Modeling the transboundary survival of foreign animal disease pathogens in contaminated feed ingredients.

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Statement of the Problem

"It's not if, it's when". Sound familiar? Swine veterinarians have been saying this for years and rightfully so. The US swine industry is under constant threat of foreign animal disease (FAD) entry. Should Foot & mouth disease virus or Classical swine fever virus ever infect our livestock populations, it would cripple our export markets, induce significant animal suffering and initiate a major domestic economic crisis. In 2013, the introduction of PEDV to the US exposed limitations in our knowledge of the global epidemiology and prevention of infectious agents. While the actual source of PEDV has not been determined, phylogenetic analysis of the original PEDV strain that was introduced to the US suggests that the virus may have originated from China. Based on the frequent importation of high volume ingredients used in swine feeding from China, we developed a model to study whether PEDV harbored in imported ingredients could remain viable over the time and under the environmental conditions encountered during a "trans-Pacific" shipment from Asia to the USA (Dee et al, 2016). The model employed ingredients imported to the USA from China, including organic & conventional soybeans and meal, lysine hydrochloride, D-L methionine, tryptophan, Vitamins A, D & E, choline, carriers (rice hulls, corn cobs) and feed grade tetracycline, were inoculated with PEDV. In addition to control ingredients, treatments

(ingredients plus a liquid antimicrobial (SalCURB, Kemin Industries (LA) or a 2% custom medium chain fatty acid blend (MCFA)) were tested. The model ran for 37 days, simulating transport of cargo from Beijing, China to Des Moines, IA, US from December 23, 2012 to January 28, 2013. To mimic conditions on land and sea, historical temperature and percent relative humidity (% RH) data were programmed into an environmental chamber which stored all containers. To evaluate PEDV viability over time, ingredients were organized into 1 of 4 batches of samples, each batch representing a specific segment of transport. Batch 1 (segment 1) simulated transport of contaminated ingredients from manufacturing plants in Beijing (day 1 post-contamination (PC)). Batch 2 (segments 1 and 2) simulated manufacturing and delivery to Shanghai, including time in Anquing terminal awaiting shipment (days 1-8 PC). Batch 3 (segments 1, 2 and 3) represented time in China, the crossing of the Pacific and entry to the US at the San Francisco, CA terminal (day 1-27 PC). Batch 4 (segments 1-4) represented the previous events, including transport to Des Moines, IA (days 1-37 PC). Across control (non-treated) ingredients, viable PEDV was detected in soybean meal (organic and conventional), Vitamin D, lysine hydrochloride and choline chloride. In contrast, viable PEDV was not detected in any samples treated with LA or MCFA. These results demonstrate the ability of PEDV to survive in a subset of feed ingredients using a model simulating shipment from China to the US. This is proof of concept suggesting that contaminated feed ingredients could serve as transboundary risk factors for PEDV, along with the identification of effective mitigation options. Based on the success of the model, we then used it to evaluate the survival of FAD viruses in ingredients using viral surrogates to represent the actual pathogens.

Objectives

1. To model if foreign animal diseases could survive in feed ingredients shipped from Asia to the USA.
2. Evaluate whether 2 chemical mitigants could reduce risk.

The study was based on the hypothesis that pathogen survival will be influenced by ingredient and treatment.

Materials & Methods

Based on the Swine Health Information Center pathogen matrix, 10 FAD viral pathogens were identified

as significant risks to the US swine industry. Due to the inability to work with these actual agents, we used “surrogate viruses”, which allowed us to study closely related and structurally similar viruses, but not the actual FAD pathogens. The designated FAD and the selected surrogate were as follows: FMDV (Seneca Virus A), CFV (Bovine Virus Diarrhea Virus), PRV (Bovine HerpesVirus-1), ASFV (Vaccinia virus), Nipah virus (Canine Distemper Virus), Swine Vesicular Disease Virus (Porcine Enterovirus) and Vesicular Exanthema Virus (Feline Calici Virus). Other selected pathogens (PRRSV 174, PCV2 and Vesicular Stomatitis Virus) did not require surrogates. Using a model previously validated to study the risk of contaminated feed ingredients for the transboundary spread of PEDV (Dee et al 2016), we selected feed ingredients known to be imported from China to the USA based on the US Govt Harmonized Tariff Schedule (hs.usitic.gov). These included organic & conventional soybean meal, soy oil cake, DDGS, lysine, choline, vitamin D, pork sausage casings, and several pet foods (dry & moist). Ingredients were inoculated with representative surrogates (5g ingredient and 100 *uL* virus). Controls consisted of complete feed inoculated with surrogate or saline (negative control) as well as stock virus alone (positive control) in the absence of feed matrix. The design involved non-treated control ingredients, along with 2 mitigants: SalCURB-treated ingredients and MCFA-treated ingredients. These samples were then incubated in an environmental chamber for 37 days programmed using actual T and % RH data recorded during a journey from China to the US (Beijing to Shanghai to San Francisco to Des Moines) in December 2012 through January 2013 (SeaRates.com). Samples were tested by PCR, VI and bioassay for porcine surrogates or on primary cells for surrogates of non-porcine origin at 2 DPI (Beijing), 8 DPI (Shanghai), 25 DPI (San Francisco) and 37 DPI (Des Moines) to represent specific points in the model.

Results

Testing of the FMDV, CSFV and PRV surrogates has been completed. Preliminary data indicate the survival of the FMDV surrogate (SVA) and the PRV surrogate (BHV-1) at all points during the 37 day shipping period from China and into the US. Both surrogates survived in conventional soybean meal and soy oil cake, while SVA also survived in lysine, pet food, Vit D, choline, complete feed and casings. Both positive controls (SVA and BHV-1 stock virus) did not survive. In contrast, the CSFV surrogate (BVDV) appeared to be less stable and did not survive the 37-day journey, independent of ingredient. It did,

however, survive until the samples theoretically entered the port of San Francisco (25 DPI) in conventional soybean meal and moist dog food.

Discussion

Under the conditions of this study, these results suggest that contaminated feed could serve as vehicles for FAD introduction to the US, supporting our previous results which focused on PEDV. The success of these data have allowed us to expand the scope of the project with the help of the Swine Health Information Center, adding several additional viruses (surrogates), including:

1. Vesicular Exanthema Virus of Swine (Feline Calici virus)
2. Nipah virus (Canine Distemper virus)
3. Vesicular Stomatitis virus
4. PRRSV
5. Circovirus
6. African Swine Fever virus

Work is underway to use the actual pathogens for several of these agents. We will keep the AASV posted on our progress. Thank you very much for your support!

References

Dee S, et al. (2016) Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus *BMC Vet Res* 12:51