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Comparative Colonization of *hilA* and Parent Strains of *Salmonella enteritidis* in Fertile Eggs

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ABSTRACT

***Background*:** Young chickens are more susceptible to *Salmonella* colonization than older ones that have developed resistance with age as native microflora become established.

***Methods*:** In this study, two groups of fertile eggs were inoculated with 20 CFU of *hilA* or parent strains of *S. enteritidis*. Presence and number of *Salmonella* cells inside the homogenized egg contents were determined on the 2nd, 5th, 8th, 12th, 17th and 21th day of incubation period.

***Results*:** High infectivity rate of *Salmonella* contamination were observed in the *hilA* group eggs, three genes for *S. enteritidis* identification were detected from isolated colonies of both groups of eggs. The gene *hilA* was only detected in isolated colonies of the standard group.

***Conclusion*:** These findings indicated that *hilA* mutant of *Salmonella* is able to rapidly multiply much higher than wild-type strain but, support more pathogenicity of wild-type strain of *Salmonella* compared to mutant strain.

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Introduction

Salmonella secretes proteins into the cytosol of the epithelial cells and inters in epithelial cells by cytoskeleton rearrangements and membrane ruffling. These events occurs via a type III secretion system [TTSS] encoded by genes of the Salmonella pathogenicity island 1 [SPI-1]. HilA, a key regulator of SPI-1, is a transcriptional activator encoded on SPI-1 and regulates the expression of the SPI-1 secretion system. (1, 2). It is not known whether SPI-1 mutants are able to colonize embryonated eggs during incubation period in a simulated vertical transmission method. In the present study, the importance of *hilA* mutation on *S.* Enteritidis colonization in embryonated eggs during the 21 days incubation period was evaluated.

S. enteritidis phage type 4, strain NIDO 76Sa88 Nalr (parent strain) and its *hilA* mutant were used in this experiment (3). Four hundred-ten fertile *Salmonella* free eggs randomly divided into two groups of 200 eggs. A hole was punched through the previously disinfected shell and 100 µl of a 10-7 dilution of the wild and mutant strains of *S. enteritidis* were inoculated into the albumen giving on average level of 20 ± 2 CFU (4). Both groups of eggs were immediately transferred into two different incubators with the same environmental conditions. A sample of 10 eggs from each group was randomly taken on the 2nd, 5th, 8th, 12th, 17th and 21th day of incubation period. Eggs aseptically cracked and placed in a sterile plastic bag. Presence and number of *Salmonella* cells inside the homogenized egg contents and embryos were determined.

Salmonella detected in egg contents of two groups from 2 days of incubation period (dip). Bacteriological analysis (Figure 1) showed peak values of *Salmonella* counts in the *hilA* group eggs.

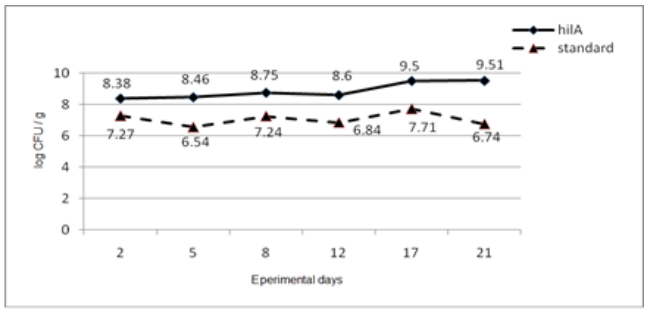

Figure 1. Recovery rate of *Salmonella* (log CFU/g) from embryonated eggs.

In this study, *Salmonella* was detected in the yolk of two group eggs after eight-days of incubation period; Viable counts of *S. enteritidis* parent strain first increased at 2 dpi and apparently decreased gradually over the experimental days and reached to 6.74 log CFU/gr at 21 days of the incubation period (Fig. 1). This decrease of SE in inoculated eggs has been described in the earlier studies. In the *hilA* group eggs, bacterial counts of S.E were generally higher than standard group eggs and tended to slight increase over the experimental days. Mastroeni et al. in a review article indicated that *Salmonella* is able to rapidly multiply in various eukaryotic cell lines, but the proliferation appears to be far less rapid within cells in tissues of infected hosts, indicating a more restrictive situation in vivo (5). The differences in results from in vivo analyses can best be explained by the presence of a large number of additional factors immune responses, different concentration gradients of nutrients in different tissues, (cytokines, etc.) that are often interrelated and affect pathogen survival and replication in host tissue (6).

Conclusion

This study initiates this hypothesis that wild-type strains of *Salmonella*, the same as viruses, kill their host cells after infection and produce low bacteria titer, whereas *hilA* mutants of *Salmonella* with low pathogenicity, replicates along with the host genome without killing the host and achieve

maximum bacteria titers. However, this result has not previously been described in the recent papers.

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Conflict of interests

The authors declare no conflict of interest.

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