RESEARCH REPORT
SWINE HEALTH

Title: An evaluation of a shipping model to investigate PEDV introduction into the USA – NPB #15-180

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Industry Summary: This study describes a model developed to evaluate the transboundary risk of PEDV-contaminated swine feed ingredients and the effect of two mitigation strategies during a simulated transport event from China to the US. Ingredients imported to the USA from China were inoculated with PEDV. Control ingredients, and 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. Historical temperature and percent relative humidity (% RH) data were programmed into an environmental chamber which stored all containers. 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. 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.

Keywords: transboundary, porcine, epidemic, diarrhea, virus, antimicrobial, ingredient, lysine, soybean meal, choline.

Scientific abstract: This study describes a model developed to evaluate the transboundary risk of PEDV-contaminated swine feed ingredients and the effect of two mitigation strategies during a simulated transport event from China to the US. 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. Control ingredients, and 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.

Introduction: Based on phylogenetic analysis, the original PEDV that infected the USA appears to have originated from China with feed and/or feed ingredients proposed as possible vehicles for virus entry. Recent publications have provided proof of concept of complete feed as a vehicle for PEDV transmission to naïve pigs, as well as the ability of specific feed ingredients to promote virus viability. This recent publication (attached) used a container model to demonstrate extended survival of PEDV in select ingredients under ambient conditions, i.e., the recovery of viable PEDV in soybean meal for 180 days post-inoculation (DPI), synthetic amino acids (14-30 DPI) and choline (14-30 DPI). Based on the frequent importation of feed ingredients, including both organic and conventional soybeans and soybean meal from China, we developed a model to study whether imported ingredients could remain viable under the time and environmental conditions encountered during a "trans-Pacific" shipment from Asia to the USA. In addition, we proposed to test the ability of a previously published intervention documented to successfully treat PEDV-contaminated feed and ingredients (SalCURB, Kemin Industries) will reduce risk during shipment.

Objectives: To model if PRSV could survive in feed ingredients shipped from Asia to USA

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Materials & Methods: Select ingredients (n=14, 30g/ingredient), known to be imported from China to the USA (organic & conventional soybeans and meal, synthetic lysine, D-L methionine, tryptophan, Vitamin A, D & E, choline chloride, carriers (rice hulls & corn cobs) and feed grade tetracycline), were inoculated with a standard quantity of cell-culture adapted strain of PEDV (Ct 16, courtesy of E. Nelson). The design involved 2 replicates of non-treated control ingredients and 2 replicates each of SalCURB-treated ingredients and ingredients treated with a 2% medium chain fatty acid blend (courtesy of Dr. Cassie Jones, Kansas State University). The shipping schedule was calculated using **SeaRates.com** and began in Beijing on December 23, 2012 and ended on January 31, 2013 in Des Moines, IA, prior to the first clinical signs of PED in the USA. In an effort to represent temperature and relative humidity cargo would experience during this period, historical data (Weather Underground, **www.wunderground.com**) over land (Beijing to Shanghai Anquing terminal and San Francisco terminal to Des Moines), as well as across the Pacific Ocean (Leinberger, Xerox Corp, 2006) were employed. Based on the collective use of all these data, we constructed a temperature & RH curve for this proposed shipment. These data were programmed into an environmental chamber which stored the containers during the 37-day shipping period. Samples were organized into 1 of 4 batches and submitted to the SDSU ADDRL for testing by PCR, VI and bioassay as described. Specifically, sample batch 1 (day 0-1 PI) represented transport for Beijing manufacturing plants to Shanghai Anquing terminal, sample batch 2 (day 2-9 PI) represented time spent in Anquing port awaiting customs clearance, sample batch 3 (day 10-27) represented time crossing the Pacific and arrival to the USA at San Francisco terminal and day 40 (day 28-40 PI) represented transport from California to Iowa, with storage in Des Moines.

Results: All feed ingredient samples were PCR negative on day 0 of the study. Successful PEDV inoculation was confirmed, as all day 1 samples were PCR-positive. The mean Ct of LA-treated samples was 24.5 (SD = 2.4) on 1 DPC and 32.5 (SD = 3.9) on 37 DPC ($p < 0.0001$). The mean Ct of MCFA-treated samples was 24.2 (SD = 4.2) on 1 DPC and 25.5 (SD = 3.7) on 37 DPC ($p = 0.25$). The mean Ct values across non-treated ingredients on day 1 was 22.9 (SD = 2.4) and 23.1 (SD = 3.5) on day 37 ($p = 0.34$). At 1 DPC, the mean Ct values of all 3 groups (non-treated, LA-treated and MCFA-treated) were not significantly different ($p = 0.14$);

however, at 37 DPC, the mean Ct of LA-treated samples was significantly higher ($p < 0.0001$) than the mean Ct of MCFA-treated and non-treated samples. Viable PEDV was recovered from organic and conventional SBM, lysine and Vitamin D across all 4 batches of non-treated samples, including batch 3 representing entry to the US at the San Francisco terminal and batch 4, representing shipment to and storage in Des Moines. No other samples harbored viable virus beyond batch 2 (Beijing and Shanghai segments) including the PEDV stock virus control. Multiple samples were VI negative on 1 DPC. All negative control samples and all LA-treated or MCFA-treated ingredients were VI negative across all batches. Samples selected for swine bioassay testing consisted of treated and non-treated ingredients from batch 4. The non-treated ingredients tested included those which were PCR- positive and VI-negative, specifically Vitamins A & E, tryptophan, D-L methionine, soybeans (organic and conventional), and choline chloride. Viable PEDV was detected in piglets administered non-treated samples of choline chloride. Affected animals displayed evidence of mild diarrhea, shed PEDV in feces and samples of small intestine were PCR and immunohistochemistry-positive at necropsy, with microscopic lesions of villous blunting, fusion and re-epithelialization. All other samples were bioassay negative. In regards to treated ingredients, LA-treated and MCFA-treated samples of soybean meal (conventional and organic), lysine, vitamin D and choline chloride were tested. All piglets inoculated with the aforementioned LA-treated or MCFA-treated ingredients were determined to be non-infectious, as piglets remained clinically normal throughout the testing period and all rectal swab and intestinal samples were negative by PCR. Results are summarized in Table 1 and 2.

Discussion: These results indicate the ability of PEDV to survive in specific feed ingredients under modeled conditions simulating shipment from China to the US. These are the first proof of concept data suggesting that contaminated feed ingredients could serve as transboundary risk factors for PEDV, along with the identification of effective mitigation options. This study also introduces a model which could enhance further transboundary research efforts, possibly employing surrogate viruses (bovine viral diarrhea virus for swine fever virus or Seneca Valley virus for foot-and-mouth disease virus) to test feed-related risks for other foreign animal diseases, further the investigation of the risk of organic ingredients, along with the continuing validation of mitigation strategies.