PIG HEALTH TODAY®

HIGHLIGHTS OF A ROUNDTABLE DISCUSSION

THE CHANGING FACE OF PCV

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Welcome

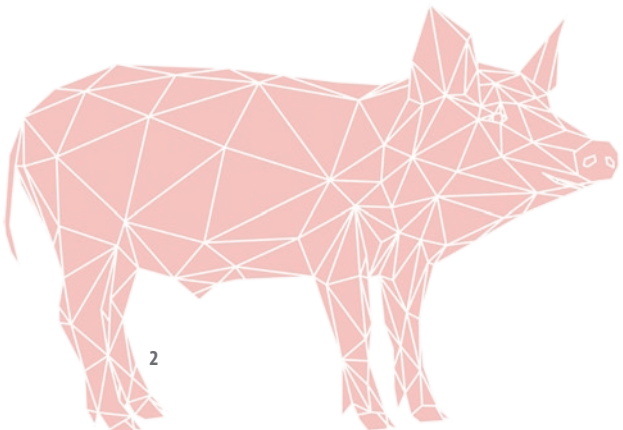
Pork producers around the world have been battling porcine circovirus (PCV), particularly PCV type 2, since the 1990s. Initially, the virus was most often associated with post-weaning multisystemic wasting syndrome.

Over the years, it became clear the clinical manifestations of the virus are much broader. Besides wasting, PCV2 is associated with porcine dermatitis and nephropathy syndrome and porcine respiratory disease complex and reproductive failure, leading to substantial losses for pork producers. The clinical manifestations of PCV2 are referred to as porcine circovirus associated disease (PCVAD).

Like other DNA viruses, PCV2 has been evolving. Globally, multiple strains of the virus have been identified. Vaccines have provided protection, although recently questions have been raised about whether they are, in fact, providing optimal protection. Most recently, another type of PCV — type 3 — has been identified. However, reports from the field indicate that it's similar to PCV2 in name only.

To help the pork industry gain an even better understanding of PCV, we organized a two-part, roundtable discussion with experts on this disease.

We are pleased to share highlights from this informative session. Special thanks to our distinguished panelists for making this possible.



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PART ONE PCV3

JOHNSON

When was PCV3 first identified? And is it a new virus or one that's been circulating but went undetected until recently?

SEGALÉS: PCV3 was found as far back as 1996 in Spain. It's been in the swine population and wild boar population. It's not new and it's widespread. It can be found in animals of different ages, but we don't know if it's pathogenic.

JOHNSON

Dr. Rovira, do you have a similar retrospective analysis for us?

ROVIRA: We haven't conducted retrospective studies, but just to confirm what Dr. Segalés said, there are reports as far back as 1996 in Spain. In Sweden, it was also found in a sample collected in 1993. It was detected in China in the late '90s, and in the UK it was found in multiple samples in 2002. I think we would all agree this is a virus that has been among us for a long time.

JOHNSON

Would you say PCV3 has been around and evolving in the US pig population — independent of what's going on with PCV3 in the rest of the world?

ROVIRA: Correct.

JOHNSON

Dr. Bai, you've also sequenced PCV3 as well?

BAI: Yes, we sequenced 51 samples, primarily from Kansas. Of those 51, we found 31 were unique. Genetic analysis indicates that among all sequences, including about 150 full-genome

BACKGROUND

sequences submitted to GenBank from different regions worldwide, the diversity level was only 2.5%. Phylogenetic analysis indicated that most of the Kansas PCV3 strains are simply merged into different clusters collected from different states in the US and from different countries worldwide, indicating these mutations are mostly random and may not be mutations under much geographic selection pressures.

SEGALÉS: Based on sequencing from the last 20 years, PCV3 doesn't have a high mutation rate compared to PCV2 because most of the sequences over the years are very similar, and sometimes, they are almost identical.

JOHNSON

Have there been situations where a veterinary practitioner feels a reproductive disease or some other clinical manifestation was due to PCV3?

MADSON: PCV3 is a finding. To my knowledge there's no infection model to reproduce the disease in pigs. Several researchers are trying to find out if PCV3 given to pigs elicits lesions that can be identified.

We need to make a clear distinction when we say the word "disease" versus "infection." As Dr. Segalés said, PCV3 is a finding. When we're in the field and investigating cases, we're looking for what could be going on and we do find PCV3. To what degree that finding is playing into clinical signs is difficult to piece out. Association is not causation. I just want to make sure that we're all on the same page from that standpoint.

BAI: I agree.

"We need to make a clear distinction when we say the word "disease" versus "infection." As Dr. Segalés said, PCV3 is a finding."

DARIN MADSON, DVM, PhD

PCV3 FIELD EXPERIENCE

JOHNSON

We tend to be fairly pessimistic in science until we see very good evidence. Dr. Bruner, I'm curious to get your perspective. What has been the experience with PCV3 in the field? What are you seeing with potential clinical cases and is there any association with PCV3?

BRUNER: I'll say we're very confused. This panel may help us work through the confusion. I can find PCV3 in almost every type of tissue. I can find it in male reproductive organs; I can find it in the fetus; I can find it in processing fluids and in post-weaning tissue cases. So, I find it a lot.

The only case I've had indicating that part of the infection was due to PCV3 involved a sow herd with a high mummy rate. We sent samples to the University of Minnesota. The diagnostic lab found high levels of PCV3 in mummy tissue as well as epicarditis and myocarditis. Other pathogens were not found. We couldn't find parvovirus or porcine reproductive and respiratory syndrome (PRRS) virus, and so it seemed as though in that case, PCV3 may have been a cause, at least for the myocarditis.

So out there in the field, whenever we have something we can't explain — poor conception rates, maybe a high abortion rate, sow death loss — we go looking for PCV3 and, unfortunately, we can find it, but we're still really confused about whether it means anything. Is it significant? Should we be looking elsewhere? Can I throw that question back to you?

JOHNSON

Apart from being a moderator, I am a practicing veterinarian. I have been fortunate, and I think probably like a majority of the veterinarians, I don't have any active cases right now where I can't find some pathogen that would cause similar clinical signs. For better or worse, I've been able to find other pathogens that I think better explain the situation. I have found some animals that were positive for PCV3, but the titers were not extremely high. To me, it's the classic question of infection versus disease. I have absolutely found infection. I have not yet found anything I would characterize as disease in my clinical judgment. Dr. Madson?

MADSON: And I would have to echo the information from practitioners. From an Iowa State Diagnostic Lab standpoint, we often find PCV3 in reproductive cases. These cases may test negative for other pathogens, but the causative pathogen for reproductive disease may be cleared at the time of submission. When we do detect PCV3 in reproductive cases, we find it in higher quantities (replicated to higher amounts).

Dr. Bruner's report reflects findings we have recognized. When we do get mummies and don't find the usual causes, we have found PCV3 to a level that raises an eyebrow. The question remains: "Is this something or is it not?"

ROVIRA: In Minnesota, we run PCR (polymerase chain reaction) in virtually all tissue cases for PCV2, PCV3 and PRRS virus. Depending on the clinical signs, we investigate further for other pathogens.

We're constantly learning and producing more data. We're analyzing data in different ways and it's a work in progress. The first thing we've found is that PCV3 is very common. We find it in over 35% of case samples that come through. I think we all agree that PCV3 infection is very common and that it's pretty much everywhere. Like Dr. Bruner said, you can find it in any sample.

Regarding the question of whether PCV3 is relevant — and with the understanding that this is an evolving question —

my opinion as of now is that it can be relevant in a very small percentage of cases.

We have a couple of clinical pictures we think might be related to PCV3. One would be abortion cases. The other would be cases of myocarditis and vasculitis lesions between 1 and 5 weeks of age. In the absence of a good model to reproduce the disease, we look at the cycle threshold (Ct) value. In some of these cases, the Ct values are low — in the teens — indicating more virus is present.

*[**Editor's note**: Using real-time PCR technology, the DNA of a virus is identified with a fluorescent signal. It can take multiple cycles to identify a virus, and the number of cycles it takes is the Ct value. When more virus is present, it takes fewer cycles to be identified with the test.]*

When we see lesions we think might be related to PCV3, we consider *in situ* hybridization, both in fetuses and in piglets with myocarditis. So these are the cases where we feel more confident that PCV3 could be a real cause of disease.

JOHNSON

So, Dr. Rovira, the clinical cases or lesions that you believe might be related to PCV3 are small in number and also infrequent?

ROVIRA: They are very infrequent. They are very occasional. There was a paper that described PCV3 in association with myocarditis. We've seen myocarditis cases associated with PCV3 two, three or four times. The cases were from different farms and different clients. Every now and then another one shows up. That builds confidence for us that the first reported case was not just a fluke. The virus is there, and every now and then you'll have a case like this.

In some of these cases, there was an influx of gilts in the herd. It could be in some cases that PCV3 is subclinical and does nothing, but if an imbalance of immunity occurs in the herd, you would start seeing some problems, then they might go away. So that's my impression about whether or not PCV3 is present and involved in any clinical cases.

JOHNSON

Dr. Segalés, can you comment on this field experience?

SEGALÉS: Yes. In fact, I fully agree with these comments. As pathologists, we would like to see lesions associated with a pathogen. Here in Europe, PCV3 hasn't been a big concern. We haven't had diagnostic labs set up to diagnose PCV3, although this has recently changed and some private labs are offering PCR for PCV3 detection.

In our lab, we are trying to develop an *in situ* hybridization technique for diagnosing PCV3, thanks in part to help from the University of Minnesota. However, the technique is expensive and difficult to implement.

It's important to note that even if PCV3 can be found in particular lesions like myocarditis or vasculitis, one must interpret this lesion and association with a pathogen within the context of a clinical problem. A clinical problem could be of certain importance, and the effects of the virus causing such lesions may not explain all the problems you may see under field conditions. That's the main reason why we cannot really assess the real importance of this virus.

Ironically — considering today's conversation about PCV3 — 20 years ago we found lesions that nobody wanted to believe were caused by PCV2. And now we're sequencing PCV3 and we're looking for associated disease. The point here is that we have to be very cautious. I'm not saying PCV3 is not able to cause disease. As others here have said, it may be important in certain cases. But we have to be very cautious.

MADSON: My question for the group — it's a thought process — is whether you think the tools we have to find PCV3 are too sensitive? We need to have a clinical syndrome or at least a working clinical syndrome for PCV3 that can be used.

On the flip side, as Dr. Segalés indicated, PCV2 is rather easy to diagnose because you have lymphoid depletion; you might have viral inclusions; you have other lesions throughout the body. PCV3 may or may not [be associated with lesions]. The technology we have for detection is sensitive. An RNA scope method is what is being used for *in situ* hybridization of PCV3. And that's literally an amplification of the DNA within the tissue. Small amounts of virus are easily visualized infected tissues.

I'm not saying I'm a non-believer, but as a diagnostician, what we really need as an industry is to come together and say, "Okay, Dr. Bruner, what have you got clinically? Dr. Johnson, what have you got clinically? Here's what we've got from a laboratory standpoint." Let's put this on paper and let's see where we're at. Because right now everything's kind of scattered out and no one's really putting the pieces together.

JOHNSON

I'd be interested in an R&D perspective. What is Zoetis hearing regarding PCV3? How much of a push are you getting from the field about PCV3?

BANDRICK: That's a great question. There's a lot of talk about PCV3, but there's also a lot of guessing and "I don't knows." Is it really a pathogen? Is it causing disease? Or is it just an infectious agent?

We see there's not much diversity in PCV3 at this time. Maybe that's due to something mentioned before — maybe there isn't a lot of immune pressure adding to PCV3 changes.

> **"There's a lot of talk about PCV3, but there's also a lot of guessing and "I don't knows." Is it really a pathogen? Is it causing disease? Or is it just an infectious agent?"**
>
> MEGGAN BANDRICK, DVM, PhD

PCV3 CO-INFECTIONS

JOHNSON

Dr. Rovira, on the diagnostic side, are there any co-infections that you see consistently in clinical cases where PCV3 is found?

ROVIRA: In the small number of clinical cases where we think PCV3 may be important and we find low PCV3 Ct values, we generally don't have co-infections, although that's not always the case.

Looking at all the PCV3-positive cases, the co-infections we find are PCV2 and PRRS virus. When we have more data, we can look at that a little better. I believe this is somewhat due to our sampling and database and the type of viruses that infect pigs at different ages, not because there is some synergistic effect.

JOHNSON

There are many confounding co-factors.

ROVIRA: Correct.

JOHNSON

As you said, you can find many things at certain ages, but it doesn't mean they're synergistic.

ROVIRA: Correct. And the other thing to consider is what we're looking for. There are many other viruses that could be there. We just don't go after all of them.

BRUNER: Have any of you seen a similarity in lesions between PCV2 and PCV3 histopathology? All of our herds are well vaccinated. Is it possible that PCV2 and PCV3 lesions could look similar enough that it could be a PCV2 problem but we're finding PCV3? I'm thinking about the mummy cases. The infection happened a while back and you might still have the lesion, but the original, causative agent is gone.

ROVIRA: I would say the lesions are not the same. The lesions I can relate to PCV3 are vasculitis and myocarditis, and I usually don't see that with PCV2.

UTRERA: I would like to comment on co-infections. There was a recent paper from China about a retrospective study from 2008 until 2017. The investigators found that 70% of PCV3-positive cases had PCV2 co-infections. Of the samples positive for both PCV3 and PCV2, 25% were also positive for porcine parvovirus. So in that study, most PCV3 cases involved another viral co-infection.

JOHNSON

Do you know if there was a noticeable difference in the titer of virus in the co-infection cases versus the single infections? Did it seem like co-infections drove the Ct value down further and there was more PCV3?

UTRERA: They didn't check for any specific titer. They just qualitatively detected the virus.

BAI: We did an animal study by inoculating piglets with PCV3 alone and PCV3 together with PRRS virus. PCV3 alone didn't produce too much disease, but together with PRRS, some piglets developed clinical signs similar to that produced by PCV2. Two animals developed neurological signs — they were turning their heads around and doing something weird. Although the animals recovered a little bit the next day, we euthanized one sick animal together with a healthy one and collected different types of tissues. The sick animal was PCV3-positive and the Ct value was a little high, but it was clearly a positive. And it was negative for PRRS,

PCV2, parvo and a few others we have tests for. So PCV3 was the only one we found in this sick animal with neurological signs. We are in the process of developing an immunohistochemistry to try and confirm if PCV3 is present in brain tissues. We hadn't gotten that far and are still working on it to confirm if that is the case. But our current data still indicates that PCV3 is maybe causing this neurological sign. We have also observed at least two diagnostic abortion cases from which PCV3 was the only pathogen identified with low Ct values.

Another project we just finished was the development of a PCR assay for PCV2 and PCV3 detection. Everybody may have an assay for circoviruses, but PCV2 is so divergent and several genotypes have been identified. Thus, we evaluated all PCV2 sequences and developed a new assay that has better coverage for field strains. During the diagnostic validation process, we tested about 1,300 samples that were submitted to us for PCV2, PRRS and swine influenza virus testing during 2016 to 2018. We found about 16% of samples with PCV2 and PCV3 co-infections.

One thing that may be interesting is that during this 3-year period, the PCV2-positive rate went down a little bit and PCV3 went up. We do not have complete PCV2 vaccination data and do not know how much of the decreased PCV2 rate can be attributed to vaccination, but PCV3 is apparently going up. It's far from reaching a conclusion, as we just had a small sample size, but we may want to keep an eye on PCV2 and PCV3 prevalence changes in the field.

During the diagnostic validation process, we tested about 1,300 samples that were submitted to us for PCV2, PRRS and swine influenza virus testing during 2016 to 2018. We found about 16% of samples with PCV2 and PCV3 co-infections.

JIANFA BAI, PhD

SAMPLING AND DIAGNOSTIC CHALLENGES

JOHNSON

We've talked a lot about infection versus disease. Tell us about submitting samples for PCV3 testing. Dr. Madson, I'm going to ask you to go first. What should be in the box I send when all it says is PCV3 PCR? Are there samples that practitioners should avoid sending?

MADSON: First off, you have to have a history. That question is important because we're finding from the literature and anecdotal reports that it's very easy to detect PCV3. We also know from experience with PCV, including PCV2 and other viruses, that PCV3 is probably shed for a long time and lasts a long time. It's probably a pretty dang hardy virus, and it's likely to be in the environment.

If you add all that together, there's a high likelihood you'd have positive PCV3 findings in oral fluids, serum, feces or tissues. If you go looking for it, you're going to find it.

You need a good investigative process — and cohorts. One thing we forget sometimes is the need for cohorts. Say we have a problem with the flow in one barn and we take samples. We can go to another barn from the same flow and the same age and do some comparisons rather than taking a one-time-point snapshot. What I would encourage you to do is ask, "Okay, do I have a problem?" Think through the situation, and say, "Can I find cohort of samples believed to be okay, and am I finding the same issue in that next group?"

SEGALÉS: Let me be the devil's advocate here regarding the submission of samples and trying to establish a potential association of PCV3 with a given problem. At this point, it's too early to answer because we do not really know if PCV3 is able to cause any particular disease. It might be it is able to cause myocarditis or vasculitis and so forth, but we don't know the real impact on the farm. It's very difficult to answer that question, and probably the wise decision here is to try to get routine diagnostics for the problem you have based on the clinical condition and possible differential diagnoses. Certainly, PCV3 can be added into the pile of pathogens you are looking for, but we don't know if PCV3 is the catalyst or not.

When we don't find what we are intending to find — PCV2, PRRS virus, parvovirus, mycoplasma, whatever — then we are happy with finding another pathogen that's around. We have to be very careful because in most of these cases, we do not really know the meaning of such findings.

If we do not have serological testing, we will not know anything about PCV3 pathogenesis. We do not know anything about immunity, etc. I'm not saying PCV3 is not causing anything, but I'm saying that it's too early to try to figure it out.

We do, however, need to keep an eye on new pathogens. In retrospective studies, PCV2 was detected as early as the '60s. Who cared because it didn't exist formally? The infectious agents of today might be the pathogens of tomorrow because things may change. But with PCV3, we still need research. We are not able to isolate the virus, which is a big problem regarding determination of its pathogenicity. I would say let's try to isolate it so we can perform experimental infections. Let's see if it can be considered a pathogen like other viruses.

So, I'm not reluctant to believe PCV3 is causing disease, but we need much more data to offer something, especially to the field practitioners.

> **Even if we are able to culture the virus, we'd need to confirm whether or not there are co-factors or co-infections associated with the reproduction of clinical signs.**
>
> VITELIO UTRERA, DVM, PhD

JOHNSON

That's a tremendous point. What I hear you saying, Dr. Segalés, is that we need to isolate the virus, intentionally infect pigs and look at the response of the potentially infected pigs because we still need to verify whether or not PCV3 causes disease. Does anybody else have a different set of criteria that would help them understand the pathogenicity question? Is there any other evidence that would prompt you to conclude PCV3 is a pathogen?

UTRERA: I agree with Dr. Segalés that it's too early to draw conclusions that would assign a pathogenic role to PCV3. Even if we are able to culture the virus, we'd need to confirm whether or not there are co-factors or co-infections associated with the reproduction of clinical signs. That's my point of view.

JOHNSON

Is growing the virus a challenge in the labs? Are there obstacles that have to be overcome for us to be able to do this work?

ROVIRA: Yes. We've tried several cell lines. Of course, the first thing you try is isolating it like we do with PCV2. But that doesn't seem to work with PCV3, so we're trying other things. There are some areas we are considering, but we're not there yet.

Going back for a minute about samples, I completely agree that samples should be selected based on the problem, not on the pathogen you have in mind. In that case, I would say send me tissue samples.

continued

SAMPLING AND DIAGNOSTIC CHALLENGES

Now, having said that, we have worked with farms that had a diagnosis of potential PCV3 in the past and they've wanted to monitor for it. That's not so much a clinical investigation as it is monitoring for one particular pathogen. In these cases, we've used serum and that was more in an effort to learn how the viremia lasts, the age of viremic pigs — that type of information. Serum seems to work well. But that's a very specific situation. To do a full workup, however, I would like to get tissues.

MADSON: To follow up, your diagnostic questions are really going to drive the samples that you take. Do you see something in the pigs? Do you need tissues or are you actually investigating epidemiological aspects? So your diagnostic question needs to be pretty precise when you're investigating these situations.

SEGALÉS: There's another issue that might be rooted in the PCV2 story. Recall that 20 years ago [in Europe] we had very severe outbreaks with high mortality and tremendous lesions with amazing amounts of virus. I've never seen a virus in such amounts with other diseases. So, I believe that at the start, PCV2 was completely different compared to PCV3. There have been three or four cases in which PCV3 has been found unequivocally in some lesions, and then you may claim that PCV3 is potentially involved in the problem. But apparently such kind of pathology with PCV3 is not a widespread problem. It seems to be rather sporadic.

PCV3 is also extremely different genetically compared to PCV2, so we do not really know if PCV3 is able to cause disease similar to that caused by PCV2. PCV type 1 is much more similar to PCV2 compared to PCV3.

Sometimes we mix up feelings and needs in the interest of looking for something new. PCV3 has been with us for long periods of time. We are simply finding it now, although of course we have to watch it to see if it is important or not, but right now it's too early for that.

“PCV3 is also extremely different genetically compared to PCV2, so we do not really know if PCV3 is able to cause disease similar to that caused by PCV2. PCV type 1 is much more similar to PCV2 compared to PCV3.”

JOAQUIM SEGALÉS, DVM, PhD

VACCINATION QUESTIONS

JOHNSON

Dr. Segalés, you are saying the situation with PCV3 feels different from the PCV2 story. While they're the same virus, PCV3 doesn't feel like a repeat of what we saw 20 years ago with PCV2, and it's genetically very different. That leads me to my next question, which is to Dr. Bandrick. As a member of the R&D team for Zoetis, which has a PCV2 vaccine, what is the genetic difference between PCV2 and PCV3, and would a PCV2 vaccine protect against PCV3?

BANDRICK: PCV2 and PCV3 are less than 40% similar genetically, based on sequencing. In my opinion, it would be unreasonable for us to expect a PCV2-based vaccine to induce protection against PCV3.

Even though they are both considered circoviruses, they're really very different, and we need to handle them differently. We can't expect PCV2 and PCV3 to behave the same.

JOHNSON

What are your thoughts about a vaccine for PCV3?

BANDRICK: Before answering any questions about a PCV3 vaccine, I keep going back to the discussion we're having right now. There's a lot of confusion about whether PCV3 is actually causing a problem. If we were to add a vaccine on top of that for mass use, we would really be adding a lot of confusion to an already confusing situation. We would be producing specific immunity and then what does that mean? What does it mean within those animals that are infected or not infected?

We need more discussion and more of the data brought together. What's the clinical picture? What are the diagnostics telling us? This conversation is one of those really important next steps to decide if we really do need a PCV3 vaccine or not.

JOHNSON

Say we end up needing a vaccine for PCV3. If we can't grow it, is there any hope of ever making a protective vaccine?

BANDRICK: In many ways it's easier to develop a vaccine if you can easily grow the virus. You have more options available to you regarding the types of vaccine that you could produce. But in the event that the virus could not be grown, there are still options in terms of vaccine development and production. There are some different technologies that could be used.

Although we need to watch and be prepared, we don't at this time have enough information to be able to answer the question of whether we even need a PCV3 vaccine. A PCV3 vaccine at this time would not be of wide benefit to the industry.

MADSON: As a diagnostician asking the practitioners in the group, do you feel a vaccine would help you? Do you feel that you need a vaccine? Tell me what you think.

BRUNER: It's funny because as you were talking, I was thinking, "If she had 2,400 doses, I'd probably try it out."

We've all said we don't know if PCV3 is causing a problem, but that being said, a PCV3 vaccine is something to consider when you have cases with a high rate of mummies and you can't figure out why, and the only thing you can find is PCV3 and maybe some lesions that go along with that.

We started testing more processing fluids, which has been really helpful understanding what a pig is born with. I find a lot of PCV3, which bothers me because a newborn pig already has a lot to deal with. It's got to figure out how to live, how to drink, how to get out of the way when the mom is lying down. I don't want my pigs to have to be fighting off a lot of virus, whether it causes a problem or doesn't.

continued

VACCINATION QUESTIONS

So — from my practitioner's viewpoint — if there was a vaccine available and we're finding a lot of PCV3 in herds with signs like a high mummy rate, I would probably be inclined to try a vaccine. Whether it causes disease or doesn't, 60% of processing fluids from one of my herds have been positive for PCV3 and the Ct value has been as low as 20. We still need to dig into PCV3 but also work on a vaccine in case it turns out to be a problem.

UTRERA: What about processing-fluid results for PCV3 in herds without any clinical problem? Drs. Bruner and Johnson?

BRUNER: The problem is that I don't know if we have many herds that are not PCV3-positive. From what I've looked at, it's on a lot of farms. We can find PCV3 in processing fluids from multiple clients and not all of them have a high rate of mummies. But at the same time, I don't like the fact that we can find PCV3 in high amounts. You know, we have PRRS virus-, mycoplasma- and parvovirus-negative farms, but the Ct value for PCV3 is 20. It feels like there's something we're missing because if there was wide exposure, you would think that with acclimation, you'd get immunity in sows and they'd pass it on to piglets, and you would not be able to find it as readily as we do.

JOHNSON

In the few times I've looked at processing fluids for PCV3, I've had a cohort farm, if you will — a farm with feed from the same feed mill and the same management. There will be a herd with reproductive failure with PCV3 in processing fluids but also a herd with excellent reproductive performance with PCV3 in processing fluids, and the Ct values would be fairly similar in both herds.

At this point, our practice isn't advocating any standard monitoring program for all the challenges we've talked about. It's the classic diagnostic question: Do you know what to do with either result? And my answer right now, is "No, I don't." So, I already have enough questions from producers that I can't answer very well.

Is anyone testing for PCV3 as part of their standard monitoring or is testing always case-based?

BRUNER: It's a bit of case-based but also research, and so if we have repro problems, or have clients that want to look into it a little bit further, then we'll test some processing fluids and maybe turn in some mummies for testing. Nothing standard as of yet, but it is part of our rule-out list for problems.

JOHNSON

Regarding the case of mummies and finding high titers of the virus, have you taken any specific intervention steps?

BRUNER: That case has been ongoing for 2 years. Anytime you're trying to diagnose the cause of mummies, we've found it to be the most beneficial to send samples in volume — as many as we can — say 30 or 40 mummies over the course of a period of time. Last summer is when we found PCV3. People were beginning to talk about it more, and at the suggestion of a colleague, we tested for it and found it in almost all of the mummies and also in almost all of the processing fluids as well.

So for backup, and not knowing for sure what to do, we tried injecting the whole herd with a commercial PCV2 vaccine. To date, I would say the mummy rate has not gone down.

It really didn't change at all. So I would concur that PCV2 vaccination does not help with PCV3 because we can still find it in samples at the same rate.

ROVIRA: Based on my interactions with veterinarians that have had cases where it was believed PCV3 could play a role, my impression is that veterinarians who are dealing with abortions or mummies would try a PCV3 vaccine on the affected farm if it was available. I don't think they would go ahead and use it throughout the whole system. I think they would be trying it in that farm to see how it works. If it did, maybe they would apply it to other farms, see what it does. For the cases where we saw pigs being affected, either before or after weaning, my feeling is that we have to wait before mass vaccination against PCV3. I understand this is just a few cases, but I don't think they — the veterinarians — would be so inclined to use a vaccine if it was available.

MADSON: I want to thank the practitioners because you are on one side of the ball and we diagnosticians are on the other. It helps us to get an understanding from you about what's going on in the field, and we realize you need to make decisions that help your producers. So I get where you're coming from, and I get the need or the want or the desire to make change and help. I appreciate that and that's what drives change as well.

If you're telling me something's wrong or something's different, we do listen. Sometimes it's hard for us because, as pathologists, we don't see a lesion or don't have a syndrome so we can't fully commit, but I think working together on these situations really helps, because you're the ones there who are actually seeing and practicing.

> **"It feels like there's something we're missing because if there was wide exposure, you would think that with acclimation, you'd get immunity in sows and they'd pass it on to piglets, and you would not be able to find it as readily as we do."**
>
> LAURA BRUNER, DVM

NEXT STEPS

JOHNSON

I always try to remind myself that we're in the business of health care, and we have clients with sick animals — whether it's a sick dog, cat, horse or pig. If you are responsible for that animal as an individual, you don't leave happy unless you feel like you have some answers. We are often between a rock and a hard place. What do we know and what do we do?

I want to wrap up with a question. There are three individuals here that got to study under Professor Carlos Pijoan of the University of Minnesota — Drs. Segalés, Rovira and Utrera. In the spirit of the Leman Conference, I would love to hear your guidance on what Dr. Pijoan would tell us to do if he were here today. What would be his direction to us on PCV3? What do we need to do between now and next year's Leman that we can come back and discuss 1 year from now?

SEGALÉS: He'd probably start with "This is crap," and he would not be very keen on this virus, at least at this point. However, he was quite reflective and when you put evidence on the table and could associate a pathogen with clinical findings such as lesions, I am sure he would reverse a little bit.

I must confess that at this point, I am not an extreme believer in PCV3 as a causative agent. Of course, it can apparently cause certain lesions.

JOHNSON

Dr. Rovira?

ROVIRA: I think Dr. Pijoan would say "Why don't you have an isolate yet?" and "Put it into pigs." He would urge us to get to the next step, and I think he would be right. So we should work on that.

JOHNSON

So it's urgent to get the isolate?

UTRERA: I agree. I had the opportunity to be at the University of Minnesota when Dr. Pijoan was having those discussions about PCV2 with Dr. Segalés. Dr. Segalés was trying to convince him that PCV2 was a real problem. I am also sure he would say we need to have the virus, we need to reproduce the symptoms and lesions, and we need to identify any associated co-factors and then record them.

JOHNSON

All right. Thanks to you all. We'll shift gears now to PCV2.

PIG HEALTH T·O·D·A·Y®

PART TWO PCV2

JOHNSON

Vaccination against PCV2 has been a success story in the industry and one we'd love to replicate with every new pathogen, but we always ask if things could be better. Subclinical PCV2 disease remains a problem. Let's start by reviewing the evolution of PCV2 genotypes in the US. Dr. Madson, you've done as much work with this as anybody, so tell us what you've learned about the evolution of PCV2 genotypes over time.

MADSON: In 2004 in North America, we think we only had the genotype PCV2a. In Europe, they already had a PCV2b at that time. By the end of 2004 and beginning of 2005, cases of PCV2b were coming from Canada. Thereafter, there was definitely an industry spike of PCVAD. That was the first PCV2 "global shift" to PCV2b, which was then the most common isolate throughout the world.

At that time, Dr. Segalés actually defined how the genotypes are laid out, based on the differences in the viral capsid ORF2 (open reading frame 2). So it was in 2006 or 2007, we know the genotypes were laid out by this mechanism, that there's a difference of 0.035% or more. We know we had PCV2a and PCV2b. And from archive samples, we know there was also a PCV2c out of Denmark. PCV2c was historical. We thought we only had a few isolated cases, but more recently, this genotype was found in the Brazilian Pantanal.

JOHNSON

And was there another global shift?

MADSON: There was. PCV2a and PCV2b were the major players from 2005 up until 2012, and 2012 is where, at least in the US, there was another shift. After some adjustments in classification and some standardization, we found that what we were calling a mutant PCV2b was PCV2d.

continued

PCV2 EVOLUTION

So, we had PCV2a and PCV2b for a few years, then PCV2d. In 2014, PCV2e was recognized, and as of 2017 — and based on how genotypes are determined — there's now a PCV2f genotype.

There will be more — i, j, k, q, r, s — because we know this virus replicates at a fairly high rate for a DNA virus. It's pretty unique to have a DNA virus mutate to the level this one has. We can only expect that with time and populations, we will be getting new genotypes based on how we classify PCV2 genotypes.

JOHNSON

Dr. Segalés, take us through the European experience and the experience in Asia.

SEGALÉS: The big outbreaks of systemic disease came with the shift in genotype from PCV2a to PCV2b. This occurred in Europe by the year 2000 or 2001, when there was a very clear-cut change in prevalence.

Now it looks like that PCV2d is increasing in prevalence in Europe as well. But it's amazing when you look at the GenBank by numbers, since there is a minimal provision of new sequences during the last 10 years. In fact, those genotype shifts are well-defined based on the GenBank sequences existing so far and their temporal availability. Certainly, there is a significant number from North America and an amazing number from Southeast Asia, especially China at present — there are a huge, huge, huge number of sequences from China and very few from Europe, so the results on the prevalence of genotypes should be considered biased. It's true that in spite of world control by vaccination, cases of systemic disease are still being diagnosed, and recently, when genotyping of these cases has been performed, PCV2d has been the most frequently found genotype. It is not known if this reflects prevalence or if this genotype is potentially more virulent.

A shift hasn't been well-established in Europe yet as it has elsewhere. There's been little genotyping in Europe. Most practitioners here aren't interested in genotyping because they don't want to pay for it. They just want to know if a herd has the disease or not. Most PCV2 sequencing has been done in North America and Southeast Asia, especially China.

JOHNSON

Dr. Bandrick, as an R&D expert, what can you say about PCV2 evolution? What are the primary mechanisms involved? Are there analogies we can use with other pathogens?

BANDRICK: We know from research by various investigators that the mutation rate of PCV2 is similar to that of an RNA virus even though it's a DNA virus. We know PCV2 is able to recombine. We also know that with PCV2 infections there are co-infections with pathogens that are not PCV2, but there are also co-infections with multiple genotypes of PCV2.

This means that an animal can have in any one of their cells PCV2a and PCV2b, for instance, and since both of those viruses are in the same cell, they have the opportunity to share some genetic material. There is agreement that PCV2 can recombine and we know it has a very high mutation rate — and a very high evolutionary rate. That contributes to the genotype shifts that we see.

In addition, we have conducted research where we look at how PCV2 viruses change over time using different data

sets. In these data sets, we can also look at possible recombination. We can use that information to look at how PCV2 has changed across time and geographies. And when we take into account factors like farm management or animal movement, this might help us understand some of the PCV2 changes. There's a lot of information published about influenza, for example, and how it changes and can shift from one location to another. PCV2 can behave similarly.

ROVIRA: I'd like to comment on a study we conducted in 2017. We sequenced 100 PCV2-positive samples and found more than 50% were PCV2d. What's interesting is that about 35% were PCV2a, about 12% were PCV2b and about 3% were PCV2e. So PCV2d is definitely the main genotype, but PCV2a and PCV2b are not gone; they are still there. What we need to do now is try to determine which cases were PCVAD or just cases where we detected the virus but we didn't think they were PCVAD.

> **"...PCV2d is definitely the main genotype, but PCV2a and PCV2b are not gone; they are still there. What we need to do now is try to determine which cases were PCVAD or just cases where we detected the virus but we didn't think they were PCVAD."**
>
> ALBERT ROVIRA, DVM, PhD

CROSS PROTECTION

JOHNSON

What do we know about cross protection across genotypes, and should we expect regular updates to PCV2 vaccines since the virus is prone to change? Or is a universally protective vaccine a reasonable goal? That question is open to anybody in the group.

SEGALÉS: This is old information, but more than 10 years ago we compared the efficacy under experimental conditions (subclinical infection), comparing the challenge of PCV2a and PCV2b using different isolates from different geographic locations and, of course, tested with a PCV2a vaccine. The results were equivalent, really. It was not possible to differentiate the protection against PCV2a or PCV2b.

So, at that time, we concluded that the PCV2a vaccines were equally or similarly protective against both PCV2a and PCV2b strains tested. Of course, when you are translating this information under field conditions, as has been said, PCV2a is still circulating as well as PCV2b, but PCV2d probably nowadays is the most prevalent one, at least in several parts of the world.

Based on field results and experimental infections, it seems clear that PCV2a vaccines also protect against PCV2d. So at this point, we must consider that there is cross protection against all existing genotypes so far — at least the most prevalent ones — as no one has tested against PCV2c or other genotypes.

It's true, however, that when you are trying to find the reason why genotypes shift their course, it's very, very difficult. Probably there is a completely multifactorial background as Dr. Bandrick indicated before. But what is amazing is that the extreme use of PCV2a-based vaccines at least coincided with some time frame difference with the increasing prevalence in PCV2d. One may speculate that PCV2a vaccines protect better against PCV2a and PCV2b and not as well against PCV2d. But this is, again, pure speculation, because we do not really have, let's say, an experimental demonstration of that. We do not have a clear-cut explanation of the reasons for genotype shifts.

So, I believe there's no cross-protection problem, and the point is whether it's important to update those vaccines introducing the new genotypes. Well, I'm not so sure that at this point there are more marketing issues rather than scientific issues behind that, because I know that in Southeast Asia especially, there are a number of vaccines based on PCV2b. It would be interesting to conduct a side-by-side comparison on the same farms with the vaccines, etc. This is something that as far as I know has not happened for most of the time and especially thinking of the current scenario with PCV2d, which is the more prevalent one, in at least some parts of the world.

For these reasons, I am not really convinced there's a need to change current existing vaccines. But this is pure opinion, because I have never seen a clear-cut case of systemic disease on vaccinated farms in which something was not wrong regarding the vaccination scheme. Based on that, I have my doubts about the lack of efficacy of PCV2a-based vaccines against current isolates.

JOHNSON

Dr. Utrera, can you comment?

UTRERA: When we consider cross protection, what are we rating? Mortality? Viremia? Infection or clinical disease? Or is it protection and what is our definition of protection?

We're also regarding all vaccines as the same. The vaccines we have today aren't all the same as they were years ago, and they will be different in the future. So, we need to be more specific about what we're rating and with each vaccine.

I think it's key that we are underestimating subclinical infection. If the vaccines are working well, that brings the genetic shift into question. If there's no immune pressure, why is the virus genetically changing?

BANDRICK: I want to take what you've said, Dr. Utrera, one step further. You and Dr. Segalés have done a lot of this research yourselves and have demonstrated cross protection, as have many others. I think that's clear. But it's a great question that you raise, Dr. Utrera, about what are we measuring cross protection against?

We know that, in general, PCV2 vaccines have been a tremendous success for the industry. But if you look closely at the data — and this is outside of mortality and production endpoints — if you use heterologous vaccine strains and challenge strains, we see a difference in terms of viremia and fecal shedding. So you may still see protection against disease where the vaccine and challenge strain are mismatched, but that subclinical disease is still there.

So while I completely agree that we have cross protection, it's not complete. It's not perfect. PCV2 virus is still circulating, and protection is at least partial. And so that also points to me that the vaccines, while very good, are not perfect.

Most PCV2 vaccines are based solely on PCV2a, and that makes sense because PCV2a was the first genotype identified. But as we go through these genetic shifts and PCV2 mutates, we see a growing gap between the vaccines and what they offer and the field strains we see today. Subclinical disease can still be there.

I would say that, based on that, maybe we do need a vaccine update so we can protect against the newer strains.

"I think it's key that we are underestimating subclinical infection. If the vaccines are working well, that brings the genetic shift into question. If there's no immune pressure, why is the virus genetically changing?"

VITELIO UTRERA, DVM, PhD

SUBCLINICAL PCV2

JOHNSON

I can measure it if pigs don't grow well, if they have poor feed conversion or if a few more of them die. But when we talk about subclinical disease, how should it be measured? Dr. Bruner, I'm going to throw it to you to get a practitioner's perspective. If a client comes to you and says, "Talk to me about subclinical PCV. Do I have it?" how would you measure and reply?

BRUNER: You've just given me an impossible question. I really struggle with defining subclinical PCV2 disease in a herd. I'm so enjoying this discussion because I'm learning a lot just listening to everyone, but I struggle with it because there are so many variables once that pig leaves the sow barn or even once the pig is born. There are so many factors affecting whether a pig is protected against PCV2 — the environment, quality of vaccination and so on.

But to answer your question, I'd say you have to have a baseline. When these pigs are born, what's the level of PCV2? What's the level when they're weaned? Without that baseline, it's hard to say. This is where processing fluids come in to play. You can monitor every week that pigs are born for the level of viremia. Once pigs leave the sow farm and go to the wean-to-finish barn, there are diagnostics for PCV2.

If we know we have good protection at the sow farm and we have a good vaccination protocol, we really shouldn't find a lot of PCV2. So, diagnostics are important and so is having good records and knowing what the potential is for those pigs regarding average daily gain, feed efficiency and mortality and your clinical observations at the farm. Do you see more fall out than normal? Do you see more mortality or more clinical signs to indicate that maybe there's some PCV2 causing some of these variances?

I'm not sure if that necessarily answers the question, but that's how I would approach it with a client who is worried about subclinical disease. Let's start with the baseline and then from that we can monitor.

JOHNSON

Yes, I think that's a great way to look at it. What's your baseline, how do you compare and is your benchmark yours or the producer's? And are there other explainable causes for that variance you see? If there's not another explanation, you start thinking subclinical disease for whatever pathogen you're talking about.

> **...diagnostics are important and so is having good records and knowing what the potential is for those pigs regarding average daily gain, feed efficiency and mortality and your clinical observations at the farm.**
>
> LAURA BRUNER, DVM

JOHNSON

We've talked a little bit about co-infections. Dr. Bai, does your lab do much sequencing for PCV2? And if so, do you find multiple viruses in the same case with the same herd?

BAI: Yes, we do more PCRs than sequencing for PCV2. We have generated some PCR data. We do have a PCR for PCV2 and PCV3 and have seen co-infection with these two viruses. However, for PCV2 alone, we don't differentiate the genotypes. In the beginning we tried to differentiate between PCV2a and PCV2b strains by PCR. But later on, as more sequences became available, the strain coverage became low if we really targeted on differentiating PCV2 genotypes. So we just try to get high coverage to make sure we do not miss too much and don't differentiate PCV2 genotypes by PCR. If somebody wants to differentiate PCV2 genotypes, we use DNA sequencing.

Since the PCV2 genome is so small, we simply sequence the whole genome. However, we don't have enough sequencing data to say if there is co-infection with different PCV2 genotypes, or how much co-infection is there. Unlike bovine viral diarrhea virus, where we see co-infections by different strains, we have not seen much co-infection with different PCV2 genotypes, but co-infections with PCV2 and PCV3 strains. From our PCR data, it's kind of true and similar to other people's observations that PCV2b and PCV2d are the most prevalent genotypes. They have taken over in recent years.

JOHNSON

Does co-infection cause more severe clinical signs or a worse outcome for the pig? Has this been shown experimentally?

continued

PCV2 CO-INFECTIONS

SEGALÉS: It has been demonstrated that co-infection with PCV2a and PCV2b causes more severe disease. We have not tested this, but it has been shown by other investigators. Although the combination of PCV2a and PCV2b causes more severe disease compared to infection with only one genotype, I don't know about PVC2a plus PCV2d or other PCV2 genotypes. I don't have experience with that.

However, getting back to comments from Dr. Utrera, I believe that nowadays, we cannot answer those questions, because at the very end, subclinical infection was discovered because of the use of vaccines. Once we applied the vaccines, we suddenly realized those animals didn't die — at least not in the proportion they used to — and they grew better. The return on investment with the vaccines was the real parameter to take into account. However, if one vaccine works better than others in terms of average daily weight gain, it's extremely difficult to say at this point since many factors should be considered (farm, study replicas, infection pressure, etc.). Because first, you need to have side-by-side calculations on the locations you want to test in different scenarios. Because at the very end, as you have discussed as well, there are co-infections. Co-infections that, of course, may worsen or may provide a better outcome of potential disease. Subclinical infection, I believe, is under good control because of vaccination, although it's not perfect — and especially if we use a single shot.

Try two doses and you will improve results from vaccination, for sure. So the bind here is that in most cases, we expect natural infection to act as a booster for the vaccine. It seems we are not up for eradicating the virus. It's just to protect the animals from clinical disease. And, of course, there's a certain level of the infection in those animals, and it's clear, as well, that with mass vaccination, infection pressure decreased. However, again, you may say this vaccine was better than another, or of two vaccines, this genotype works better than others. To me, it's certainly difficult to demonstrate that in a clear-cut way.

“So the bind here is that in most cases, we expect natural infection to act as a booster for the vaccine. It seems we are not up for eradicating the virus. It's just to protect the animals from clinical disease.”

JOAQUIM SEGALÉS, DVM, PhD

JOHNSON

Here's a question for the diagnostic group. How can diagnostic labs help us answer this question of how much mutation is enough mutation to say we need an update to the vaccines?

ROVIRA: That's what I was thinking of — kicking it back to the practitioners. One of the things we're missing in the discussion is the question of vaccine failures — whether they are real or not. And that's something that you see first-hand. We just help you with the diagnostics in cases of suspected vaccine failure.

In the last few years, I have seen a few cases of PCVAD and, at least in the cases where I could work closely with the vet and try to figure out what happened, we could always bring it back to some sort of trouble with vaccination — the vaccine was not being given or the protocol had been shifted for different reasons, maybe to address a mycoplasma issue, things like that. And adjusting that vaccine protocol would have probably yielded a better outcome. And, of course, there's the problem that I think Dr. Segalés mentioned, of half doses. So there are a lot of changes in the protocol that can be used to fix that problem.

In my experience, I haven't dealt with a case that I was confident was a real vaccine failure. That's how I'm feeling right now from what I've seen. So, I was interested in knowing if you have dealt with cases where you felt the vaccine was working, that everything is being done the same but for some reason this same vaccine now doesn't seem to work? Cases where you think there must be something with the virus — that it's changing?

MADSON: I'm not sure I can singly point to vaccine failure. There's vaccination failure. There's a difference. The piece that's sometimes missing would be the immunity aspect. Dr. Segalés alluded to that with the two-dose product, right? So if we've been monitoring and we think we have a problem, we go to the vaccine. We say, "Let's take one step back and look at our immunity and the pigs we're vaccinating and the situation we're putting them in."

However, the diagnostic lab still needs to have data and we still need to be looking at that. Granted, diagnostic cases are inherently biased because they're diagnostic cases, right? So, you know, it takes due diligence and continual monitoring as we go forward. That's how we find shifts, drifts or whatever you want to call them among different viruses.

Again, it's working together. If you don't tell me something's wrong, I'm not going to sequence. When we had the shift to PCV2d back in 2012, we thought there were vaccination failures, but that's how the new genotype was identified.

UTRERA: I have a comment about recombination. In a recent study from Chile, the findings suggested that a novel cluster of Chilean sequences emerged resulting from recombination between genotypes PCV2a and PCV2d within ORF2.

And I want to respond to a comment from Dr. Segalés. We got some clinical disease. Ten years ago, there was a paper showing that in herds that had no clinical PCV2 disease, the only effect they showed when they vaccinated was a significant improvement in average daily gain and growth performance. So in that way, they showed there was a subclinical infection associated with PCV2.

continued

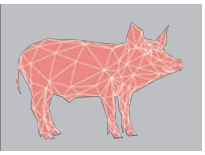
JOHNSON

One of the first things I always ask myself if I have a case of unexpected PCV2a and PCV2d is whether it's a repeat case with a flow of pigs or is it a one-time occurrence? If I walk into a barn with thousands of pigs and find five pigs with some sort of PCV-like lesions, that wouldn't completely shock us, right? That leads to a question I would pose to Dr. Bruner. Do you see, today, in 2018, flow-based PCV2a and PCV2d challenges? Or group after group with unacceptable results from a PCV-control standpoint? I know we can't always guarantee that vaccines were administered perfectly, but is it repeatable enough that it makes you concerned that it's not a vaccination problem, per se?

BRUNER: I can't say that I've seen that. We're really big on making sure the sow herd is well-vaccinated. Everything starts with the sow herd. If you have a really good PCV2 vaccination program on your sow farm, those instances of vaccine failure or vaccination failure are less just because you started with a lower amount of PCV2 on the sow farm being transmitted to piglets.

"One of the first things I always ask myself if I have a case of unexpected PCV2a and PCV2d is whether it's a repeat case with a flow of pigs or is it a one-time occurrence?"

CLAYTON JOHNSON, DVM



JOHNSON

Give me your prescription for a good sow-vaccination protocol for PCV.

BRUNER: Sure. We always want to make sure we give two doses of vaccine to young, growing gilts and then at some point pre-breeding — whether that's within 1 month or 2 months of starting the heat check and breeding of those gilts — a systematic approach to make sure the vaccination plan is automatic for those sow farms. There's a schedule and they know exactly when they're supposed to do it, and it never gets missed. And it's repeatable every month or every week.

JOHNSON

Are there any seasonal vaccinations or any mass vaccinations for your sow farms you recommend? Or any pre-farrow administration?

BRUNER: That's more on a case-by-case basis. If we have herds and for some reason we're finding more PCV2, more positive PCRs and lower CT values than we like, that might be something that gets implemented, but it's definitely on a case-by-case basis.

JOHNSON

What about the antibodies at farrowing relative to your decisions? When you vaccinate on a case-by-case basis — mass vaccination or seasonal or pre-farrowing vaccination — are you concerned about maternally derived antibodies (MDAs) and their impact on subsequent piglet vaccination?

BRUNER: I'm concerned about everything. Every time I make a decision, I hope it doesn't lead to another problem. I can say in these cases where we've implemented mass vaccination, I've not seen repercussions from mass herd vaccination.

JOHNSON

Dr. Madson, you and I have recently traded emails on the subject of MDAs. Do you or anyone else have comments on this and should MDAs be part of a circovirus-monitoring program?

MADSON: In my view, the answer to that would be yes, though not every time. Let me back up. In the literature, there are a few papers about maternal-antibody interference and vaccination. Some say there is interference and some say there isn't. Based on what I hear from practitioners, I personally believe MDAs have played a part in PCV2a occurring downstream after vaccination.

I am a personal believer in tracking when there is concern. If I were consulting, I would probably say never vaccinate pre-farrowing. I would consider mass vaccination, but I would consider delaying piglet vaccination for a few weeks or at least giving pigs two doses if you're using a one-dose product.

If you're going to vaccinate routinely, post-farrowing is probably the easiest and probably the best because you're trying to protect gestational fetal infection.

In short, I do think that there are certain situations where maternal antibodies affect what you see downstream post-vaccination.

JOHNSON

Dr. Segalés, as you look at the evolving genotypes of PCV2, is there any reason to believe that MDAs from some of the newer genotypes may be more likely to block the efficacy of PCV2 vaccination than we've seen historically with PCV2a vaccines?

continued

SEGALÉS: In general terms, there is cross protection among genotypes and this also applies to MDAs. It's true that certain monoclonal antibodies can distinguish some of the genotype epitopes. I believe cross protection is relatively efficient based on such a polyclonal response.

Of course, you may say as long as the virus is evolving (in part, thanks to the vaccination pressure), that we may create escape mutants at the very end that are not able to be covered by existing vaccines. Well, at this point I deduce that we do not have sound information that supports this issue, nowadays at least. We have seen a number of PCV2 vaccination failures, but I have yet to see a real vaccine failure, since in most cases the problem was how and when the vaccine was applied.

So from this point of view, even if there are changes in genotypes or new genotypes arise, I believe in the short term, existing vaccines will be able to cover circulating genotypes. The classification in genotypes is a rather interesting scientific topic but quite artificial, so it is always in permanent revision, and we may have more than the traditional four genotypes we are discussing mostly. At the very end, there is more than 90% similarity in most, if not all, of the sequences of PCV2, independent of the genotype. If you are taking other viruses, of course especially RNA viruses, such as PRRS virus or retroviruses, etc., these are really divergent in terms of sequencing and probably in terms of immune response, as well, compared to PCV2 isolates. With PCV2 I do not expect, at least in the short term, that we may find escape mutants. It is also important to note that protection against PCV2 is not just provided by antibodies but also by cellular immunity. So far, it looks like the major existing vaccines on the market are able to provide both, though to different degrees, humoral and cellular protection.

"With PCV2 I do not expect, at least in the short term, that we may find escape mutants. It is also important to note that protection against PCV2 is not just provided by antibodies but also by cellular immunity."

JOAQUIM SEGALÉS, DVM, PhD

LOOKING AHEAD

JOHNSON

To wrap up, let's have each of you give us your perspective. What concerns you about PCV2 evolution going forward, and tell us what you think the situation will be 5 years from now regarding new diagnostic-monitoring strategies or new control efforts in response to evolution? Dr. Bai, would you please lead off?

BAI: I'm not really involved in vaccine development too much, but we do look at pathogen sequences from time to time to make sure that our PCR tests are up to date. In recent years, the majority of the PCV2 viruses are still PCV2b-based including PCV2b and PCV2d. We have seen PCV2a and PCV2c sporadically and have not seen PCV2e strains so far. And from a technology perspective, PCR is still the main tool for molecular diagnostics, and we have recently developed a multiplex PCV2 and PCV3 real-time PCR test.

During the process, we tried to look at the published PCV2 PCR assays. Surprisingly, there are quite a few PCR papers published, but it is difficult to find one that has high strain coverage. Having high strain coverage in the design stage may be the best way to ensure high diagnostic sensitivity once an assay is developed.

There are two main reasons for the low coverage of strains. One is that some assays may not be developed with enough number of sequences — for example, only 10 or 20 sequences were used for a PCR design — then the resulting assay may not cover the rest of the sequences in GenBank. Secondly, as everybody knows, the virus is changing all the time. As molecular diagnosticians, we probably should check pathogen sequences periodically to make sure that our diagnostic assays are really picking up the majority of the viruses.

Another tricky part is that if you don't have perfect coverage for the test and you have missed identifying some strains, you wouldn't know it easily, because there are other pathogens involved in a given syndrome, like respiratory disease etc., and you may think it is caused by other pathogens. So there's no easy way to figure out false-negative results, and that may generate misleading prevalence and epidemiology interpretations. Thus, I think as molecular diagnosticians, we should try to convince people that validating a molecular diagnostic assay is an ongoing process, and a molecular assay is not something we can build and then use forever.

JOHNSON

Dr. Bruner, what concerns you about PCV evolution going forward?

BRUNER: I'm worried about subclinical disease. I wasn't worried about it before, but I think that's probably what concerns me the most now. Subclinical PCV2 disease is super-hard to find, so I worry about whether subclinical disease will become clinical.

On the diagnostic side, I worry about being able to see indicators that this virus is changing for the worse — about being able to pick it up soon enough to prevent 20% mortality like we once had with PCV2.

PCV2 wasn't a big deal at first, then all of a sudden it became a really big deal. I don't want to get back there. I want the diagnostics to identify changes that could tip us off to ensuing clinical disease. We are fortunate to have good diagnostic labs we can communicate with to relay what's happening in the field. So, I just want to stay ahead of the game.

LOOKING AHEAD

JOHNSON

I'll pause the wrap-up for a moment because Drs. Bruner and Bai brought up great points that connect to each other. Dr. Bai said we need to monitor assays and regularly update them. Dr. Bruner asked for future assays to look for leading indicators of a change that hasn't manifested itself clinically but has a high probability of doing so. Is that a realistic expectation from the field? Can diagnostic labs start to develop assays that from an evolutionary standpoint will be more predictive in trying to help identify potential virulence and, ultimately, a shift toward decreased PCV2 control?

ROVIRA: That's a great question. I think we're going to get there, and eventually we'll be able to make that type of prediction. That's something that is a little bit out of my expertise, but we do have molecular biologists working with other biologists that specialize more on immunology and protein structure modeling, and they are trying to predict how everything works. To me, it sounds a bit like science fiction, but we're starting to work with them, with all these sequences that we generate, trying to figure out what the different mutations mean for the virus, what it might mean for the pig, and how the proteins of the virus are going to interact with the immune system of the pig. And like I said, it's still a difficult concept for me to grasp, but I think in the future we'll be able to predict cross protection to some extent.

JOHNSON

Dr. Segalés, do you have any thoughts on the subject?

SEGALÉS: I must confess I'm not concerned about the virus. The virus will evolve anyway. The point is that we have to monitor and follow up to see if those changes have any effect clinically.

I'm much more concerned when producers vaccinate but don't see the expected results. Let's say there's a so-called vaccination failure or poor results associated to vaccination because, at least based on our experience in Spain, these cases are not properly investigated. That's a major problem. People get very nervous about viruses. They get a diagnosis of the systemic disease and their reaction is, "C'mon, change this, or change that, change the vaccine, change — whatever."

The proper procedure would be an investigation of immunity on the farm and determining if vaccination clears up the infection, but people are not keen about on-site investigations. This is what really concerns me because I believe that even though vaccines are not perfect, they work very well in general terms. And when something is going wrong, we should do the right investigation — not change things without any criteria, which sometimes is what I have seen under field conditions.

JOHNSON

I know that Zoetis has done a lot of work with the epitope technology from a diagnostic standpoint. Is there an opportunity to leverage that technology to investigate vaccine or vaccination failures in the field?

BANDRICK: We know PCV2 is a set of viruses, and there's a set of conserved sequences within, or conserved sequence that makes a virus a PCV2 virus. But, of course, among the different genotypes — a, b, c, d, whatever — there are specific immune-recognition sites that are different. And the immune system can tell the difference between an "a" virus versus a "b" or a "d" virus. We know based on the literature that there are specific regions of the virus that are more important in terms of the immune system being able to tell one virus from the next.

As Dr. Segalés said, much of that is based on monoclonal antibody work. But, of course, the immune system in a healthy animal works on a polyclonal system. Saying that, we also know generally what the major histocompatibility complex of pigs is. We know what the sequence of PCV viruses is, and if we can educate the pig's immune system with the right vaccine so that all of those relevant epitopes or immune-recognition sites are covered, the pig's immune system is educated such that it can respond to those relevant field viruses. That's one way that we can use the epitope work. Does that make sense? But if you look at it from the back end, it may be more closely to the question that you're asking. If there's a new PCV in town — let's just call it PCV2h — if we have PCV2h and there's a vaccine failure using a PCV2a vaccine, can we use the epitope analysis to determine what is different between PCV2h and PCV2a, for example? What is the difference that the pig is not getting from the vaccine? What does the pig need to learn such that it can be protected against PCV2h? So, yes, I think that we could use some epitope technology to deal with a bigger vaccine issue so that we can really rationally design a vaccine. Definitely.

JOHNSON

Dr. Utrera, what are your thoughts or concerns about the evolution of PCV2 over the next 5 years?

UTRERA: Twenty years ago, there was only one genotype — PCV2a. Then there was a genotype shift and we had a higher prevalence of PCV2b (after 2005). Now the most prevalent is genotype PCV2d. I don't need to be a wizard to say that 5 years from now we'll have a different genotype that will be the most prevalent. And I will say that modern vaccine design will be based more on reverse vaccinology and epitope analysis. Swine veterinarians will base their decision on what vaccine can be more efficacious against a specific strain based on tools like the epitope analysis in order to predict coverage and protection.

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"I don't need to be a wizard to say that 5 years from now we'll have a different genotype that will be the most prevalent. And I will say that modern vaccine design will be based more on reverse vaccinology and epitope analysis."

VITELIO UTRERA, DVM, PhD

LOOKING AHEAD

JOHNSON

Yes. Look upstream. That's been a consistent thing to do in the US pig industry for a long time. Dr. Segalés, your turn.

SEGALÉS: I expect we'll be finding more genotypes. Basically, that. But I do not foresee tremendously marked changes with the current vaccines present on the market. This is a different issue. Because I believe that the new defined genotypes will come along in the next year or years, so we will not have to wait for long. But so far, currently existing vaccines cover the genotypes circulating in the pig populations. I must confess that I'm not a wizard; I'm not very good forecasting the future. I prefer to go step by step and see what's going on in the future, in the immediate future. I believe I'm not the right one to make predictions.

JOHNSON

Dr. Madson?

MADSON: I'm in the same boat as Dr. Segalés. The virus is going to change. We know that. In 5 years' time, I think we're going to have new genotypes. I don't expect to see a huge change in immunity though unless we change the way we raise pigs. I think we would have seen that by now, just because of the pressures and the way pigs are raised now. We might see differences that are more drastic if there's not adequate protection. Mutants usually don't survive, so I don't expect a huge difference.

JOHNSON

Dr. Rovira?

ROVIRA: I'm not too concerned about PCV2 evolution. I'm more concerned about vaccination and how we use vaccination. We know that in most cases the vaccines work really well, if we use them well.

However, we might be getting closer to some bad times. If the swine industry is not profitable, people tend to cut corners. When we tried to cut corners on PCV2 vaccination, we started seeing it again. When times are not good, we start seeing all kinds of weird stuff in the diagnostic laboratory, and PCV2 is one that comes back.

JOHNSON

Dr. Bandrick?

BANDRICK: It's really been an honor for me to be a part of this panel with such an esteemed group. Thank you for sharing your experiences. I would say I completely agree that we need to monitor PCV2 virus evolution in the context of our global swine industry and not just focusing on one region. We know that with animal trade, the virus is in our animal populations and it's moving around the world.

We need to monitor PCV2 since the bar is changing, and we need to keep in mind a rational approach to vaccination. As we look at PCV2 mutation and PCV2 evolution over time, we know the virus is changing. We know new genotypes are emerging. We need to make sure our pigs are given vaccines that are going to induce the immunity that protects against those strains they are likely to see in their lives.

That means making a vaccine that induces the right type of immune response. If that means making a vaccine that induces protection against PCV2a, b and d, that's what we need to do.

> **We need to monitor PCV2 since the bar is changing, and we need to keep in mind a rational approach to vaccination. As we look at PCV2 mutation and PCV2 evolution over time, we know the virus is changing. We know new genotypes are emerging.**
>
> MEGGAN BANDRICK, DVM, PhD

JOHNSON: I have had a couple of herds that I'm working with that have PCVAD-like issues in growing pigs with no clinical signs of PCVAD in the breeding herds, so I asked you that question. If you completely understand it and can explain it, you're a better vet than I am, because I don't comprehend the current industry rationale and explanation. And a little bit frustratingly now the response is always "leaky sow farm," right? Well, leaky sow farms don't have infected pigs at weaning, so that's hard for me to get my head around right now. How does that work? And then the response to mass vaccination — I mean the maternal-antibody piece does concern me. It almost feels like a whack-a-mole game when you have a problem. "Well, you have to have a leaky sow farm and the only way you can fix that is mass vaccination." And that creates maternal-antibody problems for 3 months. That doesn't sound like a very good outcome. I don't have a better solution, unfortunately. But it's frustrating to me that I only have one response when it comes to PCV management. And the reaction is a PCV2 vaccine which just shifts the problem from an often undiagnosable viremia at the sow farm to maternal antibodies in the piglets.

So, when I see something that looks vaguely like PCV2 but may not actually be driven by PCV2 alone, like PCV3 or anything, right? An unusual case presentation, what do I do? I can't recommend only one reaction of PCV2 vaccination because eventually I'll have cases where PCV2 alone or the historical PCV2 isolates aren't my true root cause of PCV-like clinical signs. I'm not going to be the vet for that herd very long if that's my response to every producer with a similar clinical syndrome. I feel like we've probably inappropriately applied the tools today because we lack a complete understanding of the epidemiology of the disease and transmission within populations.

LOOKING AHEAD

On the future of it, recombination concerns me. How many decades did influenza circulate among pigs with the ability to recombine before it decided to do bad things? Eight or nine. A long time. Spanish influenza virus circulated in pigs as H1N1 until it decided to add a triple internal gene set, which really likes to reassort. And I know nothing about the genomes in PCV2, and if there are any analogies that could play out there right at their particular internal gene components that are more prone to recombination than others, I don't know. Maybe there's an analogy there or not. But rotavirus is another disease that I would throw out; for years it's had the ability to recombine but didn't do it. So I think PCV evolution would be very concerning, and I would want us to watch that closely.

Recombination will be the piece that I would want to keep the closest eye on. It makes me nervous, the fact we have them, and I think we have examples of other diseases that lay dormant and then all of a sudden changed some genes around and caused critical problems managing disease with the available control tools.

BANDRICK: I'll add one point. And so as we talked about earlier, we know the protective response to PCV2 is at least partially driven by cell-mediated immunity. And something that I see as important when you look at mutation of PCV2 is how antibodies and T cells recognize viruses differently. And so T cells of course recognize a virus in linear-sequence segments — the epitope is a sequential amino acid sequence; whereas antibodies recognize conformational epitopes — the epitope is 3-D and can be non-sequential amino acids.

You could argue that small changes within the sequence could perhaps more so affect how a T cell recognizes and responds to PCV2 than it would affect the antibody response. Changes in the PCV2 sequence may make it less likely the T cell responds to infection. And so I think that also for that reason, as we see PCV2 changing and we know that epitopes are changing, the recognition sites of emerging PCV2 viruses are different, that we also need to monitor PCV2 virus change for the sake of the pig's immune response being a protective response to PCV2.

If the immune system can already tell the difference between different genotypes of viruses and we know PCV2 will continue to change, at some point the T-cell responses aren't going to be protective. If a pig's immune system can't recognize a PCV2 virus because the epitopes are too different from what it learns from the vaccine, then we will have disease. We need to make sure the vaccines include broad enough epitopes to induce protection from these viruses.

JOHNSON
That's a good way to look at it. Thanks to all of you.

> **Recombination will be the piece that I would want to keep the closest eye on.**
>
> CLAYTON JOHNSON, DVM

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