Dynamics of *Salmonella* Typhimurium shedding from early to peak lay in laying hens

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Globally, *Salmonella* is a common cause of food-borne human gastroenteritis. It can be transmitted through consumption of contaminated eggs and chicken meat. In Australia, *Salmonella* Typhimurium (*S*. Typhimurium) is the most frequently reported serovar in egg related food poisonings. The present experiment was conducted to study the shedding of *S*. Typhimurium definitive type 9 (DT9) in eggs and faeces of laying hens, challenged before onset of lay. In addition, the effects of co-infection on the shedding of DT9 were also investigated. At 14 weeks of age, hens were orally inoculated with 10^9 colony forming units (CFU) of either DT9 or a combination of *Salmonella* Mbandaka (*S*. Mbandaka) and DT9. Faecal samples were collected on day 1 post-infection (p.i.) followed by 1, 2, 4, 6, 8, 10, 12, 14 and 16 weeks p.i.. All faecal samples were processed for enumeration of *Salmonella* by MPN (most probable number) method. All eggs laid during 6, 8, 10, 12 and 14 week p.i. were tested for *Salmonella. Salmonella* counts in faeces ranged from 1.53 ± 1.05 to 48.53 ± 16.55 MPN / g for birds infected with DT9 only and 0.78 ± 0.27 to 44.80 ± 18.30 MPN / g in the birds infected with DT9 and *S*. Mbandaka. The frequency of egg shell contamination ranged from 9.52 to 21.74 % for DT9 and 10.89 to 33.33 % in the co-infected group. Both groups had the highest rate of *Salmonella* shedding on egg shell at the onset of lay (6 weeks p.i.). Over the course of the experiment, *Salmonella* was not detected in egg internal contents in either group. The above findings suggest that there was intermittent and prolong shedding of *Salmonella* in the faeces and eggs.

Keywords: *Salmonella*; laying hens; dynamics; shedding; eggs.

Introduction

Globally, salmonellosis is one of the most important public health hazards and food-borne zoonotic diseases. Egg and raw egg products are frequently linked to outbreaks of human salmonellosis. In Australia, *Salmonella* Typhimurium (*S*. Typhimurium) is the most frequently reported serovar in egg related food poisoning (EFSA, 2010).

Salmonella enter the gastrointestinal tract by ingestion and establish persistent infection in the caeca (Barrow, 2000). Bird excreta along with environmental sources are main sources of *Salmonella* shedding. The persistence and extent of flock colonisation is dependent on several factors such as the bacterial strain, dose of initial inoculum, age, genetic line and immune status of the birds (Gast *et al*., 2005). At oviposition, 90% of eggs are free of bacteria (Board, 1966); however, egg shell surface contamination can occur during contact with any surface. Contaminated eggs are often responsible for the zoonotic transmission of *Salmonella* Enteritidis (Centers for Disease Control, 1996). Previous studies reported that an increase *Salmonella* shedding in faeces could increase the likelihood of eggshell contamination (Gole *et al*., 2014 a).

Stress has a profound impact on immune response (El-Lethey *et al*., 2003) and laying stress can alter the immune response of birds, making them more susceptible to *Salmonella* infection, thus

increasing *Salmonella* shedding. Gole *et al*.(2014 b) conducted a study on a single age layer flock for detection of *Salmonella* shedding at the onset of lay and found the highest prevalence of *Salmonella* in faeces (82.14%) was at onset of lay (18 weeks). Studies conducted under field conditions by Chemaly et al. (2009) showed that 39.3% of *Salmonella* positive flocks had at least one positive eggshell (1.05%). However, there is a paucity of literature investigating dynamics of *Salmonella* Typhimurium shedding from the early to peak lay stage in the experimentally infected birds.

The present experiment was conducted to characterise the shedding pattern of *S*. Typhimurium definitive type 9 (DT9) in eggs and faeces of laying hens, challenged before onset of lay. In addition effects of co-infection with *S*. Mbandaka, on the shedding of DT9 were also studied.

Materials and methods

Chickens: Fertile eggs were obtained from commercial Hyline brown layer parent flocks and were hatched in animal facilities. A total thirty two pullets were raised in pens up to week 10 and then divided in to three treatment groups consisting of control, *Salmonella* Typhimurium (T group) and *S*. Typhimurium + *S*. Mbandaka combination (MT group) and housed in individual cages in three separate positive pressure rooms for the duration of the experiment. Feed and water were sterilised and provided *ad libitum*. Strict biosecurity measures were maintained to ensure the birds remained free of *Salmonella* until inoculation and to avoid cross-contamination throughout the experiment. Feed, water and faecal samples were screened for *Salmonella* spp. fortnightly.

Prior to infection, all experimental birds were negative for *Salmonella* spp. All the experiments were performed according to the Australian code for the care and use of animals for scientific purposes and approved by institutional animal ethics committee of The University of Adelaide.

Bacterial inoculations: At 14 weeks of age, control birds received only sterile Luria Bertani (LB) broth, other hens were orally inoculated with 10^9 colony forming units (CFU) of either *S*. Typhimurium definitive type DT9 (T group) or a combination of *S*. Mbandaka and DT9 (MT group) suspended in LB broth (Oxoid Australia). *Salmonella* isolates used in this study were originally isolated from Australian layer farms Gole *et al*. (2014 b) and serotyped at Institute for Medical and Veterinary Science, Adelaide, Australia.

Faecal sampling for bacteriology and Most probable number (MPN): Faecal samples were collected aseptically from individual hen in Whirl- Pack plastic bags (Thermo Fisher Scientific, Australia) on day 1 post-infection (p.i.) followed by 1, 2, 4, 6, 8, 10, 12, 14 and 16 weeks p.i.. All faecal samples were cultured for *Salmonella* isolation using previously described method Gole, *et al.* (2014 b) and processed for enumeration of *Salmonella* by MPN (most probable number) method as described earlier Pavic *et al*. (2010) and Santos *et al*. (2005).

Bacteriological analysis of egg shell wash and internal contents: Eggs were collected bi-weekly from sixth week post infection (start of lay) and then on the alternate weeks till fourteen weeks p.i. from both the control and *Salmonella* infected hens. Each egg was collected in a separate sterile Whirl-Pak plastic bag to avoid cross-contamination and egg shell wash and internal egg contents were processed separately for *Salmonella* isolation as described previously Gole, *et al*.(2014 b).

Statistical analysis: All MPN values were expressed as per gram of faeces and data was analysed statistically using two way ANOVA using SAS© software 6.0 software. P values < 0.05 were considered statistically significant.

Results and discussion

***Salmonella* populations and count in the faecal samples:** Dynamics of *Salmonella* shedding for day1 p.i., followed by 1, 2, 4, 6, 8, 10, 12, 14 and 16 week p.i. hens is presented in *Figure 1*. A total of 320 faecal samples were processed for the isolation and enumeration of *Salmonella*. None of the faecal samples from control hens were *Salmonella* positive. For both the *S.* Typhimurium (T group) and *S*. Typhimurium + *S*. Mbandaka combination (MT group), no significant difference ($P > 0.05$) in

Salmonella populations was detected among the two treatment groups (Mean ± SE of 25.46 ± 4.09 and 20.90 ± 4.05 MPN/g for T and MT group, respectively) and no significant difference (P > 0.05) was found between weeks p.i.. The overall *Salmonella* counts in faeces ranged from 1.53 to 48.53 MPN / g of faeces for birds infected in T group only and 0.78 to 44.80 MPN / g in the birds infected in MT group. At one week p.i. hens showed highest MPN count which could be due to the early stage of the infection and a large number of bacteria in the intestine. After only a week p.i., the bacterial shedding of hens was reduced. Contrary to our findings, Marcq *et al*. (2011) reported reduction in *Salmonella* shedding at 22 days p.i. suggesting natural elimination of *Salmonella* Typhimurium from gut of broilers. At six weeks p.i. there was an increase in the MPN count which could be a result of stress and impaired immune response induced by onset of lay suggesting that laying stress has reactivated the bacteria. However further investigation is necessary to conclude these findings. The MPN count was increased on 12th, 14th and 16th week p.i. for the T group which indicated that *Salmonella* can persist in the intestinal tract of birds for a prolonged period of time. Similar findings were recorded by Gast *et al*. (1990) in experimentally infected hens.

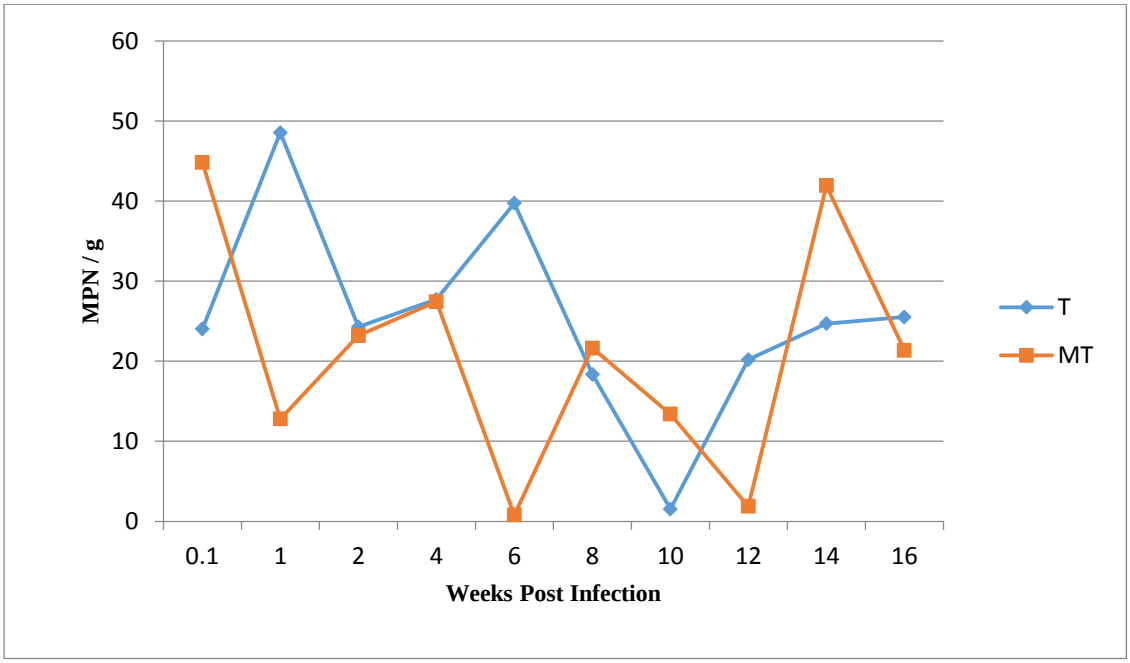

Figure 1 Populations and Most Probable Number / g of faeces at different weeks post infection in *Salmonella* Typhimurium (T) and *S*. Typhimurium + *S*. Mbandaka (MT) in experimentally infected hens.

Detection of *Salmonella* from egg shell wash and internal contents:

A total of 895 egg samples were tested for *Salmonella*. None of the samples from control hens were *Salmonella* positive. The frequency of egg shell contamination ranged from 9.52 to 21.74% for T and 10.89 to 33.33% in MT group (*Figure 2*). *Salmonella* was isolated from the start of laying i.e. sixth week post infection (p.i.) to fourteenth week p.i. from the egg shell wash of experimentally infected hens of both T and MT group. The percentage of shell contamination was highest in both the groups at six week p.i. and lowest on eight week p.i.. Over the course of the experiment, *Salmonella* was not detected in egg internal contents of either group.

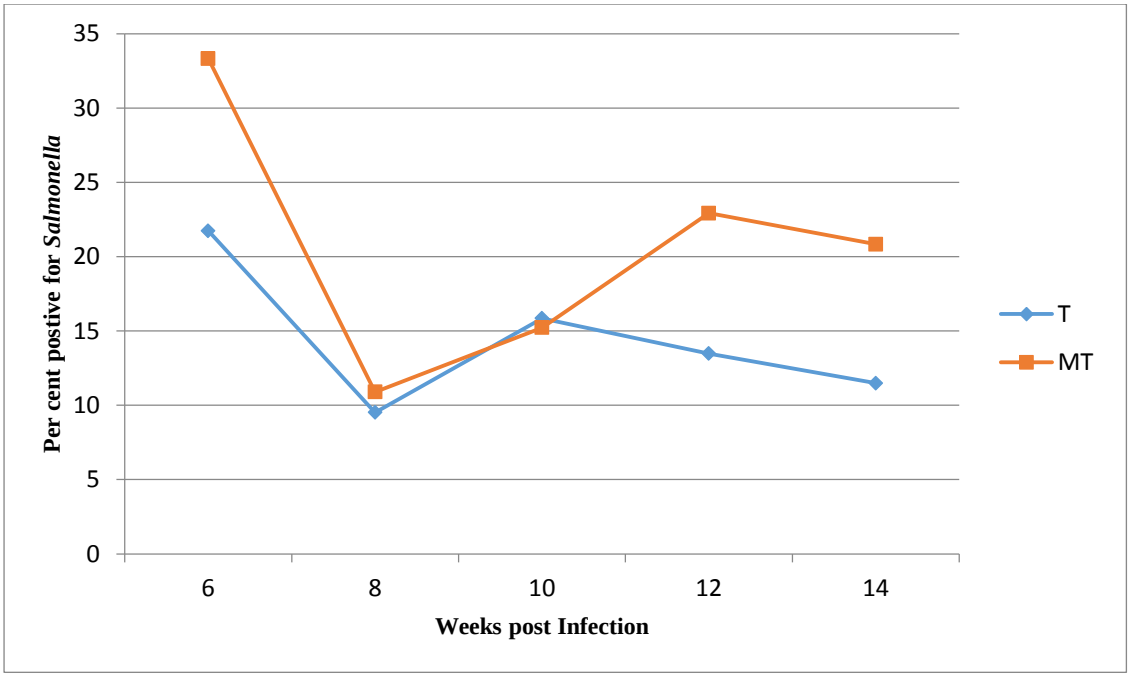

Figure 2 Per cent eggshell surface contamination at different weeks post infection by *Salmonella* Typhimurium (T) and *S*. Typhimurium + *S*. Mbandaka (MT) in experimentally infected hens.

Conclusion

The findings of current study suggests that there was intermittent and prolong shedding of *Salmonella* in the faeces of laying hens along with highest egg shell contamination at the onset of lay. Throughout the experiment, *Salmonella* was not detected in egg internal contents in either group.

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