Mycoplasma gallisepticum (MG) infections can cause significant economic losses for the layer industry due to declines in performance and production and remains an economically important pathogen (Evans et al., 2005). Controlling avian mycoplasmosis has been shown to prevent decreases in egg production in layer chickens (Carpenter et al., 1981) and is achieved by using attenuated live vaccines (Whithear, 1996). Current methods of vaccine administration include eye drop, inclusion in drinking water, and spray application; spray application has become increasingly popular as it reduces both time and labor required for vaccination (Ley, 2003).

For spray vaccination, the vaccine organism can enter the bird via three paths: inhalation, ingestion through preening or contact with other birds, and absorption through the eyes and subsequent drainage into the nasal cavity via the naso-lacrimal duct (Nickel et al., 1977). Commercially available MG vaccines were initially applied via eye drop administration to individual birds, but proved to be time and labor intensive, leading to the subsequent adoption of spray vaccination. Current methods of spray application range from backpack blowers connected to a spray wand to various “shop-built” machines which are manually pushed/pulled through the facility. The lack of constant spray paths and speed can result in non-uniform dosing of birds and inconsistent seroconversion rates (Branton et al., 2005). Seroconversion rates are the primary metric by which success for vaccination administration is determined and low seroconversion rates (< 50%) indicate that a majority of the flock did not mount an immune response to the vaccine, and remain unprotected. In addition, reactions to the vaccine can occur and result in “rolling reactions” where clinical signs of the disease occur (Branton et al., 2008) and negatively impact flock performance.

Spray application programs can prove difficult to maintain consistent levels of efficacy due to the inherent variability in dosage; inconsistent inoculation can ultimately reduce flock performance (Branton et al., 2005). Branton et al. (2005) documented the development of a spray applicator for caged layer operations (CPF® Vaccinator, Long Branch Co., West Point, Miss.) which improved seroconversion rates in a commercial layer flock from 70% to 90%. The spray applicator was self-propelled and moved at a constant speed, evenly applying vaccine to each tier of cages within the house. In addition, the labor requirements were reduced by 80% and time to apply vaccine was reduced by 84%.

Optimization of spray vaccination protocols is necessary to ensure consistent serological response in layer flocks. Parameters which may affect vaccine efficacy include physical characteristics of the spray such as droplet size, coverage, volume application, and chemical characteristics of the vaccine suspension such as pH and ionicity. System pressure may affect both application rate and droplet size generated from nozzles and may affect viability of the organism. Nozzle type can also affect spray characteristics including droplet size and application rate. The objective of this study was to determine the effects of system pressure and...
nozzle type on the delivery of MG vaccine from the CPJ® vaccinator.

METHODS AND MATERIALS

This study was comprised of two experiments to determine the effects of system pressure and nozzle type on the physical characteristics of the spray delivered from the vaccinator. The same vaccinator and group of nozzles were used in each experiment. The vaccinator (fig. 1) consists of a battery-powered self-propelled cart which travels at a constant speed of 1.6 m·s⁻¹ (5.2 ft·s⁻¹) and is equipped with six height-adjustable nozzles. A full description of the vaccinator is given in Branton et al. (2005) and a functional schematic is shown in figure 2.

Three different nozzles typical of those recommended for use by vaccine manufacturers for spray application of liquid vaccines to layer chickens were used. Nozzles used in this study include: 1553-10, 1553-08, and 1531-06 (HARDI, Taastrup, Denmark). Nozzle numbers represent nozzles with a 53° cone with a #10 orifice, 53° cone with a #8 orifice, and 31° cone with a #6 orifice, respectively. Pressures used in this study were chosen based on a limited survey of commonly reported pressures used to apply vaccines in commercial table egg producing operations. The reported pressures varied from 310.2 and 448.1 kPa (40 and 60 psi) and as such were chosen as treatments for this study. Flow rates for each nozzle and pressure combination are listed in table 1.

System pressure was measured using a precision pressure transducer (PX603-100G5V, Omega Engineering, Stamford, Conn.) and recorded on 3-s intervals with a data logger (XR440, Pace Scientific, Mooresville, N.C.). Commercial F-strain vaccine (F-VAX MG Schering-Plough Animal Health, Omaha, Neb.) was prepared according to typical commercial practice for these experiments (0.46 mL/dose). Prior to each use of the vaccinator, it was cleaned and disinfectant was circulated through the system then discharged through the nozzles, after which distilled water was used to flush the spray lines.

**EXPERIMENT 1: DROPLET SIZE MEASUREMENT**

A laser diffraction droplet sizing system (Helos, Sympatec, Inc., Clausthal, Germany) was utilized in this study. This system used a 623-nm He-Ne laser and was fitted with an R5 lens, resulting in a dynamic size range from 0.5 to 875 μm in 32 sizing bins. All droplet size measurements were taken with the system stationary, and the re-circulation pump was engaged between measurements to keep the vaccine well mixed. Spray was engaged and allowed to

![Figure 1. Self-propelled constant speed layer chicken vaccinator.](image)

![Figure 2. Piping schematic for vaccinator.](image)

<table>
<thead>
<tr>
<th>Nozzle</th>
<th>Pressure (kPa)</th>
<th>Flow (mL/min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1553-10</td>
<td>310.2</td>
<td>536.8 - 4.7</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>684.4 - 7.2</td>
</tr>
<tr>
<td>1553-08</td>
<td>310.2</td>
<td>209.2 - 1.4</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>249.5 - 1.1</td>
</tr>
<tr>
<td>1531-06</td>
<td>310.2</td>
<td>244.0 - 1.8</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>288.6 - 0.6</td>
</tr>
</tbody>
</table>
operate for approximately 30 s while the laser system mounted on a forklift stand was traversed vertically through the spray plume. Continuous measurements were made over the 30-s period after which the system software calculated the droplet spectrum statistics. Three replications were made for each nozzle and pressure combination. Droplet size characteristics and droplet size classifications follow ASAE Standard S572 (ASAE Standards, 2004).

EXPERIMENT 2: SPRAY DEPOSITION AND VACCINE VIABILITY

Water sensitive paper (Spraying Systems Co., Wheaton, Ill.) was used to determine the characteristics of the spray from the vaccinator as applied. Six strips of water sensitive paper (WSP) were attached to a wire frame, spaced 61 cm apart and suspended vertically 98 cm from the floor to correspond with the middle spray nozzle of the vaccinator. The vaccinator was moved at a constant speed of 1.6 m·s⁻¹ along a guide track to maintain a distance of 76 cm between nozzle and WSP strips to simulate the nominal distance from the sprayer to birds in a tiered-cage layer house. Exposed WSP strips were scanned and the resulting images were processed for stain size, stain diameter, droplet diameter, and deposition per area using a commercial analysis package (DropletScan™, WRK of Oklahoma, Stillwater, Okla.) to determine as-applied percent coverage, and deposition (Whitney and Gardisser, 2003; Hoffmann and Hewitt, 2005).

Flowrate from each nozzle and pressure combination was measured by capturing the nozzle discharge in bottles and timing the spray with a stopwatch. Bottles were then weighed to determine the mass of water collected, and flowrate was calculated accordingly.

Vaccine viability (survival) was measured by determining the number of color change units (CCU₅₀) per mL to determine if viability was affected by pressure or nozzle type. Mycoplasmas lack a structural cell wall and may be susceptible to injury by increased pressure. MacNaughton and MacDonald (1982) reported reduced growth at increased hydrostatic pressures for acholeplasmas, which are closely related to mycoplasmas. CCU₅₀ is a method to enumerate bacteria, similar to colony forming units, but has been shown to be more accurate in enumerating mycoplasmas (Stemke and Robertson, 1982). Serial 10-fold dilutions of bacteria are made in medium containing phenol red as a pH sensitive colorant. Bacterial growth results in the production of acid and subsequent change in color of the medium. Fifty percent endpoints can be determined from the last dilution which indicates no bacterial growth based on color change (Masover et al., 1975; Purcell et al., 1966). After each traverse along the track, 100 μL was captured from the nozzle and immediately diluted in Frey’s medium (Frey et al., 1968). Samples were also obtained directly from the reservoir prior to the first traverse along the guide track, at the mid-point of the experiment when system pressure was adjusted, and at the conclusion of the spray tests. CCU₅₀ was performed using serial 10-fold dilutions incubated at 37°C in a sealed microtiter plate (Masover et al., 1975) and the 50% endpoint was calculated as described by Reed and Muench (1938).

STATISTICAL ANALYSIS

The two system pressure settings and three nozzle types were arranged in a 2 × 3 factorial treatment structure with three replications. All data were analyzed with PROC MIXED in SAS (SAS v8.0, SAS Institute, Inc., Cary, N.C.) and differences in treatment means were separated using Fisher’s LSD. Significance for all statistical analyses was considered at P ≤ 0.05.

RESULTS AND DISCUSSION

EXPERIMENT 1

Droplet size data are presented for system pressure (table 2) and nozzle type (table 3) and include volume median diameter, Dv₀.₅, and the 10% and 90% volume diameters, Dv₀.₁ and Dv₀.₉. Volume median diameter (Dv₀.₅), the droplet diameter where 50% of the spray mass is contained in droplets smaller than this value, is typically used to describe spray droplet size spectra. The other volume diameters used to describe droplet size spectra include Dv₀.₁ and Dv₀.₉ values, which describe the proportion of the spray volume (10% and 90%, respectively) contained in droplets of the smaller than this size. The percent of spray volume consisting of respirable droplets (<10 μm diameter) is also presented in table 4. Comparisons of droplet size distribution data for each combination of system pressure and nozzle type are shown in table 4.

Mean volume diameters decreased when pressure was increased from 310.2 to 448.1 kPa, as expected. The 1531-06 nozzle consistently produces larger droplets when compared to the remaining two nozzles. Even though the orifice of 1531-06 is smaller than the other two nozzles, larger droplet sizes are produced as a result of the narrower cone angle (31° vs. the 53° of the other two). The wider cone angle produces a thinner sheet of liquid which results in greater breakup and smaller droplets than the narrower cone angle.

While the mean Dv₀.₁, Dv₀.₅, and Dv₀.₉ values are statistically different between different nozzle and pressure combinations, based on the measure droplet size data presented, all nozzle/pressure combinations result in sprays that fall into the Very Fine classification as defined by ASAE S572 (ASAE Standards, 2004).

<table>
<thead>
<tr>
<th>System Pressure (kPa)</th>
<th>Mean Volume Diameter (μm)[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dv₀.₁</td>
</tr>
<tr>
<td>310.2</td>
<td>98.2 ± 5.3ᵃ</td>
</tr>
<tr>
<td>448.1</td>
<td>90.8 ± 3.1ᵇ</td>
</tr>
</tbody>
</table>

[a] Table values represent means±standard error of the mean. Means within a column with no common superscripts differ significantly (P ≤ 0.05) and were separated using Fisher’s LSD.

<table>
<thead>
<tr>
<th>Nozzle</th>
<th>Dv₀.₁</th>
<th>Dv₀.₅</th>
<th>Dv₀.₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>1553-10</td>
<td>93.7 - 0.9ᵇ</td>
<td>160.9 - 4.5ᵇ</td>
<td>228.2 - 12.9ᵇ</td>
</tr>
<tr>
<td>1553-08</td>
<td>88.2 - 3.9ᵇ</td>
<td>157.2 - 2.3ᵇ</td>
<td>228.1 - 8.8ᵇ</td>
</tr>
<tr>
<td>1531-06</td>
<td>101.5 - 4.9ᵇ</td>
<td>182.5 - 8.0ᵇ</td>
<td>259.5 - 15.2ᵃ</td>
</tr>
</tbody>
</table>

[a] Table values represent means±standard error of the mean. Means within a column with no common superscripts differ significantly (P ≤ 0.05) and were separated using Fisher’s LSD.
Cumulative volume distribution data shown in figures 3 and 4 indicate that a negligible volume of the spray consisted of respirable droplets (< 10 μm). The maximum proportion of respirable droplets was 0.35% recorded for the 1531-06 nozzle at 310.2 kPa and the remaining nozzle and pressure combinations yielded a maximum proportion of respirable droplets of 0.01% (table 4). No respirable droplets were recorded for the 1553-10 nozzle at 310.2 kPa. However, increasing system pressure would likely increase the proportion of respirable droplets generated.

**Table 4. Mean volume diameters of spray droplets for each combination of system pressure and nozzle type.**

<table>
<thead>
<tr>
<th>Nozzle</th>
<th>System Pressure (kPa)</th>
<th>Dv0.1 (μm)</th>
<th>Dv0.5 (μm)</th>
<th>Dv0.9 (μm)</th>
<th>Respirable Droplets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1553-10</td>
<td>310.2</td>
<td>94.3 ± 1.2b</td>
<td>167.7 ± 1.5bc</td>
<td>245.1 ± 1.1b</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>93.1 ± 0.4b</td>
<td>154.2 ± 1.6d</td>
<td>208.0 ± 1.1c</td>
<td>0</td>
</tr>
<tr>
<td>1553-08</td>
<td>310.2</td>
<td>91.6 ± 4.4b</td>
<td>155.1 ± 3.0d</td>
<td>225.8 ± 13.8bce</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>84.8 ± 2.6b</td>
<td>159.3 ± 0.2cde</td>
<td>230.4 ± 1.0bce</td>
<td>0.01</td>
</tr>
<tr>
<td>1531-06</td>
<td>310.2</td>
<td>109.5 ± 3.1a</td>
<td>192.9 ± 2.9a</td>
<td>280.6 ± 3.2a</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>448.1</td>
<td>94.4 ± 1.7b</td>
<td>170.3 ± 3.0b</td>
<td>236.0 ± 5.1bc</td>
<td>0.35</td>
</tr>
</tbody>
</table>

[a] Table values represent means ± standard error of the mean. Means within a column with no common superscripts differ significantly (P < 0.05) and were separated using Fisher’s LSD.

**Figure 3. Cumulative volume distribution for all nozzles at 310.2 kPa (40 psi). Respirable droplets (those less than 10 μm) are produced only in very small proportions.**

**Figure 4. Cumulative volume distribution for all nozzles at 448.1 kPa (60 psi). Respirable droplets (those less than 10 μm) are produced in larger numbers when compared to 310.2-kPa treatments, but the maximum observed was 0.35% for the 1531-06 nozzle.**

**EXPERIMENT 2**

Data from the water sensitive paper analysis are shown in table 5. The 1553-10 nozzle provided improved coverage and deposition when compared with the 1553-08 and 1531-06 nozzles. Mean flow rates for each nozzle and pressure combination were presented in table 1. The flow rate for the 1553-10 nozzle is much larger than the remaining pair of nozzles due to the larger nozzle orifice and the differences in coverage and deposition between nozzles are a direct result of the differences in flowrate.

Vaccine viability was unchanged for the pressure and nozzle treatments tested in this study, with the exception of the 1553-10 nozzle at 310.2 kPa, as seen in figure 5. This treatment yielded lower viability than the remaining treatments resulting from insufficient flush time prior to spraying. Statistical analysis showed no significant differences between the remaining treatments. However, the effects of pressure beyond those tested here on MG viability are unknown, and require further investigation. MacNaughton and MacDonald (1982) reported reduced growth at increased hydrostatic pressures for acholeplasmas, which are closely related to mycoplasmas. Mycoplasmas lack structural cell walls and the increased shear resulting from increased pressure may negatively affect viability and consequently lead to reductions in subsequent seroconversion.

**CONCLUSIONS**

The droplet size spectra observed for all nozzles showed few respirable droplets were produced by the nozzles at the system pressures tested. Mean VMD for all nozzle and pressure treatments, while statistically different, resulted in all nozzle and pressure combinations being classified as Very Fine sprays. Given the narrow range of droplet spectra.

**Table 5. Results of coverage and deposition of vaccine solution measured with water sensitive paper.**

<table>
<thead>
<tr>
<th>Pressure (kPa)</th>
<th>Nozzle</th>
<th>Coverage[a] (%)</th>
<th>Deposition[a] (μl·cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>310.2</td>
<td>1553-10</td>
<td>23.7 ± 2.2b</td>
<td>0.91 ± 0.07b</td>
</tr>
<tr>
<td>1553-08</td>
<td>6.0 ± 1.6c</td>
<td>0.24 ± 0.07c</td>
<td></td>
</tr>
<tr>
<td>1531-06</td>
<td>0.4 ± 0.1d</td>
<td>0.01 ± 0.00d</td>
<td></td>
</tr>
<tr>
<td>448.1</td>
<td>1553-10</td>
<td>28.7 ± 2.5a</td>
<td>1.07 ± 0.08a</td>
</tr>
<tr>
<td>1553-08</td>
<td>1.7 ± 0.2d</td>
<td>0.07 ± 0.01d</td>
<td></td>
</tr>
<tr>
<td>1531-06</td>
<td>0.5 ± 0.1d</td>
<td>0.02 ± 0.00d</td>
<td></td>
</tr>
</tbody>
</table>

[a] Table values represent mean ± standard error of the mean and were separated using Fisher’s LSD. Means with no common superscripts differ significantly (P ≤ 0.05).
Figure 5. Vaccine viability as measured during Experiment 2. The
reduction in viability for the 1553-10 nozzle at 310.2 kPa was due to
insufficient flushing of the spray lines prior to spraying that treatment.
There was no statistical difference between the remaining treatments.

observed, variation in seroconversion in the field between the
various pressure and nozzle combinations is likely a product
of differences in application rate and is supported by the
water sensitive paper results showing that both coverage
and deposition are much greater for the 1553-10 nozzle.
Increased coverage and deposition resulting from higher
flow rates as observed with the 1553-10 should further reduce
variations in vaccine application when compared to other
nozzles. Further, vaccine viability appears to be impacted
little, if any, by the nozzle types or system pressures tested in
this study.

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