

1 **Genetic basis of *Campylobacter* colonisation in the broiler chicken and its impact**
2 **on intestinal health following natural field exposure.**

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15 Short title: Genetic basis of *Campylobacter* colonisation

16 ***Abstract***

17

18 *Campylobacter* is the leading bacterial cause of food-borne diarrhoeal illness in
19 humans and source attribution studies unequivocally identify handling or consumption
20 of poultry meat as a key risk factor. *Campylobacter* colonises the avian intestines in
21 high numbers and rapidly spreads within flocks. A need therefore exists to devise
22 strategies to reduce *Campylobacter* populations in poultry flocks. There has been a
23 great deal of research aiming to understand the epidemiology and transmission
24 characteristics of *Campylobacter* in poultry as a means to reduce carriage rates in
25 poultry and reduce infection in humans. One potential strategy for control is the

1 genetic selection of poultry for increased resistance to colonisation by *Campylobacter*.
2 The potential for genetic control of colonisation has been demonstrated in inbred
3 populations following experimental challenge with *Campylobacter* where quantitative
4 trait loci associated with resistance have been identified. Currently in the literature
5 there is no information of the genetic basis of *Campylobacter* colonisation in
6 commercial broiler lines and it is unknown whether these QTL are found in
7 commercial broiler lines. The aim of this study was to estimate genetic parameters
8 associated with *Campylobacter* load and genetic correlations with gut health and
9 production traits following natural exposure of broiler chickens to *Campylobacter*.
10 The results from the analysis show a low but significant heritability estimate ($0.095 \pm$
11 0.037) for *Campylobacter* load which indicates that non-genetic factors have a greater
12 influence on the level of *Campylobacter* found in the broiler chicken.

13 Furthermore, through examination of macroscopic intestinal health and absorptive
14 capacity, our study indicated that *Campylobacter* has no detrimental effects on
15 intestinal health and bird growth following natural exposure in the broiler line under
16 study. These data indicate that whilst there is a genetic component to *Campylobacter*
17 colonisation worthy of further investigation, there is a large proportion of phenotypic
18 variance under the influence of non-genetic effects. As such the control of
19 *Campylobacter* will require understanding and manipulation of non-genetic host and
20 environmental factors.

21

22 **Background**

23 *Campylobacter* is the leading bacterial cause of human foodborne illness
24 worldwide. It was estimated by the World Health Organisation to cause
25 approximately 96 million illnesses, 21 thousand deaths and loss of 2.1 million

1 disability-adjusted life years in 2010 (Havelaar et al. 2015). Human
2 campylobacteriosis is typically a self-limiting disease characterised by acute watery
3 diarrhoea which is sometimes bloody and accompanied by abdominal cramp, fever
4 and nausea. Symptoms typically persist for up to 10 days, however c. 10% of cases
5 require hospitalisation and in rare cases severe sequelae can develop including
6 reactive arthritis and inflammatory neuropathies such as Guillain-Barré Syndrome,
7 sepsis and even death (Mishu and Blaser 1993). It has been suggested that the actual
8 number of cases of campylobacteriosis in the UK community is nine times greater
9 than that captured by national surveillance (Tam et al. 2012).

10 Sources of *Campylobacter* include the environment and a range of wild and
11 domesticated animals (Penner 1988, Blaser 1997). It is widely accepted that farmed
12 poultry are a key reservoir of human infections with studies into the epidemiology of
13 *Campylobacter* outbreaks repeatedly identifying the consumption and handling of
14 undercooked and raw chicken as a major risk factor (Mullner et al. 2009, Sheppard et
15 al. 2009, Kaakoush et al. 2015). A survey in 2015-2016 by the UK Food Standards
16 Agency (FSA) demonstrated that 61.3% of fresh chicken at retail sale was positive for
17 *Campylobacter* above the minimum detection limit of 10 colony-forming units
18 (CFU)/g (Jorgensen et al. 2016). *Campylobacter* levels in the intestinal tract of
19 poultry can be in excess of 10^8 CFU/g of caecal contents and this can contaminate
20 chicken meat in the event of leakage of gut contents during the slaughter process
21 (Beery et al. 1988, Boyd et al. 2005).

22 Quantitative risk assessments have estimated that a 30 fold reduction of poultry-
23 associated *Campylobacter* human infections is achievable through a $2\log_{10}$ reduction
24 in the level of *Campylobacter* in broiler carcasses (Rosenquist et al. 2003). The UK
25 poultry industry initiated a large scale effort to find effective methods to reduce the

1 incidence and level of *Campylobacter* throughout the poultry supply chain. These
2 interventions have included reviews of farm biosecurity and subsequent optimisation,
3 processing technologies designed to kill bacteria such as steam treatment and blast
4 chilling, and the introduction of leak proof packaging and guidance to consumers.
5 One key focus for intervention is reducing the level of *Campylobacter* in poultry
6 during production and this requires a better understanding of the contribution of avian
7 and bacterial factors to colonisation. *Campylobacter* readily colonises the avian
8 intestinal tract, typically in the absence of overt pathology, and for many years has
9 been considered a commensal member of the normal chicken gut microbiota
10 (Hermans et al. 2011). In recent years it has been suggested that *Campylobacter* is
11 not merely a commensal and in some instances can be pathogenic (Humphrey et al.
12 2014). This shift in opinion is the product of published data describing innate
13 immune responses to experimental *Campylobacter* inoculation coupled with evidence
14 of inflammation and an increased influx of immune cells in some commercial broiler
15 lines (Smith et al. 2008, Meade et al. 2009, Humphrey et al. 2014). Moreover, some
16 have reported that *Campylobacter* colonisation impairs weight gain and alters gut
17 morphology (Awad et al. 2014, 2015). In contrast, other published data show no
18 evidence of gross or histopathological lesions following experimental inoculation of
19 poultry (Beery et al. 1988, Dhillon et al. 2006, Pielsticker et al. 2012). Conflicting
20 data describing the response of the chicken to *Campylobacter* inoculation is not
21 wholly unexpected as the balance between inert commensal and opportunistic
22 pathogen can be swayed depending on the strain of the bacterium, host genotype and
23 immune status, diet and co-infection (Wigley 2015).

24 Differences in *Campylobacter* levels have been described in commercial broiler
25 populations, with some data suggesting that slower growing broiler breeds harbour

1 less *Campylobacter* than standard commercial broiler breeds (Bull et al. 2008,
2 Williams et al. 2013). Conversely, Gormley *et al.*(2014) demonstrated that there were
3 no differences in *Campylobacter* levels in multiple commercial and slower growing
4 broiler breeds when reared in the same environment under commercial conditions
5 with natural exposure to field relevant populations of *Campylobacter*. Experimental
6 inoculation of inbred chicken lines with *C. jejuni* revealed heritable differences in
7 resistance or susceptibility to intestinal colonisation that were consistently observed
8 with multiple strains (Boyd et al., 2005; Psifidi et al. 2016). Through the use of
9 resistant and susceptible inbred chicken lines it has also been possible to demonstrate
10 variation in immune response through gene expression analyses following
11 experimental *C. jejuni* inoculation (Li et al. 2010, 2012, Connell et al. 2012).
12 Attempts have been made to identify loci which may explain variation in resistance to
13 *Campylobacter* with some candidate genes being identified via genome-wide
14 association studies using the progeny of crosses of lines of varying resistance
15 (Connell et al. 2013, Psifidi et al. 2016). Taken together, these findings indicate that
16 *Campylobacter* colonisation in the gut is partly under genetic control and potentially
17 provides a route by which *Campylobacter* could be controlled at the individual bird
18 level (Lin 2009). However, research on avian heritable resistance to *C. jejuni* has
19 mostly relied on inbred birds derived from layer lines, and the extent to which
20 findings apply in commercial broilers is unclear.

21 Here, for the first time, we aimed to estimate the genetic basis of *Campylobacter*
22 colonisation within an outbred pure-bred commercial broiler line reared under
23 commercial conditions with natural exposure to *Campylobacter*. To further examine
24 the influence of *Campylobacter* on the intestinal health of the chicken, the gut tissues
25 of all birds were examined using a *post mortem* gut health scoring system developed

1 by Aviagen[®]. This technique uses a severity scale to macroscopically characterise
2 enteritis and intestinal imbalance based on the appearance of the intestinal tissues and
3 contents. By analysing these phenotypes along with body weight, we aimed to
4 provide more information on the impact of *Campylobacter* on bird performance along
5 with the health and function of the intestinal tract of commercial broiler chickens
6 under relevant farming conditions with natural exposure to *Campylobacter*.

7

8 ***Materials and methods***

9 ***Birds, Housing and Management***

10 The data for this study originate from the ongoing recording of health and performance
11 traits within the Aviagen (Newbridge, UK) breeding program. The birds were housed
12 within a non-bio-secure environment referred to as sib-test environment aimed to
13 resemble broader commercial conditions and where full-sibs and half-sibs of selection
14 candidates are placed (Kapell et al. 2012). A detailed description of environmental
15 parameters can be found in Table 1. Birds were fed a standard feed ration (maize-based
16 to provide the carotenoid source) in the form of a starter, grower and finisher diet in
17 line with industry practice. All birds throughout the study received the same
18 vaccinations as per commercial regimen and were reared under the same management
19 practices. Phenotypic data were collected from 3,000 individual birds and genetic
20 parameters were estimated using five generations of pedigree. To ensure the birds from
21 each flock were exposed to *Campylobacter* during the study, the farm environment was
22 tested for the presence of *Campylobacter spp.* prior to sampling using the “boot sock”
23 method as described by Gormley *et al.* (2014).

24

25 ***Recording of traits***

1 All birds in this study were hatched in the same hatchery, fully pedigreed and
2 uniquely tagged with a barcode wingband. Sampling was performed at 35 days of age
3 with sampling occurring every two weeks in batches of 100 birds (50 males and 50
4 females) giving a total of 3,000 birds over a period of 16 months. Birds were weighed
5 and euthanised humanely by cervical dislocation by trained personnel. After euthanasia,
6 a millilitre of blood was collected from the heart for assessment of blood carotenoid
7 levels. Furthermore the intestinal tract of each bird was assessed after euthanasia and
8 scored to characterise any gross intestinal abnormalities which could indicate enteritis
9 or enteropathy. During this process the two intact caeca were aseptically removed for
10 *Campylobacter* enumeration.

11

12 **Microbiology**

13 To enumerate *Campylobacter* in intestinal contents, seven serial ten-fold dilutions
14 of caecal content were prepared in phosphate-buffered saline and 100 µl plated to
15 modified charcoal deoxycholate (mCCDA) agar supplemented with cefoperazone (32
16 mg/L) and amphotericin B (10 mg/L; Oxoid), followed by incubation for 48 h under
17 microaerophilic conditions (5% O₂, 5% CO₂, and 90% N₂) at 41°C. Dilutions were
18 plated in duplicate and colonies with morphology typical of *Campylobacter*
19 enumerated. The number of colony-forming units (CFU)/g of caecal content was then
20 calculated and the theoretical limit of detection by the method used was 100 CFU/g of
21 content. In instances where no colonies were observed after direct plating, a
22 *Campylobacter* load equal to the theoretical limit of detection was assumed, as
23 enrichment to confirm the absence of *Campylobacter* in the caecal content was not
24 performed.

25

1 **Gut health assessment**

2 The whole intestinal tracts of the birds were examined immediately *post mortem* and
3 intestinal health was evaluated based on a gut health index developed by Aviagen®.

4 The underlying principle of this gut health index is to examine each section of the small
5 intestine and assess the muscular tone of the gut wall, signs of inflammation on the gut
6 surface, the consistency of the gut contents and presence of mucus. In addition the
7 quality of the caecal contents and any evidence of infectious agents is recorded. The
8 scoring of muscular tone, inflammation and consistency is based on a scale of 0
9 (normal), 1 (mildly abnormal) and 2 (severely abnormal); for the presence of mucus it
10 is scored as 0 (absent) or 1 (present). Gut health index scoring was performed on each
11 region of the small intestine (duodenum, jejunum and ileum) and the caeca. The gut
12 health index score for each individual bird was calculated as the sum of all the scores
13 across gut sections. The maximum available score is 23 which would indicate a severely
14 affected intestinal tract; the scoring criteria for each aspect of the gut health index are
15 outlined in Table 2.

16

17 **Serum optical density**

18 The absorptive capacity of the gut can be assessed by measuring the level of carotenoid
19 levels in the blood Blood was allowed to clot at room temperature and 200µl of serum
20 was removed with a pipette and placed into a flat-bottomed 96 well plate. Carotenoid
21 levels were measured via spectrophotometry using a Tecan Sunrise microplate reader
22 at 450nm to obtain the optical density (OD₄₅₀) of the sera. Due to the fragility of avian
23 erythrocytes, haemolysis can sometimes occur and cause discolouration of the sera.
24 This discolouration interferes with the measurement of carotenoids and samples found
25 to be haemolysed were not included in the analysis. In this data set 148 samples were
26 found to be haemolysed and treated as missing values in subsequent analyses. The

1 analyses were performed both with and without the birds with the missing values and
2 no significant differences were seen in the resultant parameters.

3

4 *Statistical Analyses of Genetic Parameters*

5 The phenotypic traits of 35 days body weight (BW), gut health index score (GS), serum
6 carotenoid level (via optical density at 450nm) (OD) and *Campylobacter* load (CP)
7 were analysed in the following multivariate animal model to estimate genetic
8 parameters:

$$9 \quad \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e},$$

10 Where: \mathbf{y} is the vector of observations of the traits, \mathbf{b} the vector of the fixed effect
11 accounting for the interaction between the sex, hatch-week, pen and contributing
12 mating group. To account for the potential impact of seasonal variation on
13 *Campylobacter* load within poultry flocks the model includes the week of hatch of
14 sampled birds as a fixed effect. The vector of additive genetic effects is denoted by \mathbf{a} ,
15 the vector of permanent environmental effects of the dam is denoted by \mathbf{c} , and \mathbf{e}
16 represents the vector of residuals. \mathbf{X} , \mathbf{Z} and \mathbf{W} represent incidence matrices relating the
17 vectors \mathbf{b} , \mathbf{a} , and \mathbf{c} to \mathbf{y} . The assumed (co)variance structure was:

18

$$19 \quad V \begin{bmatrix} \mathbf{a} \\ \mathbf{c} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A} \otimes \mathbf{G} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{I} \otimes \mathbf{C} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I} \otimes \mathbf{R} \end{bmatrix},$$

20

21 Where: \mathbf{A} and \mathbf{I} are the additive genetic relationship matrix and identity matrix,
22 respectively. \mathbf{G} , \mathbf{C} and \mathbf{R} represent the variance and covariance matrices of additive
23 genetic effects, permanent environmental effects of the dam and residual effects,

1 respectively. All variance component analyses were performed using ASReml (v3.0)
2 software (Gilmour et al. 2009).

3

4 **Results**

5 **Phenotypic averages and descriptive statistics**

6 Table 3 summarises the least square means with standard errors for all the traits by
7 sex. The results show that male birds had a significantly higher *Campylobacter* load
8 ($7.145 \log_{10} \text{CFU/g} \pm 0.040$) compared to the female birds ($6.888 \log_{10} \text{CFU/g} \pm 0.040$).
9 The difference in *Campylobacter* load between the sexes, albeit significant, is small and
10 may not represent biologically relevant variation. The mean caecal *Campylobacter*
11 loads demonstrated in this study are comparable to the loads reported in Gormley *et al.*
12 (2014) where *Campylobacter* colonisation was via natural exposure as per this study.
13 There were no significant differences seen in the gut scores between males (2.197
14 ± 0.048) and females (2.210 ± 0.048), and considering the total possible cumulative
15 score of 23 both these scores are very low and indicating good intestinal health overall
16 in both sexes. Serum carotenoid levels as shown by serum $\text{OD}_{450\text{nm}}$ were significantly
17 higher ($p=0.005$) in males (0.526 ± 0.006) compared to females (0.509 ± 0.006)
18 indicating that the males, despite higher level of *Campylobacter*, have a better
19 absorptive capacity of pigments (and by inference lipids).

20

21 **Impact of *Campylobacter* on bird performance**

22 The relationship of *Campylobacter* load with body weight, gut pathology score and
23 serum carotenoid level is shown as scatter (XY) plots in Figures 1, 2 and 3 respectively.
24 These data indicate that following natural exposure of the commercial broiler line
25 studied to *Campylobacter* colonisation, the caecal *Campylobacter* load has no
26 statistically significant impact on bird performance (in agreement with Gormley *et al.*

1 (Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al.
2 2014)(Gormley et al. 2014)(Gormley et al. 2014)(Gormley et al. 2014)2014),
3 macroscopic gut health or ability to absorb carotenoid pigments (and thus lipids).

4

5 **Genetic parameters**

6 The genetic and phenotypic correlations between BWT, GS, OD and CP are
7 presented in Table 4. The phenotypic correlations (below the diagonal shown in bold
8 text in Table 4) of *Campylobacter* load with body weight, gut score and serum
9 carotenoid levels were low. There was a positive correlation between body weight and
10 serum carotenoid level indicating that those birds which have increased ability to absorb
11 carotenoid (thus lipids) grow better.

12 The heritabilities for all the traits are displayed in Table 4. The heritability for body
13 weight is moderate and in line with previously published data (Kapell et al. 2012, Bailey
14 et al. 2015). The heritabilities for gut score, carotenoid level and *Campylobacter* were
15 low with estimates of 0.074, 0.136 and 0.095 respectively.

16 Table 5 shows the proportion of phenotypic variance accounted for by environmental
17 and maternal environment effects. For all the traits analysed, the permanent maternal
18 environment accounted for 1.5-3.4% of the phenotypic variance which is similar to the
19 range reported by Kapell et al (2012) for body weight and dermatitis in the same
20 environment. The residual variance is shown to be responsible for the majority of the
21 phenotypic variance for all traits analysed in this study accounting for 57.7% of the
22 phenotypic variance of body weight and between 84.2-90.6% of the phenotypic
23 variance of gut score, *Campylobacter* load and carotenoid level (as shown by serum
24 OD₄₅₀). The genetic correlations (Table 4, above the diagonal) of *Campylobacter* load
25 with body weight and gut score were low (≤ 0.062), and moderate with serum carotenoid

1 level (0.301), however these were not statistically significant. The relationship of body
2 weight with intestinal health parameters indicated a low genetic correlation with gut
3 score (0.024) and moderate positive correlation with carotenoid level (0.244). The low
4 correlation of body weight and gut score may reflect the fact that gut health was
5 generally good across all birds leading to low phenotypic variance in the population. A
6 positive genetic correlation was seen between gut pathology score and serum carotenoid
7 level (0.482) however, since this correlation was not statistically different from zero
8 robust conclusions cannot be drawn.

9

10 **Discussion**

11 Strategies are urgently required to reduce the burden of *Campylobacter* in poultry to
12 limit the incidence of human infection. The poultry industry has already been
13 successful at reducing the presence of *Campylobacter* in chicken found in retail outlets.
14 Reports from the Food Standards Agency show 6.5% of chickens testing positive for
15 the highest level of contamination (carrying more than 1,000 cfu/g) compared to 9.3%
16 for the same period in the previous year (FSA 2017). Here we sought to evaluate if
17 genetic selection could be an additional tool to reduce *Campylobacter* levels in
18 commercial poultry. As observations of avian resistance to *C. jejuni* to date have relied
19 on inbred layer lines of questionable relevance to commercial broilers (Boyd et al. 2005;
20 Connell et al. 2013; Psifidi et al. 2016), we estimated the genetic basis of
21 *Campylobacter* colonisation in commercial broilers following natural exposure under
22 relevant rearing conditions. We also estimated the genetic correlations of
23 *Campylobacter* load with body weight and intestinal health traits in order to ascertain
24 if selecting for *Campylobacter* resistance may have adverse effects on bird performance
25 and vice-versa. The data presented shows a low but significant heritability estimate for

1 *Campylobacter* colonisation in the test population. These data indicate that whilst there
2 is a genetic component to *Campylobacter* colonisation worthy of further investigation,
3 there is a large proportion of phenotypic variance under the influence of non-genetic
4 effects. As such the control of *Campylobacter* will require understanding and
5 manipulation of non-genetic host and environmental factors.

6 The relationship between *Campylobacter* and its poultry host following exposure is not
7 fully understood. In some cases *Campylobacter* elicits a negative effect on broiler
8 performance and intestinal health (Smith et al. 2008, Meade et al. 2009, Humphrey et
9 al. 2014), whereas in other cases *Campylobacter* has no significant impact on bird
10 weight, intestinal health or immune status (Beery et al. 1988, Dhillon et al. 2006,
11 Pielsticker et al. 2012). In the current study we showed no correlation between caecal
12 *Campylobacter* load and body weight at the phenotypic or genetic level in the broiler
13 line under study. These findings are in agreement with the data from Gormley *et al.*
14 (2014) where no correlation between *Campylobacter* load and body weight was
15 reported. In this study we measured intestinal health and function using macroscopic
16 gut scoring and serum carotenoid levels as a means to investigate whether or not
17 *Campylobacter* was impacting upon the gut of the birds in this study. Typically, during
18 an intestinal challenge, the gut contents have a greater liquid fraction due to secretion
19 of immune cells into the gut lumen, reduced absorption and an increase in water intake
20 by the affected bird (Manning et al. 2007). Additionally it is common for an
21 inflammatory response to be seen on the gut surface particularly in the gut-associated
22 lymphoid tissue (Chen et al. 2015) along with thinning and loss of muscle tone in the
23 intestinal wall (Teirlynck et al. 2011). When the intestinal tract is compromised
24 malabsorption can occur resulting in the caecal microbiota becoming imbalanced
25 leading to a change from the normal dark brown pasty caecal contents to paler coloured,

1 watery and gassy contents (Wilson et al. 2005, Teirlynck et al. 2011, Sergeant et al.
2 2014). The absorptive capacity of the gut can be assessed using the level of carotenoids
3 in the blood. These naturally occurring pigments, found in many plants such as maize,
4 influence the yellow pigmentation found in the skin and legs of poultry (Rajput et al.
5 2013). Carotenoids are fat soluble and thus absorbed with lipids during digestion where
6 they enter the blood stream and can be laid down in the body tissues (Ullrey 1972,
7 Yonekura and Nagao 2007, Nagao 2011). In the event of enteric disease there is a
8 reduction in fat absorption which in turn leads to a reduction in carotenoid absorption
9 resulting in poor pigmentation; this is seen in coccidiosis, mycotoxicosis and
10 malabsorption syndromes (Tung and Hamilton 1973, Tyczkowski et al. 1991a, 1991b,
11 Zhao et al. 2006). The data presented demonstrates that there is no correlation between
12 *Campylobacter* load and intestinal health as examined by macroscopic gut scoring of
13 the intestinal tract and the ability to absorb carotenoids (through serum optical density)
14 as an indicator of intestinal function. Assuming that caecal *Campylobacter* load is
15 representative of colonisation in other parts of the intestinal tract, this result indicates
16 that in this study *Campylobacter* colonisation does not have a negative impact upon
17 intestinal health of the birds.

18 The differences seen in host response between experimental infection and natural
19 exposure may be linked, in part, to the way by which the bacterium is introduced to the
20 birds. Experimental infection of birds with *Campylobacter* is usually with a high
21 concentration of a single strain at one time point whereas natural exposure occurs
22 gradually with one or multiple strains initially at lower doses (Beery et al. 1988, Newell
23 and Fearnley 2003, Boyd et al. 2005, Psifidi et al. 2016). It is possible that in the case
24 of experimental inoculation, the introduction of a large dose of a single bacterium has
25 the potential to upset the balance of the resident microbiota resulting in dysbacteriosis

1 leading to a disruption in intestinal health and function. Furthermore, the procedure of
2 handling and dosing a bird during experimental inoculation may cause stress to the bird
3 which may have the potential to influence the physiology of the bird and the activity of
4 the bacterium once it enters the gastrointestinal tract. This could aid the proliferation of
5 *Campylobacter*, especially if there are host related factors favouring *Campylobacter*
6 colonisation such as in the case of susceptible inbred lines. At the farm level, a key risk
7 factor for increasing levels of *Campylobacter* in a broiler flock is through the process
8 of partial depopulation (also called ‘thinning’) where a proportion of the flock are
9 removed at a certain body weight and the remaining birds are kept on the farm to allow
10 them to grow for a longer period of time (Cloak et al. 2002). Opportunities for breaks
11 in biosecurity and increasing bird age may be responsible for these increases in
12 *Campylobacter* levels (Smith et al. 2016), as well as the stress associated with the
13 process of partial depopulation (Robyn et al. 2015). Catecholamines released during
14 stress, such as adrenaline and noradrenaline, can impact negatively upon the intestinal
15 environment (Siegel 1971, 1980, Virden and Kidd 2009) and promote motility and
16 growth of *Campylobacter* (Cogan et al. 2007, Xu et al. 2015). The manner and extent
17 by which a particular strain of *Campylobacter* responds to noradrenaline has been
18 shown to be highly variable (Aroori et al. 2014) and thus the outcome of an
19 *Campylobacter* challenge may be dependent on which strain is introduced to the
20 intestinal tract of the bird. The impact of *Campylobacter* on its poultry host is highly
21 variable and understanding the factors which can result in colonisation or a negative
22 interaction may inform strategies for controlling the bacterium.

23 The caecal microbiota has long been recognised influencing susceptibility to disease
24 and colonisation by zoonotic pathogens (Stanley et al., 2014). Certain bacterial species
25 are known to affect the growth of *Campylobacter* (Nishiyama et al. 2014, Mañes-

1 Lázaro et al. 2017) and there have been reports of differences in intestinal microbiota
2 composition in birds positive for *Campylobacter* (Sofka et al. 2015, Indikova et al.
3 2015). Transfer of microbiota between inbred mice differing in susceptibility to the
4 enteric pathogen *Citrobacter rodentium* resulted in a reciprocal transfer of
5 susceptibility and resistance (Willing et al. 2011). Thus, while a host genetic component
6 to resistance can exist, this may be exerted in part through differences in the microbiota.
7 Studies are therefore required to associate *Campylobacter* burden with the composition
8 of indigenous microbial communities to explore the extent to which this may explain
9 variation in *C. jejuni* colonisation phenotypes.

10 Whilst the present study provided evidence of a genetic component affecting
11 *Campylobacter* colonisation, the estimate of heritability for *Campylobacter* load in the
12 caeca is low and would mean that any progress through selection is likely to be slow
13 and very modest in impact due to a low accuracy of predicting breeding values.
14 Importantly, the lack of genetic correlation between *Campylobacter* load with body
15 weight and gut health traits indicates that any selection for *Campylobacter* would not
16 be detrimental for bird performance. Selection for disease resistance or resilience is a
17 common goal in many livestock breeding programs however success is heavily reliant
18 on two important things; firstly the animals from within the study population need to
19 be inoculated with the target organism and secondly a reliable phenotype is needed to
20 measure the presence or impact of the organism on the host (Bishop 2012). A breeding
21 strategy for reducing *Campylobacter* colonisation would need to be based on natural
22 exposure to *Campylobacter*, as experimental bacterial challenge as part of a routine
23 program has ethical and safety implications. When using natural exposure, inoculation
24 with the target organism is dependent on the seasonality of the organism and studies
25 have shown that the presence of *Campylobacter* in poultry environments is seasonal

1 (Chowdhury et al. 2012). The consequence of seasonality is that exposure will vary
2 from flock to flock thus the accuracy of the estimation of variance components is
3 compromised (Bishop and Woolliams 2014). Our results should be interpreted in the
4 context of the limitations and advantages of field studies (Bishop and Woolliams 2010,
5 Bishop et al. 2012). Compared to controlled challenge experiments, unknown and
6 uncontrolled exposure to infections, may reduce the power of a field study but does not
7 constitute a fatal flaw in demonstrating host genetic differences in resistance (Bishop
8 and Woolliams 2010). In addition, the natural infections that characterise field studies
9 offer a more realistic picture of the genetic variation and yield results that are more
10 relevant to practical genetic improvement programmes.

11 When dealing with complex traits where heritabilities are low and a reliable
12 phenotype cannot be established, molecular genomic methods may be required to
13 achieve resistance (Bishop and Woolliams 2014). The use of genome-wide association
14 studies for the identification of single nucleotide polymorphisms or QTL conferring
15 resistance to disease has been successful in a number of animal species in selecting for
16 disease resistance (Houston et al. 2008, Bermingham et al. 2014). The low heritability
17 estimate for campylobacter colonisation indicates that there doesn't seem to be any
18 QTL of large effect for resistance or any QTL present are already at a high frequency
19 in the population under study. Our ongoing research seeks to define the genomic
20 architecture of the *Campylobacter* resistance in commercial broiler chickens.

21 In conclusion this study indicates that *Campylobacter* colonisation in the broiler
22 intestinal tract following natural exposure is partially under genetic control with the
23 majority of phenotypic variance being under the influence of environmental factors.
24 Understanding the environmental factors that influence *Campylobacter* prevalence at
25 the farm level will be required to devise strategies for control of *Campylobacter* in

1 broilers and genetic selection may be only a minor part of an integrated solution to the
2 problem. Additionally by examining body weight along with macroscopic intestinal
3 health and absorptive capacity it was shown that, following natural exposure,
4 *Campylobacter* has no detrimental impact upon bird health.

5 **Acknowledgements**

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24 Table 1. Environmental parameters for the farm where birds were housed in this study.

Environmental parameter	Target
Feed days: 0-10	Starter (195g CP/kg; 12.0 MJ ME/kg)

Feed days: 11-25	Grower (170g CP/kg; 12.7 MJ ME/kg)
Feed days: 25-final weighing	Finisher (170g CP/kg; 12.7 MJ ME/kg)
Stocking density	29 to 32 kg bird weight per m ²
Temperature	Gradually reduced from 35 to 24°C
Photoperiod day 0-7	23L:1D
Photoperiod day 8-final weighing	18L:6D
Light intensity day 0-7	40 lux
Light intensity day 8-final weighing	Gradually reduced from 20 to 10 lux

1

	Score		
	0	1	2
	Normal/Healthy	Mildly abnormal	Severely abnormal
(T) Tone of intestinal wall <i>(Based on cutting into the intestinal wall longitudinally)</i>	When cutting into the gut wall the wall immediately folds back on itself	On cutting into the gut, the wall folds back but it does not occur immediately and there is a delay (more than 5 seconds) in the wall moving.	The gut wall fails to fold back on itself when cut
(C) Consistency of intestinal contents based on region of small intestine <i>(Based on quality of intestinal contents when cutting into the intestinal tract to assess tone)</i>	<i>Duodenum:</i> Typically the contents resemble coarse porridge but must be of a uniform consistency. <i>Jejunum:</i> Contents here should contain less water than the duodenal contents and the colour should be darker. <i>Ileum:</i> Contents should be starting to form firm bolus and colour should be much darker	<i>Duodenum:</i> Contents not uniform with a distinguishable fluid and solid fraction. <i>Jejunum:</i> Contents not uniform with a distinguishable fluid and solid fraction but less water than the duodenal contents. <i>Ileum:</i> Bolus is forming but it does not hold its shape but colour of contents is darker than the jejunal contents.	<i>Duodenum:</i> Distinguishable fluid and solid fraction however it is predominately fluid. <i>Jejunum:</i> Distinguishable water and solid fraction colour same as duodenal contents <i>Ileum:</i> No bolus formation with soft/wet contents. colour may be similar to contents in jejunum
(I) Mucosal inflammation	Mucosal surface light pink colour with no evidence of reddening on surface.	Localised inflammation around GALT or diffuse localised reddening of mucosa in small areas	Profuse inflammation and reddening covering extensive areas of the mucosa
(M) Mucus production <i>(Based on presence or absence)</i>	No mucus seen	Obvious layer of opaque mucus lining region of intestinal tract	(Not applicable for this criteria)
(Ca) Caecal health	Dark brown/green contents, pasty in consistency and no gas present	Pale in colour, pasty consistency and small amount of gas bubbles present	Contents are pale in colour and have a fluid consistency. Contents leak out when caeca cut. Caeca more than 50% filled with gas.

- 1 Table 2. Outline of scoring criteria for Gut Health Index – must be performed within 15 minutes of euthanasia otherwise post mortem intestinal
- 2 autolysis may interfere with the results. Scores added with a higher score indicating more severe intestinal imbalance/disturbance. Maximum

- 1 score is 23 which is obtained by assessing T+C+I+M (which has a maximum of 7) for each small intestinal region and the Ca scores which has a
- 2 maximum of 2 – these scores are then added together to give the final score $(7+7+7+2=23)$

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Table 3. Descriptive statistics for the traits for each sex.

Trait	Male Mean	Female Mean	Standard Error	P value
Body weight dg (BW) *	156.9	143.9	0.600	0.001
<i>Campylobacter</i> load (Log cfu/g) (CP) *	7.145	6.888	0.048	0.001
Gut Score (GS) *	2.197	2.210	0.048	0.088
Serum carotenoid level (OD)	0.526	0.509	0.006	0.001

Table 4. Estimates of heritabilities (bold, diagonal), genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for body weight (BW), gut score (GS), Serum carotenoid level (OD) and *Campylobacter* load (CP). Standard errors are displayed in parentheses.

BW	GS	OD	CP
0.389 _(0.063)	0.024 _(0.265)	0.244 _(0.170)	0.062 _(0.193)
-0.019	0.074 _(0.048)	0.482 _(0.358)	0.054 _(0.399)
0.136	-0.056	0.136 _(0.043)	0.301 _(0.259)
-0.023	-0.021	-0.067	0.095 _(0.037)

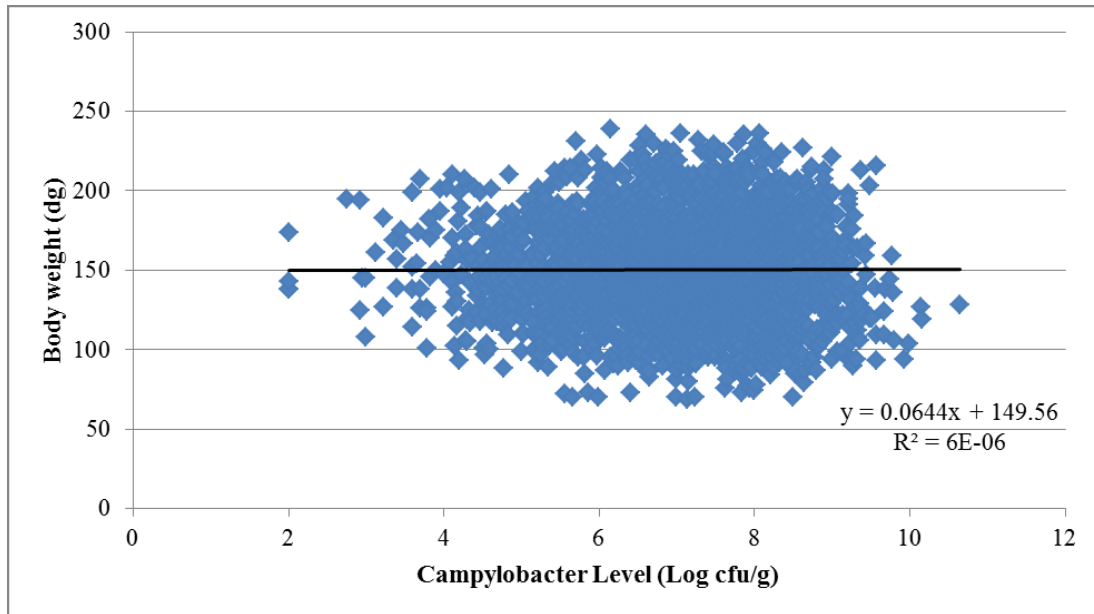
Table 5. Phenotypic (PHEN), Residual (RES) maternal permanent environmental (PEm) variances and proportions of Phenotypic variance accounted for by RES (Prop RES) and PEm (Prop PEm) for Body weight (BW), *Campylobacter* level (CP), Gut score (GS) and Carotenoid level (OD)

Line A					
Trait	PHEN	RES	PEM	Prop RES	Prop PEm
BW	402.44	232.23	13.79	0.577	0.034
GS	1.273	1.153	0.026	0.906	0.020
CP	143.19	127.41	2.164	0.890	0.015
OD	149.49	125.88	3.342	0.842	0.022

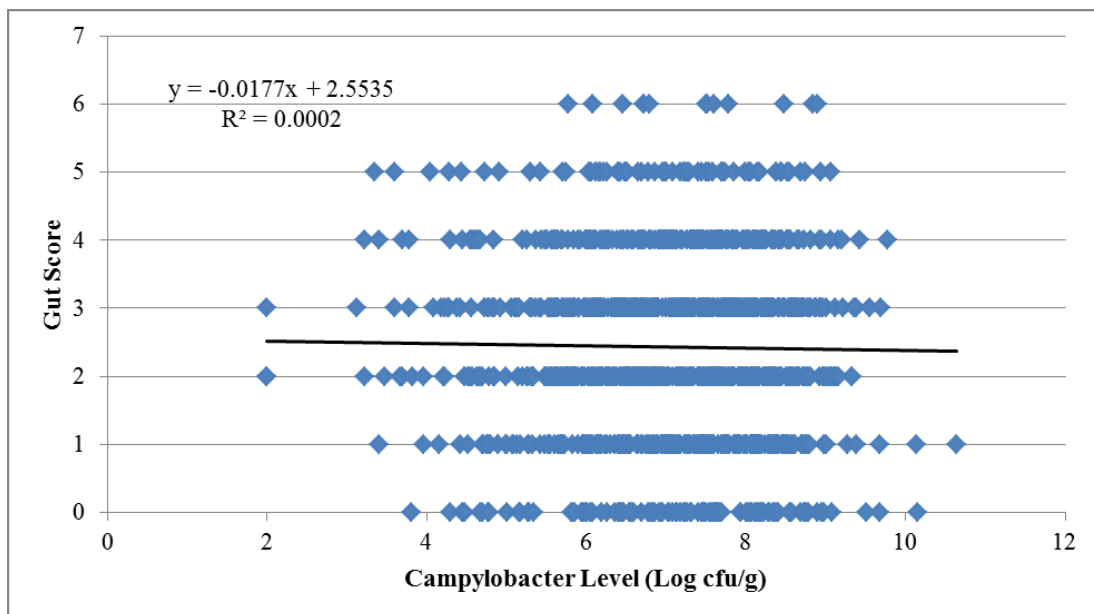
Figure Legends:

1 Figure 1. Scatter (XY) plot of *Campylobacter* load (Log₁₀ CFU/g) and bodyweight (dg)
 2 showing there is no relationship between *Campylobacter* burden and bird weight.
 3 Figure 2. XY plot of *Campylobacter* load (Log₁₀ CFU/g) and gut score showing there
 4 is no relationship between *Campylobacter* and gut pathology score.
 5 Figure 3. Scatter (XY) plot of *Campylobacter* load (Log₁₀ CFU/g) and carotenoid level
 6 (serum OD₄₅₀) showing there is no relationship between *Campylobacter* and carotenoid
 7 level, and by inference ability of the gut to absorb lipids.
 8

9 Figure 1

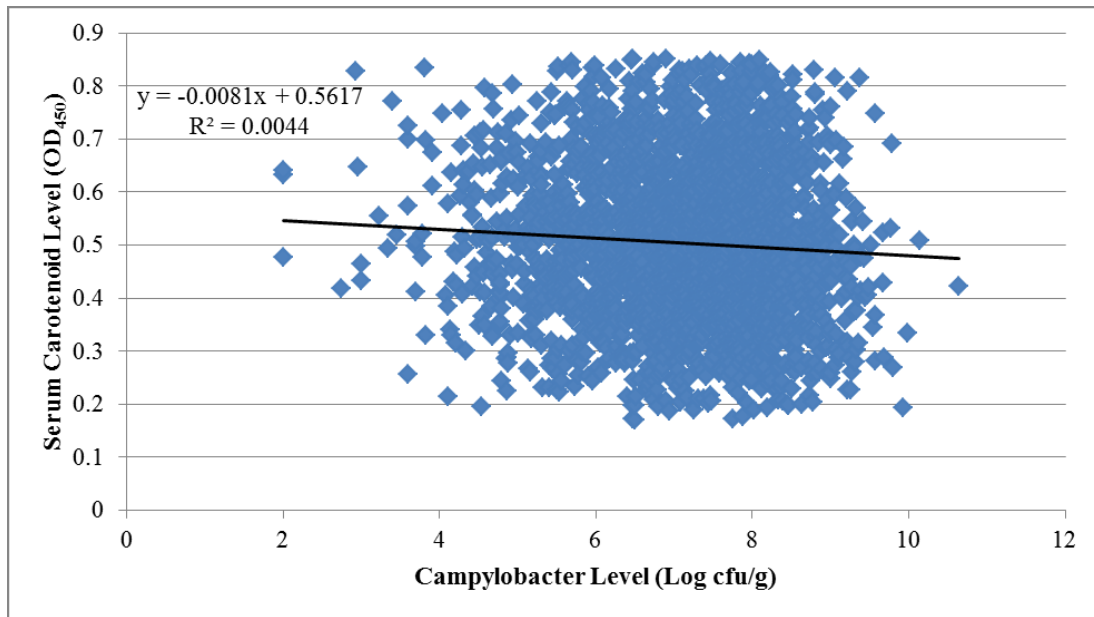


11 Figure 2



13 Figure 3

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