INFLUENCE OF EGG SHELL TRANSLUCENCY ON EGG SHELL PENETRATION BY BACTERIA

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Summary

Egg shell translucency, which can be due to changes in the mammillary cores and mammillary layer during the early phases of eggshell formation, has the potential to increase the incidence of microcracks in egg shells, and hence, may facilitate bacterial penetration. There was a significant correlation between egg shell translucency and egg shell penetration by \textit{Salmonella} Infantis. \textit{Salmonella} Infantis was able to penetrate translucent egg shells even at very low doses. The penetration, however, appeared to be hindered in both translucent and non-translucent eggs at 4°C, as compared with room temperature which highlights the importance of storage of eggs at refrigeration temperatures.

I. INTRODUCTION

Eggs produced in Australia, most of which are from cage laying systems, are considered medium to low risk food for food borne illness. The medium risk ranking is mainly because of pathogens like \textit{Salmonella} and some other enteropathogens. The egg industry in Australia is periodically implicated in cases of food poisoning and the egg’s contents are an ideal growth medium for microorganisms which are hazardous to humans. It has been observed that the microflora of the eggshell are dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defences of the egg contents (De Reu \textit{et al.}, 2008). The Australian poultry industry is considered to be free from \textit{Salmonella} Enteritidis which is of major concern for the food industry all over the world, with Cox \textit{et al.} (2002) reporting that \textit{Salmonella} Infantis (S. Infantis) was the predominant \textit{Salmonella} serovar in the Australian egg industry. It is difficult for bacteria to move across an intact good quality egg shell. However, small defects in the egg shell may provide means for the predominant bacterial spp. on the egg shell to penetrate and move into the egg contents (De Reu, \textit{et al.}, 2006). Translucency is possibly caused by irregular mammillary knobs probably due to the fusion of several mammillary cores during the early phases of eggshell formation (Bain \textit{et al.}, 2006) leaving larger spaces among the mammillary cones. It is not clear whether translucency has any role in potentiating the entry of bacteria into the egg contents and the risk that such eggs pose to product safety is uncertain. In the present study, unwashed eggs collected directly from the cage front were scored for egg shell abnormalities such as translucency. The ability of S. Infantis to penetrate translucent and non-translucent egg shells at different storage temperatures was also investigated.

II. MATERIALS AND METHODS

a) Scanning electron microscopy for studying egg shell translucency. For studying the ultrastructure of translucent egg shells, eggs were candled and areas of severe translucency were selected and marked with a pencil. Egg shell pieces of approximately 1cm\textsuperscript{2} were cut from two non-translucent and four severely translucent eggs.
After soaking the eggs shells in water, the egg shell inner membranes were removed manually. The outer membrane was removed from the dried shell by plasma ashing, using a Bio Rad RF plasma Barrel Etcher PT 7150, as described by Brackpool (1995).

The mammillary region of the egg shell was examined for ultrastructural characteristics as described by Brackpool (1995). The samples were mounted on aluminium stubs and gold-coated (Polaron E5100). Samples were then observed under a scanning electron microscope (Neoscope, Coherent Scientific, Australia). Observations regarding changes in mammillary cap arrangements, size, early fusion, late fusion depression, erosions, type A and B bodies were made and recorded as described by Solomon (1991).

b) Agar method for assessment of the egg shell penetration

An agar method described earlier by De Reu et al. (2006) was adapted to study bacterial penetration across the egg shell. Briefly, 90 fresh eggs were obtained from cage fronts of 40-week old commercial Isa Brown laying hens. Eggs were divided into three groups (n=30) in a 3 x 3 factorial arrangement. Internal contents of eggs obtained from this flock were tested for Salmonella. All eggs were candled and translucency was scored as 0 (no translucency), 1 (mild translucency), 2 (moderate translucency) and 3 (severe translucency). Each egg was dipped into 70 % ethanol for 1 min to kill any bacteria on the outside of the shell and was allowed to air dry in a biosafety cabinet. After making a hole in the egg shell, the egg contents were removed using a sterile syringe. Eggs were then washed with sterile phosphate buffered saline (PBS; pH 7.2) to remove all the albumen adhering to the membrane. Each egg was then filled with McConkeys agar. Eggs were sealed with paraffin after the agar solidified. An S. Infantis strain obtained from IVMS, Adelaide, Australia was used in this experiment. Bacteria stored at -80 C in 50% glycerol were plated onto horse blood agar and incubated overnight at 37°C. A single colony was then inoculated and grown overnight at 37°C in brain heart infusion broth (BHI; Oxoid, Australia). The broth was then diluted in PBS to a 10^-6 dilution. Enumeration of viable bacteria was performed by serial dilution, plating 100 μl of each solution onto McConkeys agar plates followed by incubation overnight at 37°C. Ten agar filled eggs were immersed for 1 minute in one of three serial dilutions for Salmonella Infantis in approximately 10^7, 10^5 and 10^3 colony forming units (CFU) per ml of PBS. After inoculation, agar filled eggs were kept at 4°C, 20°C and at 37°C. The eggs were aseptically opened in a biosafety cabinet after 72 hrs to inspect for growth of typical visible colonies. Colonies seen nearby the hole in the shell were not recorded as penetration. The shell membranes and agar with colony growth were reinoculated onto triple sugar iron (TSI) agar slopes and incubated at 37°C for confirmation. A one way analysis of variance (ANOVA) (Statview® version 5.0.1 for windows, SAS Institute Inc. Copyright © 1992- 1998) was used to study the effect of egg shell translucency on percentage of egg shells penetrated.

III. RESULTS

a) Scanning electron microscopy for studying egg shell translucency

The ultrastructural appearance of the egg shells from the non translucent egg shells were in agreement with Brackpool (1995) and showed no detrimental changes in the mammillary caps. In the translucent egg shells, mammillary caps were of good quality with extensive attachment with the shell membrane. However, there was alignment of the mammillae, where the mammillae appear to “line up” resulting in long continuous grooves between the cones (Roberts and Brackpool, 1994). Such alignment was not observed in non-translucent egg shells. There was little cuffing (additional calcium around the mammillary cones that appears to contribute to shell strength) in the translucent egg shells and late fusion of the mammillary layer was not recorded. Depression and erosion of mammillae were also observed in the
translucent egg shells. Type B bodies, which are small spherical calcified bodies which have variable contact with membrane fibres, were also found in the translucent egg shells.

b) Effects of egg shell translucency on S. Infantis penetration
At 37°C, 70% of eggs inoculated in $10^7$ cfu were penetrated by S. Infantis and 40% of eggs inoculated in $10^5$ and $10^3$ cfu/ml, were penetrated by S. Infantis. 70% of eggs, which were inoculated at the dose rate of $10^7$ cfu and incubated at 20 °C, were positive for bacterial penetration. 40% and 30% of eggs inoculated in $10^5$ and $10^3$ cfu/ml, respectively, and incubated at 20°C were found positive for bacterial penetration.

Bacterial penetration was recorded in 20% of eggs inoculated with $10^7$ cfu and incubated at 4°C. S. Infantis penetration was not recorded in any of the egg shells from the remainder of the treatments at 4°C. For the eggs incubated at 37°C, there were significant differences between egg shell transluencies of penetrated and non-penetrated egg shells inoculated in $10^5$ and $10^3$ cfu/ml (Fig. 1). Bacterial penetration was recorded along the translucent patches of the egg shell. For the eggs incubated at 20°C, there were significant differences between egg shell transluencies of penetrated and non-penetrated egg shells inoculated in $10^7$ and $10^3$ cfu/ml (Fig. 2).

Figure 1 Penetration of S. Infantis across translucent and non translucent egg shells at 37°C.

Figure 2 Penetration of S. Infantis across translucent and non translucent egg shells at 20°C

IV. DISCUSSION

Egg shell quality can be influenced by various factors like age, strain of hen, temperature and disease (Roberts, 2004). Translucency ultimately has the potential to increase the incidence of microcracks in egg shells (Bain et al., 2006). In the present study, translucent egg shells had good quality mammillary caps with extensive attachment with the shell membrane; however
there was alignment of the mammillae resulting in long continuous grooves between the cones which are thought to lower the resistance of egg shells to bacterial penetration (Solomon, 1991). Cuffing is thought to be responsible for increasing bacterial resistance to penetration and, in this study, there was poor cuffing in the translucent egg shells. Pitting can reduce the shell’s resistance to bacterial penetration (Nascimento et al., 1992). Type B bodies were found in the translucent egg shells. Type B bodies are normally present in avian egg shells, although a large number of type B bodies can disrupt the mammillary layer of the egg shell thereby potentiating the entry of bacteria (Nascimento et al., 1992). In the present study, at 4°C, only two egg shells were penetrated at high dose rates of the bacterial inoculums. The current finding highlights the importance of storing eggs appropriately throughout the supply chain. Board and Tranter (1995) reported that the extent of contamination of hatching eggs was in the range from $10^2$ to $10^7$ CFU per eggshell. In the current experiment, although the dose of bacterial inoculation was very high, it was within the normal contamination range described in earlier studies by Board and Tranter (1995). However, during the present study, eggs were washed in 70% ethanol prior to external inoculation which may have damaged the cuticle, reducing its protective properties. There is still the possibility that pathological lesions in egg shells like cuffing, type B bodies and depressions, seen in the translucent egg shells, can potentiate the entry of bacteria across the egg shell. Translucent egg shells can increase the likelihood of internal contamination of eggs. In the present study, however, the bacterial contamination of the egg shell was not quantified. It is possible that small, slow-growing colonies of S. Infantis were not detected in the present study and further research is needed in this area.

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