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Title: *Campylobacter* Succession in broiler chickens

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Running title: *Campylobacter* Succession in broiler chickens

Abstract

15 The competitive ability of *Campylobacter coli* OR12 over *C. jejuni* OR1 has been
examined in experimental broiler chickens following the observation that *C. coli* replaced an
established *C. jejuni* intestinal colonisation within commercial chicken flocks reared outdoors
(El-Shibiny, A., P.L. Connerton and I.F. Connerton 2005 Appl. Environ. Microbiol. 71:1259-
1266). Co-cultures of *C. coli* OR12 with *C. jejuni* OR1, revealed that the two species were
20 able to grow together at similar growth rates in exponential growth phase but if the disparity
of the inoculum ratios were $>\log_{10} 4$ in favour of *C. coli* OR12, *C. jejuni* OR1 was observed
to prematurely enter decline phase. Chickens were pre-colonised with *C. jejuni* OR1 at 21
days old to examine succession *in vivo*. The birds were inoculated between 2 and 12 days
later with *C. coli* OR12, to determine if the second isolate could efficiently colonise and
25 compete with an established *C. jejuni* strain. *C. coli* OR12 were able to co-colonise before
replacing *C. jejuni* OR1 as the dominant species when the birds were more than 27 days of
age at the time of administration over a 4 day period. If these criteria were met *C. coli* OR12
became the dominant isolate otherwise co-colonisation occurred until they were met. *C. coli*
OR12 was also found to displace three alternative *C. jejuni* strains from pre-colonised
30 chickens challenged with *C. coli* OR12 at 30 days of age and tested at 40 days. These data
raise the possibility of manipulating populations of *Campylobacter* colonising chickens
through competition.

Keywords: *Campylobacter*, *C. jejuni*, *C. coli*, competition, poultry.

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1. Introduction

Members of genus *Campylobacter* are frequently responsible for human cases of enteric disease. It is estimated that there were around 350,000 cases in England and Wales in the year 2000 (Adak et al., 2000), with *Campylobacter jejuni* accounting for 90% of the cases and *C. coli* contributing around 25,000 cases (Gillespie et al., 2002; Tam et al., 2003). Human
40 infections of either *C. jejuni* or *C. coli* result in intestinal inflammation leading to diarrhoea and abdominal pain (Skirrow and Blaser, 2000).

It one study of raw chicken meat sold in the UK, 80% was contaminated with *Campylobacter* (Jorgensen et al., 2002) with 98% of isolates speciated as *C. jejuni* and 2 % *C. coli*. Various studies from around the world have shown that the numbers of *C. coli*
45 isolated from poultry may range from 6 -50% (Corry and Atabay, 2001) with the highest incidence associated with free-range birds and the lowest from birds reared in conventional barns (Rivoal et al., 2005).

In a previous study (El-Shibiny et al., 2005) we reported the succession of *C. jejuni* by *C. coli* during the rearing cycle of organic and free-range broiler chickens. There were several
50 possibilities that could explain the dramatic change in dominant species from *C. jejuni* to *C. coli*. The simplest explanation being that the *C. coli* had a competitive advantage due to more efficient use of the prevailing nutrients. However, differential sensitivity to bacteriophage predation or bacteriocins or other antagonistic metabolites may also have led to the decline in numbers of one species over the other (Connerton, et al., 2004; Chaveerach et al., 2004;
55 Humphrey et al. 1989; Schoeni and Doyle, 1992). Alternatively host-associated factors such as developments in the immune system of the chicken might affect the dominant species, which could account for the common timing of the succession noted at 31 days-of-age.

Profound differences in the ability of individual strains of *C. jejuni* to colonise and persist in chickens have been demonstrated (Cawthaw et al., 1996; Ringoir and Korolik.

60 2003). The reasons for these variations are not clear but *in vivo* passage has been shown to enhance the colonisation ability of *C. jejuni* strains (Cawthaw et al., 1996, Jones et al., 2004). *C. jejuni* strains able to displace resident *C. jejuni* strains (Chen and Stern. 2001; Korolik et al., 1998) or prevent colonisation in young birds (Barrow and Page, 2000) have been reported. Of particular note fluoroquinolone-resistant strains of *C. jejuni* appear to be more fit than
65 those sensitive to the antibiotic when co-inoculated in chickens (Luo et al., 2005), and ciprofloxin resistant strains (often *C. coli* or a mixture of *C. coli* and *C. jejuni*) have been reported to persist and dominate in chicken flocks after the removal of antibiotic therapy that provoked their selection (Humphrey et al., 2005).

The aims of this work were: 1. to discover if there were any obvious differences in
70 growth between isolates *C. jejuni* OR1 and *C. coli* OR12 previously observed to undergo succession (El-Shibiny et al., 2005); 2. to investigate if the succession could be replicated in experimental birds; 3. to determine the time periods required to establish the competitive advantage of *C. coli* OR12 in order to investigate any link between the age of the birds and succession; 4. to investigate if the successful competitor *C. coli* OR12 could be used to
75 displace other well characterised *C. jejuni* isolates colonising experimental birds and therefore be used to manipulate *Campylobacter* populations in birds.

2. Materials and Methods

2.1 Bacterial strains

80 *Campylobacter jejuni* OR1 and *C. coli* OR12 were isolated from a UK organic chicken farm (El-Shibiny et al., 2005). *C. jejuni* HPC5 and *C. jejuni* GIIC8 were isolated from conventional barn-reared UK broiler chicken flocks (Loc Carrillo et al., 2005) whilst *C. jejuni* 81116, a human derived isolate, was obtained from D. Newell, Veterinary Laboratories Agency Weybridge UK (Cawthaw et al., 1996). The isolates were routinely cultured on horse

85 blood agar (BA, blood agar base No 2 CM0271, [Oxoid, Basingstoke, UK] with 5%
defibrinated horse of blood [TCS, Buckingham, UK] added) under microaerobic conditions
(5% O₂, 5% H₂, 10% CO₂, 80% N₂ produced by the evacuation and replacement technique) at
42° C for 24 h. Isolates of *Campylobacter* were stored at -80° C on Microbank storage beads
(Pro Lab Diagnostics, Cheshire, UK).

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2.2 Enumeration of campylobacters in vitro and in vivo

Serial dilutions of either broth culture from growth curve experiments or caecal
contents from chickens were made in maximum recovery diluent (MRD; CM0733, Oxoid)
and enumerated from plate counts on modified Cefoperazone Charcoal Desoxycholate Agar
95 (mCCDA) agar supplemented with cefoperazone (Pro-Lab Diagnostics) with additional agar
(L13, Oxoid) to a total of 2% added to reduce swarming. Plates were incubated under
microaerobic conditions at 42° C for 48 h before typical *Campylobacter* colonies were
counted.

100 2.3 Discrimination between strains

Where co-cultures of *C. jejuni* and *C. coli* were performed it was necessary to be able
to be able to accurately distinguish the strains from each other. Colony hybridisation was the
primary method used for this purpose. Where Hybond-N (Amersham) membranes were
placed onto the surface of agar plates with 50-200 colonies for 5 minutes and orientation
105 marks applied. The blotting membranes were then placed, colony side up, on Whatman 3 MM
paper soaked in 10% w/v sodium dodecyl sulphate and incubated for 5 minutes at room
temperature to lyse the cells. The membranes were then placed, colony side up, on successive
Whatman 3 MM papers soaked in denaturation solution (0.5 M NaOH, 1.5 M NaCl), then
neutralisation solution (0.5 M Tris-HCl [pH 7.5], 3 mM NaCl) and then wash solution (300

110 mM NaCl, 30 mM Sodium citrate [pH 7]) for 5 minutes each. The membranes were then air
dried for 30 min followed by UV cross-linking. DIG-labelled probes specific for the *hipO*
gene of *C. jejuni* were synthesised using the PCR primers described by Linton et al. (1997) in
conjunction with digoxigenin-11-dUTP (Roche) to replace dTTP in a standard PCR reaction.
The distinction between *C. jejuni* and *C. coli* was confirmed by washing off the initial probe
115 and re-hybridising with a 405bp PCR fragment based on the sequence located downstream of
the *C. coli* aspartokinase gene identified by Linton et al. (1997), which could be demonstrated
to be specific for *C. coli* OR12 in these experiments. Hybridisation and detection were carried
out using materials from Roche according to the instructions supplied. Colourmetric detection
was carried out using nitro-blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-
120 phosphate 4-toluidine (Roche) and the colonies were counted.

Isolates were also distinguished in parallel by their inherent antibiotic tolerances.
Colonies enumerated from the original mCCDA plates were replicated onto the mCCDA
plates containing the additional antibiotics (all from Sigma Aldrich, Gillingham, UK) to
suppress the competing isolates as follows: for *C. jejuni* OR1 and *C. jejuni* GIIC8,
125 Tetracycline (50 µg/ml) was added to suppress *C. coli* OR12; for *C. jejuni* HPC5, Kanamycin
(30 µg /ml) was added to suppress *C. coli* OR12; and for *C. coli* OR12 counts, Penicillin (20
µg /ml) was added to suppress *C. jejuni* OR1, *C. jejuni* GIIC8, and *C. jejuni* HPC5. The plates
were then incubated as previously described. To confirm the distinction of *C. jejuni* 81116
from *C. coli* OR12 it was necessary to perform hippurate hydrolysis on individual colonies as
130 no suitable antibiotic was available. The hippurate hydrolysis technique was validated using
the PCR amplicons of Linton et al. (1997).

In order to validate the accuracy of the three different methods, mixtures of cultures of
know cell densities were prepared, treated as normal samples and then enumerated. All 3
techniques were found to accurately distinguish between the strains (only two techniques,

135 hippurate hydrolysis and hybridisation could be used to distinguish *C. coli* OR12 from *C. jejuni* 81116 because no antibiotic selection was possible for this combination).

2.4 Growth Curves

These experiments were performed by combining *C. coli* OR12 with *C. jejuni* in broth culture. A range of inoculation ratios were used to start the cultures, from which growth
140 curves were plotted for each species. Campylobacters were grown in Nutrient Broth No 2 (CM 67, Oxoid) in conical flasks under microaerobic conditions, on an orbital shaker at 42°C. Samples were taken at intervals to obtain viable cell counts over 48 h. Growth was determined by viable count on mCCDA agar followed by replication on to antibiotic containing media to distinguish the input species from each other. The hippurate hydrolysis
145 test was used additionally to further validate the distinction between *C. jejuni* and *C. coli*.

2.5 Experimental birds

Commercial *Campylobacter*-free male Ross broiler chickens were obtained as hatchlings (P.D. Hook, Oxfordshire, UK). All experiments were performed in accordance with UK legislation and following independent ethical review. Birds were housed individually in a controlled environment in individual pens under strict conditions of biosecurity. To ensure that the experimental birds remained free of naturally occurring infection, faeces and cloacal swabs were taken each day and tested for *Campylobacter* by direct plating on mCCDA agar and for *Salmonella* by enrichment in Rappaport-Vassiliadis soya peptone broth (CM 866, Oxoid) then plating on Xylose-Lysine desoxycholate agar (XLD) agar (CM 469, Oxoid). In order to ensure that no contaminating campylobacters had infected the birds in the containment facility, pulse field gel electrophoresis (PFGE) of *Sma*I digests of genomic DNA was carried out (Ribot et al., 2001) from random isolates recovered from the chickens and compared to the known profiles of the test strains.

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2.6 Competition experiments in broiler chickens using *C. jejuni* OR1 and *C. coli* OR12

The *C. jejuni* isolate, OR1 was suspended in phosphate buffered saline (PBS, BR14a, Oxoid) and diluted to contain $\log_{10} 7$ CFU/ml. Each chicken was dosed with 1 ml of bacterial suspension in PBS or PBS alone for the controls, by oral gavage at 21 days of age. The *C. coli* OR12 strain was administered in the same way to the pre-colonised chickens at different points during the rearing period from 25 days to 35 days. Chickens were killed at various intervals from 26 days to 46 days of age and the numbers of each *Campylobacter* species determined from the contents of the caeca as described previously (Loc Carrillo et al. 2005). The effect of administering *C. jejuni* OR1 at day 27 to birds that had been pre-colonised with *C. coli* at 21 days then sampling after 1, 5, 9 and 12 days to examine the short medium and

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long term effects was also determined and campylobacters discriminated and enumerated by colony hybridisation and antibiotic resistance as described above.

Analysis of variance was performed using the software package Statistica (StatSoft Ltd, Bedford, UK).

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2.7 Competition of *C. coli* OR12 with additional *C. jejuni* isolates

Experiments to determine competitive interaction between *C. coli* OR12 and *C. jejuni* strains HPC5, GIIC8 and 81116 were carried out with each isolate being administered as above but at 20 days of age to mimic natural infection since *C. jejuni* is usually first detected at around 3 weeks of age. *C. coli* OR12 was then administered to these birds at 30 days of age. These birds were culled at 40 days of age around the age at which they would normally be culled in commercial situations and the numbers of each *Campylobacter* species determined from the contents of the caeca as described previously above.

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3. Results

3.1 *In vitro* Competition between *C. coli* OR12 and *C. jejuni* OR1

The *in vitro* competition experiments were carried out to determine if the succession *in vivo* could be attributed to any simple metabolic difference, for example one isolate might have a faster growth rate than the other. Growth curves were prepared using viable counts obtained from the different input ratios over 48 h. Figure 1 shows representative growth curves from some of the different input ratios tested. The exponential phase growth rates of *C. coli* OR12 ($0.94 \pm 0.2 \text{ h}^{-1}$) and *C. jejuni* OR1 ($0.96 \pm 0.4 \text{ h}^{-1}$) in co-culture were similar, and this was independent of their relative inoculum ratios.

When the input ratios were equal no significant differences in the growth curves were observed over the entire experimental period (Fig 1A). However, a significant change in the

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behaviour of *C. jejuni* OR1 with *C. coli* OR12 was noted at higher input ratios, where *C. jejuni* OR1 counts started to fall at 12 h akin to death phase without any notable stationary phase, showing declines of \log_{10} 1.6 CFU/ml, and \log_{10} 4.3 CFU/ml at respective *C. coli* OR12:*C. jejuni* OR1 input ratios of 10^2 and 10^6 (Fig. 1D and 1E). This did not occur when the situation was reversed with ratios of 10^2 :1 and 10^6 :1 *C. jejuni* OR1:*C. coli* OR12 (Fig 1 B and 1C), implying that *C. jejuni* OR1 is sensitive to excess numbers of *C. coli* OR12 or its products in stationary phase.

3.2 *In vivo* competition of *C. jejuni* OR1 and *C. coli* OR12 in broiler chickens

Significant variation in the ability of *Campylobacter* isolates to colonise chickens has been reported (Cawthaw et al., 1996; Ringoir and Korolik, 2003). The colonisation potential and ability to persist over time of each isolate were therefore determined individually in broiler chickens before commencing competition experiments. The first five columns of Fig. 2a and 2b show the mean caecal counts of *C. jejuni* OR1 and *C. coli* OR12 respectively recovered from the control chickens (n=9) at time points over the course of the experiment. The chickens remain colonised with either *C. jejuni* OR1 or *C. coli* OR12 through out the experimental period. The mean caecal counts for all control birds were \log_{10} 7.8 ± 0.7 CFU/ g for *C. coli* OR12 and \log_{10} 7.8 ± 0.8 CFU/g for *C. jejuni* OR1. Precautions were taken to prevent experimental birds being colonised by campylobacters, other than those under study, through stringent biosecurity measures, and by the routine examination of antibiotic tolerances and PFGE profiles of isolates recovered from the chickens.

A series of experiments were designed in order to study the competitive ability of the *C. coli* OR12 over *C. jejuni* OR1 in relation to the age of the birds. The effect on established *C. jejuni* OR1 populations of introducing the competitor *C. coli* OR12 into birds of different ages was assessed by sampling the birds at 1, 4 and 11 day intervals following competitor

challenge by enumerating the total and species populations of the intestinal contents of the birds. The ages of the birds at the point of challenge that were selected to span the days leading up to the 35 day time point at which time we observed profound changes in the dominant species in our previous work. The challenge days were therefore chosen as 25 days, 27 days, 31 days and 35 days. Once *C. coli* OR12 had been administered orally it was able to establish and co-colonise to produce recoverable counts within 24 h from the ileum, caeca and colon of all the birds pre-colonised with *C. jejuni* OR1. The mean caecal counts presented in Fig. 2A show that irrespective of the age of the bird to which *C. coli* OR12 was introduced, the strain was able to co-colonise with the existing *C. jejuni* OR1, to establish *C. coli* OR12 populations comparable with those recorded for controls birds colonised by *C. coli* OR12 alone (the first five columns of Fig 2B). However, after 11 days there was a striking reduction in the *C. jejuni* counts of $>4 \log_{10}$ CFU per gram of caecal contents, to leave *C. coli* OR12 as the dominant species. At 4 days post challenge the ability of *C. coli* OR12 was dependant on the age of the birds but not on the time between administration and sampling. In the youngest birds (those administered with *C. coli* OR12 at 25 or 27 days of age) the counts of *C. jejuni* OR1 remained at normal levels compared with the controls. However, there was a greater than $2 \log_{10}$ CFU /g reduction in *C. jejuni* OR1 in those birds administered with *C. coli* at 31 and 35 days of age and sampled 4 days later at 35 and 39 days of age respectively. Multivariate analysis of these data reveals the changes observed to be highly significant with respect to the change in species caecal count ($p=5.8 \times 10^{-11}$) and the time post challenge ($p=0.002$), with clear interaction between these terms ($p=5.5 \times 10^{-13}$). Partitioning of the data collected at 4 days post challenge demonstrated the importance of the age of the birds at this time with a significant fall in the *C. jejuni* caecal count for birds post 31 days of age ($p=0.0001$). To conclude, in order for *C. coli* OR12 to become dominant over *C. jejuni* OR1, a period of time of more than one day was necessary after introduction of the new species and

the birds needed to be greater than 31 days of age for this to occur. If these criteria were met *C. coli* OR12 became the dominant isolate otherwise co-colonisation occurred.

Following this dramatic decline in *C. jejuni* counts after the introduction of *C. coli* we elected to perform the reverse experiment to examine if the primary colonisation of chickens with *C. coli* at day 21 could prevent or hinder subsequent challenge with *C. jejuni* at day 27. Birds were sampled after 1, 5, 9 and 12 days to examine the effects of the challenge on 27 day old birds. Figure 2B shows that when birds were pre-colonised with *C. coli* OR12 this did not prevent initial co-colonisation with *C. jejuni* OR1, although this was always at a reduced level compared to controls (first five columns of Fig2A). It was also notable that the caecal counts of *C. coli* OR12 were reduced as compared to unchallenged controls (Fig. 2B). However, at 12 days post administration, the *C. jejuni* OR1 caecal count was observed to show a significant decline to $\log_{10} 2.9 (\pm 0.9)$ CFU/g ($p=0.02$) accompanied by a rise in the *C. coli* OR12 mean caecal count from $\log_{10} 5.4$ after 9 days to $\log_{10} 7.0$. Thus following a period of co-colonisation *C. coli* OR12 became the dominant species, as had been observed in the previous experiments.

3.3 *The displacement of alternative C. jejuni isolates in competition with C. coli OR12*

It was our aim in this series of experiments to examine if *C. coli* OR12 could compete with independent isolates of *C. jejuni* capable of efficiently colonising broiler chickens. The timing of infection was selected to mimic intensively rearing of birds rather than free-range rearing that we had studied in the previous experiments. Chickens were pre-colonised at 20-days-of-age with $\log_{10} 7$ *C. jejuni* from one of the following *C. jejuni* isolates: GIIC8, 81116 or HPC5. They were then challenged with $\log_{10} 7$ *C. coli* OR12 at day 30. The birds were culled at day 40 and the *Campylobacter* species enumerated from their intestinal contents. These results recorded in Fig. 3 show that control chickens maintained *C. jejuni* colonisation until day 40 in

the absence of *C. coli* but following inoculation with *C. coli* OR12 at day 30 the birds showed a dramatic fall in their caecal populations of *C. jejuni* compared to the controls. In contrast the *C. coli* OR12 caecal counts were not significantly different to the chickens colonised with *C. coli* OR12 alone.

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4. Discussion

Chickens exposed to the environment are likely to encounter more than one strain or species of *Campylobacter* capable of establishing a persistent commensal relationship during the lifetime of the bird. Previously we and others have shown that the dominant strain may
280 change several times throughout the rearing cycle of organic and free-range birds exposed to environmental sources (El-Shibiny et al., 2005; Rivoal et al., 2005). Barn reared broiler chicken flocks are likely to be exposed to a somewhat more limited set of *Campylobacter* types but these have also been reported to exhibit the dominance of one *Campylobacter* type only to be replaced with another (Bull et al., 2006). We currently have little idea of the factors
285 that enable one species or strain to become the dominant type in the chicken gut but it appears to be more complicated than being just a matter of being the first strain to establish colonisation. It is also not unusual to isolate more than one species or subtype from the same bird (Schouls et al., 2003; Höök et al., 2005). In these experiments we have shown that the *C. coli* OR12 was able to co-colonise chickens with *C. jejuni* isolate OR1 until the birds were 35
290 days of age, at which stage *C. coli* OR12 began to dominate the intestinal population. It does not appear to be possible to identify a potential dominant strain on the basis of its growth rate *in vitro*, since in broth co-cultures *C. coli* and *C. jejuni* grew in a compatible manner with similar growth rates. However, we did note a significant difference in behaviour of *C. jejuni* OR1 compared to *C. coli* OR12, which was observed to go into decline after peaking at the

295 end of the exponential growth phase when cultured as a minority population with *C. coli*
OR12.

A good deal of effort has gone into the identification of *Campylobacter* genes that
are necessary to enable the colonisation of poultry (Hendrixson and DiRita, 2004). These
studies have provided some intriguing insights into the structures and metabolic processes that
300 support growth in the avian intestine but they have not shed light on the key competition
factors that enable *Campylobacter* species and subtypes to dominate populations, or indeed
the strategies that are adopted so that minority sub-populations be retained in the presence of
a dominant competitor in order that they may proliferate again later. The enigmatic nature of
the processes in operation may be exemplified by the recent observations of the high
305 frequency loss of *C. jejuni* signature-tagged transposon mutants at random from chickens of
signature-tagged pool members that later proved to be colonisation proficient (Grant et al.,
2005).

The overall conclusions from the series of *in vivo* experiments in broiler chickens
were that, both *C. jejuni* OR1 and *C. coli* OR12 could colonise and compete with each other
310 equally until the birds were 35 days old, regardless of the order or day of exposure. Beyond
this time point the numbers of *C. jejuni* OR1 declined leaving *C. coli* OR12 as the dominant
isolate. Without competition *C. jejuni* OR1 did not decline at this time point so the observed
decline must at least in part be due to competition with *C. coli* OR12 but is also likely
influenced by developmental changes occurring in the birds at that time.

315 Having established that *C. coli* OR12 was a particularly good competitor against *C.*
jejuni OR1 it was important to determine if it could compete against other *C. jejuni* isolates in
a time scale more typical of intensively reared birds. Our aim was to see if it might be
possible to manipulate the dominant strain present in commercially reared birds at the time
they are sent for slaughter by using a particularly competitive isolate. *C. coli* OR12 was found

320 to be able to replace *C. jejuni* broiler chicken isolates HPC5 and GIIC8 as well as a *C. jejuni*
isolate of human origin (81116), all of which were efficient colonisers of broiler chickens
beyond 35 days-of-age without challenge.

Changes in host immunity may contribute to the competitive succession of *C. coli*
OR12 either through responses directed at the *C. jejuni* isolate or through host-mediated
325 changes in the gut flora that result in the antagonism of *C. jejuni*. Studies demonstrate that
there are profound changes in the intestinal flora of chickens during development (Lu et al.,
2003), and how these bacterial communities vary are likely the result of interactions between
the diet and immunological competence of the birds. These studies were carried out on male
Ross broiler chickens but it would be of interest to examine if the succession of these *C. jejuni*
330 by *C. coli* isolates could also occur with in-bred chicken lines, and if so whether the
succession occurred at a similar stage of development. From these studies it might be possible
to assess if a genotypic basis can be ascribed to the intolerance of the birds to the selected *C.*
jejuni isolates when co-colonised with *C. coli* OR12.

Our experimental findings on chickens reared under conditions of strict biosecurity
335 and fed *ad libitum* indicate that it is possible that the organic chickens in our original study
(El-Shibiny et al., 2005) may have demonstrated the succession of *C. coli* OR12 independent
of any external factor, and that this event could have occurred whether both species were
acquired from the same source at the same time or through later exposure to *C. coli* OR12
from another source. We were prompted to perform these experiments since our original
340 study (El-Shibiny et al., 2005) demonstrated that bacteriophage appeared at the same time as
the change in dominant species leading to the possibility that they could have been involved
in, or indeed even provoked the succession process. This appears not to be the case; instead
we have highlighted a more general process whereby chickens co-colonised by *C. coli* OR12
and one of several *C. jejuni* isolates tested showed a marked change in their respective

345 populations at around 35 days-of-age that resulted in the dominance of *C. coli* OR12 at the
expense of the *C. jejuni* isolate. In this context it is notable that adult breeding hens have been
reported to be readily colonised by *C. coli* (Peterson et al., 2001).

The timing of the change in species dominance is of particular note, as conventional
barn-reared broilers are often slaughtered from 35 days old. Organic and free-range birds are
350 reared over a longer period and are at the same time exposed to a wider variety of
environmental campylobacters, and other microbes producing metabolites that may be
differentially prejudicial to the campylobacters colonising the intestinal tracts of the chickens.

The ability to understand which factors allow one species or strain to dominate over
others may lead to strategies to reduce colonisation of chickens by campylobacters. We have
355 studied a *C. coli* isolate based on the observation of its dominance in our previous work and
this allowed the competition between pairs of strains to be examined as they were easily
discriminated on the basis of DNA hybridisation, antibiotic sensitivity and ability to
hydrolyse hippurate. It is possible that there are also successful strains of *C. jejuni* that are
destined to dominate. Our experiments have demonstrated that replacement of one isolate by
360 another is not always a random event but can be readily repeated in experimental birds using
specific strains. It may be feasible to replace antibiotic resistant *C. jejuni* colonising strains
with sensitive ones (either *C. jejuni* or *C. coli*) thus reducing their presence on poultry meat
and the likelihood of them infecting humans. In the future, if specific human pathogenic traits
can be identified, strains that express these traits might be replaced with more benign strains.

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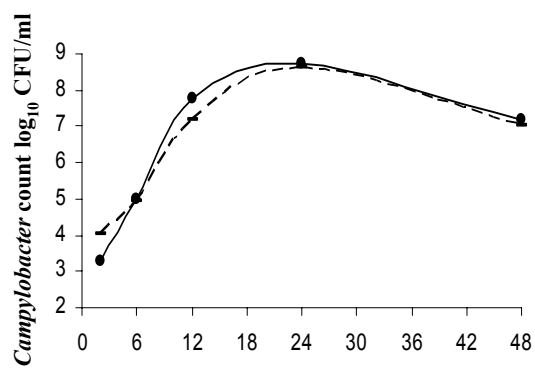
Figure Legends

Fig. 1 Growth *in vitro* of *C. jejuni* OR1 (dashed lines) in competition with *C. coli* OR12 (solid lines) with the following starting ratios: A) 1 *C. coli*:1 *C. jejuni*; B) 1 *C. coli*:10² *C. jejuni*; C) 1 *C. coli*:10⁶ *C. jejuni*; D) 10² *C. coli*: 1 *C. jejuni*; E) 10⁶ *C. coli*: 1 *C. jejuni*.

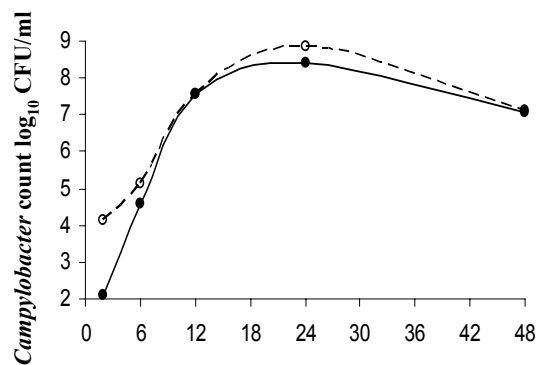
Fig. 2 Competitive colonisation of broiler chickens with campylobacters: A) Effect of introducing *C. coli* OR12 (black columns) at: 25, 27, 31 and 35 days old and sampled after 1, 4 and 11 days, to chickens that had been pre-colonised with *C. jejuni* OR1 (unshaded columns); B) Effect of introducing *C. jejuni* OR1 (unshaded columns) at 27 days old and sampled at 1, 5, 9 and 12 days to chickens that had been pre-colonised with *C. coli* OR12 (black columns) at 21 days of age. Each sample point point post-challenge represents the means and standard deviations of the caecal counts for each *Campylobacter* species from n=12 independent birds. For unchallenged birds with single *Campylobacter* species colonisation n=9.

Fig. 3. The displacement of alternative *C. jejuni* isolates GIIC8, 81116 or HPC5 used to pre-colonise chickens at 20 days, then challenged with *C. coli* OR12 at 30 days and sampled at 40 days of age. The data are recorded as the means and standard deviations of the caecal counts for each *Campylobacter* species from n=9 independent birds.

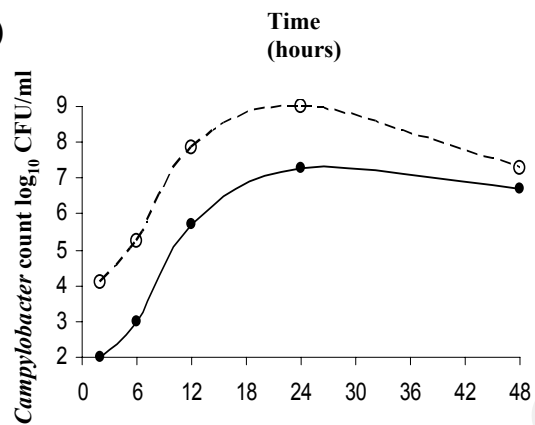
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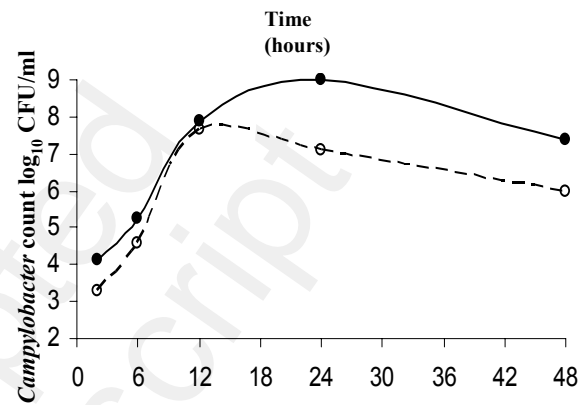
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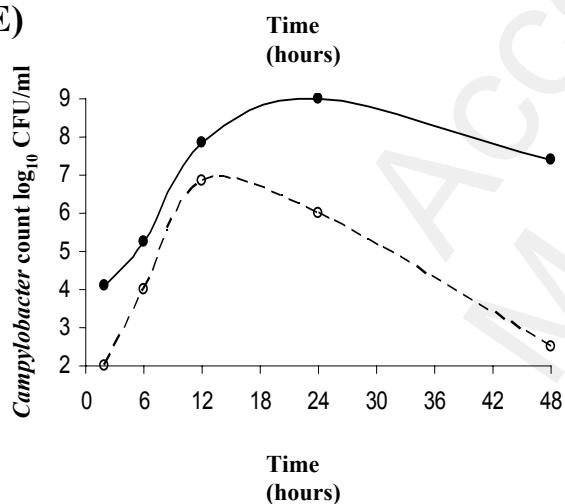
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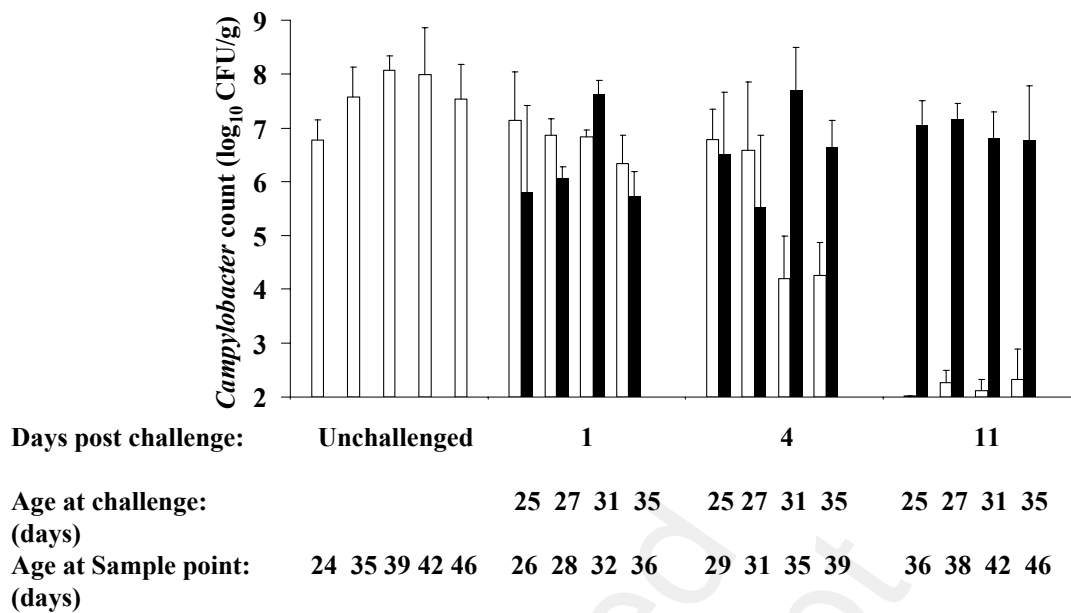
D)



E)



A)



B)

