"A Challenge Study to Determine Whether In-feed Flavophospholipol Can Reduce Salmonella Shedding and Colonization in Nursery Pigs."

Jane Newman
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Hannah Golightly

Thank you for joining us for the second 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 researcher 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 futures 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, but feel free to comment throughout.

So this afternoon I am pleased to introduce today's speaker, Jane Newman. To give you a bit of background, Jane completed her Bachelor of Science degree here at the University of Guelph, and then entered the DVM program.

Jane has an interest in epidemiology, swine research and public health, which aligns nicely with this project, and she had the opportunity to give this presentation just a few weeks ago at the American Association of Swine Veterinarians Annual Conference where she place in the top 10 student speakers.

With that, I will turn it over to Jane to take you through her presentation entitled, A Challenge Study to Determine Whether In-feed Flavophospholipol Can Reduce Salmonella Shedding and Colonization in Nursery Pigs.

Thank you, Jane.

Jane Newman

Thank you Hannah, and thank you for everyone joining us today.

So as Hannah said, what I'm here to talk about today is the project I had the pleasure of working on this last summer, which Dr. Friendship and Dr. Farzan. We were able to develop a study examining the effects of an infeed antibiotic called flavophospholipol, and its effects on Salmonella shedding in colonization in nursery-age pigs.

So to set an agenda for what I'll be talking about today, so to start off we'll be talking about Salmonella in swine. So why it's relevant to us and to the Swine Research Network.

Next I'm going to talk about flavophospholipol and what it is and why it's relevant for the project we did and for swine producers. And finally I'll be talking about the trial we did and just giving a bit into our design and our results that we got.

So to start, Salmonella in swine.

So as you may know, salmonellosis is an enteric illness that can infect and animals. Salmonellosis cases in humans are usually the result of consumption of contaminated food products.

Salmonella is becoming an increasing health concern in pigs and humans, so it's the emergence of multi-drug-resistance strains. In addition, it has been shown in recent studies that Salmonella is prevalent on the majority of swine farms across North America. So pigs are often asymptomatic carriers of the bacteria, and during periods of stress, such as weaning, may shed Salmonella in their feces. This allows for transmission from pig to pig and eventual colonization in the tissue.

In addition, if swine manure is used as fertilizer we run the risk of contaminating produce, crops and groundwater, all of which can lead to further contamination in animals and in humans. In addition, another popular site of transmission in asymptomatic shedding pigs involves transport and slaughter. So pigs will shed the bacteria in their feces during their time on the truck or in holding at abattoir. So this allows for transmission, again, to humans.

So as was said before, human cases of salmonellosis are often the result of a consumption of contaminated pork. Now this can be pork where it has been undercooked and Salmonella has colonized in the tissue, or much more commonly what happens is the pork has been cross-contaminated at the abattoir through contaminated personnel or even equipment.

So all this being said, Salmonella in swine is becoming an increasing public health and food safety concern.

So in comes flavophospholipol. So flavophospholipol is an antibiotic that does not meet the minimum requirements to be considered a therapeutic agent in human or veterinary medicine, and this is due to a variety of factors, such as its lack of bio-availability, it's high concentrations, its poor absorption with an oral administration and its slow

excretion. As a result, the last 30 years it has primarily been used as an in-feed, antimicrobial growth promoter in swine and poultry populations.

So as you may know, Salmonella is a gram negative rod-shaped bacteria. By inhibiting peptidoglycan synthesis in the cell wall, flavophospholipol functions predominantly against gram positive bacteria, however, research has shown that it does seem to have some effects against members of the Enterobacteriaceae family, such as Salmonella and including E-coli.

So flavophospholipol has been shown to alter gut microflora in pigs. By doing so, this inhibits Salmonella colonization in the tissue, due to a reduced available intestinal binding sites or a reduced overall intestinal ph. As a result, there is a reduction in Salmonella due to a reduced colonization and an overall reduced shedding.

So for our study, we had two main objectives. The first was to compare Salmonella shedding and colonization as well as the antibody response to Salmonella in pigs receiving flavophospholipol compared to control pigs.

Now the second objective was simply to determine the time course of Salmonella shedding in any antibody response in pigs challenged with Salmonella Typhimurium DT 104.

So for our study, we brought 32 newly-weaned piglets to a level-2 isolation unit at the Ontario Veterinary College. Upon arrival the piglets were isolated into two separate groups. A treatment group and a control group. So these pigs were split between four rooms with there being eight pigs per room and two control rooms and two treatment rooms overall.

On the day of arrival half of the piglets received a diet containing a four-parts-per million of infeed flavophospholipol. The other half of the piglets received an identical diet, but without the added medication.

On day seven, we challenged the pigs with an oral dose of Salmonella colony forming units of Salmonella Typhimurium DT 104 with nalidixic acid resistance. The Typhimurium serotype was chosen as it is commonly seen in swine populations. The resistance to nalidixic acid was for laboratory purposes so that when we cultured the bacteria from the piglets we were confident that it was the same strain that we had inoculated our population with and not an environmentally acquired strain. We repeated the oral challenge on day eight in an attempt to maximize and ensure infection in our population.

So over the course of 36 days, fecal and tissue samples were collected several times. Blood samples were collected nine times and fecal samples were collected 10 times. On day 36 of the trial the pigs were euthanized and liver, spleen and ileocecal lymph nodes samples were taken.

The fecal and tissue samples were cultured for Salmonella in processes I will describe in further slides. The blood samples were used to detect antibodies against Salmonella using a commercial ELIZA kit.

So in terms of our Salmonella culturing, we cultured the fecal and tissue samples simply to determine the presence of bacteria in the feces. So to do so, firstly the samples were processed with an enrichment agent called Tetrathionate Broth or TTB. Nine grams of feces was added to TTB, stomached and incubated for 24 hours.

Next, this was transferred to another enrichment called Rappaport-Vassiliadis. This, again, was then incubated for 24 hours.

Finally, these samples were streaked onto XLT4 agar, which contains nalidixic acid. And then the following day they were examined for colonies.

So this plate on the slide here represents what a positive Salmonella plate would look like with the black streaks representing the bacterial colonies.

So in addition to culturing the bacterial samples to examine Salmonella prevalence, we decided to count the number of Salmonella colony-forming units per gram of feces. The purpose of this was to examine the number of bacteria between the treatment and control groups so we could better understand Salmonella infection occurring in our pigs.

So in order to do this, the fecal samples were diluted with a 0.1 percent buffered peptone water to make our serial dilutions of 10 to the minus one, 10 to the minus two, and 10 to the minus three. Then these serial dilutions were plated onto XLT4 agar with resistance to nalidixic acid.

So this diagram explains how this looks, with each test tube containing our dilutions of 10 to the minus one, two and three, and each of the plates receiving a separate dilution. So then the XLT4 agar plates were incubated and the following day the number of colonies on each plate were counted.

In terms of our data analysis, a mixed effects multi-level linear regression method was used to compare Salmonella colony-forming units in flavophospholipol and control groups, and in mixed effects multi-level logistic regressions method was used to compare seropositivity between the treatment and control groups.

So informal teacher leader our results, from the fecal and tissue samples culturing, as you can see from this graph, our prevalence of infection was quite high throughout the trial with it being 100 percent on almost every single sampling day, and 97 percent on only two sampling periods.

We decided to culture fecal samples one day prior to the challenge to determine if there was any residual Salmonella infection from the sow barn to which we found none. As you can see, one day post challenge the prevalence of Salmonella on our herd had risen to 100 percent and remained high through the entirety of the trial.

Interestingly, two pigs died prematurely in the trial. These pigs were sent for a diagnostic workup, and it was determined that one pig died due to a respiratory infection, and the other pig had salmonellosis with Salmonella Typhimurium, DT 104 recovered from one tissue.

In terms of our shedding profile, as you can see there did appear to be a slight decrease in trend in infection as the trial progressed, but remember from the previous slide that our prevalence remained high the entire time.

We decided to examine the difference in the number of bacteria between the treatment and control groups, and what we found was there was a significant difference in Salmonella colony-forming units per gram of feces between the two, with the flavophospholipol group having a higher number of Salmonella in feces overall.

In terms of our tissue samples, we were able to culture Salmonella from five out of the 31 pigs that we tested. We found positive pigs in both the flavophospholipol group and the control group, with there being three positive pigs in the flavophospholipol group and four in the control group. One pig from the control group was positive in all three tissue types, which indicates to us that there was a high level of infection in that pig.

In terms of our second objective of examining the time course of our Salmonella infection over the trial, this graph correlates to that. So as you can see, this is the trend of antibody response to Salmonella as the trial progressed.

So on the first day of the trial, 21 out of our 32 pigs had antibodies against Salmonella. By day 12 of our trial, which corresponded to around 33 days of age in the pigs, this had fallen to only nine out of the 32 pigs. Then as you see, over the next two weeks, the seropositivity trend increases and we assume this was due to the acquired immunity beginning to appear.

We found that there was no significant difference in Salmonella seropositivity between the treatment groups on any sampling day, though.

So this graph can be a bit difficult to understand on first glance, so I'll walk you through so that it makes more sense. So the red boxes represent pigs that are seropositive for Salmonella, and the white boxes are pigs that were negative. Each row represents a separate pig. The column on the left, or the counts, represents the number of times that a pig tested positive from a blood sample, and the number of brackets is simply the number of pigs to which this is the case.

So as I said before, on the first day of our trial, 21 out of our 32 pigs were seropositive for antibodies against Salmonella. By do you 36 this had risen to 26 out of the remaining pigs.

Interestingly five pigs were seropositive on all nine blood collection periods, indicating to us that we had a high level of infection in our population.

So to summarize some of our key results, so we found that four parts per million of infeed flavophospholipol had no impact on the prevalence of Salmonella shedding and colonization in nursery-aged pigs challenged with Salmonella Typhimurium DT 104.

However, there did appear to be an impact on the number of colony-forming units per gram of feces. It is possible that this is due to the random assignment of pigs to the treatment and control groups so that some of the high-shedding pigs were more likely in the flavophospholipol group, resulting in a higher transmission between animals. However, this result does contradict previous literature reports that suggest that flavophospholipol has positive therapeutic effect.

And finally we found there was no significant difference in Salmonella seropositivity between the treatment groups.

So some future directions for where we could apply this research, so although both premature deaths occurred from the control group, more trials would need to be completed to evaluate if flavophospholipol has any impact on morbidity and mortality rates in pigs.

And finally the Salmonella infection model we used for this trial appeared to work quite well and would be a good model for future challenge studies.

With that I'd like to thank my advisors, Dr. Friendship and Dr. Farzan as well as the help I received from Saranya Nair and Jordan Buchan. In addition to the Swine Research Team here, the OVC as well as the Centre for Public Health and Zoonoses, in addition to the variety of funding sources that helped me throughout the trial.

So now I will open the floor to any questions.

Karen Richardson

Thank you. Thank you Jane for a nice webinar presentation. I don't have a question, but congratulations on your award at the AFC.

Jane Newman

Well it looks like that's it for -

Karen Richardson

No. There's two more questions come in.

Jane Newman

Sorry about that. We actually have two questions on the way it looks like.

Danielle Hopkins

Great presentation and also wanted to say Happy Birthday.

Karen Richardson

Okay, here is a question from Abby. How does the Salmonella infection affect the pig's welfare, or is it more of a human health risk?

Jane Newman

Okay, so I'm just going to repeat the question so everyone can hear. The question was, how does themselves Salmonella infection affect pig welfare, or is it more of a human health risk?

So in our scenario what we saw at least, was that most of the pigs appeared to be sub-clinically infected with Salmonella. We did take rectal temperatures and we evaluated fecal scores while we challenged the pigs, and although there was some diarrhea and some fever on the first day that went away very quickly. So I would say that we saw that it was more of a human health risk in the sense that because the pigs are asymptomatically shedding the bacteria they weren't showing clinical signs of disease, and just on general examination appeared fairly fine. So yeah, the issue is more in the feces that can come into contact with humans or other animals.

Hannah Golightly

So that looks like that's it for the questions. Thank you to everyone for attending this afternoon, and thank you again to Jane for making that great presentation for us. We'll be circulating information for our next webinar taking place in April shortly. And as soon as our website is up and running we will have recordings of our webinar presentations posted there as well. So that's it, and hope everyone has a great day.

Thank you.