Technical Bulletin

Information from Phibro Technical Services

Reviewing a novel approach to classifying porcine reproductive and respiratory syndrome virus—MJPRRS® Grouping Technology

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSv) has remained highly elusive. Breakthroughs in the science of PRRSv immunization often do not hold up for the long term when applied to populations and ever-changing biological systems. This is apparent when evaluating diagnostic descriptors and methods of virus identification and comparison used today. Since the late 1980s producers and veterinarians alike have battled PRRSv and struggled as to how to best catalogue and compare these viruses within and between farms and systems. The methods available to this point, Restriction Fragment Length Polymorphisms (RFLP), dendrograms and Nucleotide (Nt) % Homology comparisons fall short when attempting to explain large clinical presentations when only small genetic differences have been identified in the PRRSv ORF 5 sequence.

There exists today a technology that is not only capable of describing differences in clinical presentations (population's response to the disease regardless of sequence homology) but also of more clearly identifying new introductions versus internal mutations. **MJPRRS** Grouping Technology, developed by MJ Biologics, is a unique means of comparing and classifying PRRSv based on ORF 5 amino acid sequences and on-farm clinical presentations. This technology, which is the subject of this article, was invented by Dr. BK Kim of MJ Biologics and licensed to Phibro Animal Health Corporation in January 2015. This method of "grouping" PRRS viruses has helped many producers and systems better understand their key biosecurity intervention points as well as to better control pig flow health and maximize system throughput. In scenarios where virus elimination has proven to be both difficult and costly, the understanding of common viral mutations through application of **MJPRRS** Grouping Technology affords an option for addressing these challenging farms.

PRRS Diagnostics

The most common strategies for identifying PRRSv outbreaks and the specific viral characteristics involved have been dominated by Polymerase Chain Reaction (PCR), virus sequencing, RFLP, dendrograms and Nt homology tables. This is not to infer that any of these are poor methodologies for comparing PRRSv strains, but most provide insufficient information because they often fail to distinguish between PRRSv strains, particularly when small changes in the viruses equate to large clinical changes in the infected population.

MJPRRS Grouping Technology utilizes clinical presentations and Open Reading Frame (ORF 5) nucleotide and amino acid sequences to categorize different physical viral presentations. This identification, while not as visually impressive as a list of RFLP or nucleotide homology tables, can be highly accurate when describing a clinical presentation on a farm. This is especially the case when the farm has a history of prior PRRSv exposure and highly genetically similar ORF 5 viral sequences.

MJPRRS vaccine is unique

There are at least two unique parts to this technology. The first is a patented method for producing an immune response in a subject (US Patent No. 8,142,788). The immune response is produced by evaluating and choosing viral vaccine candidates based on differences in physical presentations ("how the pig sees PRRSv" vs. "how we as scientists see PRRSv"). The second is a method of preparing a vaccine containing PRRS virus proteins and antigens from PRRS

virus infected cells (US Patent No. 7,776,537), which was patented by the University of Minnesota and has been sublicensed by MJ Biologics to Phibro. The vaccine composition is prepared by virus selection, preparation and antigen harvest. We will not focus on vaccine preparation here, but rather on the selection of the correct virus for inclusion in the vaccine.

Unlike RFLP, dendrograms, or Nt homology comparisons, highly similar viruses as measured by these methods are not always similar enough when viewed through the lens of clinical signs in a population (the pig's immune response to specific antigens). From our basic understanding of molecular biology, we recall that DNA is transcribed into RNA, and RNA is translated into an amino acid sequence (or protein structure). A portion of these protein structures form exposed envelope proteins that serve the virus by seeking out host cell receptors. Although there is a high level of conservation in DNA-based organisms, RNA viruses are not as highly conserved or proofread. So, errors are common and often advantageous to strain survival. PRRS is an RNA virus. A single Nt change, if in the right position(s), can change the resulting protein structure and the virus' physical presentation. See the example in Table 1. Table 1 demonstrates the effect of changing one Nt in a nucleotide sequence. In some cases, a change in one Nt makes no difference in the resulting amino acid. In other cases, a single Nt change can result in production of a totally different amino acid, thus modifying the resulting protein. This Nt change is called the Wobble Base Effect.

The Wobble Base Effect can result in a highly variable amino acid with the simple change identified in Table 1. If the Nts in the first or second positions of this codon are changed, then the possibilities are even more dramatic. A change in the wobble base occurring in a critical area could make a significant change in the resulting protein, thus changing the physical presentation of PRRSv to this population of animals, even if this is the only change to occur. This change is small numerically, 1 Nt out of 603 Nts or 0.17% difference in nucleotide homology. Although likely to not have changed this virus' RFLP or position on a production system's dendrogram sufficiently so as to catch the viewer's attention, the possibility still exists that the clinical effects are catching the producer's attention in this situation.

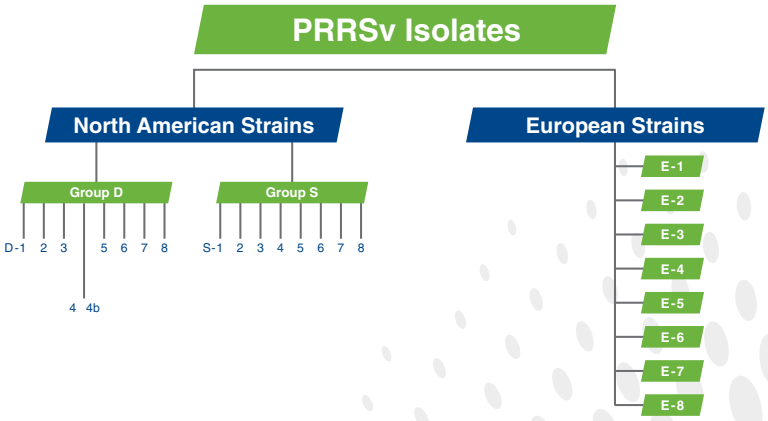
The analysis of these physical changes to the protein structure is known as **MJPRRS** Virus Grouping. Through evaluation of 10,000-plus viral sequences by MJ Biologics, all viruses have fit neatly into one of 25 physical viral presentations: 3 major groups D, S, and European (E), and 8 or 9 individual subgroupings of these (D-1 thru D-8, including D4b, S-1 thru S-8, and E-1 thru E-8). These group relationships are shown in Figure 1.

These **MJPRRS** virus groupings are independent of both virus Nt homology and RFLP. This evaluation occurs at the amino acid (protein structure) level. For example, the same RFLP will often represent 3-5 different **MJPRRS** virus groups while a single **MJPRRS** viral group may contain viruses with 5-8 (or more) different RFLP patterns. Viruses immediately neighboring each other on a system's dendrogram may represent different **MJPRRS** viral groups. At the same time, those on one end or the other of the dendrogram may be more physically alike in appearance to the pig. **MJPRRS** virus grouping helps to explain how small, seemingly irrelevant, changes in Nt homology, or in sequence proximity in an Nt homology table or dendrogram, can appear clinically different in a population of animals.

Table 1. Wobble Base Effect

1st Nt Base	2nd Nt Base	3rd Nt Base	Resulting Amino Acid
A	T	T	Isoleucine
A	T	C	Isoleucine
A	T	A	Isoleucine
A	T	G	Methionine
A	A	T	Asparagine
A	A	C	Asparagine
A	A	A	Lysine
A	A	G	Lysine

Figure 1.
MJPRRS Immunological Groupings



How to utilize MJPRRS grouping technology

Producers and their veterinarians who are using **MJPRRS** vaccine have embraced the technology of virus group identification. The main reason behind this acceptance is that the previously unexplainable is now explained. Historical comparisons, RFLP analysis, and dendrograms failed to describe why the virus clinically appeared different when it was highly genetically related to the last disease break. Matching a clinical picture with historical PRRSv challenges based off no more than **MJPRRS** grouping analysis often helps to obtain acceptance of the technology. Evaluating the previous viral challenges and associated clinical pictures from a farm with a difficult PRRSv situation is often a good place to start for those unfamiliar with **MJPRRS** Grouping Technology. A farm, or system, with a rocky history of PRRSv stability will often allow the linkage of epidemiologic description and clinical signs.

Characteristics of a farm (system) with PRRSv stability issues are as follows:

1. Weaned piglet numbers vary frequently;
2. Tested pools of piglet serum oscillate between PRRSv PCR positive and negative;
3. Piglet populations fail to test PRRSv PCR negative despite lengthy farm closures;
4. Clinical disease in sows/gilts despite exposure to 98% + similar PRRSv by Nt homology.

All of the above factors are further complicated by: 1) the need for replacement animals, 2) sustained poor production and 3) increasing costs of production.

By gathering 18–24 months of the most recent PRRSv ORF 5 ORF sequence information, much of this story can be elucidated. There is nothing magical about the 18–24 month period of time except that this will often catch at least one PRRS break with a new introduction and an internal virus mutation or two. ORF 5 sequences can be obtained and grouped by MJ Biologics typically within 48–72 hours, and discussion can begin as to why this population of animals may not be responding clinically as would be expected.

Differentiation between new viral introductions and internal viral mutations is critical to maintaining a PRRSv control program. New viral introductions usually fuel a full-blown investigation as to how it happened: "How did the virus get here?" Mutations, in spite of being a natural phenomenon, add the element of unpredictability to an already problematic situation. To

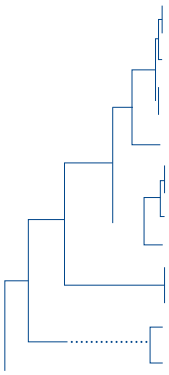
date, controlling these mutations has not been possible. If common mutations could be identified, PRRSv control programs could be tailored to reduce the clinical severity of these mutations as well as to control the current virus challenge within the farm.

Comparisons of amino acid sequences using **MJPRRS** Grouping Technology have identified mutations that are likely to have occurred within the farm population versus those that occurred with a new viral introduction (different origin ORF 5 amino acid sequence). This differentiation is possible even within the same physical groupings of a virus. For example, two D-4 viruses can be of varied origin. The alternative is also true, that a small mutation can give rise to a different **MJPRRS** group from the original virus — for example a D-4 virus becoming a D-5 virus with a small Nt change (refer to Table 1).

MJPRRS Grouping Technology can also help identify patterns (viral group partnerships), or mutations that are likely to occur from one specific virus group to another. One instance of this is the relationship of D-4, D-5 and D-6 viral groups. These viral groups can be introduced independently of one another. But, they are equally or more likely to be found as mutations in the same population over time (see Figure 2).

Figure 2 shows the progression of PRRS outbreaks in one farm over a period of time from December 2010 to December 2012. The actual dendrogram is attached without scale on the left and corresponds with the date of clinical signs and RFLP rows across the top. Some variant groups are positioned immediately adjacent to one another. This dendrogram was produced by the supporting diagnostic laboratory in this case with all the historical farm information attached. The only farm contributing to this comparison of viruses was the farm in question.

Figure 2. Internal mutations over time



Date	RFLP	Jan 2012	Dec 2012	Dec 2011	Dec 2010	May 2010	MJ Group
Dec 2012	1-2-2	93.2%	100.0%	99.7%	98.7%	99.3%	D-4
Dec 2011	1-2-2	93.0%	99.7%	100%	98.7%	99.3%	D-5
Dec 2010	1-18-2	92.9%	98.7%	98.7%	100.0%	99.3%	D-4b
May 2011	1-2-2	92.5%	99.3%	99.3%	99.3%	100.0%	D-4
Jan 2012	1-18-2	100.0%	93.2%	93.0%	92.9%	92.5%	D-6

MJPRRS Grouping Technology explains occurrence of clinical signs:

D-4b → D-4 → D-5 → D-6 → D-4

The farm was experiencing clinical signs related to PRRSv circulation every 5–8 months. Neither RFLP nor Nt homology could explain this clinical phenomenon.

The only way to explain each occurrence of these clinical presentations was the **MJPRRS** Grouping Technology. As is demonstrated in Figure 2, these viral mutations are highly similar and non-distinguishable by all other comparison methods, but each date identified represented a new clinical presentation. The **MJPRRS** Grouping Technology was able to differentiate the breaks as caused by D-4b, D-4, D-5, D-6 and D-4.

The most powerful, and likely most beneficial, capability of **MJPRRS** Grouping Technology is the ability to track viral movements epidemiologically throughout a large geographic region. Many of the most devastating PRRS breaks in the last 18–24 months may have been connected to 1-7-4 RFLP patterns. However, there were many of these breaks that were identified as involving PRRS viruses with the RFLP patterns of 1-7-3, 1-21-4, 1-10-4 or even 1-4-4. These cases were similar in clinical appearance but did not match exactly by RFLP. These viruses were dominant in many regions of the United States. Using the **MJPRRS** Grouping Technology, approximately 60-65% were identified as D-7. Despite the high percentage of 1-7-4 viruses also being D-7s, not all 1-7-4 RFLPs are D-7 MJ grouped viruses. **MJPRRS** Grouping Technology provides a method for identifying and monitoring virus movement in a more defined manner than a combination of RFLP, dendrograms and Nt Homology. When Porcine

Epidemic Diarrhea virus (PEDv) went from a low (zero) prevalence situation to a high prevalence situation in a short period of time, it was considered an epidemic. The H1N1 "Swine Flu" of 2009 was very similar. Both instances were classified as epidemics. If the identification of a specific PRRS virus presentation (D-7 in this case) can be correlated by similar monitoring, shouldn't the same epidemic status be applied?

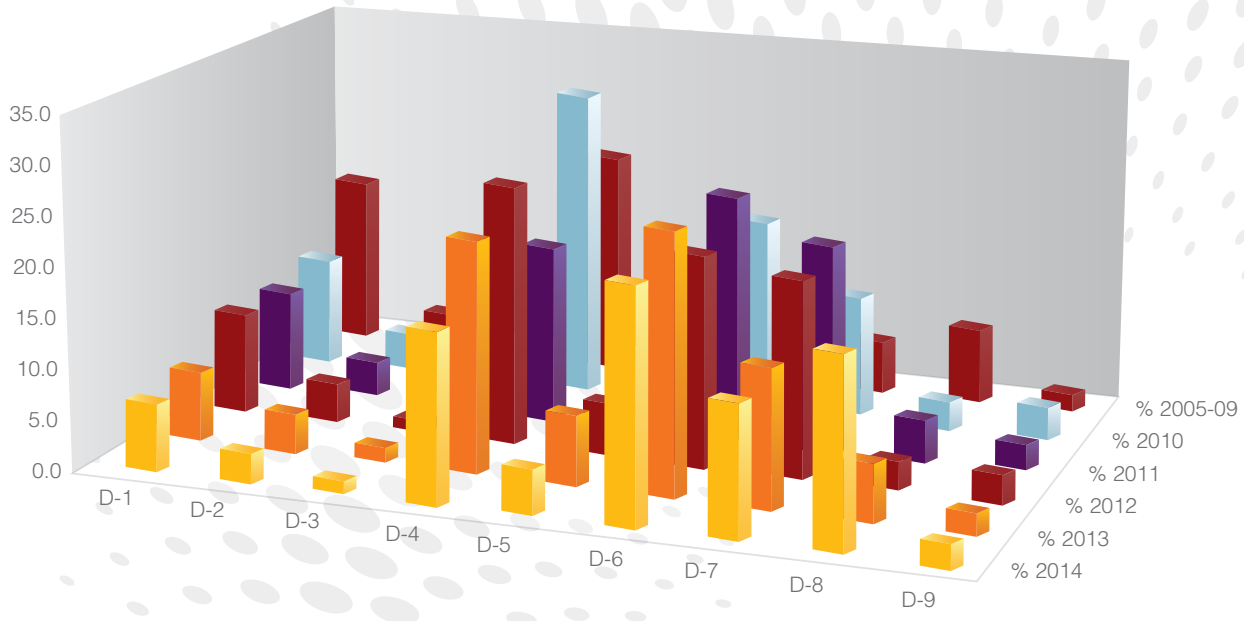
Figure 3 shows a historical picture of PRRSv groups from 2005 to 2014. From back to front, D-7 prevalence as a total of all D-groups identified in the U.S. from 2005–2013 was very low. By the end of 2014, D·7 was the second most frequently identified D-group virus and rising. Current monitoring would suggest that this trend has continued at an average of 35-40% monthly. In nearly all cases identified, D-7 has been associated with a new clinical break. Projects tracking RFLP as a method for viral classification and comparison may miss this subtlety.

Presently the D-7 virus group has spread quickly throughout the United States. Only the **MJPRRS** Grouping Technology has been able to demonstrate the similarity of the virus causing these outbreaks. The strength of **MJPRRS** Grouping Technology is the ability to identify similarities where traditional methods of comparisons have failed to classify PRRS viruses as being related. This can have a meaningful impact on population (as well as regional) virus control programs through recognition of common virus movement and potentially source of origin.

Conclusions

MJPRRS Grouping Technology is a tool for classifying and differentiating PRRSv based on their different physical properties — as identified by the ORF 5 amino acid sequence. To date, all PRRSv that have been analyzed have fit reasonably well into one of the 25 established MJ groupings. This grouping technology has allowed for some degree of predictability with regard to potential mutations and has facilitated tracking of viral epidemics even where Nt % Homology comparisons and RFLP analysis would suggest that different viruses may be involved. **MJPRRS** Grouping Technology also offers an explanation in situations where small changes in Nt % Homology equate to clinical presentations on a farm.

Figure 3: Historical grouping percentages 2005-2014 – % D group



Taken alone, any one of these aspects helps to better grasp PRRSv control in a region, system or on an individual farm. All facets being used concurrently can not only aid in the selection of viruses for future autogenous vaccine production but can also predict which mutations are likely, and can improve monitoring of new introductions to a given region or across the country. This capability allows producers and veterinarians alike to aggressively and precisely address the PRRS virus on their farms. As well, appropriate interventions can be implemented with respect to their internal and external biosecurity measures. **MJPRRS** Grouping Technology best prepares for the next PRRSv epidemic wave.

Acknowledgments

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Please contact your Phibro Animal Health representative and ask how you can have your PRRS virus grouped free of charge.

Potency and efficacy of autogenous biologics have not been established.

MJPRRS Autogenous Vaccines are manufactured by and distributed to veterinarians by Phibro Animal Health Corporation.