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Swine Enteric Coronavirus Introduction to the United States: Root Cause Investigation Report

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Swine Enteric Coronavirus Introduction to the United States: Investigation Report

Executive Summary

Cases of porcine epidemic diarrhea (PED) were first diagnosed in the United States (U.S.) beginning in April 2013. The swine industry and associated professionals responded on many fronts with the Veterinary Services (VS) branch of Animal and Plant Health Service (APHIS) initially engaging in laboratory diagnostics, analytic support, epidemiology expertise, and data management. Multiple investigations and studies were conducted in attempt to answer questions about the epidemic; at the top of the list was how the virus arrived in the United States and whether there was risk of another disease following the same path.

In late spring of 2014, the U.S. Department of Agriculture (USDA) formed an investigative group (Root Cause Group-RCG) that was tasked with revisiting the mass of information that had accumulated following the initial outbreak of PED. In addition, the RCG initiated studies, analyzed data, and conducted follow-up investigations of early-affected farms. Information was gathered from manuscripts published in peer review literature; data from research projects; consultation with swine industry and veterinary specialists familiar with the individual outbreaks; collaboration with U.S. Government partners, information published on university, industry, and laboratory websites; U.S. Customs and Border Protection (CBP) data on imported products; data from illegal product seizures at U.S. ports; and the collated testing data from affected pigs. The group also reached out to international partners that had experienced outbreaks of swine enteric coronavirus diseases (SECD).

In many instances, information gathering was complicated because records and recall were not available or not collected at the time of the initial veterinarian's herd examinations. Although many people were eager to help solve the problem, some in the laboratory, feed, and swine industries had concerns about sharing intellectual property or individual information with the Federal Government.

During 2014, APHIS-VS prepared a pathway entry assessment entitled, *Pathways Assessment: Entry Assessment for Exotic Viral Pathogens of Swine*, as the first step towards determining whether significant gaps exist in import regulations that may result in infections of U.S. domestic swine with exotic viral pathogens of swine. The RCG used an epidemiological approach to the pathways assessment to more specifically address the entry of PED into the United States. After researching information that had been published, meeting with first-responder veterinary consultants, and analyzing data, the RCG and State/industry partners revisited many of the early farms and associated feed mills. Collaborations were established with other government and non-government entities, including Food and Drug Administration (FDA) units, other APHIS units (Wildlife Services (WS) and Plant Protection and Quarantine (PPQ)), the U.S. Department of Homeland Security National Biodefense Analysis and Countermeasures Center (NBACC), and CBP, as well as universities and industry organizations.

Between April and August 2013, there were three novel swine enteric coronavirus disease viruses (SECDv) that appear to have entered the U.S. at the same time or within a few months. These may have arrived together or separately, but likely via the same mechanism. While possible that the introduction of SECDv was a random one-time event, the scenarios described below do not preclude a similar occurrence in the future without implementation of mitigations.

The investigation did not uncover incontrovertible proof for any route of entry, but did arrive at a small number of scenarios described in detail later in this report. For the purposes of this investigation, a scenario had to explain transit through four segments of travel in order to be plausible: 1) The product or person carrying the U.S. outbreak virus had to be contaminated in the origin country, 2) the virus had to remain viable and infectious in transit to the United States, 3) the virus had to have means of dispersion to at least six geographically distinct locations in the United States in approximately two weeks, and 4) the virus had to reach farms and infect pigs. The scenario also had to be compatible with the herd investigation data, the consultant observations, and the epidemiology data as well as explain why the epidemic occurred in the United States but not Canada or the European Union (EU), given their similar industries, travelers, and international imports.

The results of the APHIS investigation indicate that the use of Flexible Intermediate Bulk Containers (aka: FIBC or “tote bags”) best fit the criteria established for entry in to the United States, rapid and wide spread across the country, and introduction onto individual farms. FIBCs are commonly used to transport many types of material including sand for flood control, soybeans, pet treats, or almost any kind of bulk material including pig feed. The FIBCs come in various sizes, usually with 1,000 to 3,000 pound capacity, and are designed to be reused. Several of the farm investigations as well as an early case-control study suggested feed or feed delivery as the source of the outbreak; however, there were no common feed manufacturers, products, or ingredients in the initially infected herds. In addition to meeting the investigation criteria, the contaminated FIBC scenario explains the apparent anomalous association of the epidemic to feed.

In this scenario, the FIBCs may be contaminated in the origin country by transport in contaminated trucks, by exposure to irrigation or flood waters containing organic fertilizer (i.e., pig manure), by organically grown soybeans, by birds, or via various other products and uses. Upon arrival in the United States, a contaminated FIBC may be reused for many purposes including transport of bulk feed or ingredients. The most probable route of dissemination is in the context of recycled food or feed products through distribution companies who generally service a large network of feed mill customers across the Midwest and beyond. Once a contaminated FIBC or its contents are delivered to a local mill that manufactures pig rations, the FIBC or its contents would contaminate feed or ingredients destined for delivery to the farm. A slight variation of the scenario would involve products that could be contaminated prior to shipment, and waste or scrap material from them carried in FIBCs, thus contaminating them. The FIBCs could then be reused to transport and deliver swine feed and ingredients.

Other scenarios were considered less likely to have occurred due to lack of supporting evidence or evidence contrary to them, and did not explain the apparent association of the outbreaks to feed and near simultaneous appearance of the disease in multiple locations. Many were deemed negligible risk in

the pathways entry assessment and were not further investigated. Others of interest included accidental or intentional introduction by people, contaminated feed supplements (antibiotics, vitamins, and minerals), spray dried porcine plasma, release from a diagnostic laboratory or research facility, contaminated biologicals or injectable medications, contaminated semen or germ plasm, birds, or prohibited product import. Another scenario investigated was the existence of a reservoir of PED virus in feral swine. The scenarios are described in more detail in the body of this report.

In light of these findings, VS initiated further testing in attempt to provide additional empirical evidence for the primary scenario(s). These are: 1) testing of organic soybeans at ports of entry, 2) testing of archived jerky pet treats from SECD endemic areas, 3) testing of FIBCs in a field environment, 4) measuring the survival time of PED virus in the FIBC material to determine if viruses can remain infectious for long transit times, and 5) testing of archived serological samples from feral swine.

Follow-up testing results summary:

1) As of 9/2/15, samples from 25 shipments of imported soybeans had been received with no detection of SECDv.

2) No virus was detected from 40 samples of imported jerky pet treats archived prior to April, 2013.

3) As of 8/10/15, no virus was detected from 60 FIBCs provided by participating feed mills. (Note: The samples submitted were from FIBCs that had not been reused.)

4) Results for survival of PEDv on FIBC material are suggestive that the FIBC scenario has merit. The woven fabric was treated with a preset amount of cultured PED virus. The virus remained stable through the 10-week time point for both the 4^0C or minus 80^0C temperatures. Viable virus was detected after five weeks but not six weeks at room temperature.

5) Serologic tests on 368 feral swine samples archived prior to April 2013 were negative.

The RCG did not recommend or delve into mitigation measures; however, since plausible scenarios must explain all four segments of transit to U.S. farms, interventions might be focused on the link that almost all scenarios required, which is the capability of the FIBCs to facilitate dispersement to multiple locations.

Breaking any one of the four segments of the virus journey, would suffice to mitigate the risks of this type of event. Contamination of products in an origin country is largely out of government regulatory control and likely outside the realm of industry management. Inspections at entry ports are vital, but unable to identify products containing miniscule amounts of contagious virus.

If the fomite moving the virus is indeed the FIBC, not reusing or sanitary management prior to reusing the bags could be an effective intervention. Further study is necessary to identify cleaning and disinfection procedures that might be appropriate, but the answer could be as simple as not reusing the bags or yet to be determined disinfection procedures such as dry heat prior to reusing the containers.

SECD Root Cause Investigation: Report of Findings

Background of project

Group tasking: *identify the route that SECD entered the United States or the most plausible scenarios that describe how the viruses could have arrived and infected U.S. pigs.*

The RCG was formed in the summer of 2014 and tasked with reviewing and compiling the mass of information that accumulated following the initial outbreak of PED in April 2013. In addition, the RCG initiated studies, analyzed data, and conducted follow-up investigations of farms affected early in the outbreak. Information was gathered from manuscripts published in peer review literature; data from research projects; consultation with swine industry and veterinary specialists familiar with the initial outbreaks; collaboration with United States Government partners; information published on university, industry, and laboratory websites; CBP import data and data from prohibited product seizures; and collated laboratory data from testing affected pigs.

The scope of the RCG investigation was limited to novel SECDs and approached the question from an epidemiological perspective. This process initially involved evaluation of published research that had accumulated about the viral agent, the host population and swine industry practices, as well as the micro and macro-environmental conditions that influenced the epidemic. It followed with an in-depth analysis of CBP data and laboratory testing data that had accumulated since the beginning of the outbreak. These data were combined with aggregated information from each individual farm investigation, study reports and manuscripts, and expert interviews. Several hypothetical scenarios were then generated to explain the evidence that was identified. The hypotheses led to further questions, studies, and collaborations, and finally to a small number of possible scenarios.

In many instances, information gathering was complicated because records and recall were not available or not collected at the time of the veterinarian's initial herd examinations. Although many people were eager to help solve the problem, some in the laboratory, feed, and swine industries had concerns about sharing intellectual property or individual information with the Federal Government.

Criteria to narrow the scope of scenarios

1. Virus survival: In order to travel from another country-particularly from Asia, environmental and carrier matrix conditions must be adequate for virus stability; or alternately, the travel time must be short; i.e., by airline (see epidemiology section).
2. Country comparison: The scenario must explain why the epidemic occurred in the United States and not Canada or the EU given their similar industries, travelers, and international imports. (see United States-Canada comparison section)
3. Herd investigations: The scenario must be compatible with the outbreak investigation data (see investigation summary section). That is, a product or person is not likely responsible for the epidemic if never in contact or linked in some way to the index farms.

4. Virus travel: The scenario must explain transit through four segments of travel, all necessary and none sufficient alone: 1) person or fomite became contaminated in the source country, 2) it entered the United States, 3) it was dispersed to separate geographic locations in a short time, and finally, 4) pigs were exposed and infected (see virus movement overview section).
5. Legal imports: If the scenario involves legal imports, the product must have record of being shipped to the United States in the time prior to the outbreaks (e.g., CBP data and APHIS import permits).

Overview: Virus movement from affected country to U.S. pigs

There are four segments of travel between the foreign origin of the virus and the domestic destination in affected pigs. Each link is necessary for the epidemic to occur, but not sufficient alone.

1. Contamination: Product or people are contaminated with virus in the origin country.
2. Entry: The agent leaves origin country and arrives in the United States
3. Dispersion: The agent moves from its entry point to multiple locations at nearly the same time.
4. Exposure: The agent is delivered to swine farms and pigs are exposed.

Initial contamination of product or people

Pathogenicity and genetic analysis of viruses isolated in the United States are highly similar to those identified in China between 2010-2013, and many reports suggest China as the country of origin of the viruses that appeared in the United States (Chen et al., 2014; Huang et al., 2013; Stevenson et al., 2013; LY Wang, Byrum, & Yan, 2014; S. Wang et al., 2014). This conclusion was also reached after analysis of Genbank data by the DHS-NBACC; however, a definitive source of the viruses identified in the United States has not been determined.

For the viruses to travel to the United States, people, animals, or products first become contaminated or affected at the origin. Two products that have opportunity to be contaminated in other countries and exported to the United States were considered as significant candidates by the RCG. The first is organic soybeans that may have had contact with water or trucks contaminated by organic fertilizer prior to shipment. The second product is pet treats composed of pork, or pork products, or commingled with pork or pork products during processing or transport. Another type of fomite that could be contaminated and travel to the United States is the FIBC (aka totes) used to transport bulk material. Prior to the SECD epidemic, they were often reused for different products and, in the United States, generally not cleaned or disinfected between uses or products. It is not known whether FIBCs that are used to export products from other countries are new or sometimes reused after transporting other products.

Many persons associated with swine production travel regularly between the United States and Asia, and may come in contact at some point with SECD infected pigs. Although people have the potential for being contaminated during travel, herd investigation data to date have not supported people as an entry pathway. Scenarios in this document describe more detail of how these products, people, or other virus carriers could become contaminated and travel to the United States.

Entry to the United States

In 2014, APHIS VS conducted a pathway entry assessment for exotic viral pathogens of swine (USDA-APHIS, 2014). The assessment was not targeted specifically to the SECD epidemic, but had the objective to identify plausible pathways for any exotic pathogenic virus to enter the United States. The assessment identified several pathways as having non-negligible risk for entry routes of exotic virus into the United States. The data and conclusions of the entry pathway assessment were complementary and supportive to the SECD-specific epidemiology investigational approach. With current mitigations, pathways determined to have non-negligible risk included:

- dietary supplements and traditional medicines;
- veterinary vaccines and miscellaneous biological products;
- unprocessed animal feed ingredients derived from plants or plant products;
- commercial swine meat and meat (by-) products for human consumption;
- non-rendered pet food treats and chews;
- bush meat;
- non-regulated garbage;
- livestock and germplasm;
- humans;
- and other live animals

SECDv must transit through four segments of travel to infect U.S. pigs:

1) A person or product is contaminated at the origin country.
2) Virus remains viable for the time of travel, and enters the United States.
3) There is a mechanism for rapid dispersal to widely separated locations.
4) It reaches the farm, and breaches its exclusionary biosecurity measures, and infects pigs.

After evaluating epidemiological evidence, a few scenarios for entry of SECD emerged as most plausible. These include reused FIBCs, pet treats, and organic soybeans (further detail in scenario section of document). Other scenarios are plausible, but evidence supporting them is limited, lacking, or in some cases not supportive. These include: virus movement associated with people or clothing, a reservoir in feral swine, intentional introduction by humans, contaminated amino acid products, carriage in human nasal passages, contaminated rice hulls used as filler in antibiotic products, and vitamin and mineral premixes. Others included: contaminated vaccines and pharmaceuticals, illegal or smuggled products, entry via wild migratory birds, semen and germ plasm, blood products used in feeds, and non-regulated garbage.

Dispersion within the United States

After entry in the United States, PED was discovered in six locations within approximately two weeks. The locations were geographically separated and did not have epidemiological links through common age groups, production types, companies, ration formulations, feed mills, feed products, vehicles, veterinarians, or other visitors. However, one factor is common to most feed mills and represents a potential mechanism for moving SECD virus across various parts of the country. This is the practice of using recycled feed or food products in the ration formulations. These products are varied and include dairy products such as cheese or whey, dried distiller grains, wheat midds, bakery products, human food products such as breads and pasta, soybean hulls, scrap pet food, and many others.

Although no single product was identified as common to all early affected farms, standard operations of recycling companies include brokering of a wide variety of salvage ingredients at any time. They generally own or are associated with trucking and transport partners. Their networks cover wide expanses of the United States, and products are shipped in bulk by truck, train, or in FIBCs. The FIBCs are large bags made of various materials such as woven polypropylene, and are used to transport almost any bulk material such as vitamin and mineral mixes, dried distiller grains, pet food, or soybean hulls. They are also designed for other purposes (for example; carrying sand for flood control barriers or transporting wood shavings).

The FIBCs represent a mechanism of dispersion across multiple states in a short time, since they are reusable and may carry different products at different times. The woven material provides protection from sunlight and ultra-violet radiation as well as having small spaces between fibers that could harbor virus particles.

Exposure of U. S. pigs

The investigations of swine farms did not identify any single common source of infection, yet evidence from on-the-ground investigations, an early case-control study, and several swine consultants indicated that the outbreaks may be associated with feed or feed components. Interpretation of the association is not as simple as feed products carrying viruses because no ingredient, brand, or feed company was identified as unique among the early-farm outbreaks. This implies that the carrier was more likely a fomite associated with feed or feed delivery. Although vehicles were suspected in some cases and ruled out in others, there were no common vehicles identified that delivered feed, hauled pigs, or provided services to these farms. Several studies have reported finding viruses at feed mills, on trucks, or in feed products (Davies et al., 2014; Dee, 2014b; Dee et al., 2014; Sampedro et al., 2015; Yeske, 2014), and provides evidence that feed and feed mills can be intermediate waypoints of virus movement, yet the early farms had no common feed mill. Follow-up information on ration formulation demonstrated that rations used on the earliest affected farms included salvaged/recycled products. Assuming that dispersion was via fomites associated with the recycle and transport network, contamination of local feed delivery trucks, FIBCs, or the feed mills would be a highly plausible source of exposure to individual farms.

Epidemiology

1. Interviews with consultants and swine experts

Background for interviews

A series of interviews was conducted with swine experts to provide insight into the emergence of SECD in the United States. The consultant group included swine subject matter experts representing academia, the swine industry, and veterinary specialists who had first-hand experience with the SECD outbreaks in the United States, Dominican Republic, and Puerto Rico. Puerto Rico had no confirmed cases of SECD at the time of the interviews. Although conversations were ongoing during the investigation, the interviews summarized below occurred during the summer of 2014.

Collectively, the consultants had experience in veterinary practice, swine farming, pork production, and processing as well as first-hand experience with the first outbreaks. The data collected from the interviews are arranged in common themes for plausible scenarios for SECD entry. These themes include People/Feed/Minerals/Vitamins as a Pathway, and Other Pathways.

Discussion and hypotheses from the consultants

Some of the interviewees have established consulting practices in China and are experienced with management practices, biosecurity, and travel between the countries.

Opinions on the merit of people as a pathway of PEDv introduction into the United States varied between consultants. Most U.S. swine farms require strict biosecurity measures such as showering in when entering and although it is possible for people to become lax in following the procedures, most of this panel thought it unlikely that a person entered the United States with the virus in tow. One consultant thought it likely.

One veterinarian mentioned that there are a lot of visitors from Korea and Japan to the United States, and virus could have moved from China through these countries. He indicated that while the United States enjoys many visitors from China, there were probably few Chinese coming to U.S. swine farms and those that did would have presumably gone through biosecurity measures prior to swine contact. There also was no major influx of travelers in 2013 compared to 2012 or 2011. Although one large U.S. company was purchased by a Chinese firm, personnel from China reportedly did not visit the farms and none of the Chinese-owned U.S. farms were involved in the initial cases.

The format of the meetings was informal semi-structured discussion and most conversations were via prescheduled conference calls. The consultants were provided the following background:

"We do not think there is culpability or intentional error on anyone's part, and the interview is not intended for retribution or faultfinding. We think there is or has been an open window, possibly related to feed components, and want to close it. A lot of documents, studies, and other information have become available in the last year, but we would also like your expert opinions, speculations, and first hand impressions."

Objectives:

1. *Investigation of the "root cause" of SECD outbreak(s); how did SECD get into the United States and then how did it get into operations; or*
2. *How <u>could</u> it have happened?*

One interviewee did not support the people pathway since the virus was identified in multiple locations while farms that shared technicians, trucks, and other inputs were not affected. He also suspected that it was the sheer size of the U.S. swine industry that increased risk compared to EU and Canada.

Interviewees considered other introduction routes including feed-grade antibiotics; a major percentage of which come from China. They suggested it could have been post production contamination of antibiotics, or contamination of products used in finished injectables such as vaccines. Few finished injectables are bought from China, but reagents involved in a finished product may come from there. Additionally, some systems disinfect all incoming products, but it is possible the disinfection chambers could have been overloaded.

Interview questions: General

Population/demographic

- *Were there any other age groups on the premises?*
- *How old were the first pigs with signs?*
- *How did it start? Do you think it started in a few pigs and spread, or did it start everywhere at once?*
- *What was the source of the pigs? Integrated farms? Any from outside this farm?*

Facility(s)

- *One location? Multiple facilities? Start in one and spread?*
- *How are units set up? What kind of physical separation between pigs/ages?*

Human introduction. *Were there any visitors immediately prior to the outbreak? Any temporary workers or students? Consultants or sales people? Maintenance people?*

- *Any people or products that came from China? Other country? Gifts, mail order, pet treats for dog, other?*
- *Was there any contact (link) between truck drivers or maintenance people with pigs?*

Do you think this is the index farm?

- *Have you had any suspicion of any earlier operations affected?*
- *Describe the source farms of the piglets. Are they vertically integrated or any outside sourcing?*
- *Was there any evidence of GI disease in the sow farms or in any associated finishers immediately prior to outbreak?*

Introduction from trucks or vehicles.

- *Can you think of any way that infection may have come on a vehicle? Rendering trucks, feed trucks, utility vehicles, other?*

Biosecurity

- *Review survey information: What were the exclusionary practices? Foot baths, shower, air filters? Who is required to use them? Other?*
- *Exposure to birds or rodents? Is there possibility of exposure to feral swine? Do any employees have back yard pigs?*
- *Was any new or used equipment introduced prior to outbreak? How was it sanitized?*

One veterinarian described inventorying products at one company and finding 58 imported items, including artificial insemination catheters, from countries identified as having PED outbreaks. Producers often do not know the country of origin for imported items and some distributors may substitute other countries' products when an original supplier is unable to fulfill a product need. He speculated that numerous importers coupled with few import inspections may be underlying the origin of PEDv.

Background information uncovered during the interviews points to differences in opinion about which swine industry sector first exhibited clinical signs. Opinions also varied as to whether there was a single or multiple PEDv introductions initially. Observations were also made comparing PEDv disease behavior in the United States to other countries such as Canada, the EU, Brazil and Japan.

One consultant stated that originally TGE was thought to be the disease occurring in April 2013 since clinical signs are similar to SECD. Therefore, he suggested that PEDv might have gone unnoticed in finishers because diarrhea in these pigs is

frequent and often not diagnosed. Fecal samples would then not have been collected from the animals based on the premise the disease was most likely TGE.

Another expert believed that it was unlikely SECD clinical signs would have gone unobserved, as the disease did not display clinically like any other disease event seen before. For example, in his experience, if one farm was affected, neighboring farms were affected within 1-2 days.

SECD also presented with milder clinical signs in the grower-finisher sector than would be seen in cases identified in farrowing sows. One interviewee did not see any clinical signs until it reached the sow farms. A question was asked whether cases also could have been missed in the nursing piglets. He commented that sows exhibit diarrhea three days prior to piglets, so original estimates for when diarrhea first occurred could have been off. Whether grower-finishers, sows, piglets, or another swine production type were affected first, SECD would spread quickly to other farms and detection would have had to be more rapid.

Interview questions: Feed topics

Can you clarify the business structures and explain who is who and their role in the operation?

- *Does company have its own nutritionists? Who formulates their rations?*
- *Who mixed the feed?*
- *What is the protein source of the ration (especially if nursery/pregrowers)? Whey? Pet food? Other?*
- *Where was the protein sourced? Were salvage/repurposed products used?*
- *Was there one farm/facility that broke? Or more than one related location? Timing of when different groups of pigs started showing signs?*

Ration formulation

- *Where did ingredients come from?*
- *Was there any change in formulation? Or in sourcing?*
- *Were there any components of the ration that did not come through the feed mill?*

Some interviewees were not convinced there was more than one introduction accounting for the first PEDv identification made in April 2013 followed by a PEDv variant in June 2013. One considered this event as a sporadic introduction. Another thought it most likely that we had one "catastrophic" event and that "all four" viruses introduced were at same time. One panel member said he had given up on ever figuring out how it got into this country and didn't believe the answer would ever be known.

Feed/Minerals/Vitamins

Feed, feed components, and feed containers used to transport feed were identified as risks for a PEDv U.S. introduction. As the interviews were conducted in a free flowing format, discussion of feed as a carrier of PEDv often blurred between feed as a method of U.S. introduction and that of lateral spread.

Some experts did not believe feed was a likely source since processing methods and long shipping times would not be conducive for virus survival. However, interviewees believed feed contamination could occur at or during feed component processing or via trucks picking up affected sludge off of highways. One veterinarian noted that viral introduction was most likely through feed or supplies introduced to

the inside of swine barns since many farms in the United States maintain high biosecurity and had been able to keep other contagious viral diseases but not PED out (e.g., PRRS).

There were four modes of introduction to a site that are part of feed transport and were considered in this interview series: contaminated feed rations, contaminated feed components, contaminated feed containers, and contaminated feed trucks.

Feed in general was thought of as a sporadic risk, which agrees with the "one mouthful" concept (i.e., due to non-uniform mixing of feed, a small dose of SECD virus might be present in one bite of feed but not in the remainder of the volume). Contaminated components of feed bolster the reasoning behind the one mouthful idea in that a contaminated lot of a component may not be homogeneously mixed in the end ration and the contamination itself may only be a small nidus of the original component. Components of a premix generally contain low moisture products such as vitamin and mineral compounds. Some also contain plasma components or simple amino acids or peptides. There was lack of agreement between consultants as to whether certain components such as a premix can sustain a virus for any time. Vitamins and minerals are generally dry inhospitable materials for virus stability, but one interviewee believed a "matrix" within this dry material could sustain a virus (i.e., small bit of material like manure that is moist enough to stabilize a virus).

Several consultants indicated that as much as 90 percent of vitamin and mineral premixes come from China due to supply and price. Large amounts of amino acids also come from China (e.g., lysine, methionine). Premix entering the United States may be contaminated in a variety of ways during the mixing and bagging processes. They related that most premix products come straight from China, and then are diluted to a customer specific concentration in the United States. Some products such as antibiotics are diluted in China with rice hulls. One consultant had seen rice hulls being dried on a roadside where they are likely driven over by contaminated pig trucks. Contamination may also occur through a reused container (e.g., FIBC aka "tote"). The flow of product in China is from small plants to large plants using tote bags that might have held rice hulls or other contaminated material previously. Tote bags may be used for premix or premix components and are a way of transoceanic transportation of product as well. These tote bags or pallets that store feed or feed ingredients may have been contaminated.

There are many buyers and blenders of Chinese products in the United States that ship to mills. One veterinarian had spent years in the feed industry and believes that the initial introduction came from a contaminated premix. The interviewee postulated that it is difficult to get micronutrients concentrated evenly throughout a feed mix which could explain why only select farms had outbreaks.

Besides premixes, one interviewee thought it was possible that we brought non-premix feed ingredients into the country in 2013 that we did not in 2010-12. For example, corn prices were high here during that time span so an alternative energy source could have been imported. Another interviewee thought there might have been some porcine plasma imported as well (Note: United States import records do not show any imports of porcine plasma). Another suggested that farms may have also received the virus through a rendered product shipped here. Pet food ingredients are also manufactured in China and the contamination potential is thought to be high. Unused waste dog food may be sold to swine producers. One consultant said that waste dog food is usually fed to smaller farms since it is harder to

control the nutrient consistency required by larger farms for uniform growth. Larger farms rely on tight scheduling when emptying and filling barns. Dog food also takes less pressure and heat to make than pellets so it may retain virus infectivity when fed.

Questions raised by the consultants

Questions were raised about the intensity (e.g., number of farms affected) and spread of SECD in the United States versus other countries. These questions and other random statements are listed below.

- Why have the new strains not been reported in Europe?
- Canadian and EU manufacturers that share possible common feed/feed ingredient or other inputs as those in the United States may shed light on the disease origin.
- Western Canada and Brazil have not broken with SECD at the time of this report. Why?
- United States provides biosecurity advice to Canada so practices are similar. Why didn't the outbreak occur in Canada at the same time as in the United States?

Further investigations suggested

- Identify and compare feed/mineral/vitamin lot numbers used in Canada and the United States.
- Improved understanding of the animal feed manufacturing process and identify risk pathways.
- Reexamine the first reported PEDv cases in April 2013 to validate them in the grow-finish sector and not the wean-finish sector.
- Return to the first cases and make sure they were grow-finish and not wean to finish. Also, examine in more detail types of premixes and feed ingredients, particularly creep feed that is used. There are questions as to how we found the initial cases in older pigs, and whether we could have missed cases in wean to finishers or piglets.
- Examine vaccine manufacturers and the manufacturing process (vaccines are common across farms; we know some vaccines such as mycoplasma vaccine uses serum).

Interviews with officials from Dominican Republic and Puerto Rico

The RCG met with officials from the Dominican Republic (D.R.) to discuss any mechanisms of introduction of SECD viruses that might be informative to the U.S. investigation. The (D.R.) outbreak started in November 2013 at an integrated premise. A second outbreak did not occur until February of 2014 about 12 miles away in a group of farms that were similar in terms of biosecurity and movements. About 30 miles away from the second set of farms, a cluster of 14 producers remained free from SECD. These farms were a close co-op reported to have very high biosecurity and thorough truck cleaning and disinfection procedures, which suggested that feed may not have been the common source. The source of the outbreak was not identified, but spread theories included feed, transportation of live swine to markets, and trucks.

The RCG also interviewed officials from Puerto Rico in attempt to evaluate either source of SECD introduction or means by which Puerto Rico excluded it. Puerto Rico had no confirmed cases of SECD at the time of interview. Both corn and pre-starter feed is purchased from the United States. There are no restrictions on movement of semen.

2. USDA Data: Introduction date of PED virus (presumed index cases)

The USDA reports information weekly, which summarizes test results from the National Animal Health Laboratory Network (NAHLN). Samples reported prior to June 5, 2014 were voluntarily submitted and reported, while those made after that date were mandated by USDA Federal Order (U.S. Department of Agriculture, 2014). Prior to the Federal Order, only prevalence of sample submission was available, but after the Federal Order, site level prevalence in terms of number of positive premises became available (USDA-APHIS, 2015a).

Initial introduction is suspected to be March or early April 2013

- *Over 1,000 archived samples targeted to diarrheal disease did not identify cases prior to April 15, 2013*
- *Validation studies on serological samples from2011-2012 for a new test were negative*
- *Feral swine testing of archived samples were negative*
- *After initial farms were affected in April, the disease spread exponentially to other herds*

Herds found to be PEDv positive prior to the early part of May 2013 were identified through retrospective testing of archived samples at State diagnostic laboratories. These samples had been originally submitted for various diarrheal diseases and served as highly targeted samples for SECDs. Targeting samples to populations with clinical signs provides substantially higher information value than would be expected from a random selection of the swine population and are much more likely to detect disease if present (Wilesmith et al., 2004) (Wells et al., 2009; Williams, Ebel, & Wells, 2009). In the retrospective laboratory investigations, Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) reports testing approximately 800-1,000 samples prior to April 15, 2013 for PEDv with no additional accessions testing positive (ISUVDL personal communication). Although the testing was not that of a structured study protocol, the laboratory provides services for a large cross section of the Midwest. This helps limit testing bias and provides a reasonably representative sample of U.S. swine operations.

An additional ISUVDL data set suggesting that PEDv was not previously prevalent was collected in a validation study for a new PEDv ELISA test. In this study, serum samples from December 2011 and January 2012 were tested with a new test and resulted in a specificity of 98.5 percent with a 1.5 percent false positive rate. Likewise, serologic testing of archived feral swine serum did not identify any positive PEDv samples prior to April 2013 (see Feral Swine Scenario, page 34), and further suggests that PEDv was not circulating among the feral population or small domestic herds that would have contact with the feral animals.

Swine testing data collected by VS from NAHLN laboratories provides additional information showing an explosive propagation of the disease following an initial introduction. This also suggests that there were not cases of the original highly virulent PEDv prior to the first cases detected. The earliest two cases began showing clinical disease on or about April 15, 2013.[1] Figure 1 shows herd identification dates and

[1] Two April 15, 2013 farms were identified by VS through the retrospective testing and interviews with swine consultant veterinarians

number of submissions testing positive. Data through May 2014, were fit to an exponential distribution with a goodness-of-fit Chi square[2] statistic $p<0.05$. This type of epidemiology curve is suggestive of a point source introduction(s) followed by a rapidly propagated infectious disease (Smith, 1995).

Because reporting was voluntary until the USDA Federal Order was issued, it remains possible that a herd(s) could have been affected prior to April 2013 and was the source of infection to the earliest cases detected shown in Figure 1. Although possible, the available evidence is not supportive.

After introduction of the disease in April 2013, it spread explosively. The virus is extremely contagious and rapidly broke through biosecurity defenses of many of the most secure herds in the United States (Stevenson et al., 2013), many of which had been previously able to exclude other contagious diseases such as porcine reproductive and respiratory syndrome (PRRS) (personal communication: interviews with U.S. swine consultants). This supports an epidemiological conclusion that a reservoir for infection would be unlikely to persist for more than several days or at most a few weeks without spreading widely and being observed. The exception might be the feral swine population, where the population may be adequately isolated for it to circulate undetected for a longer period. As stated, tests of archived samples conducted in collaboration with APHIS Wildlife Services and ISUVDL make this of low probability (see **Scenario for feral swine**).

Further evidence indicates that milder strains of PEDv (with DNA insertions or deletions described as INDEL variants) were not circulating prior to April 2013. Pigs affected with the INDEL viruses have been shown to exhibit milder clinical signs but also provide protective immunity against the more virulent PEDv (Goede et al., 2015). If this clade of viruses had been circulating, we would not be likely to see the exponentially propagated epidemiology curve described in Figure 1. Instead, in a partially immune population, clinical signs would have been more subdued with fewer herds showing the high mortality rates and explosive herd outbreaks reported by Stevenson (2013) (Stevenson et al., 2013).

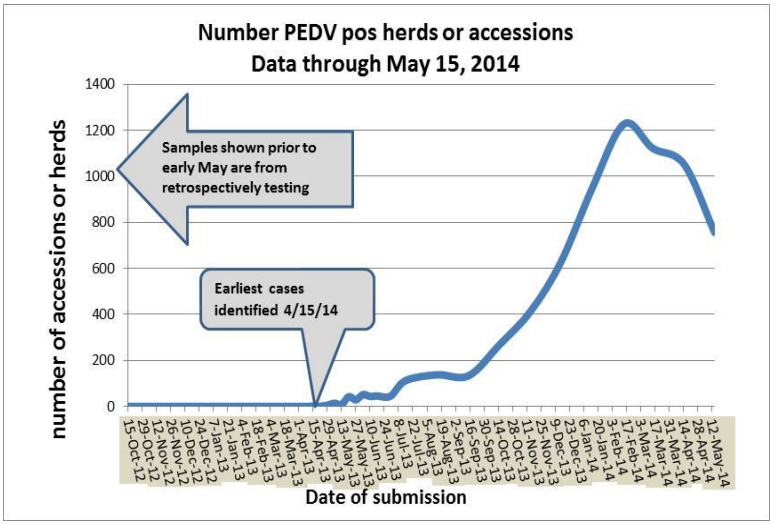

Figure 1. Sample accessions from October 2012 through May 2014 are shown. Sample dates prior to May 2013 were tested retrospectively from laboratory samples banked from previous diarrheal disease outbreaks. The earliest positive herds identified were on April 15th. The epidemic curve is more flat during the summer months of 2013, probably due to summertime environmental conditions.

[2] Distribution fitting function using software: Palisade Corporation, @Risk version 6.3.0

Porcine deltacornavirus (PDCoV)

Argument may be made that the cases shown in Figure 2 were new introductions, or alternately that PDCoV was circulating in the United States for a long time prior to detection.

Veterinary Services, in collaboration with four large swine diagnostic laboratories servicing a large portion of the U.S. commercial swine industry, tested 2,000 samples by polymerase chain reaction (PCR) for Porcine deltacoronavirus (PDCoV). These samples represented multiple production types (Table 1) and were primarily in calendar year 2013, although several were collected in 2010-2012. They had originally been submitted to the laboratories for gastrointestinal disease, and were therefore high value targeted samples for identifying SECD. Although PDCoV was first reported in January 2014, the retrospective testing of archived samples revealed multiple cases in December, one in October 2013, four in August 2013, and none prior to August 2013. The earliest of these in August and October did not propagate beyond the initial few herds, possibly due to the warm summer and fall weather. More than 1,200 of the retrospective targeted samples were tested using PCR for PDCoV RNA between the August and October 2013 cases, and more than 600 tested between the October 2013 case and the epidemic curve in December 2013.

Table 1 Production type of swine in 2,000 PDCoV archived samples.

Production type	Percentage of samples
Grower finisher	14.2%
Nursery	18.1%
Sow/boar	5.1%
Suckling	22.4%
unknown	40.2%

The data for PDCoV after December 2013 demonstrate a similar exponential epidemiology curve as seen with PEDv (see Figure 2), typical of a point-source origin followed by highly contagious propagated spread (Smith, 1995).

An alternate school of thought is that the virus was silently circulating prior to and in-between these times and unobserved because it is clinically of lower virulence with milder signs than the initial PEDv outbreak. Thachil et al. (2015) presented data at the 2015 American Association of Swine Practitioners Meeting describing a new ELISA test for PDCoV. The authors state that retrospective serological testing of 395 banked samples submitted for gastrointestinal disease with the new assay “confirms that PDCoV has been present in the United States since 2010,” and is therefore supportive of suspicions that PDCoV has been present in the United States for some time (Thachil et al., 2015). The AASV presentation reported that the results are based on the test having 95 percent specificity based on a negative population cohort of 30 animals; the confidence interval was not reported. The report and validation data have not been published in the peer reviewed literature.

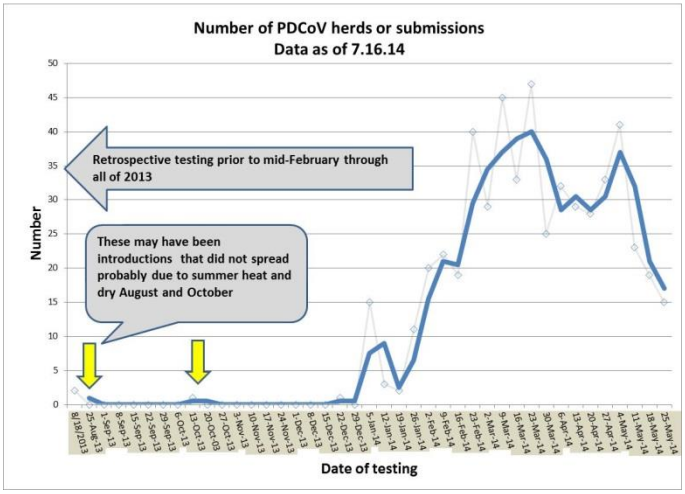

Figure 2. The epidemiology curve for Porcine deltacoronavirus accessions in 2013-2014 is similar in shape to PED in Figure 1. Three separate PDCoV events appear in the testing data, but it is unclear whether these represent separate introductions or an undetected circulation of PDCoV.

3. Descriptive and inferential epidemiology of initial cases

This section will review the basic epidemiology of the first few cases of PEDv that were detected in this country. With the exception of Stevenson et al. (Stevenson et al., 2013), there has been little published on facts surrounding the earliest cases. The RCG interviewed first responding veterinarians and producers in addition to revisiting affected farms.

***Summary of herd investigations**: The earliest swine herds identified with PEDv were revisited by USDA epidemiologists. Herds were also visited if there were unusual circumstances such as nurseries that became affected but source sow farms remained free of disease. Initial investigations in 2013 were conducted by company's consultant veterinarians and a herd survey was administered. The RCG acquired individual results of the surveys for each farm prior to revisits. Most were done face-to-face with additional communications by email or telephone as needed.*

The investigations found that no personnel or veterinarians had visited the different farms. They were owned by different companies and received feed of different brand names and manufacturers. Different feed mills prepared the rations and each used different sources of ingredients. There were no common trucks or vehicles owned or operated by the different farms. Where feed tickets and information were available, product names and lot numbers were collected and analyzed. There were no lots of vitamin or feed premixes that were in common to any two farms. No farm reported visitors from other countries immediately prior to the break and none had employees or consultants that had recently traveled out of the country. Feed mills frequently received ingredients in bulk FIBC ("totes") and in many cases delivered feed to farms in bulk.

In one of the first cases identified on April 15, 2013 in Ohio, clinical signs were observed in nursery pigs. Feed was suspected by the investigating veterinarian, but not proven to be the method of introduction. A second index case occurred in Indiana on approximately the same day, but clinical signs manifested in older growing pigs. Feed samples collected in May 2013 in response to this outbreak were negative for PEDv on PCR. Neither farm used the same brands of feed and was not linked by ownership, workmen, veterinarians, or visitors.

The first farm in Iowa (week of April 28, 2013) was, to the best of our knowledge, the first instance of piglets getting sick and dying (95 percent mortality). These pigs died within two days (Stevenson et al., 2013). Sows and gilts also developed diarrhea and would not eat. The authors report that four geographically separated herds were infected with genetically identical viruses within ten days, but were unable to identify any epidemiological link between them. Biosecurity on these farms was considered good to excellent.

Beginning May 9, 2013, and over the next week, six sow farms in Colorado developed clinical signs consistent with PED. Sows exhibited fever, vomiting and would not eat. Piglets died within 8 to 24 hours of birth. PEDv spread to nursery and finishing pigs as well. At the time of the initial investigation in June, the timing of the infections due to pigs, truck, and people movement was unknown. Contaminated feed was suspected as the source of introduction because only the pigs receiving rations mixed at one of two feed mills became infected. From May 15-25, 2013, complete feed and vitamin premix were sampled and tested yielding one PCR positive result. Further testing failed to confirm the finding and it was determined to be a false positive (UMN Veterinary Diagnostic Laboratory).

Feed has been implicated in several of the first outbreaks including strong relationship between feed and infection in a case control study; however, the association with feed does not rule out feed delivery vehicles or transport containers as the source of virus.

Feed was suspected in cases subsequent to this as well and became a focus of the first epidemiological study of multiple farms. This was a collaborative effort between the American Association of Swine Veterinarians (AASV), National Pork Board (NPB), National Pork Producers Council (NPPC), and VS' National Animal Health Monitoring System (NAHMS). Through the National Center for Foreign Animal and Zoonotic Disease Defense (FAZD),[3] a case-control study was conducted with 25 case and 18 control farms and completed by June 20, 2013. Univariate regression analysis on the probability of being a case (i.e., presence of PEDv RNA plus animals with clinical signs) revealed feed factors that were associated with higher odds of having PED. Feed that was custom mixed off-farm, increased number of meal/mash rations fed to nursery or finisher pigs, and whether vitamins and minerals were in the same as opposed to separate premixes increased the odds of PED on a farm between 1.5 and 3.5 times. These variables suggest the potential for contamination of feed where complete feed mixed off-farm is related to an effect where more ration types could mean more chances to get a contaminated batch. Additionally, when grain was mixed with an amino acid source and a base mix in sow feed compared to grain mixed

[3] Currently retitled: Institute for Infectious Animal Diseases (IIAD), a DHS Center of Excellence at Texas A&M University

with an amino acid source, salt, calcium, phosphorus, and a premix , the odds of being a case was 2.3 times higher.

4. Genetic epidemiology

Quantifying the relatedness of viral whole genome sequences (WGS) has been central to the attempt to detect the geographic origin and timing of divergence of PEDv and PDCoV isolates. In addition to the whole genome, there has been sequencing focused on several open reading frames that constitute four structural proteins of the virus. Of these, the spike (S), nucleoprotein (N) and membrane (M) genes have been examined for diversity (Snelson, 2014). Analyses of the genetic epidemiology of SECD viruses have focused on several key questions:

1) What is source of the emergent strains of SECD viruses in the United States?
2) What is the pattern and timing of genetic divergence of U.S. SECD viruses with potential foreign sources and within the U.S. outbreak?
3) What constitutes a new strain of the virus rather than just another isolate collected and sequenced?

For the purposes of this section, the term strain will be used synonymously with clade to refer to a grouping of isolates with sufficient genetic similarity denoting a common ancestor. The term isolate will be used to denote individual virus genomes collected from individual animals or from pooled samples from animals with the same exposure event. The determination of what is sufficient similarity is often made on a statistical basis.

What is source of the emergent strains of SECD viruses in the United States?

The genetic epidemiology to date provides evidence that the first isolates of PEDv had a single genetic ancestor closely related PEDv isolated in China in 2011 and 2012. The first sequence of PEDv appearing after the confirmation of PEDv in the United States in April 2013 was reported by Marthaler et al. (Marthaler et al., 2013). An isolate (called a strain by Marthaler), USA/Colorado/2013 (CO/13), was completely sequenced and found to have the highest identity with Chinese isolate AH2012 (99.5 percent sequence identity). AH2012 was an isolate from the Anhui Province in eastern China. Subsequently, Huang et al. (2013) sequenced three isolates from Minnesota and Iowa (called MN, IA1, IA2) that were obtained shortly after the first confirmed PEDv cases in the United States and determined they were closely related to AH2012 as well (99.5-99.6 percent identity). These three isolates together with other isolates from China formed a clade, termed Genogroup 2a, based on a complete genome sequence phylogeny (Huang et al., 2013). Phylogenetic analyses of whole genomes and several gene sequences from additional U.S. PEDv isolates further supports a U.S. clade of PEDv with a common ancestor related to isolates obtained during the recent Chinese outbreak (S. Wang et al., 2014) (Vlasova et al., 2014).

Genetic epidemiology studies indicate that the U.S. viruses are most closely related to strains reported to be in China in 2010-2012.

Phylogenetic analysis of PDCoV isolates from the United States and Asia also provide evidence of a common ancestor, with a whole genome phylogeny supporting a distinct U.S. clade sharing a common ancestor with isolates collected from domestic swine in Hong Kong in 2009 and 2010 (Marthaler et al., 2013; Woo et al., 2012). Relative to the phylogenetic relationship among PEDv clades from the United States and Asia, PDCoV and PEDv are distantly related and considered to be in different proposed genera of the *Coronaviridae* family with an estimated time of most recent common ancestry on the scale of thousands of years (Deltacoronavirus and Alphacoronavirus, respectively) (Woo et al., 2012).

Pattern and timing of genetic divergence of SECD viruses

Due to the impact of PEDv in China over the last decade, Chinese researchers have reported much sequencing work prior to 2014, and sequencing in the United States has increased since the 2013 outbreak. Most of the isolate sequences published to date have been from PEDv with few available for PDCoV. The remaining discussion will only deal with patterns of PEDv genome phylogeny because too few PDCoV isolates from Asia and the United States have been reported in the literature to identify detailed phylogenetic relationships.

Initial phylogenetic analyses of limited sets of PEDv isolates identified that U.S. isolates shared >99.5 percent nucleotide identity and had high support for a common ancestor (U.S. Clade) based on whole genome sequences, as well as sequences of the S, N, and ORF3 genes (Huang et al., 2013) (Chen et al., 2014) (S. Wang et al., 2014). Subsequently, researchers at the University of Minnesota and Ohio State University sequenced and analyzed 74 isolates (whole genome) in the United States to determine phylogenetic relationships among them (Vlasova et al., 2014). These isolates clustered into two distinct clades (termed North American Clades 1 and 2) with strong genetic evidence that these clades shared a common ancestor which, in turn, shared a common ancestor with PEDv isolated from the 2010-2011 outbreak in China. Vlasova et al. (2014) and (Oka et al., 2014) identified isolates from the United States with distinct insertion and deletion of nucleotides in the S gene (S-INDEL) that differentiated them from the grouping of all other United States PEDv isolates in North American Clades 1 and 2 (Oka et al., 2014). The origin of the S-INDEL clade is currently uncertain as its phylogenetic relationship with the United States clades and Chinese isolates differs depending on whether the entire genome or S-gene sequences are examined and the number of serial passages through in-vitro cell culture (Oka et al., 2014) (Vlasova et al., 2014). Analysis by Wang et al. (2014) suggests that the INDEL represented a recombination event in the S-gene between the AH2012 and another strain isolated in China (L Wang, Bryum, & Zhang, 2014)

Huang et al (2013) (Huang et al., 2013) analyzed three early isolates of PEDv from Iowa and Minnesota and isolates from the 2010-2011 Chinese outbreaks (Huang et al., 2013) and used a molecular clock analysis to estimate the time of divergence of virus strains (Drummond, Pybus, & Rambaut, 2003). They estimated that the most recent common ancestor of the Chinese and the three early United States isolates was 2007-2008, and the most recent common ancestor of the three U.S. isolates was 2011-2012.

A second molecular clock analysis with 120 PEDv whole-genome sequences (most from the U.S. outbreak isolated in 2013-2014; the rest from China, Mexico, and South Korea 2011-2013) was conducted at the DHS-NBACC. The investigators obtained sequences from GenBank and estimated the

most recent ancestor of the North American isolates at 2008 (+/- 5 years) and the most recent ancestor for the U.S. and Chinese isolates at 2004 (+/- 5 years). They found that the INDEL and original U.S. viruses were significantly different and that the mutations between them were not likely to have occurred in the time between identification of the first PEDv (April 15, 2013) and first INDEL (late May 2013) (DHS-NBACC, unpublished).

If the April 15th cases were indeed the index herds in the United States, these changes would have happened outside the United States (i.e., two distinct viruses entered the United States). The interpretation of both of these molecular clock analyses is currently very limited because of reported uncertainty estimates of divergence dates and because the time-scale of available isolates is very short.

What constitutes a new strain of the virus?

As of the end of 2014, there were considered to be three clades of PEDv in the United States. These included the North American clades 1 & 2 identified by Vlasova (Vlasova et al., 2014) and the S-INDEL clade (Hao, Xue, He, Wang, & Cao, 2014), (Tokach, 2014), (Vlasova et al., 2014). There has been increasing interest in sequencing the S (spike) gene as a way to measure variability among isolates rather than using whole genome comparisons. The S-gene was found to change even when passing through Vero cells in a laboratory setting (Lawrence PK, 2014). This research and the work of Chen (Chen et al., 2014) were twofold in impact: cell lines were needed for vaccine research and to gain insight into rate of change between earlier isolates so that time of entry of an "original" isolate into the country could be approximated . It was through sequence analysis of the S-gene of isolate OH851 that Wang (2014) declared a new variant that would be later termed the S-INDEL clade (L Wang, B Bryum, et al., 2014). Additional analysis of S-gene sequences in 2014 by the University of Minnesota has identified a new divergent set of S-gene nucleotide substitutions and a deletion in an isolate from Minnesota (Marthaler, Bruner, Collins, & Rossow, 2014). These S-gene changes are different from the S-INDEL sequences and cluster with the North American clade 2. Thus, while Marthaler et al. (2014) term this a new strain, it is an isolate with a most likely common ancestor shared with North American Clade 2, and may represent an early signal of a new clade evolving from novel S-gene changes during the U.S. outbreak.

5. Number of virus introductions

Three genetically distinct swine enteric coronaviruses that appear to have evolved outside the United States have been identified and described in scientific literature (LY Wang et al.) (L Wang, Byrum, & Zhang, 2014) (Stevenson et al., 2013). In addition to research publications, data compiled by USDA, and laboratory testing of archived samples show the earliest identified dates of outbreaks from these viruses as April 15, 2013[4]; June 5, 2013 (Vlasova et al., 2014); and August 9, 2013[5]. Additionally, as stated, data

> *At least three viruses appear to have arrived in the United States within a four month period in 2013. They may have come together or individually.*

[4] Retrospective testing of archived diarrhea samples at NAHLN laboratories
[5] Retrospective testing of archived diarrhea samples at NAHLN laboratories

from retrospective testing of 2,000 samples suggest identification points for PDCoV at August 9, 2013; October 6, 2013; and December 2013, although the possibility remains that it was previously circulating in populations showing milder clinical signs and samples not submitted.

Researchers evaluating the genetic epidemiology have postulated that the different SECD viruses likely came into the United States at the same or similar time (Vlasova et al., 2014), which is compatible with the clinical epidemiology data. However, it is unclear whether the entry of the three SECD viruses came in a single bolus of contamination, or arrived in the United States separately during the spring and summer of 2013. Likewise, it is unclear whether the three different appearances of PDCoV shown in Figure 2 were separate introductions or the result of circulating virus that had been previously undetected. Although testing data may have been inadequate to precisely pinpoint entry dates, the data described above suggest the possibility of multiple entries at very close to the same time. Further, SECD outbreak herds have previously been reported to be concurrently affected with more than one SECD virus (Ge et al., 2013; USDA-APHIS, 2015b); however, the earliest herds identified in the United States were only reported with a single SECD virus. If the single bolus introduction were correct, it would seem likely that the first outbreaks would have had dual or triple SECD virus infection.

6. Virus survival

Transit time of one to two weeks is required for sea cargo to travel from Asia to the United States. Processing of products at ports of entry and distribution to destinations take additional time. Combined, the time that a virus must survive from point of contamination to exposure of swine is at least two weeks and likely more.

SECD viruses remain infectious for prolonged periods when the temperature is low (e.g.; 4^0C) and may remain longer when in wet feed, cell culture media, or manure slurry.

In one study using a cultured cell model, PEDv was dried in a petri dish and reconstituted at various time intervals and temperatures. Viable virus was present for three but not four weeks at room temperature, and over 49 days at 4^0Celsius or -80^0Celsius. The samples at the cooler temperatures showed less than a two log reduction over the entire period (Nelson et al., 2014).

Follow-up testing for a survival study was initiated by VS to evaluate the stability of PEDv in FIBC material[6]. The woven fabric was spiked with a preset amount of cultured PED virus. Samples were taken weekly and evaluated in the cell model for viability. There was negligible reduction in infectious virus concentration in samples stored at either 4^0Celsius or -80^0Celsius at ten weeks. Infectious virus could be detected at five weeks but not six.

Studies have shown that feces-contaminated feed and water can carry PEDv. The virus remained viable in wet feed for more than four weeks, and one but not two weeks in dry feed. In drinking water, survival has been documented at two weeks and in recycled water, one week. Temperature and time studies to assess survival of PEDv in feed have been conducted at different levels of relative humidity. At 200°F

[6] Study was conducted collaboratively with South Dakota State University

and 30 percent, 50 percent, and 70 percent relative humidity, PEDV was inactivated in 7.2, 11.5 and 2.1 minutes, respectively (Verma, Erber, Goede, Morrison, & Goyal, 2014).

Feed components that are of animal product origin, such as hydrolyzed porcine protein (HPP) and spray dried porcine plasma (SDPP) can be contaminated with PEDv (Alonso et al., 2014). The latter has been evaluated after experimental contamination and heating to 200°C (temperatures similar to that used in its evaporative process processing step) or not (remaining in liquid form) and Vero cell passage. No SDPP samples remained infectious (Pujols J, 2014). In other studies, PEDv RNA recovered from SDPP was not infectious (Opriessnig, Xiao, Gerber, Zhang, & Halbur, 2014).

Initial research into PEDv survival in feces was done at the University of Minnesota. Feces were divided to simulate two groups: fresh feces as one might find above ground anywhere at swine facilities and slurry which may be found in collection pits beneath the facility or in another storage container such as a lagoon. Transmissible Gastroenteritis virus (TGEv), an *Alphacoronavirus* endemic to the United States, was used for comparison of survival times. At three environmental temperatures: 104°F, 122°F, 140°F and three levels of relative humidity: 30 percent, 50 percent and 70 percent, PEDv survived up to seven days in fresh feces at all three temperatures at 70 percent relative humidity. This is roughly half the time TGEv survived. In slurry, PEDv survived more than 28 days in 40°F slurry at 30 percent, 50 percent, and 70 percent environmental relative humidity. The result was the same at -4°F and 30 percent relative humidity. At 77°F survivability was halved at 30 percent and 50 percent relative humidity. It appears that PEDv can survive longer in a colder environment or in a liquid form (Verma et al., 2014).

7. Infectious dose

The Swine Vet Center in St. Paul, MN supplemented existing laboratory work with field trials to determine presence and infectivity of PEDv from deep pits in 30 barns in southern Minnesota and Northwest Iowa. In the trials, infectious PEDv was detected in 93 percent of barns six months after clinical signs of PED were in the herd and in 86 percent barns sampled four months after infection. Among the latter set of farms, pits from two barns held virus that could infect pigs by stomach tube administration in a dose of 20 ml. It is important to note that this 20 ml came from a volume of 1 million or more gallons, demonstrating that high dilution is no bar to infection (Tousignant, 2014). One researcher calculated that only a teaspoon of virus laden feces in 1,300 gallons of water is sufficient to infect swine (Henry, 2014).

The infectious dose of PEDv is extremely small, and exposure to a small number of virions is capable of infecting pigs.

Research at the University of MN (Goyal, 2013) was conducted in 2013 on PEDv to estimate the minimum infectious dose. The project used a small intestinal mucosal extract as a base and demonstrated viral RNA in inoculated piglets when dosed with 0.5 ml of base diluted 10^{-7} times. They further used intestine from piglets inoculated with 10^{-9} (billion-fold) dilutions to inoculate naïve piglets, and demonstrated viral RNA in the second set of piglets indicating active infection was present in the inoculated piglets.

Together, these studies demonstrate that the infectious dose of PEDv is extremely small, and exposure to a small number of virions is capable of infecting pigs.

8. Viral transference or transmission

Recently, research was published by Pipestone Veterinary Services that demonstrates proof of concept for the multitude of sources of infectious virus during a PEDv outbreak. Although the main focus of the research was to show that contaminated feed could infect pigs, preliminary on-farm assessments in the affected system found PEDv nearly everywhere they looked. These places included: concrete pads, farm personnel, vehicles, and walls of feed bins (Dee et al., 2014). Briefly, paint rollers were used to collect material from feed bins. The material tested positive by PCR, and was pooled and fed to three week old PEDv negative pigs. Clinical signs of PEDv infection and virus shedding were observed by day four post ingestion (Dee, 2014b). Another large veterinary clinic mixed a small PEDv inoculum in 12 tons of feed held in a 30 ton feed bin. Four days after beginning to feed (8 to 10 tons of feed dispensed) clinical signs were observed in breeding pigs (Yeske, 2014). Nugent et al. (2015) in a "state of knowledge" paper stated that lateral fecal contamination of feed components is more probable than vertical contamination where the virus survives processing (Nugent, 2015).

Lateral transmission via feed is a likely mode of farm-to-farm viral transmission but a questionable way for the virus to enter the country. Perhaps the most frustrating aspect of feed transmission is its unpredictability. Contaminants are not necessarily mixed within feed uniformly, and samples of bulk feed may not include the contaminated material. A bolus of contaminated feces may be inadvertently incorporated into a feed ingredient during manufacturing. Virus could survive within that bolus for a length of time depending on environmental conditions and ingredient characteristics. As the ingredient is manufactured into a final feed, the bolus may evade blending processes such that one individual animal consuming the feed could ingest the bolus while others remain uninfected. Such a singular event would be sufficient to ignite an industry-wide epidemic.

Vehicles used to haul pigs or feed may readily transfer virus from place to place, although not likely a mechanism for trans-oceanic travel. Nonetheless, the lateral spread studies in the United States suggest the ease with which products could become contaminated by truck traffic. Any part of the vehicle may be contaminated including the tires, floor mats, personal protective equipment, and pig-handling equipment. The initial vehicle contamination may occur at farms, feed mills, packing plants or any collection point. In one study during the peak of the U.S. epidemic, 575 trailers were sampled at six packing plants over a few days' time. PEDv was detected in 17.3 percent of the trailers on arrival, and for each contaminated trailer that arrived, an additional trailer was contaminated during the unloading process (Lowe J et al., 2014). Presumably, similar cross contamination of vehicles or cargo could occur in any country where PEDv was prevalent.

Airborne transmission of PEDv has also been theorized as a means of lateral spread. This type of spread is not a likely means for virus to travel from Asia, but may provide insight to ways that products imported to the United States could be contaminated at their origin. A large scale study in Oklahoma revealed that the direction of spread of the disease roughly followed the prevailing wind vector over the time of an outbreak. The investigators suspected transmission associated with air movement, and were

able to detect viral RNA in air as far as ten miles distant from an affected herd. The RNA laden samples, however, failed to infect pigs in the follow-up bioassay (Goede, Robbins, & Dufresne, 2014). The researchers followed up the field investigation with a controlled study where air samples were taken various distances from experimentally affected pigs. In this study, viral RNA was detected and shown to be infectious by bioassay. The investigators speculated that transmission was associated with air particulates, and that the arid warm environment in Oklahoma may explain the lack of infectivity in air samples, despite the presence of PEDv RNA (Alonso et al., 2014).

In a similar outbreak described by one of the swine consultants interviewed by the RCG, a group of Colorado farms otherwise isolated were affected along the path of predominant wind currents. The consultant's conclusion was that the virus was most likely carried in dust particles in air. Others have reported similar findings (Sun, 2014).

9. Feed comparison of United States, Canada, and EU (a virtual study)

A question that remains unanswered is why the outbreak occurred in the United States and not in other countries with similar commercial swine industries and importation trends. Consider three populations of pigs: United States, Canada, and EU. The large commercial industry is similar in each, and basic rations are comparable based on least cost products that provide highest performance. The sample size is very large in terms of pigs and farm units and likely accounts for minor variations in base rations.

China holds a large market share of vitamin and mineral pre-mixes, and most swine feed-producing companies purchase large volumes. Other feed components such as antibiotics, amino acids, enzymes are also often sourced from China based on cost (U.S. swine consultants-see interview summaries).

Two country differences in swine industries are total number of pigs and legislation in Canada and EU regulating use and species- specific labeling of feeds.

In spite of similar industries and swine feed rations, herds in the United States became affected in spring of 2013, while Canada and the EU remained free.

Two possibilities can explain the difference in status. Either there was one event that introduced all of the virus variants into the United States at one time; a one-time random event that affected the United States; or there were multiple introductions involving three different viruses over the four month period between April and August 2013. If the former, the United States has considerably more pigs and would have a higher probability of being impacted by a one-time event. If the latter, it is unlikely that only the United States would be affected multiple times by chance alone and the cause would then likely be unique to this country. Although it is unclear whether the viruses arrived all at one time, they were identified in different herds and U.S. locations in mid-April, late May, (Vlasova et al., 2014) and in mid-August 2013[7].

[7] Veterinary Services commissioned testing of 2,000 archived diarrhea samples that had been submitted to four major diagnostic laboratories prior to January 2014. Four herds were identified with PDCoV on August 9, 2013

In addition to number of pigs, a difference between the United States, Canada, and the EU exists in regulations. In the United States, feed mills and livestock producers may use pet food "scrap or salvage" material such as waste, damaged, or outdated pet food when prices are economically beneficial. Regulations in Canada prohibit this practice (CFIA, 2012), and the EU has strict labeling rules for feed ingredients appropriate for a given species (USDA-FAS, 2010).

Although pet food is used in U.S. swine rations, the RCG was not able to determine whether related products such as pet treats have opportunity to contaminate the salvage pet food products, processing equipment, or transport containers. The pet treat scenario section of this document describes possible mechanisms where viable PED virus could have contaminated pet treats from China and arrived in the United States.

10. Evaluation of U.S. CBP data

The RCG epidemiologists reviewed metadata files for CBP data (http://hts.usitc.gov/) and identified products that were believed to meet criteria as potential fomites for SECD viruses. These were products that could be contaminated in the source country with an ultimate use that might expose U.S. pigs (e.g., organic grains). Detailed CBP data were then accessed and risk-evaluated to further narrow the list of likely products. Those that had non-negligible risk are listed in Table 2, and were further assessed to determine if they had been imported to the United States during the first three months of 2013 prior to when the initial detection of PED occurred. Further evaluation was conducted to determine possible scenarios where the product could have facilitated virus transit through the four segments of travel from the origin country to end up in the initial locations where PEDv was initially identified. Product shipments were considered less likely to have resulted in the U.S. epidemic if the quantity was very small (e.g., a few kilograms), or if the product was consigned to companies in the western part of the United States, specifically those without nationwide distribution networks. Products were considered more likely when the consignor was located in the swine-dense geographic area of China near where the closest known ancestors of the U.S. viruses were reported in the international literature, and less likely if they originated in more distant areas of China.

Products identified from the CBP data that met these criteria as likely candidates were organic grains (e.g., soybean), pet treats, lysine, or containers (FIBC) that could have carried contaminated products. These are discussed in more detail under individual scenarios.

Table 2 provides a summary of products identified in CBP metadata that were deemed to be capable of carrying SECD viruses if contaminated. The risk evaluation of each is described in the respective columns. Those with non-negligible risk ratings were further evaluated to determine if the product was imported during the pre-outbreak months, and if it had been used in the herds that were investigated.

Table 2, page 1: Summary of CBP data risk evaluation

Risk of feed ingredients imported from China serving as a pathway for infecting swine in the U.S. with PEDV.								
Product Code	**Feed Ingredient**	**Imported from China 2010-2013?**	**Likelihood of swine, tissues or fluids contact with raw ingredients**	**Likelihood processing will not inactivate coronaviruses**	**Likelihood swine, tissues or fluids have contact with ingredient post-processing**	**Likelihood that virus survives transport**	**Likelihood that swine will be exposed to the ingredient**	**Overall risk**
1204.00.00.25	Organic flaxseed	No; imported from Canada only in 2012 and 2013 (Data not collected prior to 2012.)						Negligible
2309.90.10.35	Swine feed, prepared	No; only imported from Ireland between 2010 and 2013						Negligible
0511.99.40.30	Dried blood	No						Negligible
1501.20	Other pig fat	No; Canada and Cayman Island						Negligible
0209	Pig fat free of lean meat and poultry fat	No; Canada, Spain, and Mexico						Negligible
1503	Lard stearin, lard oil, oleostearin, oleo-oil, and tallow oil	No; Canada and Egypt						Negligible
0404.10	Whey and modified whey	No; Canada, EU, Mexico, others (not China since						Negligible
1005.90.20.15	Organic corn	No; Argentina, Brazil, Canada, Romania						Negligible
3004.50.5005	medicaments containing vitamins/ products for veterinary use	No; Canada, Europe, Australia						Negligible

Page 2: Risk of feed ingredients imported from China serving as a pathway for infecting swine in the U.S. with PEDV.								
2930.40.0000	Methionine	Yes; on average 200 metric tons annually between 2010 and 2013	Negligible; chemically synthesized from sulphur, methanol, ammonia, propylene, sulphuric acid	Negligible; manufacturing involves heat, pressure, and extremes of pH	Low; Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean vessels, and stored for weeks to months until need to mix ration (2 year shelf life for product)	High; commonly used to balance swine rations	Negligible
1201.90.00.10	Organic soybeans	Yes; 49,000 metric tons in 2012 and 57,000 metric tons in 2013. (Data not collected prior to 2012.)	Low; Possible contact on farm, bins, vehicles, other equipment	High; Beans may be imported raw	Low; Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean vessels, and stored in bulk bins until need to mix ration	Moderate; Imported organic soybeans are used for human consumption and for animal feed. Data are unavailable to estimate proportion going to each intended use.	Low
1109	Wheat gluten	Yes; 4400 metric tons on average between 2011 and 2013	Low; Possible contact on farm, bins, vehicles, other equipment	Low; Gluten is manufactured via washing, water separation, and hot air drying to >90%DM	Low; Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean vessels	Low; Most imported wheat gluten is used for food for human consumption due to cost of ingredient and lower cost protein sources	Low
1507	Soybean oil	Yes, average of 5 metric tons annually from 2011-2013	Low: Possible contact on farm, bins, vehicles, other equipment	Negligible; manufacturing process involves high temps and chemicals [flaking, extraction, solvents, oil separation, hexane removal, and evaporation]	Low to medium if processing of oil for human use is co-located with animal soybean meal production, if processors bake the high protein fiber left after the oil is removed and sell for animal feed	Low; Typically shipped via ocean cargo vessels	Low to moderate; oil for human consumption (soy milk, soy flour, tofu, etc.), oil for cooking and other edible uses, or sold for biodiesel and industrial uses.	Low

Page 3: Risk of feed ingredients imported from China serving as a pathway for infecting swine in the U.S. with PEDV.								
3503.00.55.10	Edible gelatin	Yes, average of 3,900 metric tons annually between 2010-2013.	High; obtained from pigskins, cattle hides and bones	Negligible; processing involves degreasing (hot water), roasting (30 min at 200F), acid/alkali (4% HCL), boiling, and sterilizing (375F 4 sec)	Low; Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean cargo vessels	Low; gelatin is used primarily for human consumption, some used as a meat preservative	Low
2309.90.1050	Mixed feeds for animal feeding (containing milk products)	Yes, average of 26,000 metric tons between 2010-2013. However, most of this category is pet food. No APHIS permits for swine feed from China have been issued in at least the last three years.	Low to medium; Possible contact on farm, bins, vehicles, other equipment	Neglible; processing involves heat, pressure and/or steam, common feed processing includes grinding, pelleting, extruding and roasting	Low: Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean cargo vessels	Low; most feed in this category is pet food. Exposure not been documented, although possible through waste pet food feeding.	Low
2309.90.70	Vitamin B12 for animal feeding	Yes, 2 metric tons in 2012	Low; fermentation process to extract bacteria	Negligible; manufacturing involves anerobic fermentation, 2nd step aerobic fermentation, 3rd replace part of volume with fresh culture medium, repeat all at least once	Low; possible incidental contact with vehicles and other equipment	Low; Typically shipped via ocean cargo vessels	High; commonly used to balance swine rations; [Historically, feed samples positive for infectious virus have rarely been documented]	Low
2922.41.0090	Lysine	Yes; increasing from 8,000 metric tons in 2010 to 19,000 metric tons in 2013	Low; Plant carbohydrate source (often corn) used as feedstock for mictrobial fermentation	Negligible; manufacturing involves, heat, microbiological kill step, filtration, drying	Low; Possible incidental contact via vehicles and other equipment	Low; Typically shipped via ocean vessels, and stored for weeks to months until need to mix ration (2 year shelf	High; commonly used to balance swine rations	Low

Scenarios

The RCG evaluated several scenarios that describe how SECD could have moved from an origin country to infect pigs in the United States. For a scenario or combination of scenarios to be plausible, it must explain how contamination occurred in the origin country, how the virus was transported to the United States, how it was dispersed across geographically diverse locations in a very short time, and finally how it arrived at the index farms to infect pigs. Each step along this pathway is essential and all must have happened successfully for the outbreak to occur.

1. Flexible Intermittent Bulk Container (FIBC aka "tote") as a fomite

Feed totes (FIBC) are large container sacks that are commonly used to transport bulk animal feed as well as many other products. A commonly used variety is made of woven polypropylene and may also have an internal liner. The interior of the FIBCs are designed with reinforcing material, various folds, and exit chutes as well as protected area between the woven fibers that could provide protection from environmental conditions such as flushing by product, desiccation, heat, and UV radiation from sunlight. In the United States prior to the SECD epidemic, they were frequently reused, and are not likely to have been cleaned or disinfected in a manner to eliminate viruses. In addition to reuse at feed mills, recycled FIBCs are available for sale and may be purchased for use with any number of products (Figure 3 taken from: http://www.repurposedmaterialsinc.com/store/products/used-tote-bags-bulk-bags/). Similarly, reusable FIBCs are advertised for sale in other countries (www.alibaba.com) for a multitude of uses.

The Root Cause Investigation found that most U.S. feed mills receive products and transport feeds in FIBCs including soybeans, scrap pet food, grains, or bulk feed. Likewise, products are often shipped to the United States in them, including bulk organic soybeans and prepackaged pet treats. Because they are widely used for many products, and reused for other products, they may function as fomites (mechanical carrier of virus) for SECD viruses. For example, Figure 4 shows an advertisement for FIBCs on http://alibaba.com, a large distribution company with a worldwide customer base. The image shows FIBCs being used for bulk transport of wood shavings in an open sided warehouse.

There were no Federal regulations prohibiting reuse of FIBCs for importing products in 2013, although the number of imported products that arrive in recycled FIBCs is unknown. Inspectors at U.S. ports visually inspect the FIBCs for overt contamination, but are not likely to detect less obvious contaminants or viruses (personal communication, APHIS-PPQ official). California, Idaho, Michigan, Minnesota, Nevada, New Mexico, and North Dakota have state regulations pertinent to use of feed containers.[8] The FDA Food Safety Modernization Act of September 2015 requires animal food facilities, required to

[8] http://www.cdfa.ca.gov/is/ffldrs/feedlvstkdrugs.html
http://legislature.idaho.gov/idstat/Title25/T25CH27.htm
http://www.michigan.gov/documents/mdard/Michigan_Commercial_Feed_Law_amended_2015_496531_7.pdf
https://www.revisor.mn.gov/statutes/?id=25&format=pdf
http://agri.nv.gov/
http://www.nmda.nmsu.edu/wp-content/uploads/2013/10/New-Mexico-Commercial-Feed-Act.pdf
http://legis.nd.gov/cencode/t19c13-1.pdf?20150901114632

register with FDA as food facilities, to develop a food safety plan and perform a hazard analysis to identify known or reasonably foreseeable hazards associated with the animal food and the facility.[9]

Figure 3. The following is a product description from an advertisement of recycled FIBCs for sale. Note the diversity of products and large number of uses suggested for them.

Advertisement for recycled totes available in the United States for sale (site accessed 2/6/15):

"…These extra tough, Tyvek-like Bulk Bags can be hung in place or moved around with a forklift, Bobcat, etc. In their previous un-used life, they were able to hold up to 2,000 lbs of bulk materials. We have access to sacks that can be completely closed or ones that are left open like a typical sack. They don't tear easily, but can be cut with scissors or a knife if needed. The extra-large FIBC bags sacks are breathable, yet impermeable to liquids…

"**Possible Repurposes:** Landscape Debris – Compost Carriers – Large Sand Bags for Levees – Used Flexible Intermediate Bulk Containers - Used FIBC Bags – Used Bulk Bags – Used Polypropylene Bulk Bags – Used One Ton Sack – Used One Ton Bag – Used Tote Bag …"

Figure 4. FIBC "totes" are commonly used, tough, versatile bags made to transport many bulk products but are not specifically designed to exclude environmental contaminants that could harbor viruses. This image shows totes filled with wood shavings in an open sided warehouse. Products carried in them could easily be exposed to bird traffic, flood water, or other sources of virus.

[9] *http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm366510.htm*

FIBC: Contamination in Source Country

We can only speculate on how an FIBC may have been contaminated, but recognize that it would be plausible for a FIBC to carry a contaminated material and later be used to carry another product. Blomme et al. (2014) describes transmission of PEDv by European starlings, which can easily contaminate any material by their droppings that is in open and unprotected containers (Blomme, 2014). The wood shavings shown in Figure 4 are an example of one type of material that could easily contaminate FIBCs. Other products that could contaminate FIBCs would include grains, fertilizer, compost, animal parts, or bulk rendered products. An additional opportunity for FIBCs or products to become contaminated is through untreated water either used for washing or accidental exposure to wastewater from a crop or animal farm.

An alternative pathway for FIBC contamination would be via contaminated products transported in the FIBC and imported into the United States. After the product is removed, the FIBC could remain contaminated and its reuse would potentially transmit virus to the next product it contained. The second product would then arrive at a feed mill or farm to infect pigs.

FIBC: Introduction into the United States

FIBCs carrying a variety of products could become contaminated; however, they would need to provide a hospitable environment for virus survival and later carry a product slated for use on a pig farm. Virus survival studies are described in detail in the epidemiology section of this report, but most intriguing is a report by Nelson et al. (Nelson et al., 2014). The investigators added cultured virus in culture medium to a petri dish. They started with a known concentration of virus and measured infectivity over time and temperature with a PEDv cell culture model. Virus survival in the dry petri dish was between three and four weeks at room temperature and only a log 10 reduction by 49 days. Similar results were seen in data from a follow-up study conducted by USDA in collaboration with South Dakota State University (see virus survival section of document). This was in a cell model, but suggestive that field virus could last for several weeks within the protective weave of the FIBC and presumably longer at lower-than-room temperature during transit on a cargo ship.

FIBC introduction scenario:

1. *FIBC contamination in the source country,*
2. *Carry products into the United States and provide protection to the virus from environmental conditions,*
3. *Used by companies for delivery of many products to diverse geographic locations,*
4. *Reused to deliver bulk feed to swine operations*

FIBC: Dispersion within the United States

Based on retrospective laboratory testing, after arrival in the United States, SECD viruses appeared rapidly in at least six farms in two weeks. On investigation, there were no epidemiological links found between the operations and no common production types, product brands, or ration ingredients. One common factor that joins feed mills across the Midwestern part of the Nation is the use of recycled products and associated transport companies (see transport scenario for detail). Products such as dried

distiller grains, soybean hulls, salvage human food products, scrap pet food, and others are often used in rations when economically feasible. The transport networks cover multiple states and do not necessarily have dedicated trucks or FIBCs for hauling any product.

FIBC: Pig exposure

Feed mills that formulate swine rations receive and process ingredients using various types of equipment, such as grinders and mixers, and send the final product to farms. Investigations have shown feed to be a potential vehicle for SECD, and feed mills a possible transit point (Dee, 2014b; McCluskey, 2014; Yeske, 2014). Exposure in the FIBC scenario could happen easily due to contaminated finished ration or a contaminated FIBC reused to deliver feed.

2. Recycle/transport/warehousing network scenario; dispersion in United States

Several companies in the Midwest provide valuable services to swine producers by recycling various products and by-products for use in formulating rations. Although no fault was identified or suspected in biosecurity practices and operations of the companies, they inadvertently provide a mechanism that may quickly move the highly contagious SECD viruses to many locations. Company websites advertise trucking networks that service areas having a radius of several hundred mile; areas that easily encompasses all of the early SECD affected farms. In addition to trucking, they often provide services for trading grain, feed ingredients, by-products, and recycled human food products.

Salvage product companies deliver products on a routine basis to feed mills across the Midwestern United States. Although no fault was found or suspected, the delivery networks provide a mechanism for rapid movement of bulk products to many locations.

Warehousing facilities are available as well as multiple kinds of trucks, trailers, and rail delivery. With a large volume of trucks and trade, they have opportunity to visit many locations in a short time.

The recycle scenario describes a mechanism for rapid dispersion of virus to many locations. In the scenario, contaminated product or contaminated FIBCs would have been warehoused temporarily, and then shipped to feed mills for recycling into swine feed. Because of the efficient network, this may occur within a few days to weeks.

3. Pet treats

In this scenario: Pet treats (chicken jerky, pork, pig ears, or other animal/ plant origin treats) are contaminated in the origin country post-processing. The virus load is not adequately inactivated by irradiation or travel time. Treats arrive in the United States and are processed in a pet food plant for sale. Scrap pet treat material contaminates waste pet food when dispersed with salvage products or when reusing FIBC (totes) for transporting the material. The salvage material is warehoused and/or resold for swine rations by companies that specialize in food product recycling. FIBC containers are used to transport the material, are contaminated, and subsequently reused to carry any of a variety of products eventually to pig farms.

Pet treats are not manufactured for swine feed, but provide a protein source that could be combined with waste/salvage pet food and repurposed into swine feed. There are two reasons that this might have happened in the United States and not Canada or the EU. First, use of pet food including pet treats as a swine ingredient is allowed in the United States; both Canada and the EU prohibit this practice. Second, the United States is the primary distribution market for pet treats manufactured pet treats in China (FDA-CVM, 2012a, 2012b). Although the U.S. Swine Health Protection Act[10] provides strict regulations for feeding recycled food to swine, it exempts processed products (e.g., those that are precooked). In the pet treat scenario, the contamination would occur post-processing.

Pet treats scenario:

- *Cross- contaminated after cooking.*
- *Irradiation designed to eliminate bacterial contamination is inadequate for virus*
- *After entry to the United States, scrap material transported in FIBCs*
- *Contaminated FIBCs then reused for other feed products*

During 2012 and 2013, the FDA investigated pet deaths that were associated with various types of pet jerky treats. In January 2013, the FDA issued a warning to pet owners cautioning them on the use of Chinese origin treats. Although the FDA has tested for various toxins and bacteria, the exact cause of animal death has not yet been identified and the FDA investigation and testing continues today (FDA-CVM, 2013). In January 2015, FDA in collaboration with USDA began a pilot study to test pet treats from China to determine if they had been contaminated by PEDv. FDA tested approximately 40 jerky pet treats that had been archived in 2013.[11] No virus was detected.

Pet treat: contamination

A large portion of pet treats used in the United States prior to 2013 originated from manufacturers in China. CBP data indicate that approximately 4.6 million kilograms (4,600 metric tons) of various types of pet treats were shipped to the United States in the first quarter of 2013. The consignors were companies with website addresses located in areas adjacent to the swine dense parts of China near Anhui, Shandong, and Henan provinces, where the closest ancestors of U.S. strains of SECD virus were first reported (Huang et al., 2013; Zhengfeng et al., 2013). The companies reported to FDA that their product source locations for raw material (meat and animal parts) include Shandong, Henan, and adjacent provinces (FDA-CVM, 2012a, 2012b). Many companies advertise treats made of pig parts as well as chicken jerky and sweet potato, although it is unknown whether opportunities for commingling of raw material may happen in trucks or manufacturing facilities.[12] Finished products included chicken jerky, sweet potato treat combinations, pork, “meat treats”, pig ears, rawhides, and others.

[10] Federal Register: http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/shp_garbage_feeding_final_rule.pdf

[11] Dr. Yancy laboratory group, FDA, Center for Veterinary Medicine

[12] Search alibaba.com for example of variety of products available

FDA inspected five pet treat manufacturing plants in 2012 because of pet deaths associated with their products. We do not have specific data on biosecurity practices that may have been implemented in trucks carrying the raw material for pet treat manufacturer, but we assume from the experience in the United States that it is likely for trucks to move the virus if visiting contaminated farms or locations (Lowe J et al., 2014). FDA inspections of pet treat manufacturers indicate that trucking of raw and finished products are frequently done by third party companies and therefore, are not dedicated to a specific product or source location. The FDA reports also indicate that material is transported in "plastic bags inside woven bags" (FDA-CVM, 2012a, 2013). Presumably these are FBICs. It is likely that transport vehicles would have opportunity to contact affected swine farms during 2012-2013 because the prevalence of SECD was widespread and high in these areas (Feng, 2014).

Biosecurity practices in the plants are described in FDA reports (FDA-CVM, 2012a, 2013) and appear suitable to exclude gross contamination and bacterial pathogens, but are unlikely to control the spread of the highly contagious SECD virus. For example, employees that enter the semi-clean or clean areas are required to wash their hands and step through a boot bath; procedures which have been inadequate to prevent U.S. swine herds from becoming affected. The cooking process would likely inactivate viruses in raw product; however, there are opportunities for cross contamination from raw to finished product before packaging.

Most treats exported to the United States are cold-pasteurized after cooking prior to export. According to FDA inspectors who are familiar with the facilities and processes, finished products are usually irradiated with doses of gamma radiation similar to FDA standards for pasteurization; i.e., approximately 5-9 kGy. This is a voluntary, not mandatory process, and is designed as a pasteurization procedure and not adequate for sterilization.

Sullivan et al. (1971) conducted studies to measure the radiation required to reduce virus infectivity by one D-value[13], and reported D-values for nine virus genera that ranged from 3.9 to 4.6 kGy (0.39 to 0.46 Mrad) (Sullivan, Fassolitis, Larkin, Read Jr, & Peeler, 1971). Assuming all equipment functioned properly, the pet treat irradiation would provide a one to two D-value reduction (ten to one hundred-fold). This is unlikely to eliminate virus contamination when considered in terms of the very low infectious dose of PEDv where a 10^{-8} dilution was able to infect pigs (Goyal, 2013). However, all equipment in the irradiation facilities may not function properly since there is no regulatory oversight of the facilities and no batch monitoring. These and other factors were described and cited as issues that resulted in failure of approval of the irradiation facilities by EU standards (DG(SANCO), 2009).

Pet treats: entry into the United States

Customs and Border Protection data report approximately 4.6 million kg of treats imported from China to the United States in the first quarter of 2013. Of these, many went to consignees in the Midwest. In January 2013, FDA issued a warning to consumers related to the pet death issue (FDA-CVM, 2013) which prompted some companies to switch sources to pet treats sourced within the United States. It is unknown if there was an excess of unsold pet treats, or if so what the disposition of them was.

[13] A D-value is a measure of reduction in concentration for a bacterial or viral agent equal to one $\log_{10}$. This represents a 90 percent decrease in the amount of agent present in the material.

Pet treats: dispersion and exposure of swine herds

Although the pet treat scenario describes plausible mechanisms for pet treats to carry infectious SECD viruses into the United States, cross contamination of pet food by pet treats is speculative. Due to the extremely low infectious dose, cross-contamination could occur by means unsuspected for other disease causing agents. Mechanisms might include: Reuse of FIBCs used to haul damaged or scrap material from treats; incorporation of waste material from pet treats into pet food salvage products; or contamination of mixing or grinding equipment used to process salvage product for pig feed.

Although some swine operations and feed mills use salvage pet food in rations, none of the rations fed to the pigs that were first affected with PED included pet food; however, the feed mill serving the earliest case in Ohio was utilizing salvaged pet food in other pig diets for the farm. If pet food was indeed capable of carrying PED virus, the possibility of cross-contamination of feed or transit containers such as FIBCs could be a source of the virus in the outbreak. Some salvage/warehousing/transport companies advertise that they specialize in recycling pet food and have large distribution networks across the Midwest (see Recycle/transport/warehousing network scenario). The salvaged pet food is often transported in FIBCs (personal communication with VS officer investigating early farms) to destination feed mills.

For contaminated pet treats to represent a viable scenario for SECD spread, distribution would need to have occurred rapidly across multiple States by contaminated trucks or FIBCs used to transport the recycled treats. The trucks or contaminated FIBCs would have been unloaded at feed mills servicing the respective farms. At this point, either contamination of feed handling equipment or rations to be delivered or reused FIBCs would carry virus directly to the farms.

4. Organic Soybeans

The scenario is: Soybeans are contaminated with swine manure in the origin country → shipped to the United States → contaminate a secondary fomite such as FIBCs, or contaminate other feed, or by-product soybean hulls used in are swine ration → the FIBC is reused or a secondarily contaminated ration is delivered and infects pigs.

The United States is a major soybean exporter, but due to high demand for organically grown and non-genetically modified organism (GMO) beans, the U.S. imports organic soybeans from several countries including China. For the period in 2013 between January 1st and April 15th, imports from China totaled approximately 31,000 metric tons of soybean and 1,000 tons of soy flours. These were imported for animal feed and human products. The largest proportion of this went into organic chicken food; however, a significant amount was used directly for organic swine feed or indirectly as by-products (soybean hulls) that may have been used swine rations (Note: not all of the early farms investigated used soybean hulls).

Soybean: contamination at origin

Organic soybean production requires non-synthetic fertilizers. Schmitt and Rehm (1992) describe procedures and recommendations for use of swine manure to fertilize soybean and other crops.

Methods of application of liquid manure include broadcasting, injection, and irrigating (Schmitt & Rehm, 1992). Because of the very low infectious dose and multiple routes of virus movement (see detailed description in epidemiology section), contamination of the beans, or FIBC containers carrying them may occur at various points including via machinery, trucks, and water supplies. Specific soybean production data from China were not available; however, USDA officials familiar with marketing practices described a wide variety farming practices that exist in China. Many smaller farmers deliver crops and animals to market in small trucks or trailers and these may be used for multiple purposes. China encourages use of pig manure on crops to help control widespread contamination of waterways by pig manure. (Personal communication with USDA officials- Economic Research Services).

Although contamination is plausible, exact mechanisms are only speculative. Of note: the USDA National Organic Program Standards prohibit direct application of untreated manure to soybean crops within three months of harvest.[14]

Soybeans: entry to the United States

The United States imports organic soybeans in bulk from both India and China. Although port inspections are thorough for pests and invasive plant species seeds, inspectors are not able to screen for viruses, and can only visually inspect for gross contamination (personal communication, APHIS-PPQ, Port officials). Import shipments may take two weeks or more for trans-oceanic travel. Temperature and humidity conditions would likely be favorable during transit in the springtime, and soybeans are oil laden, which may provide a matrix that would enhance survival of SECD virus.

Soybean: Dispersement and exposure of swine herds

Soybean scenario:

- *Organic soybeans imported into the United States become contaminated at their origin,*
- *The index farms were <u>not</u> organic farms and viruses do not survive soybean processing so not likely a direct source of infection*
- *Either soybean FIBC is cross contaminated or contaminated soybean hulls used in ration*

The early-farm outbreaks were not organic swine farms and all used domestically grown soybeans due to cost. Because soybeans processing requires heating for extraction of oil or processing of flour and feed, any movement of virus to different locations in the United States would likely require cross-contamination of another product or fomite. Nonetheless, some of the first detected farms did use soybean hulls as ration filler, and nearly all included one or more product that would have been delivered via a salvage/transport network company. There are several possibilities for dispersement, although all are conjecture based on common practices.

In the first quarter of 2013, some soybeans were delivered in FIBCs to mills for use in organically produced poultry or swine. When reviewing websites of companies that imported organic soybeans, it

[14] http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateN&rightNav1=NOSBlinkNOSBMeetings&topNav=&leftNav=&page=NOPOrganicStandards&resultType=

was apparent that many also handled non-organic feed products, and it is not unlikely that FIBCs would be reused for various feed products. Alternately, the contaminated bean hulls could be recycled into swine rations and contaminate grinders or mixers.

Other organic soybeans were shipped to human food wholesalers in 2013 for processing into flour, oil, tofu, or other products. The destinations were located in the Midwest. Soybean hulls have little if any use in human food and would likely be recycled through one of the salvage/warehouse/transport companies (see scenario for these companies). Exposure of swine herds would occur through feed deliveries and involve either virus carrying FIBCs or contaminated feed.

5. Feral swine SECDv reservoir

Scenario for feral swine

In this scenario: Virus enters the United States through human food waste, landfill contamination, or other means. Feral swine become affected and the disease circulates through this population for a period of time without detection. The feral animals have little if any contact with larger swine operations and only occasionally contact smaller farms. Farms with only a few pigs may be less likely to submit samples and may not recognize or report the disease. The large commercial farms that were identified as the first cases would then have been exposed via trucks, birds, fomites, SDPP, or visitors who had contact with either feral pigs or pigs exposed to feral pigs.

Because the first PEDv proved to be very virulent, its unnoticed circulation in swine prior to April 2013 would be unlikely unless in an isolated population. Detecting PEDv in feral swine samples archived prior to April 2013 would suggest that the initial introduction into the United States was earlier than indicated by reported test data, and that the path of introduction differed significantly than what might be expected in commercial swine.

Feral swine disease reservoir:

- *For SECD to have been circulating in the United States prior to April 2013, it would likely have been in isolated populations; e.g., feral swine.*
- *Serological testing of archived samples did not identify evidence of SECD viruses.*

To test the hypothesis of whether there was a reservoir of PEDv in feral swine, VS conducted a study in collaboration with APHIS-Wildlife Service and ISUVDL to further investigate this possibility. The purpose of retrospective testing of feral swine was to produce evidence to inform the hypothesis that SECD-related virus was circulating in feral swine prior to the initial detection of clinical signs in domestic swine in April 2013. Testing of the samples was conducted with a Whole Virus ELISA for PEDv recently developed by ISUVDL. Initial performance testing on domestic swine indicated that the diagnostic specificity was 98.5 percent and the sensitivity was greater than the indirect immunofluorescence assay (IFA) from 2-7 weeks post inoculation (diagnostic sensitivity point estimate 99.2 percent). Serum samples from 368 feral swine were provided from the Wildlife Services Wildlife Disease Program archive for fiscal years 2011, 2012, and 2013. The samples were collected opportunistically from various locations in Iowa, Indiana, Michigan, Ohio, Illinois, and Hawaii. Although considerable uncertainty exists in estimating feral pig population numbers, six post-

hoc groups of pigs were identified by VS and WS biologists. With the assumption that sampling was proportional to population size and the sampled pig units represented 25 to 90 percent of the population in each group, estimates of minimum detection levels ranged from 5 to 30 percent. All results were negative, and provide no evidence to support the hypothesis that PEDv was present in the United States prior to the first detection in domestic swine in April 2013.

The first positive test accessions for PEDv in commercial swine were recorded in April 2013 in Ohio, Indiana, and Iowa. These three States were also among the first to have positive test accessions recorded for PDCoV. Additionally, the timing of six commercial farms breaking with PEDv infection over a two week period would make direct feral swine exposure of herds unlikely unless the source was feral swine blood finding its way into SDPP. Later investigations were unable to identify feed products containing SDPP that were common to the early farms. While these data do not entirely rule out the feral swine scenario, they make it unlikely.

6. Birds as virus carriers

A scenario where birds carried the SECD viruses to the United States was considered. Airborne spread is an unlikely means for PEDv to have entered the United States, but Blomme (2015) presented evidence that flying birds have the capability of transmitting the virus. Three nursery sites within a few miles of each other broke with PEDv the spring of 2014. Traffic on and off the site and feed were ruled out as the transmission mechanisms. A migration of red wing blackbirds arrived about two weeks before the outbreak. The bulk bin feed lids on all sites were coated with birds' feces, and at one site the feces tested positive for PEDv RNA. It was noted that for some of these sites, lids had been open so contamination with bird feces could have happened directly or later when the lids were opened (Blomme, 2014).

These species do not migrate from Asia, and the species that do migrate follow a long route through Alaska and Canada. The length of transit time would make virus survival unlikely for mechanical transmission. An alternate scenario where the virus moves from pigs to migratory fowl, to other bird species, and then back to the porcine species is also unlikely because of the adaption necessary for virus to cross species lines and back again (Dr. Darrel Styles, personal Communication).

Birds do provide an interesting possibility for contaminating products in open containers in the origin country, and moving virus locally as described in the FIBC scenario.

7. Semen or live animals

Semen may be a medium of transference as well. PEDv RNA has been reported in swine semen (Sun, 2014), and has the potential for transmitting virus. Two reasons make this an unlikely means of entering the country:

- The United States prohibits import of live animals or swine semen from countries with foreign animal diseases including China and Southeast Asia.
- None of the early herds that were investigated had record of any imported semen or new entry of pigs to the herd.

8-10. Introduction by humans

Postulating introduction of SECD viruses into the United States by people provides several compelling scenarios; however, there is little data to support this actually happening. International movement of people in the swine industry and veterinary professions has been substantial for the last decade as China modernizes its swine industry, but there was no known change in numbers of travelers before or after April 2013. It is possible that veterinary consultants and swine industry professionals coming to meetings in the United States, or individuals buying swine breeding stock were in contact with affected pigs before traveling to the United States because of the high prevalence of the disease (Feng, 2014); however, several of the swine consultants that frequently travel to China were interviewed by the RCG, and explained that travel biosecurity measures have been adequate to prevent disease introduction in the past. Standard practices in the U.S. commercial swine industry for visitors or employees include down time after travel, isolation of visitors from pigs, and segregation of clothing (personal communication swine consultants).

Introduction of SECDs by people is possible, but there is little evidence to support this as a likely scenario.

PEDv was able to enter farms that were considered to have excellent biosecurity through unknown mechanisms (Stevenson et al., 2013), and infect farms that had been able to exclude other contagious viruses such as PRRS (personal communication swine consultants). In these examples, there was no history of visitors or travel to foreign countries.

Human nasal passage carriage scenario

One scenario that has been suggested is the possibility that swine enteric coronaviruses may inhabit human nasal passages after a veterinarian or industry person visits an affected farm. There have been no reports of SECD viruses affecting humans; however, there have been reports of antigenic cross-species reactivity and cross-species infection by other coronaviruses (Han, Cheon, Zhang, & Saif, 2006; Li, 2013). Although a possible route for introducing SECD to the United States, the question remains as to why this would happen during spring and summer of 2013 and not before, and not in Canada or EU countries with similar movement patterns for international consultants and industry representatives.

USDA funded a study through the University of Minnesota to further investigate the question of PED virus persisting within human nasal passages. As of August 10, 2015, the study had not been concluded.

This scenario provides a plausible route of contamination in the origin country, mechanism for entry into the United States, and potentially, a means of infecting pigs. However, it does not explain the dispersion of virus across several states. Neither the farm consulting veterinarians nor Root Cause follow-up investigations of early farms identified visitors or company travelers that may have exposed pigs.

Human: Intentional introduction

Intentional introduction is possible but there is minimal evidence of a person directly infecting herds. First, most of the first-identified herds had excellent, or at least good biosecurity practices for visitor control. None of the investigations or consultant interviews identified visitors from other countries or

unusual events associated with outbreaks that might suggest intentional exposure of swine, while much of the evidence collected suggests an association with feed or feed delivery.[15] Although the sudden appearance of disease in multiple farms at close to the same time might suggest an intentional introduction, access of a route for direct introduction to the farms by a person seems unlikely. Although someone contaminating a central location such as a feed distribution network as described above is possible, simpler explanations seem more likely.

Human: Contaminated clothing/shoes

Contaminated clothing or shoes provide similar caveats as the previous two scenarios. There were no known visitors to the farms or employee travel to other countries near the time of the herd outbreaks. One might postulate that that someone contaminated a product in a distribution network (see #2) or that the first identified farms were not actually the index cases; however, the investigation did not find evidence to support these possibilities.

11. Spray Dried Porcine Plasma (SDPP)

Spray dried porcine plasma used in swine started rations has been implicated as the source of the PED outbreak that occurred in Canada in late January 2014 (Pasick et al., 2014). The RCG evaluated SDPP as a potential source of introduction to the United States, but ruled it as unlikely for two reasons: 1) There has been no recorded importation of this product for the last decade (USDA-APHIS, 2014). 2) Herd investigations did not identify SDPP as an ingredient in the ration for several of the first herds.

12. Release from a research or diagnostic laboratory

The release of PEDv through inadequate treatment of laboratory waste streams or removal from the laboratory on a fomite such as shoes or clothing is another scenario that was considered. In the United Kingdom 2007 FMD outbreak, breach of biocontainment was cited as the likely origin of the disease agent (Health Safety Executive, 2007). Proximity to the farms initially identified during the outbreak added validity to the assessment. In the United States, a permit for organisms and vectors is required for the importation or interstate movement of animal disease agents [9 CFR § 122] and would have been required for PED viruses. Laboratory release as the source of the outbreak is unlikely since the National Veterinary Services Laboratories held the only permits for PEDv prior to the outbreak and at the time of the outbreak and did not use the permits. Additionally, this scenario fails to fit with the herd investigations because none of the earliest farms were proximate to diagnostic laboratories.

13. Contaminated biological

A contaminated lot of a biological such as a vaccine or oral medication could be distributed to multiple locations and affect different herds. The evidence of the investigation discounts this scenario because there was no common biological product found in the investigation of early affected herds. Further,

[15] Case control study described in section (7) of the epidemiology section of this report; three of four consultants that were interviewed suspected feed and one believed people were the source; epidemiologist conclusions from several of the index herd investigations were that the outbreak was likely associated with feed or feed delivery.

because the production type of the pigs varied from farm to farm and biologicals are generally indicated for specific age groups, it is not likely that any product was used in all of the different outbreaks.

14. Antibiotic filler (e.g., rice hulls) scenario

Products such as ground rice hulls are incorporated in oral antimicrobial products as filler for the active ingredients. These ingredients serve an additional purpose to maintain low moisture content of the products and aide in uniform dispersal of the anti-microbial product in the feed mix. A scenario that has been proposed is that rice hulls are dried on roadsides in China and contaminated by passing trucks. The product is then used as filler for oral antimicrobials, distributed in the United States, and given to pigs that broke with disease.

The investigations of index farms found that antimicrobial were used in some but not all rations. Although virus survival in this type of product has not been researched, it is a dry matrix and would likely not support infectious virus over a long period and virus survival would likely be similar to that found in dry feed. For these reasons, the scenario was considered unlikely; however, rice hulls could be a source of contamination for FIBCs. The scenario would involve rice hulls dried on a roadside, contaminated by passing trucks, and then transported in FIBCs which are later used for exporting a product. This would assume that the contaminated FIBCs were reused for export to the United States.

15. Importation of prohibited products

APHIS-PPQ and CBP monitor ports of entry for prohibited products. Data collected by PPQ officers were reviewed for types and quantity of prohibited material seized from import shipments and described within *Pathways Assessment: Entry Assessment for Exotic Viral Pathogens of Swine*(USDA-APHIS, 2014). Illegal entry of various products into the United States occurs as demonstrated by the number of shipments that are refused entry at ports. For 2013 and 2014, this amounted to over 1,400 shipments[16]. Undoubtedly, some prohibited imports are not detected; however, the majority of those that were detected were very small quantities or were products not associated with livestock production or feed. If a prohibited product entry occurred, it would be unlikely for that product to have arrived at the widespread locations in multiple brands of feed as seen in the index herd outbreak investigations. One exception for this scenario would be the possibility that transport containers (FIBCs) were contaminated by an animal or plant product (for example a root vegetable could have been washed with untreated water, and then exported). In this scenario, the contaminated FIBCs would have to be reused to transport animal feed ingredients.

16. Vitamin and mineral premix

Vitamin and mineral mixes have been suggested as a source of virus, and one of the early investigations reported a PCR test positive sample from a bag of premix. The test was not confirmed by repeat testing and cataloged as a false positive (UMN Veterinary Diagnostic Laboratory, 2014). Further testing was unable to repeat the initial finding, but detecting virus in feed samples is difficult because of the volume of material that may dilute a sometimes miniscule bit of contamination. The RCG evaluated a scenario

[16] Emergency Action Notification database, APHIS-PPQ

that a product lot of premix was contaminated, imported to the United States, and disseminated to swine farms resulting in multiple affected herds.

Several factors undermine this scenario. First, China holds a large share of the market for this type of product used in the United States and other countries; the United States received at least three SECD viruses, other countries such as Canada and EU remained free. Secondly, the low moisture content of vitamin and mineral mixes (Darroch, 2001; DSM Corporation) provides an inhospitable environment for virus survival and raises the question of whether virus could survive the length of travel time. Furthermore, vitamin products are extracted from plants, synthesized by microbial fermentation, or chemically synthesized with minimal if any opportunity to become contaminated (microbial fermentation requires strict attention to sterile procedure). Minerals provide a harsh environment for virus survival (DSM Corporation).

To further follow up this scenario, product and lot numbers were gathered from the index farm investigations. No farms had common product or lot numbers. There was no common brand, distributor, or feed manufacturer that provided these products to the early farms. Although possible, the evidence of the Root Cause Investigation does not support it as a scenario.

17. Amino acid supplement

Amino acids are common components of swine rations and those such as lysine are an essential addition to rations. In most cases, amino acids are produced by bacterial fermentation processes where the cultures require sterile procedures to operate. Although they are usually shipped in sealed bags rather than open containers, post-production contamination could be possible if pallets were exposed to contaminated water from irrigation or flooding. The VS report: The *Pathways Assessment: Entry Assessment for Exotic Viral Pathogens of Swine* (USDA-APHIS, 2014) determined the risk of virus entering the United States associated with amino acids and vitamin products to be of "negligible risk with low uncertainty." In addition, a contaminated lysine product would seem likely have infected herds in Canada or the EU as well as the United States.

- *Vitamins and amino acids are generally produced by microbial fermentation and packaged; i.e., a sterile procedure.*
- *If contaminated, the product would have to be compromised in some unknown way after packaging.*
- *The RCG examined lot and product numbers for early outbreaks and was unable to identify any two farms using the same product.*

However, crystalline lysine imported from China into the U.S. market increased dramatically in the three months immediately preceding the earliest cases of SECD, more than tripling as compared to previous periods (United States International Trade Commission data). During the first quarter of 2013, it is estimated that approximately 13 percent of U.S. swine were consuming feed containing lysine imported from China, triple the number in previous periods.

While crystalline lysine or vitamin/mineral premixes represent an inhospitable environment for survival of SECD viruses, the possibility remains that adulteration of product with a bolus of contaminated feces

or fecal contamination of product packaging could have occurred. An Indiana herd owner whose pigs were infected was an end-user of lysine imported from China in the period immediately prior to the earliest known outbreak of SECD in the United States. He reported concerns with inconsistency in color and texture of this product and apparent water-damage, indicating dubious quality or negligent handling and was suspicious that it may have been the source of his outbreak. Other producers did not observe or, at least, did not report similar information. Altogether, evidence supporting the amino acid scenario is limited.

Conclusions and discussion

The specific route and travel of SECD viruses that came to the United States is unlikely to be uncovered; however, a common theme runs through the most plausible scenarios described above. The viruses must have been carried through four segments of the journey: contamination in the country of origin, entry to the United States, dispersion to multiple locations, and exposure and infection of pigs.

The FIBC scenario describes a mechanism for each segment of the journey, as well as explaining the apparent anomalous association of feed with the outbreaks. The early case-control study provided significant association of feed to infected herds and several investigations and consultants also pointed to feed as the source. At the same time, the investigations were unable to identify any ingredient or manufacturer that was common to all farms, which discounts the possibility of feed as a source. Because FIBCs are used for transporting many types of potentially contaminated product, initial contact with SECD viruses could come through various routes, and the reusable design allows transmission of virions from contaminated to clean products. The FIBC scenario describes the containers as being fomites able to carry the virus and also used to transport various feed products, thus explaining the statistical association of disease with feed. Follow-up testing to evaluate the stability of the virus in the FIBC material further supports the hypothesis that PED virus could easily remain stable through the time needed to travel to the United States and infect pigs.

It is possible that pet treats entered the United States carrying the virus, but unlikely that they affected pigs without a secondary fomite to carry the virus such as the FIBCs. Similarly, organic soybeans could also be contaminated with viruses, but they too must have a secondary carrier to achieve the dispersal and exposure parts of the journey to infect pigs. The same conclusion, that a secondary fomite is necessary, exists for almost any of the plausible scenarios that the RCG investigated.

Breaking any one of the four segments of virus transit would suffice to mitigate the risks of this type of event. Contamination of products in an origin country is largely out of U.S. Government regulatory control and likely outside the realm of industry management. Inspections at entry ports are vital, but unable to identify products containing miniscule amounts of infectious virus. If the fomite moving the virus is indeed the FIBC, not reusing or yet to be determined methods of sanitary management prior to reusing the bags could be an effective intervention.

Collaborations and acknowledgements

During the SECD investigations, VS partnered, consulted, and collaborated with multiple agencies, universities, individuals, and organizational groups.

Industry groups (NPB/NPPC/AASV)	Weekly calls, individual interviews and consults, on-the-ground investigations, surveys
South Dakota State University, Animal Disease Research and Diagnostic Laboratory (ADRDL) Drs. Eric Nelson, Travis Clement, and Aaron Singrey	• Collaboration on soybean testing, field tote testing, and PEDv stability (Tote) studies
University and laboratories	• UMN nasal carriage study • ISUVDL feral swine testing study and retrospective testing of archived samples • NAHLN testing of herd plan samples • SDSU ADRDL testing of FIBCs, soybeans and follow-up stability study
Swine consultant veterinarians: • Dr. Bill Minton • Dr. Terry Specht • Dr. Joe Connor • Dr. Jim Lowe • Dr. Harry Snelson • Dr. Max Rodibaugh • Dr. Luc Dufresne • Dominican Republic: Dr. Uciel Duran Puerto Rico: Dr. Jose Urdaz, Dr. Carlos Soto (VS epi-retired), Dr. Jose Acosta, Ing Jesus Santiago Agronomist	Providing expertise and insight about SECDs and early SECD outbreaks
FDA	• Feed testing at outbreak herd • Consultation on feed products and compliance • Testing of archived jerky pet treats at the FDA Center for Veterinary Medicine (Dr. Haile Yancy group)
• USDA-APHIS-Wildlife Services National Wildlife Disease Program • USDA-APHIS National Feral Swine Damage Management Program	Feral swine study
State Veterinarians	• Collaborative funding through Federal Order • Collaborative investigations
USDA-APHIS-PPQ	Collaboration to sample soybeans at ports
CBP	Collaboration to sample soybeans at ports
DHS/NBACC	Genetic analysis

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