

Study of *Salmonella typhimurium* infection and vaccination in laying hens

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Thesis submitted for the degree of

Doctor of Philosophy in Veterinary Science



THE UNIVERSITY
of ADELAIDE

School of Animal and Veterinary Sciences

The University of Adelaide

Australia

June 2017

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Thesis Abstract

Salmonella Typhimurium is responsible for the majority of egg-associated foodborne outbreaks in Australia. It colonises sub clinically in commercial laying hens, and these hens can shed bacteria in the poultry environment resulting in egg contamination; hence, it is of significant public health concern. Therefore, understanding the biology of *S. Typhimurium* shedding, host-pathogen interaction, and its management in laying hens is essential. In our study we conducted a 16-week infection trial, using layer hens reared free from exogenous *Salmonella*. The results highlighted that *S. Typhimurium* continued to persist in the internal organs for 16 weeks post challenge and infection was not eliminated despite measurable antibody response from the asymptomatic *S. Typhimurium* carrier adult birds. The stress of onset of lay led to the recrudescence of *S. Typhimurium*, further leading to its proliferation, intermittent shedding and causing eggshell contamination.

Live attenuated *Salmonella* vaccine is commonly used for the vaccination of chickens to control the contamination of poultry products. Vaxsafe[®] ST; (Strain STM-1, $\geq 10^{7.0}$ cfu/dose, Bioproperties Pty Ltd) is the only commercially available live attenuated *aroA* deletion mutant *S. enterica* serovar Typhimurium vaccine in Australia and was used in this study. The antibody response to vaccine and efficacy of Vaxsafe[®] ST during pullet rearing and early production were investigated. The pullets vaccinated after intramuscular injection at 12 weeks produced significantly higher antibody response ($p < 0.001$) to *S. Typhimurium* vaccine strain. The vaccine strain STM-1 successfully colonised the chicken gut but did not induce a systemic antibody response until after parenteral administration. The load of STM-1 in litter samples increased gradually and was significantly higher at week 13, highlighting that Vaxsafe[®] ST has a potential as an antigen delivery system.

During laying, Vaxsafe[®] ST was tested in naturally infected *S. Typhimurium* laying hen flocks. At the onset of lay there was no significant difference in prevalence of *Salmonella* spp. in faeces in vaccinated and unvaccinated groups, although antibody titre was significantly higher in vaccinated than unvaccinated group at all sampling points during this study. The prevalence of wild-type *S. Typhimurium* did not vary significantly in subsequent samplings. *S. Typhimurium* was consistently found in dust and shoe cover samples, throughout the study. Given that *S. Typhimurium* and other serovars can survive/persist in the shed environment (such as in dust), regular cleaning, disinfection and or removal of dust from shed is necessary. *Salmonella* spp. can form biofilms on various surfaces hence cleaning of the shed could be challenging.

Three commercial disinfectants (Product A, B and C: containing a chlorinated compound, quaternary ammonium compounds (QAC), and twin-chain QAC; respectively) tested in this experiment significantly reduced viable biofilm cells; however, none of the product eliminated the biofilm cells. The results of this study showed that biofilm age was associated with the increased resistance to disinfectant treatments. These findings may have future implications for the use of disinfectants such as required concentration and exposure time in the poultry industry to control biofilm.

From this work, it can be concluded that *S. Typhimurium* persists in the internal organs of hens for a prolonged period and these hens act as a latent carrier with a continuous source of egg and environmental contamination. Vaccination of poultry, which would decrease *S. Typhimurium* contamination of flocks, is considered as an effective measure to reduce human cases of salmonellosis. However, use of the Vaxsafe[®] ST vaccine in laying hens is “not an ultimate intervention” for reduction of *S. Typhimurium*, hence, implementation of more than one or several interventions strategies is essential.

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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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Pardeep Sharma

Acknowledgements

With an overwhelming sense of gratitude, I thank the Almighty for showering his blessings for successful completion of this work. Achieving an advanced degree is no large feat; rather, the cumulative effect of little accomplishments that had significant impacts. Moreover, rarely are great accomplishments earned with one big stroke of luck or genius. Instead, it is the cumulative effect of hard work, persistence, patience, diligence, humbleness, and a general respect for all challenges at hand. We are not at our best every day, but, there is indubitably love and support of people who make this all possible.

To this degree, I owe thanks to a number of people who guided me and helped mould the person I am today. First and foremost, I would like to thank my supervisor Dr Kapil Chousalkar for giving me the opportunity to do PhD at The University of Adelaide and for the unflinching support, knowledge, and sense of curiosity you instilled in me. You always had an open door come to any issue or concern. Without your great contribution, this thesis would not be possible. I thank, Dr Andrea McWhorter, my co-supervisor for her constructive criticism, valuable suggestions and precious inputs in reviewing this thesis.

I would like to acknowledge, the University of Adelaide, for providing an International Postgraduate Research Scholarship, and Australian Egg Corporation limited and Poultry CRC for funding their research projects. My special thanks to Drs Gordon Howarth, Milton McAllister, Darren Trott, Farhid Hemmatzadeh, Kiro Petrovski and Bec Forder for the guidance during my research.

I would like to thank Dr Margaret Sexton for liaising with egg producers for sample collection during my PhD candidature. I am thankful to Drs Gregory Underwood and Karen Holden from Bioproperties Pty Ltd for facilitated discussions between industry and research

personnel. I thank, Dr Charles Caraguel for reading my manuscript and assisting in the interpretation of vaccine trial data.

I would like to acknowledge the technical help provided by Dr Vivek Pande, Dr Vaibhav Gole and Ms Talia Moyle during experimental work. Along with my supervisory committee members, I thank each of you for guiding me through the many struggles in putting this research and thesis together. It was a team effort.

I am also thankful to Marie Kozulic, Angela Mills and all other staff of Library at Roseworthy Campus, The University of Adelaide for their help with Endnote. Many thanks to staff members, at School of Animal and Veterinary Sciences, for their help and assistance. I would especially like to thank student support services and accommodation team of David, Lesley, Sally, Alyson and Rebecca for their support throughout my stay at Roseworthy campus. My thanks to my lab mates and friends, I will forever treasure the times of graduate school and sharing more than a few laughs. Janet, Sugi, Saad, Wei, Sadeesh, Dom, Liza, Farida, Abid, Liz, Jess, Bec, Amanda, Jay, Tim, Hui, Sarah, Danny, Wesley, & many others for their refreshing friendship.

My thanks to Mohinder, Sapna, Riya, Abhay, Leena, Poonam, Mahesh, Jaya, Hasmukh, Jignya, Gaurav and Bindu for their wonderful family support. A special thanks to all my mentors and professional colleagues in CSKHPKV, Palampur, India for their tremendous motivation for continued encouragement during my research work.

A big thank to my father, who taught me the value of hard work and to my mother, who provided unquestioned love and support to me and for all my dreams and aspirations. I also extend loving thanks to our son Aryan for his innocent gestures. Lastly, most importantly, I extend my gratitude to my life partner, Dr Madhu Sharma, for her patience, encouragement and continual morale boost during the entire period of this research and thesis.

List of Publications

Sharma, P., Pande, V. V., Moyle, T. S., McWhorter A. R., and Chousalkar, K. K. (2017).

Correlating bacterial shedding with fecal corticosterone levels and serological responses from layer hens experimentally infected with *Salmonella* Typhimurium. *Veterinary Research*, 48:5, 1-11.

Pande, V.V., Devon, R. L., **Sharma, P.**, Moyle, T. S., McWhorter A. R., and Chousalkar,

K. K. (2016). Study of *Salmonella* Typhimurium Infection in Laying Hens. *Frontiers in Microbiology*, 7:203.

Manuscripts submitted for Publications

Sharma, P., Moyle, T. S., Sexton, M., McWhorter A. R., Holden, K., Underwood, G. and

Chousalkar, K. K. (2017). Shedding of a live attenuated *Salmonella* Typhimurium vaccine Vaxsafe[®] ST (strain STM-1) during pullet rearing - a field study. *Avian Pathology* (Submitted).

Sharma, P., Pande, V. V., Moyle, T. S., Caraguel, C., Sexton, M., McWhorter A.R. and

Chousalkar, K. K. (2017). Shedding of *Salmonella* Typhimurium in vaccinated and unvaccinated hens during early lay: a randomised controlled trial. *Vaccine* (Submitted).

Sharma, P., Pande, V. V. and Chousalkar, K. K. (2017). Efficacy of commercial

disinfectants against biofilms formed by *Salmonella enterica* serovars Typhimurium isolates. *Animal* (Submitted).

Conference Oral and Poster Presentations

Sharma, P., Pande, V. V., Devon, R. L., McWhorter, A. R. & Chousalkar, K. K. (2015), Dynamics of *Salmonella* Typhimurium shedding from early to peak lay in laying hens (Oral). <<http://www.saysoft.net/meat15/proceedings/papers/100075.pdf>>. "XVI European Symposium on the Quality of Eggs & Eggs Products & XXI European Symposium on the Quality of Poultry Meat, 10-14 May, 2015 at Nantes, France".

Pande, V. V., Devon, R. L., **Sharma, P.**, McWhorter, A. R. & Chousalkar, K. K. (2016). *Salmonella* Typhimurium shedding and egg contamination in experimentally infected laying hens (Oral). The 43rd Annual General Meeting of the Australian Society for Microbiology, 12-15 July 2015, Canberra, Australia.

Sharma, P., Pande, V. V., Devon, R. L., McWhorter, A. R. & Chousalkar, K. K. (2016). Serological responses to *Salmonella* Typhimurium infection in laying hens. (Poster) <http://www.asap.asn.au/wp-content/uploads/abstract-2015/272/attach_brief.pdf>. Animal Production 2016 joint conference of the Australian Society of Animal Production and the New Zealand Society of Animal Production held on July 4 -7, Adelaide, Australia.