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David Brown
www.davidhbrown.us

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Ruth Cronje, and Jan Swanson;
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A case study on *Mycoplasma hyopneumoniae* diagnostics

Keith Kinsley, DVM

Mycoplasma hyopneumoniae verification in a naïve population should be straight-forward to determine by serology right? Many times there are one or two false positives from a group of 30-60 head using the IDEXX ELISA serology as the primary surveillance test. Most times, these suspects or positives will be cleared (deemed negative) by use of the DAKO serology as a secondary test, but in cases where they cannot be, or the positives/suspects are more numerous than expected on both serology tests, what should be believed – clinical signs or the test results?

The attempt in this text was to place as many of these events in chronological order of testing progression as possible. It may seem a little busy, but the entire span of these events was 24 days from the first test results of the isolation barn until the animals were tested for the final time.

The following is a case summary of a *Mycoplasma hyopneumoniae* testing incident encountered in May 2011. At no point in time did clinical signs ever suggest that disease was present, but two late April tests suggested that there was an outbreak in an isolation barn which had been filled four weeks prior from a naïve multiplication finisher. The initial testing resulted in 11 of 30 (36.7%) positive or suspect IDEXX ELISA samples. All 11 were retested using the DAKO serology and found positive.

After finding these results, the isolation barn was immediately retested. Efforts were made not to test the same animals as there was the possibility that some *Mycoplasma hyopneumoniae* vaccine could have been used on the group nearly six months prior. The second testing revealed 5 of 30 as suspect or positive using the IDEXX ELISA. All of which tested negative on the DAKO serology. With the possibility that vaccination had occurred errantly, the gilt recipients requested that all animals be individually tested and tagged prior to the next isolation turn being filled.

From the multiplier's finishing barn, 204 head were tagged and tested for *Mycoplasma hyopneumoniae*. Of this tested group, 55 of 204 (27%) were either suspect or positive by IDEXX ELISA. There had been no clinical signs in either group during the testing periods and so the decision was made to sacrifice three of the highest ELISA positives from the finishing site and three gilts from the isolation (near the location of the original positives) and perform

tissue PCR. Surely there had to be a PCR positive in these groups to yield these results.

The same day the gilts were posted, the multiplier sow farm was bled as were the recipient sow farms. Sixty head were tested from all three sow farms as follows – 60 random head from the multiplication farm and 60 of the most recently introduced gilts at the recipient sow farms. This had gone from a possible test error scenario to needing to find out how widespread this disease may be.

While delivering the tissues for PCR and sow samples serum for IDEXX ELISA, the 55 multiplication finisher samples (suspect or positive by IDEXX ELISA) were run with the DAKO serology and 45 came back negative and the remaining 10 came back, "Retest in 2 Weeks." This did not help bring any finality to the situation, but most certainly the sow herd tests and the six sacrificed gilts would, but this had to be the beginning of an outbreak.

All six of the posted gilts came back PCR negative on tissue and no lesions were identified on histopathologic examination suggestive of an infection with *Mycoplasma hyopneumoniae*. The three sow farms came back with similar results, 3-4 IDEXX ELISA suspects or positives, all of which were negative by DAKO serology. There had been no clinical signs at any of the sow farms at the time of testing, so maybe they dodged a bullet.

Only two obstacles, it appeared, remained in the way of deeming these animals clear:

1. The initial test results on the isolation barn (both IDEXX ELISA and DAKO positives), and
2. The 10 (at present 8) remaining "Retest in 2 Weeks" head at the multiplication finisher.

The decision was made at minimum to wait two weeks and retest the multiplication finisher before moving forward with a refill of the isolation barn from this site. While waiting for this time to pass, the isolation barn was rope tested for final confirmation prior to entry. The attempt was made to maximize the number of animals contacting these ropes and as a result the number of head tested by salivary PCR.

As if to get one last little jab in from this group of gilts, one of the 12 ropes hung returned PCR positive. This was an

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extremely late cycle positive, but reported as positive none the less. This sample was rerun and found negative, and re-extracted and found negative twice, but what is truth at this point. There were still no clinical signs and no death loss in either group (isolation or gilt finisher). The only avenues left were to dump the entire group in isolation, or test all of the individuals serologically and by nasal PCR in those pens that contributed to the positive rope.

Ultimately 35 head were rebled and individually nasal swabbed for PCR analysis from the two isolation pens contributing to the PCR positive rope. The finishing barn had not been bled for nearly two weeks at this about this same time and eight DAKO “Retest in 2 Weeks” animals still remained. All eight were retested by nasal swab PCR and serology as well as 22 random serologies and nasal swabs of the other 194 head that had previously been IDEXX ELISA or DAKO negatives.

Every animal from these final two samplings were IDEXX ELISA and DAKO serology negative with the exception of one animal in the finishing barn that still came back “Retest in 2 weeks”. All animals were nasal PCR negative.

With these findings the decision was made to move the gilts from the isolation barn into the recipient sow herds and to move the multiplier finishing group into the isolation barn. Groups originating from this finisher both before and after this testing exercise have tested IDEXX ELISA negative to *Mycoplasma hyopneumoniae* or if suspect or positive have tested DAKO negative.

At the point of this paper’s submission, at least three additional groups have been run through the isolation barn with no adverse testing results, and the source sow farm and the recipient sow farms have shown no clinical signs of respiratory disease. Also by the point of submission,

there is no identified explanation as to why this group of animals tested the way that they did and why only the first sample pull from each site reacted the way they did.

What was learned from this experience:

- If clinical signs do not match diagnostic findings investigate further – this could have been an extremely costly clean-up
- Never use *Mycoplasma hyopneumoniae* in a herd represented as naïve – during this many *Mycoplasma hyopneumoniae* vaccine technical veterinarians have been consulted, most if not all suggest that seroconversion from vaccine alone (especially one shot) would be unlikely to produce a measurable level in a population.
- Testing over multiple time points may be more representative of what is actually happening in the population than a snapshot – this can go either way
- Always identify isolation animals prior to leaving the multiplication finisher or before any testing is done in the isolation – it is never known when samples need to be paired, as in this case, to verify developing seroconversion and disease or lack thereof.

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