"An investigation into the risk factors contributing to Streptococcus suis outbreaks in nursery pigs"

Danielle Hopkins
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Hannah Golightly

Okay. Good afternoon everyone, and thank you for joining us for the third presentation in a multi-part webinar series by the newly formed Ontario Swine Research Network. My name is Hannah Golightly, and I'm a second-year veterinary student here at the Ontario Veterinary College, who has been assisting with the OSRN activities.

The OSRN has been formed by faculty at the University of Guelph and representatives from Ontario Pork, OMAFRA and the Swine Veterinary Community. The goal of the network is to enhance and improve the timeliness and accuracy of the U of G research results and activities to end-users. We also aim to highlight the on-going collaborative work, taking place with other institutions and research partners in order to capture the provincial, national, and international impact of the U of G Swine Research Program.

We intend to provide a platform where producers, veterinarians, industry, students and others can go for current and archived research results. Our new website is currently under development, and we hope to launch it soon.

Before I introduce our speaker, I would like to inform you of some of the features of Adobe Connect, our webinar platform. You may enter a comment under the chat window, which should be at the bottom right hand side of your screen. To keep the webinar flowing we will be taking questions at the end of the presentation.

So, this afternoon I am pleased to introduce our speaker, Danielle Hopkins. To give you a bit of background, Danielle completed her Bachelor of Science degree at the University of Guelph, and then entered her Master's Degree in Epidemiology, which she successfully defended this past Wednesday. Congratulations Danielle. Danielle has interest in epidemiology, swine research and outbreak analysis, which aligns nicely with her thesis work. Danielle has had the chance to give this presentation at the conference for research workers in animal diseases in Chicago and has additionally won two poster presentations on here research at the Mike Wilson Day conferences in Guelph, both in 2015 and 2016. With that, I will turn it over to Danielle to take you through her presentation entitled, An investigation into the risk factors contributing to Streptococcus suis outbreaks in nursery pigs.

Danielle Hopkins

Wonderful, well thank you so much Hannah, I was just saying how this webinar series is such a great idea. So, I'll get right into my thesis work, and if you've heard this presentation yesterday, thank you for – or Wednesday, thank you for joining me again. As Hannah said it, it's entitled; An investigation into the risk factors contributing to

Streptococcus suis outbreaks in nursery piglets. And first I'll just go over some background information on Streptococcus suis, then I'll go over some objective results and conclusions of my second chapter, and finally, put it all together with some future steps.

So, what exactly is Streptococcus suis? It's considered to be one of the most important post-weaning pathogens or a pathogen of the nursery. There are 35 different stereotypes, and even more unknown. So, what I mean by this is that S. suis is encapsulated by a capsular polysaccharide and there are 35 different compositions of these, and even more unknown and also some encapsulated versions that haven't been identified. The exact pathogenesis of S. suis is unknown or how it causes clinical disease in pigs, and it seems to be present on all swine farms. Is any microbiologists in the room or at home? This is what Strep. suis looks like under the microscope.

So, it's specific to nursery pigs, it causes disease in about 0-5% of pigs in the nursery, while others simply remain healthy carriers. It can also transition into outbreak situations affecting over 20% of the herd in some cases. There's still a lot of unknowns involved with this bacteria, which makes it a great area of research, especially for prevention and control methods, so we can stop this 0-5% that occurs sporadically, and even these outbreak situations that can cause severe economic losses on the farm.

Which leads me to the core research question. Here, to illustrate this, I have a scenario where we have Pig 1 and Pig 2. They're alike in all aspects, so they're from the same sow, which I arbitrarily named Sow234, they're the same week of age, so four weeks old, they're from the same farm and within the same pen. If I were to go on this farm, I would be able to culture S. suis from the tonsils of both of these pigs, however, let's say about one week passes and for some reason Pig 1 remains healthy, while Pig 2 becomes clinically ill. Which leaves us with the question; What causes this transition from healthy carrier to clinical disease. And we attempted to analyse different risk factors to tackle this question.

Those objectives were: By determining sow and litter level factors that were associated with the hazard of dying due to Strep. suis within the nursery, and it was done using a Cox's hazard regression model, that was run on retrospective data to determine the hazard of mortality within the nursery during this outbreak situation. So, just to briefly go over what this data set entailed. It was from a 300sow farrow-to-finish farm, where all-cause mortality was recorded from October 2011 to March 2012 or over a six-month duration.

Two data sets were merged together; one was a sow data set containing 297 sows and 24 weaning cohorts. The other was a pig mortality data set. So, over this six month duration, 483 pigs had died during that time period, however, we wanted the time at risk to be specifically during the nursery phase of life. So, we removed the pigs that died in nursery, sorry, in the farrowing room, and also the pigs that died in the grower-finisher phase, so we can get the risk factors specific to the nursery. Then I merged and expanded these data sets, so I get not only the pigs that died, but also the pigs that

survived the duration of the nursery. We ended up with a full data set of 2,779 observations or pigs, and that time at risk or the time within the nursery was set to be 63 days or nine weeks. So, I continually all-cause mortality and this is because it was a retrospective data set and the deaths were recorded based on clinical signs.

These clinical signs were specific to S. suis and the fact that they were due to acute meningitis, which is a very characteristic cause of S. suis in some cases. This is when the pigs have a head tilt initially, then they are unable to walk, they end up on their side paddling, and eventual death can occur within one to two days. This is a picture taken from an outbreak situation on the farm, and you can see that these are very characteristic signs due to S. suis, so we assumed the majority of the deaths were due to S. suis. In addition, a sub sample was taken and 100% of those samples were confirmed cases of S. suis, so we can be pretty confident that this was a good measure. This is what the data set looks like. There was percentage litter level mortality on the Y-axis and the month of weaning or date of weaning on the X-axis, so about 24 cohorts that were involved. And you can see that there are some dramatic peaks and falls in the data, however, there was consistently high mortality levels throughout this entire six months, some cases almost reaching 30%, which makes a great level, or a great data set for analysis of risk factors for outbreak situations.

So, these are some of the factors that we looked at. One of them, if they're not quite intuitive to you I will explain them in the upcoming slides, but we'll start with the left and work our way to the right. So, there was nursery mortality within the same litter, previous litter mortality, parity of the sow, the number of piglets weaned to each sow, the age of weaning, pre-weaning mortality, and also potential seasonal affects.

The methods were to take these risk factors of interest, run them through univariable analysis and remove any non-significant risk factors. Then we put it through a model building process where we built a Cox's hazard regression model, which is a survival analysis or time-to-event or a time-to-death data set. Interaction terms and quadratic terms were tested, and also a proportionality assumption was tested. So, using Cox's hazard or Cox's proportional hazard regression, we assumed that all the risk factors remained constant over time, so you have to test to make sure this is true and adjust if it's not. Then we went through residual analysis to see if the model fits the data, identify any outliers and adjust for a potential effect of clustering. With this we looked at clustering within litters and potentially cohorts, as they contain similar factors.

We ended up with this final model based on Cox's regression, and it was adjusted for clustering within litter in addition, there was a time bearing component with age of weaning. So, the ones that remained significant in the model were age of weaning with this time bearing component, nursery mortality within the same litter, previous litter mortality, seasonal effects, and also the number of piglets weaned. Cox's hazard regression is used, analysed using this hazard ration or this HR, if it's above one, this is a risk factor, if it's below one this actually has protective effects on nursery mortality within, on nursery mortality. So, for time, I'm going to go over the first four risk factors of

interest as they had some interesting findings, and additionally, some support in the literature.

We'll start off with age of weaning. Now age of weaning isn't 100% intuitive as there's this time bearing coefficient. In order to analyze how this hazard changes over time within the nursery, I created this scenario. So, if a piglet was aged or weaned seven days later, so from day 21 to 28, what is the hazard this older piglet once it enters the nursery to when it leaves the nursery. And we can see that apparently the hazard has increased up until about day 21, or that hazard of mortality has increased. Then after day 21 it seems to become protective over their time within the nursery, and this may not be intuitive as you would think an older piglet may be stronger or hardier and able to handle the stress of a nursery. However, it has to do with this common pattern of mortality that was seen within this nursery outbreak.

By this I've used a graph to illustrate this pattern. This time there's percentage mortality on the Y, and the week of mortality once the piglet enters, so from week one up until week nine. And we can see that over a 70% of the mortalities appear occur in those first four weeks. So, this initial hazard ratio or risk, happen within those initial four weeks, or that's the reason for that first 21 day increased hazard within the nursery. This is also seen with our other Streptococcus suis outbreaks, where there's this common pattern or timing of death that seems to be experienced by pigs.

Moving on to nursery mortality within the same litter. So, this means that if one piglet has died within the litter, what is the risk or hazard of an additional pig dying within the litter. I have hazard ratio in a red, as it's a very high hazard or risk, and I'm using Cox's regression survival probability to illustrate this hazard. The survival probability curve essentially is if it's higher up on the probability curve or that Y-axis, there's higher probability of survival. If it's lower, there's lower survival probability, and again that time in days or the analysis time in days, is simply the time within the nursery, that 63 days.

So, the blue line represents that no other pig within that litter died, while the red line represent at least one additional pig within that litter has died, and you can see that there's very low survival probability with that. And what are some hypothesis for these? It could be due to within pen transmission. So, a study was done by Dekker in 2009 where they had an experimental trial, and they experimentally infected pigs within the same pen and measured the direct transmission rate between susceptible pigs in direct contact to these experimentally infected pigs, in addition, they measured the transmission rate to indirect pigs or pigs in neighbouring pens. And they actually found that pigs with direct contact, they had 3.58 per pig per day infected versus 0.001 per pig per day infected indirectly. So, interact would be through aerosol or vectors just as flies, versus direct transmission which would be nose to nose contact or fecal matter within the same pen. So, it is possible that their, since the litter mate seem to follow each other into the nursery, that just their proximity has led them to be more susceptible.

In addition, they have similar passive immunity, so they're from the same sow, and this has may have led them to have the similar set of factors that may have made them

more susceptible to disease than other piglets. In addition, since they are in the same environment, it may be similar pathogen exposure or potential for concurrent infection.

Now, I also want to talk about previous litter mortality. This one is slightly different as the hazard ratio is actually below one or protective effect and I'll explain what that means. So, previous litter mortality has to do with if there was a sow that had two litters within the data set, what is the hazard of mortality of their second litter if their first litter had mortality as well. And we can see here that the blue line or the referring category, is a sow with the previous litter having 0% mortality, their second litter actually has a lower survival probability versus a risk factor. Which is a sow with a previous litter, having greater than 0% mortality, their second litter actually has increase survival probability or is higher up on that curve. And we also have some hypotheses for these, however, it's important to note just quickly that that Y-axis has changed, and although I have expanded this to see this comparison, it is a very small difference, but it is still interesting to explore.

So, what are some hypotheses? It could potentially be a build-up of immunity. So, since those sows were initially exposed to S.suis, they may have developed some antibodies, which they were then able to pass on to their piglets. This is seen with vaccinated sows that had been vaccinated with a vaccine for S. suis, and using this vaccine, it was actually seen that piglets did show this passive immunity and zero positivity up until about six weeks of age. However, it does need to be taken with this limitation as only 15% sows had two litters within the data set, as it was only a six-month duration. So, more research is needed as sow vaccination or the sow effect would be a great target for potential prevention and control methods.

Finally, I just want then to briefly go over the seasonal effects. So, here's that same graph that we saw initially, however, I just want to illustrate that it seems to be fairly consistently high mortality within the colder months of December and January compared to the warmer months. So, this was taken as a potentially compounding effect to the data, and that it might have influenced the results during those cohorts.
So, just wrapping it all up. We first talked about this age of weaning, where in this nursery, pigs experienced increasing levels of mortality up until week four when the mortality level seem to dramatically drop. Then we had this within litter mortality effect that had a very high hazard ratio of 9.21. Where pigs are more likely to die if at least one additional pig from the litter also died. And there's also this previous litter mortality effect where pigs seem to be less likely to die if a sow had deaths associated with S. suis in their previous litter.

So, that brings me back to this main research question we started with. With what causes this transition from healthy carrier to clinical disease? And like all great research, I believe it's still under investigation. We, to this point, believe it might be a potentially a multi factorial set of circumstances that has led one pig to be a healthy carrier versus the other pig transitioning to clinical disease, whether this is environmental or maybe even potentially a genetic effect, more research is still needed.

So, putting it all together, what are some future steps and how can we use this research for prevention and control methods. I believe that it would be great to have more research into the effect of sow's immunological response that it has on piglet survival in the nursery. Whether this is identifying how long the passive immunity lasts, and also its effect on mortality.

It would also be good to look at the impact within litter spread. If this finding is due to environmental pressure, or maybe there's a genetic impact involved as well. Also, additional field study should be done on Streptococcus suis vaccination, so we can apply these to swine farms and potentially prevent the outbreaks from happening before they occur. This could be done by using maybe, exploring a different type of vaccine, certain vaccination protocol whether this is vaccinating sows or even piglets or maybe even both.

So, with that, I just want to thank my advisory committee, Dr. Robert Friendship, Dr Zvonimir Poljak and Dr Vahab Farzan, also the Population Medicine Department, Emily Arndt for all of her help with Streptococcus suis research, the Pig Research Group and of course the producers and funders that made this research possible. And with that, I'll take any questions or comments.

So, since I did just do my defence on this, I did get a couple of questions with regards to this idea that we used all-cause mortality instead of Streptococcus suis deaths specifically. And maybe there was an impact of Glässer's disease, as they also follow very similar clinical signs, however, this farm was vaccinated for Glässer's disease and it did not appear that this had any, it was not apparent on the farm or not present. So, we can pretty confident that this was not a potential factor or reason for deaths on this farm, so that may also have strengthened our case selection, just in case this was a question that anyone may have been thinking of.
Perfect.

Karen Richardson
You do have a comment that it was a great presentation.

Danielle Hopkins
Oh, well, thank you.

Hannah Golightly
All right, so it looks like we don't have any questions, so we'll wrap it up now. Thank you for everyone for attending this presentation this afternoon, and that you Danielle for giving it, it was great.
Please look out for the next webinar information that will be circulating soon. Our next one will be taking place at the beginning of April, and so that's all and have a great day everyone.