**Assessing Mycoplasma hyopneumoniae aerosol movement at several distances**

**A. C. Cardona, C. Pijoan, S. A. Dee**

RECENT changes in modern pig production, such as the application of segregated production, early weaning and strategic medication, have attempted to eliminate pathogens from pig populations (Alexander and others 1980). However, despite these efforts, significant diseases such as enzootic pneumonia still play a major role in reducing pig performance. The primary aetiological agent of enzootic pneumonia is Mycoplasma hyopneumoniae (Ross 1999). *M. hyopneumoniae* is classified in the taxonomic group Mollicutes (Tully and others 1993) and is an extremely fastidious organism, requiring special conditions to grow in the laboratory (Friis 1975). The difficulty in culturing *M. hyopneumoniae* has led to the development of nested PCR (nPCR) technology to aid in the detection of the organism in tissue and air samples (Calsamiglia and others 1999).

The routes of transmission of *M. hyopneumoniae* include infected pigs and possibly aerosols. Experimentally, aerosol infection with *M. hyopneumoniae* has been used to reproduce the disease successfully (Jakob and others 1991). Under field conditions, the transmission of *M. hyopneumoniae* has been speculated to occur at a distance of up to 3-2 km (Goodwin 1985). Distance between farms, farm size and the proximity of farms to roadways used to transport pigs have been identified as risk factors for the spread of the organism (Jorsal and Thomsen 1988, Thomsen and others 1992). However, these data have only been based on retrospective epidemiological studies, and no attempts have yet been made to assess scientifically whether *M. hyopneumoniae* can actually be transported in the air over various distances.

This short communication describes a study to develop a laboratory model to determine whether *M. hyopneumoniae* could be transported over distances of 1, 75 and 150 m.

The model was based on a protocol previously described to evaluate the ability of porcine reproductive and respiratory syndrome virus to be transported by aerosols (Dee and others 2005), and consisted of three components: dispersal, dissemination and detection. The dispersal phase entailed aerating a suspension of Friis medium containing 10<sup>7</sup> colour-changing units of *M. hyopneumoniae* strain 232, using a cooking oil spritzer, a utensil resembling a pressurised aerosol spray can used in the cooking industry to apply oil to frying pans: 3 ml of *M. hyopneumoniae*-inoculated Friis medium was poured into the flask of the utensil, followed by a manual injection of 200 ml of air (Dee and others 2005). Throughout the study, this mixture was considered to be one aliquot of aerated *M. hyopneumoniae*. For the dissemination of each aliquot over the three distances, a Dayton split capacity blower was used to pull aerated *M. hyopneumoniae* down a straight plastic tube 10 cm in diameter. Finally, a portable air centrifuge (Spin Con 45B; Camber) was used to collect *M. hyopneumoniae*-inoculated air samples exhausted from the blower following transport through the tube (Fig 1). This instrument was capable of collecting 450 litres of air per minute. During the process of air collection, sterile saline was automatically injected into the air centrifuge, rinsing the interior of the centrifuge drum and pooling any particles in a 10 ml aliquot for diagnostic evaluation.

The study consisted of a total of six replicates, two replicates being conducted at each distance. During each replicate, one aliquot of aerated *M. hyopneumoniae* was dispersed and a 60 minute air-collection period was employed. Each replicate also included a positive control, a protocol control and a sanitation control. The positive control consisted of collecting aerated *M. hyopneumoniae* dispersed directly from the uterus without the use of the tube model, while the protocol control consisted of testing *M. hyopneumoniae*-free Friis medium to ensure that the aforementioned procedure itself did not result in cross-contamination. Both controls were conducted between each replicate that utilised *M. hyopneumoniae*-positive aerosols.

Between each replicate, the model and the air centrifuge were sanitised. To thoroughly sanitise the pipe, it was disassembled into separate 10 m sections and swabbed using a rag immersed in 10 per cent bleach solution and another immersed in sterile water, which were attached to a 10 m extension pole and applied to the inner surface of each section of pipe. The air centrifuge was disinfected by an automated process that involved rinsing the interior of the instrument with 10 per cent bleach solution according to the manufacturer’s instructions. Following completion of the sanitisation of the centrifuge, its inner workings were rinsed with sterile saline and a 10 ml sample was collected for diagnostic testing to ensure the absence of *M. hyopneumoniae* (the sanitation control).

During all replicates, the temperature, relative humidity and velocity of the air transported through the tube were measured using a Kestrel weather meter (Nielsen-Kellerman). The instrument’s impeller was inserted 5 cm into the lumen of the pipe and readings were taken at 1, 75 and 150 m. All air samples and the sanitation controls were tested for the presence of *M. hyopneumoniae* DNA by the nPCR assay as previously described by Calsamiglia and others (1999).

The results of air flow parameters are summarised in Table 1. Air velocity readings of 11.75 m/s (42 km/hour) were recorded over the 150 m distance, while the mean temperature and relative humidity recorded over this distance were 9.2°C and 49 per cent, respectively. All air

![FIG 1: Diagram of the pipe model used to disperse, disseminate and detect aerated Mycoplasma hyopneumoniae at 1, 75 and 150 m](image-url)
samples collected at 1, 75 and 150 m (two of two replicates per distance measured) were positive for *M. hyopneumoniae* DNA by qPCR. All positive controls were qPCR positive, while all protocol and sanitation controls were negative. Isolation of *M. hyopneumoniae* from the air samples was also attempted (Friis 1975); however, all the samples were heavily contaminated with non-specific bacteria, making it impossible to accurately detect the presence of *M. hyopneumoniae*-like colonies.

To the authors’ knowledge, this is the first report describing the experimental aerosol movement of *M. hyopneumoniae* over distances of 1, 75 and 150 m. While these are interesting results, under the conditions of the study, it is not known whether the organisms that were collected were capable of travelling over greater distances or could successfully infect pigs. Further studies are required to determine the maximum distance that aerated *M. hyopneumoniae* could travel, the climactic conditions that promote maintained viability of the organism over distance, and possibly to employ more sensitive measures such as a swine bioassay or quantitative PCR to better determine the concentration and infectivity of the *M. hyopneumoniae* in the recovered samples.

### References


TULLY, J. G., BOVÉ, J. M. & LAIGRET, F. (1993) Revised taxonomy of the class Mollicutes: proposed elevation of a monophyletic cluster of arthropod-associated Mollicutes to ordinal rank (Entomoplasmatales ord nov), with provision for familial rank to separate species with nonhelical morphology (Entomoplasmataceae fam nov) from helical species (Spiroplasmataceae), and emended descriptions of the order Mycoplasmatales, family Mycoplasmataceae. *International Journal of Systematic Bacteriology* **43**, 378-385

### Table 1: Mean (range) airflow parameters across all replicates

<table>
<thead>
<tr>
<th>Distance (m)</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Velocity (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.7 (8.7-10.8)</td>
<td>55 (47-56)</td>
<td>11.75</td>
</tr>
<tr>
<td>75</td>
<td>10.3 (8.8-11.7)</td>
<td>46 (40-53)</td>
<td>Not tested</td>
</tr>
<tr>
<td>150</td>
<td>9.2 (8.3-10.8)</td>
<td>49 (42-54)</td>
<td>11.75</td>
</tr>
</tbody>
</table>
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