Research Note: MHCY haplotype impacts *Campylobacter jejuni* colonization in a backcross [(Line 6_1 x Line N) x Line N] population

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ABSTRACT MHCY is a candidate region for influencing immune responses in chickens. MHCY contains multiple specialized, polymorphic MHC class I loci along with loci belonging to 4 additional gene families. In this study, MHCY haplotypes were tested for association with cecal colonization after *Campylobacter jejuni* infection of a backcross [(Line 6_1 × Line N) × Line N] population derived from 2 White Leghorn research lines, Line 6_1 and Line N, that were previously shown to exhibit heritable differences in colonization. Samples were obtained for 51 birds challenged with 10^8 CFU *Campylobacter jejuni* at 3 wk of age. Viable *C. jejuni* in the ceca were enumerated 5 d postinfection and counts were log-transformed for analysis. Birds were assigned to either low or high colonization groups based on the individual count being below or above the mean bacterial count for all birds. The mean bacterial count of the low infection group differed significantly from the high infection group. Sex and MHCB haplotype had similar distributions within the 2 groups. Overall, 7 MHCY haplotypes were found to be segregating. Two were significantly associated with *C. jejuni* colonization. MHCY *Y18* was associated with low colonization ($P = 3.00 \times 10^{-5}$); whereas MHCY *Y11a* was associated with high colonization ($P = 0.008$). The MHCY haplotype impacted the mean bacterial count among all birds with MHCY *Y18* having the lowest bacterial count compared with MHCY *Y11a* and all other MHCY (*Y5, Y7, Y8, Y11b*, and *Y11c*) haplotypes. These findings support further investigation of the contribution of chicken MHCY in resistance to *Campylobacter* colonization.

Key words: MHCY, *Campylobacter jejuni*, cecal colonization, Line 6_1, Line N

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INTRODUCTION

Campylobacter infection is a major cause of foodborne illness in humans. Poultry meat products can carry *Campylobacter* that can cause human disease due to improper food preparation. New methods are needed to reduce *Campylobacter* colonization of chickens in order to limit human illness. Genetic selection is considered a possible option for reducing the level of *Campylobacter* in chickens raised for food. Multiple studies provide evidence that chicken genetics influence *Campylobacter* colonization, but the genes of greatest influence still need to be identified. A selective breeding study carried out with commercial grandparent broiler lines established the role of a major gene in cecal colonization by *C. jejuni* (Stern et al., 1990). The role for chicken genetics in *Campylobacter* colonization was further demonstrated in a study of 4 white leghorn chicken research lines (Lines 6_1, 7_2, 15I, and N) (Boyd et al., 2005). This study showed a single autosomal dominant locus was likely influencing the level of *Campylobacter* colonization. Line 6_1 carried a lower number of bacteria compared to lines N, 7_2 and 15I. MHCB was excluded as a major contributing region since Line 6_1 and Line 7_2 have the same MHCB type (*B2*). A subsequent study involving *C. jejuni* challenge of a backcross [(Line 6_1 x Line N) x Line N] population and an advanced intercross of Line 6_1 and Line N revealed several quantitative trait loci and implicated pathways and networks governing immune responses in resistance to colonization (Psifidi et al., 2016). Recently, transcriptomic analyses of the cecal tonsils of Line 6_1 and Line N chickens

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identified differential gene expression for MHCY, MHCB, and genes encoding antimicrobial peptides (Russell et al., 2021). Higher MHCB and MHCY gene expression occurred in Line N. Overall, these data suggest that the outcome of *Campylobacter* infection may be linked to intrinsic differences in immune responses in genetically different chickens with MHC genes as possible contributors. This idea is further supported by a recent genome-wide association study of 2,750 broilers in which high quality genotypes, cecal transcriptomic data and cecal colonization phenotypes were available, and in which QTL on chromosome 16 and differentially transcribed genes within the MHCB locus, as well as *cis*-acting variation in MHCB class I and II and BG genes, were identified (Psifidi et al., 2021).

MHCY is a complex polymorphic gene region on chicken chromosome 16. MHCY haplotypes vary both in gene sequence and in gene number. The contribution of the MHCY gene region to immune responses and disease resistance has not been rigorously tested. Until recently the complex nature of the MHCY region made it extraordinarily difficult to define MHCY genotypes for large numbers of birds. Previously restriction fragment patterns in Southern hybridizations were the only means available for MHCY typing. Typing is easier now with a PCR method based on short tandem repeat (**STR**) sequences (Zhang et al. 2020). The STR patterns reliably reflect haplotypes assigned by restriction fragment patterns. MHCY haplotypes can now be scored more readily for large sample sets.

MHCY is located near MHCB on chicken chromosome 16, but it is separated from MHCB by a region supporting highly frequent recombination. MHCY and MHCB haplotypes assort as if located on separate chromosomes (Miller and Taylor, 2016). MHC class I-like genes in MHCY are highly polymorphic, encoding molecules possibly binding lipid ligands. Different MHCY haplotypes contain different numbers of MHCY class I loci. It is reasonable to consider MHCY class I genes as a genetic source contributing to the immune response exhibited by different chickens in response to invading microbes. Members of additional gene families are also present in MHCY (Miller and Taylor, 2016) and are also candidate contributors to the immunogenetics of the MHCY region. Early tests for MHCY effects on viral diseases showed mixed results. Rous sarcomas and Marek's disease were affected by MHCY in some experiments but not in others (Miller and Taylor, 2016). We focused on individuals in the backcross [(Line 6_1 x Line N) x Line N] population challenged with *C. jejuni* at 3 wk of age. The hypothesis tested is that MHCY haplotype has a role in defining the level of *Campylobacter* colonization.

MATERIAL AND METHODS

Experimental Lines

Line N was developed at Cornell University. Line N became fixed for MHCB21 through repeated selection over multiple generations for resistance to Marek's disease. It is maintained as a closed population but is not considered an inbred line. Line N was defined at the USDA-ARS Avian Disease and Oncology Laboratory (**ADOL**), East Lansing, MI, USA as having Y5, Y7 and Y8. ADOL developed inbred Line 6_1. The MHCY type for Line 6_1 was not previously determined, but the related ADOL Line 6_3 is defined as homozygous for Y11. Lines 6_1 and N were imported to the Institute for Animal Health in Compton UK in 1972 and 1982, respectively. Each generation has been produced by random mating within the lines at the National Avian Research Facility (**NARF**), The Roslin Institute, University of Edinburgh, UK.

Campylobacter Colonization Phenotypes

Cecal colonization phenotypes for the chickens analyzed in the present study are derived from a published study using the progeny of a backcross [(Line 6_1 x Line N) x Line N] population (Psifidi et al., 2016). This experiment was performed in 2013 in 3 replicate trials. The chickens were inoculated by oral gavage at 3 wk of age with 10^8 colony-forming units (**CFU**) of the 11168H strain of *C. jejuni*. Birds were sacrificed 5 d following inoculation and *C. jejuni* in the ceca were enumerated as described (Psifidi et al., 2016). The animal experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986, with the approval of the Ethical Review Committee of The Pirbright Institute (under project license PPL 30/2462) and the Animal Welfare and Ethical Review Body of The Roslin Institute (under PPL 60/4420).

MHCY and MHCB Genotyping

The recently developed MHCY STR-based typing method was used for MHCY genotyping (Zhang et al., 2020). Genotyping patterns were produced with 2 primer pairs, FAM897/899 and YY916/899, as previously described. A third primer pair, FAM944/899, was used to verify genotype assignments. The Primer 944 sequence is AAAGGGGCGGAGGCACCA. The primers produce PCR products from heritable short tandem repeat (STR) regions found immediately upstream of the MHCY class I loci. Because MHCY class loci are located throughout the haplotypes, the STR typing surveys broadly across the MHCY region. The STR regions vary in number and type among different MHCY haplotypes and hence produce distinctive patterns. The PCR products from all 3 primer pairs resolve well in capillary electrophoresis. They provide distinctive patterns in chromatograms generated in an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and are suitable for analysis with Peak Scanner 2.0. The patterns are highly reproducible. The primers were previously tested extensively with DNA from fully pedigreed families.

MHCY typing was completed for samples in a *Campylobacter* trial using archived DNA and blinded to

colonization scores. The samples provided were chosen to reflect a balanced range of colonization scores ranging from low to high within the same trial. Four haplotypes (*Y5, Y7, Y8,* and *Y11*) were assigned based on identity with previously defined STR patterns (Zhang et al. 2020). Line 6_1 provided 3 patterns. In addition to the pattern typical of *Y11*, 2 similar patterns with additional distinctive peaks were present. These were named *Y11b*, and *Y11c*. For clarity in this report, *Y11* has been designated *Y11a*. The typing patterns for haplotypes *Y11b* and *Y11c* appear to be variations of the *Y11a* pattern. These may have arisen through recombination events that occurred in the years intervening following the restricted matings initially used to produce the Line 6 series at ADOL. In Line N parents and segregating in the backcross were *Y5, Y7, Y8*, and *Y18*. The *Y18* pattern was new. It was assigned the next number available for naming of MHCY haplotypes (Miller et al., 2004). The source of this haplotype is presently unknown. MHCB genotypes were determined using the microsatellite marker LEI0258 which is located within the chicken MHCB region (Fulton et al., 2006).

Data Analysis

Postinfection counts of *Campylobacter* in the ceca, sex, MHCB haplotype, MHCY haplotype, and DNA concentration and quality (260/280) were recorded for each bird. *Campylobacter* counts were log-transformed to normalize their distribution (Psifidi et al., 2016). The mean *Campylobacter* count across all samples was used as the dividing point for forming low and high colonization groups. The transformed counts for the low- and high-count groups were evaluated by analysis of variance. Differences in the distribution within the groups for sex, MHCB haplotype, and MHCY haplotype, were evaluated for statistical significance using the Chi square test. In a second step, *Campylobacter* counts overall were analyzed by MHCY haplotype and groups formed based on *Campylobacter* counts. The haplotype impact on bacterial count was analyzed by assigning 0.5 x the bacterial count to each haplotype comprising a genotype. The 0.5 count was log transformed and evaluated by analysis of variance. Significantly different means were identified by Tukey's HSD.

RESULTS AND DISCUSSION

The samples MHCY and MHCB typed here are from an earlier investigation focused on identifying quantitative trait loci contributing to resistance to *Campylobacter jejuni* colonization (Psifidi et al., 2016). *Campylobacter* colonization of the ceca, sex, MHCB genotypes, and MHCY genotypes were determined for 51 individuals within a single hatch of a backcross [(Line 6_1 x Line N) x Line N] population. The average colonization count for all samples, 60.5×10^6 bacteria, was used to define the boundary between low (n = 31) and high (n = 20) colonization groups (Table 1). Colonization counts differed significantly between these 2 groups ($P = 4.66 \times 10^{-15}$). The distribution of sex and MHCB genotypes did not differ significantly between the high and low groups (Table 1). One individual, not identified for sex, was excluded from that chi-square analysis for the sex distribution. The haplotypes identified include *Y5, Y7, Y8, Y11a, Y11b, Y11c*, and *Y18*. Among the 7 haplotypes, 2 had highly significant distribution differences. Haplotype *Y18* was more frequent in low colonization birds whereas haplotype *Y11a* was more frequent in high colonization birds (Table 1).

The significant difference observed in the distribution of the 2 MHCY haplotypes raised questions about the MHCY effect on bacterial counts overall. To address this issue, each haplotype was assigned 0.5 x bacterial counts of its genotype. The effect of each MHCY haplotype in the genotype may not be equal but this method allowed an assessment. The bacterial count calculated for each haplotype was transformed by ($\log_{10}$(bacterial count+1)). MHCY haplotypes with distribution differences were placed in separate groups. Haplotypes whose distributions lacked significant differences (*Y5, Y7, Y8, Y11b*, and *Y11c*) were pooled into a group identified as *others*. Analysis revealed that the *Y18* haplotype had lower bacterial counts that differed significantly from *Y11a* and *others* (Figure 1).

This study brings forward the polymorphism inherent in MHCY and its potential role in the interactions between the chicken host and microbes. It is appealing to consider that MHCY gene region has a general role in detection and early signaling of the presence of microbes including bacteria and viruses. The genetic differences affecting bacterial colonization and viral diseases that

Table 1. Mean bacterial colonization count, sex, MHCB, and MHCY frequencies in high and low colonization groups after *Campylobacter* infection in a backcross [(Line 6_1 x Line N) x Line N] population trial.

Colonizationrate (n)	Mean *C. jejuni* count (x 10^6)	Sexfrequency[1] (n)		MHCB haplotype frequency (n)		MHCY haplotype frequency (n)						
		F	M	*B2*	*B21*	*Y18*	*Y5*	*Y7*	*Y8*	*Y11a*	*Y11b*	*Y11c*
Low (31)	0.59 +/- 1.56	16	14	17	45	41	1	1	10	2	2	5
High (20)	153.33 +/- 55.33	10	10	9	31	11	1	0	11	12	2	3
P[2]	4.66×10^{-15}	0.239	0.414	0.177	0.108	3.00×10^{-5}	1.00	na	*0.827*	*0.008*	*1.00*	*0.479*

[1]Sex not identified in one bird.

[2]*P* for *Campylobacter* count from ANOVA and *P* for sex, MHCB, and MHCY are from Chi-square tests.The overall mean 60.5×10^6 colonization count was the discriminator for defining the low (<mean) and high (>mean) groups. The low- and high-colonization groups differed significantly in colonization. Distribution by sex or MHCB type according to colonization rate showed no significant difference. A significant difference in MHCY type was found between the low and high colonization groups. Haplotype *Y18* is statistically more frequent in the low colonization group ($P = 3.00 \times 10^{-5}$) and haplotype *Y11a* is statistically more frequent in the high colonization group ($P = 0.008$).

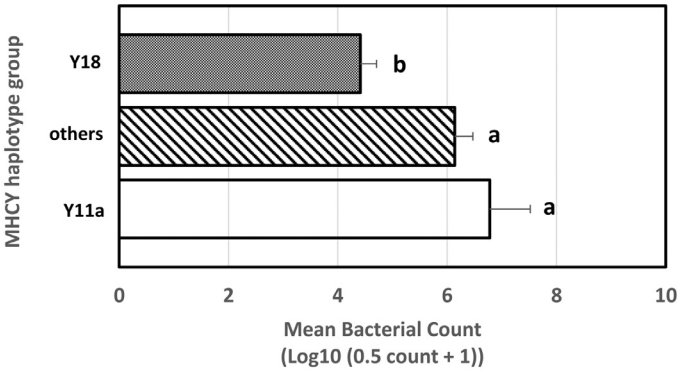

Figure 1. Graphical depiction of MHCY haplotype effect on *Campylobacter* cecal colonization in a backcross [(Line 6_1 x N) x Line N] population trial. Each MHCY haplotype was assigned 0.5 x the genotype bacterial count which was then transformed using (Log_{10}(bacterial count+1)). Data were assigned to one of 3 MsHCY haplotype groups: *Y11a* (n = 14), *others* (less frequent haplotypes – *Y5, Y7, Y8, Y11b* and *Y11c* (n = 36), and *Y18* (n = 52). The MHCY groups were evaluated using ANOVA followed by Tukey's HSD mean separation. Means having no common letter differ significantly ($P < 0.01$).

have been observed could be the result of structural differences among MHCY haplotypes. MHCY haplotypes vary in complexity (Miller et al., 2004). It could be that effective loci are present in some haplotypes but not others. Allelic differences at particular loci might also be the basis for the differences observed among the MHCY haplotypes associated with disease responses. The highly polymorphic MHCY class I genes are candidate genes for conferring the observed differences. Within the MHCY class I gene sequences, variation often results in differences in the amino acids on the MHCY class I molecular surface (Miller and Taylor, 2016). This contrasts with the variation observed among classical MHC class I molecules where amino acid variation occurs most frequently within the antigen binding groove. The surface variation might be the basis for specificity in signaling to receptors on specialized effector cells. MHCY signaling could be similar to interactions between MR1 (MHC class I-related molecule) and CD1 with their cognate receptors (Ogg et al., 2019), but with added specificity provided by the polymorphic nature of MHCY class I. It could be that the MHCY class I molecular interactions result in immune cell inhibition in some instances, as suggested from earlier experiments (Miller and Taylor, 2016). The MHCY is intriguing. The data reported here support further investigation into the role of MHCY haplotype in *Campylobacter* colonization and in the interactions of chickens with other microbes.

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DISCLOSURES

The authors declare no conflicts of interest.

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