INTRODUCTION

For the last 25 yr, Salmonella enterica subspecies enterica serotype Enteritidis has been a continuous worldwide threat to public health because of the ability of this serotype to contaminate eggs (Braden, 2006; EFSA, 2012). Monitoring and control programs have been set up in many countries with varying success. As an example, in the European Union, national control programs have been implemented to reduce the Salmonella contamination on the farm and at the retail level. Although the number of human cases and outbreaks has decreased the last years, and efforts in the poultry industry have been successful in decreasing the flock prevalence, Salmonella Enteritidis still remains a major bacterial pathogen causing a significant fraction of human foodborne disease (EFSA, 2012).

Eggs can be contaminated either on the shell or internally. Intact eggs can become internally contaminated with Salmonella by 2 different routes: (1) penetration of the eggshell during or after oviposition and (2) direct contamination of the internal egg during formation, originating from infection of the reproductive tract (Okamura et al., 2001; De Reu et al., 2006; Gantois et al., 2009a). Although it is difficult to define which egg contamination route is most frequently associated with human food poisoning, some authors claim that internal contamination of eggs after oviduct colonization is the most important route (Gast and Holt, 2000; Gantois et al., 2009a). If it is supposed that internal egg contamination after oviduct colonization is the principal route causing human infections, this would mean that Salmonella Enteritidis would be more capable than other serotypes to (1) colonize the oviduct tissue after systemic spread or (2) survive better in the antimicrobial egg white compartment (or both 1 and 2). Indeed, although strains from many different Salmonella serotypes can be isolated from the litter in layer flocks, most predominantly strains from the Salmonella serotype Enteritidis are found in eggs and are isolated from human cases caused by egg consumption (EFSA, 2007). Several experimental studies demonstrated that, apart from Salmonella Enteritidis, other strains belonging to different serotypes of Salmonella were also able to colonize the oviduct tissue and to contaminate eggs internally, making the oviduct colonization process not the reason for the preferential association of the serotype Enteritidis with eggs (Okamura et al., 2001; Gantois et al., 2008; Gast et al., 2011). Egg white contains numerous antimicrobial molecules and whereas forming eggs in the oviduct can be highly positive for Salmonella, only few laid eggs are positive, indicating a potent antimicrobial activity of egg white (Keller et al., 1997).
Salmonella Enteritidis and Typhimurium have been shown to be more capable to survive in egg white compared with Escherichia coli (Clavijo et al., 2006), but a large study comparing multiple strains from different Salmonella serogroups and serovars has not yet been carried out. Therefore, in the current study, 89 Salmonella enterica subspecies enterica strains from multiple serovars were tested for their ability to survive in egg albumen at 42°C, to evaluate whether strains from the egg-contaminating serotype Enteritidis have a superior egg white survival compared with other serotypes.

MATERIALS AND METHODS

Bacterial Strains

Eighty-nine strains of Salmonella enterica subspecies enterica, belonging to 5 different serogroups, were used in this study. All strains were isolated from poultry, poultry-associated environments or products, or from human food poisoning cases. The strains were kindly provided by the Belgian Reference Laboratory for Salmonella, Animal Health (H. Imberechts, Veterinary and Agrochemical Research Centre, Ukkel, Belgium) and by the Belgian Scientific Institute for Public Health (K. Dierick, WIV, Ukkel, Belgium) and were from poultry and human origin. Twenty-five strains belonged to serogroup B (3 Salmonella Agona, 4 Salmonella Braenderup, 2 Salmonella Derby, 1 Salmonella Heidelberg, 3 Salmonella Paratyphi B var. Java, and 12 Salmonella Typhimurium strains), 28 to serogroup C (2 Salmonella Blockley, 1 Salmonella Bovismorbificans, 4 Salmonella Hadar, 3 Salmonella Indiana, 4 Salmonella Infantis, 1 Salmonella Kentucky, 1 Salmonella Livingstone, 3 Salmonella Mbandaka, 1 Salmonella Montevideo, 3 Salmonella Rissen, and 5 Salmonella Virchow strains), 30 to serogroup D (1 Salmonella Dublin, 1 Salmonella Gallinarum, 1 Salmonella Saintpaul, 1 Salmonella Stanley, and 26 Salmonella Enteritidis strains), 4 to serogroup E (1 Salmonella Give and 3 Salmonella Senftenberg strains), and 2 to serogroup G (1 Salmonella Rubana and 1 Salmonella Worthington strain). The choice of serotypes and serogroups was based on the frequency of isolation (i.e., most isolates from poultry and humans were from serogroups B, C, and D) and partly arbitrarily. As a control, a Salmonella Enteritidis rfbH mutant strain, shown in a previous study not to be able to survive in egg white, was used (Gantois et al., 2009b).

In Vitro Survival of Salmonella in Albumen at 42°C

Freshly laid eggs were obtained from a local farm, which was screened negative for Salmonella by the national monitoring program. Within 24 h after oviposition, egg shells were disinfected by immersion in Lugol solution (Sigma-Aldrich, St. Louis, MO) followed by immersion in 70% ethanol. Egg yolk and albumen were separated and collected into a sterile recipient. Egg albumen from 150 eggs was pooled and mixed during 10 min using a vortex.

All Salmonella isolates were grown overnight in Luria Bertani broth (LB, Difco, Sparks, MD) at 37°C while shaking at 125 rpm, centrifuged at 1,800 x g for 10 min at 4°C, and resuspended in PBS to ensure that all LB medium was removed before inoculation in egg albumen. Serial dilutions of the suspensions were made on LB agar to determine the number of cfu per milliliter. The suspension was stored overnight at 4°C and further diluted with PBS to a concentration of 10^4 cfu/mL. Two hundred microliters was then added to 1.8 mL of egg albumen to obtain a final concentration of approximately 1 × 10^3 cfu/mL. This concentration is used because at higher concentrations the albumen loses its bactericidal activity (Kang et al., 2006; Gantois et al., 2008). From each bacteria-egg albumen mixture, 150 µL was incubated in triplicate in a 96-well plate at 42°C for 24 h. After incubation, enrichment was performed by adding 150 µL of LB broth to each well. The 96-well plates were further incubated for 24 h at 37°C. Thereafter, 1 drop of 20 µL of each well was plated on LB agar and incubated for 20 h at 37°C. The measured parameter was thus whether or not at least 1 cfu could be detected in 3 × 20 µL of the final suspension of each strain. The experiment was performed in triplicate with fresh bacterial cultures.

Statistical Analysis

To avoid cells with zero observations and subsequent computational difficulties, for each category one observation was added to both the positive and negative cell. Based on this data set, data were analyzed using logistic regression analysis with SPSS 20.0 (SPSS Inc., Chicago, IL). Statistical results were considered to be significant when P-values were lower than 0.05.

RESULTS AND DISCUSSION

Egg white is a nonfavorable medium for bacterial growth because of its antimicrobial characteristics, including its alkaline pH, iron deficiency, and the presence of a wide variety of antibacterial compounds, of which ovotransferrin and lysozyme are the most abundant (Baron et al., 2004; Kang et al., 2006). Because forming eggs in the oviduct of Salmonella infected laying hens are more frequently positive for Salmonella whereas laid eggs are mostly negative, it was suggested by Keller et al. (1997) that the survival capacity of Salmonella Enteritidis in egg albumen at 42°C may have contributed to the worldwide emergence of Salmonella Enteritidis as important cause of human salmonellosis. In some small-scale studies, it was shown that specific Salmonella strains can survive in egg white at 42°C, whereas the tested Escherichia coli strains were not able to survive (Okamura et al., 2001; Lu et al., 2003; Guan
et al., 2006; Gantois et al., 2008). A large comparison on the survival of Salmonella serotypes and strains in egg albumen was still missing. In the current study, 89 Salmonella strains from different serogroups and serotypes were incubated for 24 h in egg white at chicken body temperature (42°C) and tested for their survival. Table 1 shows the ratio of the total number of positive Salmonella strains from different serogroups and serotypes and strains in egg white for 24 h at 42°C.

Table 1. The number of Salmonella positive egg albumen samples is shown relative to the total number of incubated egg albumen samples, after incubation of Salmonella strains belonging to different serotypes and serogroups in egg white for 24 h at 42°C^1

<table>
<thead>
<tr>
<th>Serotype/serogroup</th>
<th>Number of Salmonella positive albumen samples/total samples (%)</th>
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<tbody>
<tr>
<td>Serogroup B</td>
<td>8/75b (10.67)</td>
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<tr>
<td>Serogroup B isolates excluding Salmonella Typhimurium</td>
<td>0/39b (0)</td>
</tr>
<tr>
<td>Salmonella Typhimurium isolates</td>
<td>8/36b (22.22)</td>
</tr>
<tr>
<td>Serogroup C</td>
<td>4/84b (4.76)</td>
</tr>
<tr>
<td>Serogroup D</td>
<td>41/90b (45.56)</td>
</tr>
<tr>
<td>Serogroup D isolates excluding Salmonella Enteritidis</td>
<td>3/12 (25)</td>
</tr>
<tr>
<td>Salmonella Enteritidis isolates</td>
<td>38/78b (48.72)</td>
</tr>
<tr>
<td>Serogroup E+G</td>
<td>0/18b (0)</td>
</tr>
</tbody>
</table>

^1Values are statistically significant different from the results of the group consisting of Salmonella Enteritidis isolates (P < 0.05).

^bValues are statistically significant different from the results of the group consisting of Salmonella Typhimurium isolates (P < 0.05).

^The results are the sum of 3 independent experiments. The threshold of detection was whether or not at least 1 cfu was detected in 60 µL of the egg white suspension after enrichment.

All these data add evidence to the hypothesis that egg white survival is one of the reasons why Salmonella Enteritidis is more predominantly isolated from contaminated eggs, and helps explain why most reported egg-borne Salmonella outbreaks in humans are caused by Salmonella Enteritidis and in some cases Salmonella Typhimurium.

REFERENCES


