Campylobacter Colonization and Proliferation in the Broiler Chicken upon Natural Field Challenge Is Not Affected by the Bird Growth Rate or Breed

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The zoonotic association between *Campylobacter* bacteria in poultry and humans has been characterized by decades of research which has attempted to elucidate the epidemiology of this complex relationship and to reduce carriage within poultry. While much work has focused on the mechanisms facilitating its success in contaminating chicken flocks (and other animal hosts), it remains difficult to consistently exclude *Campylobacter* under field conditions. Within the United Kingdom poultry industry, various bird genotypes with widely varying growth rates are available to meet market needs and consumer preferences. However, little is known about whether any differences in *Campylobacter* carriage exist across this modern broiler range. The aim of this study was to establish if a relationship exists between growth rate or breed and cecal *Campylobacter* concentration after natural commercial flock *Campylobacter* challenge. In one investigation, four pure line genotypes of various growth rates were grown together, while in the second, eight different commercial broiler genotypes were grown individually. In both studies, the *Campylobacter* concentration was measured in the ceca at 42 days of age, revealing no significant difference in cecal load between birds of different genotypes both in mixed- and single-genotype pens. This is important from a public health perspective and suggests that other underlying reasons beyond genotype are likely to control and affect *Campylobacter* colonization within chickens. Further studies to gain a better understanding of colonization dynamics and subsequent proliferation are needed, as are novel approaches to reduce the burden in poultry.

Elucidating the sources and transmission dynamics of the bacterial pathogen *Campylobacter* has never been more important as laboratory-confirmed cases in the United Kingdom reach an all-time high (1). *Campylobacter jejuni* and *Campylobacter coli* cause the majority of human infections (campylobacteriosis), which are characterized by acute, often bloody diarrheal episodes and stomach cramping, persisting for up to 10 days. Some 10% of cases require hospitalization, and further complications in the form of reactive arthritis or the neurodegenerative disorder Guillain-Barré syndrome may occur in rare circumstances (2). While confirmed cases place a significant burden on the health service, case underascertainment estimates suggest that as many as 9 times more cases go unreported (3), thus contributing to the significant morbidity associated with *Campylobacter*.

Campylobacter is a diverse microorganism in terms of both its genomic variation and ubiquitous nature. Its ability to adapt to new environments and occupy distinct niches facilitates the latter, and as such, the organisms are found in a range of wild and domesticated animals and in the environment. Overall, it is widely accepted that poultry is the predominant reservoir. Epidemiologically, outbreak reports and case-control studies have repeatedly identified consumption and handling of undercooked and raw chicken as a major risk factor, while a United Kingdom Food Standards Agency (FSA) survey in 2007–2008 (being repeated in 2014) demonstrated that 65% of chicken at retail sale was contaminated with *Campylobacter* (4). Furthermore, a survey by the European Food Safety Authority (EFSA) described that approximately 86% of broiler carcasses across Europe harbored *Campylobacter* in 2008 (5).

Source attribution modeling using molecular subtyping has corroborated such observations, attributing up to 40% to 80% of human cases to the broiler chicken (6, 7), but it is acknowledged

that additional sources of campylobacters for humans exist, the extent of which may vary depending on receptive cohorts and geography (8). Despite these observations, from the chicken's perspective, the presence of *Campylobacter* within poultry does not guarantee a negative impact on the bird's health or performance, although there is much to learn about the biological basis of this interaction.

From a consumer perspective, the appropriate cooking of poultry meat is recognized as the most effective practice to reduce the risk of campylobacteriosis, and the United Kingdom FSA continues to advocate such food safety practices. At the same time, the United Kingdom poultry industry has invested heavily in tackling *Campylobacter* throughout the supply chain to reduce the prevalence and concentration of *Campylobacter* within this reservoir. Interventions have included extensive farm biosecurity practice review and optimization, processing treatments, including steam and hot water and blast chilling, and nearer the consumer end, the introduction of leak-proof packaging in supermarkets. However, despite extensive and open cooperation between poultry producers, retailers, and government regulators to facilitate such interventions, the “silver bullet” has not been found. As a result, the United Kingdom FSA's *Campylobacter* target was recently deemed unachievable, and as such, a revised strategy was outlined (9).

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Within this revised strategy, the FSA proposed improvements in availability of information throughout the supply chain and continued support for research initiatives while encouraging the industry to maintain focus on *Campylobacter* reduction as well as developing novel intervention initiatives.

It is generally accepted within the scientific community that vertical transmission does not contribute to the colonization of poultry flocks (10, 11, 12), and birds typically do not “acquire” *Campylobacter* until 2 to 3 weeks of age (13). As such, there are major gaps in the understanding of the factors leading to *Campylobacter* colonization and subsequent transmission in poultry. Nonetheless, control is challenging, and following seeding, the spread of *Campylobacter* within a flock is rapid (14), aided by behavioral factors, such as coprophagia.

Effective flock biosecurity has been consistently recognized as the most important “on-farm” intervention for reducing flock colonization, and the specific factors contributing to good biosecurity have been previously reviewed (15, 16). While biosecurity would appear to be effective to a point, it is imperative that best practice be adhered to since the slightest lapse can facilitate introduction of the bacteria to a flock.

Recently, differences between production system and chicken breed has been investigated in order to better understand other potential underlying reasons for susceptibility of a flock to *Campylobacter* colonization (17). It has also been suggested that “faster-growing” broiler chicken genotypes may harbor higher levels of *Campylobacter* (18), which is an important factor given the currently large market share of the faster-growing broiler chicken. The United Kingdom poultry industry is structured to meet consumer demand and as such produces broiler chickens of various growth rates, from conventionally reared birds to slower-growing alternatives. If birds of a particular growth profile were more susceptible to *Campylobacter* colonization, this would present an increased risk to the consumer. It is therefore pertinent to ask whether the risk to the consumer is affected by the choice of broiler chicken type.

Here, we present the results of two independent investigations examining the effect of the host (chicken) genotype and growth rate on *Campylobacter* colonization under commercial flock conditions with a natural *Campylobacter* challenge. A natural challenge to the growing birds from the litter was chosen in contrast to an artificial challenge with a high dose of a pure culture of *Campylobacter* to ensure the experiment was representative of more-realistic *Campylobacter* challenge in the commercial farm environment. In the first scenario, we examined whether birds grown with a faster-growth profile harbored higher levels of *Campylobacter*. Second, we examined the impact of slowing the growth rate through feed composition on *Campylobacter* levels in a range of commercial broiler breeds.

MATERIALS AND METHODS

Investigation 1: growth rate and *Campylobacter* load. (i) Environment. Trial 1 was performed on an Aviagen company “sib-test” farm, a facility designed to replicate broader commercial broiler conditions, where chickens are placed at a day old and grown to 42 days. At this facility, siblings of chicken selection candidates are placed but never reintroduced to the breeding program. The facility is less biosecure than standard production farms and operates a built-up litter system, which was supplied in the form of a layer of wood shavings and supplemented with fresh litter as required (19).

Prior to bird placement, the farm environment (house litter without

birds) was screened for carriage of *Campylobacter* spp. using the “boot sock” method. Briefly, premoistened boot socks were walked around the flock and were then enriched in Exeter broth under microaerophilic conditions (2% H₂, 5% CO₂, 5% O₂, and 88% N₂) for 48 h at 41.5°C, followed by plating on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubating as before.

Colonies consistent with *Campylobacter* morphology (small, greyish, translucent, irregular colonies, often spreading) were purified on blood agar, and genus and species confirmation was performed as described previously (20). Control strains *C. jejuni* (NCTC 11168) and *C. coli* (NCTC11353) were used for microbiological validation of growth conditions.

(ii) Birds. Four different Aviagen genotypes were placed at a day old and were chosen to represent a range of growth rates (genotype 1, 34 g/day; genotype 2, 51 g/day; genotype 3, 19 g/day; genotype 4, 18 g/day). Genotypes 2 and 4 were modern lines (a fast-growing line and a slow-growing line), while genotypes 1 and 3 were “control lines” of the faster-growing line (genotype 2) from 1972 and 1996. Control lines were discrete populations, randomly selected to maintain the characteristics of the 1972 and 1996 commercial broilers, respectively. Thirty birds from each of the four genotypes were penned together (in duplicate pens), resulting in 120 birds per pen. This was repeated 2 weeks subsequently to examine potential hatch-by-hatch variation. On each occasion, environmental *Campylobacter* exposure was determined before bird placement, as described previously.

Birds were grown with a standard feed ration (maize based) to 42 days, and body weights were measured prior to slaughter. At 42 days of age, 15 birds per genotype were humanely killed by cervical dislocation, and intact ceca were removed aseptically.

(iii) Microbiology. Pooled cecal contents (from each cecum from the same bird) were homogenized in sterile saline and subsequently serially diluted, plated on mCCDA, and incubated under microaerophilic conditions (2% H₂, 5% CO₂, 5% O₂, and 88% N₂) for 48 h at 41.5°C. Numbers of *Campylobacter* CFU per gram of cecal content were then calculated.

Investigation 2: feed composition effect on *Campylobacter* load between commercial broiler breeds. (i) Environment. Trial 2 was performed on an Aviagen trials farm. This facility is designed to replicate standard commercial broiler conditions in the United Kingdom, where chickens are placed at a day old and grown to 42 days. Additionally, the facility is used to optimize nutritional specifications of feed and facilitates comparisons between feed composition and form.

On this farm, houses undergo litter replacement following bird depletion and cleaning and disinfection before birds are again placed in the houses.

As described previously, the *Campylobacter* status of the farm was determined prior to bird placement and was found to be positive.

(ii) Birds. Eight different genotypes (comprising both Aviagen and commercial genotypes of different breeds) were penned individually, with each genotype exposed to two different feed compositions (16 pens collectively). Genotypes comprised birds of contrasting growth rates up to 42 days (from 32 g/day [slow growth] to 71 g/day [fast growth]) across four different breeds (five Aviagen genotypes and three additional commercial genotypes). In terms of growth rate, genotypes were ranked from fastest growing to slowest growing as follows: genotypes 2, 3, 1 (all Aviagen), 8, 4 (Aviagen), 7, 5 (Aviagen), and 6. Subpopulations of all genotypes were fed two types of diets: 100% balanced protein (wheat based) and 90% balanced protein (maize based) (Table 1), based on Aviagen specifications (21). Feed differed in terms of cereal base as well as crude protein content. The higher-protein feed was a standard ration using wheat as the main grain source, providing a good pellet quality. The alternative feed ration contained 10% less protein, used maize as the main grain source, and produced a poorer pellet quality.

After 42 days of growth and following recording weights at this age, 10 birds per genotype per feed treatment were humanely killed by cervical

TABLE 1 Diet formulations showing percent compositions in wheat- and maize-based feed^a

Raw material	% composition in feed					
	100% BP (wheat)			90% BP (maize)		
	Broiler starter	Broiler grower	Broiler finisher	Broiler starter	Broiler grower	Broiler finisher
Maize				59.74	63.27	68.14
Wheat	56.81	59.53	63.19			
Fish meal	3.50	1.50	—	—	—	—
Soybean meal, 49% crude protein	32.75	29.67	26.73	33.26	27.84	23.10
Vitamin + trace mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine liquid	0.21	0.20	0.21	0.26	0.24	0.25
DL-Methionine	0.31	0.28	0.26	0.29	0.26	0.23
L-Threonine	0.09	0.08	0.08	0.08	0.07	0.06
Choline chloride, 50%	0.10	0.10	0.10	0.15	0.15	0.15
Xylanase enzyme product	0.01	0.01	0.01	—	0.01	0.01
Phytase enzyme product	0.01	0.01	0.01	0.01	0.60	0.60
Organic acid mixture	0.60	0.60	0.60	0.60	—	—
Limestone flour	1.04	0.84	0.82	1.09	—	—
Dicalcium phosphate 18	0.98	1.02	1.10	1.46	1.23	1.10
Salt	0.07	0.12	0.16	0.18	0.17	0.17
Sodium bicarbonate	0.15	0.17	0.17	0.14	0.16	0.16
Soya oil	2.13	2.50	2.50	2.39	0.96	1.09
Fat Hispec (Veg)	1.00	3.12	3.81	0.09	3.90	3.80

^a BP, balanced protein; Fat Hispec (Veg), fatty acid blend of vegetable origin. “Broiler starter” is feed provided from day 0 to day 10; “broiler grower” is feed provided from day 11 to day 24; “broiler finisher” is feed provided from day 25 to final weighing.

dislocation, ceca were removed aseptically, and *Campylobacter* bacteria were enumerated as described earlier.

Statistical analysis. Data from both trials were analyzed using REML (restricted maximum likelihood) variance component analyses using the software program GenStat, 14th edition (22). The model fitted included fixed effects of genotype, feed (where applicable), and interaction term, plus the covariate of body weight. *Campylobacter* levels in the ceca were presented on a log scale in the results.

RESULTS AND DISCUSSION

Natural *Campylobacter* challenge. Environmental sampling on both farms detected *Campylobacter* spp. via conventional bacteriological culture. Genus and species testing confirmed the presence of *Campylobacter jejuni* in all pens tested on both farms. Relying on a natural challenge as opposed to an artificial challenge with a high dose of a pure culture of *Campylobacter* enabled a more representative challenge in the commercial environment. It is known that it takes very few *Campylobacter* cells to colonize a chicken (23, 24), and subsequent shedding can increase the colonization potential significantly (25). Furthermore, while not examined in this study, it is known that flocks may harbor multiple *Campylobacter* subtypes (10, 26). Therefore, given the number of variables that affect *Campylobacter* transmission dynamics, it was deemed appropriate that the natural challenge route was used.

Investigation 1: bird growth rate does not affect *Campylobacter* carriage in the ceca. Investigation 1 was performed on two separate occasions (to account for hatch-by-hatch variation) and used duplicate pens in each trial. In the first, the mean *Campylobacter* concentrations across genotypes 1 to 4 in the two pens did not significantly differ (7.0×10^6 CFU/g and 5.4×10^6 CFU/g; $P = 0.264$), and all birds analyzed harbored *Campylobacter* at slaughter age (Fig. 1A). In the repeated investigation, again the mean *Campylobacter* concentrations between pens, across genotypes 1 to 4, did not significantly differ (2.6×10^7 CFU/g and 2.0×10^7 CFU/g, respectively [$P = 0.540$] [Fig. 1B]).

FIG 1 (A) Mean *Campylobacter jejuni* levels by bird genotype (trial 1). a, pen 1; b, pen 2. (B) Mean *Campylobacter jejuni* levels by bird genotype (trial 2). a, pen 1; b, pen 2.

FIG 2 Scatter plot of individual birds’ *Campylobacter jejuni* cecal loads (all four genotypes, $n = 240$) against individual body weights at slaughter (42 days). Regression line shown to demonstrate lack of correlation ($r^2 = 0.0075$).

In both trials and pens, *Campylobacter* concentrations did not differ significantly between genotypes collectively ($P = 0.504$). Overall bird body weight was not correlated with *Campylobacter* cecal load at slaughter age (Fig. 2) ($r^2 = 0.0075$).

Investigation 2: no evidence of differences in *Campylobacter* cecal carriage between broiler breeds. Birds of eight different broiler breed genotypes were penned separately (to appropriate

stocking densities) in duplicate, with one fed a high-protein diet and one with a lower-protein diet, as described in Methods.

On average, birds fed a higher-protein diet gained more weight than those on a reduced-protein diet ($P < 0.001$), and this was evident for all genotypes analyzed (Fig. 3A). At slaughter, mean *Campylobacter* concentrations in the ceca did not significantly differ between bird genotypes ($P = 0.989$) (Table 2 and Fig. 3B), and there was no association of *Campylobacter* load with feed type ($P = 0.180$) (Table 2), suggesting little or no effect of these specific feed variations on cecal *Campylobacter* concentrations. It is known that modifying the upper gastrointestinal tract can influence the colonization of the lower tract (including the ceca) by *Campylobacter*. Stimulation of the gizzard has been shown to reduce *Salmonella* in growing broilers and can reduce the horizontal spread of *Campylobacter* (27, 28). This is a consequence of lowering the pH and creating a barrier for gastrointestinal bacteria (including *Campylobacter*), suggesting a potential role for feed composition and structure in *Campylobacter* reduction in the gut. However, the comparator feed types used in the current study did not deliver a significant difference based on their current compositions, with each differing primarily in crude protein content and cereal base.

Furthermore, although not explored in the current study, *Campylobacter* “subtypes” have been shown to have variability in colonization ability (29), and it is not known whether this colonization may vary across intestinal sites within the chicken.

Concluding remarks and looking to the future. Here we have demonstrated that in two independent investigations, following natural exposure to *Campylobacter*, the growth rate and/or breed of commercial broiler chicken did not have any influence on the colonization and subsequent proliferation within the ceca. Fur-

FIG 3 (A) Mean genotype body weights for each feed treatment group. Dark shading, 100% balanced protein (wheat based). Light shading, 90% balanced protein (maize based). (B) Mean *Campylobacter jejuni* levels by genotype and feed treatment. a, 100% balanced protein (wheat based); b, 90% balanced protein (maize based).

TABLE 2 Significance of genotype, feed type, and their interaction when analyzing body weight and *Campylobacter* load (investigation 2)

Variable or interaction	Degrees of freedom	P value	
		Body wt	<i>Campylobacter</i> load
Genotype	64	<0.001	0.989
Feed type	64	<0.001	0.180
Breed × feed	64	0.619	0.218

thermore, in seeking to determine the differences in *Campylobacter* concentrations after a natural challenge plus the impact of a slowing growth rate through feed composition specifically, no significant differences between chicken genotypes were observed.

In food animal production, ensuring food safety is a top priority, and determining the relative risks of food-borne infection aids targeted interventions throughout the system. The chicken growth rate and/or broiler breed did not contribute to a greater risk of *Campylobacter* carriage in the chicken ceca, which is important from a public health perspective. Intervention strategies should continue to explore and exploit novel research areas, involving both the birds and bacteria, while optimizing approaches already known to help lower the *Campylobacter* prevalence and concentration during processing (through best hygiene practices and effective carcass treatment) and on farms (through effective biosecurity). It is likely that adopting multiple approaches will be more effective than one solution alone. Some primary breeder broiler flocks can remain *Campylobacter* negative during the 5 weeks of the growing phase (Aviagen data, unpublished), and while this could be attributable to the extremely high level of biosecurity, it is unlikely that biosecurity alone will prevent commercial broiler flock colonization. Furthermore, the source of *Campylobacter* in chickens and specific mechanisms for flock entry have yet to be elucidated.

Chickens can harbor a considerable number of campylobacters within the ceca with seemingly little damaging effect on the individual. But while it has historically been considered a commensal, the induction of an innate immune response by the bacteria may suggest otherwise (30, 31). This implies that control of *Campylobacter* in the chicken host may be achievable. Such research should not be disregarded as an area of further investigation, as exemplified by Buckley and others (32), who demonstrated the potential for intestinal control of *C. jejuni* by expressing CjaA and Peb1A (solute-binding and aspartate/glutamate-binding proteins, respectively) in *Salmonella enterica* serovar Typhimurium, which protected against subsequent *C. jejuni* colonization.

The possibility of genetic resistance has been investigated through work with inbred chicken lines and has indeed revealed line resistance, independent of *C. jejuni* strain, bird age, or body weight. Backcrossing experiments have demonstrated heritability, suggesting specific gene involvement in resistance (33). Quantitative trait loci (QTL) involved in resistance have been identified, and current work attempts to refine these loci to potentially detect individual genes, locate these in modern commercial chickens, and investigate the variance these might prompt in *Campylobacter* colonization between individual birds within a population (34).

While continued collaborative effort is required to ensure that the burden of *Campylobacter* is minimized within poultry production, an equally important role must be filled by regulators and consumers in dealing with a human pathogen that remains to be better understood scientifically. Nonetheless, we have shown that when the chicken growth rate is reduced genetically and/or nutritionally, *Campylobacter* levels within the ceca are not affected. Therefore, bird genotype and growth rate cannot be perceived as risk factors from a public health perspective. Further investigation into factors governing colonization and transmission of *Campylobacter* within poultry flocks is needed, as are novel approaches to better understand the unique relationship between poultry and *Campylobacter*. To this end, the possibility of breeding for natural

resistance to *Campylobacter* carriage in poultry is an intervention that calls for investigation.

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