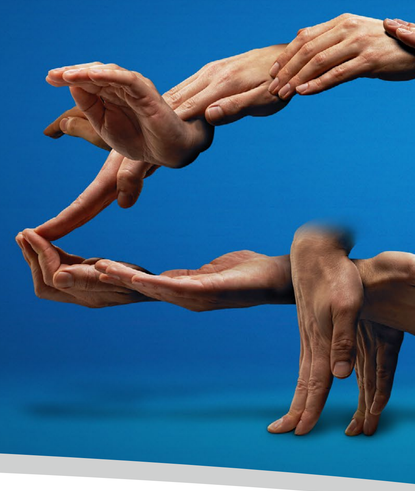
Influence of pooling on artificial laryngeal swab sample PCR results in low *Mycoplasma hyopneumoniae* prevalence scenarios



A. Sponheim[1], E. Fano[1], D. Polson[1], K. Doolittle[1], M. Pieters[2]
[1] Boehringer Ingelheim Vetmedica, Inc., MO, USA; [2]University of Minnesota, MN, USA

INTRODUCTION

A recent study comparing ante-mortem versus post-mortem samples by individual and pooled samples for detection of *Mycoplasma hyopneumoniae* (Mhp) by PCR suggested that utilizing pooling and increasing sample size via ante-mortem laryngeal swab sampling (LS) allows for a higher herd detection rate while pursuing the most economical testing approach in a high prevalence population[1].

The following study was designed to determine the extent pooling samples from an artificially created low Mhp prevalence ante-mortem population scenario lowers test diagnostic sensitivity (DxSe).

MATERIALS AND METHODS

Due to the power required for the study, artificial positive and negative samples comparable to those found in low Mhp prevalence scenarios were created in vitro.

Treatment groups were chosen for the study based off of a histogram created from field LS results. To mimic LS, an artificial Mhp positive stock solution for each treatment group was created using Mhp strain AP 414 (Ct 20) stored at the University of Minnesota, PBS, and known Mhp negative oral fluids.

Dilutions were made to create the desired Ct value for each treatment group:

- low (L = 26)
- middle (M = 31)
- high (H = 36)

Artificial negative samples were made using PBS and known Mhp negative oral fluids.

L, M, and H treatment groups were tested in 3:1 and 5:1 pools.

The remaining samples in each pool were composed of the known negative samples.

Ninety PCR tests (VetMAX™-Plus qPCR Master Mix) were run for each pool and Ct value for a total of 540 PCR tests.

Six PCR plates were used to test all of the samples.

Fifteen PCRs for each Ct value and pool were run per plate.

To reduce potential variation, one technician was responsible for all extractions (MagMAX-96 Viral RNA Isolation Kit) and the amplification process. All PCRs were tested on one day using 3 machines (2 plates / machine).

RESULTS

DxSe results are reported in Table 1.

L and M had similar DxSe results for pools of 3:1 (100 % and 98.89 %) and 5:1 (100 % and 100 %). L and M were more sensitive than H pools of 3:1 (90 %) and 5:1 (72 %).

95% and 99% confidence intervals (CI) were determined for H pools

- 3:1 (81.9 – 95.3 and 79.1 – 96.4)
- 5:1 (61.8 – 81.1 and 58.5 – 83.5)

Table 1: Diagnostic sensitivity results

	High (H) Ct (36)		Middle (M) Ct (31)		Low (L) Ct (26)	
Pools of	3	5	3	5	3	5
DxSe (positive/ expected positive)	90 81/90	72. 65/90	100 90/90	100 90/90	98.89 89/90	100 90/90
95 % Confidence Interval	81.9 – 95.3	61.8 – 81.1				
99 % Confidence Interval	79.1 – 96.4	58.5 – 83.5				

DISCUSSION AND CONCLUSION

As expected in a low Mhp prevalence and high Ct value scenario, there is a risk of not detecting one positive animal in a pool when the rest of the pool is negative.

DxSe and CI obtained from this study have been used in a stochastic model to develop novel LS tables to provide veterinarians guidance in determining number of animals along with number of times to sample herds to most economically detect Mhp in low prevalence scenarios.

REFERENCES

1. C. Sievers et al. AASV 2015 Conf. Proc. Pages 115 – 116

MycoLab
MYCOPLASMA RESEARCH LABORATORY