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Eggshell penetration by *Salmonella* Typhimurium in table eggs: Examination of underlying eggshell structures by micro-computed tomography and scanning electron microscopy

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ABSTRACT

Horizontal infection of table eggs by food borne, human infection causative agents such as *Salmonella* is a serious concern for consumers and industry. In this study, we investigated the relationship between eggshell translucency, mammillary layer abnormalities and pore structure using Computed Tomography (CT) and scanning electron microscopy. The effects of eggshell pore structure, size and number on *Salmonella* Typhimurium penetration was also investigated. The eggs were infected with *S.* Typhimurium and were incubated at 37 °C for 3 or 6 days. Micro CT results comparing shell features to shell translucency found that there was a significantly increased incidence of externally branching pores found in the high translucency score eggshell group, and more straight pores found in the low translucency score group. Different pore structures, the total number of pores and the shell thickness do not appear to play a role in the horizontal infection of eggs by the *S.* Typhimurium strain used in this study. While it is likely that the presence of shell pores is responsible for shell penetration, other unknown shell factors must also play a role, and eggshells with a higher incidence of shell pores are not penetrated at a higher rate.
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1. Introduction

Table eggs are an important part of the human diet (Lublin & Sela, 2008); they are a cheap, easily available (Aboonajmi et al., 2010) and generally considered a safe foodstuff. Eggs are, however, periodically implicated in cases and outbreaks of food borne illness in Australia and around the world (OzFoodNet Working Group, 2010). Eggs in Australia are considered to constitute a medium to low risk of food borne illness (Food Standards Australia New Zealand, 2009). The eggshell is a significant physical barrier to penetrating microorganisms, providing both contamination protection and a suitable container for the egg contents. There are two pathways of microbial contamination of the egg, vertical and horizontal. Vertical (trans-ovarian) contamination occurs when the eggs are infected during their formation, either in the ovary or oviduct. Horizontal transmission occurs after lay when the egg is exposed to bacteria and the bacteria enter through the shell (Gantois et al., 2009). Eggs may become infected with *Salmonella* either as a result of infection of the oviduct or by faecal contamination (De Reu et al., 2008). In Australia, where *Salmonella* Enteritidis is not endemic in commercial poultry industry, *Salmonella* Typhimurium is the most common serovar associated with egg-borne *Salmonella* human infection (OzFoodNet Working Group, 2010). The course of microbial infection of shell eggs can be considered in three stages, penetration of the cuticle and shell, colonization of the underlying membranes, and contamination of the albumen leading to generalised infection of egg contents (Clay & Board, 1991). The physical defences of the egg comprise the cuticle filling the pores of the shell, the shell itself, the shell membranes and the physical and chemical attributes of the egg albumen (Lifshitz, Baker, & Naylor, 1963). The eggshell is the first barrier to pathogenic microorganisms but the shell has been reported as being an ineffective barrier to bacterial penetration as the pores are wide enough to allow entry of bacteria (Berrang, Frank, & Buhr, 1999). De Reu et al. (2006) found that the shell thickness, number of pores and area of the eggshell had no statistical effect on bacterial eggshell penetration, and Bain et al. (2011) found that the presence of microcracks did not make a significant difference in the rate of penetration by *S.* Enteritidis. Bacterial penetration across eggshell could also be influenced by eggshell translucency (Chousalkar, Flyn, Sutherland, Roberts, & Cheetham, 2010). Eggshell translucency is the appearance of lighter coloured regions of the shell that can be seen when an egg is candled over a light source (Chousalkar et al., 2010). Talbot and Tyler (1973) suggested that the translucent areas of the shell are due to the presence of liquid and Solomon (1986) added that structures within the shell allow for the entry of more water into the shell and that this results in translucency. Eggshell pores play an important role in egg embryo development, facilitating gaseous exchange and can be viewed in a number of forms

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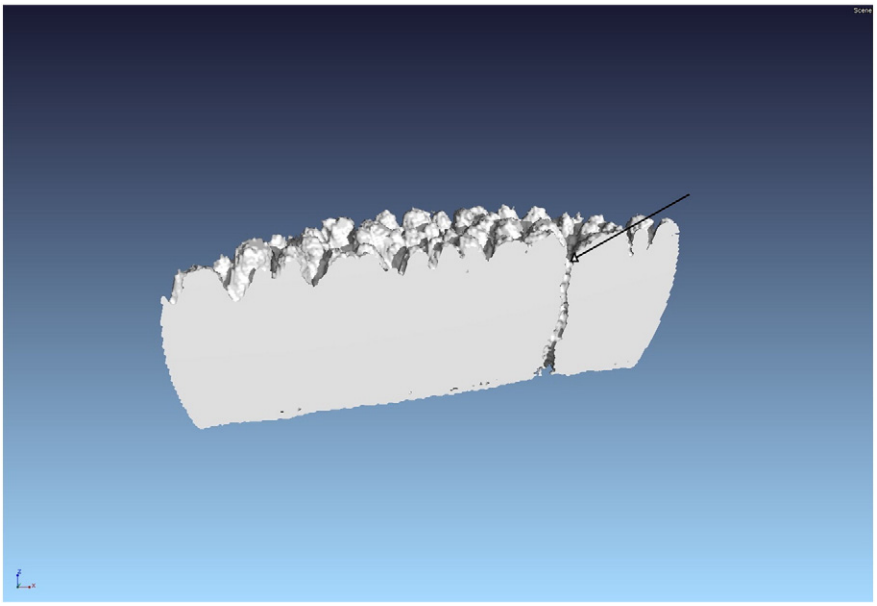

Fig. 1. Three-dimensional CT reconstruction, showing the shells mammillary layer and straight pore structure (arrow).

(Tullet, 1984). In the vast majority of avian species, the shell pores are funnel shaped with a wider orifice facing externally. Board (1980) identified a number of pore types which include both straight unbranched pores and externally branching pores.

The appearance of eggshell translucency has previously been associated with increased bacterial penetration (Chousalkar et al., 2010) and it is important to determine if highly translucent eggs are more likely to be penetrated by bacteria originating on the outside of the egg shell. The effect of eggshell pores on the penetration of *S.* Enteritidis has been investigated (De Reu et al., 2006); however the effects of eggshell pore numbers and structure on the *S.* Typhimurium penetration remain unclear. This experiment utilises micro-CT as a novel way of imaging and classifying pores. The aim of this study was to investigate the effects of eggshell translucency, mammillary layer structures and pore structure on *S.* Typhimurium penetration. Candling was used to identify translucent regions. Micro-computed tomography (CT) involved placing the sample on a rotating stand between an X-ray emitter and detector. Examining the penetrated regions of eggshells is a novel use of the CT technology (Hausherr, Fischer, Krenkel, & Altstädt, 2006). CT and scanning electron microscopy (SEM) offer high resolution images of the mammillary layer and other structures, which may allow the identification of the structural basis of translucency. Hence during this study, CT and SEM were used to investigate the effects of increased eggshell translucency and pores on penetration of *S.* Typhimurium through the egg shell.

2. Materials and methods

2.1. Eggs

Eggs (240) were collected from a commercial layer operation in South Australia, within one day of lay. All eggs with cracked shells or

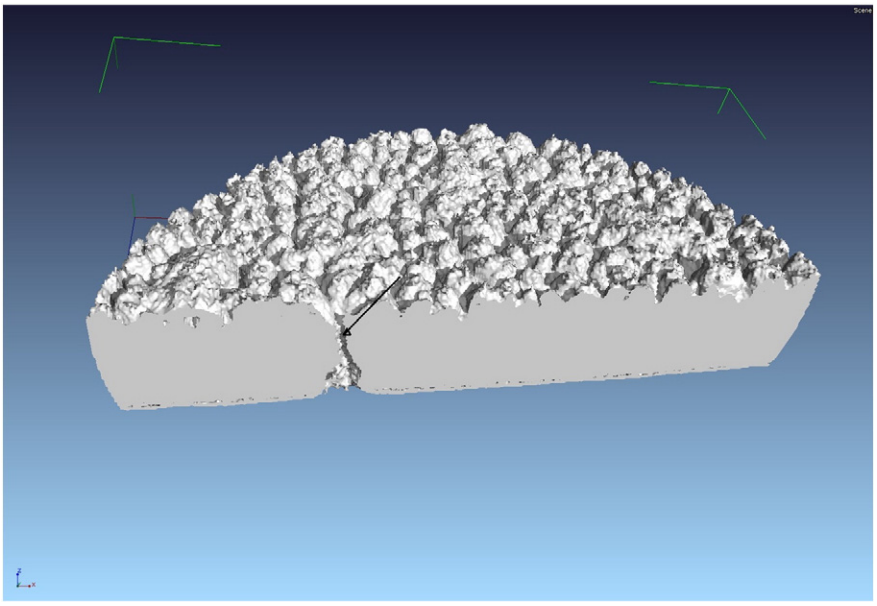

Fig. 2. Three-dimensional CT reconstruction, showing the shells mammillary layer and externally branching pore structure (arrow).

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