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Applying PRRSv sequencing to field cases

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Background

PRRSv was identified as a single-strand RNA virus (Weensvoort, 1991). Single-stranded RNA viruses have high mutation rates and strain diversity results (Domingo, 1996; Murtaugh, 1997; Yuan, 1999). Diversity is advantageous to viral survival as alternative prototypes allows rapid adaptation to avoid host immunity (Domingo, 1989). Different PRRSv strains exhibit marked difference in susceptibility to immunity (Lager, 1997; Lager, 1999; Mengeling, 1996; Mengeling, 1998). The least immunogenic strain dominates other strains as it can propagate large quantities of virions (Domingo, 1997; Sevilla, 1998). PRRSv strains are very different when compared across unassociated sow herds (Yuan, 1999; Lager, 1997). The mutational variations of closed herds were minor when compared to strain shifts noted after introduction of infected animals from unassociated herds (Roberts, 1999; Faaberg, 2001). Current control programs seek simultaneous protective immunity of an entire sow herd to avoid subpopulations of nonimmune animals (Dee, 2000; Bautista, 2000; Baker, 2000). Control relies on immunity to a resident PRRSv strain. Control is impossible when foreign PRRSv strains can gain entry into a sow herd.

Application

Two issues arise in production application of the background information.

- PRRSv control in a production system cannot be effective if management tolerates random introductions of "foreign" PRRSv strains.
- Sow herds within a system must be monitored to identify "resident" PRRSv strains over time.

It is necessary to know the location and the relationship of various PRRSv strains within a system. Acute sow farm episodes must be identified to ascertain if infection was a "foreign" versus a "resident" strain. Control program effectiveness is impossible to ascertain when sources of infection remain unrecognized. Successful PRRS epidemiology does not allow the luxury of approaching PRRS as a single entity like the pseudorabies virus. Success is realized by considering the collective of PRRSv entities in a system.

PRRSv strains are identified with DNA sequencing. Phylogenetic analysis of sequences can compare strains with homology charts and tree diagrams (Andreyev, 1997). Strains located within herds must be compared to other herds in the same or different pyramids. Resident strain shifts are apparent when sequences are collected over time and compared using phylogenetic tree diagrams. A single tree analysis affords multiple comparisons of many sequences making the tool superior to simple "sequence-to-sequence" homology chart interpretations. Tree analysis is an important tool for those who attempt PRRS control.

Passage of time

Replacement animals flow slowly through systems. Female replacements that are infected as piglets on a multiplier farm usually do not reach a commercial sow farm until they are 30 weeks of age. PRRSv can also be slow to transmit (Benfield, 1999). Sequencing may yield information quickly in some situations. However, PRRS isolates may need to be captured for a year to adequately identify strain variability and successfully estimate the rate of strain shift.

Deriving sequences

Sequences are derived from two types of sampling. Some sequences are the product of diagnostic sampling from cases of active PRRS expression. Other sequences are found during routine sampling of herds, usually nursery pigs. PRRSv is difficult to recover steadily from an endemic sow herd. Allied nursery pig flow offers a predictable point to recover the PRRSv. Sampling in the nursery assumes a dedicated, unmixed, and uncontaminated flow. A routine 4-month testing interval has been used as shorter intervals increase testing with little enhancement of strain variation. Longer testing intervals could miss sequential strain shifts. PRRSv often replicates when pigs are between 6 and 12 weeks of age. PRRSv is usually found in the serum of a pig that is experiencing a replicating infection. Fever and lethargy are associated with a replicating PRRSv infection with in a pig (Benfield, 1999). The best nursery age to assure PRRSv isolation is narrowed by taking the fevers (≥103.5° F.) of lethargic pigs to find age groups with high fever prevalence. Alternatively, the age at onset of acute disease can be determined serologically.

Sample collection, handling, and storage

A low prevalence of fevered pigs infers the probability of PRRSv isolation success will be limited. Pig selection and sample quantity are important to assure isolations. Twenty-four samples are commonly drawn. It is not required that all samples undergo PCR isolations. PCR is run until the quantity of isolates needed for a monitor is fulfilled. Establish the required quantity of PCR product isolates related to suspicion of multiple strains. Two to five PCR isolates are a common goal. Pooling can reduce cost, but may inhibit isolating multiple strains.

It is best to chill blood samples constantly, especially if conducting live isolations simultaneously. A small cooler of ice is carried while collecting to chill samples immediately after they are drawn. Keep samples refrigerated after collection. Attention is needed during clot separation to assure serum is warmed as little as possible. Some centrifuges run warm. Separate serum from the clot if samples are held over 48 hours after collection. Send samples on ice by overnight air or delivery. Do not freeze the samples or use dry ice.

PCR isolation

It is advantageous to isolate PRRSv directly from serum with PCR, avoiding tissue culture. Common tissue cultures preferentially grow vaccine strains (Umthun, 1999). The important portion of the PRRSv genome to sequence for strain differentiation is ORF 5. It is the least conserved area of the genome and the area most likely to differentiate strains (Kapur, 1996). Nested-set PCR is the method preferred to amplify ORF 5. The technique is sensitive to detect low quantities of virus and yield an ORF 5 product for sequencing (Umthun, 1999).

Sequencing

Sequencing is available through many diagnostic and genetic laboratories. Capillary sequencers are preferred. Results are usually reported as sets of 500 base pairs. One thousand and 500 base pair sequences can be interpreted together by sizing all to 500 base pairs. ORF 5 is about 600 base pairs. ORF 6 is often included even though it is less variable than ORF 5. ORF 6 translation may influence the immunogenic quality of ORF 5 translations. It is best to receive digital reports of sequence information via e-mail.

Interpreting sequence information

Free software is available free through the Internet to aid interpretation. Reported sequences are downloaded into a Chromas 1.45(tm) program (McCarthy, 1998). Reported sequences often have unidentified and misreported nucleotides. Chromas(tm) is used to correct, format, and name sequences. The resulting sequence is accurate.

It is important to give a sequence a name that is easily recognized on interpretative charts. It is easier to find meaningful viral family relationships when sequence names indicate farm of origin. A shift of strain or strain drift is easier to notice when sequences are dated. One naming scheme involves naming a sequence by isolate type, sample number, farm code, and collection date. Isolation type includes diagnostic isolates (X), random isolates (no code), or autogenous vaccine isolates (V). For example, XRP2(4104B)101000 was a diagnostic isolate from an aborted sow at the Ross Price Sow farm. It was the second isolate from submission, D00-4104B. The sample that derived the sequence was taken on October 10, 2000.

Sequences are aligned by statistical relationship with ClustalX(tm) (Higgins, 1994) and reported in a PHYLIP format for plotting as a tree diagram. The PHYLIP information can be opened with a NJ Plot(tm) program (Perriere, 1996) or the TreeView(tm) program (Page, 2000) to display a phylogenetic tree diagram. Diagram interpretation focuses on the length of the horizontal branches that connect sequences. The branch lengths indicate the magnitude of strain variation. A thumb rule interprets branch lengths less than 0.02 to indicate isolates are of the same strain. Different strains project values of 0.04 and greater.

Clustal alignment files saved in a MSF format are loaded into GeneDoc(tm) (Nicholas, 1997) to create consensus sequences and homology charts. A consensus sequence is the "average" sequence calculated from a group of sequences. A consensus sequence is saved and used in ClustalX and tree programs to represent a family of similar viruses to consolidate tree interpretation and reduce complexity. GeneDoc(tm) is also capable of doing enzyme fragmentation of sequences and amino acid translations.

The programs are not difficult. Remember that the subject is merely 500 to 1000 base pairs of one virus with many strains. The task is quite simplistic considering that some of the programs were designed to compare the sequences of animal species.

Anecdotal findings

- PRRS control is impossible when management tolerates random entries of foreign viral strains into a system.
- Pyramids that exhibit the same dominant viral strain (master sequence) from multiplier sow farm, to commercial sow farms, to commercial nurseries and finishers appear to have fewer clinical problems than pyramids whose sow herds contain differing master sequences.
- Modified live vaccine strains can remain for extended periods with in populations. Some isolates are closely related to vaccine, but appear modified by mutational drift. It is not unusual to find 30% of isolates similar

to MLV vaccines when a producer has ardently used vaccine.

- Clinical herds are more likely to yield multiple related strains on one routine sampling at a nursery as compared to nonclinical herds.
- Replacement flow management may affect the rate of genetic drift and direction of drift. Continuous flow replacement acclimation appears to be associated with a high level of strain divergence between sow farms.

Proposed questions

- Strain variation is not the entire "picture" of clinical disease in endemic sow herds. Population dynamics related to exposure prevalence and the time since prior exposure also fail to describe some cases. Strain virulence, prevalence of replication, and viral fitness are suggested alternative causes. Should investigation probe deeper? Should investigation focus on translated ORF 5 protein? Would strain comparison be more informative if protein products were compared rather than nucleotide sequences? Would digital evaluation of protein epitopes be revealing?
- Does reduction of incidental PRRS cases in high seroprevalence sow herds lead to viral "bottle necking" and reduced viral fitness that possibly precludes the persistent state? (Does PRRSv persistence increase after "sow-to-sow" viral transfer is reduced? If so, is persistence due a genomic strain expression at an ORF 1 regulatory site that suppresses ability of the strain to replicate?)

References

Andreyev VG. Wesley RD. Mengeling WL. Vorwold AC. Lager KM. 1997. Genetic variation and phylogenetic relationships of 22 porcine reproductive and respiratory syndrome virus (PRRSV) field strains based on sequence analysis of open reading frame 5. Arch Virology 142: 993-1001.

Baker RB. 2000. Health management of growing pigs. Proc. NC Healthy Hogs. Greenville, Fayetteville, NC. November 00: 35-43.

Baustista L. Pjoan C. 2000. PRRS control by gilt replacement exposure in the nursery. Seminar at 2000 Amer. Assn. Swine Prac. Conf. March.

Benfield DA. Collins JE. Dee SA. Halbur PG. Joo HS. Lager KM. Mengeling WL. Murtaugh MP. Rossow KD. Stevenson GW. Zimmerman JJ. 1999. Section 18: Porcine Reproductive and Respiratory Syndrome in Diseases of Swine. Straw, B.E.; Editor. 8th Edition, Iowa State University Press, Ames, Iowa.

Dee SA. 2000. PRRS eradication through test and removal with a stabilized herd. Seminar at 2000 Amer. Assn. Swine Prac. Conference. March.

Domingo E. 1989. RNA virus evolution and the control of viral disease. Prog. Drug Resist. 33: 93-133.

Domingo E. Escarmis C. Sevilla N. Moya A. Elena SF. Quer J. Novella IS. Holland JJ. 1996. Basic concepts in RNA virus evolution. FASEB Journal 10: 859-864.

Domingo E. Holland JJ. 1997. RNA virus mutation and fitness for survival. Ann. Review Microbio. 51: 151-178.

Faaberg K. Mahlum CE. Murtaugh MP. Roberts J. 2001 Porcine Reproductive and Respiratory Syndrome Virus: A case Study of Virus Variation in Different Swine Production Systems. Proc. 2001 Amer. Assn. Swine Prac. Conf. March: 322-323.

Higgins DG. 1994. CLUSTAL V: Multiple alignment of DNA and protein sequences. Methods in Molecular Biology. 25: 307 – 318.

www.hgmp.mrc.uk/Registered/Options/clustalx.h(tm)l

Kapur V. Elam MR. Pawlovich (tm). Murtaugh MP. 1996. Genetic variation in porcine reproductive and respiratory syndrome virus isolates in the Midwestern United States. J. Gen. Virol. 77: 1271-1276.

Lager KM. Mengeling WL. Brockmeier SL. 1997. Duration of homologous porcine reproductive and respiratory syndrome virus immunity in pregnant swine. Vet. Microbiol. 58: 127-133.

Lager KM. Mengeling WL. 1999. Significance of PRRS mutations on the farm. Proc. 1999 Amer. Assn. Swine Prac. Conf. March: 423-424.

McCarthy C. 1998. Chromas 1.45. Technelysium Pty. Ltd. Helensvale, Queensland, Au.

www.technelysium.com.au/chromas14x.h(tm)l

Mengeling WL. Lager KM. Vorwald AC. 1998. Clinical consequences of exposing pregnant gilts to strains of porcine reproductive and respiratory syndrome (PRRS) virus isolated from field cases of "atypical" PRRS. Amer. Journal of Vet. Research 59: 1540-1544.

Mengeling WL. Vorwald AC. Lager KM. Brockmeier SL. 1996. Comparison among strains of porcine reproductive and respiratory syndrome virus for their ability to cause reproductive failure. Amer. Journal of Vet. Research 57: 834-839.

Murtaugh MP. Faaberg KS. Yuan S. Kapur V. 1997. Interrelatedness of PRRS virus isolates in North America. Proc. Allen D. Leman Conf. September: 146-149.

Nicholas KB. Nicholas HB. 1997. GeneDoc: Analysis and visualization of genetic variation. Pittsburgh Supercomputing Center. Pittsburgh, Pa.

www.psc.edu/biomed/genedoc/

Page RD. TreeView (Win32). 2000. Institute of Biomedical and Life Sciences. University of Glasgow. Glasgow, UK.

http:/taxonomy.zoology.gla.ac.uk/rod/treeview/.h(tm)l

Perriere G. Gouy M. 1996. An on line retrieval system for biological sequence banks. Biochimie 78: 364-369.

http://pbil.univ-lyon1.fr/software/njplot.h(tm)l

Roberts J. 1999. PRRS management suggestions derived from sequencing. Proc. NC Healthy Hogs. Greenville, Fayetteville, NC. November 99: 75-78.

Sevilla N. Jarabo CM. Mariano G. Baranowski E. Domingo E. 1998. An RNA virus can adapt to the multiplicity of infection. Jour. of Gen. Virology 79: 2971-2980.

Umthun AR. Mengeling WL. 1999. Restriction fragment length polymorphism analysis of strains of porcine reproductive and respiratory syndrome virus by use of a nested-set reverse transcriptase-polymerase chain reaction. Amer. Jour. of Vet. Res. 60: 802-806.

Weensvoort G. Terpstra C. Pol JM. ter Laak EA. Bloemraad M. De Kluyver EP. Kragten C. van Buiten L. Den Besten A.

Wagenaar F. 1991. Mystery swine disease in the Netherlands: isolation of the Lelystad virus. Vet. Q. 13: 121-130.
Yuan S. Nelsen CJ. Murtaugh MP. Schmitt BJ. Faaberg KS. 1999. Recombination between North American strains of porcine reproductive and respiratory syndrome virus. Virus Research 61: 87-98.