Serological responses to *Salmonella* Typhimurium infection in laying hens

Pardeep Sharma, Vivek V. Pande, Rebecca L. Devon, Andrea R. McWhorter and Kapil K. Chousalkar

School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy SA 5371 Australia
Presenting author: Pardeep Sharma paredeep.sharma@adelaide.edu.au

Summary

*Salmonella* detection by bacteriological culture along with serology is a common practice in the poultry industry. The present experiment was conducted to study the antibody response of laying hens to *Salmonella* Typhimurium infection using an Enzyme-linked immunosorbent assay (ELISA). At 14 weeks of age, hens were orally inoculated with 10⁶ colony forming units (CFU) of either S. Typhimurium Definitive Type 9 (DT9) or a combination of S. Mbandaka and DT9. Serum samples were collected at day 0 followed by 1, 2, 4, 6, 8, 10, 12 and 14 weeks post infection (wks p.i.). Serological analysis revealed a strong immune response to *S.* Typhimurium infection. IgG antibody titres started to rise from 1 wk p.i., peaked at 6 wks p.i., and persisted throughout the course of this study (14 wks p.i.) in both the infected groups. The results of this study suggest that serology can be used as a preliminary screening of *S.* Typhimurium infected birds for further bacteriological examination.

Introduction

Globally *Salmonella* is one of the major zoonotic foodborne pathogens. Amongst more than 2,500 serovars of *Salmonella*, serotypes, such as Enteritidis and Typhimurium, have been responsible for the majority of food-borne outbreaks in humans (Voetsch et al. 2004). In South Australia, *Salmonella* Typhimurium DT9 (S. Typhimurium) is an important food borne pathogen and has been reported from several food borne outbreaks following the consumption of contaminated egg and egg products (OzFoodNet 2015). Despite on-farm control strategies *S.* Typhimurium, is a major concern for the Australian egg industry. Therefore, detection and prevention of *Salmonella* infections within poultry flocks is important. Bacterial culture method is routinely used for the *Salmonella* identification (Hsu et al. 2011) however, low and intermittent shedding of *S.* Typhimurium in the faecal and or environmental samples can provide false positive results (Ishola 2009). Moreover, the faecal sample analysis by the culture method is laborious and time consuming. Monitoring birds for *S.* Typhimurium specific antibodies is an initial screening step used to identify flocks that have been exposed to this pathogen. To overcome false positive results by culture method, monitoring of antibody titres to *S.* Typhimurium could be a better alternative. The aim of the current experiment was to characterise the serological response to *S.* Typhimurium infections in hens from early to peak lay.

Materials and Methods

Fertile eggs were obtained from a commercial layer parent flock. Eggs were fumigated and incubated for 21 days at 37.7°C. A total of 32 birds were hatched and raised in pens in positive pressure rooms at Roseworthy campus, at the University of Adelaide.Strict biosecurity measures along with fortnightly testing of feed, water and faecal samples were followed to maintain birds *Salmonella* free until the start of the challenge experiment. At week 10, birds were divided in three groups and transferred in to cages in separate rooms. At 14 weeks of age, the C (control) group received only sterile Luria Bertani (LB) broth, other hens were orally inoculated with 10⁶ colony forming units (CFU) of either *S.* Typhimurium definitive type DT9 (T group) or a combined total of 10⁶ CFU of *S.* Typhimurium DT9 and *S.* Mbandaka (MT group) suspended in LB broth (Oxoid Australia). Blood samples were collected at day 0 followed by 1, 2, 4, 6, 8, 10, 12 and 14 wks p.i. Serum was separated by centrifuging the blood samples at 1500g for five minutes and samples were stored at -20°C until further analysis. Serum samples were analysed by the Chicken *S.* Typhimurium Antibody Kit LPS Group B (BioChek, Holland) and titres were calculated, according to the manufacturer’s instructions. Absorbance of controls and test samples was measured at 405 nm (Multiskan Ascent pathtech). All data generated in this study was analysed statistically using GraphPad Prism version 6 (Graph Pad inc, CA, USA) using two-way ANOVA or Student’s t-test to compare groups. p < 0.05 were considered statistically significant.

Results and Discussion

Specific antibody response to *S.* Typhimurium
None of the birds were seropositive prior to infection with *Salmonella*. Overall, the mean antilog of antibody titers in T group were higher (without any significant difference) than the MT group. Serum IgG antibody titers started to rise from wk 1 p.i. and peaked at the onset of lay (6 wks p.i.). The antibody titers persisted and birds were seropositive till the end of experiment i.e. 14 wks p.i. in both T and MT group (FIG. 1). These findings are in agreement with the previous finding (Hassan et al. 1991) in which four day old chickens infected with S. Typhimurium strain showed a strong IgG antibody response with peak titers at 4 wks p.i. Similar to our findings Gast and Beard (1990) reported a rapid and early antibody response in *Salmonella* infected birds and found that most birds were seropositive at 10 weeks p.i. There were a significantly (p < 0.05) higher antibody titers in T group at 6, 10, 12 and 14 wks p.i. in comparison to the MT group. It is difficult to compare our results to those obtained by other researchers because of the paucity of such reports in the literature.

**FIG. 1. Antibody Titters for S. Typhimurium at different weeks of post infection in S. Typhimurium (T) and S. Typhimurium + S. Mbandaka (MT) groups.**

Mean antilog of log10 antibody titers ± standard error. Bars with the different lower case letters in the same wks p.i. are significantly different (p < 0.05).

The findings of the present study suggest persistent antibody response to *S. Typhimurium* infection in laying hens from early to peak period of lay. In conclusion, serology can be used as a preliminary screening for *S. Typhimurium* infected birds for further bacteriological screening.

**Acknowledgement**

This research was funded by Australian Egg Corporation Limited (AECL) Australia.

**References**
