Campylobacter colonisation and proliferation in the broiler chicken under natural field challenge is not affected by bird growth rate or breed.


Aviagen Ltd, 11 Lochend Road, Newbridge, Midlothian EH28 8SZ, UK.

Running title: Campylobacter in chickens not affected by growth rate or breed.

Address correspondence to Fraser J Gormley fgormley@aviagen.com
The zoonotic association between *Campylobacter* in poultry and humans has been characterised 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 focussed 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 UK poultry industry, various bird genotypes with widely varying growth rates are available to meet market needs and consumer preference. 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 caecal *Campylobacter* concentration, after natural commercial flock *Campylobacter* challenge. In one investigation, four pure line genotypes of varying growth rates were grown together while in the second, eight different commercial broiler genotypes were grown individually. In both studies *Campylobacter* concentration was measured in the caeca at 42 days of age and revealed no significant difference in caecal load between birds of different genotypes, both in mixed and single genotype pens. This is important from a public health perspective and suggests other underlying reasons beyond genotype are likely to control and affect *Campylobacter* colonisation within chickens. Further studies to gain a better understanding of colonisation dynamics and subsequent proliferation are needed as are novel approaches to reduce the burden in poultry.

Keywords: Campylobacter, poultry, genetics, growth rate, food safety.
Introduction

Elucidating the sources and transmission dynamics of the bacterial pathogen Campylobacter have never been more important as laboratory confirmed cases in the UK reach an all-time high (1). Campylobacter jejuni and Campylobacter coli cause the majority of human infections (Campylobacteriosis), which is characterised by acute, often bloody diarrhoeal episodes and stomach cramping persisting for up to 10 days. Some 10% of cases require hospitalisation and further complications in the form of reactive arthritis or the neurodegenerative disorder Guillain-Barre Syndrome may occur in rare circumstances (2). While confirmed cases place significant burden on the health service, case under-ascertainment estimates suggest as many as nine times more cases go unreported (3) thus contributing to the significant morbidity associated with Campylobacter.

Campylobacter is a diverse microorganism both in terms of 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 UK Food Standards Agency 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 harboured Campylobacter in 2008 (5).
Source attribution modelling using molecular subtyping has corroborated such observations, attributing up to 40%-80% of human cases to the broiler chicken (6, 7) but it is acknowledged that additional sources of *Campylobacter* 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 recognised as the most effective practice to reduce the risk of *Campylobacteriosis* and the UK Food Standards Agency (FSA) continue to advocate such food safety advice. At the same time the UK poultry industry has invested heavily into 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 optimisation, 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 UK FSA’s *Campylobacter* target was recently deemed unachievable and as such a revised strategy was outlined (9). 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 colonisation of poultry flocks (10, 11, 12) and birds typically do not ‘acquire’ *Campylobacter* until 2-3 weeks of age (13). As such, there are major gaps in the understanding of the factors leading to *Campylobacter* colonisation and subsequent transmission in poultry. Nonetheless, control is challenging and following seeding, the spread of *Campylobacter* within a flock is rapid (14), aided by behavioural factors such as coprophagia. Effective flock biosecurity has been consistently recognised as the most important ‘on-farm’ intervention for reducing flock colonisation 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 as 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* colonisation (17). It has also been suggested that ‘faster-growing’ broiler chicken genotypes may harbour higher levels of *Campylobacter* (18) which is an important factor given the currently large market share of the faster growing broiler chicken. The UK poultry industry is structured to meet consumer demand and as such produces broiler chickens of varying growth rates; from conventionally reared birds to slower growing alternatives. If birds of a particular growth profile were more susceptible to *Campylobacter* colonisation, this would present an increased risk to the consumer. It is therefore a pertinent question 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 host (chicken) genotype growth rate on *Campylobacter* colonisation 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 harboured higher levels of *Campylobacter*. Secondly we examined the impact of slowing 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

**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 day old and grown to 42 days. At this facility, siblings of chicken selection candidates are placed but never re-introduced to the breeding programme. 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, pre-moistened boot socks were walked around the flock and were then enriched in...
Exeter Broth under microaerophillic conditions (2% H₂, 5% CO₂, 5% O₂ and 88% N₂) for 48 hours at 41.5°C, followed by plating onto modified charcoal cefoperazone deoxycolate 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 performed as described previously (20). Control strains *C. jejuni* (NCTC 11168) and *C. coli* (NCTC11353) were used for microbiological validation of growth conditions.

**Birds**

Four different Aviagen genotypes were placed at day old and were chosen to represent a range of growth rates (Genotype 1: 34 grams/day; Genotype 2: 51 grams/day, Genotype 3: 19 grams/day, Genotype 4: 18 grams /day. Genotypes 2 and 4 were modern lines (a fast growing and slow growing) 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 two 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 measured prior to slaughter. At 42 days of age, 15 birds per genotype were humanely killed by cervical dislocation and intact caeca removed aseptically.
Pooled caecal contents (from each caeca from the same bird) were homogenised in sterile saline and subsequently serially diluted, plated onto mCCDA and incubated under microaerophillic conditions (2% H₂, 5% CO₂, 5% O₂ and 88% N₂) for 48 hours at 41.5°C. *Campylobacter* colony forming units (cfu) per gram of caecal content were then calculated.

**Investigation 2 – Feed composition effect on *Campylobacter* load between commercial broiler breeds**

**Environment**

Trial 2 was performed on an Aviagen trials farm. This facility is designed to replicate standard commercial broiler conditions in the UK where chickens are placed at day old and grown to 42 days. Additionally, the facility is used to optimise 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 into the houses.

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

**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 rate up to 42 days [from 32 grams/day (slow grow) to 71 grams/day (fast grow)] 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: genotype 2, 3, 1 (all Aviagen), 8, 4 (Aviagen), 7, 5 (Aviagen) and 6. Sub-populations 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 dislocation, caeca removed aseptically and *Campylobacter* enumerated as described earlier.

Statistical analysis

Data from both trials were analysed using REML (restricted maximum likelihood) variance components analyses using 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 caeca 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 colonise a chicken (23, 24) and subsequent shedding can increase the colonisation potential significantly (25). Furthermore, while not examined in this study, it is known that flocks may harbour 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 caeca*

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 concentration across genotypes 1-4 in the two pens did not significantly differ (7.0 x 10^6 cfu/g and 5.4 x 10^6 cfu/g, \( p=0.264 \)), and all birds analysed harboured Campylobacter at slaughter age (Figure 1a). In the repeated investigation, again the mean Campylobacter concentration between pens, across genotypes 1-4 did not significantly differ [2.6 x 10^7 cfu/g and 2.0 x 10^7 cfu/g respectively (\( p=0.540 \)) (Figure 1b)].

In both trials and pens, Campylobacter concentration did not significantly differ between genotypes collectively (\( p=0.504 \)). Overall bird body weight was not correlated with Campylobacter caecal load at slaughter age (Figure 2) (R^2=0.0075).

**Investigation 2**
No evidence of differences in Campylobacter caecal 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 the 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 analysed (figure 3a). At slaughter, mean Campylobacter concentration in the caeca did not significantly differ between bird genotypes ($p=0.989$) (Table 2, Figure 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 caecal Campylobacter concentration. It is known that modifying the upper gastrointestinal tract can influence the colonisation of the lower tract (including the caeca) 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 on 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 primarily differing in crude protein content and cereal base. Furthermore, although not explored in the current study, Campylobacter ‘subtypes’ have been shown to have variability in colonisation ability (29) and it is not known whether this colonisation may vary across intestinal sites within the chicken.

Concluding remarks and looking to the future
Here we demonstrate that in two independent investigations, following natural exposure to *Campylobacter*, growth rate and/or breed of commercial broiler chicken did not have any influence on the colonisation and subsequent proliferation within the caeca. Furthermore, in seeking to determine the differences in *Campylobacter* concentration after a natural challenge plus the impact of 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 foodborne infection aids targeted interventions throughout the system. Chicken growth rate and/or broiler breed did not contribute to a greater risk of *Campylobacter* carriage in the chicken caeca, which is important from a public health perspective. Intervention strategies should continue to explore and exploit novel research areas, involving both the bird and bacteria; while optimising approaches already known to help lower the *Campylobacter* prevalence and concentration during processing (through best hygiene practice and effective carcass treatment) and on farm (through effective biosecurity). It is likely that adopting multiple approaches will be more effective that one solution alone. Some primary breeder broiler flocks can remain *Campylobacter* negative during the five 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 colonisation. Furthermore, the source of *Campylobacter* in chickens and specific mechanisms for flock entry has yet to be elucidated.

Chicken can harbour a considerable number of *Campylobacter* within the caeca with seemingly little damaging effect on the individual. But while historically 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 Typhimurium*, which protected against subsequent *C. jejuni* colonisation.

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 (QTLs), 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* colonisation between individual birds within a population (34).

While continued collaborative effort is required to ensure the burden of *Campylobacter* is minimised 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 by reducing chicken growth rate genetically and / or nutritionally, *Campylobacter* levels within the caeca 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 colonisation and transmission of *Campylobacter* within poultry flocks are 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.

Acknowledgments

Authors would like to extend gratitude to Mr Shaun Russell for technical assistance in the project.

References


Campylobacter infection has different outcomes in fast- and slow-growing broiler 

Jorgensen F. 2008. Flock health indicators and Campylobacter spp. in commercial 

2012. Genetic parameters of foot-pad dermatitis and body weight in purebred 

Has retail chicken played a role in the decline of human Campylobacteriosis? 

http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross_308_Broiler_Nutriti
on_Spec.pdf.

Hemel Hempstead, UK. GenStat.co.uk.

Colonisation of chicks by motility mutants of Campylobacter jejuni demonstrates 

infectivity of several strains of Campylobacter jejuni in chickens. Risk Anal. 26: 
1613-1621.

colonization potential of Campylobacter jejuni strain 81116 after passage through


Campylobacter antigens for control of C. jejuni in poultry. Vaccine. 28: 1094-1105.


Table 1. Diet formulations showing percentage compositions in wheat and maize based feed.

Table 2. Significance of Genotype, Feed Type and their interaction when analysing Body Weight and Campylobacter load (Investigation 2).

Figure 1a. Mean Campylobacter jejuni levels by bird genotype (trial 1). a, pen 1; b, pen 2.

Figure 1b. Mean Campylobacter jejuni levels by bird genotype (trial 2). a, pen 1; b, pen 2.
Figure 2. Scatter plot of individual birds’ *Campylobacter jejuni* caecal load (all four genotypes, n=240) against individual body weights at slaughter (42 days). Regression line shown to demonstrate lack of correlation ($R^2=0.0075$).

Figure 3a. Mean genotype body weights for each feed treatment group. Dark shading, 100% balanced protein (wheat based). Light shading, 90% balanced protein (maize based).

Figure 3b. Mean *Campylobacter jejuni* levels by genotype and feed treatment. a, 100% balanced protein (wheat based); b, 90% balanced protein (maize based).
Table 1. Diet formulations showing percentage compositions in wheat and maize based feed.

<table>
<thead>
<tr>
<th>Raw Materials (%)</th>
<th>100% BP* (Wheat)</th>
<th>90% BP* (Maize)</th>
<th>Raw Materials (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broiler starter(^1)</td>
<td>Broiler Grower(^1)</td>
<td>Broiler Finisher(^1)</td>
</tr>
<tr>
<td>Wheat</td>
<td>56.81</td>
<td>59.53</td>
<td>63.19</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3.50</td>
<td>1.50</td>
<td>-</td>
</tr>
<tr>
<td>Soybean meal 49% crude protein</td>
<td>32.75</td>
<td>29.67</td>
<td>26.73</td>
</tr>
<tr>
<td>Vitamin + trace mineral premix</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Lysine Liquid</td>
<td>0.21</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.31</td>
<td>0.28</td>
<td>0.26</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Xylanase enzyme product</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Phytase enzyme product</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Organic acid mixture</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Limestone Flour</td>
<td>1.04</td>
<td>0.84</td>
<td>0.82</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>18</td>
<td>1.02</td>
<td>1.10</td>
</tr>
<tr>
<td>Salt</td>
<td>0.07</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>0.15</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Soya Oil</td>
<td>2.13</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Fat Hispec (Veg)</td>
<td>1.00</td>
<td>3.12</td>
<td>3.81</td>
</tr>
</tbody>
</table>

\(^*\)BP, balanced protein. \(^1\)Broiler starter: feed provided from days 0-10. \(^2\)Broiler grower: feed provided from days 11-24. \(^3\)Broiler finisher: feed provided from days 25-final weighing.
Table 2. Significance of Genotype, Feed Type and their interaction when analysing Body Weight and *Campylobacter* load (Investigation 2).

<table>
<thead>
<tr>
<th></th>
<th>Degrees of freedom</th>
<th>Body weight</th>
<th><em>Campylobacter</em> load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>64</td>
<td><em>p</em>&lt;0.001</td>
<td><em>p</em>=0.989</td>
</tr>
<tr>
<td>Feed type</td>
<td>64</td>
<td><em>p</em>&lt;0.001</td>
<td><em>p</em>=0.180</td>
</tr>
<tr>
<td>Breed x feed</td>
<td>64</td>
<td><em>p</em>=0.619</td>
<td><em>p</em>=0.218</td>
</tr>
</tbody>
</table>
Campylobacter jejuni concentration (cfu/g caecal content)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>1^*</th>
<th>1^c</th>
<th>2^*</th>
<th>2^c</th>
<th>3^*</th>
<th>3^c</th>
<th>4^*</th>
<th>4^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>