A STUDY OF METHODS TO ENUMERATE THE MICROBIAL FLORA OF THE AVIAN EGG SHELL

K. W. B. Gunaratne and John V. Spencer
Department of Animal Sciences, Washington State University, Pullman, Washington 99163
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ABSTRACT

Experiments were conducted to compare results from three different methods used to enumerate the microbial flora of avian egg shells. The methods compared were: (a) swabbing the shell surface, (b) rinsing the shell surface, and (c) blending the entire shell and membranes after removal of the contents. Sanitized shells held at 25°C were inoculated with a 24-hr old culture of Pseudomonas fluorescens by immersion for 5 min at 25°C. In the swab method, the entire surface of the inoculated egg was swabbed by rolling the swab in either direction twice as each area was swabbed. For the rinse method, the inoculated egg was held using a metal holder and rinsed with 100 ml of 0.1% peptone solution. With the blending method, the entire emptied inoculated egg shell and shell membranes were blended using a Sorvall Omni-Mixer to which a presterilized Mason jar was attached. As expected, the blender method gave significantly higher counts (P<0.05) than either the swab or rinse methods. There was no significant difference (P<0.05) between counts obtained by the swab and rinse methods.

This study was undertaken to compare a newly devised rinsing method with that of the swabbing method used by Rizek et al. (4) and a modification of the blending method used by Board et al. (2) to enumerate the microbial flora of the avian egg shell.

MATERIALS AND METHODS

The experiments consisted of four replicates conducted during four different weeks. For each replicate large eggs were gathered from the Washington State University White Leghorn flock and those weighing 61 g were stored at 1.1°C for 24 hr before using for inoculation. The shell surface of a 61-g egg was calculated using the formula of Bosch et al. (1). Eggs were sanitized by immersing in 70% ethyl alcohol for 5 min. They were then aseptically transferred onto a sterile board which had pointed wire nail ends to support the eggs, and were allowed to air dry at room temperature (25°C). The eggs were then flame-sterilized in a 24-hr old culture of Pseudomonas fluorescens by immersion for 5 min at 25°C. These eggs were then transferred to the sterile board as described earlier and left to air dry at 25°C.

The inoculum was prepared in 100 ml of nutrient broth (Difco) and incubated at 25°C for 24 hr. It was then diluted with sterile distilled water until a transmittance reading of 44% was observed. The average number of organisms at this transmittance was 4.4 x 10^7 organisms/ml.

Swab method

The inoculated egg was aseptically held in position between the left thumb and index finger using sterile surgical gloves. A swab made as described by Mallman et al. (3) and moistened in 0.1% sterile peptone solution was used to swab the entire surface of the egg by rolling the swab in either direction twice for each area swabbed. The swab was then broken into 99 ml of 0.1% peptone solution. Serial dilutions were then made and plated using Standard Methods Agar (BBL). Plates were incubated at 25°C for 72 hr.

Rinse method

One inoculated egg was aseptically held small end downwards using a sterile metal holder. The holder was made from stainless steel wire 1.5 mm in diameter bent into a triangular shaped base with a handle extending upward from one corner of this triangle. For rinsing, the holder and egg were held over a sterile funnel leading into a sterile bottle. The egg was rinsed with 100 ml of sterile 0.1% peptone solution which was dispensed using a Brewer automatic pipetter, the whole delivery system of which had been previously sterilized. Serial dilutions were made and plated using Standard Methods Agar (BBL) and then incubated at 25°C for 72 hr.

Blending method

One hundred milliliter dilution blanks of 0.1% peptone solution were made in six regular one-pint Mason jars. A 0.1 N solution of sodium thiosulfate was prepared and 240 ml of this solution were placed in each of six one-pint Mason jars. Also, 240 ml of distilled water were placed in each of six one-pint Mason jars. All the above 18 Mason jars were autoclaved at 15 psi for 15 min. At the same time 240 ml solution containing 200 ppm available chlorine were transferred to each of six previously sterilized one-pint Mason jars which were free of any organic material.

A Sorvall Omni-Mixer with Mason jar adaptor was used for blending in this method. The rotor knife blade was sanitized before use and between counts in the following manner. Using sterile disposable tissue wipers any dust particles and any organic material on the knife blade assembly were physically removed. The Mason jar containing 200 ppm available chlorine was attached to the adaptor ring. The mixer was operated for 1 min at maximum speed. Next, the jar containing 0.1% sodium thiosulfate solution was attached to the mixer and the mixer operated for 1 min at maximum speed. This was repeated after attaching the Mason jar containing sterile distilled water.

Each egg was aseptically broken and the contents were removed. The shell and shell membranes were then placed in a one-pint Mason jar containing the sterilized 0.1% peptone solution. The jar was attached to the blender and the blender operated for 45 sec at maximum speed. The Mason jar was removed, its mouth flamed, and its lid replaced. Using sterile disposable tissue wipers all adhering shell membranes and
Table 1. Average* number of *Pseudomonas Fluorescens* organisms per square centimeter of avian egg shell per replicate

<table>
<thead>
<tr>
<th>Replicate number</th>
<th>Method</th>
<th>Swab</th>
<th>Rinse</th>
<th>Blender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(× 10^4)</td>
<td>5.9</td>
<td>8.2</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>7.7</td>
<td>6.8</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>31.0</td>
<td>23.0</td>
<td>190</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>10.0</td>
<td>11.0</td>
<td>170</td>
</tr>
</tbody>
</table>

*Average of five eggs.

shell were removed from the rotor knife blade which was then sanitized in the manner described above. A sample from the final rinse of distilled water was plated using Standard Methods Agar (BBL) to test for complete sanitization. The blended sample was serially diluted and plates were poured using Standard Methods Agar (BBL). Plates were incubated at 25°C for 72 hr.

**Results and Discussion**

Results obtained in this study (Table 1) show that the blender method gave higher counts than the rinse or swab methods. The results were statistically analysed using an analysis of variance. When Dunncan’s new multiple range test was applied, results showed a significant difference between the blender method and the other two methods at the 5% level, but there was no significant difference between the swab method and rinse method at the 5% level.

The blender method is useful to enumerate the microbial flora of the entire shell and membranes, while the swab and rinse methods are useful for making counts of the shell exterior only. Therefore, the marked difference between the results of the blender method and the other two methods can be attributed largely to the fact that the entire shell and shell membranes were macerated when using the blender method, thus giving a higher recovery of microorganisms. This seems reasonable, because by swabbing and rinsing, all organisms may not be removed from the surface of the egg shell. When swabbing, there is a possibility of error from rolling the swab in the same area or from not swabbing a particular area. In the rinse method microorganisms which may tend to adhere to the shell surface may not be removed. Also, the removal of organisms may depend on the force with which the egg is rinsed.

**References**


**AMENDMENT TO THE**

**3-A SANITARY STANDARDS FOR**

**INSTRUMENT FITTINGS AND CONNECTIONS**

**USED ON MILK AND MILK PRODUCTS EQUIPMENT**

**Serial #0906**

Formulated by

International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The “3-A Sanitary Standards for Instrument Fittings Used on Milk and Milk Products Equipment, Serial #0900” as amended are hereby further amended as indicated below.

The following is substituted for the paragraph on page 1 preceding the list of drawings:

The following is a list of drawings included in this standard. Dimensions and the contour of fittings and connections shown on the drawings are for reference only and may be changed if they do not affect cleanability. Instrument fittings and connections not illustrated in the drawings shall be considered as being included in this standard, provided they conform to the provisions of this standard with respect to material, finish, construction and use of gaskets and have no special requirements for construction or installation.

This amendment is effective April 22, 1973.