

Studies on Salmonella Enterica spp Isolated From Egg Farm Environment

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A Thesis submitted for the fulfilment of the requirements of
the Doctor of Philosophy



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Adelaide, South Australia, Australia

August 2016

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Thesis Abstract

Infection with *Salmonella* spp is among the most common causes of foodborne outbreaks of human gastrointestinal disease. Humans acquire *Salmonella* through the consumption of contaminated food items, including eggs and undercooked egg products. There are over 2500 *Salmonella enterica* serovars but globally, *Salmonella* Enteritidis and *Salmonella* Typhimurium are responsible for causing the majority of human disease. *S. Enteritidis* is, however, not endemic in Australia; strains of *S. Typhimurium* have filled this niche and are frequently isolated during cases of egg related foodborne illness. Over the last decade, cases of salmonellosis have increased, representing a significant public health issue. The experiments presented in this thesis were designed to investigate aspects of antimicrobial resistance, layer hen infection, biofilm forming ability of *Salmonella* spp on eggshell and the efficacy of organic acids on controlling *Salmonella* biofilms.

Antimicrobial resistance was characterised for multiple *Salmonella* isolated from the layer hen environment. The majority of *Salmonella* isolates (91.72%; 133/145) were susceptible to all antimicrobials tested. This finding indicates that there is minimal public health risk associated with emergence of antibiotic resistant *Salmonella* within the Australian layer industry.

An infection trial investigated the ability of *S. Typhimurium* to colonise reproductive organs and contaminate developing eggs. Intermittent but persistent faecal shedding of *Salmonella* after oral infection was observed for 15 weeks post infection. Further, *S. Typhimurium* caused eggshell contamination and colonised the reproductive tissue however, *S. Typhimurium* was not isolated from the internal egg contents. These findings indicate that horizontal transmission through contaminated faeces is the main route of egg contamination with *S. Typhimurium* in laying hens.

Biofilm formation was dependent on both temperature and serovar. At ambient temperature, *Salmonella* isolates attached and formed biofilm on eggshells however, differences between strains of *Salmonella* serovars were evident in eggshell biofilm formation.

The anti-bacterial and anti-biofilm activity of commercial organic acid products against *S. enterica* isolates was further investigated. The result of this experiment showed no significant differences between isolates of representative *Salmonella* serovars in respect of inhibitory and bactericidal dilutions of each product. Two out of three commercial organic acid products tested in this study significantly reduced viable cells from 3 and 5 day old biofilms in a dose and time dependent manner. None of the tested products completely eliminated biofilm cells under any of the tested conditions.

In conclusion, the results presented here improve our understanding of the risks of egg associated *S. enterica* spp. Knowledge gained from these experiments could contribute to the development of improved guidelines for food safety and public health in Australia.

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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Acknowledgements

“I may not have gone where I intended to go, but I think I have ended up where I intended to be” –Douglas Adams

First and foremost, I would like to express my sincere gratitude to my supervisor Dr Kapil Chousalkar, for giving me an opportunity to pursue my PhD. Kapil has been a brilliant mentor and without his continuous guidance, this work would not have been possible. Kapil, you believed in me and my ability to complete this daunting task. I shall remain in your debt throughout my life for making my dream of PhD come true.

I would like to express my profound indebtedness to my co-supervisor Dr Andrea McWhorter, for her encouragement, critical evaluation, valuable suggestions, and excellent co-operation throughout my thesis.

My special thanks to Dr Gordon Howarth, Dr Milton McAllister, Dr Darren Trott, Dr Ryan O'Handley, Dr Bec Forder, Dr Farhid Hemmatzadeh, Dr Manouchehr Khazandi, and Dr Charles Caraguel for the guidance during my research work.

I would like to acknowledge The University of Adelaide for providing the Postgraduate Research Scholarship to pursue my higher degree research.

I am thankful to Angela Mills, Marie Kozulic, Jeremy Winchester, and all other staff of Library at Roseworthy campus, The University of Adelaide for their help with Adobe and EndNote.

Words are few to owe my special and heartiest thanks to my friends for their moral support and refreshing company; Vaibhav Gole, Pardeep Sharma, Talia Moyle, Sasikumar, Vinod Kadam, Lesley Menzel, Rebecca Dunbar, David Purdie, Saad Gilani, Rebecca Devon, Janet Pandi, Michael Dom, Jane McNicholl, Noor Haliza Hasan, Sugiyono Saputra, Saad Gilani, Rebecca Abraham, Sam Abraham, Amanda Kidsley and Elizabeth Hickey.

I do not have words to express my sincere thanks to Leena, Mahesh, Jaya, and Vaibhav for the wonderful family support. All of you have made my journey of PhD very joyful and a memorable one.

My special thanks to Hasmukh and Jignya Patel for their wonderful family support during critical periods of thesis writing.

I would also like to thank Dr Jaswinder Kaur and Dr Emad Ehsan from Gawler medical clinic for providing the best health care service to my family during PhD.

A special thanks to all my mentors and my professional colleagues in India for their tremendous motivation; Dr Kurkure, Dr Kalorey, Dr Vishal Pavitrakar, Dr Jaiprakash Bhelonde, Dr Pankaj Shelar, Dr Swapnil Chilgunde, Anand Bokare, Dr Amol Fiske, and Dr Jitendra Ramgaokar. My heartfelt thanks to Dr Umesh Wankhade, Dr Pradyumna Baviskar and Dr Nitin Kamble for continued encouragement during my research work.

A big thank you to my family members who have always been supportive and proud of what I am today. My special thanks to my wife Poonam, my beautiful daughters Unnati and Aastha, my mother, father, brother and his wife for their great understanding, sacrifice, unconditional love and belief in my abilities. My all-family members have been my real pillars of emotional strength while pursuing this degree. My special thanks to my in-laws, Nilesh Tayde, Rucha Kangte and Deepak Kangte for great support and love during my stay in Australia.

Finally, thanks to God for the joy and opportunity to worship him through learning more about this small part of his creation.

List of Publications

Pande, V. V., Gole, V. C., McWhorter, A. R., Abraham, S., & Chousalkar, K. K. (2015). Antimicrobial resistance of non-typhoidal *Salmonella* isolates from egg layer flocks and egg shells. *International Journal of Food Microbiology*, 203, 23-26.

Pande, V. V., Devon, R. L., Sharma, P., McWhorter, A. R., & Chousalkar, K. K. (2016). Study of *Salmonella* Typhimurium Infection in Laying Hens. *Frontiers in Microbiology*, 7, 203.

Pande, V. V., McWhorter, A. R., & Chousalkar, K. K. (2016). *Salmonella enterica* isolates from layer farm environments are able to form biofilm on eggshell surfaces. *Biofouling*, 32(7), 699-710.

Pande, V. V., McWhorter, A. R., & Chousalkar, K. K. (2016). Anti-bacterial and anti-biofilm activity of commercial organic acid products against *S. enterica* isolates recovered from layer farm environment. *Journal of Applied Microbiology* (Submitted)

Conference Oral Presentations

Vivek V. Pande, Andrea R. McWhorter, Kapil K. Chousalkar. *Salmonella enterica* spp isolated from egg farm environments are able to form biofilm on the eggshell. The Australian Society for Microbiology annual scientific meeting, 3-6 July 2016, Perth, Australia.

Vivek V. Pande, Rebecca Devon, Pardeep Sharma, Andrea R. McWhorter, and Kapil K. Chousalkar. *Salmonella* Typhimurium shedding and egg contamination in experimentally infected laying hens. The 43rd Annual General Meeting of the Australian Society for Microbiology 12-15 July 2015, Canberra, Australia.