

A comparison of alternative media for *Mycoplasma hyopneumoniae* aerosol exposure

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Introduction

Mycoplasma hyopneumoniae (Mhp) causes significant economic losses to the swine industry.¹ Effective planned Mhp exposure of replacement breeding animals aids Mhp elimination protocols. Friis medium (FM) is the current gold standard to dilute lung homogenate (LH) in Mhp exposure protocols.² Less costly media are available, but their effect on Mhp viability is unknown. The goal of this study was to evaluate alternative media used in Mhp LH preparation on aerosol exposure efficacy.

Materials and methods

Four barns were used for the trial, each having 10 pens housing 125 pigs/pen. The finishing site selected was part of a porcine reproductive and respiratory syndrome virus (PRRSV) and Mhp negative flow. At -14 days post-infection (DPI), tracheobronchial (TB) swabs from 30 pigs chosen randomly were confirmed Mhp-negative by polymerase chain reaction (PCR). There were no observed clinical signs indicating the presence of other respiratory pathogens prior to the start of the study.

Four media were evaluated: Friis medium (FM), phosphate buffered saline (PBS), purchased saline (PS), and homemade saline (HS). Each barn was assigned a single medium. For HS preparation, 37 grams of table salt was dissolved into 1 gallon of distilled water. Two routes of administration (ROA) were evaluated per medium: intra-tracheal (IT) inoculation and aerosol exposure (AE) (8 total treatments).

All exposures were completed at 0 DPI (pig age approximately 22 weeks). Inoculum was prepared using a ratio of 80 mL of known-Mhp-positive LH diluted into one gallon of medium. After mixing, inoculum used for IT exposure was stored on ice packs until administered; inoculum used for AE was stored at room temp (approximately 80 degrees F) for 60 minutes before use. Ten pigs in each barn's southwest-most pen (40 total) were ear tagged and inoculated via IT administration of 10 mL diluted chilled inoculum delivered via a rubber urethral catheter (positive controls). Pigs in the northeast-most pen were exposed using 1 gallon of aerosolized inoculum mist delivered via two Hurricane foggers per pen over 15-20 minutes.

At 4 DPI, 30 random pigs from each AE pen (120 total) were ear tagged for repeat sampling. TB swabs were collected individually from tagged pigs at 4 DPI (all groups, N = 160) and 14 DPI (AE only, N = 120) using a sterile swab attached to a modified post-cervical artificial insemination catheter. Swabs were transported in 5 mL falcon tubes containing 1 mL PBS then tested for Mhp in pools of 5 by PCR at Iowa State University Diagnostic Laboratory (ISU-VDL).

At 57 DPI, lungs from a subset of tagged pigs were examined post-harvest at a packing plant.

Results

By 4 DPI, Mhp was detected by PCR from all groups, regardless of ROA (Table 1); 4 DPI IT samples were 100% PCR Mhp-positive when tested individually; these were not retested at 14 DPI.

At 14 DPI, 100% of pooled samples from each aerosol group were PCR positive. All samples were tested individually and every sample from each media type was confirmed PCR positive.

At harvest, 87.5% of 96 examined lungs showed gross lesions of pneumonia, predominately in the anterior-ventral lobes.

In this study, all media and ROA conferred rapid and complete Mhp colonization within 14 DPI, indicating Mhp viability was not adversely affected by media type or ROA. Storage for 60 minutes did not hamper efficacy inoculum used for aerosol ROA. More rapid colonization was seen with IT ROA versus aerosol. Homemade saline proved to be as effective as the other media as a diluent for LH. HS advantages include cost savings and ease of formulation in most veterinary clinics.

References

1. Maes D., Segales J., Mynes T., Silbila M., Pieters M., Hasebrouck F., Control of *Mycoplasma hyopneumoniae* infections in pigs. *Rev Vet Microbiol.* 2008; 126:297-309
2. Robbins RC, Betlach AM, Mondragon-Evans MR, et al. Development of a herd-specific lung homogenate for exposure to *Mycoplasma hyopneumoniae* under field conditions. *J Swine Health Prod.* 2019;27(4):221-227.

Table 1: Detection of *Mycoplasma hyopneumoniae* by PCR in tracheobronchial swabs on days 4 and 14 post-inoculation

ROA and media	Price per gallon	4 DPI				14 DPI			
		Pooled		Individual		Pooled		Individual	
		Positive/ Total	Average Ct	Positive/ Total	Average Ct	Positive/ total	Average Ct	Positive/ total	Average Ct
Intra-tracheal									
Friis medium media	\$366.16	2/2	27.9	10/10	29.5				
Phosphate buffered saline	\$143.85	2/2	26.1	10/10	26.9				
Purchased saline	\$49.21	2/2	23.3	10/10	27.6				
Homemade saline	\$0.89	2/2	23.3	10/10	24.6				
Aerosol									
Friis medium media	\$366.16	3/6	32.6			6/6	26.4	30/30	27.6
Phosphate buffered saline	\$143.85	6/6	31.2			6/6	23.6	30/30	23.4
Purchased saline	\$49.21	5/6	32.7			6/6	24.4	30/30	23.5
Homemade saline	\$0.89	5/6	28.6			6/6	22.8	30/30	23.3

Tracheobronchial swabs were not collected for intra-tracheal inoculated pigs on 14 DPI because all swabs were individually PCR positive on 4 DPI.

