Vaccination of laying chickens with an inactivated Salmonella vaccine reduces Salmonella growth in eggs.

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INTRODUCTION

Studies of eggs laid by naturally or experimentally infected hens have shown that contaminated eggs contain few bacteria, these being distributed throughout the yolk and albumin (2,3,4). Thus, given a minimal infectious dose for humans of ca. 1 x 10^5 (5), multiplication of S. Enteritidis in contaminated eggs is a necessary prerequisite for human infection. This multiplication is likely to occur as a consequence of improper storage or inappropriate processing or handling.

In this study the ability of specific anti-Salmonella egg yolk antibody to restrict the multiplication of S. Enteritidis in egg contents is examined using a commercially available vaccine of proven efficacy (1) and of relevance to the current situation in the field.

METHODS AND MATERIALS

Homogenised egg contents were tested for the presence of specific antibodies by enzyme-linked immunosorbent assays (ELISA). LPS and flagella antigen were prepared using standard methods. Antibody titres obtained from eggs from Salenvac T-vaccinated 26 week (+/- 1 week) old hens were given a nominal value of 100% and all other titres are expressed relative to this value.

To determine S. Enteritidis growth in egg contents, eggs were obtained from commercial laying hens of 26 weeks of age (+/- 1wk) vaccinated with Nobilis Salenvac T. As controls, eggs were obtained from unvaccinated specific pathogen-free (SPF) hens and from commercial, age-matched, laying hens vaccinated in the field with a live attenuated vaccine administered via drinking water. Eggs from each flock were pooled and homogenised to mix the yolk and albumin. A fresh overnight culture of S. Enteritidis strain Se857 (6) was diluted in TSB and used to inoculate each 100 ml pool with low numbers of bacteria. Growth of S. Enteritidis in each homogenised egg pool was determined by plating serial decimal dilutions of the culture in TSB onto BGA plates and enumeration of colonies after overnight growth at 37°C. Log_{10} transformed data (cfu/ml) were analysed by Analysis of Variance (ANOVA) using SAS version 8.2 to determine differences between treatment groups.

RESULTS

Abundant antibodies to S. Enteritidis flagella (Fig.1) and LPS antigens (data not shown) were detected in the egg contents from Nobilis Salenvac T vaccinated hens. Antibody levels were relatively unaffected by storage of the eggs for up to 3 weeks at 4°C (Fig.1). Moreover, relative antibody levels in eggs obtained from 63 week old layers were comparable to those observed in eggs from younger birds (Fig.1 and data not shown).

In contrast, S. Enteritidis-specific antibodies were virtually undetectable in the egg contents from unvaccinated SPF hens. Levels of specific antibody in the egg contents from the live vaccine group were marginally higher than the negative control group but remained on average 55-fold (LPS) and 23-fold (flagella) lower than those observed in eggs from the bacterin vaccinated hens (Fig.1).

As naturally infected eggs contain few bacteria, pools of homogenised eggs were seeded with less than 1 cfu/ml (mean 0.19 +/- 0.22). After overnight growth at 37°C, S. Enteritidis grew to a mean density of 1.04 x 10^{10}/ml in the contents of eggs obtained from SPF birds and 3.24 x 10^9/ml in the contents of eggs obtained from flocks that received the live attenuated vaccine (Fig.2). In contrast, S. Enteritidis growth was noticeably reduced (mean density 1.99 x 10^5/ml) in the contents of eggs obtained from the Nobilis Salenvac T vaccinated hens (Fig.2). Statistical analysis of the data revealed a significant reduction in bacterial multiplication in eggs from Salenvac T-vaccinated hens when compared to either unvaccinated SPF hens ($p = 0.0016$) or live vaccine treated hens ($p = 0.0019$). No significant differences in bacterial multiplication were observed in eggs from these latter two treatment groups ($p = 0.96$). Further analysis of the data confirmed the treatment group to be the primary variable affecting growth, with little variability between different flocks in each treatment group (data not shown).

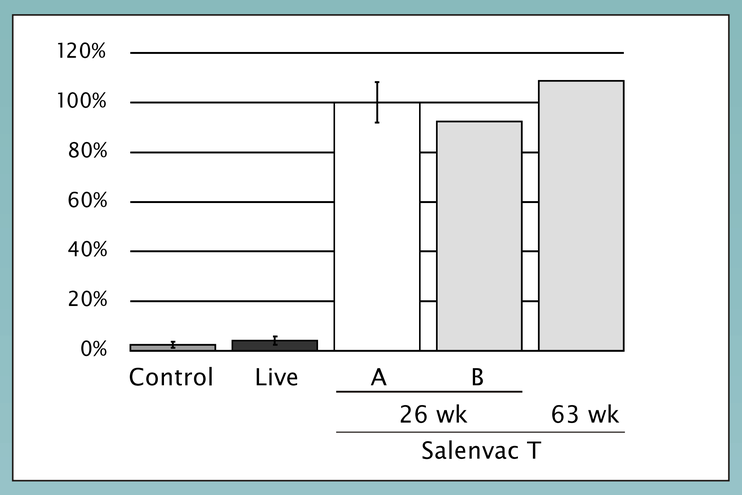

Figure 1: Relative abundance of antibodies to S. Enteritidis flagella antigen in eggs from vaccinated and unvaccinated hens. For Salenvac T vaccinated flocks, antibody levels were determined in eggs from 26 and 63 week old hens. Eggs obtained from 26 week old hens were tested during the first week (A) and during the third week (B) of storage at 4°C.

DISCUSSION

In this study, we found substantial quantities of anti-Salmonella antibodies in eggs from hens vaccinated with Salenvac T and our data suggest a role for this antibody in restricting Salmonella growth in egg contents.

Anti-Salmonella antibody was present throughout the recommended shelf life (3 weeks) of the eggs and similar levels of antibody were detected in eggs from young hens and from 63 wk old hens nearing the end of their laying cycle. In contrast, eggs from hens vaccinated with a commercial live attenuated S. Enteritidis vaccine contained little anti-Salmonella antibody, the levels of antibody in these eggs being essentially indistinguishable from those in eggs obtained from unvaccinated SPF hens.

S. Enteritidis growth was significantly impaired in the eggs from Salenvac T vaccinated hens, whereas the bacteria grew vigorously in eggs from unvaccinated and in eggs from hens treated with a commercial live attenuated S. Enteritidis vaccine. Vaccination with Salenvac T reduced bacterial growth in the homogenised egg contents by more than 99% compared to the unvaccinated or live attenuated vaccine groups. In contrast, there were no significant differences in bacterial growth in eggs from unvaccinated hens or hens treated with a commercial live attenuated S. Enteritidis vaccine.

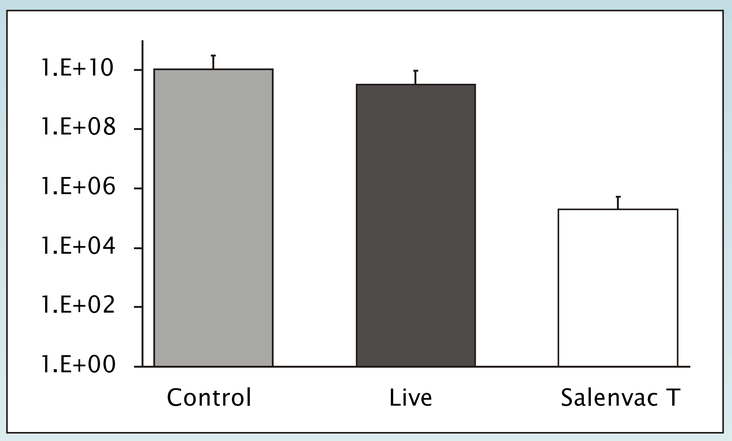

Figure 2: Effect of egg antibodies on growth of S. Enteritidis in homogenised egg contents. (mean of 11 experiments with eggs from at least 3 different flocks per treatment group).

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