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Epidemiological analysis of outbreaks

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Molecular techniques have become a very powerful tool in veterinary medicine. A number of such techniques have been used for epidemiological studies as well as diagnostics, including RFLP, PCR, Multilocus Enzyme Electroforesis, Ribotyping, Restriction Enzymes, and others.

Most of the epidemiological studies have been directed to the study of the clonal expansion of microorganisms in human and veterinary populations and also to track epidemic strains in outbreaks. Several investigators have used some of these techniques to perform studies relevant to swine production. Using DNA fingerprinting our group was the first to demonstrate that within farms, disease outbreaks of *S. suis* meningitis were caused by a single—or, at most, very few—strains. We later showed the presence of these epidemic strains with *Pasteurella multocida* isolated from pneumonic lungs. Some other researchers used DNA fingerprinting to demonstrate the toxigenic strains of *P. multocida* within a region belonged to very few genotypes. Rubies et al., used fingerprinting to study vertical transmission within a swine production pyramid, sampling the maternal and paternal multipliers as well as the production herds. They found a wider dispersion of epidemic strains in the production herds probably due to the fact that these were open instead of closed (as those from the previous studies had been). They also found a disproportionate prevalence of strains originating from the paternal multiplier. Amass et al., also showed vertical transmission of *S. suis* strains. All these studies have highlighted the following facts:

- Within-herd bacterial disease is generally associated to one or few strains ("virulent" or "epidemic strains").
- Open herds have more strains present but still show the prevalence of few predominant strains.
- Vertical transmission occurs, but is not proportionate to the number of animals imported from a given source.

However, most of these studies had the important shortcoming of using DNA fingerprinting, which is a difficult, time-consuming technique that allows the study of only a small part of the genome due to the numerous bands that result. Ribotyping has also been used with good results, but it is even more time-consuming and expensive than DNA fingerprinting. In order to address these problems our group has been using Rep-PCR as an alternative to study within-farm or within-pyramid epidemic patterns of bacterial disease.

Rep-PCR or repetitive element sequence-based polymerase chain reaction enables the generation of DNA fingerprints that discriminate bacterial species and strains. Some of the primers used in this reaction—referred to as ERIC—are complementary to enterobacterial repetitive intergenic sequences. These sequences are distributed throughout the bacterial genome; inter-ERIC distances and patterns are specific for bacterial species and strains within species.

The following cases are given as an example of the use of Rep-PCR in farm studies.

Within farm studies

Case 1

The objective in this farm was to determine if mortality observed in nursery pigs was caused by a single strain or if there were several strains involved in the outbreak. The study lasted two years during which we also tried to show persistence of the epidemic strains.

Material and methods

In a 1000 sow herd divided between two production sites that—one year after the beginning of the study—moved to a three-site production system and doubled its inventory:

- *weaning age:* average of 18 days
- *mortality rates in the nursery stage:* very variable (2–18%)
- *mortality due to: Streptococcus suis* serotype 2
- home-grown replacement gilts
- piglet and sow vaccination

Results

There was only one strain responsible for mortality in that farm (epidemic strain). This strain was naturally estab-

lished and persisted for a long period. This strain remained in the farm even though important management changes had been taken (doubling sow inventory, changing from two-site to three-site production system, and vaccination).

Case 2

The objective in this study was to see if the *S. suis* mortality observed in two farms was caused by the same strain. These farms were unrelated except for the breeding stock.

Material and methods

Farm A had *S. suis* mortality in nursery pigs and provided gilts regularly to farm B.

Both farms were more than 100 miles away. Farm B had no other incoming animals except for pigs from Farm A. Both farms were in a three-site production system.

Results

The strains isolated from both farms were the same. Mortality observed in Farm B was probably due to the purchase of positive gilts. We also showed persistence of this epidemic strain in Farm B for almost two years.

Case 3

The objective of this study was to see the distribution of *H. parasuis* and *S. suis* strains in a production pyramid and to determine if those strains were the ones included in the autogenous vaccine in use.

Material and methods

A swine production company with numerous production farms. Some production farms had high nursery mortalities whereas others had almost no mortality.

Results

There was high homogeneity among the *H. parasuis* isolates collected from several farms. Only one strain was observed, but this strain was not the one included in the autogenous product that was being used.

In the case of *S. suis*, more than 10 different genetic patterns were observed, suggesting high variability among the *S. suis* population. Again, different patterns were observed both between farms and within farms. The autogenous vaccine product had a strain different from the ones observed, but the question is whether an autogenous product would be useful to control the problem in that system due to the high variability of strains observed.

The cases given here are examples of the use of rep-PCR in farm epidemiological studies. This technology can be used to:

- detect epidemic strains within a herd;
- develop autogenous vaccine and antibiotic sensitivity profiles;
- assess transmission between farms;
- assess colonization profiles and define risk factors based on these profiles.

In order to access this technology practitioners must:

- sample affected internal organs (in the case of *S. suis* it would be the brain and in the case of *H. parasuis*, the pleural or ascitic liquid); avoid tonsillar or respiratory isolates.
- determine the epidemic strains present in the herd by submitting the samples for Rep-PCR analysis.

If an autogenous product is desired, confirm that the epidemic strain is included.