Modelling porcine reproductive and respiratory syndrome virus dynamics to quantify the contribution of multiple modes of transmission: between-farm animal and vehicle movements, farm-to-farm proximity, feed ingredients, and re-break

Jason A. Galvis [1], Cesar Corzo[2], Gustavo Machado[1]*

[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, Raleigh, North Carolina, USA.

[2] Veterinary Population Medicine Department, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA.

Summary

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to cause substantial economic losses for the North American pork industry. Here we developed and parameterized a mathematical model for transmission of PRRSV amongst the swine farms of one U.S. state. The model is tailored by eight modes of between-farm transmission pathways including: farm-to-farm proximity (local transmission), networks comprised of different layers contacts here considered the number of batches of pigs transferred between-farm (pig movements), transportation vehicles used for -- feed delivery, transferring live pigs to farms and to markets, and personnel (crew), in addition to the quantity of feed with animal by-products within feed ingredients, and finally we also accounted for re-break probabilities for farms with previous PRRSV outbreaks. The model was calibrated on weekly PRRSV outbreaks data. We assessed the role of each transmission pathway considering the dynamics of specific types of production. Our results estimated that the networks formed by transportation vehicles were more densely connected than the actual network of pigs moved between-farms. The model estimated that pig movements and farm proximity were the main route of transmission in the spread of PRRSV regardless of production types, but vehicles transporting pigs to farms explained a large proportion of infections (sow = 17.2%; nursery = 11.7%; and finisher = 29.5%). Animal by-products delivered via feed contributed principally to finisher farms, with a significant impact on PRRSV outbreaks on sow farms. Thus, our results support the consideration of transport vehicles and feed meals in order better to understand the transmission dynamic of PRRSV and establish more robust control strategies.

Keywords: network modelling, swine disease dynamics, surveillance, secure business continuity.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major economic burden in North America (Holtkamp et al., 2013), which continue to spread across multiple pig-producing companies (Sanhueza et al., 2019; Jara et al., 2020; Galvis et al., 2021). A recent study developed a new mathematical model to reconstruct the between-farm PRRSV dynamics, revealed the role of between-farm pig movements, indirect contacts by farm-to-farm proximity, and the continued circulation of PRRSV within infected sites (re-break) (Galvis et al., 2021). Despite the promising results, the study did not fully consider indirect contacts formed by the between-farm transportation vehicles networks, which has been previously described as one the major modes of the between-farm transmission of disease in swine (Büttner and Krieter, 2020; Porphyre et al., 2020; Niederwerder, 2021), and also in other systems such as cattle (Yang et al., 2020).

Detailed data about the flux of transportation vehicles coming in and out premises can be difficult to obtain, which indeed explain lack of mathematical models considering this transmission pathway (Bernini et al., 2019; EFSA Panel on Animal Health and Welfare (AHAW) et al., 2021). Yet, previous studies have approached indirect transmission by transportation vehicles, such as the studies by Thakur et al., 2015 and Wiltshire, 2018, using probabilities to define indirect contact between farms (Thakur, Sanchez et al., 2015; Wiltshire, 2018), or the study by Buttner, 2020 that used observed truck movements between pig farms in Germany (Büttner and Krieter, 2020). The networks formed by transportation vehicles have been also described earlier as potential transmission routes of PRRSV (Dee et al., 2002; Dee et al., 2004; Pitkin et al., 2009; Thakur, Sanchez et al., 2015). The potential of viral survivability on external vehicles surfaces has been demonstrated, briefly being dependent on environmental conditions such as external temperature, pH, moisture, and vehicle disinfection procedures (Dee et al., 2002, 2003; Jacobs et al., 2010). For example, Dee et al., isolated PRRSV under field conditions from surfaces such as concrete, floor mats and fomites between 2h and 4h after inoculation (Dee et al., 2002). On the other hand, PRRSV is more stable under low temperatures, surviving for long periods (>4 months) at frozen conditions (-20° C to -70° C) (Benfield et al., 1992) and unstable as the temperature increases (Jacobs et al., 2010). On the other hand, dry condition and or low pH between5 and 7 (Benfield et al., 1992), and iodine, quaternary ammonium compounds or chlorine used in vehicle disinfection were successful in inactivating PRRSV (Shirai et al., 2000). Thus, if the environmental conditions favor PRRSV survivability, under a poor vehicle disinfection scenario and highly connected vehicle networks (Büttner and Krieter, 2020), it has the potential to propagate pathogens to a large number of farms.

Contaminated feed ingredients have been recognized as possible routes for between-farm pathogen transmission (Niederwerder, 2021), however the probabilities of PRRSV transmission through them have been described as relatively low (Cochrane et al., 2017; Ochoa et al., 2018). Dee et al. 2020, experimental

study demonstrated that pigs that consumed pellet feed contaminated with 1×10^5 TCID50/ml of PRRSV exibeted clinical signs (Dee et al., 2020). Thus, it is worth noting that cross-contamination of feed ingredients may occur also after the pelleting process by the direct contact of contaminated fomites or feed mill workers (Niederwerder, 2021). In addition, it is important to acknowledge that inadequate pressure in combination or alone with temperature applied during pelleting process could reduce the probability to inactivate PRRSV, as observed in an experimental study with Porcine Epidemic Diarrhea Virus (PEDV) (Cochrane et al., 2017). In North America, most feed formulations include some animal by-products in order to increase growth performance (Lewis and Southern, 2001), which include fat, dried plasma, meat and bone, for example, and without a correct process to inactivate PRRSV, these ingredients could potentially be source of feed contamination and infection. Magar and Larochellle in 2004 surveyed two Canadian slaughterhouses and found 4.3% of animal serum samples and 1.2% of the meat samples were positive for PRRSV by Polymerase chain reaction (PCR), the same study also demonstrated that the consumption of those animal by-products infected pigs experimentally (Magar and Larochelle, 2004).

The dramatic change in the global epidemiological situation of African swine fever (ASF) spread has resulted in concerns about its spread via feed and transportation vehicles moving between-farms and countries (Lee et al., 2017; Elijah et al., 2021; Mighell and Ward, 2021; Niederwerder, 2021; Shurson et al., 2021). However, there is limited information about the contribution of both vehicle movements and the delivery of specific feed ingredients (i.e. meat and bones) in the between-farm spread of disease among swine farms worldwide. Here we have built a novel mathematical model of PRRSV transmission, tailored to eight modes of between-farm propagation: local transmission by the farm-to-farm proximity, between-farm animal and vehicles movements (feed delivery, shipment of live pigs between farms and to slaughterhouses, and personnel (crews)), the quantity of animal by-products in pig feed ingredients and re-break for farms with previous PRRSV outbreaks. The model provides the contribution of each transmission route, the weekly estimates of new PRRSV outbreaks, and model performance in identifying the spatial distribution of PRRSV outbreaks.

Material and Methods

Databases

In this study, we used weekly PRRSV records captured by the Morrison Swine Health Monitoring Program (MSHMP) (MSHMP, 2020). Data included outbreaks between January 22, 2009 and December 31, 2020, from 2,294 farms from three non-commercially related pig production companies (coded as A, B, and C) in a U.S. region (region not disclosed by confidentiality terms) (Galvis et al., 2021). Individually, each PRRSV record was classified as new or recurrent according to the time between consecutive outbreaks per

farm (Galvis et al., 2021). A list of pig farms was available from the MSHMP database (MSHMP, 2020), which included individual national premises identification number, farm type (sow [which included farrow, farrow-to-wean, and farrow-to-feeder farms]), nursery, finisher [which included wean-to-feeder, wean-to-finish, feeder-to-finish], gilt development unit [which could be either part in finisher or sow farms depending upon production type used by pig production company], isolation and boar stud, pig spaces per farm, and geographic coordinates. Between-farm pig movement data from January 01, 2020, to December 31, 2020, was used to reconstruct the directed weekly contact networks. Each movement batch included movement date; farm of origin and destination; the number of pigs transported; and purpose of movement (e.g., weaning). Movement data missing either the number of animals transported, production type, farm of origin, or destination were excluded prior to being analyzed. In addition, four networks formed by transportation vehicles were recorded from the global positioning system (GPS) vehicle tracker for one company for 2020, these movements comprehended: (i) the records of the movement between feed mill and each farm, (ii) the movements records of vehicles transporting live pigs between farms, (iii) the movements records of vehicles transporting pigs to the market (slaughterhouse), and (iv) the movements records of vehicles transporting crew to help in the loading or other activities in the farms (Figure 1). Each movement batch included a unique identification number, speed, date and time along with the coordinates of each vehicle location recorded each minute. A vehicle visit was defined as: a vehicle coordinate (latitude & longitude) and speed of zero km/h for at least 5 minutes in a farm or in a cleaning station within a radius of 1.5 km of any farm or cleaning station (time and distance radius selected after discussion with personnel in charge of vehicle logistics and data observation). We calculated the time in minutes the vehicle remained within each farm's perimeter (1.5 km) and the vehicle contact networks between the farms was built considering the elapsed time a vehicle visited two or more different farms (Figure 1). To accommodate PRRSV survivability in the environment, we considered two seasons (cold and warm weather) based on previously literatures (Dee et al., 2002; Jacobs et al., 2010). Under laboratory conditions, it was reported that PRRSV preserved stability for more than 72h when temperatures oscillated between 4° and 10° C (cold temperatures) and less than 24h when temperatures were higher than 20° C (warm temperatures) (Jacobs et al., 2010). Thus, an edge between farms was recorded among all farms each vehicle visited without a limit time of 72 hours or 24 hours, for the cold and warm season, respectively. However, no edges were recorded after the vehicle visited a clean station (Figure 1). The edges for all four vehicle networks were weighted by the elapsed time the same vehicle visited two different farms, which was later transformed to a probability assuming a decreasing linear relationship of PRRSV stability on the environment (Figure 1 and Supplementary material Figure S1). Additionally, we collected feed load outs records from three feed mills of company A for 2020, each feed record included feed mill identification with individualized feed formulation (ingredients), amount delivered, and destination farm identification and delivery data to

destination farms. From the feed formulation data, we collected the amount of animal by-products of each formulation (fat, meat, plasma and bone) in pounds (lbs) received by the farms for each week of 2020 (Supplementary material Figure S2 and S3).

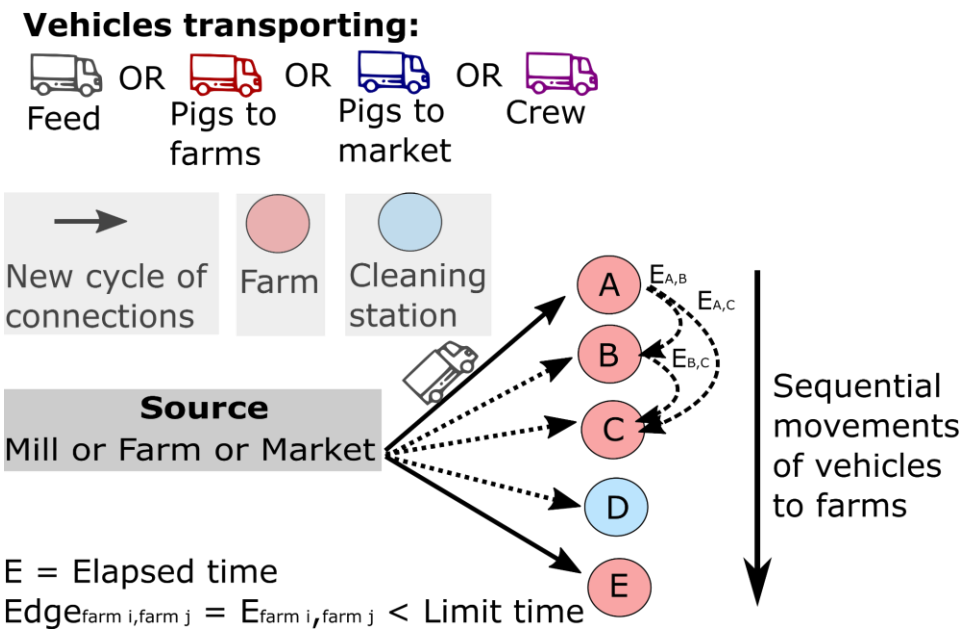

Figure 1. Framework of the indirect farm contacts formed by vehicle movements. For this study, the limit time was defined as 72 hours for cold months (October-March) and 24 hours for warm months (April-September) considering the normal seasonal patterns from the United States.

Descriptive analysis

Between farm animal movement and the movement of transportation vehicles

The networks formed by movement of live animals between farms and four types of vehicles visiting farms were reconstructed and analyzed descriptively. A set of network metrics including: size, properties, and heterogeneity at node-level were evaluated from each directed static and temporal network (visit supplementary material Table S1 for terminology and network metric description). To determine if the static network of pig and vehicle movements could represent the temporal variation of the between farm contacts over a year, we calculated the causal fidelity (Lentz et al., 2016). Briefly, causal fidelity quantifies the error of the static representation of a temporal network through a ratio among the number of paths between both representation, thus casual fidelity of 100% means that a temporal network is well represented by its static counterpart, on the contrary values close to 0 means the network should not be considered as a static system (Lentz et al., 2016). We estimated if farms with PRRSV outbreaks records were more

frequently connected with other infected farms through the ingoing and outgoing contact chain, compared with farms without PRRSV records. Finally, we estimated if the time the vehicles stay in the farms was higher in the farms with PRRSV outbreaks records compared with farms without PRRSV records. The association for the contact chain and the time the vehicles stays in the farms with PRRSV outbreaks were evaluated through t test or Mann-Whitney test.

Animal by-products in the feed ingredients

We calculated the total amounts of animal by-products present in each of the 23 feed formulations sent to farms with and without PRRSV outbreaks in 2020 (Supplementary material Figure S2 and S3). We performed a logistic regression analysis using a generalized linear model and generalized linear mixed model in which the output was the positive or negative for PRRSV. The fixed effect was the amount of animal by-products, and both the farm's pig capacity and production type accounted for the random effects.

Transmission model

In order to perform the analysis of spatiotemporal distribution of farm-level PRRSV outbreaks, we extended a previously developed between-farm transmission model (Galvis et al., 2021), including additional transmission routes. The model was calibrated to the weekly PRRSV outbreaks and accounted for eight transmission modes including (1) contact network of discrete pig movements, (2) the local transmission events between neighboring farms driven by distances among farms, indirect contact by vehicles coming into farms, including for (3) feed delivery, animal delivery to (4) farms and (5) market, and (6) vehicles used by loading and unloading pigs personnel (crew), (7) amount of animal by-products in feed formulation delivery to farms, and (8) re-break by a previous exposure to the PRRSV (Figure 2). The model simulates between farm transmission among 3 farm infectious states, Susceptible-Infected-Outbreak, with farms at states of susceptible (i) receive the force of infection of infected and outbreak farms (j) in each time step *t* and turn infected at rate Y_{it} (Figure 2). It is worth noting that the latent period is not explicitly modeled, as it is typically a few days after infection, and often viral shedding starts within 7 days post-infection (Pileri and Mateu, 2016; Chase-Topping et al., 2020), thus it is embedded in the weekly timestep. Local transmission was modelled through a gravity model where the probability of infection is proportional to the animal capacity of the farms and inversely related to the distance between the two farms (i.e. lower transmission at longer distances), with maximum distance set at 35km, based on previous study (Galvis et al., 2021). Local transmission is also dependent on the enhanced vegetation index (EVI) around the farm *i* (Jara et al., 2020; Galvis et al., 2021), such that the probability of transmission decreases with high EVI values (Supplementary material Figure S4). The transmission between farm pig movements is modeled by the number of all infected and outbreak farms sending pigs to susceptible farms. The transmission of

between farm vehicle movements is modeled by the edge weight (E) and the time the vehicle stays on the susceptible farm (Z_{it}) (Figure 1 and Supplementary material Figure S1 and S5). The transmission by animal by-products is dependent on the amount delivered to susceptible farms (A_{it}). The re-breaks rate was implemented to sow farms that reported outbreaks two years before the initial date of the simulations, and the probability was based on a survival analysis evaluating the time farms re-break after recovering (W_{it}) (Holtkamp et al., 2010)(Supplementary material Figure S6). The force of infection (λ) of infected and outbreak farms varies with a seasonality derived from analysis of the PRRSV records from 2015 to 2019 (Supplementary material Figure S7). In addition, farms without PRRSV outbreaks records since 2009 were assumed to have high biosecurity levels (*H*) that reduce the force infection received by infected and outbreak farms, this value was calibrated by farm type (Supplementary material Table S2), otherwise, farms with outbreaks records within that time were assumed to have low biosecurity levels and *H* was defined as 0.

The transition from infected to an outbreak farm is estimated through a detection rate $f(x)$ (Figure 2), thus the probability that a farm becomes an outbreak was assumed to be dependent on the maximum detection probability (*L*), considered equal to cases reported to MSHMP (MSHMP, 2020), and the average time it takes a farm to detect the disease ($x0$), assumed to be 4 weeks (best guess based the information provided by the local swine veterinarians and literature) (Neira et al., 2017). The proportion of recovered sow farms that turn on susceptible again is drawn from a Poisson distribution with a mean of 41 weeks, which is the average time to stability (Sanhueza et al., 2019). Nursery and finisher farms' transition to susceptible status were driven by pig production movement scheduling of all-in all-out management of closeouts or by incoming or outgoing movements, whatever came first. Briefly, downstream farms become susceptible within 7 and 25 weeks of pig placement, respectively, or when a movement is recorded before reaching the scheduled production phase timeline. A detailed description of the model can be found in Figure 2 and a previous work describes in greater detail other model parameters (Galvis et al., 2021). Finally, we used an Approximate Bayesian Computation (ABC) rejection algorithm (Minter and Retkute, 2019), to estimate the posterior distribution of unknown model parameters (Supplementary material Table S3) by selecting the particles that fitted better the temporal and spatial distribution of observed PRRSV outbreaks (Supplementary material section 2).

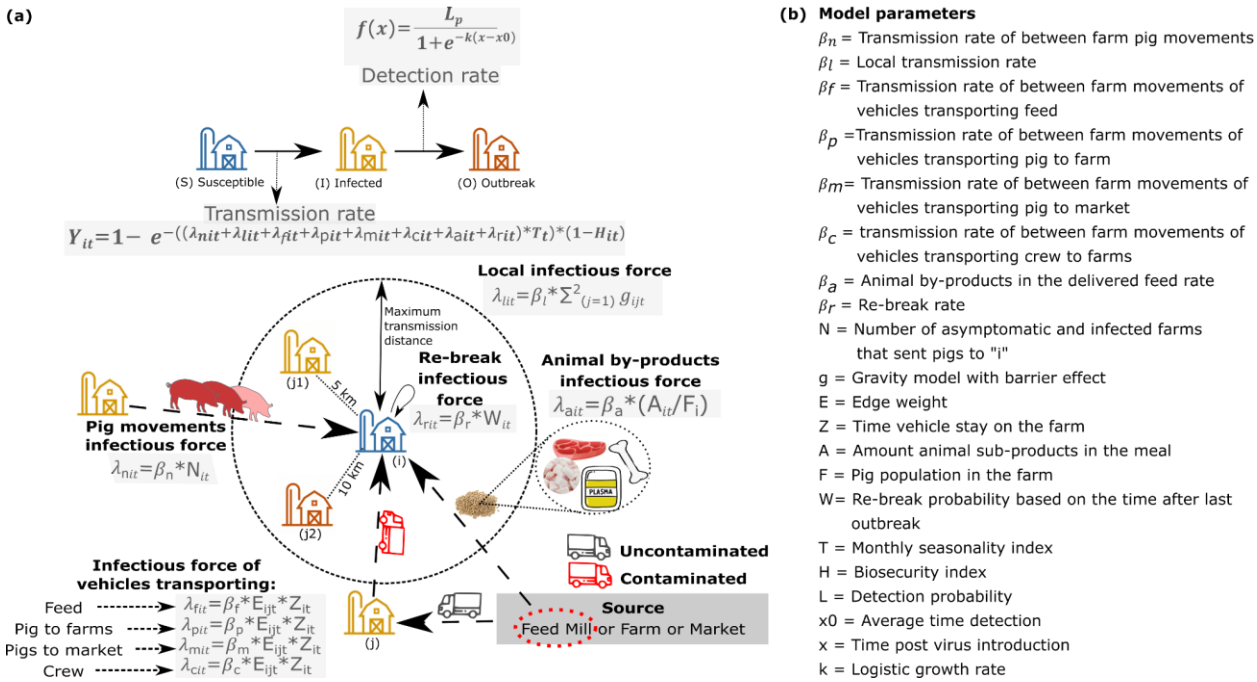

Figure 2. Transmission model framework. (a) Model flowchart of the farm's infectious status and routes of transmission and (b) Description of the model transmission parameters

Model outputs

The model outputs were derived from a random sample of 27 accepted particles in the ABC rejection algorithm. The model outputs included (a) the force of infection for each farm type and transmission route, (b) the weekly number of infected undetected and detected farms (outbreaks) and (c) the sensitivity to detect PRRSV outbreaks in the space (Supplementary Material section 2). We carried out 1,000 runs to estimate the relative contribution of each transmission route. Here, we evaluated the number of infected farms resulting from each transmission route individually, which were then divided by the number of simulated infected farms from all the combined routes and commercial companies.

Results

The comparison among the multiple networks showed that vehicles transporting feed, pigs to farm, pigs to market and movement of crews were significantly more connected than the pig movement network (Table 1). The network density formed by vehicles transporting feed exhibited the highest density (edge density = 0.2). Comparing the paths between the static and temporal networks of pig and vehicle movements, the causal fidelity of all networks was above 32%, which in turn means that 32% or more of causal paths in the static networks can be found in the temporal networks. The networks of vehicles transporting feed and pigs

to farms exhibited the highest causal fidelity values (causal fidelity >90%), which means the closest representation of causal paths among the static and temporal network. Analyzing the network components of each vehicle network for company A, we found that the Largest Giant Strongly Connected Component (LGSCC) had between 976 and 1591 farms, thus these vehicle movement networks connected between 55% and 90% of company A farms (Table 1). On the other hand, in the pig movement network of company A, 11 farms were part of the LGSCC, which represented less than 1% of the farms, in company B the LGSCC had 61 farms which represented 27% of the farms and company C had only 1 farm in the LGSCC, indicating a low connectivity among these farms. Comparing pig movement networks also showed that company B had the highest edge density of 0.013, followed by company C (edge density of = 0.005) and company A the lowest (edge density of = 0.002)

The feed delivery static network had a median in-degree and out-degree of 319 and 304, respectively, which was the highest in comparison with vehicles transporting pigs to farms, pigs to market and vehicles used in the transportation of crew (Table 1, Supplementary Material Figure S11 and S12). The networks of vehicles transporting pigs to farm and crew exhibited a median in-degree and out-degree ranging between 14 and 21, while vehicles transporting pigs to market and pig movements for all three companies showed median in-degree and out-degree less than 7 (Table 1). The network of vehicles transporting pigs to farms showed the highest median betweenness centrality, followed by vehicles transporting feed, vehicles transporting pigs to market and then crew networks, while pig movements had the lowest betweenness (Supplementary Material Figure S13). It is interesting to notice that in spite of the network of vehicles transporting feed was more densely connected, the network of vehicles transporting pigs to farms showed the highest median betweenness centrality values, indicating that this network has the highest number of shortest paths by farm to connect other farms (Table 1 and Supplementary Material Figure S13). Considering the result from the temporal networks, vehicles transporting feed showed the highest median ingoing (ICC) and outgoing contact chain (OCC), thus this means that feed delivery routes create the largest sequential paths over time that allow connecting more farms than any transportation network or pig movements (Table 1 and Supplementary Material Figure S14 and S15). The ICC and OCC from vehicles transporting crew showed the second highest values, followed by vehicles transporting pigs to farms and then vehicles transporting pigs to market. For pig movements, company A had a median ICC of 34, which was the highest median ICC among the different companies, while it had a median OCC of 0. The companies B and C showed lower ICC values, but a higher median OCC of 15 and 4, respectively (Table 1 and Supplementary Material Figure S14 and S15).

Table 1. Summary of the network metric of pig movements and vehicle movements of the three different pig producing companies, with data from January 2020 until December 2020.

Company	A					B	C
	Pig movement*	Vehicles transporting:				Pig movement *	Pig movement *
Network metric / type		Feed to farms	Pigs to farms	Pigs to market	Crew		
Number of nodes	1.745	1.745	1.745	1.745	1.745	228	321
Number of edges - temporal network	34.833	3.182.144	386.730	35.854	109.675	4.344	3.882
Number of edges - static network	5.664	603.977	43.968	18.836	53.880	692	553
Network density	0.002	0.2	0.014	0.006	0.018	0.013	0.005
Causal fidelity	43.2%	98.7%	89.6%	58%	76.9%	32.9%	61.9%
Number of SCC groups[1]	9	2	6	7	3	1	0
Number of farms in the Largest GSCC	11	1591	1479	976	1058	61	1
In-degree[2]	3	319	19	5	14	2	1
Out-degree[2]	1	304	21	6	16	1	0
Betweenness centrality[2]	0	552.3	920.66	111.25	51.32	0.53	0
Ingoing contact chain[2]	34	1515	27	12	131	15	4
Outgoing contact chain[2]	0	1502	32	14	119	15	4

[1]Number of SCC groups with more than 2 farms.

[2]Median values

*Pig movement stands for the movement of live animals between farms.

Furthermore, we evaluated the association of PRRSV outbreaks frequency within both ICC and OCC from infected and non-infected farms. The results found that PRRSV outbreaks were more frequently located in the ICC and OCC of other infected farms for the networks of vehicles transporting feed, pigs to farms and pigs to market networks ($p<0.05$), while such association was not observed for vehicles transporting crew, and for pig movements networks it was only significant for OCC (Supplementary Material Figures S16 and S17). We also evaluated the association between the time transportation vehicles remained within infected and non-infected farms. The vehicles transporting feed and crew to nursery farms showed a higher time on infected farms compared with the non-infected farms ($p<0.05$), but no associations were found for sow and finisher farms ($p>0.05$) (Supplementary Material Figures S18). In addition, no differences were found for the vehicles transporting pigs to farm and to market for any production type ($p>0.05$).

From the data of animal by-product delivered to farms, we found that infected sow farms received slightly more by-products in delivered meals than non-infected sow farms, while no differences were observed in nursery and finisher farms (Supplementary Material Figure S19). However, through a univariate mixed-effects logistic regression in which we included the farm's pig capacity and production type as a random effect, we identified that the amount of animal by-products received by the farms was not significantly associated with the farm's PRRSV status ($p>0.05$).

We first estimated the average number of infected farms in the simulated period which considered January 1, 2020 until December 31, 2020, results showed that from the 1.683 (95% CI: 1665–1701) PRRSV farm infections, corresponding to 109 (95% CI: 107–110) infected sow farms, 678 (95% CI: 665–691) nursery farms and 894 (95% CI: 889–900) finisher farms (of note, this result considered multiple times infections by farm over one simulated 52 weeks, for example a single nursery farm could become infected more than one time over the 52 weeks). Overall, results showed a good agreement between the weekly observed number of PRRSV outbreaks and simulated outbreaks (Supplementary Material Figure S9). The model inferred that at the end of the 52 weeks on average 145 or 8% of the infected farms would be detected, in which 90% of the infected sow farms were detected, while a much lower proportion of detection was estimated for nurseries and finishers farms (>4%). The model performance to estimate the weekly spatial distribution of observed PRRSV outbreaks showed an area under the ROC curve of 0.69 (More details are available in Supplementary Material section 2).

Evaluating the contribution of eight transmission routes over the simulated PRRSV spread for company A's farms. Results demonstrated that for sow farms the most important route was the local transmission contributing with an average of 34.8% of the farm infections, followed by pig movements

with 29.4%, vehicles transporting pig to farms with 17.2%, vehicles transporting feed 11.6%, re-break 3.2%, animal by-products in the feed meal 2.8%, vehicles transporting pigs to market 0.47% and, vehicles transporting crew 0.22% (Figure 3). For nursery farms, pig movements was the most important route contributing with 78.4% of the farm infections, followed by vehicles transporting pig to farms 11.7%, local transmission with 6.9%, vehicles transporting feed 2%, animal by-product in the feed meal 0.5%, vehicles transporting pigs to market 0.4% and vehicles transporting crew 0.1%. For finisher farms, local transmission was also the most important route contributing with 36.8% of the farm infections, followed by vehicles transporting pigs to farms with 29.5%, pig movements 15.9%, vehicles transporting feed 8.1%, animal by-product in the feed meal 5.8%, vehicles transporting pigs to market 3.3% and vehicles transporting crew 0.56%. For companies B and C, because transportation vehicles data was not available, results are restricted to three transmission pathways. Thus, for company B, re-break was the main source of farm infections in sow farms with a contribution of 36.9% on PRRSV transmission, followed by local transmission with 29.3% and pig movements with 28.1%, for nursery 80% was related to pig movements and 20% to local transmission, while in finishers 52% was related to local transmission and 48% to pig movements (Figure 3). Finally, for farms of company C, local transmission was the most important route for sow farms contributing with 70% of the farm infections, followed by re-break with 24.8% and pig movements 1.2%, in nursery 52% was related to local transmission and 48% to pig movements, and for finishers 79% was related to local transmission and 21% to pig movements (Figure 3).

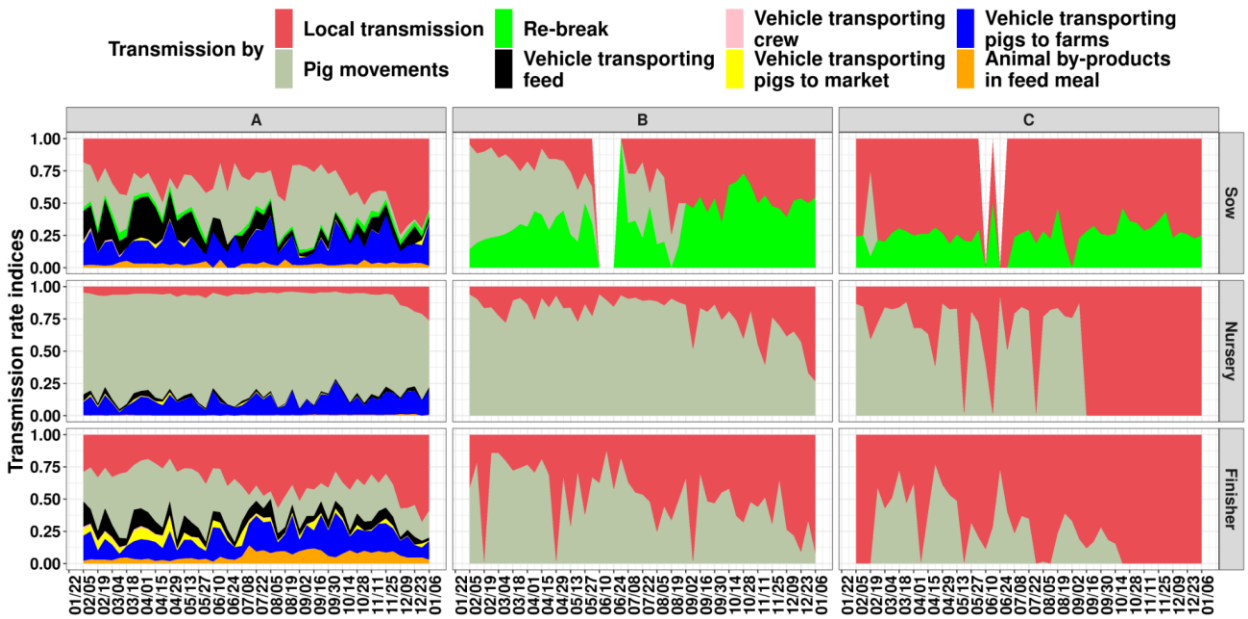

Figure 3. Force of infection for each transmission route of each company (columns) and production types (rows). The *y*-axis represents the proportion of transmission by transmission routes for each of the simulated

weeks (*x*-axis) represented by the different colors. Weekly rate indices were calculated by dividing the number of simulated infected farms for each transmission route by the number of simulated infected farms by the total number of routes combined.

Discussion

In this study, we demonstrated the relevance of eight pathways involved in the between-farm PRRSV transmission dynamic. Our model accounted for: pig movements network, local transmission, different types of transportation vehicle networks, the delivery of animal by-products in the feed meal and re-break. All four transportation vehicle networks were strongly more connected than the pig movement network, thus between farm contacts by vehicles have the potential to propagate pathogens widely and faster than moving live animals between-farms. Thus, we remark that the greater potential that transportation vehicles showed could potentially explain the continued and long standing frustration of swine veterinarians in controlling PRRSV spread, and importantly pose a great challenge to surveillance and effective control of endemic disease in North America and eradication of for example ASF (Brown et al., 2020).

Our study addresses a major gap in better understanding how PRRSV propagates between-farms, given that so far most of the studies have only focused on between farm contacts throughout animal movements in isolation (Thakur, Revie et al., 2015; Lee et al., 2017; Makau et al., 2021). Based on our previous study (Galvis et al., 2021) and accumulated knowledge about the transmission dynamics of PRRSV (Dee et al., 2002; Thakur, Revie et al., 2015; Perez et al., 2015; Pileri and Mateu, 2016; Silva et al., 2019; Jara et al., 2020), it is likely that farm-to-farm proximity (local transmission) and pig movements between-farms may not be sufficient to explain PRRSV transmission dynamics. In this study, we have further extended the previous transition model and included five additional sources of infection, including the contact networks formed by transportation vehicles and animal by-products delivered in the feed meal. Our results demonstrate that pig movements and local transmission were the main transmission routes, regardless of farm types (i.e. sow, finisher) (Figure 3). However, the contribution of transportation vehicles used to transfer pigs to farms explains a significant number of outbreaks. Our examination to the contribution of animal feed delivery to farms, more specifically the volume of animal by-products (i.e. meat and bones meals) confirmed that such mode of transmission was not the dominant one, but still our modelling showed that it was relevant for sow and finisher farms, with an average contribution of 2.8% and 8.1% of the force of infection, respectively. This result should be interpreted with caution given the limited knowledge and great uncertainties surrounding contamination of feed ingredients in the transmission of infectious pathogens, with some studies indicating a low risk of transmission (Cochrane et al., 2017). Even though the literature on PRRSV and feed ingredients remains limited, the discussion about swine diseases such as ASF being transmitted through feed, water and fomites is of great interest. In the U.S. and in other

countries, a widely used product of animal origin is spray-dried plasma and meat and bones (Supplementary Material Figure S2), however, neither experimental or field condition studies have evaluated the entire chain of animal sub-product processing, from the source of ingredients all the way through the mill processing until the animal consumption. Therefore, in the future we remark the need for such studies before further conclusions can be drawn for the contribution of such a route in the propagation of PRRSV and other diseases. Finally, for companies B and C, the transmission dynamic was similar, with pig movements with the greatest contribution to infect nursery farms, and local transmission affecting mainly finishers (Figure 3). However, in the absence of other transmission routes, re-break contributed to a high number of infections that was not observed in our previous study (Galvis et al., 2021) and highlight elsewhere this could be attributed to within-farm persistence of PRRSV (Duinhof et al., 2011; Pileri and Mateu, 2016). These results reinforced the findings of previous studies about the role of vehicles in the transmission of infectious diseases that affect the swine industry (Dee et al., 2003; Dee et al., 2004; Dee et al., 2007; Melmer et al., 2020) and the potential risk of contaminated animal by-products in the feed meals to the between-farm transmission of PRRSV (Dee et al., 2020; Niederwerder, 2021).

This study used individual vehicle GPS movement data to reconstruct networks while considering the elapsed time among farm-to-farm visits, and the time the vehicle spent on the farm to define effective contacts among the farms. In addition, we considered when each truck drove through a cleaning station to avoid links among the farms after that event (Figure 1). The inclusion of cleaning stations to reconstruct the vehicle networks reduced around half of the edges in the temporal network for all the four types of transportation vehicles, when cleaning was not considered as expected. Here, we assumed that the cleaning process was always effective to inactivate PRRSV, even though there are several products that have shown high effectivity to inactivate PRRSV (Shirai et al., 2000), we still lack appropriate data about the probability of PRRSV survival on vehicle surfaces after cleaning and disinfection (Dee et al., 2004), thus further studies evaluating the presence and infectivity of PRRSV after cleaning are necessary.

Previous studies' attempts to consider transportation vehicles were limited to either static or simulated networks, thus limiting our ability to compare our results (Thakur, Sanchez et al., 2015; Wiltshire, 2018; Sterchi et al., 2019; Porphyre et al., 2020; Yang et al., 2020). However, our result demonstrated that for the transportation data we have collected, that static networks provided a close representation of the temporal networks (Table 1), this was especially high for the vehicles transporting feed and pigs to farms, with more than 90% of paths in the static network found in the temporal network, indicating the network could be considered as a static system, since most paths are causal (Lentz et al., 2016). Although these results were only evaluated comparing the static and temporal network from one single commercial company and for one period of time (one year), these results in part support the use of transportation data even if researchers only have access to a static view of a network.

The network density of transportation vehicles was between 3 and 100 more densely connected than the networks of between-farm pig movements. Descriptive studies have also found that vehicle movements between-farms used haulage vehicles increased the indirect contacts among farms by more than 50% (Porphyre et al., 2020), or increasing more than 50% the size of the network weak component (Sterchi et al., 2019) or infect 10 times more farms than animal movements when included in simulated epidemics scenarios (Yang et al., 2020). Among the transportation vehicle networks analyzed in our study, the network of feed deliveries was the most connected network, for instance more than 85% of the farms were connected through sequential paths in the temporal network (ICC and OCC Table 1) or simply because a direct path existed among the farm in the static network (LGSCC Table 1). On the contrary, the network of trucks transporting live hogs between-farms was less connected, but in our transmission model it represented between 11% and 29% of the total transmissions. In turn, the probability of transportation vehicles being contaminated with PRRSV is more likely in vehicles transporting animals than feed, mainly because of the direct contact with infected animals, but also by the indirect contacts by visiting infected sites and becoming contaminated (Bigras-Poulin et al., 2007; Thakur et al., 2016). Vehicles transporting pigs to market could in the same way have a similar risk of vehicle contamination as vehicles transporting pigs to farms, however the former vehicles tend to visit finisher farms with pigs ready for slaughter, thus less likely to re-introduce PRRSV back into the transmission chain. It is worth noting that this is not the case for farms with complete production cycles, farrow to finisher, in which movements returning from slaughterhouses pose a great risk. We expected to see a higher contribution of vehicles transporting crew on the PRRSV transmission, because sharing workers among farms has been reported as a risk factor for PRRSV transmission (Dee et al., 2002; Pitkin et al., 2009), and enhance the pathogen propagation among farms (Rossi et al., 2017). However, in this route we evaluated the individual vehicle connecting the farms, rather than the crew members, who can vary at each farm visit. Therefore, the risk of infection by vehicles transporting crew could be more related to the vehicle itself, which in turn is likely to represent a lower risk of infection compared to the risk of infection related to the personnel. In general, all the four transporting vehicle networks represent a risk of infection for the farms at different levels of disease transmission, which is consistent with previous studies (Thakur, Sanchez et al., 2015; Rossi et al., 2017; Porphyre et al., 2020; Yang et al., 2020), and at the same time this risk is probably influenced by the biosecurity of the farms and vehicle decontamination protocols (Dee et al., 2004; Dee et al., 2007; Silva et al., 2019), which were not thoroughly evaluated in this study and require further research.

The potential propagation of infectious diseases via feed ingredients has been of concern; more recently studies have attempted to relate different feed categories: blood products (animal by-products), cereal grains (non-pig-derived feed materials), oil seeds, forage, pellets (complete compound feed) and straw (bedding material) with the propagation of ASF (Gordon et al., 2019; EFSA Panel on Animal Health

and Welfare (AHAW) et al., 2021; Niederwerder, 2021). Despite the concerns about this transmission route, there are still many uncertainties, including questions about where in the feed processing contamination is more likely to occur, the necessary infection doses, and the effectiveness of feed processing such as pelleting at high temperatures and the use of feed additives (Dee et al., 2020; Niederwerder, 2021). In this study we assumed that all feed meals with any amount of animal by-products were infected with PRRSV, and that pelleting was not enough to eliminate contamination, and feed was delivered with enough virus loads to cause pig infection. Because there is insufficient information and uncertainty about the effects of high temperatures applied during pelleting over the survivability of PRRSV, such as described in (Cochrane et al., 2017), with the potential to inactivate PRRSV (Benfield et al., 1992; Van Alstine et al., 1993; Bloemraad et al., 1994) and other viruses (Cochrane et al., 2017), we note that our model assumption for this dynamics represents the worst case scenario. The contribution of feed meals modulated by the amount of animal by-products transmission as a route of infection was highly influenced by whether or not we used the farm population to modulate the amount of animal by-products. It is likely that farm sizes decrease or increase the contribution of animal by-product due to its association with PRRSV incidence, with large farms presenting a higher frequency of PRRSV outbreaks (Evans et al., 2008; Arruda et al., 2017), and at the same time receiving a high amount of animal by-product in the feed meals. Despite the fact that our model used the volume of animal by-product and the number of animals per premises, we cannot roll out the influence of external factors such as farm type, the performance of each groups with farms with greater average gain weight would tend to consume less feed than farms at limited or compromised performance, indeed when such details production data becomes available those should be taken in consideration. Finally, it is important to mention that feed contamination can also occur within the feed mill facility either by contaminated environments, personnel, equipment, birds, or rodents, or even contaminated trailers (Dee et al., 2020; Niederwerder, 2021). In the presence of detailed information on the environment as a source of feed contamination of feed ingredients, our model could be run with the appropriate parameter values to produce an updated measure of the relevance of feed as disease transmission route.

Limitations and final remarks

There are some important limitations to our study. The lack of vehicle movements and feed ingredients records of companies B and C limited our ability to account for and explain the role of those routes in the outbreaks of PRRSV among their farms. Even without transportation data for company B and C, the data on farm location and PRRSV outbreak of both companies was essential while modelling the local transmission dynamic among farms from different companies, this is important because of the high flux of company-to-company spread of PRRSV, described in details elsewhere (Jara et al., 2020). The way on-farm was considered in our modelling was simply given by the historical records of PRRSV outbreaks, in

which farms with fewer infections were considered to have better biosecurity-level. However, this model simplification may not capture farm biosecurity levels. Thus, future models considering the presence of specific on-farm biosecurity practices and infrastructure such as the presence of cleaning and disinfection stations, could potentially contribute to the transmission dynamic of PRRSV. Another important limitation of our modelling work was the lack of information about the immunization strategies used by each farm, which could have reduced the probability of infection. Despite the limitations, this is the first study that considered several sources of information at farm level to reproduce the transmission dynamic of PRRSV, which provide robust results to the swine industry and regulatory agencies with needed information for better control of PRRSV spread, importantly also help in the identification of most important vulnerabilities offering an unique opportunity to enhance the control of endemic disease while also in preparation of responses to future threats (Herrera-Ibatá et al., 2018; Jurado et al., 2019; Brown et al., 2020).

Conclusion

This work has focused on the development of a mathematical model of PRRSV transmission dynamics to quantify the contribution of eight different routes of between-farm transmission. Indeed, this is the first time that four different transportation vehicle networks and the quantities of animal derived by-products within feed ingredients are simultaneously considered in the dynamics of PRRSV spread. Our results demonstrate that transportation vehicle networks were more likely to spread PRRSV to a greater number of farms when compared with the movement of live pigs between-farms. Our results demonstrate that the network of trucks delivering feed represented the highest risk of possible disease propagation, because it had the largest ICC and OCC, which on average translated in each truck connected >1500 farms over one year. Overall, we conclude that pig movements and local transmission were still the main routes of PRRSV transmission regardless of production types, but vehicles transporting pigs to farms explained a large proportion of infections (sow = 17.2%; nursery = 11.7%; and finisher = 29.5%). As expected, vehicles transporting pigs to market were more important for PRRSV infection in finisher farms (3.3%), while vehicles transporting feed showed the highest impact in sow farms (11.6%), and the vehicles transporting crew had minimum contribution in the propagation of PRRSV. The animal by-products delivered via feed were more relevant in sow and finisher farms, in which 2.8% and 8.1% were explained by this route, respectively. Ultimately, our results described and compared pig and vehicle movement networks which can provide valuable information to other studies using similar databases, and identified the most important routes of PRRSV transmission, granting to the swine industry an opportunity in prioritizing the allocation of resources towards the most risky modes of transmission.

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Authors' contributions

JAG and GM conceived the study. JAG and GM participated in the design of the study. CC coordinated the disease data collection by the Morrison Swine Health Monitoring Program (MSHMP). JAG and GM conducted data processing, cleaning, designed the model, and simulated scenarios with the assistance of NC. JAG and GM designed the computational analysis. JAG and GM wrote and edited the manuscript. All authors discussed the results and critically reviewed the manuscript. GM secured the funding.

Conflict of interest

All authors confirm that there are no conflicts of interest to declare

Ethical statement

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page. Since this work did not involve animal sampling nor questionnaire data collection by the researchers, there was no need for ethics permits.

Data Availability Statement

The data that support the findings of this study are not publicly available and are protected by confidential agreements, therefore, are not available.

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