Research Notes

Genetic Line Differences in Survival and Pathogen Load in Young Layer Chicks after *Salmonella enterica* Serovar Enteritidis Exposure[1]

M. G. Kaiser and S. J. Lamont[2]

Department of Animal Science, Iowa State University, Ames Iowa 50011-3150

ABSTRACT Early infection may result in long-term colonization of layers with *Salmonella enterica* sv. *enteritidis* (*S. enteritidis*, SE), resulting in shedding into table or hatching eggs. To evaluate genetic factors underlying early response to SE, genetic line differences in mortality and pathogen load at two sites (cecal lumen and spleen) were investigated. At day of hatch, chicks of four genetic lines were intra-esophageally inoculated with one of three doses of SE phage type 13a. There was a significant effect ($P < 0.001$) of genetic line on chick 6-d survival. The effect of genetic line was significant ($P < 0.05$) on survivors' SE burden in cecal content but not on SE burden per gram of spleen. The SE pathogen load of the spleen and the cecal content were not significantly correlated, indicating that independent host mechanisms are partly responsible for these two traits. Genetic line differences in chick survival and SE colonization of cecal content were demonstrated in young layer chicks.

(*Key words*: layer chick, *Salmonella enteritidis*, bacterial colonization, survival)

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INTRODUCTION

In the United States, an estimated 500,000 cases of human illness are annually attributed to *Salmonella enterica* sv. *enteritidis* (*S. enteritidis*, SE)-contaminated food products (Schlosser and Ebel, 1998). The primary route for salmonellae transmission in poultry is vertical transmission via contaminated eggs, but lateral transmission also occurs via contaminated feed, water, and facilities or via host reservoirs such as wild birds, rodents, pet, and humans (Hafez, 1999). The SE serotypes colonized at an early age are maintained throughout adulthood in layer chickens (Gast and Holt, 1998). SE contamination in layer hens can result in decreased egg production, and furthermore, the bacteria can contaminate the eggs laid (Gast and Beard, 1990). Gast and Holt (1998) have suggested that reduction of SE pathogen load in chicks reduces the SE-contaminated eggs produced by the hens.

Management and pharmaceutical approaches such as vaccination, competitive exclusion, and antibiotic treatments can help reduce the SE burden in poultry (Gast, 1999). Additional reduction in pathogen load in poultry may be obtainable through genetic selection for disease resistance (Lamont, 1998). Previous studies have demonstrated the polygenic nature of disease resistance to SE in poultry (e.g., Bumstead and Barrow, 1993; Lindell et al., 1994; Protais et al., 1996; Cotter et al., 1998). Others (e.g., Gorham et al., 1991; Guillot et al., 1995; Beaumont et al., 1999) have measured mortality and frequency of colonization in young chicks, but little is known regarding the quantifiable pathogen load of SE in the internal organs. Genetic factors involved in early resistance and immune response in layer chicks are not well understood. The objective of this study was to evaluate genetic line effect on survival and pathogen load after SE exposure in young layer chicks.

MATERIALS AND METHODS

Experimental Animals

We used 144 chicks from four layer lines, equally represented for sex and genetic line. Three pure lines and one experimental cross were used. The experimental cross was produced from the three pure line strains. All lines were from the same commercial egg-layer breeding company. To ensure that the maternal immune status of all hens producing the chicks was equivalent and would not interfere with testing their chicks for salmonella response, all hens were kept under the same biosecure management conditions and were from breeding flocks that were tested weekly for freedom from *Salmonella* spp. The chicks were equally divided, with regard to genetic line, sex, and

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[2]To whom correspondence should be addressed: sjlamont@iastate.edu.

Abbreviation Key: BGA = brilliant green agar; LB = Luria-Bertani; SE = *Salmonella enteritidis*.

inoculation dose, into three biosafety level-two animal rooms. The chicks were given access to water and food ad libitum, meeting or exceeding all National Research Council (1994) requirements. The feed contained standard feed additive amounts of Amprolium (0.0125%).[3] All procedures were approved by the Institutional Animal Care and Use committee.

SE Inoculation and Challenge

The SE phage type 13a[4] was brought into the exponential growth phase by incubating in Luria-Bertani (LB) broth for approximately 3.5 h at 37 C with shaking. Inoculation dose dilutions of SE were made based on a concentration estimated from the optical density at 600 nm applied to an SE growth curve regression equation. Actual SE inoculation doses were then confirmed by serial plate dilutions of the inoculum. Chicks (d of hatch) were intraesophageally inoculated with SE via a syringe equipped with an infusion teat. Each chick received one of three inoculation doses (1×10^3, 1×10^5, or 1×10^7 cfu/chick in 0.25 mL of LB broth). Chicks were monitored twice daily for clinical expression of disease. Morbid chicks were euthanized by cervical dislocation.

All 99 birds surviving to Day 6 were euthanized (approximately half each on Days 6 and 7) to determine SE burden in spleen tissue and cecal content. The spleen and one cecum were aseptically removed and rinsed with sterile PBS. The spleen was aseptically weighed and minced with a sterile scalpel.

Bacteriology

The spleen (1 g/10 mL) and cecal content (sterile swab collection of content from 2.5-cm section/10 mL) were enriched in selenite broth,[5] for 24 h at 37 C. Each enrichment culture was screened for presence or absence of SE (plus-minus screening) by plating on brilliant green agar (BGA)[5] plates containing 100 μg/mL nalidixic acid[6] and then incubating for 24 h at 37 C. If colony morphological identification of SE was questionable, the colony identity was confirmed by *Salmonella* antiserum group D agglutination.[5] The enrichment cultures were held at 4 C during the plus-minus screening. For individuals with plus-minus plates that showed SE growth after 24 h at 37 C, the enrichment cultures were then serially 10-fold diluted in selenite broth, and three different dilutions were plated in duplicate on BGA plates for bacteria quantification. After 24 h at 37 C, the colonies on the serial dilution plates were counted.

Statistical Analysis

All analyses were performed by using tests contained in the JMP® statistical program package (SAS Institute, 1995). Six-day survival (alive vs. euthanized or dead) with variables of genetic line, inoculation dose, and sex were analyzed by contingency tables (2×4, 2×3, and 2×2, respectively). Effects of interactions on survival were tested by the Wald method. The numbers of SE colonies $\times 10^{-6}$ were used for analysis. Samples exhibiting no SE growth on plus-minus plates were designated as zero SE and were included in the analysis. Minor room effects on SE colony counts were adjusted as described by Yonash et al. (1999). ANOVA was performed with variables of genetic line, sex, and inoculation dose and with all two- and three-way interactions. Pair-wise comparison rankings were performed by contrast. Pearson's correlation test was used to determine the correlated relationship between spleen and cecal content SE pathogen load. If not otherwise indicated, $P \leq 0.05$ was considered significant.

RESULTS

Pre-Exposure Scans for SE in Chicks and Environment

Environmental scans of the animal and laboratory rooms, bedding, water, and feed confirmed the absence of environmental SE prior to the experimental exposure. Four chicks (one from each genetic line) were euthanized prior to inoculation with SE, and bacterial cultures of spleen and cecal contents confirmed them to be SE free.

Chick Survival after SE Challenge

All 144 chicks were included in the survival analysis. Chicks were categorized as alive or dead (euthanized and spontaneous mortality) at 6 d. The highest inoculation dose of SE (1×10^7 cfu/bird) had a significantly ($P < 0.03$) lower chick survival than the other two inoculation doses. Survival for SE inoculation doses of 1×10^3, 1×10^5, and 1×10^7 cfu/bird were 79.2, 72.9, and 54.2%, respectively. There was no significant effect of sex, interaction of sex with either inoculation dose or genetic line, or interaction of inoculation dose with genetic line on chick survival; therefore, inoculation doses and sexes were pooled for analysis of genetic line effect. There was a significant effect ($P < 0.001$) of genetic line on chick 6-d survival post-SE challenge. Survival rates were 47.2, 72.2, and 55.6% in the three pure lines (Lines 1, 2 and 3, respectively) and 100% in the experimental three-way cross line (Line 4).

SE Burden of Cecal Content and Spleen

The SE pathogen load was quantified in the 99 chicks that survived to Day 6, at which time the chick numbers were no longer equal for genetic line, sex, inoculation dose, or room. There was no significant effect of inoculation dose or sex on SE numbers in the cecal content or spleen. Data of all three inoculation doses and both sexes were, therefore, pooled for analysis of genetic line effect and adjusted for room effect. Genetic line had a significant effect ($P < 0.05$) on SE burden in the cecal content but not

[3]Purina Mills Inc., St. Louis, MO 63166.
[4]Gift of H. M. Opitz, University of Maine, Orono, ME 04469.
[5]DIFCO, Detroit, MI 48232.
[6]Sigma, St. Louis, MO 63178.

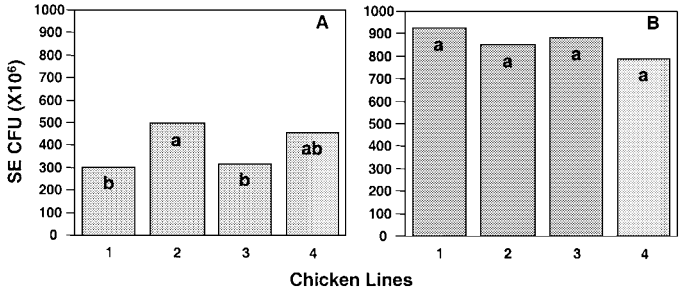

FIGURE 1. *Salmonella enterica* serovar *enteritidis* pathogen load of cecal content (A) and spleen (B) by genetic line. Lines 1 (n = 17), 2 (n = 26), and 3 (n = 20) are pure layer lines. Line 4 (n = 36) is an experimental three-way layer cross. Bars not sharing letters (a, b) are significantly different at $P < 0.05$.

the spleen tissue (Figure 1). The SE pathogen loads in the cecal content and the spleen tissue were not correlated (r = 0.077, $P = 0.457$).

DISCUSSION

Early contamination of young layer chicks has long-lasting negative impact on egg production and food safety (Gast and Holt, 1998). The role of genetics in reducing the SE pathogen load in young chicks is not clearly understood. In this study, we demonstrated a significant effect ($P < 0.001$) of genetic line on young layer chick 6-d survival after SE challenge. Guillot et al. (1995) have also reported varied degrees of genetic line effects on resistance to SE-induced mortality rate of young chicks. They ranked the chicks from broiler, layer, and experimental lines according to the level of SE resistance to i.m. SE challenge, as evaluated by lethal dose 50 results at 15-d postchallenge. The three commercial layer lines and one experimental White Leghorn line in the study of Guillot et al. (1995) were all ranked as susceptible (lethal dose $50 \leq 10^2$ cfu/bird) to SE relative to the other lines in the study. The current study demonstrates a wide range of genetic line responses in survival of young layer chicks to oral SE challenge. The experimental three-way cross had survival significantly superior to each of the pure lines, consistent with expression of heterosis for this trait.

Inoculation dose may affect pathogenicity of SE and the resulting resistance or susceptibility categorization of young chicks (Guillot et al., 1995). In the current study, inoculation dose had a significant effect on chick 6-d survival but not on SE burden of the cecal content or spleen tissue of survivors. This result suggests that a wide range of exposure doses might all result in sustained colonization of survivors.

There are conflicting reports regarding the effect of genetic line on resistance to SE colonization and on which internal organs are differentially colonized (Lindell et al., 1994; Guillot et al., 1995; Protais et al., 1996; Girard-Santosuosso et al., 1998). In layer hens, a significant genetic line difference in SE isolation from eggs is consistently reported; however, significant differences in cecal colonization have been observed in some studies (Protais et al., 1996) and not in others (Lindell et al., 1994). By using identical chicken lines and SE strain, Guillot et al. (1995), Protais et al. (1996), and Girard-Santosuosso et al. (1998) each reported a different ranking of resistance among genetic lines. The three studies differed most notably in age of exposure as well as route and dose of inoculation, all of which might have contributed to the apparent differences in resistance rank (Girard-Santosuosso et al., 1998). Despite variation in experimental protocols, chicken genetic line is clearly a contributing factor in the current study as well as in previous studies on SE resistance.

In the current study, there were significant genetic line differences in the surviving chicks for SE burden in the cecal content but not the spleen tissue. Cecal content and spleen SE burden were not correlated, indicating independent host genetic mechanisms are partly responsible for pathogen load at the two sites. The SE quantification data did not include chicks that died or that were euthanized before Day 6. The data set, therefore, does not include some of the presumably more heavily SE-burdened chicks and, as such, is probably an underestimate of SE pathogen load in susceptible chicks.

Duchet-Suchaux et al. (1995) reported that high mortality rate in SE-challenged, 1-d-old chicks was incompatible with a persistent carrier state, thus suggesting that susceptibility to mortality is directly related to carrier state potential. In contrast, Gast and Beard (1992) were unable to establish a strong correlation between chick mortality and frequency of production of SE-contaminated eggs. A previous study also indicated that the extent and persistence of internal organ bacterial load are not direct factors related to SE-contaminated egg production (Gast and Beard, 1990).

Future SE contamination control mechanisms in poultry may be basically the same as are currently being utilized, but for long-term sustainability, genetic resistance should be pursued (Hafez, 1999). The agricultural industry should be prepared for the potential phasing out of antibiotics for use in controlling bacterial pathogens (Ferber, 2000). Previous studies have identified genetic line differences in resistance of layer hens (e.g., Lindell et al., 1994; Protais et al., 1996; Cotter et al., 1998) and young chicks (Guillot et al., 1995) to SE challenge. A heritability estimate of 0.20 for SE burden of enriched cecum culture was reported for 1-wk-old chicks that were orally inoculated with SE phage type 4 (Berthelot et al., 1998). The current study is unique in that it quantified SE burden from enrichment cultures of the gastrointestinal tract and internal organ (cecal content and spleen tissue, respectively) of young layer chicks. The current study demonstrated significant differences among genetic lines in resistance to SE-induced mortality and in the SE burden in cecal contents of young layer chicks. Sufficient genetic line variation exists to suggest that it is feasible to effectively choose among pure breeder lines for those that exhibit reduced SE-induced mortality and cecal content SE pathogen load in young layer chicks.

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