ONTARIO PORK

Ontario Pork Research Final Report (14-007) Executive Summary

Reporting Date: May 11, 2019

Introduction: In January 2014, the first case of porcine epidemic diarrhea (PED) was reported in an Ontario swine herd; which was soon followed by a series of other primary and secondary cases. Feed from a single feed company (FC) as a source of the virus was implicated during the early phase of the outbreak. The detailed observational epidemiological studies presented in this reported were required to further investigate the role of feed in the spread of porcine epidemic diarrhea virus (PEDV), while adjusting for other potential confounding factors.

Objectives: The overall objective of this study was to conduct an epidemiological evaluation of the role of feed and other possible factors in the early phase of the PED outbreak in Ontario swine herds that occurred in 2014. A case-control (C-C) approach, cohort (CO) approach, and network analysis (NA) were utilized to meet the objectives.

Materials and Methods: The study period-of-interest was Jan 22nd to Mar 1st, 2014. A questionnaire was administered to herd managers of participating swine herds with questions focused on herd demographics, biosecurity protocols, live animal movements onto and off sites, deadstock movements, feed and people movements during the period-of-interest. Three hypotheses were evaluated with NA: 1) whether feed supplier, semen supplier and/or animal transportation company networks contained a higher proportion of case (PED) herds, 2) whether the proportion of case herds differed from randomly permuted networks, and 3) whether external herd biosecurity, was different between case and control herds. Data from 3 sources were utilized for the CO study and Kaplan-Meier estimates of survival curves and the Log-rank test were used to evaluate differences in survival probability between different levels of exposures to feed and contaminated spray dried porcine plasma (SDPP).

Results and Discussion: Multivariable exact conditional logistic regression and mixed multivariable logistic regression models documented that the odds of a PED occurrence was 38.1 (95% CI: 2.7–531.3) times greater for herds receiving feed from FC that provided contaminated feed (P=0.007) than herds that did not. Interpretation of the Cox's proportional hazard model indicated that herd exposure to contaminated SDPP from FC had an increased hazard (HR= 36.6, 95%CI: 2.5-529.6, P=0.008) of experiencing PED outbreak in the early phase of PED incursion. Both the C-C and CO studies supported that the frequency of movements was not associated with the odds of PED outbreak in the period-of-interest. The NA also indicated little significance in the roles of animal movement, animal transportation companies, and semen suppliers during the initial phase of PED incursion.

Conclusions: The epidemiological analytical studies presented in this report provides strong epidemiological evidence that contaminated feed, specifically the contaminated ingredient SDPP, from FC was a significant risk factor for PEDV transmission during the early phase of the PED outbreak in Ontario swine herds in 2014.

Overall take-home scientific messages:

- Feed containing contaminated SDPP was a risk factor for PED during the initial phase of the outbreak
- A specific feed supplier (supplying contaminated feed ingredient) was identified as a risk for PEDV transmission
- Non-feed service suppliers did not have an impact on PEDV spread even though connections among swine herds and service suppliers were identified during the initial PED outbreak
- Animal and human movement on and off of farms did not have an impact on PEDV spread during the initial phase

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Introduction: In January 2014, the first case of porcine epidemic diarrhea (PED) was reported in an Ontario swine herd; which was soon followed by a series of other primary and secondary cases. An early investigation into this outbreak suspected that the probable source of the porcine epidemic diarrhea virus (PEDV) was contaminated feed[1]. This early finding prompted a voluntary recall of feed containing the suspected ingredient by the supplying feed company (FC). The Canadian Food Inspection Agency (CFIA) collaborated with the FC to trace the feed shipments. Subsequently, a shipment of spray-dried porcine plasma (SDPP) that was utilized by the FC, tested positive for PEDV nucleic acid, and bioassay results indicated that pigs could be infected via this SDPP plasma following oral exposure[1]. These findings from the CFIA investigation were very suggestive of the role of feed in the outbreak but were not conducted under field conditions. Hence, detailed observational epidemiological studies were required to further investigate the role of feed in the spread of PEDV, while adjusting for other potential confounding factors such as amount and frequency of feed delivered that contained the SDPP, animal movements on and off the farm, human movements, and other potential fomites. The epidemiological studies presented in this report aimed to document different aspects of risk of a swine herd developing PED following the initial outbreak of PED in Ontario in January 2014.

Objectives: A case-control (C-C) study was conducted in which an array of different factors considered important in the transmission of PEDV was investigated including: feed, biosecurity and transportation practices, and movement of animals, people and potential fomites. A secondary general objective that was associated with the C-C study data was to conduct a network analysis (NA) to describe the contact structure and animal movement patterns of PED case herds and matched control herds during the early phase of the outbreak. Hence, the overall objective of the NA was to describe the contact structure and animal movement patterns of PED case herds and matched control herds, during the initial incursion, and to evaluate possible mechanisms (connections) of spread during this period. Three hypotheses about possible mechanisms of spread during this period were evaluated and included transmission through as: (i) common-

source, (ii) herd-to-herd transmission, and (iii) transmission due to low biosecurity. The CO study focused specifically on whether farms receiving feed (classified as exposed vs. non-exposed farms) containing the contaminated SDPP (that was implicated at the time as a mechanical vector for the PEDV) resulted in the increased risk of PED outbreak in the early reported cases.

More specifically, and collectively, the objectives of all these studies aimed to:

1. Evaluate the association between the use of specific feed type and the occurrence of a herd outbreak of PED in swine herds between Jan 9th and Mar 1st 2014 after adjusting for other confounding factors using a C-C control approach,

2. Investigate other potential risk factors for infection with PEDV in the early phase using a C-C approach and network analysis,

3. Determine the likelihood of developing PEDV infection after feeding a shipment of feed containing contaminated SDPP, and;

4. Evaluate whether there is difference in risk/hazard of PEDV infection between herds which received the batch of contaminated SDPP and the herds which received only a plant-based diet using a CO study.

Materials and Methods: A questionnaire was administered to the farm manager from each participating case and control herd and focused on published risk factor categories for PED and other infectious diseases for pigs in general[1,2,3] Questions focused on three main areas: 1) herd demographics, 2) movements of animals, people and fomites entering the premises, and 3) movements of animals, people and fomites leaving the premises. A 31-day period of interest was utilized. This period included the initial day of onset of clinical signs for a case herd, which was also used as the reference point for the matched control herd, along with the 30 days prior to this day. This 30-day time period (i.e. prior to herd outbreak) was considered important in order to understand the history of the case herds prior to being diagnosed with PED and to properly evaluate potential risk factors and to determine temporal sequence of potential risk factors. Control herds were matched with their case herds based on time and herd demographics. Multivariable exact conditional logistic regression and mixed multivariable logistic regression models, with the matched stratum as a random effect, were used to assess the association between various risk factors and the odds of PED introduction into a herd.

Network measures were analyzed in the statistical software R version 3.3.0 using the 'igraph" library and multiple two-mode networks with swine herds and service suppliers were constructed. This resulted in networks constructed

between herds and: feed suppliers, animal movement, animal transportation companies, semen suppliers and a complete network with all service providers. The three hypotheses that were evaluated using NA included: 1) whether feed supplier, semen supplier and/or animal transportation company networks contained a higher proportion of case herds compared to randomly permuted networks, 2) whether the proportion of case herds in the giant weak component differed from randomly permuted networks, and 3) whether external herd biosecurity, defined as the number of mean contacts with other herds in a one-mode network, was different between case and control herds (questionnaire data).

Three sources of data were used for the CO study:

1. Diagnostic data on positive PEDV submissions provided from the Animal Health Laboratory (AHL) of the University of Guelph for the period of Jan-Apr 2014. A dataset of customers was produced together with the date of submission to the AHL, and the date when clinical signs suggestive of PED were first observed.

2. A database of voluntary feed recall data was obtained from the feed company (FC) that was involved in the voluntary recall. This data source had two components, recall-level information and the customer-level information with PED status,

3. A dataset consisting of all invoices from the FC with pig feed aimed to be fed to production classes until the end of nursery period and that was delivered from the company to between Jan 2nd-Feb 28th 2014. To define exposure status the dataset of invoices was manually compared to the dataset of voluntary feed recalls in order to establish when the contaminated SDPP was used in the pig feed. The feed associated with each invoice date was subsequently classified as exposed or non-exposed.

Kaplan-Meier estimates of survival curves and the Log-rank test were used to evaluate differences in survival probability between different levels of exposures. Following this, system-level analysis was performed using Cox's proportional hazard model. Final models were fitted by considering exposure to potentially contaminated SDPP, defined as binary (yes/no) or quantitative variables (i.e. tonne) as the main exposure of interest and other factors as potential confounders.

Results and Discussion: The most important finding from the C-C study was that the odds of a PED occurrence was 38.1 (95% CI: 2.7-532.3) times greater for herds receiving feed from FC than herds that did not (*P*=0.007). The number of live pigs delivered onto sites, semen deliveries and the frequency of deadstock pickups were not associated with PED status during the initial phase of the outbreak. Animal movement, biosecurity practices related to transportation, and contacts between farms due to movement of people were expected to play a role in the transmission of this virus. However, the C-C study supported that the frequency of movements was not associated with the odds of PED outbreak including: (i) frequency of pig movement onto and off premises (OR=1.04, *P*=0.70), (ii) frequency of deadstock (OR =0.86,

P=0.50) and culled sow movements (OR= 1.63, *P*=0.45) off premises, and (iii) total number of visitors (OR=1.08, *P*=0.40) and the total number of staff (OR=1.08, *P*=0.40) in the period-of-interest.

The most important finding from the CO cohort study was that herd exposure to contaminated SDPP (from FC) significantly increased the hazard (HR= 36.6, 95%CI: 2.5-529.6, *P*=0.008) of experiencing PED outbreak in the early phase of PED incursion to Canada (Table 1). This was evident regardless of which way that exposure was defined, or the analytical approach utilized (e.g. binary or quantitatively). A dose-response was clearly demonstrated however, indicating that the more SDPP the herd was exposed to (via delivery of feed) the increased hazard of PED developing in the herd (Table 1). The total number of deliveries or total feed received however, was not associated with the hazard of a PED outbreak suggesting that the initial outbreak was not spread by transportation vehicles. Taken together these results support that the contaminated SDPP from FC was the primary mechanical entry of PEDV onto swine farms in Ontario during the initial phase of the outbreak.

The complete network consisted of 145 nodes (unique stakeholder units) and there was a total of 765 edges (connections) in the complete network. The majority of these connections were between feed suppliers and primary (PED) herds 29.8% (228/765). The proportion of case herds in the largest feed supplier network was higher than what was expected using the randomly permuted networks, suggesting that the likely mechanism of spread during this phase was a common-source thorough the feed network. To further support this hypothesis, a single feed supplier (identified as FC) had the highest out-degree and outgoing contact chain (most connections) indicating its importance in potential disease spread through the feed and complete networks. These networks descriptive measures, as well as the results of the hypothesis testing, indicated little significance in the roles of animal movement, animal transportation companies, and semen suppliers during the initial phase of the 2014 PED outbreak in Ontario.

Conclusions: Regardless of the epidemiological and analytical approach taken (C-C, CO, and NA), the findings independently and collectively, provide strong epidemiological evidence that contaminated feed, specifically the contaminated feed ingredient SDPP, from FC was a significant risk factor for PEDV transmission during the early phase of the outbreak in Ontario swine herds. As further evidence to support this major finding, the results (independently by analytical style and collectively), support that the frequency of contacts through people, animals, and different fomites was not found to be associated with the development of PED cases at the herd level during the early aspect of outbreak.

The data and analytical approach taken for the CO study represents the closest we can get to achieving the counterfactual effect for this type of study. For example, the non-exposed group consisted of herds that received feed from the same feed company and with the same plant-based ingredients, delivered through same feed trucks. The only difference between (counterfactual state) the non-exposed herds and exposed herds was the inclusion of the contaminated SDPP. A significant result stemming from the CO study was the analytical approach took into consideration different perspectives of exposure including time-varying covariates, and their time-varying effect. In addition, this analysis has resulted in conservative estimates of the hazard ratio e.g. all the factors contributing to the hazard ratio results in an underestimate of the measure of associations (hazard ratio) for exposures that were identified in this study. In other words, the association (hazard ratio) is likely underestimated by this study.

References:

1. Pasick, J., et al (2014). Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. *Transbound. Emerg. Dis*. 61, 397–410.
2. Lee, C., (2015). Porcine epidemic diarrhea virus: an emerging and re-emerging epizootic swine virus. Virol. J. 12, 193–209.
3. Lowe et al., (2014). Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. Emerg. Infect. Dis. 20(5):872–4.

Knowledge Transfer: Presentation, poster or abstract from a scientific or industry meeting (please provide a copy or the link). *Highlight any communications of the research to date*

**Provided a PDF copy of published manuscript/abstract that represent the completed work. Provided a variety of different publication styles targeted at different audiences (i.e. some presentations and posters were of the same material but pitched to different audiences – scientific, lay).*

Peer-reviewed publications

1. *Perri, A.M., Poljak, Z., Dewey, C., Harding, J.C.S., O'Sullivan, T.L. 2018. An epidemiological investigation of the early phase of the porcine epidemic diarrhea (PED) outbreak in Canadian swine herds in 2014: A case-control study. *Prev. Vet. Med.* 150(1):101-109.

2. *Perri, A.M., Poljak, Z., Dewey, C., Harding, J.C.S., O'Sullivan, T.L. 2019. Network analyses using case-control data to describe and characterize the initial 2014 incursion of porcine epidemic diarrhea (PED) in Canadian swine herds. *Prev. Vet. Med*. 162(1): 18–28.

Oral Presentations (abstracts)

3. *Using network analysis to evaluate the role of feed suppliers during the early months of the 2014 porcine epidemic diarrhea Canadian outbreak. 15th International Symposium of Veterinary Epidemiology and Economics (ISVEE). November 12-16, 2018. Chiang Mai, Thailand

4. *Network analysis on the contact structure between swine herds and feed suppliers during the 2014 porcine epidemic diarrhea outbreak in Canada. International Pig Veterinary Society Congress (IPVS). June 11-14, 2018. Chongqing, China

5. A case-control study on the early outbreak of porcine epidemic diarrhea (PED) in Canadian swine herds. University of Guelph Swine Research Day. May 17th 2017. Guelph, Canada

6. How network analysis can help us understand the 2014 Canadian porcine epidemic diarrhea

outbreak. University of Guelph Swine Research Day. May 16, 2018. Guelph, Canada

7. Contact network analysis between swine herds and feed suppliers during the early phase of the porcine epidemic diarrhea outbreak in Canada. Conference of research workers in animal diseases (CRWAD). December 3-5th, 2017. Chicago, USA

8. *Investigation of factors that led to emergence of Porcine Epidemic Diarrhea virus through feed delivery during early phase of Canadian outbreak. 7th International Symposium on emerging and re-emerging pig diseases. June 21-24, 2015. Kyoto, Japan.

9. Investigation of factors that led to emergence of Porcine Epidemic Diarrhea virus through feed delivery during early phase of Canadian outbreak. 14th International Symposium of Veterinary Epidemiology and Economics (ISVEE). November 3-7, 2015. Yucatan, Mexico

Poster Presentations (abstracts)

10. Network analyses on swine herds and service suppliers during the early months of the 2014 porcine epidemic diarrhea outbreak in Canada. International Pig Veterinary Society Congress (IPVS). June 11-14, 2018. Chongqing, China

11. *Visualizing and describing the 2014 porcine epidemic diarrhea outbreak in Canada using network analysis: a contribution to big data. Arrell Food Summit, University of Guelph. May 22, 2018. Guelph, Canada

12. A case-control study investigating the early outbreak of porcine epidemic diarrhea (PED) in Canada 2014. Conference of research workers in animal diseases (CRWAD). December 3-5th, 2017. Chicago, USA

13. A case-control study investigating the early months of the porcine epidemic diarrhea (PED) outbreak in Canadian swine herds. North American PRRS symposium. December 1-3rd 2017. Chicago, USA

14. A case-control study: an investigation of the early phase of the porcine epidemic diarrhea (PED) outbreak in Canada 2014. Graduate Student Research Symposium. November 16, 2016. Guelph, Canada

15. How network analysis can help us understand the 2014 Canadian porcine epidemic diarrhea outbreak. Graduate Student Research Symposium. November 16, 2016. Guelph, Canada

Popular Press Articles and communications:

16. *Pigs of Instagram? Using network analysis to understand swine disease outbreaks. Ontario Swine Research Network (OSRN) Swine Research Snapshot (Infographic). Aug 2018.

17. *How network analysis can be used to help understand swine health problems and disease outbreaks. OMAFRA Pork news and views (Better Pork magazine). June 2018.

18. OSRN Researcher Profiles 2017: Amanda Perri (Media interview for the OSRN). June 29, 2017.

OP14-007 Tables

Table 1. Final models* based on customer-level analysis comparing exposed vs non-exposed swine herds during the initial incursion of porcine epidemic diarrhea in Ontario swine herds in 2014

	Final Model 1 (binary)		
Variable	HR	95% CI	p
Exposed	36.6	(2.5, 529.6)	0.008
tot_tonnes	1.2	(1.1, 1.3)	<0.001
Model statistics:	LR χ^2(2 df) = 21.2, p<0.001, AIC=160.5, n=132		
GOF	Score χ^2(4 df) = 2.8, p=0.59		

	Final Model 2 (quantitative kg)		
Variable	Coefficient	95% CI	p
kg_exp_plasma	3.8E-02	(2.0E-02, 5.5E-02)	<0.001
kg_exp_plasma2	-8.7E-05	(-1.6E-04, -1.0E-05)	0.027
Model statistics:	LR χ^2(2 df) = 33.20, p<0.001, AIC= 148.4, n=132		
GOF	Score χ^2(4 df) = 7.6, p=0.11		

*Cox's proportional hazard model