SUPPLEMENTARY MATERIAL

Acute *Mycoplasma hyopneumoniae* infection in a naive breed-to-wean herd

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sister 1950-sow breed-to-wean herd (unit 2) was populated with **M**ycoplasma hyopneumoniae-(MHP) and porcine reproduction and respiratory syndrome virus- (PRRSV) naive animals in late 2001 and early 2002. The monthly disease monitoring program used was identical to the program used by unit 1 as described in the paper. Replacement animals for unit 2 were raised in a wean-to-finish site several kilometers away from either unit 1 or unit 2. The replacement gilts were selected at around 20 to 24 weeks of age and delivered to an isolation building attached by a hallway to the sow unit of unit 2. The animals were tested prior to and after delivery using polymerase chain reaction on oral fluid samples for PRRSV and random enzyme-linked immunosorbent assay on serum samples for both PRRSV and MHP. Acclimatization events alongside boar exposure activities occurred within this building. The criteria for opening isolation and allowing replacement animals to enter the sow herd were negative retesting results for PRRSV and MHP and monitoring for clinical signs. Positive test results for MHP occurred after a group of gilts were placed into the isolation building on Friday of week 50, 2016. The gilts were infected with MHP and asymptomatic, although a detectable cough did start soon after placement. The infection was transmitted into the unit 2 adult animals over the weekend, before the isolation building could be depopulated. Positive laboratory diagnosis on coughing animals at unit 2 was confirmed week 51, 2016.

System-wide biosecurity audit activities

Immediately after the acute MHP infection was confirmed in unit 2, a systemwide biosecurity audit occurred. The goal was to review all aspects of the biosecurity program including, but not limited to, people movement, feed deliveries, weaned piglet loading procedures, and delivery of replacement animals. The existing biosecurity program included using bio-sheds located by the county road and entry drive, spraying off tires on incoming vehicles, sporadic environmental monitoring of trucks and trailers, and using a fumigation room after the bioshed for incoming supplies, all with the goal of achieving high biosecurity standards in all areas to protect from further pathogen introductions. The following procedures were used to mitigate people risks: compulsory use of shoe covers and disinfectant mats for all entering the unit, limiting and documenting visitors, monitoring maintenance personnel and vehicles, proper downtime documented, and shower with farm clothes provided. Minor changes were implemented with timing of loading piglets and cleaning inner-sanctum vehicles. The source of MHP infection in unit 2 was the replacement gilts delivered from a separate nursery-to-finisher site that became infected but was not clinically detected until after the replacement gilts were moved to the unit 2 isolation building. Following confirmation of an acute MHP infection in unit 2, large bales of hay were placed approximately 1 m from the exhaust fans to deflect exhaust air upward, although this did not occur immediately after the confirmed positive

diagnosis. The objective of this activity was to contain the infection on this site and not allow area spread. A review of the timeline between the acute MHP infection in unit 2 and detection of the first clinical signs at unit 1 indicates that the critical time was 4 weeks.

A system-wide biosecurity audit was performed during this same 4-week time between the acute MHP infections of unit 1 and unit 2, which included the feed mill and all deliveries. The goal with this exercise was to heighten the need to maintain proper procedures already outlined in written protocols, and to find any lapses in daily routines that would influence success of the biosecurity programs. The review did not find any major concerns in the biosecurity program that may have contributed to the spread of infection from unit 2 to unit 1.

MHP sequence information

The MHP sequences from both unit 1 and unit 2 infections were compared using Disease BioPortal (University of California-Davis). The MHP sequences were identical, which supports the hypothesis that area spread had occurred.

Weather information for week 51, 2016 to week 2, 2017

The daily weather conditions¹ for the 4-week period between week 51, 2016 to week 2, 2017 are illustrated in Table S1. The maximum relative humidity for most days during the 4-week period were near or equal to 100%. Likewise, the average relative humidity during the same

TG: Performance Health, PC, Battle Ground, Indiana.

OGD: Boehringer-Ingelheim Vetmedica GmbH, Ingelheim, Germany.

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Supplementary material to: Gillespie T, Gomez Duran O. Acute *Mycoplasma hyopneumoniae* infection in a naïve breed-to-wean herd. *J Swine Health Prod.* 2022;30(5):298-307. https://doi.org/10.54846/jshap/1279

Supplementary material DOI: https://doi.org/10.54846/jshap/1279suppl1.

time was near or over 90% on several days. Slow wind speed is another factor for potential aerosol transmission. Due to favourable weather conditions and the lack of other obvious biosecurity breaches, it was suspected that the MHP infection in unit 1 had been introduced via airborne transmission.

Reference

*1. West Lafayette, IN Weather History. Weather Underground. Accessed September 2021. https://www. wunderground.com/history/monthly/ us/in/west-lafayette/KLAF/date/2017-1

* Non-refereed reference.

	Minimum temperature, °F	Mean temperature, °F	Maximum temperature, °F	Dew point, °F	Precipitation, in	Snow depth, in	Wind, mph	Gust wind, mph	Sea level pressure, in
Week 51, 2	016 (Dec 18-24)								
Minimum	-6	4.71	34	-9	0.00	0.00	0	0	29.17
Mean	4.71	24.45	36.51	19.81	0.024	0.00	6.44	2.17	29.64
Maximum	15	36.51	39	39	0.12	0.00	21	30	30.13
Total	NA	NA	NA	NA	0.124	0.00	NA	NA	NA
Week 52, 2	016 (Dec 25-31)								
Minimum	22	29.97	35	18	0.00	0.00	0	0	29.04
Mean	27.71	36.23	44.14	29.94	0.11	0.00	11.3	9.05	29.38
Maximum	33	51.48	62	58	0.74	0.00	26	39	29.72
Total	NA	NA	NA	NA	0.77	0.00	NA	NA	NA
Week 1, 20	17 (Jan 1-7)								
Minimum	-4	4	9	-7	0.00	0.00	0	0	29.06
Mean	12.86	21.36	29.14	17.02	0.07	0.00	8.23	3.28	29.46
Maximum	31	39.86	49	46	0.35	0.00	23	33	29.95
Total	NA	NA	NA	NA	0.46	0.00	NA	NA	NA
Week 2, 20	17 (Jan 8-14)								
Minimum	1	9.83	18	-5	0.00	0.00	0	0	28.83
Mean	21.29	30.62	40.14	25.7	0.14	0.00	9.55	7.25	29.58
Maximum	33	45.7	61	56	0.45	0.00	38	55	30.09
Total	NA	NA	NA	NA	1.01	0.00	NA	NA	NA