Penetration Pattern of *Salmonella* Typhimurium in Artificially Inoculated Desi and White Shelled Eggs at Ambient Temperature

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**ABSTRACT**

An attempt was made to study the penetration pattern of *Salmonella* Typhimurium in desi and white shelled eggs by artificial inoculation at ambient temperature (30±2°C). A total of 120 shelled eggs comprising of 60 white shelled eggs and 60 desi eggs were distributed into 5 lots containing 12 eggs each. Out of these, 10 eggs were kept for surface inoculation with *S*. Typhimurium and 2 eggs as control and analyzed at 2, 24, 48, 72 and 96 h after artificial inoculation. The shell surface was decontaminated with 65 ppm of polyhexamethylene guanidine (PHMG) before analysis. The penetration of *S*. Typhimurium in desi eggs was found to be within 2 h with an average count of 2.17±0.79 log cfu/ml, whereas in white shelled eggs, the penetration was after 24 h with an average bacterial count of 0.71±0.63 log cfu/ml. The penetration percentage and *S*. Typhimurium count inside both shelled eggs further increased from 24 h to 96 h. After 96 h, all eggs had 100% penetration with an average count of 4.72±0.01 and 4.56±0.05 log cfu/ml for desi and white shelled eggs, respectively. It can be concluded that both desi and white shelled eggs allowed penetration of *S*. Typhimurium with slight variation in percentage and duration for penetration at ambient temperature.

**Keywords**: Artificial inoculation, desi eggs, eggshell, penetration, *Salmonella* Typhimurium, white shelled eggs

Egg is a nutrient-dense food, containing high quality protein with highest biological value as it contains all the essential amino acids and a wide range of essential vitamins, minerals and trace elements which helps to maintain normal body functions of human beings. It is easily digestible by people of all age groups (FSA, 2002). But it can also transmit important foodborne zoonotic diseases if the hygienic managemental practices are not followed in food supply chain. Salmonellosis is one such important foodborne disease throughout the world for which raw shell eggs act as an important source (OIE, 2002). Shell eggs may be contaminated externally with *Salmonella* spp. mainly through droppings of infected layers, litter, water, egg trays, etc. (Stadelman and Cotterill, 2002). Desi and white shelled eggs are produced and marketed under different management systems and vary in their external qualities (Parmar et al., 2006).

Several workers have studied the factors responsible for the bacterial penetration through the eggshell. Cox *et al.* (1999) observed that the most likely area on the shell to be penetrated was the air cell end. Miyamoto *et al.* (1998) found that *S*. Typhimurium readily penetrated through the shell of freshly laid eggs, but this penetration was suppressed by cooling the eggs at 4°C for 15 min. Hence, an experiment was conducted to study the penetration pattern of *S*. Typhimurium inside desi eggs and white shelled eggs at ambient temperature (30±2°C).
Good quality fresh shell eggs free from external stains were purchased from the market and candled in the laboratory to eliminate poor shelled and cracked eggs. All the eggs were disinfected by dipping in 70% alcohol solution for 15 min. A total of 120 eggs comprising of 60 white shelled eggs and 60 desi eggs were distributed into 5 lots each containing 10 eggs for surface contamination and 2 eggs as controls. The distribution of eggs for artificial inoculation and subsequent analysis at different time intervals are shown in Table 1.

The surface inoculation or challenge studies in raw shell eggs were carried out as per United States Food and Drug Administration (USFDA, 2009) protocol with slight modifications as described by Madhavaprasad (2009). The shell surfaces were inoculated by dipping the eggs for 10 min in (1:10 dilution) normal saline solution containing 24 h peptone water culture of *S. Typhimurium* ATCC 51812 having concentration of 5.78 log cfu/ml for 10 min and then air dried and placed in sterile polythene bags individually and sealed. The lots were analyzed at different time intervals viz., 2, 24, 48, 72 and 96 h for the recovery of *S. Typhimurium*. The shell surface was decontaminated by spraying 65 ppm of polyhexamethylene guanidine (PHMG) for 10 min and air dried (Madhavaprasad, 2009). Then egg shells were broken aseptically and homogenized. Internal contents were used for the recovery of *S. Typhimurium* using buffered peptone water (BPW) for pre enrichment, Rappaport Vassiliadis broth for selective enrichment and xylose lysine deoxycholate (XLD) agar for plating as per USDA standard isolation procedures (USFDA, 2011). The colonies obtained on XLD agar were enumerated. Shell thickness was measured with the help of disc micrometer after breaking the eggshell.

It was observed that penetration of *S. Typhimurium* was seen early inside desi eggs, when compared with white shelled eggs. Sixty per cent of desi eggs showed penetration after 2 h of surface inoculation, whereas white shelled eggs showed penetration after 24 h (and an average count of 0.71±0.63 log cfu/ml in the internal contents). After 48 h, 80% desi eggs and 40% white shelled eggs revealed penetration of *Salmonella*. Hundred per cent penetration of white shelled eggs was observed after 96 h incubation. All the controls were negative.

This indicated that *S. Typhimurium* penetrated in all the desi eggshells on 3rd day, while the white shelled eggs had 100% penetration on 4th day at ambient temperature. The results were in accordance with the study conducted by Schoeni *et al.* (1995) who observed that at 25°C, *S. Typhimurium* penetrated the shell in 3 days and increased in internal contents by 3 logs or more in number.

The average shell thickness of white shelled eggs was 0.41±0.01 mm and that of desi eggs was 0.42±0.05 mm. De Reu *et al.* (2006) found that the

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Time intervals (hours)</th>
<th>White shelled eggs inoculated eggs</th>
<th>White shelled eggs controls</th>
<th>Desi eggs inoculated eggs</th>
<th>Desi eggs controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>24</td>
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<td>2.</td>
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<td>10</td>
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<td>2</td>
<td>2</td>
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<tr>
<td>3.</td>
<td>48</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>4.</td>
<td>72</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>24</td>
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<tr>
<td>5.</td>
<td>96</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>50</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>120</td>
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</table>
eggshell characteristics such as surface area of the eggshell, shell thickness and porosity of eggshell did not influence the bacterial penetration, but the mean cuticle deposition was lower for penetrated as compared to non-penetrated eggshells. Hence, there is scope for evaluating the cuticle deposition on desi eggshells.

From this study, it can be concluded that desi and white shelled eggs allowed penetration of S. Typhimurium with slight variation in penetration per cent and duration for penetration at ambient temperature. Hence, both the type of shell eggs is easily contaminated internally with Salmonella spp. at ambient temperature by external shell surface contamination. Desi eggs were more prone to internal penetration as compared to white shelled eggs. Further, storage at ambient temperature allowed the growth and multiplication of S. Typhimurium in the internal contents of shell eggs. Hence immediate collection, sanitation and refrigeration of raw shell eggs is advised which will limit the contamination, growth and multiplication of Salmonella spp. in the raw shell eggs.

REFERENCES


