Variation in Avian Pathogenic *Escherichia coli* Colonization Levels in Chickens

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Summary and Implications

Colonization levels in five tissues after avian pathogenic *Escherichia coli* (APEC) inoculation were investigated in chickens to generate phenotypic data for a genome wide association study (GWAS). Bacterial loads were measured in 370 birds and varied among individuals and tissues. Mean bacterial levels were significantly different between tissues (right lung > spleen > left lung and liver > blood). There were also significant correlations in bacterial load between tissues. These data suggest that colonization levels could be used as phenotypes in GWAS and could help identify markers associated with poultry resistance to APEC infections. After verification, these markers could be used for genetic selection for more resistant chickens.

Introduction

Extra-intestinal infections with avian pathogenic *Escherichia coli* in poultry lead to multimillion dollar losses for the US poultry industry each year. Exposure to APEC occurs primarily through the respiratory tract, followed by systemic colibacillosis and, in severe cases, septicemia and death. Due to the diversity of APEC strains, most vaccines provide only homologous protection from infection. In contrast, increasing host resistance to APEC colonization could provide broader protection against multiple serotypes. For this to be possible, markers for genomic selection need to be developed. This study was designed to measure APEC colonization in an advanced intercross line (AIL) of chickens and generate phenotypic data for a GWAS. The AIL was derived from a cross between an outbred broiler line and an inbred disease-resistant Fayoumi line. The GWAS will use genetic variation to link regions of the genome to higher resistance to APEC and is expected to identify candidate genes and selection markers.

Materials and Methods

Animals

The F_{24} generation of the AIL (n = 324 chicks) was used in this study; chickens from the broiler (n = 23) and Fayoumi (n = 23) parental lines were also included. Birds were obtained on day of hatch and blood was collected for genotyping at 10 days of age.

Bacterial Challenge and Sample Collection

At 14 days of age, chicks were inoculated with APEC O1:K1:H7 by injection of $1x10^7$ colony forming units (CFU) into the right air sac. After one day of exposure, blood was collected and each bird euthanized. Tissue samples from the liver, spleen, left lung, and right lung were then collected.

Bacteriology

Ten-fold serial dilutions of blood and homogenized tissue samples were spread on MacConkey agar plates and incubated overnight at 37 °C. Bacterial colonies on each plate were counted the following day. Log transformed CFU per gram of sample ($\log_{10}$(CFU/g)) were used for statistical analysis in JMP Pro 11.0.0 software.

Results and Discussion

Colony counts were obtained from 1,573 tissue samples and ranged from 1-11.8 $\log_{10}$(CFU/g). Within each tissue, bacterial loads were widely distributed between samples (Figure 1) and covered at least 2 standard deviations from the mean. Bacterial loads were distributed similarly in each line, illustrating that the AIL captures the range of variation in the parental lines. Significant differences in the least-squares mean (lsmean) were identified between tissues (Figure 1), but not between lines. On average, right lung samples had significantly higher levels of APEC, followed by spleen samples, than left lung or liver samples. Blood had significantly lower bacterial loads than any other tissue. However, levels of APEC in each tissue were positively correlated with all other tissues (0.35-0.64; Table 1).

These data suggest that bacterial loads are variable but related phenotypes and represent differences in APEC colonization levels between individuals and between tissues. Genotypes will be generated for these birds using the Affymetrix Axiom 600K Chicken Genotyping array. Together the genotypic and phenotypic data will be used for GWAS to identify genomic regions involved in resistance to APEC colonization. Markers and candidate genes in these regions will provide targets to improve poultry health during colibacillosis.

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Figure 1. Distribution of bacterial load in each tissue. Least-squares means (lsmeans) are shown by red circles. Different letters indicate tissues with significantly different lsmeans (p-values < 0.02).

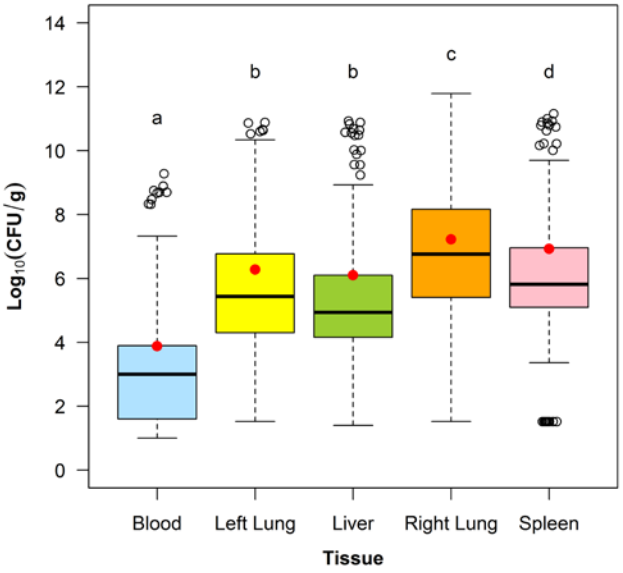

Table 1. Correlation in bacterial load across tissues[1].

Tissue	Left Lung	Liver	Right Lung	Spleen
Blood	0.55	0.55	0.35	0.64
Left Lung		0.44	0.54	0.42
Liver			0.42	0.63
Right Lung				0.42

[1] Pearson pairwise correlations based on $\log_{10}$(CFU/g). All correlations are significant (p-value < 0.0001).