Mycoplasma hyopneumoniae genetic variability within swine production flows

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Abstract

The aim of this study was to assess the genetic variability of *Mycoplasma hyopneumoniae* within various swine production flows. Four *M. hyopneumoniae* positive production flows, composed of 4 production stages, were selected for this study. Laryngeal and/or bronchial swabs were collected from each production stage within a flow, for a period of 4 months up to 3 years. A multiple-locus variable-number tandem repeat analysis was performed to assess the genetic variation of *M. hyopneumoniae* within and across production flows through the identification of variable-number tandem repeat (VNTR) types. A maximum of 6 *M. hyopneumoniae* VNTR types were identified in a single flow, in which VNTR types appeared to be flow specific. An identical VNTR type was detected across several production stages for up to 3 years. In this study, minimal *M. hyopneumoniae* genetic variation was evidenced within and across production flows.

Résumé

L'objectif de cette étude était d'évaluer la variabilité génétique de Mycoplasma hyopneumoniae *au sein de différents flux de production porcine. Quatre flux de production positifs pour* M. hyopneumoniae, *composés de quatre stades de production, furent sélectionnés pour cette étude. Des écouvillons laryngés et/ou bronchiaux furent prélevés de chaque stade de production à l'intérieur d'un flux, pour une période de 4 mois jusqu'à 3 ans. Une analyse multi-locus du polymorphisme des séquences répétées en tandem fut effectuée afin d'évaluer la variation génétique de* M. hyopneumoniae *au sein et à travers les flux de production par l'identification des types de polymorphismes de séquences répétées en tandem (VNTR). Un maximum de six types de VNTR de* M. hyopneumoniae *fut identifié dans un flux unique, dans lequel les types de VNTR apparaissaient être spécifiques de flux. Un type de VNTR identique fut détecté à travers plusieurs stades de production et jusqu'à 3 ans. Dans cette étude, une variation génétique minime de* M. hyopneumoniae *fut notée au sein et à travers des flux de production.*

(Traduit par Docteur Serge Messier)

Mycoplasma hyopneumoniae is the primary agent of enzootic pneumonia (EP) in swine (1,2). Enzootic pneumonia is a prevalent disease known to have a significant negative impact in swine health and production worldwide (3). Variability in *M. hyopneumoniae* virulence has been presumed due to demonstrated differences in clinical course (4) and disease severity among strains (5). Molecular characterization techniques have been developed and thus have described genomic differences among strains (6). Molecular findings have increased interest among veterinarians and researchers who want to understand the influence of *M. hyopneumoniae* variability on disease epidemiology.

Using multiple-locus variable-number tandem repeat analysis (MLVA), studies have shown the presence of multiple *M. hyopneumoniae* variable-number tandem repeat (VNTR) types at a geographic, production flow, herd, and individual pig level (7–10). However, the potential source of VNTR type variation has not been fully elucidated. Insight on understanding the potential origins and drivers of genetic variation is critical for *M. hyopneumoniae* epidemiological investigations and to help tailor control strategies based on VNTR type(s) and diversity. Therefore, the aim of this study was to assess the genetic variability of *M. hyopneumoniae* within various swine production flows.

Four production flows (A to D) located in the Central United States were selected based on positive status for *M. hyopneumoniae* in the sow farms and their downstream herds, history of clinical signs suggestive of infection, and similar management protocols within one production system. The herd veterinarian assessed differences in the onset and severity of clinical signs suggestive of *M. hyopneumoniae* infection

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across flows. Historically, flows B and C experienced a slower onset and decreased severity of clinical signs compared to flows A and D. In this study, a production flow was defined as a group of sites sharing pig sources and health status, and encompassed at least one herd from each production stage: gilt developing unit (GDU), sow farm, nursery, and finisher. All farms were single sourced by flow. Herds that belonged to each flow were located across different geographical states, except for flow A. The breeding stock source for all flows was presumed to be *M. hyopneumoniae* negative gilt multipliers that originated within the production system and their health status was confirmed through routine diagnostics. Upon arrival into the breeding herd, gilts were acclimated through a seeder-to-naïve model by introducing *M. hyopneumoniae* positive culled sows and gilts into flow specific GDUs. Gilts were vaccinated with a commercial *M. hyopneumoniae* bacterin at arrival into the sow farms. Moreover, all suckling pigs in each flow received a dose of commercial *M. hyopneumoniae* bacterin at 3 wk of age. Pigs were humanely cared for and adequate housing and nutritional demands were met or exceeded. All health and welfare standards were approved by the attending veterinarian(s).

A total of 262 samples, consisting of either laryngeal or bronchial swabs, were collected from the 4 production flows. In each flow, swabs were obtained from 1 to 4 herds per production stage (i.e., GDU, sow farm, nursery, and finisher) for a period of 4 mo up to 3 y. Flow sample sizes were estimated to detect at least one test-positive pig in the sample with a group sensitivity of 95%. Thus, at least 1 VNTR type per production stage for each flow was estimated to be detected. Laryngeal swabs were obtained from pigs expressing clinical signs suggestive of *M. hyopneumoniae* infection. In the case that clinical signs were not observed, pigs to be sampled were randomly selected from the population. Bronchial swabs were collected if mortalities were observed. Laryngeal and bronchial swabs were collected using a sterile collection swab (BBL CultureSwab; Becton, Dickinson and Company, Sparks, Maryland, USA) as previously described (11,12). Samples were refrigerated immediately after collection and were stored at approximately −20°C until processed.

DNA extraction (MagMAX-96 Viral RNA Isolation Kit and MagMAX Express-96 Magnetic Particle Processor; Life Technologies, Grand Island, New York, USA) and species-specific *M. hyopneumoniae* real-time polymerase chain reaction (RT-PCR; VetMAX qPCR Master Mix and VetMAX *M. hyopneumoniae* Reagents kit; Life Technologies) were performed at the University of Minnesota Veterinary Diagnostic Laboratory. Samples with a Ct value ≤ 37 were considered positive for *M. hyopneumoniae* and those with a Ct value ≤ 32 were selected for genetic typing (personal communication from Dos Santos LF, University of Minnesota, 2015). To assess the genetic variability of *M. hyopneumoniae*, VNTR typing was performed as previously described (7). The *M. hyopneumoniae* ATCC 25095 reference strain and molecular grade water were used as a positive and a negative control, respectively.

Results were analyzed using a bioinformatics analytic software (BioNumerics version 7.1; Applied Maths, Austin, Texas, USA) according to the parameters described by Dos Santos et al (7). A minimum spanning tree was created to illustrate the relationship between VNTR types within and across flows.

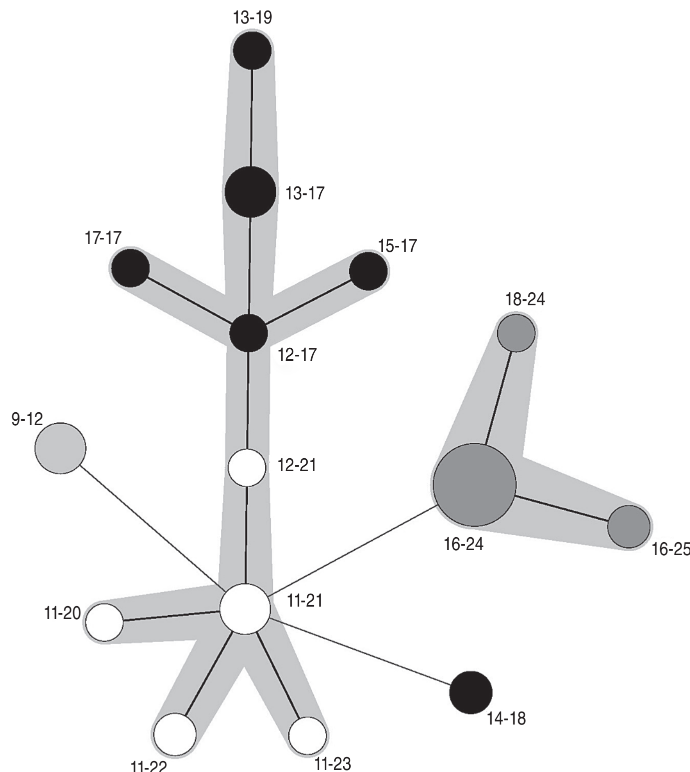

Figure 1. Minimum spanning tree of *Mycoplasma hyopneumoniae* variable-number tandem repeat (VNTR) types. Circle color corresponds to production flow (i.e., dark gray = Flow A; light gray = Flow B; white = Flow C; black = Flow D). Grey shading illustrates clonal complex of VNTR types. Lines connecting 2 circles show a relationship between the VNTR types, whereas darker lines represent a clonal complex. The 2 numbers outside of each circle correspond to the VNTR type in the sample. Each number represents the number of tandem repeats for P97 and P146 loci, respectively. Circle size corresponds to sample size (the larger the circle, the more samples with the specific VNTR type). For example, *n* = 1, 2, 4, and 15 for MLVA types 11-23, 11-22, 13-17, and 16-24, respectively.

Overall, 45.4% (119/262) of samples were positive for *M. hyopneumoniae* with RT-PCR. Within 2 flows (B and D), a higher proportion of bronchial swabs (100% and 88.9%, respectively) were positive compared to laryngeal swabs (55.5% and 46.7%, respectively). A VNTR type was obtained in 83.7% (41/49) of samples in which MLVA was attempted. A graphic representation of VNTR types from all flows over time is presented in Figure 1. Across the 4 flows, a total of 15 VNTR types were identified, in which a single flow consisted of 1 to 6 VNTR types. During a single sampling event, up to 3 VNTR types were identified within a herd. A dominant type (i.e., 16-24, 9-12, 11-21, 13-17) was observed in flows A-D, respectively (Figure 1). In flows A, B, and C, an identical VNTR type was identified in the GDU and grower sites (i.e., nursery or finisher) from 4 mo up to 2 y (Figure 2). In addition, an identical VNTR type was identified in 2 finishers in flow D, for approximately 2 y (Figure 2). Based on sample type, an identical VNTR type (i.e., 9-12 and 11-22) was detected in laryngeal and bronchial swabs for flows B and C, respectively. In flow D, 2 VNTR types (i.e., 14-18 and 13-19) were only identified in bronchial swabs.

In this study, 3 to 6 VNTR types were identified in 3 of the 4 flows, in which the predominant type(s) varied by 1 to 3 tandem

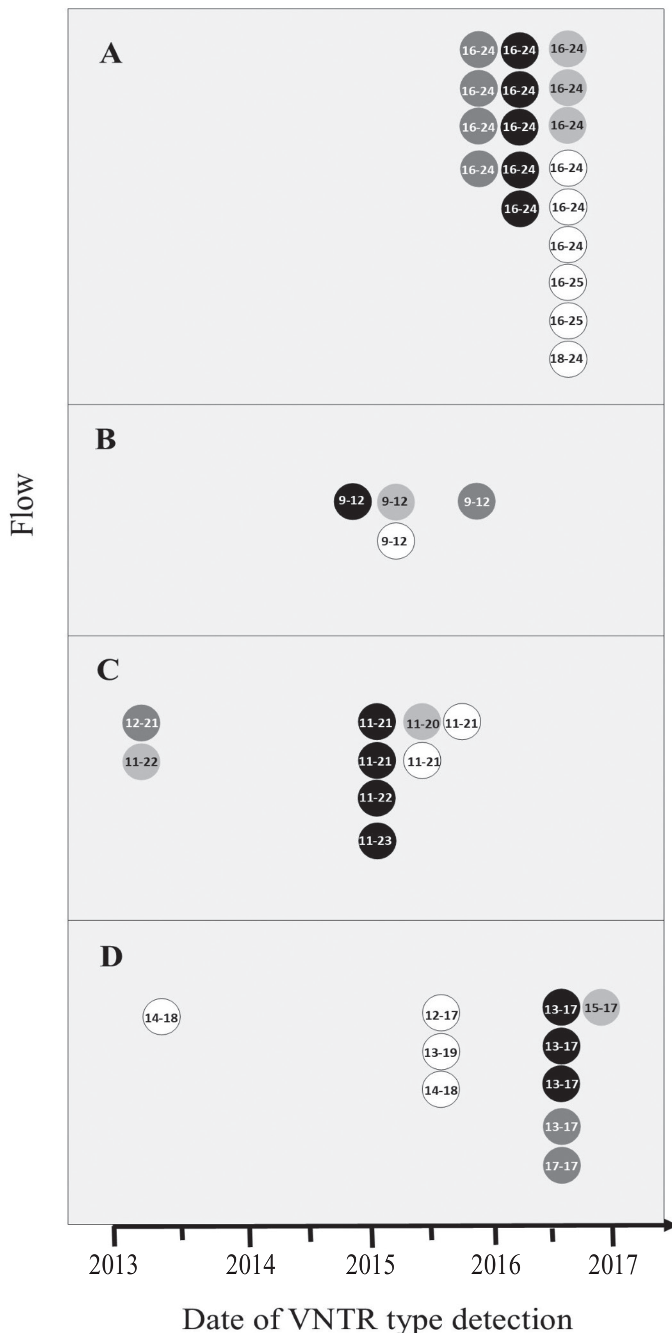

Figure 2. ***Mycoplasma hyopneumoniae*** **variable-number tandem repeat (VNTR) types identified over time by flow and production stage. Each box corresponds to a production flow (A to D). Each circle represents a single VNTR type detected in a given herd on a given date. Circle color corresponds to production stage (i.e., black = gilt developing unit, dark gray = sow farm; light gray = nursery; white = finisher). Within each circle, the first and second number correspond to the number of tandem repeats identified in P97 and P146 loci, respectively.**

repeats. Previous literature has shown the detection of multiple *M. hyopneumoniae* VNTR types in a single site and group of pigs (9,10). Moreover, the number and diversity of VNTR types present in a herd may be potential factors for disease outcome as recent work has shown that lung severity may be influenced by the existence of more than one VNTR type in a herd (10,13). However, the etiologic cause and importance of genomic modifications evidenced in VNTR length differences by MLVA typing has not been fully described. Since these flows were similarly managed, the potential impact of swine management and control methods, namely co-sourcing flows and different vaccine and antimicrobial programs, on *M. hyopneumoniae* genetic variation, could not be investigated. Therefore, further information is needed on this topic to help explore potential drivers of genetic diversity.

An identical VNTR type was identified over several years and production stages within a flow, which is comparable to results by Rebaque et al (8). In each flow, the VNTR types identified in nursery and finisher herds appeared to be derived from the breeding herds (i.e., GDU and sow farms), from which those pigs were sourced. This statement is further supported by the detection of flow specific types, since a common VNTR type was not detected between flows. In endemic populations, the circulation of *M. hyopneumoniae* in swine production flows has been thought to originate from incoming gilts and shedding sows through dam-to-piglet transmission (14,15). Lateral transmission of this microorganism has been suggested to occur at long distances (16,17). However, recent field data proposes that the likelihood of *M. hyopneumoniae* lateral transmission in high density swine populations may be minimal (18). With this knowledge, the origin of *M. hyopneumoniae* infection and VNTR type in grower-finisher sites appeared to be dependent on the sourcing herd regardless of sampling time. Such information is important to consider when structuring management of flows and *M. hyopneumoniae* control and acclimation strategies.

In conclusion, *M. hyopneumoniae* genetic variability within swine flows was minimal over time and flow-specific under the conditions of this study. The circulation of existing *M. hyopneumoniae* VNTR types appeared to be derived from breeding herds, potentially through dam-to-piglet transmission. Further research focused on determining the effect of different management strategies (e.g., vaccination, antimicrobials) and geographical location on *M. hyopneumoniae* genetic variability should be implemented to provide additional insight.

Conflict of Interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Drs. Fano and Sponheim are employed by Boehringer Ingelheim Animal Health USA Inc. However, no commercial products were evaluated in this investigation. Dr. Singer has received funding from Boehringer Ingelheim Animal Health USA Inc.

References

1. Mare CJ, Switzer WP. New species: *Mycoplasma hyopneumoniae*; A causative agent of virus pig pneumonia. Vet Med Small Anim Clin 1965;60:841–846.
2. Goodwin RF, Pomeroy AP, Whittlestone P. Production of enzootic pneumonia in pigs with mycoplasma. Vet Rec 1965;77:1247–1249.
3. Pieters MG, Maes D. Mycoplasmosis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, eds. Diseases of Swine. 11th ed. Hoboken, New Jersey: John Wiley & Sons, 2019:863–883.

4. Vicca J, Maes D, Thermote L, Peeters J, Haesebrouck F, de Kruif A. Patterns of *Mycoplasma hyopneumoniae* infections in Belgian farrow-to-finish pig herds with diverging disease-course. J Vet Med B Infect Dis Vet Public Health 2002;49:349–353.
5. Vicca J, Stakenborg T, Maes D, et al. Evaluation of virulence of *Mycoplasma hyopneumoniae* field isolates. Vet Microbiol 2003; 97:177–190.
6. Betlach AM, Maes D, Garza-Moreno L, et al. *Mycoplasma hyopneumoniae* variability: Current trends and proposed terminology for genomic classification. Transbound Emerg Dis 2019; 66:1840–1854.
7. Dos Santos LF, Sreevatsan S, Torremorell M, Moreira M, Sibila M, Pieters M. Genotype distribution of *Mycoplasma hyopneumoniae* in swine herds from different geographical regions. Vet Microbiol 2015;175:374–381.
8. Rebaque F, Camacho P, Parada J, Lucchesi P, Ambrogi A, Tamiozzo P. Persistence of the same genetic type of *Mycoplasma hyopneumoniae* in a closed herd for at least two years. Rev Argent Microbiol 2018;50:147–150.
9. Pantoja LG, Pettit K, Dos Santos LF, Tubbs R, Pieters M. *Mycoplasma hyopneumoniae* genetic variability within a swine operation. J Vet Diagn Invest 2016;28:175–179.
10. Michiels A, Vranckx K, Piepers S, et al. Impact of diversity of *Mycoplasma hyopneumoniae* strains on lung lesions in slaughter pigs. Vet Res 2017;48:1–14.
11. Pieters M, Daniels J, Rovira A. Comparison of sample types and diagnostic methods for *in vivo* detection of *Mycoplasma hyopneumoniae* during early stages of infection. Vet Microbiol 2017;203:103–109.
12. Sievers C, Dalquist L, Sponheim A, Fano E, Wetzell T. Comparison of ante-mortem sampling procedures to post-mortem bronchial sampling for the detection of *Mycoplasma hyopneumoniae* by PCR. Proceedings of the American Association of Swine Veterinarians Meeting, Orlando, Florida, 2015:115–116.
13. Vranckx K, Maes D, Calus D, Villarreal I, Pasmans F, Haesebrouck F. Multiple-locus variable-number tandem-repeat analysis is a suitable tool for differentiation of *Mycoplasma hyopneumoniae* strains without cultivation. J Clin Microbiol 2011;49:2020–2023.
14. Fano E, Pijoan C, Dee S, Deen J. Effect of *Mycoplasma hyopneumoniae* colonization at weaning on disease severity in growing pigs. Can J Vet Res 2007;71:195–200.
15. Maes D, Sibila M, Kuhnert P, Segalés J, Haesebrouck F, Pieters M. Update on *Mycoplasma hyopneumoniae* infections in pigs: Knowledge gaps for improved disease control. Transbound Emerg Dis 2018;65 Suppl 1:110–124.
16. Goodwin RF. Apparent reinfection of enzootic-pneumonia-free pig herds: Search for possible causes. Vet Rec 1985;116:690–694.
17. Otake S, Dee S, Corzo C, Oliveira S, Deen J. Long-distance airborne transport of infectious PRRSV and *Mycoplasma hyopneumoniae* from a swine population infected with multiple viral variants. Vet Microbiol 2010;145:198–208.
18. Yeske P, Morgan J, Fano E. Assessment of the likelihood of rate of *Mycoplasma hyopneumoniae* lateral transmission. Allen D. Leman Conference, St. Paul, Minnesota, 2017.