaning and disinfection programs. Due to the concern of PEDV transmission during movement of raw materials, major renderers in Canada and the U.S. have implemented enhanced biosecurity procedures in dead stock and offal collections from swine operations. Some of these procedures are summarized in the National Pork Board website under PEDV resources under the section "Biosecure Mortalities Removal for PED Control"(National Pork Board, 2014).

For the management of protein based products such as meat and bone meal, two main facility types were identified: 1) 'open handling systems' where the unblended 'crax' product is stored in open hoppers and transferred to the blending equipment or transport vehicles using loaders; and 2) 'closed handling systems' where the unblended 'crax' product is transferred within a closed loop system from the cooker to blending equipment or transport vehicles. For fat based products such as choice white grease, the handling, storage, and distribution system is always a closed loop liquid handling system.

An observed example of the risks associated with 'open handling systems' was the use of area specific vehicles, such as loaders, used for the handling of 'crax'. These types of vehicles may cross-contaminate when they exit their dedicated processed product handling area for fueling and servicing purposes and then return to handle processed product. During these types of activities, the likelihood of tire and undercarriage contamination increase if not managed properly. This risk is elevated during epidemic conditions when PEDV may be at higher concentrations in the environment and when conditions (e.g.; ambient temperature) enhance virus survivability.

The risk of cross-contamination for integrated renderers is likely to be greater since, in most cases, the associated animal harvest facility will include live animal handling facilities. This facility arrangement increases the risk of cross contamination for rendered products as a result of the potential for an ongoing source of recently shed microorganisms (e.g.; PEDV). Proximity and volume of any source is normally expected to increase the opportunity for viable organisms to be cross-contaminated in an infectious form. In addition to an increased risk of cross-contamination due to equipment and personnel carriage, aerosol transmission would likely become a more feasible mode of cross-contamination for PEDV within integrated rendering facilities where the aerosolization of the PEDV from the live animal handling areas is more likely. A recently published study, demonstrated the presence of infectious PEDV in aerosol particles (Alonso et al, 2014). As noted by Alonso et al., (2014) the survivability of PEDV is influenced by temperature and ultraviolet light. Currently, the assessment of the practical importance of these potential routes of contamination is limited by the scarcity of research data.

Likelihood of PEDV cross-contamination after processing of spray dried ingredients

Summary

This risk assessment evaluated the likelihood for the cross-contamination of spray-dried porcine plasma products at spray-drying production facilities between processing and the point of distribution. The goal was to identify and evaluate the potential pathways for PEDV to contaminate the final product during this phase of production. *Likelihood estimation: The likelihood that spray-dried ingredients would be cross-contaminated with PEDV after processing is negligible to low.*

PEDV cross-contamination pathways after processing

In general, the production systems used to produce spray-dried porcine plasma are closed loop in nature and impressively hygienic. By definition, closed loop systems lack open access points that might allow cross-contamination to occur. Internal sanitation (i.e.; CIP – Clean in Place) of the dedicated raw material transport vehicles and production system is completed between individual batches of production (Personal Communication – APC 2014). During the UMN risk assessment visit to spray-drying facilities, the observed production stages (i.e.; raw material receiving and storage, processing, packaging, and the handling and storage of the final product), were physically separated into distinct

production areas within the facility. As well, biosecurity GMPs were in place that restricted personnel movement flows and required any personnel moving through the facility to utilize approved PPE (Personal Protective Equipment) (e.g.; footwear coverings, smocks, etc.) to prevent cross-contamination within the facility. These facility design and management characteristics greatly reduce the potential for the cross-contamination of finished product by indirect fomite transfer.

The only potential point of cross contamination observed for the final spray-dried product was at the stage of packaging. Since PEDV can be transmitted via the movement of aerosolized particles in an infectious form (Alonso et al., 2014), there exists the theoretical potential for cross-contamination at this point. Within this closed loop system, the only identified source of aerosolized PEDV would be exhausted air from the spray-dryer. However, typically plants use valve bags or totes that fill directly from the packaging machine, limiting contamination risk. Further assessment of the risk associated with this pathway would require research related to the potential for infectious PEDV survival in air exhausted from spray-dryers and the likelihood that PEDV contaminated exhaust air could significantly contaminate final product at packaging. This does not appear to be warranted.

Likelihood of PEDV cross-contamination after processing of hydrolyzed porcine protein products

Summary

This risk assessment evaluated the likelihood for the cross-contamination of hydrolyzed porcine protein products at production facilities between processing and the point of distribution. The goal was to identify and evaluate the potential pathways for PEDV to contaminate the final product during this phase of production. Two main production systems were identified and a separate analysis was done for each one. *Likelihood estimation: The likelihood that hydrolyzed porcine protein products would be contaminated with PEDV after processing is negligible for both system A and system B.*

PEDV cross-contamination pathways after processing

Two distinct processing systems were evaluated for this risk assessment. For both systems, the initial processes were conducted at heparin production facilities. During the heparin extraction, the possibility of cross-contamination from external sources is minimal due to the closed loop system used for product flow. After heparin extraction, liquid peptone product is transferred to dedicated transport trucks minimizing the risk for cross-contamination from external sources. At this point, the two systems deviate in their processing steps and will be considered separately.

System A keeps the liquid peptone product in an entirely closed system until the drying stage. During drying, there is a single open access point to the product as it flows through the system. The access point is situated greater than 3 feet (~ 1 meter) above the floor level and functions for the visualization of the product during the drying process. The potential for cross-contamination at this particular step

could occur by the hands and clothing of the personnel or aerosol contamination. After this step, the product is again returned to a closed loop system until it reaches the mechanical bagging system. The bagging occurs under a hood that prevents direct employee access. Triple layered paper bags with a plastic moisture barrier are used. The bags are mechanically stacked on a pallet and shrink wrapped to prevent external contamination and exposure to moisture. The product is inventoried in an indoor warehouse until shipping.

In system B, the liquid product is processed through a closed loop system until just before the drying stage when it is mixed with a carrier agent. The carrier agent is brought into the production line in one of two ways. The first method utilizes hopper bottom trailers. The carrier agent is off loaded onto the ground and then vacuumed into a hopper. From the hopper, it is fed into the system through a semi-closed set of augers. The potential for cross-contamination during this step is possible due to direct exposure to external ground surfaces. For the second method, the carrier agent is delivered in bulk tote bags which are handled using a forklift. From the tote bags, the carrier agent is then drop fed into an elevated hopper from where the carrier is augured into the main production system.

After the liquid peptone is mixed with the carrier, the mixture is moved to the dryer. After the drying step, the product is kept in a closed system until bagging and storage. This closed system moves the product to a warehouse where bagging and distribution happens. In system B, the majority of the final product is inventoried in hoppers and shipped via independent haulers. Any final product that is bagged is placed into triple layered paper bags with a plastic moisture barrier. Plant employees manually hold the bag during filling and heat sealing. The bags are palletized, shrink wrapped, and stored in an indoor warehouse.

Editor's Note: The GMP for system B stated that, if the dryer malfunctioned, any improperly dried product would be discarded and stored in an open pit within the facility where the final product is bagged and stored before distribution. Due to the concern of aerosol transmission from the discarded product, the GMP has been recently updated and indicates that any discarded material should be stored in a separate facility to decrease the potential for cross contamination.

Discussion

The potential for exposure to physical, chemical or biological hazards via ingestion is self-evident and most developed countries regulate animal feeding due to concerns about introduction of foreign animal pathogens via feed. In the USA, feeding of 'garbage' (defined as waste derived from meat or other animal materials) to pigs is prohibited under the Swine Health Protection Act (1980) except under license and after heating the materials to 212°F for 30 minutes. However, processed products of animal origin are legal and valuable components of swine diets, notably rendered products, SDPP, peptones, and pet food byproducts. Furthermore, reports implicating feed or feed ingredients in transmission of endemic swine viruses are rare (Kim et al., 2008), thus the question of PEDV transmission in feed has little scientific precedent. For producers, feed is over 50% of the cost of production, and nutritional programs constrained by ill-defined disease risks may negatively affect cost of production and environmental impact.

Unless pathogen free sources of pigs are identified, the presence of endemic pathogens in raw materials of porcine origin is inevitable, and substantial research has been done to establish the safety of rendered and spray-dried products destined for animal feed (Franco, 2006; Polo et al., 2005; Pujols et al., 2007, 2008; Gerber et al 2014; Pujols and Segalés, 2014). The paucity of published reports linking such products to disease outbreaks in the USA indicates that existing procedures for mitigating risk have delivered an acceptable level of protection over an extended period. However, relatively well documented investigations of field outbreaks in North America suggest that both ingredient contamination (Pasick et al., 2014; Aubry et al., 2015) and cross-contamination (Dee et al., 2014) with PEDV may have contributed to the transmission of the virus in North America. However, these case studies, which strongly indicate the possibility of feedborne transmission of PEDV, give little insight into the probability of such events. A retrospective case-control study of 2,117 premises receiving feed in Indiana, Illinois, Iowa, and Ohio did not find evidence of an association between feeding of products of animal origin and occurrence of PEDV (Neumann et al., 2014). Furthermore, industry data indicate that large amounts (corresponding to the consumption by 3.4 to 4 million pigs) of PCR positive SDPP (mean Ct = 31.1) were exported and fed to pigs in western Canada and Brazil without PEDV outbreaks occurring (Crenshaw et al., 2014), although the lag between production and consumption may have been extended (particularly for Brazil). Both these sources of information point to ingredients of porcine origin having a minor or no role in PEDV transmission respectively in those particular settings. On the other hand, the routes of introduction of PEDV into several countries of the Western Hemisphere over a relatively brief period of time remains unexplained and warrant further investigation.

As anticipated, availability of relevant data remains an important constraint to this project, and the data used in the assessment were derived from a small number of unreplicated experimental studies. Estimated D-values based on recent experimental studies at the University of Minnesota were used to assess the impact on PEDV survival of thermal inactivation as employed in the respective processes (rendering, spray-drying, hydrolysates). The D-values (time to reduce numbers of pathogen by 90% or 1 log) indicated relatively high thermal resistance of PEDV. It is important to note that D-values are

temperature and matrix specific. Although no data were available on raw material contamination entering the rendering and hydrolysate processes, the large log reductions predicted for thermal inactivation with the time-temperature combinations employed in these processes indicated negligible risk of PEDV survival even at high levels of contamination of raw materials. In contrast, owing to the much shorter time-temperature profiles employed in spray-drying, assessment of thermal inactivation alone (scenario 1) led to predictions of potential survival of PEDV in some circumstances (proportion of viral RNA in infective virions greater than 0.1%). However, it is recognized that observations on thermal resistance alone do not accurately predict survival during spray-drying (Licari and Potter, 1970; Ghandi et al., 2012). Therefore, important caveats regarding the simulations performed in Scenario 1 relate to: 1) uncertainty surrounding the accuracy of the D-values used due to scarcity of data; 2) that the simulation parameters employed do not adequately reflect the thermal profile of particles during spray-drying, and 3) that non-thermal mechanisms of inactivation may be important in spray-drying (Ghandi et al., 2012). More extensive evaluation of the D-values of PEDV in appropriate matrices would be valuable to obtain more precise data at relevant temperatures. For comparison, studies of thermal inactivation by pasteurization of avian viruses in egg products have been based on comprehensive assessment of D-values across a narrow range of relevant temperatures and in the specific matrix of interest (Chmielewski et al., 2013). Similar refinement in understanding the thermal inactivation kinetics of PEDV would enable more definitive assessment of risk in all ingredients of porcine origin, and particularly in SDPP.

Unlike the assessments of the rendering and hydrolysate processes, where no data other than the experimental D-values were available, during the course of the project two studies which investigated the inactivation of PEDV using laboratory-scale spray-dryers were published. These data (observed log reduction in PEDV) were used for the simulations in scenario 2 (Gerber et al., 2014; Pujols and Segalés, 2014) which predicted negligible survival of PEDV during the spray-drying process across the range of assumptions regarding virus viability. Together with the absence of PEDV in both Brazil and Western Canada, despite feeding large numbers of pigs with SDPP that was PCR positive for PEDV (Crenshaw et al., 2014) these studies suggest that the likelihood of PEDV surviving the spray-drying process and commercial storage periods is negligible.

Beyond the philosophical issues that zero risk may be unattainable, and it is not possible to ‘prove a negative’, inference of ‘zero risk’ from current data would require assumptions that the two experimental studies modeled in Scenario 2 are adequately representative of the universe of current commercial spray-drying operations for porcine plasma. Indeed, Gerber et al (2014) concluded that use of different spray-drying conditions or lapses in the consistency of the processes could potentially lead to incomplete viral inactivation. Similarly, an extensive review published recently by the European Food Safety Agency concluded that the influence of variations in spray-drying processes has not been validated sufficiently for PEDV (EFSA, 2014). Specific caveats regarding the simulations performed in Scenario 2 relate to 1) the data were obtained using laboratory-scale spray-dryers that are not equivalent to commercial scale-dryers; 2) both experimental studies were small in scale and have not been replicated; 3) the study end points to indicate virus inactivation (i.e., lack growth in cell culture or failure to infect 4 pigs with 0.9g of product) are not equivalent to an industry setting where thousands of

pigs are fed product. 4) The two cell-adapted variants used in the studies may not represent the universe of PEDV strains with respect to inactivation kinetics.

The complexity of 'scaling up' of spray-drying processes is well recognized and differences in the physical environment surrounding particles and their thermohydric profiles vary between laboratory, pilot, and commercial scales (Thybo et al., 2008; Ghandi et al, 2012). The outlet temperature (80°C) used in both experimental studies was typical for commercial scale spray-drying of porcine plasma. The retention time (time in the dryer) used by Gerber et al (2014) was apparently less than 1 second based on data in a cited reference (Patterson et al., 2010). Similarly, the retention (dwell) time in the laboratory-scale spray-dryer used by Pujols and Segalés (2014) was stated to be 0.41s, after which samples were held at 90°C for 30 to 60 seconds to mimic commercial conditions. Both studies observed reductions of approximately 4 logs based on different end points (i.e.; bioassay and cell culture), which were the maximum achievable based on the initial virus concentrations. These reductions were much greater than predicted based on the experimental D-values for thermal inactivation alone. In particular, the 4 log reduction observed by Gerber et al (2014) at an outlet temperature of 80°C suggests either that PEDV is substantially more labile than the coronaviruses evaluated by Nims and Plavsic (2013), or that non-thermal mechanisms of inactivation may be important in this system (Ghandi et al., 2012). Although it can be argued that inactivation may be greater in commercial compared with laboratory-scale dryers based on the greater residence time for thermal inactivation (Gerber et al., 2014; Pujols and Segalés, 2014), the processes of protein denaturation and pathogen inactivation during spray-drying are complex, multifactorial and incompletely understood (Gerber et al., 2012; Bernard et al., 2013). There are no data comparing inactivation kinetics of different PEDV strains, yet this possibility cannot be ignored. Substantial differences in inactivation of different strains of the same virus are well documented, including cell culture adapted versus clinical isolates (Farcet et al., 2012).

Another caveat for both of the scenarios that were modeled relates to the assumption of infectivity of virus that has been quantified as genomic copies using quantitative PCR. This is compounded by the fact that the oral infectious dose of PEDV is not well defined but appears to be low. In the study of Pujols and Segalés (2014) data on $TCID_{50}$ and Ct values indicated that a Ct value of approximately 29 corresponded with a $TCID_{50}$ of one (10^0). By comparison, the concentration of PEDV RNA in SDPP used to reproduce PEDV by bioassay in Canada was considered to be at the limit of detection of the assay, with a Ct of approximately 37 to 39 (Pasick et al., 2014). This would suggest that apparently complete inactivation assessed by cell culture methods is not equivalent to complete biological inactivation of the virus. By extension, if spray-drying leads to very low risk of virus survival in large scale production, such minimal risk will not be detectable with small scale bioassays but could result in infection if fed to large numbers of animals. Research to better define the infectious dose response curve for PEDV would provide more insight into the assessment of risks at low doses of exposure (Haas, 1983). Efforts to quantify the relationship between genomic copies and intact viral particles in natural infections would also give insight into the interpretation of Ct values with respect to virus concentration (but not infectivity).

Under commercial conditions, a lag of approximately one month or more will typically elapse between production and consumption of SDPP. Simulation of storage conditions based on both D-values and

inactivation experiments indicate that post-production storage provides conditions that will substantially inactivate PEDV. Extension of post processing storage periods by two weeks at room temperature was implemented in the North American spray-drying industry during 2014. A more conservative approach requiring 6 weeks of storage has been adopted by the European Union for imports of blood products of porcine origin (EFSA, 2014). The available data on virus inactivation at room temperature indicate that these measures to extend storage prior to feeding provide additional assurance that residual virus should be inactivated (Goyal, 2013, Pujols and Segalés, 2014; Thomas et al., 2014). However, it is noted that in the study of Pasick et al (2014), which reproduced disease, SDPP had been stored for at least an additional 4 weeks beyond the time it was fed to commercial pigs. Further research may be warranted to verify and refine the required time and temperature conditions for storage, and particularly in colder climates where product may be stored at relatively low temperatures.

Unlike the assessment of the respective processes, assessment of cross-contamination risk at industry facilities was entirely subjective as no relevant data are available. In all scenarios, potential avenues of post processing contamination were identified and discussed with industry partners. In some cases this led to revision of procedures. The issue of post-processing contamination is not new, particularly in the rendering industry with respect to *Salmonella spp.*, and substantial effort has been directed at this problem. The addition of PEDV as another potential feedborne hazard should provide some impetus to review and comply with existing best management practices in handling processed products. It is our view that post-processing cross contamination with PEDV at production facilities is unlikely to present a substantial risk for introduction of PEDV to farms relative to other biosecurity risk inherent to the industry. Again, this judgment is constrained by the absence of relevant field data.

In summary, the assessments made in this project are constrained by a paucity of specific data on several aspects that are germane to the risk of PEDV transmission in feed ingredients of porcine origin. Available data on thermal inactivation of PEDV indicate that risk of virus surviving the processes of rendering and hydrolysis (peptone production) are negligible. The time and temperature profiles used in spray-drying are much less severe, and therefore, the possibility of virus survival is inherently greater if non-thermal mechanisms are ignored. Currently available data indicate that probability of PEDV surviving the spray-drying process and current commercial storage periods is extremely small.

Research recommendations

An important outcome of risk assessment is identification of data gaps that introduce uncertainties and to direct research strategies to fulfill these data gaps in order to enable more robust risk estimation.

The following data gaps and research needs were identified:

- Need for more extensive and relevant data on the inactivation kinetics of PEDV at different combinations of temperature and moisture content in feed matrices.
- Quantitative data on PEDV occurrence in raw materials and during processing. Quantitative data related to PEDV prevalence and concentration throughout feed processing from raw materials

to storage. These data should be obtained for relevant feed ingredients such as rendered products, spray-dried plasma and hydrolyzed products.

- PEDV inactivation kinetics during spray-drying simulating industrial conditions. Data related to the survival of different strains of PEDV (culturable and wild) after spray-drying conditions simulating the industrial setting.
- Infective dose of PEDV. The minimal amount of PEDV able to produce infection in pigs at different growing stages via feed. Especially the susceptibility of finisher pigs to PEDV.
- The relationship between quantities of genomic copies estimated by PCR and virus particles in both *in vivo* and *in vitro* (cell culture) systems, and their relationship with measures of infectivity (e.g., TCID, bioassays)

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Appendices

Appendix A: Environmental resistance of PEDV (Verma and Goyal, 2014)

Type of sample	RH (%)	Temp (°C)	Clinical signs	Post-mortem (Intestine)
Fresh feces	30	40	0 Days-Yes (ct=24.13) 3 Days-Yes (ct=27.29) ≥ 7 Days –No	0 Days-ct=16.48 3 Days-ct=32 7 Days-No virus
	50	40	1 Day-Yes (ct=27.59) 3 Days-Yes (ct=28.70) ≥ 7 Days -No	1 Day-ct=16.35 3 Days-No virus 7 Days-No virus
	70	40	0 Days-Yes (ct=27.36) 3 Days-Yes (ct=32.74) 7 Days-Yes (ct=37.92) 14 Days-No	0 Days-ct=13.79 3 Days-ct=13.30 7 Days-ct=15.16 14 Days-No virus
	30	50	0 Days-Yes (ct=22.68) 1 Day-Yes (ct=26.99) ≥ 3 Days-No	0 Days-ct=15.65 1 Day-ct=15.75 ≥ 3 Days-No virus
	50	50	0 Days-Yes (ct=21.85) 3 Days-Yes (ct=31.63) 7 Days-Yes 14 Days-No	0 Days-ct=16.93 3 Days-ct=19.08 7 Days-No virus 14 Days-No virus
	70	50	0 Days-Yes (ct=20.24) 3 Days-No (ct=32.30) 7 Days-No (ct=33.79) 14 Days-No	0 Days-ct=16.12 3 Days-ct=14.99 7 Days-ct=35.33 14 Days-No virus
	30	60	1 Day-Yes (ct=23.58) 3 Days-No (ct=29.08) 7 Days-No	1 Day-ct=13.26 3 Days-ct=35.10 7 Days-ct=37.24

Appendix B: Experimental data on PEDV thermal inactivation in feed and feed ingredients at 30, 50 and 70% relative humidity

Extent of PEDV inactivation in complete feed (June 10,2014)

SOP used:

1. Prepare several 5 gm aliquots of feed in 25 mL beakers.
2. Maintain ovens at temperatures: 120°C, 130°C, 140°C and 145°C, and place the feed aliquots to reach at the certain temperatures.
3. After feed temperature attained, remove 5 gm aliquots of feed from the oven. Then spike with 1mL of PEDV and incubate the beakers for 0, 5, 10, 15, 20, 25, and 30 min.
4. Remove the aliquots from each temperature then elute the virus by adding 10 mL/aliquot of an eluent solution (3% beef extract-0.05M glycine, pH 7.2).
5. Transfer the contents from beakers to the scintillation vials and centrifuge at 2510 xg for 10 min then collect supernatants.
6. Prepare serial 10-fold dilutions of the elutes and inoculate in Vero-81 cells.
7. Incubate at 37^0C and examine daily for 8 days for CPE.
8. Calculate titers for all samples as $TCID_{50}$/mL by Karber method.

Effect of high heat on PED virus (5 minute intervals).

Temperatures	Incubation periods in min.	Virus titer $TCID_{50}$/mL
120°C	0	$6.8x10^3$
	5	$6.8x10^2$
	10	$3.2x10^2$
	15	$2.3x10^2$
	20	$1.1x10^2$
	25	≤ 1
	30	≤ 1
130°C	0	$6.8x10^3$
	5	$3.2x10^2$
	10	$1.5x10^2$
	15	≤1
	20	≤1
	25	≤ 1
	30	≤ 1
140°C	0	$6.8x10^3$
	5	$4.1x10^2$
	10	$0.7x10^2$
	15	≤1
	20	≤1
	25	≤ 1

	30	≤ 1
145°C	0	$5.0x10^3$
	5	$3.2x10^2$
	10	≤ 1
	15	≤1
	20	≤1
	25	≤ 1
	30	≤ 1

Extent of PEDV inactivation in common feed ingredients under 30% RH (June 30, 2014)
SOP used:

1. Weigh 5 gm each of the following in scintillation vials.
    - Complete feed (obtained from RAR)
    - Spray-dried porcine by-products: plasma
    - Meat meal
    - Meat and bone meal
    - Blood meal
    - Grow finish premix
    - Corn
    - Soybean meal
    - DDGS
2. Add 1 mL of PEDV (NVSL strain, p-19) to each tube, mix well.
3. Keep all the vials at four different temperatures 60°C, 70°C, 80°C and 90°C at 30%, 50% and 70% humidity levels for 0, 1, 5, 10, 15 and 30 min.
4. Add 10 mL of sterile, 3% beef extract-0.05 M Glycine solution at pH 7.2 to the tubes after various incubation periods
5. Vortex for a few seconds and then centrifuge at 1,200 xg for 10 min, collect the supernatants.
6. Titrate the added virus immediately in Vero-81 cells.
7. Incubate the cells at 37°C under 5% CO_2 for 8 days until the CPE appears.
8. Calculate titers for all samples as $TCID_{50}$/mL by Karber method.
9. Results are shown in tables below.

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Complete feed.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$3.2x10^4$	*NA
	5	$1.5x10^4$	53.1
	10	$6.8x10^2$	97.9
	15	$3.2x10^2$	99.0
	30	$1.5x10^2$	99.5
70°C	0	$6.8x10^4$	NA

	5	$3.2x10^3$	95.3
	10	$1.5x10^3$	97.8
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^3$	95.3
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
90°C	0	$1.5x10^4$	NA
	5	$3.2x10^3$	78.7
	10	$3.2x10^2$	97.9
	15	$1.5x10^2$	99.0
	30	<1	>99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Plasma.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	*NA
	5	$6.8x10^3$	90.0
	10	$6.8x10^2$	99.0
	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^3$	95.3
	10	$6.8x10^2$	99.0
	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
80°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$6.8x10^1$	99.8
90°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$1.5x10^1$	99.5

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Meat meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	NA*
	5	$3.2x10^3$	95.3
	10	$3.2x10^2$	99.5
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9
70°C	0	$6.8x10^4$	NA
	5	$1.5x10^3$	97.8
	10	$3.2x10^2$	99.5
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9
80°C	0	$6.8x10^4$	NA
	5	$6.8x10^3$	99.0
	10	$3.2x10^2$	99.5
	15	$6.8x10^1$	99.9
	30	$6.8x10^1$	99.9
90°C	0	$3.2x10^4$	NA
	5	$6.8x10^3$	78.8
	10	$6.8x10^1$	99.8
	15	$6.8x10^1$	99.8
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Meat and bone meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$3.2x10^4$	*NA
	5	$6.8x10^3$	78.8
	10	$1.5x10^2$	99.5
	15	$6.8x10^1$	99.8
	30	<1	>99.9
70°C	0	$6.8x10^4$	NA
	5	$1.5x10^3$	97.8
	10	$1.5x10^2$	99.8
	15	$6.8x10^1$	99.9
	30	<1	>99.9
80°C	0	$3.2x10^4$	NA
	5	$3.2x10^2$	99.0

	10	$3.2x10^2$	99.0
	15	$6.8x10^1$	99.8
	30	<1	>99.9
90°C	0	$1.5x10^4$	NA
	5	$3.2x10^2$	97.9
	10	$6.8x10^1$	99.5
	15	<1	>99.9
	30	<1	>99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Blood meal.

Temperatures	**Incubation period (in min)**	**Virus titer TCID$_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	NA*
	5	$6.8x10^3$	90.0
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
70°C	0	$3.2x10^4$	NA
	5	$1.5x10^4$	53.1
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$1.5x10^2$	99.5
80°C	0	$6.8x10^4$	NA
	5	$1.5x10^3$	97.8
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
90°C	0	$6.8x10^4$	NA
	5	$3.2x10^3$	95.3
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 30% RH on Grow finish premix.

Temperatures	**Incubation period (in min)**	**Virus titer TCID$_{50}$/mL**	**Percent virus reduction**
60°C	0	$3.2x10^4$	*NA
	5	$6.8x10^3$	78.8
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0

	30	$1.5x10^2$	99.5
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^3$	95.3
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
80°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	$6.8x10^1$	99.8
90°C	0	$3.2x10^4$	NA
	5	$3.2x10^2$	99.0
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	<1	>99.9

*NA: Not Applicable

Extent of PEDV inactivation in common feed ingredients under 50% RH (June 30, 2014)

SOP used:

1. Weigh 5 gm each of the following in scintillation vials.
    - Complete feed (obtained from RAR)
    - Spray-dried porcine by-products: plasma
    - Meat meal
    - Meat and bone meal
    - Blood meal
    - Grow finish premix
    - Corn
    - Soybean meal
    - DDGS
2. Add 1 mL of PEDV (NVSL strain, p-19) to each tube, mix well.
3. Keep all the vials at four different temperatures 60°C, 70°C, 80°C and 90°C at 30%, 50% and 70% humidity levels for 0, 1, 5, 10, 15 and 30 min.
4. Add 10 mL of sterile, 3% beef extract-0.05 M Glycine solution at pH 7.2 to the tubes after various incubation periods
5. Vortex for a few seconds and then centrifuge at 1,200 xg for 10 min, collect the supernatants.
6. Titrate the added virus immediately in Vero-81 cells.
7. Incubate the cells at 37°C under 5% CO_2 for 8 days until the CPE appears.
8. Calculate titers for all samples as $TCID_{50}$/mL by Karber method.
9. Results are shown in tables below.

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Complete feed.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$3.2x10^2$	99.5
70°C	0	$6.8x10^4$	NA
	5	$6.8x10^3$	90.0
	10	$6.8x10^2$	99.0
	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
90°C	0	$3.2x10^4$	NA
	5	$6.8x10^3$	78.8
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$6.8x10^1$	99.8

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Plasma.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^3$	95.3
	30	$6.8x10^2$	99.0
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$1.5x10^3$	97.6
	15	$3.2x10^2$	99.5
	30	$3.2x10^2$	99.5
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$1.5x10^3$	97.6

	15	$3.2x10^2$	99.5
	30	$6.8x10^1$	99.9
90°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	$6.8x10^1$	99.8

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Meat meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	*NA
	5	$6.8x10^3$	90.0
	10	$6.8x10^2$	99.0
	15	$3.2x10^2$	99.5
	30	$3.2x10^2$	99.5
70°C	0	$3.2x10^4$	NA
	5	$3.2x10^2$	99.0
	10	$3.2x10^2$	99.0
	15	$6.8x10^1$	99.8
	30	≤ 1	≥99.9
80°C	0	$1.5x10^4$	NA
	5	$3.2x10^3$	78.7
	10	$6.8x10^1$	99.5
	15	$6.8x10^1$	99.5
	30	≤ 1	≥99.9
90°C	0	$6.8x10^4$	NA
	5	$1.5x10^3$	95.3
	10	$6.8x10^1$	99.9
	15	$6.8x10^1$	99.9
	30	≤ 1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Meat and bone meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^3$	95.3
	30	$3.2x10^2$	99.5

70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$1.5x10^3$	97.8
	30	$6.8x10^2$	99.0
80°C	0	$6.8x10^4$	NA
	5	$1.5x10^4$	77.9
	10	$1.5x10^3$	97.8
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
90°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$1.5x10^2$	99.8
	30	$1.5x10^2$	99.8

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Blood meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$1.5x10^3$	97.8
	30	$6.8x10^2$	99.0
70°C	0	$3.2x10^4$	NA
	5	$1.5x10^4$	53.1
	10	$3.2x10^3$	90.0
	15	$3.2x10^3$	90.0
	30	$6.8x10^2$	97.9
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$1.5x10^3$	97.8
	30	$6.8x10^2$	99.0
90°C	0	$3.2x10^4$	NA
	5	$6.8x10^2$	97.9
	10	$6.8x10^1$	99.8
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 50% RH on Grow finish premix.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	NA*
	5	$3.2x10^4$	52.9
	10	$6.8x10^3$	90.0
	15	$3.2x10^3$	95.3
	30	$3.2x10^2$	99.5
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$1.5x10^3$	97.8
	15	$3.2x10^2$	99.5
	30	$3.2x10^2$	99.5
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$1.5x10^3$	97.8
	30	$3.2x10^2$	99.5
90°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8

*NA: Not Applicable

Extent of PEDV inactivation in common feed ingredients under 70% RH (June 30, 2014)

SOP used:

1. Weight 5 gm each of the following in scintillation vials.
    - Complete feed (obtained from RAR)
    - Spray-dried porcine by-products: plasma
    - Meat meal
    - Meat and bone meal
    - Blood meal
    - Grow finish premix
    - Corn
    - Soybean meal
    - DDGS
2. Add 1 mL of PEDV (NVSL strain, p-19) to each tube, mix well.
3. Keep all the vials at four different temperatures 60°C, 70°C, 80°C and 90°C at 30%, 50% and 70% humidity levels for 0, 1, 5, 10, 15 and 30 min.

4. Add 10 mL of sterile, 3% beef extract-0.05 M Glycine solution at pH 7.2 to the tubes after various incubation periods
5. Vortex for a few seconds and then centrifuge at 1,200 xg for 10 min, collect the supernatants.
6. Titrate the added virus immediately in Vero-81 cells.
7. Incubate the cells at 37°C under 5% CO_2 for 8 days until the CPE appears.
8. Calculate titers for all samples as $TCID_{50}$/mL by Karber method.
9. Results are shown in tables below.

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Complete feed.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$3.2x10^4$	*NA
	5	$3.2x10^4$	NA
	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$3.2x10^2$	99.0
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
80°C	0	$6.8x10^4$	NA
	5	$6.8x10^3$	90.0
	10	$3.2x10^2$	99.5
	15	$3.2x10^1$	99.95
	30	≤1	≥99.9
90°C	0	$6.8x10^4$	NA
	5	$3.2x10^3$	95.3
	10	≤1	≥99.9
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Plasma.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^3$	95.3
	30	$3.2x10^2$	99.5

70°C	0	$3.2x10^4$	NA
	5	$6.8x10^3$	78.8
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	$6.8x10^1$	99.8
80°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9
90°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^2$	99.5
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Meat meal.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$3.2x10^4$	*NA
	5	$3.2x10^4$	NA
	10	$3.2x10^3$	90.0
	15	$3.2x10^2$	99.0
	30	$3.2x10^2$	99.0
70°C	0	$6.8x10^4$	NA
	5	$6.8x10^3$	90.0
	10	$3.2x10^3$	95.3
	15	$1.5x10^2$	99.8
	30	$6.8x10^1$	99.9
80°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	$6.8x10^1$	99.8
90°C	0	$1.5x10^4$	NA
	5	$6.8 x10^3$	54.7
	10	≤1	≥99.9
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Meat and bone meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	*NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
70°C	0	$6.8x10^4$	NA
	5	$6.8x10^3$	90.0
	10	$6.8x10^2$	99.0
	15	$6.8x10^1$	99.9
	30	≤1	≥99.9
80°C	0	$1.5x10^4$	NA
	5	$3.2x10^3$	78.7
	10	$3.2x10^2$	97.9
	15	≤1	≥99.9
	30	≤1	≥99.9
90°C	0	$3.2x10^4$	NA
	5	$3.2x10^3$	90.0
	10	≤1	≥99.9
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Blood meal.

Temperatures	Incubation period (in min)	Virus titer $TCID_{50}$/mL	Percent virus reduction
60°C	0	$6.8x10^4$	*NA
	5	$6.8x10^3$	90.0
	10	$3.2x10^3$	95.3
	15	$6.8x10^2$	99.0
	30	$3.2x10^2$	99.5
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^2$	99.5
	30	$3.2x10^2$	99.5
80°C	0	$3.2x10^4$	NA
	5	$1.5x10^4$	53.1

	10	$3.2x10^2$	99.0
	15	$3.2x10^2$	99.0
	30	$6.8x10^1$	99.8
90°C	0	$6.8x10^4$	NA
	5	$1.5x10^3$	97.8
	10	≤1	≥99.9
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Effect of 60°C, 70°C, 80°C, 90°C and 70% RH on Grow finish premix.

Temperatures	**Incubation period (in min)**	**Virus titer $TCID_{50}$/mL**	**Percent virus reduction**
60°C	0	$3.2x10^4$	*NA
	5	$6.8x10^3$	78.8
	10	$3.2x10^3$	90.0
	15	$3.2x10^3$	90.0
	30	$3.2x10^2$	99.0
70°C	0	$6.8x10^4$	NA
	5	$3.2x10^4$	52.9
	10	$3.2x10^3$	95.3
	15	$3.2x10^2$	99.5
	30	$1.5x10^2$	99.8
80°C	0	$3.2x10^4$	NA
	5	$3.2x10^4$	NA
	10	$3.2x10^2$	99.0
	15	$1.5x10^2$	99.5
	30	$6.8x10^1$	99.8
90°C	0	$6.8x10^4$	NA
	5	$1.5x10^4$	77.9
	10	$3.2x10^2$	99.5
	15	≤1	≥99.9
	30	≤1	≥99.9

*NA: Not Applicable

Appendix C: D values of PEDV in feed and feed ingredients Trials were conducted adding 1 ml of virus culture to 5g of fed/feed ingredient

D values and correlation coefficient for PEDV in complete feed under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
120	-	7.94	0.79
130	-	4.26	0.88
140	-	4.11	0.95
145	-	2.71	0.92
60	30	12.79	0.71
70	30	10.97	0.75
80	30	11.52	0.68
90	30	7.20	0.99
60	50	12.79	0.44
70	50	10.47	0.80
80	50	10.97	0.57
90	50	11.52	0.74
60	70	13.55	0.42
70	70	10.97	0.57
80	70	6.22	0.93
90	70	2.07	0.87

D values and correlation coefficient for PEDV in plasma under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
60	30	10.47	0.80
70	30	10.97	0.77
80	30	12.12	0.73
90	30	9.60	0.89
60	50	15.35	0.82
70	50	12.12	0.63
80	50	9.60	0.84
90	50	12.12	0.69
60	70	12.79	0.90
70	70	11.52	0.70
80	70	9.60	0.64
90	70	9.60	0.64

D values and correlation coefficient for PEDV in meat meal under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
60	30	10.97	0.65
70	30	11.52	0.60
80	30	10.47	0.70
90	30	6.77	0.88
60	50	14.39	0.54
70	50	7.43	0.88
80	50	7.20	0.91
90	50	6.77	0.86
60	70	13.55	0.67
70	70	10.01	0.80
80	70	12.12	0.69
90	70	2.40	0.62

D values and correlation coefficient for PEDV in meat and bone meal under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
60	30	6.58	0.93
70	30	6.77	0.91
80	30	7.43	0.88
90	30	3.78	0.95
60	50	12.79	0.90
70	50	14.39	0.76
80	50	11.52	0.77
90	50	10.97	0.53
60	70	10.47	0.79
70	70	6.22	0.97
80	70	3.71	0.86
90	70	2.21	0.81

D values and correlation coefficient for PEDV in blood meal under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
60	30	10.97	0.71
70	30	12.79	0.60
80	30	13.55	0.48
90	30	12.79	0.55
60	50	14.39	0.76
70	50	17.72	0.89
80	50	14.39	0.76
90	50	3.44	0.98
60	70	13.55	0.77
70	70	12.12	0.69
80	70	10.97	0.72
90	70	2.07	0.93

D values for PEDV in grow finish premix under the experimental conditions

Temperature (°C)	RH (%)	D value (min)	Adjusted R^2
60	30	13.55	0.61
70	30	12.79	0.55
80	30	12.12	0.69
90	30	7.68	0.87
60	50	12.79	0.97
70	50	12.12	0.63
80	50	12.79	0.87
90	50	10.47	0.79
60	70	16.45	0.92
70	70	10.47	0.79
80	70	10.47	0.63
90	70	3.11	0.90

Appendix D: Thermal inactivation kinetics of swine and non-swine viruses

Appendix D-1: Thermal inactivation kinetics of other swine-related viruses

Virus	**Type**	**Type of sample**	**Process conditions**	**Detection method**	**Inactivation (log reduction)**	**Reference**
Pseudorabies (PRV) Porcine Reproductive Respiratory Syndrome (PRRS)	Enveloped	Bovine plasma (3 batches for each virus)	Spray-drying Inlet T: 240°C Outlet T: 90°C Dwell time: 0.41 s	PRV: Microtiter assay in PK cell cultures PRRS: Microtiter assay in MARC cell cultures	PRV (3 batches) Initial concentration:$10^{5.3}$ $TCID_{50}$/mL **5.3 log** PRSS Initial concentration: 10^4 (1 batch), $10^{3.5}$ (2 batches) $TCID_{50}$/mL **4 log, 3.5 log**	Polo et al., 2005
Porcine Reproductive Respiratory Syndrome (PRRS)	Enveloped	Eagle's Minimum Essential media with 10% Fetal Bovine Serum	4°C, 5 days Water bath: 37°C, 5 days 56°C, 5 days	Crandell feline kidney (CRFK), monkey kidney (MA-104), and CL2621a cells	Initial concentration: 10^6 $TCID_{50}$/mL **3 log** (37°C, 12 h) **6 log** (37°C, 48 h; 56°C, 45 min)	Benfield et al., 1992
Classical Swine Fever Virus (CSFV)	Enveloped	-Defibrinated pig blood	Water bath: 60-97°C, 0-60 min	Inoculation to live pigs; clinical signs were a proxy	Initial concentration: 10^5 $TCID_{50}$/mL Defibrinated blood 66°C, 60 min	Torrey and Prather, 1963 (In Edwards, 2000)

		-Blood diluted with 80% NaCl -Swine serum		to determine if the virus was not inactivated, inactivated or attenuated.	68°C, 45 min 69°C, 30 min **5 log (Inactivated)** Diluted blood 64°C, 30 min 66°C, 30 min 68°C, 30 min **Did not inactivate** Swine serum 60°C, 30 min 64°C, 30 min 66°C, 30 min **Did not inactivate** 68°C, 30 min **Attenuated** 97°C, 0 s **5 log (Inactivated)**	
Foot and Mouth Disease Virus (FMD) Pseudorabies	Non-Enveloped Enveloped	Lab scale - Slurry -Glasgow Eagles medium Pilot plant - Slurry+Virus suspension	Lab scale and pilot plant thermal processing at 55-70°C for 1-15 min		FMD-lab scale Initial concentration: $10^{7.3}$ pfu/mL - Glasgow medium: 70°C for 1 min **4.5 log** 65°C for 10 min **>6.6 log** - Slurry: 70°C for 1 min **>6.6 log** 67°C for 3 min **>6.6 log** 65°C for 10 min >**6.6 log** ADV-lab scale Initial concentration:	Turner et al., 2000

Classical Swine Fever (CSFV)	Enveloped	- Water+Virus suspension			$10^{7-8.2}$ $TCID_{50}$/mL - Medium: 65°C for 4 min **>5.8 log** 60°C for 15 min **>7 log** - Slurry: 65°C for 1 min **>5.8 log** 60°C for 15 min **>7 log** CSFV-Lab scale Initial concentration: 10^{7} $TCID_{50}$/mL - Medium: 70°C for 1 min $\geq$5.2 log 60°C for 5 min 4.9 log - Slurry: 70°C for 0 min >5.2 log 60°C for 3 min >5.2 log FMD-pilot scale Initial concentration: $10^{6.7-7.8}$ pfu/mL - Water: 64.5-65.5°C, ~3h **>6 log** - Slurry: 60.3-61.6°C, ~3h 64.3-65.4°C, ~3h **>6 log** ADV-pilot scale Initial concentration: $10^{6.6-8.8}$ $TCID_{50}$/mL - Water:	

					60.3-61.5°C, ~3h **>5.4 log** - Slurry: 60.8-62.8°C**,** ~3h **>5.4 log** 56.0-57.8° **6.5 log** CSFV-pilot scale Initial concentration: $10^{6.8-7}$ $TCID_{50}$8-63.1°C, ~3**g**lur:-5.8°C, ~**log**9.0 ~3	
African swine fever virus (ASFV) Swine vesicular disease virus (SVDV)	Enveloped Non-enveloped	-Pig slurry: Raw Acidified pH 6.4 - Water	Pilot plant reactor: Continuous thermal processing using hot water (40.7-60.1°C, 5 min)	ASFV- Microtitre plate system and haemadsorbing cells (if present, positive) SVDV- Plaque assay in IB-RS2 cells	ASFV Initial concentration: $10^{3.3}$ HAD50/mL 52.2°C (outflow) for 5 min **1.5 log** SVDV Trial 1 Initial concentration: $10^{5.2-5.8}$ PFU/mL 60.1°C (outflow) for 5 min 53.2°C for 5 min 53.5°C for 5 min **~4.5 log** Trial 2 Initial concentration: $10^{5.4}$PFU/mL 50.4°C for 5 min **1.6 log** Trial 3 Initial concentration:	Turner et al., 1999

					$10^{5.4}$PFU/mL 40.7°C for 5 min **0.2 log**	
Porcine circovirus 2 (PCV2)	Non-enveloped	Dulbecco's Eagle medium	Water bath: 75-80°C for 15 min	RT-PCR and IHC.	Initial concentration: $10^{3.6}$ $TCID_{50}$/mL 75°C for 15 min **Retained infectivity** 80°C for >15 min **3.6 log**	O'Dea et al., 2008
Porcine circovirus 2 (PCV2) Chicken anemia virus (CAV)	Non-enveloped	- Human albumin - Human factor VIII	Water bath: 60°C for 10h Dry heating: 80°C for 72h 120°C for 30 min	PCR	Water bath 60°C for 24 h **1.3 log** (PCV2) **1.4 log** (CAV) 65-75°C for 30 min **0.25-1.92 log** (PCV2) **0.16-3.5 log** (CAV) Dry-heat 80°C for 72 h **0.75 log** (PCV2) **1.25 log** (CAV) 120°C for 30 min **1 log** (PCV2 and CAV)	Welch et al., 2006
Porcine Parvovirus (PPV)	Non-enveloped	Human albumin solution	Dry heat: 80-100°C for 30 min-72 h	Cytopathic effect at $CCID_{50}$	Initial concentration: $10^{6.5}$ $CCID_{50}$/mL 100°C for 30 min	Blumel et al., 2008

					2-3 log 80°C for 72 h **5 log**	
Foot and Mouth Disease Virus (FMD)	Non-enveloped	PBS medium	Water bath: 50-100°C	50% tissue culture infective dose	D values (time to achieve 1 log inactivation) 50°C: 732 to 1,275 s 60°C: 16.3 to 42 s 70°C: 6 to 10.8 s 90°C: 3.1 to 1.6 s 100°C: 2.9 to 1.9 s	Kamolsiripichaiporn et al., 2007

Appendix D-2: Thermal inactivation kinetics from non-swine related viruses

Virus	**Type**	**Type of sample**	**Process conditions**	**Detection method**	**Inactivation (log reduction)**	**Reference**
Canine Coronavirus (CCoV)	Enveloped	CCoV strain S378 10% Bovine fetal serum	Water bath: 56, 65 and 75°C	50% tissue culture infectious dose assay $TCID_{50}$/50 mL	Initial concentration: $10^{6.75}$ $TCID_{50}$/50 mL- 56°C for 30 min **4.75 log** 65°C for 5 min **4.75 log** 75°C for 5 min **5.75 log**	Prateli et al., 2008

Severe Acute Respiratory Syndrome (SARS) Coronavirus	Enveloped	Sputum from human placed in Vero Cell cultures	Water bath: 56°C or 60°C with or without 20% FCS as protein additive for 30 min	Confluent cells in 96-well microtitre plates ($TCID_{50}$)	Initial concentration: $10^{7.18}$ $TCID_{50}$/mL 56°C**,** 60°C for 30 min **^{3}5.01 log** 56°C, with 20% Foetal Calf Serum for 30 min **1.93 log**	Rabenau, 2005
Severe Acute Respiratory Syndrome (SARS) Coronavirus	Enveloped	Vero E6 cells with virus in Dulbecco's modified Eagle's medium	Heating block: 56, 65 and 75°C	Vero cell monolayers on 24 and 96-well plates using a 50% tissue culture infectious dose assay ($TCID_{50}$)	Initial concentration: $10^{6.33}$ $TCID_{50}$/mL 56°C for 10 min **4 log** 56°C for 20 min **5 log**	Darnell et al., 2006
Severe Acute Respiratory Syndrome (SARS) Coronavirus	Enveloped	Vero E6 cells in Minimum Essential Medium (Virus): 1)Immunoglobulin preparation 2)Anti-thrombin III preparation	Water bath: 60°C for 0.5-2h	Karber's method to measure infectivity (Karber et al., 1931)	1) Initial concentration: ~$10^{4.8}$ $TCID_{50}$/mL 60°C for 30 min **~3.8 log** 2) Initial concentration: ~10^{6} $TCID_{50}$/mL 60°C for 30 min **~3.5 log** 60°C for 60 min **~4.5 log** 3) Initial concentration: ~10^{7} $TCID_{50}$/mL	Yunoki, 2004

		3) Haptoglobin preparation 4)Human serum albumin			60°C for 30 min **~5.5 log** 4) Initial concentration: ~$10^{6.2}$ $TCID_{50}$/mL 60°C for 30 min **~4.7 log**	
Sindbi virus	Enveloped	FVIII protein	60°C for 1-30 h	Method described by Reed and Muench (1938) CCID50 test	Initial concentration: $10^{11.7}$ $CCID_{50}$ /0.1 mL - Aqueous solution 60°C for 1 h **~6.5 log** 60°C for 5-30 hn) **>9 log** - Lyophilized 60°C for 1 h **~5.95 log** 60°C for 10 h **>9 log** - Reconstituted 60°C for 10 h **~8.5 log** 60°C for 20 h **>9 log**	Espindola et al. 2006

Poliovirus type 1 Adenovirus type 5 Polyomavirus SV40 Bovine parvovirusVaccinia virus Bovine viral diarrhea virus	Non-enveloped	Cell culture+ Media (different for each virus)	Dry heat: 40-95°C	Micro-plate wells with cell culture for measuring the TCID50	Initial concentration $10^{5.5\text{-}10.2}$ $TCID_{50}$/mL Polyovirus type 1 40°C for 1h **No change** 75°C for 1h **4.3 log** 85°C for 1 h **^{3}4.8 log** 95°C for 1h **^{3}5.6 log** 75°C for 2 h **4.3 log** Polyomavirus SV40 95°C for 1h **5.2 log** 95°C for 2 h **^{3}5.1 log** Adenovirus type 5 85°C for 2 h **^{3}5.5 log** 95°C for 2 h **^{3}6.2 log** Vaccinia 95°C for 2 h **^{3}4.3 log** Bovine viral diarrhea virus 95°C for 2 h **^{3}4.0 log**	Sauerbrei et al., 2009

					Bovine parvovirus 95°C for 2 h **1 log**	
Hepatitis A virus Canine Parvovirus	Non-enveloped	- Factor VIII protein - IX Factor concentrate	1) Freeze-drying 2) Dry heat: 80°C, 24 h 90°C, 2 h		Hepatitis A 1) **2 log** 2) **4.3 log** C. Parvovirus 1) **No change** 2) **2.1 log** (48 h, 80°C; 10 h, 90°C)	Hart et al., 1994
Poliovirus Sabin Adenovirus type 5 Parechovirus 1 Influenza A (H1NI) Murine Norovirus (MNV1)	Non-enveloped Non-enveloped Non-enveloped Enveloped Non-enveloped Non-enveloped	- 1% Human stools - Control Medium (Dulbecco's modified Eagle's medium) *H1N1 only in the control medium	1) Preheat 30°C, Heat block 2) Water bath: 56°C for 0-30 min,73°C for 0,30 s, 1, 3 min	Cell culture assays	1) No reduction 2) Poliovirus Sabin Initial concentration: $6.3x10^8$ $TCID_{50}$/mL 56°C for 10 min **2.8 log** 56°C for 30 min **3.5 log** 73°C for 1 min **3 log** Adenovirus type 5 Initial concentration: $6.3x10^7$ $TCID_{50}$/mL 56°C for 10 min **3.5 log** 73°C, for ~40 s **~0.8 log** Parechovirus 1	Tudlahar et al., 2012

Human Norovirus (NoV)					Initial concentration: $1.3x10^8$ $TCID_{50}$/mL 56°C for 30 min **No reduction** 73°C for 3 min **~3.8 log** Influenza A (H1N1) Initial concentration: $1.3x10^6$ $TCID_{50}$/mL 56°C for 30 min ~**2.2 log** 73°C for 1 min **~2 log** MNV1 Initial concentration: $1.7x10^{ô}$ PFU/mL Human NoV Initial concentration: $1x10^8$ PCRU/mL 56°C for 10 min **~ 2 log** 73°C for 1 min **2 log**	

Appendix E: Thermal profile used to simulate thermal inactivation PEDV during a generic spray-drying process, based on experimental D values for damp plasma at static temperatures

Time (s)	Temperature (°C)	Inactivation (log)	moisture	Heating stage
0	30.0		4	**Adiabatic**
1	42.2	0.01	2.1	
2	53.3	0.02	0.9	
2.6	60.0	0.03	0.3	
3	64.4	0.03	0.185	**Dry kill**
4	75.6	0.03	0.135	
5	80.0	0.04	0.13	
10	80.0	0.05	0.105	
15	80.0	0.06	0.08	
20	80.0	0.07	0.08	
30	80.0	0.09	0.08	
40	83.3	0.11	0.08	
50	88.9	0.14	0.08	
60	94.4	0.17	0.08	
70	100.0	0.20	0.08	
80	105.6	0.23	0.08	
90	111.1	0.26	0.08	

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