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Genetic Capability of Young Layers for Survival after *Salmonella entritidis* Challenge

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Abstract: Problem statement: Genetic line differences in resistance of layer hens and young chicks to *Salmonella entritidis* have been identified through a lot of studies. That is why the agricultural industry was prepared for the potential phasing out of antibiotics for use in controlling bacterial pathogens. Early infection may result in long term colonization of layers with *Salmonella entritidis*, resulting in shedding into table or hatching eggs. **Approach:** This study was carried to evaluate the genetic factors underlying early response to *Salmonella entritidis*, 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 *Salmonella entritidis* phage type 13 A. **Results:** There was a significant effect ($p \leq 0.001$) of genetic line on chick 6 days survival. The effect of genetic line was significant ($p \leq 0.05$) on survivors' *Salmonella entritidis* burden in cecal content but not on *Salmonella entritidis* load per gram of spleen. *Salmonella entritidis* 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. **Conclusion:** Future *Salmonella entritidis* control mechanism in poultry may be the same as it is used these days but it has to be for longer term sustainability, genetic resistance should be pursued. Sufficient genetic line variation should exists to suggest that it is feasible to effectively choose among pure breeder lines for those exhibits reduced *Salmonella entritidis* induced mortality and cecal content *Salmonella entritidis* pathogen load in young layer chicks.

Key words: Susceptibility, layers, *Salmonella enteritidis*, genetics

INTRODUCTION

In the United States, an estimated 500,000 cases of human illness are annually attributed to *Salmonella entritidis* contaminated food products[17]. 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[13]. The *Salmonella entritidis* serotypes colonized at an early age are maintained throughout adulthood in layer chickens[8]. *Salmonella entritidis* contamination in layer hens can result in decreased egg production and furthermore, the bacteria can contaminate the laid eggs[6]. Gast[9] has suggested that reduction of *Salmonella entritidis* pathogen load in chicks reduces the *Salmonella entritidis* contaminated eggs produced by the hens. Management and pharmaceutical approaches such as vaccination, competitive exclusion and antibiotic treatments can help reduce the *Salmonella* burden in poultry[9]. Additional reduction in pathogen load in poultry may be obtainable through genetic selection for disease resistance; Lamont[14]. Previous studies have demonstrated the polygenic nature of disease resistance to *Salmonella entritidis* in poultry; Bumstead and Barrow[3,4,12,15,16]. Others Gorham *et al*.[1,11] have measured mortality and frequency of colonization in young chicks, but little is known regarding the quantifiable pathogen load of *Salmonella entritidis* in the internal organs. Genetic factors involved in early resistance and immune

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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 *Salmonella entritidis* exposure in young layer chicks.

MATERIALS AND METHODS

Birds: Two hundred 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. 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 biosecurity measures and mangemental conditions and were from breeding flocks that were tested weekly for freedom from *Salmonella* sp. The chicks were equally divided, with regard to genetic line, sex and inoculation dose, into three biosafety level two animal rooms. The chicks were given access to water and food *ad libitum*. The feed contained standard feed additive amounts of Amprolium (0.0125%).

***Salmonella entritidis* challenge:** The *Salmonella entritidis* phage type 13A was brought into the exponential growth phase by incubating in Luria-Bertani (LB) broth (Fisher Sci) for approximately 4 h at 37°C. Inoculation dose dilutions of *Salmonella entritidis* were made based on a concentration estimated from the optical density at 600 nm. Actual *Salmonella entritidis* inoculation doses were then confirmed by serial plate dilutions of the inoculum. Chicks were intraesophageally inoculated with *Salmonella entritidis* 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 sacrificed. All 99 birds surviving to day 6 were euthanized to determine *Salmonella Entritidis* burden in spleen tissue and cecal content. The spleen and one cecum were aseptically removed and rinsed with sterile PBS (Ambion, Fisher Int.). The spleen was aseptically weighed and minced with a sterile scalpel.

Bacteriological analysis: The spleen (1 g/10 mL) and cecal content (sterile swab collection of content from 3 cm section/10 mL) were enriched by adding the samples into tryptic soy broth (Remel, Fisher Int.) and incubated at 37°C for 18-20 h. One loopful was taken and inoculated into Tetrathionate-iodine broth (BD Diagnostic System, Fisher Int.) and then incubated at 27°C for 20-24 h. After incubation a loopful is streaked on XLT4 medium plates (BD Diagnostic System, Fisher Int.) containing 100 μg mL^{-1} nalidixic acid and incubated at 37°C for 20-24 h. If colony morphological identification of *Salmonella entritidis* was questionable, the colony identity was confirmed by Salmonella antiserum group D agglutination (BD Diagnostic System, Fisher Int.). The enrichment cultures were held at 4°C during the plus-minus screening.

Statistical analysis: All analyses were performed by using SAS 9.2.0. Effects of interactions on survival were tested by the Wald method. The numbers of *Salmonella entritidis* colonies × 10^{-6} were used for analysis. Minor room effects on *Salmonella entritidis* colony counts were adjusted[18]. ANOVA was performed with variables of genetic line, sex and inoculation dose and with all two and three way interactions. Pearson's correlation test was used to determine the correlated relationship between spleen and cecal content *Salmonella Entritidis* pathogen load. If not otherwise indicated, $p\leq0.05$ was considered significant.

RESULTS

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

Layers survival post-challenge: All 200 chicks were included in the survival analysis. Chicks were categorized as alive or dead (euthanized and spontaneous mortality) at 6 days. The highest inoculation dose of *Salmonella Entritidis* (1×10^7 CFU/bird) had a significantly ($p\leq0.03$) lower chick survival than the other two inoculation doses.

Survival for *Salmonella entritidis* 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 ($p\leq0.001$) of genetic line on chick 6 days survival post *Salmonella entritidis* challenge. Survival rates were 47.2, 72.2 and 55.6% in the three pure lines (Line 1-3, respectively) and 100% in the experimental three way cross line (Line 4).

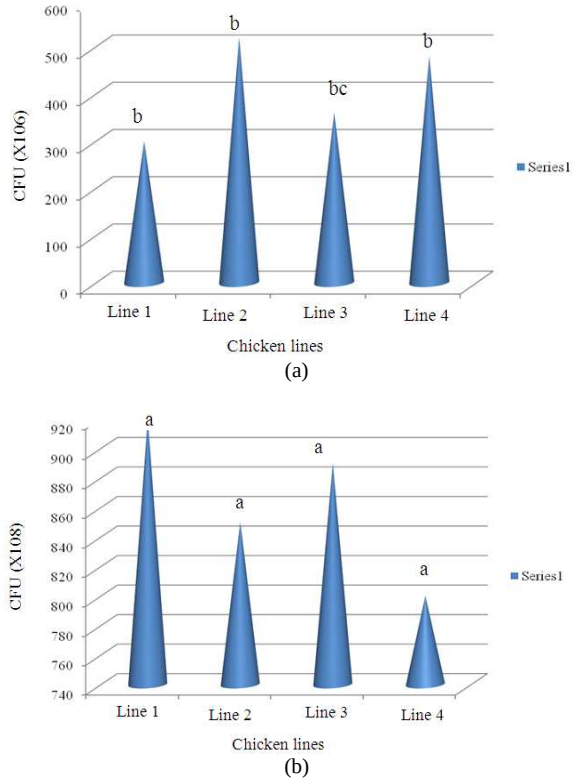

Fig. 1: *Salmonella entritidis* pathogen load of cecal content A. liver (a) and B. spleen (b) by genetic lines

Bacterial count in ceca and spleen: The *Salmonella entritidis* pathogen load was quantified in the 135 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 *Salmonella entritidis* 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 ($p \leq 0.05$) on *Salmonella entritidis* burden in the cecal content but not the spleen tissue, Fig. 1. The *Salmonella entritidis* pathogen loads in the cecal content and the spleen tissue were not correlated ($r \leq 0.077$, $p \leq 0.457$).

DISCUSSION

The role of genetics in reducing the *Salmonella entritidis* 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 days survival after *Salmonella entritidis* challenge. Guillot *et al*.[12] 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 *Salmonella entritidis* resistance to IM *Salmonella entritidis* challenge, as evaluated by lethal dose 50 results at 15 days post-challenge. The three commercial layer lines and one experimental White Leghorn line in the study were all ranked as susceptible (lethal dose $50 \leq 10^2$ CFU/bird) to *Salmonella entritidis* 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 *Salmonella entritidis* 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 *Salmonella entritidis* and the resulting resistance or susceptibility categorization of young chicks. In the current study, inoculation dose had a significant effect on chick 6 days survival but not on *Salmonella entritidis* 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 *Salmonella entritidis* colonization and on which internal organs are differentially colonized[10,12,15,16].

Despite variation in experimental protocols, chicken genetic line is clearly a contributing factor in the current study as well as in previous studies on *Salmonella entritidis* resistance. In the current study, there were significant genetic line differences in the surviving chicks for *Salmonella entritidis* burden in the cecal content but not the spleen tissue. Cecal content and spleen *Salmonella entritidis* burden were not correlated; indicating independent host genetic mechanisms are partly responsible for pathogen load at the two sites. Duchet-Suchaux *et al*.[5] reported that high mortality rate in *Salmonella entritidis* challenged, 1 day 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[7] were unable to establish a strong correlation between chick mortality and frequency of production of SE-contaminated eggs. Future *Salmonella entritidis* 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[13]. A heritability estimate of 0.20 for *Salmonella entritidis* burden of enriched cecum culture was reported for 1 week old chicks that were orally inoculated with *Salmonella entritidis* phage type 4[2].

CONCLUSION

The current study was able to quantified *Salmonella entritidis* laod from enrichment cultures of the gastrointestinal tract (cecal content) and internal organ (spleen tissue) of young layer chicks.

The current study demonstrated significant differences among genetic lines in resistance to SE-induced mortality and in the *Salmonella entritidis* burden in cecal contents of young layer chicks.

The genetic line variation exists to effectively choose pure breeder lines that characterized with exhibition of reduced *Salmonella entritidis* load.

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