

Food Borne Pathogens and Commercial Eggs in Australia

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Abstract

A low level of bacterial contamination on an eggshell is important from both food safety and storage perspectives. A high number of bacteria present on the eggshell surface increases the chances of eggshell penetration and contamination of internal contents. Overall, food quality and sanitary processing conditions can be judged by *Enterobacteriaceae* populations. In chapter 2, no significant differences were detected in the *Enterobacteriaceae* counts of the eggshell surface or in the eggshell pores, of visibly clean eggs collected from Australian layer flocks at various stages of lay. Out of all eggs tested, 4.51% (14/310) eggshell samples were reported *Salmonella* positive.

The rate at which *Salmonella* infected flocks produce *Salmonella* contaminated eggs is unclear. In chapter 3, the possible transmission of *Salmonella* from the environment to the egg was investigated with the help of longitudinal studies on commercial egg farms. Results indicated that the odds of an eggshell being positive for *Salmonella* were 91.76, 61.5 and 18.2 times higher when faecal, egg belt and dust samples, respectively, tested positive for *Salmonella*. On other hand, a one log increase in the load of *Salmonella* detected in faecal, egg belt and in the floor dust samples, respectively, resulted in 35%, 43% and 45% increase ($p < 0.001$) in the odds of an eggshell testing positive for *Salmonella*.

In chapter 4, the shedding of *Salmonella* in a single age commercial egg layer flock was also investigated at the onset of lay (18 weeks) followed by two longitudinal samplings at 24 and 30 weeks. At the age of 18 weeks, when the first sampling was performed, the prevalence of *Salmonella* in faeces was 82.14%. However, in later samplings, at the age of 24 and 30 weeks, the prevalence of *Salmonella* in faeces was significantly reduced ($p < 0.001$) to 38.88% and 12.95% respectively. The prevalence of *Salmonella* in faeces collected from the low tier

cages was significantly higher ($p=0.009$) as compared samples from the high tier cages.

There are various methods to decontaminate the eggshell surface; egg washing is one of them. Egg washing can reduce the level of bacteria on the eggshell surface and horizontal transmission across the eggshell. However, egg washing can damage the cuticle which is the outmost layer on the eggshell surface. The effect of egg washing on *Salmonella* Typhimurium (*S. Typhimurium*) penetration was investigated using agar and whole egg penetration techniques. The results in chapter 5 indicated that eggshell penetration was higher in washed eggs as compared to unwashed eggs. Hence, appropriate attention is essential to make sure eggs are kept at appropriate storage and drying conditions after washing. Statistical analysis also indicated that eggshell penetration by *S. Typhimurium* was related to the incidence of various eggshell ultrastructural features such as cap quality, alignment, erosion, confluence, Type B bodies and cuticle cover. All the *S. Typhimurium* strains used in this study were able to survive on the eggshell surface and in egg internal contents 21 days after infection. Other egg industry associated *Salmonella* serovars such as *S. Singapore*, *S. Adelaide*, *S. Worthington* and *S. Livingstone* had the capacity to penetrate the eggshell. However, these serovars had little or no capacity to survive in the egg internal contents 21 days after inoculation (Chapter 6).

Eggshell quality and safety are important for the consumer's impression of the product. A good quality eggshell protects the egg internal contents from bacterial penetration. A cracked or damaged egg encourages bacteria to move across the eggshell. *Mycoplasma synoviae* (*M. synoviae*) have been found to be associated with poor egg shell quality. The association between egg shell quality parameters and the seroprevalence of *M. synoviae* in eggs collected from Australian commercial layer flocks was investigated in chapter 7. Seroprevalence of *M. synoviae* was found to be

high at 69% (95% confidence interval (CI) = 41.3–89.0). Statistical analysis showed an association between serological status for *M. synoviae* and the incidence of egg quality parameters such as translucency, shell breaking strength, % shell reflectivity and shell deformation. Thus, *M. Synoviae* infection could compromise eggshell quality and as a consequence the eggs from *M. Synoviae* positive flocks may be at risk of becoming contaminated by potentially pathogenic bacteria.

Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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List of Abbreviations

%	Percent
°C	Degree Celsius
<	Less than
µL	microlitre
µm	Micrometre
AECL	Australian Egg Corporation Limited
ANOVA	Analysis of variance
BHI	Brain heart infusion broth
bp	Base pair
BPW	Buffered peptone water
BSA	Brilliance Salmonella agar
CFU	Colony forming unit
CI	Confidence interval
cm	Centimetre
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
dNTP	Deoxy-nucleotide triphosphate
DT	Definitive type
EAA	Egg apex abnormalities
EDTA	Ethylene diamine tetra acetic acid
EFSA	European food safety authority
ELISA	Enzyme-linked immune-sorbent assay
FSANZ	Food Standards Australia New Zealand
gm	Gram
h	Hour
INVA	Invasion Gene A
IPC	Internal positive control
ISR	Infantis-specific (genomic) region
KW	Kauff-White-Le Minor
LDC	Lysine decarboxylase
min	Minute
mL	Mililitre

MLST	Multi-locus sequence typing
MLVA	Multi-locus variation number tandem repeat analysis
mm	Milimetre
N	Newtons
NA	Not applicable
ND	Not detected
nm	Nanometre
NSWFA	New South Wales Food Authority
ONPG	Ortho-nitrophenyl- β -D-galactopyranoside
p.i.	Post inoculation
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PFGE	Pulse field gel electrophoresis
PT	Phage type
Q-PCR	Quantitative Polymerase Chain Reaction
RH	Relative humidity
RT-PCR	Real time polymerase chain reaction
RV	Rappaport Vassiliadis
Se	Sensitivity
sec	Second
SEM	Scanning electron microscopy
Sp	Specificity
SPI	<i>Salmonella</i> Pathogenicity Island
spp	Species
STTR	<i>Salmonella</i> Typhimurium tandem repeat
TAE	Tris-borate-EDTA
TBGB	Tetrathionate brilliant green broth
TG-ROC	Two graph receiver operating characteristics
TSI	Triple sugar iron
TSR	Typhimurium-specific (genomic) region
VNTR	Variable number tandem repeat
XLD	Xylose lysine deoxycholate

List of Publications

Journal articles

- Gole, V. C., Chousalkar, K. K., & Roberts, J. R. (2012). Prevalence of antibodies to *Mycoplasma synoviae* in laying hens and possible effects on egg shell quality. *Preventive Veterinary Medicine*, *106*, 75-78.
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