Commensal or pathogen bacteria could penetrate the pores of egg shell cause reducing the quality and safety of eggs. The good handling practice of eggs in household level should be introduced to consumer. The aim of this study was to investigate the impact of disinfection and storage in refrigerator against egg’s bacterial amount. Multivariate ANOVA was applied on the four groups of disinfection treatments (gas fumigation 2x strength, KMNO₄ solution 0.5%, acetic acid 1.25%, detergent solution 0.5%) and the control group (without cleaning up). All of groups were stored at refrigerator and bacteria was counted at 2 hours, 2 days, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after treatment. There was significant in difference on total bacterial amount between disinfection treatment and control groups at day 4th, 8th, 14th, and 18th after treatment. Total bacteria amount (TPC) in which under Indonesia National Standard (SNI) of TPC (10^6 cfu/ml) were produce by the treatment and control group having strong at 4 day and by the gas fumigates grew at 6th gas fumigation 2.39 X 10^4 CFU/ml, KMNO₄ solution 0.5% 2.09 X 10^4 CFU/ml, acetic acid 1.25% was 3.98 X 10^4 CFU/ml, detergent solution 0.5% was 3.02 X 10^5 CFU/ml; and a control group was 5.75 X 10^5 CFU/ml. Until day 6th, only gas fumigated group (8,91 X 10^5 CFU/ml) inhibited bacteria growth under SNI. The impact of disinfection treatment on egg shell will effectively inhibited bacteria growth up to 6 days in refrigerator storage.

Key words: egg, TPC, disinfection, refrigerator
Introduction

The egg is the poultry product which mostly consumes everyone because it's very cheap and easy to find. The high demanding of this commodity need guarantee of good and food-safety supply. When the egg laid in the nest, the shell contaminated by the environment, in litter system the bacteria amount reached $1.75 \times 10^9$ cfu/ml, nest system $6.96 \times 10^4$ cfu/ml, and in the slate system $3.87 \times 10^5$ cfu/ml. Coliform, *Escherichia coli*, *Staphylococcus*, and some other bacteria pathogen i.e. *Salmonella sp* could be isolated from egg shell (Sander *et al.*, 2003).

Usually chlorine 30-220 ppm were used to sanitized water for any purpose, in 100 ppm used to washing poultry, and 250 ppm to disinfected equipment (Gracey & Collins, 1992). Ockerman *et al.* (2001) showed that chlorine could not killed *Staphylococcus* effectively, but lactic acid 1 % inhibited *Staphylococcus* and *Salmonella*. Some sanitizers eventually have been using for cleaning up egg-shell ex. quaternary ammonium, phenol solution, iod, and formaldehyde. All of those compounds known as a toxic material and will left the residual in yolk (Leeson and Summers, 2000).

Egg consumers in household level need more information how to keep the eggs correctly and safe for their health. If they should clean up the eggs before storing, The aim of this study was to investigate the impact of disinfection and storage in the refrigerator against egg’s bacterial amount.

Method

One hundred sixty five fresh eggs were taken from layer farm and divided into five groups. Four groups were treated using disinfectant (gas fumigation 2x strength, KMNO4 solution 0.5%, acetic acid 1.25%, detergent solution 0.5%) and the control group without clean up. After disinfected, all groups were stored in the refrigerator ($4^\circ$C) then total bacterial were counted at 2 hours, 2 days, 4 days, 6 days, 8 days, 10 days, 12 days, 14 days, 16, days, 18 days, and 20 days after storing. Three samples (mixed of yolk and albumin) of each treatment unit were taken in every observation period. All samples were serial diluted in sterile ringer solution (Oxoid) and plating with Plate Count Agar (Oxoid) then incubated at $37^\circ$C for 24 hours.

Results and Discussion

There were significant differences on total bacterial counted between disinfection treatment and control groups at day $4^{th}$, $8^{th}$, $14^{th}$, and $18^{th}$ after treatment. Total bacteria amount of all groups under Indonesia National Standard (SNI) of TPC ($10^6$ cfu/ml) only first 4 days storage, the average bacterial amount of each group: gas fumigation $2.39 \times 10^4$ CFU/ml, KMNO4 solution $0.5\%$ $2.09 \times 10^4$ CFU/ml, acetic acid $1.25\%$ was $3.98 \times 10^4$ CFU/ml, detergent solution $0.5\%$ was $3.02 \times 10^5$ CFU/ml; and a control group was $5.75 \times 10^5$ CFU/ml. Until day $6^{th}$, only gas fumigated group ($8.91 \times 10^5$ CFU/ml) inhibited bacteria growth under SNI (Table 1).

Bacteria in egg comes from transovary and egg shell contamination. When contaminated egg lay down from hen's body, indicated that chicken suffered a certain disease. Egg shell was contaminated bacteria whenever contact with the environment. Sander *et al.* (2003) reported, where an egg is laid has significant influence on the degree of surface bacterial contamination. Bacterial counts on shell surfaces from the eggs of broiler breeder hens housed in partial slat pens revealed that the eggs laid in litter material ($1.75 \times 109$ cfu/mL) were significantly dirtier than eggs laid in the nest ($6.96 \times 104$ cfu/mL) or on the slats ($3.87 \times 105$ cfu/mL). The type of raised slat used in broiler breeder houses influenced the degree of bacterial contamination on the slats over the life of the flock. Wood and plastic slats harbored more bacteria than polyvinyl chloride (PVC) coated wire slats before and after the slats were washed and disinfected.
But wood and plastic slat materials also had a greater surface area. There was no difference in effectiveness of bacterial reduction when a quaternary ammonium compound ($4.37 \times 10^7$ cfu/mL) or a phenol compound ($6.43 \times 10^6$ cfu/mL) was used to disinfect the slats. Eggs laid on slats with square openings, regardless of surface area, were significantly cleaner than eggs laid on wooden slats.

Table 1 Average of bacterial counted of eggs content in every observation period (log/ml)

<table>
<thead>
<tr>
<th>Time</th>
<th>2 hours</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>10 days</th>
<th>12 days</th>
<th>14 days</th>
<th>16 days</th>
<th>18 days</th>
<th>20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanitizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas</td>
<td>3.2</td>
<td>3.9</td>
<td>4.4</td>
<td>5.9</td>
<td>12.7</td>
<td>16.9</td>
<td>15.5</td>
<td>15.8</td>
<td>7.8</td>
<td>11.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Fumigation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KMnO4</td>
<td>3.6</td>
<td>3.9</td>
<td>4.3</td>
<td>9.5</td>
<td>13.4</td>
<td>10.8</td>
<td>15.6</td>
<td>14.2</td>
<td>3.7</td>
<td>11.5</td>
<td>6.7</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>3.9</td>
<td>3.4</td>
<td>4.6</td>
<td>9.6</td>
<td>13.4</td>
<td>17.8</td>
<td>15.1</td>
<td>15.5</td>
<td>7.6</td>
<td>11.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Detergent sol</td>
<td>3.0</td>
<td>3.3</td>
<td>4.5</td>
<td>8.3</td>
<td>13.8</td>
<td>17.4</td>
<td>15.5</td>
<td>15.2</td>
<td>7.1</td>
<td>11.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Control</td>
<td>3.9</td>
<td>2.3</td>
<td>5.8</td>
<td>6.3</td>
<td>12.8</td>
<td>5.6</td>
<td>15.4</td>
<td>15.5</td>
<td>6.6</td>
<td>11.7</td>
<td>10.7</td>
</tr>
</tbody>
</table>

NB: the same superscript in the same column showed the significant deference between groups ($P<0.05$)

Eggs from conventional litter floor environments are typically more contaminated than those from cage systems. One study found the total number of bacteria on eggs from deep litter production systems to be 15 times greater than that found on eggs from battery production systems. It was noted that bacteria from the litter were transferred to the nest linings on the feet and the feathers of laying birds and that the bulk of the bacteria on deep litter eggs consisted of types found in the litter itself. It was also concluded that cycling of salmonellae between litter and the intestinal tract of birds appeared to be significant in maintaining intestinal infection. Litter floor systems also have a risk of contamination of the litter by rodents. The point can be made that poultry and rodents cannot exist so closely in cage systems and therefore the potential for cross-infection with salmonellae may not be as great in cage systems as in floor or free-range systems (Dawson et al. 2001).

The ability of spoilage bacteria to penetrate the egg shell pore could be happened in 5 minutes (Sauter and Peterson cited ICMSF, 2005). According to this statement, if the surface of egg shell were contaminated microorganism more than $10^7$ cfu/mm², then bacteria through the egg-shell then contaminated albumin and yolk. The high nutrition of egg content will be supported bacteria multiplication and understandable in a few hours we found so many bacteria in it as good as this study result. Even though disinfection method and refrigerate stored had been done in household level to handling egg but the issue of eggs distribution was more than 5 minutes so there were a moment for bacteria to penetrate and multiply in the cell. Storage egg in refrigerator (4°C) for 6 days could resist bacterial growth in 6 days effectively (picture 1) because after 6 days the bacteria amount were raised up more than $10^6$ cfu/ml the number of maximum TPC in egg (SNI 2000). This result parallel with Steele et al. 1965 (cited ICMSF, 2005) who reported that bacteria could grew eventhough stored at 4°C for 8-10 days (3.10^6 cfu/ml (ICMSF, 2005)). According to this study the critical stage of stored of egg in refrigerator not more than a week (black arrow in figure 1). Disinfection treatment in poultry farm could reduced bacterial contamination from environment/equipment on egg-shell surface and resisted of bacterial growth as long distribution phase.

Figure 1 Total plate count of bacteria in egg which stored in refrigerator (log 10/ml).
Conclusion

This study showed, in household level, cleaned-up egg-shell by disinfectants was not effectively inhibited bacterial growth eventhough the eggs were stored in refrigerator. Consumers were recommended keep eggs in refrigerator not more then a week.

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