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Mycoplasma hyopneumoniae strain variation being observed in the field

Simone Oliveira¹; Montserrat Torremorell²; Clint Been¹

¹University of Minnesota, St. Paul, Minnesota; ²Genus/PIC, Hendersonville, Tennessee

M*ycoplasma hyopneumoniae* is one of the most important pathogens affecting the swine industry. The role of this pathogen in swine enzootic pneumonia was first reported in 1965, and it continues to be a major player in the respiratory disease complex affecting pigs (Mare et al, 1965). Significant progress has been made towards molecular characterization of *M hyopneumoniae*. Whole genome sequences are available for at least 3 different strains (232, J, and 7448) and comparative genomics is now being used to search for putative virulence genes (Minion et al 2004, Vasconcelos et al, 2005).

Mycoplasma hyopneumoniae has a small genome compared with other bacterial pathogens. Its genome size averages 900,000 bp, which is four times smaller than the *Escherichia coli* genome (4–5 million bp), for example. *Mycoplasma hyopneumoniae* has reduced redundancy and has conserved genes that are necessary for its core functions and survival. This limited set of genes limits adaptation to *in vitro* growth and is one of the reasons why this organism is not easily isolated from clinical samples.

Comparative genomics has confirmed the existence of several regions of the genome that are specific to different *M hyopneumoniae* strains. More importantly, comparative genomics has identified candidate genes that can be sequenced and used as markers to identify and compare strains. Whole genome sequencing is considered the gold standard for strain comparison; however, the workload involved in sequencing and assembling 900,000 bp is unsuitable for routine characterization and comparison of field isolates. The ideal method for *M hyopneumoniae* strain typing should be fast, inexpensive, and highly discriminatory.

Several alternative methods have been used to identify and compare *M hyopneumoniae* strains. These methods can be divided into gel-based and sequence-based approaches. Some of the gel-based techniques that have been successfully used to identify *M hyopneumoniae* strains are pulsed field gel electrophoresis (PFGE), arbitrary primed PCR (AP-PCR), and amplified fragment length polymorphism (AFLP). These techniques utilize different methods to interrogate the genome, generating a genomic fingerprint (Frey et al, 1992; Artiushin et al, 1996; Kokotovic et al,

1999). Although these techniques are able to characterize *M hyopneumoniae* to the strain level, they all require isolation of *M hyopneumoniae* from clinical samples, which is labor intensive and often unsuccessful.

Sequence-based approaches that do not require *M hyopneumoniae* isolation and that allow detection and typing of strains directly from clinical samples are ideal for characterizing strain variability and studying the molecular epidemiology of this pathogen. The whole genome sequence of *M hyopneumoniae* reference strain 232 revealed the presence of at least 22 regions with a variable number of tandem nucleotide repeats (VNTRs) within the genome. These repeated sequences are distributed in 12 different proteins, including known surface adhesins (Minion et al., 2004).

Some of the genes coding for surface adhesins that contain VNTRs are P97, P76, P146, and P216. The value of VNTR for strain typing has been evaluated for at least two of these proteins: P97 and P146. The adhesin encoded by the P97 gene is known to be an important factor in *M hyopneumoniae* attachment to host cells. The P97 gene contains two repeat sections, R1 and R2, and the R1 sequence has been evaluated as a target for strain typing. This is an important region because it is directly associated with cell attachment and it contains epitopes recognized by the host. Although sequencing of the P97 R1 repeat region was successfully used to differentiate *M hyopneumoniae* field isolates, it was found to be less discriminatory than P146 sequencing (Wilton et al, 1998; de Castro et al, 2006, Stakenborg et al., 2006).

The P146 lipoprotein was first described and used for strain typing in *M. conjunctivae* (Belloy et al, 2003). A homolog of this gene was recently identified in *M hyopneumoniae*. This adhesin-like lipoprotein contains a series of serine repeats and is located on the surface of the bacterial cell. These serine repeats, and the DNA region that codes for these amino acid repeats, were recently used for characterization and comparison of *M hyopneumoniae* isolates from different European herds and from herds from different continents. Sequencing of the P146 gene of *M hyopneumoniae* allowed the identification of herd-specific strains and demonstrated that there is high variability among isolates from different herds and geographical locations (Mayor et al, 2007; 2008).

At the University of Minnesota Veterinary Diagnostic Laboratory (MVDL), we have utilized P146 sequencing to characterize the variability of *M. hyopneumoniae* strains circulating among U.S. swine herds. We have sequenced 60 isolates so far and have identified at least 15 different strain groups (Figure 1). Isolates evaluated in this study originated from 28 herds distributed in 13 states. Single strains were identified in 21 of the evaluated herds and strain variability was observed in 7 herds. The number of strains present in herds with more than one strain ranged from two to five. These preliminary results suggest that most herds have a single *M. hyopneumoniae* strain, which facilitates source tracking. It also confirms that co-infections with different *M. hyopneumoniae* strains within one herd are infrequent, but possible.

Our results confirm that *M. hyopneumoniae* is highly variable and that most swine herds are affected by single, and many times, unique strains. Translation of DNA sequences into amino acid sequences demonstrates that most of the variability observed at the nucleotide level is not translated into major differences in the P146 lipoprotein sequence. At this time, we feel confident that P146 sequencing is a useful tool to study the molecular epidemiology of *M. hyopneumoniae*. This technique fits all the requirements for the ideal typing system: it is discriminatory, rapid, relatively inexpensive, and produces unambiguous data that can be shared between laboratories.

In conclusion, molecular characterization of *M. hyopneumoniae* isolates is currently available at the MVDL. This represents an innovative tool for producers and veterinarians to study the epidemiology of *M. hyopneumoniae* infections in the field and it will provide insight into *M. hyopneumoniae* pathogenesis and control strategies.

References

1. Artiushin S, Minion FC. Arbitrarily primed PCR analysis of *Mycoplasma hyopneumoniae* field isolates demonstrates genetic heterogeneity. *Int J Syst Bacteriol.* 1996;46(1):324–328.
2. Belloy L, Janovsky M, Vilei EM, Pilo P, Giacometti M, Frey J. Molecular epidemiology of *Mycoplasma conjunctivae* in Caprinae: transmission across species in natural outbreaks. *Appl Environ Microbiol.* 2003;69(4):1913–1919.
3. Djordjevic SP, Cordwell SJ, Djordjevic MA, Wilton J, Minion FC. Proteolytic processing of the *Mycoplasma hyopneumoniae* cilium adhesin. *Infect Immun.* 2004;72(5):2791–2802.
4. de Castro LA, Rodrigues Pedrosa T, Kuchiishi SS, Ramenzoni M, Kich JD, Zaha A, Henning Vainstein M, Bunselmeyer Ferreira H. Variable number of tandem amino acid repeats in adhesion-related CDS products in *Mycoplasma hyopneumoniae* strains. *Vet Microbiol.* 2006;116(4):258–269.
5. Frey J, Haldimann A, Nicolet J. Chromosomal heterogeneity of various *Mycoplasma hyopneumoniae* field strains. *Int J Syst Bacteriol.* 1992;42(2):275–80.
6. Kokotovic B, Friis NF, Jensen JS, Ahrens P. Amplified-fragment length polymorphism fingerprinting of *Mycoplasma* species. *J Clin Microbiol.* 1999;37(10):3300–7.

Figure 1: *Mycoplasma hyopneumoniae* strain variability based on sequencing of the P146 gene. Sample identification: Sequence ID (CB), herd ID (H), location of the swine herd (state), age of affected pig (weeks), presence or absence of lesions (+ or -).



7. Mare CJ, Switzer WP, (1965) New species: *Mycoplasma hyopneumoniae*; a causative agent of virus pig pneumonia. *Vet Med Small Anim Clin.*;60:841–6.
8. Mayor D, Zeeh F, Frey J, Kuhnert P. Diversity of *Mycoplasma hyopneumoniae* in pig farms revealed by direct molecular typing of clinical material. *Vet Res.* 2007;38(3):391–398.
9. Mayor D, Jores J, Korczak BM, Kuhnert P. Multilocus sequence typing (MLST) of *Mycoplasma hyopneumoniae*: a diverse pathogen with limited clonality. *Vet Microbiol.* 2008;127(1–2):63–72.
10. Minion FC, Lefkowitz EJ, Madsen ML, Cleary BJ, Swartzell SM, Mahairas GG. The genome sequence of *Mycoplasma hyopneumoniae* strain 232, the agent of swine mycoplasmosis. *J Bacteriol.* 2004;186(21):7123–33.
11. Stakenborg T, Vicca J, Maes D, Peeters J, de Kruif A, Haesebrouck F, Butaye P. Comparison of molecular techniques for the typing of *Mycoplasma hyopneumoniae* isolates. *J Microbiol Methods.* 2006;66(2):263–275.
12. Vasconcelos AT, et al. Ferreira HB, Bizarro CV, et al. Swine and poultry pathogens: the complete genome sequences of two strains of *Mycoplasma hyopneumoniae* and a strain of *Mycoplasma synoviae*. *J Bacteriol.* 2005;187(16):5568–5577.
13. Vicca J, Stakenborg T, Maes D, Butaye P, Peeters J, de Kruif A, Haesebrouck F. Evaluation of virulence of *Mycoplasma hyopneumoniae* field isolates. *Vet Microbiol.* 2003;97(3–4):177–90.
14. Wilton JL, Scarman AL, Walker MJ, Djordjevic SP. Reiterated repeat region variability in the ciliary adhesin gene of *Mycoplasma hyopneumoniae*. *Microbiology.* 1998;144 (Pt7):1931–1943.

