

Determination of the Optimum Time for Preparation of Half-Boiled Eggs Free from *Salmonella Enterica* Serovar Enteritidis

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Received

14th November 2016

Received in revised form

10th April 2018

Accepted

16th April 2018

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ABSTRACT

Introduction: Consuming half-boiled eggs during breakfast is popular among Malaysians. The eggs are usually prepared by submerging them in freshly boiled water for about 20 minutes duration. As chickens have the likelihood of harbouring *Salmonella enterica* Serovar Enteritidis in their eggs, it is feared that consumption of half-boiled eggs contaminated with *S. Enteritidis* could lead to gastroenteritis. Hence, this study was carried out to determine the optimum time required for boiled water to kill *S. Enteritidis* in half-boiled eggs. **Methods:** Grade C chicken eggs (average weight 54.5 g) were obtained from a local sundry shop and were confirmed to be *Salmonella*-free prior to the experiment. Fifteen eggs that were previously spiked with 10⁶ colony-forming units (CFU) of *S. Enteritidis* were completely submerged into freshly-boiled water (≈100°C) for a maximum of 20 min. Positive controls consisted of eggs that were inoculated but were not treated. At every 5 min interval, three eggs were taken out and checked for presence of viable *S. Enteritidis* on MacConkey agar, and the respective water temperature was recorded. **Results:** Results of two independent trials showed that after 10 min of submerging the eggs in the hot water, there were an average of 3 log reductions in the CFU of *S. Enteritidis* but after 15 min, no viable *S. Enteritidis* was detected. **Conclusion:** A half-boiled egg should be prepared by submerging it in freshly boiled water for at least 15 min to ensure that it is safe to be consumed.

KEYWORDS: *S. Enteritidis*, half-boiled egg, optimum time

INTRODUCTION

Salmonella enterica serotype Enteritidis is a rod-shaped, enteric Gram-negative bacterium capable of causing gastroenteritis in humans with signs and symptoms of watery diarrhoea, vomiting, abdominal cramps and fever which could last for two to five days. Some individuals infected with *S. Enteritidis* have mild symptoms or remain asymptomatic, however, others with particular predisposition as the children and immunocompromised patients, the infection with this pathogen can become invasive. The reported mortality rate with *S. Enteritidis* infection was 3.6%, of which most patients were elderly [1].

There are an estimated 1 million cases of foodborne illness in human caused by *S. Enteritidis* in the United States with >350 death annually. However, in Malaysia, 30% of *Salmonella* cases, last reported in 1995, were due to *S. Enteritidis* [2]. A retrospective study on *Salmonella* cases from 1991 to 2001 in University Malaya Medical Centre and Kota Bharu

Hospital involving children also revealed *S. Enteritidis* to be the most common serotype isolated [3].

Human acquire infection with *S. Enteritidis* by consumption of contaminated food, especially poultry eggs, because the bacteria can be found in the gastrointestinal tract of healthy chickens. Eggs laid by infected hens contain *S. Enteritidis* and through consumption of raw or uncooked eggs and unhygienic food handling can lead to *S. Enteritidis* infection. In the United States, a surveillance data revealed that most of the *S. Enteritidis* infections were from domestic sources, of which chicken meat and eggs are the major source [4].

Like in other countries, many Malaysians take half-boiled eggs in their daily breakfast. Half-boiled eggs are usually submerged in freshly boiled water and left for approximately 10 to 15 min to 'cook'. The clear albumen usually thickens while the yolk still remains runny when left in the hot water for this duration. Because of the simplicity, convenience and

the short-time required in preparing half-boiled eggs, they are very popular dish for breakfast. However, if the eggs are contaminated with *S. Enteritidis*, consumption of half-boiled eggs poses health risk. It was reported that a five-minute duration of boiling eggs inoculated with 10^4 CFU/mL *Salmonella enteritica* serovar Typhimurium was sufficient to completely eliminate the organism [5]. To our knowledge, there has not been any published guideline for the preparation of half-boiled eggs that is guaranteed safe for consumption of eggs free from pathogens such as *S. Enteritidis*.

Therefore, the aim of this study was to determine the optimum time that is required to kill *S. Enteritidis* in eggs during the preparation of half-boiled eggs. The information gathered from this work would provide scientific evidence on the optimum time for preparation of half-boiled eggs that are safe for consumption and thus, will help to reduce incidence of gastroenteritis due to *S. Enteritidis* infection.

METHODS

Bacterial Strain and Preparation of Inoculum

Briefly, stock culture of *S. Enteritidis* ATCC strain 13076 was subcultured onto MacConkey agar (Oxoid, UK) and incubated overnight at 37°C. A suspension containing 10^7 CFU/ml of the bacterium was freshly prepared in phosphate buffer saline (PBS, pH 7.2; Oxoid, UK) and used as inoculum.

Specimen and inoculation of *S. Enteritidis* into eggs

A total of 30 Grade C eggs (Nutriplus^R), with average weight of 54.5 g were purchased from a local sundry shop and used before the expiration date. Prior to the experiment, three eggs were sampled for the presence of *S. Enteritidis* and were confirmed to be free from any bacteria by culture method. Each of the remaining eggs was candled to locate the air sac and marked for inoculation site. The shell of the eggs was cleaned with tap water and air dried. The eggs were firstly swabbed with 70% ethanol in order to remove any microorganisms present on the shell. A tiny hole was then pierced at the marked area on the shell using a sterilised pin and finally inoculated with 0.1 ml of the

bacterial suspension through the hole into the yolk by using a 1 ml sterile syringe and 24 mm, 27-gauge needle. The hole was then sealed using superglue. In each trial, a total of 15 eggs were inoculated with *S. Enteritidis*

Preparation of Half-Boiled Eggs

Of the 15 inoculated eggs, 12 eggs were gently placed into a 1 L glass beaker and were subsequently added with freshly boiled ($\approx 100^\circ\text{C}$) distilled water until all the eggs were completely submerged. Three eggs which were not treated served as positive controls. The top of the beaker was covered and the eggs were placed in the hot water for 20 min. At every 5 min interval and including at zero time, the temperature of the water was measured and three eggs were taken out to determine the number of viable *S. Enteritidis* in each egg.

Counting of *S. Enteritidis* in eggs

The shell of each egg was cracked using a sterile glass rod and the content of the egg was poured into a sterile container and was gently stirred by using another sterile rod. Ten-fold serial dilution was performed by adding 0.1 ml of egg content to 0.9 ml of PBS. At each dilution, 0.1 ml of the suspension was homogeneously spread onto MacConkey agar for viable count. Following a 24 h of incubation at 37°C, the number of colonies was counted by using a colony counter.

RESULTS

It was observed that at 0 time, the initial average number of viable colonies in each egg was 10^6 CFU (positive controls), but after placing in the hot water for 10 min, the number of viable colonies was reduced to 6×10^2 CFU (Figure 1). After the eggs were treated for 15 min, there was no viable bacterium detected in any of the eggs. On average, there were 3.2 log reductions of viable *S. Enteritidis* after 10 min of treatment but after 15 min, all the inoculated *S. Enteritidis* were killed. The temperature of the boiling water which was initially 100°C , dropped rapidly to 83°C when it was added to the eggs and continued to drop to 63°C after 10 min, after which it remained constant (approx. 60°C) for another 10 min.

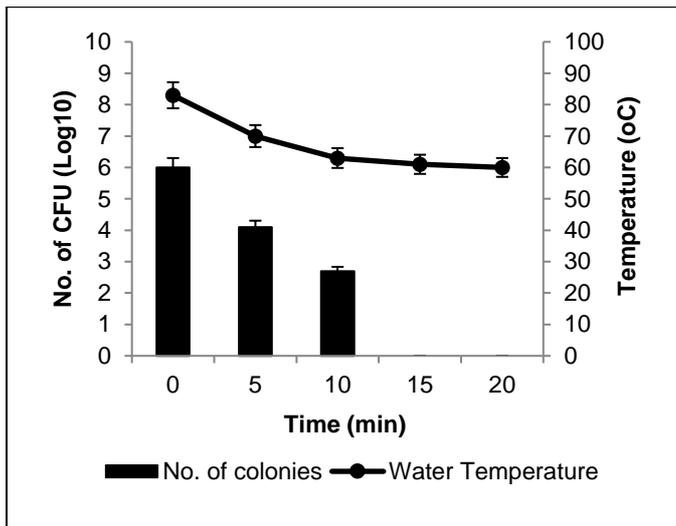


Figure 1 Average number of viable *Salmonella enterica* serovar Enteritidis in chicken eggs after exposure to freshly boiled water. Each error bar represents \pm standard error means.

DISCUSSION

In this study, 10^6 CFU of *S. Enteritidis* were used to simulate the infective dose that can cause gastroenteritis in healthy person. However, in normal condition, the number of infective dose depends on the immune status of the host. In an immunocompromised person, lower infective dose would exacerbate the condition [6].

The result obtained in this study showed that *S. Enteritidis* is heat labile and could be killed by using freshly boiled-water, crucially for appropriate duration of treatment. It is important that the treatment should be for at least 15 min to ensure that all the organisms are killed.

Fifteen minutes of hot water treatment was the optimum duration to ensure that no *S. Enteritidis* survived because from our observation, when the eggs were treated for 10 min, 50% of the bacterial population was still viable. When the boiling water was added to the eggs, the shell and the content of the eggs immediately absorbed some of the heat, thus not all of the organisms were instantly killed. Because of the rapid drop in the temperature, it took a longer time to kill all of the organisms. Nevertheless, at 70°C , the heat was high enough to kill the remaining organisms because *S. Enteritidis* is sensitive to heat. This finding supports an earlier report that temperature of $62.3 \pm 2^{\circ}\text{C}$ is effective in killing *Salmonella* spp. in yolk [7]. In another study, it was revealed that *S. Typhimurium* in eggs required 7 min of boiling for its complete

elimination, whereas *Staphylococcus aureus* required 12 min of boiling [8].

CONCLUSION

The results presented here therefore, suggest that half-boiled eggs need to be placed in freshly boiled-water for at least 15 min to ensure they are free from the bacteria.

Conflict of Interest

Authors declare none.

Acknowledgements

The authors thank the Director of the Institute of Medical Molecular Biotechnology (IMMB) for the permission and the use of the facilities, and the staff of the Microbiology Unit of the Centre for Pathology Diagnostic and Research Laboratory, UiTM for their technical support advices.

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