



# **Influence of production system on the rate of onset of Campylobacter colonisation in chicken flocks reared extensively in the United Kingdom.**

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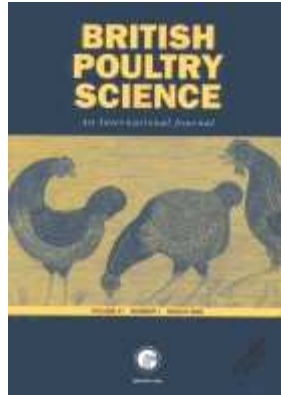
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**Note to scientific Editor.** Et al needs italicising. Journal titles need formatting. The Tables need some tidying up. There is just one Figure, which is a combined electrophoretic gel sequence and associated text – the Legend strikes me as a bit cursory – if you agree then I guess you have to consult the authors and get it expanded.

**Influence of production system on the rate of onset of *Campylobacter* colonisation in chicken flocks reared extensively in the United Kingdom**

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Short title: - *CAMPYLOBACTER* IN EXTENSIVE FLOCKS

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**Abstract.**

1. Because thermophilic *Campylobacter* spp. are common in chicken flocks reared extensively, cross-sectional and longitudinal studies were carried out on organic and free-range farms to determine the onset of colonisation (lag phase) and likely sources of flock infection.

2. For 14 organic and 14 free range flocks, there was a difference in lag phases, with the former being colonised at a mean of 14.1 d in comparison with 31.6 d for the latter. Whereas most free-range flocks became colonised when released on to pasture, those reared organically were usually colonised at the housed brooding stage.

3. Further study of organic flocks on three farms over 7 successive crop cycles confirmed that colonisation was strongly influenced by the prevailing husbandry conditions and was not a consequence of the length of the rearing period.

4. Molecular epidemiological investigations on a farm showing the shortest lag phase, using PFGE typing with two different restriction enzymes (*Sma*I and *Kpn*I) and *flaA* SVR sequence typing, revealed that potential sources of colonisation for organic chickens were already present on the farm at the time of chick placement. Such sources included the ante area of the brooding house, surrounding pasture and other livestock being kept on the farm.

5. Overall, the study demonstrated that, under UK conditions, the prevalence of colonisation was greater in extensive flocks (95 – 100%)

49 than it was for conventional broilers (55%), similar to the situation in  
50 other countries, but all three management systems showed comparable  
51 levels of caecal carriage in positive birds ( $\log_{10}/g$  6.2 – 6.7).

53 INTRODUCTION

54 Thermophilic *Campylobacter* spp. are environmentally widespread and a  
55 major cause of acute bacterial gastro-intestinal infection in man (Kramer et  
56 al., 2000; Newell and Fearnley, 2003). During 1992 – 2000, these organisms  
57 were responsible for an estimated 359 000 cases in England and Wales (Adak  
58 et al., 2002). Although more than 90% of cases of campylobacteriosis are  
59 attributed to *Campylobacter jejuni*, there is increasing recognition of the role  
60 of *C. coli* in this disease (Tam et al., 2003). In the developed world, most  
61 *Campylobacter* infections result from the ingestion of contaminated food,  
62 including raw milk (Fahey ei1995) and red meat (Kramer et al., 2000);  
63 however, raw and undercooked poultry meat are considered to be the principal  
64 sources of human campylobacteriosis (Rivoal et al., 1999; Pearson et al.,  
65 2000; Adak et al., 2002) and approximately 80% of raw poultry being sold in  
66 the United Kingdom (UK) was found to be contaminated with *Campylobacter*  
67 (Jørgensen et al., 2002).

68 In the UK, most poultry meat comes from birds that are reared in  
69 controlled-environment houses and at relatively high stocking densities.  
70 Such birds are usually slaughtered at 35 – 42 d of age (El-Shibiny et al.,  
71 2005). Nevertheless, there is a growing market for free-range and  
72 organically-produced food products (Sato et al., 2004). Poultry flocks of this

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3 73 type are grown at lower stocking densities and, for the organic birds, there are  
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5 74 strict rules governing the use of antimicrobials and organically-produced  
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7 75 feedstuffs. Both types of extensively-reared birds are relatively slow-  
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9 76 growing and are given low-energy, low-protein feeds; consequently, they are  
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11 77 often slaughtered in the UK at around 56 and 73 d for free-range and organic  
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13 78 flocks respectively. During the brooding period of up to 24-28 d, the flocks  
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15 79 are continuously housed. However a key feature of the production systems  
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17 80 used is the access given to pasture land, so that a proportion of the rearing  
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19 81 period in each case is spent outside the house (El-Shibiny et al., 2005).

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22 82 Conventionally-reared poultry have been studied widely in relation to  
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24 83 colonisation by *Campylobacter* spp., which are generally carried  
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26 84 asymptotically, mainly in the caeca and colon (Corry and Atabay, 2001;  
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28 85 Newell and Fearnley, 2003). However, several studies have shown a  
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30 86 significantly higher prevalence of *Campylobacter* infection in organic chicken  
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32 87 flocks than in those reared conventionally (Heuer et al., 2001; Luangtongkum  
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34 88 et al., 2006; Van Overbeke et al., 2006) and a similarly high prevalence in  
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36 89 other free-range chickens (Kazwala et al., 1993; Rivoal et al., 1999; El-  
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38 90 Shibiny et al., 2005; Huneau-Salaün et al., 2007). Likely factors are thought  
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40 91 to include the greater exposure of extensively-reared birds to environmental  
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42 92 challenge, rearing for longer periods, which increases the risk of colonisation,  
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44 93 and differences in susceptibility to *Campylobacter* infection among different  
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46 94 breeds of chicken. However, recent work has questioned that the importance  
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48 95 of environmental exposure (Colles et al., 2008).

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Three separate studies were carried out to investigate *Campylobacter* colonisation in extensively-reared flocks. Twenty eight farms studied during 2004 and 2005 were associated with two (Company A and Company B) of the three major UK companies that specialise in the production of free-range and organic table chicken (16 and 12 farms from Company A and B respectively).

Study (i) and (ii) examined the time taken for *Campylobacter* colonisation to be first detected; 14 free-range flocks and 14 organic flocks from 28 farms were compared in study (i), while three organic farms from company A that had participated in study (i) were investigated over a further 7 crop cycles in study (ii), with environmental sampling being carried out on one of these farms. In addition, whole caeca were collected over a 4-month period (August to October) at a single processing plant in Company A to compare conventional and extensively-reared flocks for carriage of *Campylobacter* (study (iii)).

### Rearing of the birds

The free-range flocks were reared in conventional-type broiler houses with access to an associated paddock and in accordance with European Union Poultrymeat Marketing Regulations (no. 1538/9). For organic flocks, the husbandry conditions were based on the requirements of Council Regulation (EEC) no. 2092/91, as amended. These flocks were brooded in static houses and moved into mobile houses at approximately 28 d of age except for one farm in Company A where they remained throughout in the same mobile house. In Company A, the mobile grower houses were situated on the same farm as the brooding houses. In contrast, the flocks in Company B were moved, by farm staff using their own crates and transport or by catching teams, to grower farms on different sites between 5 and 30 miles away.

**Deleted:** However the flocks in Company A's farms remained in the same house but let out onto pasture at 24-28 days while flocks in Company B were moved at 28 days from brooder houses to grower houses.

Three organic farms belonging to the same company were studied over seven crop cycles (study (ii)). Farm 1 brooded the birds in Modark modular houses (Creedy Carver Ltd, Crediton, Devon, UK). There were 4 such



houses, adjacent to each other in an open field, each containing about 1250 chicks. All these houses were filled at the same time. All houses had a concrete base and a gravel surround. Mains water was provided via a nipple drinking system and feed in pan feeders. The chicks were kept on wood shavings.

Farm 2 also had 4 Modark brooder houses, two of which were inside an old barn with a concrete floor and two outside in a concrete yard. Nipple drinkers supplied mains water to the birds and feed was provided in pan feeders. Wood shavings were used as litter. There was a weekly intake of approximately 1500 chicks, with one of the 4 brooding houses being filled each week.

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Deleted: with approximately 1500 chicks.

At Farm 3, in a field, were two brooding houses constructed of wood on a steel frame, with a raised wooden floor. Each contained about 1000 chicks on wood shavings, with bell feeders and mains water provided in fountain-type drinkers. The brooding houses were filled alternately every two weeks and for the first week of each crop cycle an area was separated off by bales of straw at the front and rear of the house (designated ante area in this study). On this site, there were 12 separate growing houses, as well as some cattle, a free-range pig and two dogs.

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**Collection and handling of samples**

Study (i): To compare the time taken for the onset of *Campylobacter* colonisation between organic and free-range flocks, 14 flocks of each management type were sampled from Company A and Company B (8 and 6 flocks of each type respectively). Farmers used Amies Charcoal Transport

168 swabs (Medical Wire, MW171) to sample 4 caecal or faecal droppings at each  
169 of 5 locations within a house (near each corner and at the centre). The 5  
170 pooled samples were taken twice-weekly and sent by post overnight to the  
171 laboratory where they were processed individually.

172 For study (ii), three farms from Company A were sampled daily by  
173 taking ten samples of caecal or faecal droppings from the parts of the house  
174 described previously. These were transported to the laboratory under chill  
175 conditions and examined within two hours of receipt as pools of 5 samples  
176 from each house.

177 Samples were taken from the environment surrounding the two  
178 brooder flocks on one of these farms (Farm 3) during the first 5 d of the third  
179 crop cycle using a standardised sampling plan. Surfaces were sampled by  
180 swabbing an area of approximately 100 cm<sup>2</sup> from each site using a sterile  
181 Radiwipe (Robinson Healthcare Ltd, Chesterfield, UK) pre-moistened in a  
182 small amount of Maximum Recovery Diluent (MRD, CM733, Oxoid,  
183 Basingstoke, UK.). Boot swabs in the form of gauze overshoes (Mike  
184 Bowden Livestock Service, Attleborough, Norfolk, UK) were used for  
185 sampling large areas of grass or concrete. These were pre-moistened in MRD  
186 and worn over plastic overboots (A547, Arnold, Shrewsbury, UK). Both  
187 swabs and overshoes were aseptically transferred, on the farm, to labelled  
188 containers with 150 ml modified Exeter broth (Humphrey et al., 1995).  
189 Faecal droppings from other animals on the farm were collected by inverting  
190 sterile plastic bags using sterile disposable gloves and were immediately  
191 transferred into Exeter broth as above. Water present in drinkers or puddles

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192 was collected with the aid of a sterile pipette and dispensed into a sterile  
193 container with an equal volume of double-strength Exeter broth.

194 To compare standard housed and extensively-reared flocks for carriage  
195 of *Campylobacter*, in study (iii) whole caeca from 40 standard housed flocks  
196 and 41 extensively-reared flocks were sampled randomly on 20 separate days.  
197 These were transported to the laboratory under chill conditions and examined  
198 within two hours of receipt as pools of 10 from each flock.

199 **Isolation, enumeration and confirmation of *Campylobacter***

200 Whole caeca were opened aseptically, the contents removed and pooled as  
201 required. Each pooled sample was mixed well using a sterile, cotton-tipped  
202 swab and serial 10-fold dilutions prepared in MRD. Duplicate aliquots (50  
203 µl) of each dilution were plated on Modified Charcoal-Cefoperazone-  
204 Deoxycholate Agar (mCCDA, Oxoid CM739 and SR155) for microaerobic  
205 incubation at 37°C for 48 h. Presumptive colonies of *Campylobacter* were  
206 subjected to the confirmatory tests described below and counts expressed as  
207 log<sub>10</sub> cfu/g. In addition, 2 g of pooled contents were used to prepare  
208 enrichment cultures in 20 ml amounts of modified Exeter broth. These were  
209 incubated aerobically, with a small headspace and tightly-closed caps, at 37°C  
210 for 48 h, and then used to inoculate mCCDA.

211 Caecal or faecal droppings and swab samples were pooled, where  
212 required, and used to inoculate plates of mCCDA for isolation purposes only.  
213 Enrichment cultures were also prepared and inoculated on to mCCDA after  
214 incubation as were the environmental samples.

Presumptive colonies of *Campylobacter* spp., up to three per sample, where available, were subcultured onto Oxoid Blood Agar Base No 2 (Oxoid CM0271) and incubated microaerobically at 42°C for 48 h. All isolates were tested for Gram-stain reaction, production of oxidase and absence of aerobic growth. A selection of isolates was also confirmed with the Oxoid *Campylobacter* Test Kit. (DR0150M).

#### Identification of isolates

Species identification of presumptive *Campylobacter* isolates was carried out by a real-time PCR assay according to the method of Best et al. (2003), using the MX3005p (Stratagene, Amsterdam, The Netherlands), as described previously (Ridley et al., 2008a).

#### Storage of isolates

Confirmed isolates were stored in cryovials containing porous beads and glycerol. After inoculation with a fresh culture suspension from an agar plate, the vial was closed and inverted four to five times to emulsify the suspension. Excess cryopreservative was then removed, leaving the inoculated beads as liquid-free as possible. Vials were stored at -80°C while awaiting typing.

#### Typing of strains

On farm 3, environmental isolates and isolates from two brooder flocks (n=58) and from two previous flocks (n=6) were characterised by pulsed-field gel electrophoresis (PFGE). Firstly, each isolate was plated on Blood Agar containing Skirrow's Supplement (Oxoid CM0271 and SR0069) plus actidione (100 µg/ml) and cefoperazone (30 µg/ml). Plates were incubated

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239 microaerobically at 42°C for 24 h. To prepare chromosomal DNA, cells  
240 harvested in phosphate-buffered saline were lysed in 1.2% agarose blocks  
241 (Gibson et al., 1995). The DNA was digested overnight at 25°C using 20 U  
242 *Sma*I. PFGE was performed on a DRIII apparatus (BioRad, Hemel  
243 Hempstead, UK) at 6.6 V / cm for 22.5 h, with pulse times increasing from  
244 0.5 to 40 s. Standardised parameters were as proposed by CAMPYNET  
245 (<http://www.medvetnet.org/cms/templates/doc.php?id=99&searchstring=CA>  
246 MPYNET). Gels were stained with ethidium bromide and the images  
247 captured using UV illumination with a video system (Alpha-Imager HP,  
248 Genetic Research Instrumentation Ltd., Braintree, UK). The macrorestriction  
249 profiles were analysed using Bionumerics Software (version 5.0, Applied  
250 Maths, Kortrijk, Belgium). Similarities between profiles were derived using  
251 the Dice coefficient, with position tolerance values set at 1.5%. Cluster  
252 analysis was performed using the unweighted pair group method with  
253 arithmetic averages (UPGMA). Thirty isolates recovered from samples taken  
254 at days 1 and 2 as well as the 6 isolates from the previous flocks were also  
255 typed by PFGE using *Kpn*I at 6.0 V / cm for 24 h, with pulse times increasing  
256 from 0.2 to 20 s and analysed as above. To confirm flock and environmental  
257 matches, *flaA* SVR PCR and subsequent sequencing was performed on a total  
258 of 23 isolates with the primers FLA242FU and FLA625RU (Meinersmann et  
259 al., 1997).  
260 A 321 bp sequence containing the *flaA* SVR nucleotide sequence was  
261 compared with the database at <http://pubmlst.org/campylobacter/flaA/> (Dingle

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et al., 2005). To provide strain type designation the following nomenclature was adopted: *Sma*I PFGE type/ *Kpn*I type /*fla* SVR type.

#### Statistical analysis

Analysis of variance (ANOVA) was undertaken where appropriate using 'Minitab' software.

## RESULTS

### Time to onset of *Campylobacter* colonisation and possible sources of infection

*Campylobacter* colonisation was studied by regular monitoring of 14 free-range and 14 organic flocks from Companies A and B, study (i). For each flock, the time at which *Campylobacter* colonisation was first detected was noted. The results for all flocks are given in Table 1. With organic flocks, the onset of colonisation was detected at a minimum of 4 d and a maximum of 32 d. The corresponding figures for free-range flocks were 7 and 57 d, indicating that organic flocks were usually colonised earlier than their free-range counterparts ( $P<0.01$ ). The former were colonised mainly at the brooding stage (mean 14.1 d) in comparison with a mean of 31.6 d for the latter. Only one organic flock was colonised post-brooding. When Company A and B were compared, results for the two sets of organic flocks were similar, with means of 14.6 d for Company A and 13.5 d for Company B. There was a greater difference between the two companies with respect to free-range flocks. In this case, the onset of *Campylobacter* colonisation occurred at a mean of 35.4 d for Company A and 26.5 d for Company B. However these

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286 were not statistically different since time to colonisation ranged from 7 to 48 d  
287 in Company B, with 4 of the flocks becoming positive at the brooding stage.

288 In study (ii), three organic farms from Company A were studied over 7  
289 successive crop cycles (Table 2). The results confirmed the relative rapidity  
290 with which organic flocks become detectably colonised, with means of 6.7 d  
291 for Farm 1 and 5.9 d for Farm 3, although there was usually a longer delay in  
292 colonisation at Farm 2 (mean 14.6 d).

293 In order to investigate the possible role of environmental  
294 contamination in the infection of brooder chicks, samples were collected from  
295 Farm 3 following placement of a single flock in each brooding house (Houses  
296 A and B) on the third crop cycle. One hundred and eighty one samples were  
297 taken from in and around the designated target flock in house A and the flock  
298 in the adjacent house (B) over the first 5 d following placement to identify  
299 possible sources of infection that might explain such early colonisation (Table  
300 3). A total of ~~52 isolates from 45 samples recovered from the surrounding~~  
301 environment and an additional 6 isolates ~~(6 samples)~~ from the previous flocks,  
302 sampled during the second crop cycle, were typed by PFGE and ~~compared~~  
303 ~~with the strains recovered from the chicks. *flaA* SVR was performed on 23 of~~  
304 ~~the isolates covering the present flocks, surroundings and the previous flocks~~  
305 ~~for confirmation of genotypic identity. Faecal samples from both flocks were~~  
306 positive by day 2 (Table 3). The 6 isolates recovered from ~~the 4 different~~  
307 ~~faecal samples~~ in the target flock and the adjacent flock (House B) belonged  
308 to two different genotypes (*Sma*I PFGE type 3-1 / *Kpn*I type I / *fla* SVR type  
309 497 (3-1/I/497) and 3-8/I/208), (PFGE types are shown in Figure 1). Isolates

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310 | belonging to 3-1/I/497 were predominant (4/6) in faeces and were identified  
 311 | as *C. coli*; those of 3-8/I/208 were *C. jejuni*. Multilocus sequence typing  
 312 | (MLST), carried out using the method of Dingle et al. (2001) by the Dept of  
 313 | Zoology at the University of Oxford, demonstrated that all 9 isolates of type  
 314 | 3-1/I/497 examined belonged to MLST sequence type (ST) 829, part of clonal  
 315 | complex 828, while both isolates of type 3-8/I/208 ~~were~~ identified as *C. jejuni*  
 316 | MLST type 877. Chick isolates belonging to the predominant genotype, 3-  
 317 | 1/I/497, were indistinguishable by *SmaI* PFGE and *flaA* SVR from 4 isolates  
 318 | recovered from 3 different samples collected from the adjoining field, around  
 319 | the brooding houses and from the ante area of the house on day 1. However,  
 320 | using the additional enzyme *KpnI*, only 3 of these environmental isolates  
 321 | (from between the two houses and those from the ante-area of House A)  
 322 | matched by all genotypic methods investigated (Table 3, Figure). One of 6  
 323 | isolates from the two previous brooding flocks in the same house also  
 324 | matched by *SmaI* PFGE (a mix of 3-1 and 3-3) and *flaA* SVR (497), but not  
 325 | by *KpnI*, the colonising strain in the target flock. The relationship between  
 326 | these genotypes with identical *SmaI* PFGE and *flaA* SVR types but not *KpnI*  
 327 | is unclear and requires further investigation. Nevertheless, the combined  
 328 | PFGE/SVR typing indicated that the ante-area and ground between the two  
 329 | tested houses were the most likely sources of flock contamination.

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330 |       The second chick strain, (3-8/I/208) was only detected in House B  
 331 | flock samples but was also identified in an in-house overshoe sample from  
 332 | House A prior to placement (Figure 1). Thus, it appears as though the strains  
 333 | first colonising the chicks were present in and around the brooder house



environment at the time of chick placement. In this flock the chicks were colonised by day 2, thereafter strains that genotypically matched the predominant flock type (3-1/I/497) were isolated from the main drive and cow faeces and from faeces from older poultry, which had been let out on to pasture in an adjoining field (Table 3, Figure 1) as well as from drinking water within the house, a foot-dip, water collected from old fountain drinkers stored outside the house, puddles on the main drive, and from a stile located between the area of the brooding houses and the farmhouse. Thus, once colonisation was detectable in the chicks, rapid dissemination by this strain occurred throughout the surrounding environment. For some environmental isolates, only minor changes in the size of a single band of the *Sma*I and/or *Kpn*I PFGE profiles were observed, indicating the presence of subtypes. Such minor variations in band size are well recognised in *Campylobacter* and are indicative of genomic instability (Wassenaar et al., 1988), possibly as a consequence of exposure to environmental stresses.

In contrast, the second campylobacter strain found in the flock, 3-8/I/208, while detected in two environmental sites at day 2 (anteroom of house A and heater of house B), was not recovered from any of the 19 positive environmental samples investigated on day 5 suggesting poor environmental dissemination.

**Species present, levels of colonisation and strain diversity**

A total of 86 *Campylobacter* isolates obtained from the 28 organic and free-range flocks, study (i), was identified to species level. Of these, 47 (55%)

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3 357 | were identified as *C. jejuni* and 39% as *C. coli*, while 5 (6%) belonged to  
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5 358 | neither species and were not studied further.

7 359 | **Comparison of standard housed and extensively reared flocks for**  
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9 360 | **carriage of *Campylobacter***

11 361 | From within a single poultry company, (Company A), organic (n=21), free-  
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13 362 | range (n=20) and conventionally reared (n=40) flocks were sampled by  
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15 363 | randomly collecting 10 caeca per flock from the common processing plant.  
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17 364 | The sampling of flocks from each type of rearing regime took place on 20  
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19 365 | occasions equally spread over a 4-month period from August to October. The  
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21 366 | caecal contents were analysed for *Campylobacter* in positive birds as  
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23 367 | described. The data given in Table 4 show that there were no significant  
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25 368 | differences in carriage levels (mean log<sub>10</sub>/g 6.2 - 6.7) between the three  
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27 369 | production systems, despite the differences in flock age at slaughter.  
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29 370 | However, whereas 95 – 100% of the 41 organic and free-range flocks tested  
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31 371 | were *Campylobacter*-positive, the organism was found in only 55% of 40  
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33 372 | flocks that had been reared conventionally ( $P < 0.001$ ).  
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39 374 | **DISCUSSION**

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41 375 | In this study, attention has been largely focussed on the lag phase, the period  
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43 376 | that precedes the onset of *Campylobacter* colonisation in poultry. This  
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45 377 | phenomenon has been partly attributed to the protective effect of maternal  
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47 378 | antibodies that are present initially in chicks obtained from infected parent  
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49 379 | stock (Cawthraw et al., 1994; Sahin et al., 2001, 2003), but other host factors,  
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51 380 | such as changes in gut-flora composition during development, could play a  
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381 part in resistance to early colonisation. Coliform bacteria and enterococci  
382 with antagonistic activity against *Campylobacter* have been isolated from  
383 chickens (Mead et al., 1996) within the lag phase and these microbial groups  
384 are among those known to be abundant in the young bird (Mead, 1989).  
385 Also, with conventional broiler flocks, it has been shown that the length of the  
386 lag phase can be usefully extended by improving flock biosecurity (Gibbens et  
387 al., 2001).

388 For extensive flocks in particular, it appears that the lag phase can be a  
389 useful indicator of the stage in rearing where a flock has become colonised by  
390 *Campylobacter*. It is clear from the results obtained that some free-range  
391 flocks remained uninfected for relatively long periods, up to 57 d, but organic  
392 flocks were colonised much earlier and this occurred mainly at the housed  
393 brooding stage that has a duration of 24 – 28 d.

394 At Farm 3, an organic farm that was studied in detail, the predominant  
395 colonising strain had matching *Sma*I PFGE and *fla*A SVR types to that  
396 associated with the previous brooding flock, suggesting either a poor standard  
397 of biosecurity or inadequate cleaning and disinfection of the house after the  
398 previous birds were transferred to growing houses. The farm had no concrete  
399 area on which to contain the chicks and used fountain drinkers that are more  
400 prone to microbial contamination than the nipple drinkers used on the other  
401 two farms studied. Also, Farm 3 contained a mixture of other animals, which  
402 may have been reservoirs of *Campylobacter*, thus increasing the risk of flock  
403 infection. Supportive evidence for this was obtained from PFGE typing,  
404 which showed that a strain isolated from a cow on the site on day 2 was

indistinguishable from those found in the study flock. Genotypic evidence to support the role of cattle as a possible source of infection of broiler flocks has recently been reported (Ridley et al, 2008a; Zweifel et al, 2008). Although the proximity of pig holdings has been associated with an increased risk of broiler flocks testing positive for *Campylobacter* (Lyngstad et al, 2008), *Campylobacter* was not recovered from the pig faecal samples collected during this study. Once the brooding flock had been colonised, strain 3-1/1/497 spread rapidly in the vicinity of the house, again an indication of poor biosecurity.

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Among the organic farms, the lag phase was longest at Farm 2, where chicks were held initially within an enclosed concrete area that would have facilitated post-crop cleaning and disinfection, and there was a clear separation between the brooding area and the growing houses that were situated some distance away. Although the potential for practising appropriate biosecurity was seen to vary between the farms being studied, it is evident that more attention to this aspect of bird husbandry could be beneficial in reducing the risk of *Campylobacter* colonisation, at least during the stage at which the birds are housed. In a Belgian study (Van Overbeke et al., 2006), organic flocks were detectably colonised after a much longer period, between weeks 7 and 10, suggesting that farm biosecurity measures were more effective in this case.

In contrast to the organic flocks studied here, those designated free-range were mostly colonised after the brooding stage and therefore showed a significantly longer lag phase. Several factors may have affected the extent

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429 of this lag phase. Firstly, the birds were kept in the same houses and  
430 adjoining paddocks throughout the rearing period, thus limiting exposure to  
431 environmental organisms. Secondly, the management system allowed a  
432 longer cleaning period between crops, with the surrounding area being free  
433 from poultry for at least 5 weeks at a time perhaps enabling a significant  
434 reduction in *Campylobacter* contamination of the environment. Although  
435 both free-range and organic birds were of the same slower-growing breed  
436 (Hubbard), the free-range farms were more like those used for conventional  
437 flocks, with comparable standards of cleaning and disinfection, and the birds  
438 were subject to the same degree of biosecurity while they were housed.  
439 These flocks were of a single age, whereas the organic flocks of Company A  
440 were kept on “multi-age” sites and, in the case of Company B, the intake of  
441 chicks was staggered over a 6-d period, thereby increasing the risk of  
442 exposure to *Campylobacter*. The importance of biosecurity during the initial  
443 stages of rearing was evident from a French study (Huneau-Salaün et al.,  
444 2007) where 71% of free-range flocks studied were colonised by  
445 *Campylobacter* before being released on to pasture.

446       Once the birds are released from the house, standard biosecurity  
447 measures are thought to be largely irrelevant, because of the unavoidable  
448 exposure to environmental campylobacters (Rivoal et al., 2005). More  
449 recently, however, this view has been challenged and Colles et al. (2008), in a  
450 study of free-range chickens, found no evidence of flock-to-flock  
451 transmission, no association of ranging behaviour with the likelihood that the  
452 birds would shed *Campylobacter*, and a clear distinction between the

population of *C. jejuni* carried by certain wild birds and the chickens. Nevertheless, it seems most likely that transmission into all broiler flocks is horizontal from the environment and this is supported by convincing evidence using highly discriminatory typing methods reporting the presence of identical strains in the farm environment prior to colonisation in the flock (Bull et al., 2006; Johnsen et al., 2006).

Given the opportunity for challenge of extensively-reared flocks by environmental strains a wide diversity of colonising strains would be anticipated. Strain tracking indicated, like those reared conventionally in the UK (Ayling et al., 1996), the two target flocks on Farm 3 carried a restricted number of predominant strain types. However, these belonged to different species (*C. jejuni* and *C. coli*).

The presence of both species in extensively-reared flocks has been described elsewhere (Rivoal et al., 2005), but significant biodiversity among strains of *Campylobacter* was observed. In the present study almost 40% of caecal isolates examined from 28 extensively reared flocks were identified as *C. coli*, which was consistent with data from another study of organic flocks in the UK by El-Shibiny et al. (2005). One suggestion is that *C. coli* has a colonisation advantage over *C. jejuni*, over time, thus accounting for its frequent presence in extensively-reared birds at the time of slaughter. One interesting observation was diversity in strains recovered from flock and environmental samples, as detected by minor changes in PFGE patterns, as a result of apparent genetic rearrangements. Similar observations were reported by Rivoal et al., (2005). Such rearrangements may be a consequence of

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477 environmental stresses during colonisation (Ridley et al., 2008b), which over  
478 the longer period of chicken rearing might become more evident.

479        Whatever the risk of flock infection from exposure to pasture, the  
480 present study has confirmed previous work from other countries showing that  
481 chicken flocks reared under extensive conditions are particularly susceptible  
482 to *Campylobacter* colonisation and often have a higher prevalence of  
483 colonisation at slaughter than, conventionally-reared birds (Kazwala et al.,  
484 1993; Heuer et al., 2001; Luangtongkum et al., 2006; Van Overbeke et al.,  
485 2006). However, this could not be explained entirely by environmental  
486 exposure or the longer rearing period, and appeared to be more to do with in-  
487 house husbandry conditions than any other single factor. With the growing  
488 popularity for the consumption of organic and free-range chickens in the UK,  
489 it appears that any associated public health risk from *Campylobacter* would be  
490 related primarily to the high prevalence of flock infection rather than  
491 increased levels of intestinal carriage, which were found to be similar in  
492 samples from organic, free-range and conventionally-reared flocks. Work is  
493 now being done to establish the most effective biosecurity measures for use in  
494 extensive poultry production.

495  
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## REFERENCES

- ADAK, G.K., LONG, S.M., O'BRIEN, S.J., (2002). Trends in indigenous foodborne disease and deaths, England and Wales: 1992 – 2000. *Gut* 51, 832 – 841.
- AYLING, R.D., WOODWARD, M.J., EVANS, S.J. NEWELL, D.G., (1996). Restriction length polymorphism of polymerase chain reaction products applied to the differentiation of poultry campylobacters for epidemiological investigations. *Res. Vet. Sci.* 60, 168 – 172.
- BEST, E.L., POWELL, E.J., SWIFT, C., GRANT, K.A., FROST, J.A., (2003). Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiol. Lett.* 229, 237 – 241.
- BULL, S.A., ALLEN, V.M., DOMINGUE, G., JORGENSEN, F., FROST, J.A., URE, R., WHYTE, R., TINKER D., CORRY J.E., GILLARD-KING, J., HUMPHREY, T.J. (2006). Sources of *Campylobacter* spp. colonising housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72, 645-652.
- CAWTHRAW, S.R., AYLING, R., NUIJTEN, P., WASSENAAR, T., NEWELL, D.G., (1994). Isotype, specificity, and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. *Avian Dis.* 38, 341 – 349.



COLLES, F.M., JONES, T.A., MCCARTHY, N.D., SHEPPARD, S.K., CODY, A.J.,  
DINGLE, K.E., DAWKINS, M.S., MAIDEN, M.C.J. (2008). *Campylobacter*  
infection of broiler chickens in a free-range environment. *Environ. Microbiol.*  
doi: 10.1111/j.1462 – 2920.2008.01623.x

CORRY, J.E.L., ATABAY, H.I. (2001). Poultry as a source of *Campylobacter* and  
related organisms. *J. Appl. Microbiol.* 90, 96S – 114S.

DINGLE, K.E., COLLES, F.M., FALUSH, D., MAIDEN, M.C.J. (2005). Sequence  
typing and comparison of population biology of *Campylobacter coli* and  
*Campylobacter jejuni*. *J. Clin. Microbiol.* 43, 340 - 347.

DINGLE, K.E., COLLES, F.M., WAREING, D.R.A., URE, R., FOX, A.J., BOLTON  
F.J., BOOTSMA, H.J., WILLEMS, R.J.L., URWIN, R., MAIDEN, M.C.,  
(2001). Multilocus sequence typing system for *Campylobacter jejuni*. *J. Clin.*  
*Microbiol.* 39, 14-23.

EL-SHIBINY, A., CONNERTON, P.L., CONNERTON, I.F., (2005). Enumeration  
and diversity of campylobacters and bacteriophages isolated during the rearing  
cycles of free-range and organic chickens. *Appl. Environ. Microbiol.* 71, 1259  
– 1266.

FAHEY, T., MORGAN, D., GUNNEBURG, C., ADAK, G.K., MAJID, F.,  
KACZMARSKI, E., (1995). An outbreak of *Campylobacter jejuni* enteritis  
associated with failed milk pasteurization. *J. Infect.* 31, 137 – 143.

FEARNLEY-WHITTINGSTALL, H., (2008). Hugh Fearnley-Whittingstall in  
farmyard fight with Tesco over chicken welfare:  
[http://www.timesonline.co.uk/tol/life\\_and\\_style/food\\_and\\_drink/article4231173](http://www.timesonline.co.uk/tol/life_and_style/food_and_drink/article4231173)  
.ece

- 548 GIBBENS, J.C., PASCOE, S.J., EVANS, S.J., DAVIES, R.H., SAYERS, A.R.,  
549 (2001). A trial of biosecurity as a means to control *Campylobacter* infection of  
550 boiler chickens. *Prev. Vet. Med.* 48, 85 – 99.
- 551 GIBSON, J.R., FITZGERALD, C., OWEN, R.J, (1995). Comparison of PFGE,  
552 ribotyping and phage-typing in the epidemiological analysis of *Campylobacter*  
553 *jejuni* serotype HS2 infections. *Epidemiol. Infect.* 115, 215 – 225.
- 554 HEUER, O.E., PEDERSEN, K., ANDERSEN, J.S., MADSEN, M., (2001).  
555 Prevalence and antimicrobial susceptibility of thermophilic campylobacters in  
556 organic and conventional broiler flocks. *Lett. Appl. Microbiol.* 33, 269 – 274.
- 557 HUMPHREY, T., MASON, M., MARTIN, K., (1995). The isolation of  
558 *Campylobacter jejuni* from contaminated surfaces and its survival in diluents.  
559 *Int. J. Food Microbiol.* 26, 295 – 303.
- 560 HUNEAU-SALAÜN, A., DENIS, M., BALAINE, L., SALVAT, G., (2007). Risk  
561 factors for *Campylobacter* spp. colonization in French free-range broiler-  
562 chicken flocks at the end of the indoor rearing period. *Prev. Vet. Med.* 80, 34 –  
563 48.
- 564 JOHNSEN G., KRUSE H., HOFSHAGEN M., (2006). Genetic diversity and  
565 description of transmission routes for *Campylobacter* on broiler farms by  
566 amplified-fragment length polymorphism. *J Appl. Microbiol.* 101, 1130-1139.
- 567 JØRGENSEN, F., BAILEY, R., WILLIAMS, S., HENDERSON, P., WAREING,  
568 D.R., BOLTON, F.J., FROST, J.A., WARD, L., HUMPHREY, T.J., (2002).  
569 Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole  
570 chickens in relation to sampling methods. *Int. J. Food Microbiol.* 76, 151 – 164.

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571 KAZWALA, R.R., JUVA, S.F., NKYA, A.E., (1993). The role of management  
572 systems in the epidemiology of thermophilic campylobacters among poultry in  
573 the eastern zone of Tanzania. Epidemiol. Infect. 110, 272 – 278.

574 KRAMER, J.M., FROST, J.A., BOLTON, F.J., WAREING, D.R.A., (2000).  
575 *Campylobacter* contamination of raw meat and poultry at retail sale:  
576 identification of multiple types and comparison with isolates from human  
577 infection. J. Food Prot. 63, 1654 – 1659.

578 LUANGTONGKUM, T., MORISHITA, T.Y., ISON, A.J., HUANG, S.,  
579 MCDERMOTT, P.F., ZHANG, Q., (2006). Effect of conventional and organic  
580 production practices on the prevalence and antimicrobial resistance of  
581 *Campylobacter* spp. in poultry. Appl. Environ. Microbiol. 72, 3600 – 3607.

582 LYNSTAD, T.M., JONSSON, M.E., HOFSHAGEN, M., HEIER, B.T. (2008).  
583 Risk factors associated with the presence of *Campylobacter* species in  
584 Norwegian broiler flocks. Poultry Sci. 87(10):1987-94

585 MEAD, G.C., (1989). Microbes of the avian cecum: types present and substrates  
586 utilized. J. Exp. Zool. 3, S48 S54.

587 MEAD, G.C., SCOTT, M.J., HUMPHREY, T.J., MCALPINE, K., (1996).  
588 Observations on the control of *Campylobacter jejuni* infection of poultry by  
589 ‘competitive exclusion’. Avian Pathol. 25, 69 –79.

590 MEINERSMANN, R.J., HELSEL, L.O., FIELDS, P.I., HIETT, K.L., (1997).  
591 Discrimination of *Campylobacter jejuni* isolates by *fla* gene typing. J. Clin.  
592 Microbiol. 35, 2810 - 2814.

593 MORRIS, V.K., ALLEN, V.M., EDGE, S., CANNING, P. (2009). Review of  
594 current practices and recommendations for *Campylobacter* reduction in

- 595 [extensively reared flocks, report to Food Standards Agency UK.](#)  
596 [http://www.foodbase.org.uk/admintools/reportdocuments/361-1-  
597 624\\_B15014\\_Final\\_Report.pdf](http://www.foodbase.org.uk/admintools/reportdocuments/361-1-624_B15014_Final_Report.pdf)  
598  
599 NEWELL, D.C., FEARNLEY, C., (2003). Sources of *Campylobacter* colonization in  
600 broiler chickens. Appl. Environ. Microbiol. 69, 4343 – 4351.  
601 PEARSON, A.D., GREENWOOD, M.H., DONALDSON, J., HEALING, T.D.,  
602 JONES, D.M., SHAHAMAT, M., FELTHAM, R.K.A., COLWELL, R.R.,  
603 (2000). Continuous source outbreak of campylobacteriosis traced to chickens.  
604 J. Food Prot. 63, 309 – 314.  
605 RIDLEY, A.M., ALLEN, V.M., SHARMA, M., HARRIS, J.A., NEWELL, D.G.,  
606 (2008a). Real-time PCR approach for detection of environmental sources of  
607 *Campylobacter* strains colonizing broiler flocks. Appl. Environ. Microbiol. 74,  
608 2492 – 2504.  
609 RIDLEY, A.M., TOSZEGHY, M.J., CAWTHRAW, S.A., WASSENAAR, T.M.,  
610 NEWELL, D.G. (2008b). Genetic instability is associated with changes in the  
611 colonisation potential of *Campylobacter jejuni* in the avian intestine. J. Appl.  
612 Microbiol. 105, 95-104.  
613 RIVOAL, K., DENIS, M., SALVAT, G., COLIN, P., ERMEL, G., (1999). Molecular  
614 characterization of the diversity of *Campylobacter* spp. isolates collected from a  
615 poultry slaughterhouse: analysis of cross contamination. Lett. Appl. Microbiol.  
616 29, 370 – 374.  
617 RIVOAL, K., RAGIMBEAU, C., SALVAT, G., COLIN, P., ERMEL, G., (2005).  
618 Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates

1  
2  
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619 recovered from free-range broiler farms and comparison with isolates of various  
620 origins. Appl. Environ. Microbiol. 71, 6216 – 6227.

621 SAHIN, O., ZHANG, Q., MEITZLER, J.C., HARR, B.S., MORISHITA, T.Y.,  
622 MOHAN, R., (2001). Prevalence, antigenic specificity, and bactericidal activity  
623 of poultry anti-*Campylobacter* maternal antibodies. Appl. Environ. Microbiol.  
624 67, 3951 – 3957.

625 SAHIN, O., LUO, N., HUANG, S., ZHANG, Q., (2003). Effect of *Campylobacter*-  
626 specific maternal antibodies on *Campylobacter jejuni* colonization in young  
627 chicks. Appl. Environ. Microbiol. 69, 5372 – 5379.

628 SATO, K., BARTLETT, P.C., KANEENE, J.B., DOWNES, F.P., (2004).  
629 Comparison of prevalence and antimicrobial susceptibility of *Campylobacter*  
630 spp. isolates from organic and conventional dairy herds in Wisconsin. Appl.  
631 Environ. Microbiol. 70, 1442 – 1447.

632 TAM, C.C., O'BRIEN, S.J., ADAK, G.K., MEAKINS, S.M., FROST, J.A., (2003).  
633 *Campylobacter coli* – an important foodborne pathogen. J. Infect. 47, 28 – 32.

634 VAN OVERBEKE, I., DUCHATEAU, L., DE ZUTTER, L., ALBERS, G.,  
635 DUCATELLE, R., (2006). A comparison survey of organic and conventional  
636 broiler chickens for infectious agents affecting health and food safety. Avian  
637 Dis. 50, 196 – 200.

638 WASSENAAR, T.M., GIELHAUSEN, B., NEWELL, D.G., (1988). Evidence of  
639 genomic instability in *Campylobacter jejuni* isolated from poultry meat. Appl.  
640 Environ. Microbiol. 64, 1816 – 1821.

641 ZWEIFEL, C., SCHEU, K. D., KEEL, M., RENGGLI, F., STEPHAN, R., (2008).  
642 Occurrence and genotypes of *Campylobacter* in broiler flocks, other farm

643 | animals, and the environment during several rearing periods on selected  
644 | poultry farms. Int. J. Food Microbiol. 125, 182-187.

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Table 1. Comparison of free-range and organic farms: time taken for *Campylobacter* colonisation to be first detected in each flock studied.

Free-range farm/Flock No	Time to detectable colonisation (d)	Organic farm/Flock No	Time to detectable colonisation (d)
<b>Company A</b>			
A / FR 1	57	A / OR 1	32
A / FR 2	37	A / OR 2	16
A / FR 3	46	A / OR 3	20
A / FR 4	30	A / OR 4	20
A / FR 5	27	A / OR 5	6
A / FR 6	37	A / OR 6	4
A / FR 7	26	A / OR 7	8
A / FR 8	23	A / OR 8	11
<b>Company B</b>			
B / FR 1	18	B / OR 1	14
B / FR 2	7	B / OR 2	7
B / FR 3	42	B / OR 3	14
B / FR 4	28	B / OR 4	18
B / FR 5	48	B / OR 5	7
B / FR 6	16	B / OR 6	21
Mean (d)	31.6 ± SD 13.8	14.1 ± SD 7.7 ( $p<0.001$ )	

Table 2. First detection of *Campylobacter* colonisation: comparison of data from seven successive crop cycles on each of three organic farms belonging to Company A.

Farm	Time to detectable colonisation (d)							Mean $\pm$ SD
	1	2	3	4	5	6	7	
1	4	9	10	5	5	6	8	6.7 $\pm$ 2.3 <sup>a</sup>
2	10	20	18	11	15	11	17	14.6 $\pm$ 3.9 <sup>ab</sup>
3	9	6	2	3	12	2	7	5.9 $\pm$ 3.8 <sup>b</sup>

<sup>a</sup> & <sup>b</sup> significant difference  $p=0.001$



Table 3. Samples positive for *Campylobacter* spp. on Farm 3.

Bird age (days)	No. of positive samples	Total no. of samples	Description of culture-positive environmental samples	combined PFGE matches to flock <sup>d</sup>
1	7	53	Overshoes in adjoining field	1/2
			Overshoes around houses	1/2
			Overshoes in ante area	1/1
			Overshoes in house A <sup>b</sup>	0/1
			Rat faeces outside house A	0/1
2 <sup>a</sup>	23	70	Overshoes around houses	1/3
			Overshoes front drive	1/1
			Cow faeces in adjoining field	1/1
			Swab from stile	1/1
			Puddle water on front drive	0/1
			Old water from drinkers	1/1
			Overshoes and faeces from 'older' chickens	2/3
			Ante area house A	2/3
			Litter from house A	1/1
			Litter from house B <sup>c</sup>	2/3
			Swab from under roof outside house A	0/1
			Foot-dip water house A	1/1
			Drinkers in house B	2/2
			Heater in house B	1/1
5	19	58	Faeces from cow in adjoining field	1/1
			Faeces from 'older' chickens in adjoining field	1/1
			Overshoes between house B and field	2/2
			Ante areas in house A	3/3
			Feed house A	1/1
			Swabs from drinkers in house A	1/1
			Swabs from drinkers in house B	2/2
			Litter from house A	2/2
			Overshoes from house A	2/2
			Litter from house B	4/4
Previous flocks	6	10	Previous crop in house A	0/3
			Previous crop in house B	0/3

<sup>a</sup> Flocks found to be colonised at this visit.

<sup>b</sup> Target brooder house.

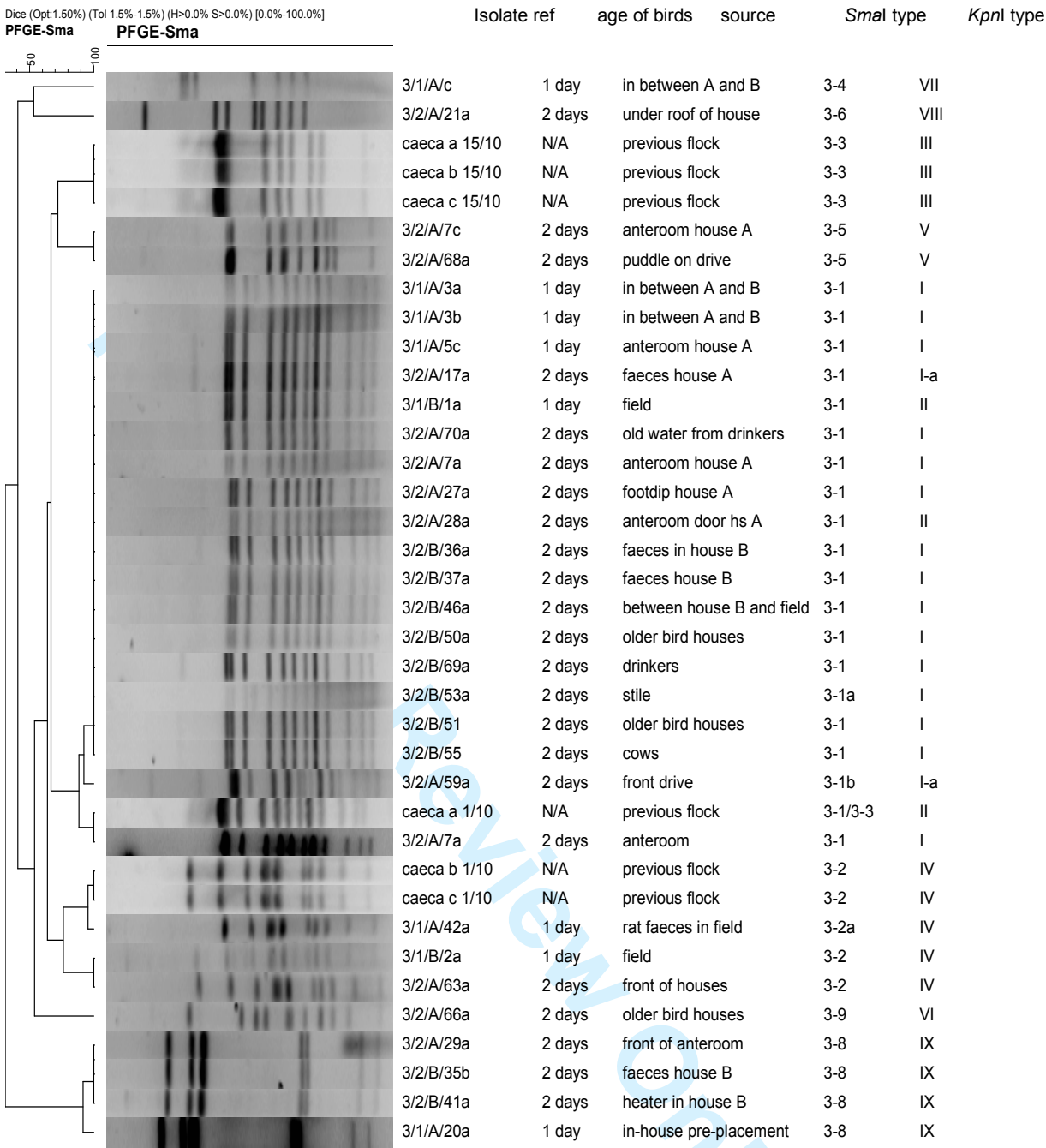
<sup>c</sup> Adjacent brooder house.

<sup>d</sup> Number matching by both *Sma*I and *Kpn*I types/Total number tested

Table 4. Prevalence and levels of *Campylobacter* spp. in pooled caecal samples\* from colonised flocks reared under different systems within a single company (company A).

Flock type	Free-range	Organic	Conventional
Slaughter age	54-56days	72-74days	41-43 days
No. examined	20	21	40
No. positive (%)	19 (95)	21 (100)	22 (55)
Geometric mean count (log <sub>10</sub> cfu / g)	6.70 ± SD 1.27	6.52 ± SD 1.21	6.23 ± SD 1.85
Median	7.15	6.35	6.74

\* Ten samples taken from each flock.



1

2 Fig. 1. Relationships of *SmaI* and *KpnI* PFGE types for *Campylobacter*  
3 isolates from brooder houses and surrounding environment at Farm. 3,  
4 initially (day 1) and at the time the flock was found to be positive (day 2)

## REFERENCES

- ADAK, G.K., LONG, S.M., O'BRIEN, S.J., (2002). Trends in indigeous foodborne disease and deaths, England and Wales: 1992 – 2000. *Gut* **51**: 832 – 841.
- AYLING, R.D., WOODWARD, M.J., EVANS, S.J. NEWELL, D.G., (1996). Restriction length polymorphism of polymerase chain reaction products applied to the differentiation of poultry campylobacters for epidemiological investigations. *Research in Veterinary Science* **60**: 168 – 172.
- BEST, E.L., POWELL, E.J., SWIFT, C., GRANT, K.A., FROST, J.A., (2003). Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. *FEMS Microbiology Letters* **229**: 237 – 241.
- BULL, S.A., ALLEN, V.M., DOMINGUE, G., JORGENSEN, F., FROST, J.A., URE, R., WHYTE, R., TINKER D., CORRY J.E., GILLARD-KING, J., HUMPHREY, T.J. (2006). Sources of *Campylobacter* spp. colonising housed broiler flocks during rearing. *Applied and Environmental Microbiology*. **72**: 645-652.
- CAWTHRAW, S.R., AYLING, R., NUIJTEN, P., WASSENAAR, T., NEWELL, D.G., (1994). Isotype, specificity, and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. *Avian Diseases*. **38**: 341 – 349.
- COLLES, F.M., JONES, T.A., MCCARTHY, N.D., SHEPPARD, S.K., CODY, A.J., DINGLE, K.E., DAWKINS, M.S., MAIDEN, M.C.J. (2008). *Campylobacter* infection of broiler chickens in a free-range environment. *Environmental Microbiology* **10**: 2042-2050.
- CORRY, J.E.L., ATABAY, H.I. (2001). Poultry as a source of *Campylobacter* and related organisms. *Journal of Applied Microbiology* **90**: 96S – 114S.

DINGLE, K.E., COLLES, F.M., FALUSH, D., MAIDEN, M.C.J. (2005). Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. *Journal of Clinical Microbiology* **43**: 340 - 347.

DINGLE, K.E., COLLES, F.M., WAREING, D.R.A., URE, R., FOX, A.J., BOLTON F.J., BOOTSMA, H.J., WILLEMS, R.J.L., URWIN, R., MAIDEN, M.C., (2001). Multilocus sequence typing system for *Campylobacter jejuni*. *Journal of Clinical Microbiology* **39**: 14-23.

EL-SHIBINY, A., CONNERTON, P.L., CONNERTON, I.F., (2005). Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens. *Applied and Environmental Microbiology* **71**: 1259 – 1266.

FAHEY, T., MORGAN, D., GUNNEBURG, C., ADAK, G.K., MAJID, F., KACZMARSKI, E., (1995). An outbreak of *Campylobacter jejuni* enteritis associated with failed milk pasteurization. *Journal of Infection* **31**: 137 – 143.

FEARNLEY-WHITTINGSTALL, H., (2008). Hugh Fearnley-Whittingstall in farmyard fight with Tesco over chicken welfare:  
[http://www.timesonline.co.uk/tol/life\\_and\\_style/food\\_and\\_drink/article4231173.ece](http://www.timesonline.co.uk/tol/life_and_style/food_and_drink/article4231173.ece)

GIBBENS, J.C., PASCOE, S.J., EVANS, S.J., DAVIES, R.H., SAYERS, A.R., (2001). A trial of biosecurity as a means to control *Campylobacter* infection of boiler chickens. *Preventive Veterinary Medicine* **48**: 85 – 99.

GIBSON, J.R., FITZGERALD, C., OWEN, R.J., (1995). Comparison of PFGE, ribotyping and phage-typing in the epidemiological analysis of *Campylobacter jejuni* serotype HS2 infections. *Epidemiology and Infection* **115**: 215 – 225.

HEUER, O.E., PEDERSEN, K., ANDERSEN, J.S., MADSEN, M., (2001). Prevalence and antimicrobial susceptibility of thermophilic campylobacters in organic and conventional broiler flocks. *Letters in Applied Microbiology* **33**: 269 – 274.

- HUMPHREY, T., MASON, M., MARTIN, K., (1995). The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *International Journal of Food Microbiology* **26**: 295 – 303.
- HUNEAU-SALAÜN, A., DENIS, M., BALAINE, L., SALVAT, G., (2007). Risk factors for *Campylobacter* spp. colonization in French free-range broiler-chicken flocks at the end of the indoor rearing period. *Preventive Veterinary Medicine* **80**: 34 – 48.
- JOHNSEN G., KRUSE H., HOFSHAGEN M., (2006). Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. *Journal of Applied Microbiology* **101**: 1130-1139.
- JØRGENSEN, F., BAILEY, R., WILLIAMS, S., HENDERSON, P., WAREING, D.R., BOLTON, F.J., FROST, J.A., WARD, L., HUMPHREY, T.J., (2002). Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *International Journal of Food Microbiology* **76**: 151 – 164.
- KAZWALA, R.R., JUVA, S.F., NKYA, A.E., (1993). The role of management systems in the epidemiology of thermophilic campylobacters among poultry in the eastern zone of Tanzania. *Epidemiology and Infection* **110**: 272 – 278.
- KRAMER, J.M., FROST, J.A., BOLTON, F.J., WAREING, D.R.A., (2000). *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. *Journal of Food Protection* **63**: 1654 – 1659.
- LUANGTONGKUM, T., MORISHITA, T.Y., ISON, A.J., HUANG, S., MCDERMOTT, P.F., ZHANG, Q., (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology* **72**: 3600 – 3607.

- LYNGSTAD, T.M., JONSSON, M.E., HOFSHAGEN, M., HEIER, B.T. (2008). Risk factors associated with the presence of *Campylobacter* species in Norwegian broiler flocks. *Poultry Science* **87**: 1987-94.
- MEAD, G.C., (1989). Microbes of the avian cecum: types present and substrates utilized. *Journal of Experimental Zoology* **3**: S48-S54.
- MEAD, G.C., SCOTT, M.J., HUMPHREY, T.J., MCALPINE, K., (1996). Observations on the control of *Campylobacter jejuni* infection of poultry by 'competitive exclusion'. *Avian Pathology* **25**: 69 –79.
- MEINERSMANN, R.J., HELSEL, L.O., FIELDS, P.I., HIETT, K.L., (1997). Discrimination of *Campylobacter jejuni* isolates by *fla* gene typing. *Journal of Clinical Microbiology* **35**: 2810 - 2814.
- MORRIS, V, K., ALLEN, V.M., EDGE, S., CANNING, P. (2009). Review of current practices and recommendations for *Campylobacter* reduction in extensively reared flocks, report to Food Standards Agency UK.  
[http://www.foodbase.org.uk/admin/tools/reportdocuments/361-1-624\\_B15014\\_Final\\_Report.pdf](http://www.foodbase.org.uk/admin/tools/reportdocuments/361-1-624_B15014_Final_Report.pdf)
- NEWELL, D.C., FEARNLEY, C., (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology* **69**: 4343 – 4351.
- PEARSON, A.D., GREENWOOD, M.H., DONALDSON, J., HEALING, T.D., JONES, D.M., SHAHAMAT, M., FELTHAM, R.K.A., COLWELL, R.R., (2000). Continuous source outbreak of campylobacteriosis traced to chickens. *Journal of Food Protection* **63**: 309 – 314.
- RIDLEY, A.M., ALLEN, V.M., SHARMA, M., HARRIS, J.A., NEWELL, D.G., (2008a). Real-time PCR approach for detection of environmental sources of *Campylobacter* strains colonizing broiler flocks. *Applied and Environmental Microbiology* **74**: 2492 – 2504.



- RIDLEY, A.M., TOSZEGHY, M.J., CAWTHRAW, S.A., WASSENAAR, T.M., NEWELL, D.G. (2008b). Genetic instability is associated with changes in the colonisation potential of *Campylobacter jejuni* in the avian intestine. *Journal of Applied Microbiology* **105**: 95-104.
- RIVOAL, K., DENIS, M., SALVAT, G., COLIN, P., ERMEL, G., (1999). Molecular characterization of the diversity of *Campylobacter* spp. isolates collected from a poultry slaughterhouse: analysis of cross contamination. *Letters in Applied Microbiology* **29**: 370 – 374.
- RIVOAL, K., RAGIMBEAU, C., SALVAT, G., COLIN, P., ERMEL, G., (2005). Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range broiler farms and comparison with isolates of various origins. *Applied and Environmental Microbiology* **71**: 6216 – 6227.
- SAHIN, O., ZHANG, Q., MEITZLER, J.C., HARR, B.S., MORISHITA, T.Y., MOHAN, R., (2001). Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Applied and Environmental Microbiology* **67**: 3951 – 3957.
- SAHIN, O., LUO, N., HUANG, S., ZHANG, Q., (2003). Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chicks. *Applied and Environmental Microbiology* **69**: 5372 – 5379.
- SATO, K., BARTLETT, P.C., KANEENE, J.B., DOWNES, F.P., (2004). Comparison of prevalence and antimicrobial susceptibility of *Campylobacter* spp. isolates from organic and conventional dairy herds in Wisconsin. *Applied and Environmental Microbiology* **70**: 1442 – 1447.
- TAM, C.C., O'BRIEN, S.J., ADAK, G.K., MEAKINS, S.M., FROST, J.A., (2003). *Campylobacter coli* – an important foodborne pathogen. *Journal of Infection* **47**: 28 – 32.
- VAN OVERBEKE, I., DUCHATEAU, L., DE ZUTTER, L., ALBERS, G., DUCATELLE, R., (2006). A comparison survey of organic and conventional broiler chickens for infectious agents affecting health and food safety. *Avian Diseases* **50**: 196 – 200.



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WASSENAAR, T.M., GIELHAUSEN, B., NEWELL, D.G., (1988). Evidence of genomic instability in *Campylobacter jejuni* isolated from poultry meat. *Applied and Environmental Microbiology* **64**: 1816 – 1821.

ZWEIFEL, C., SCHEU, K. D., KEEL, M., RENGGLI, F., STEPHAN, R., (2008). Occurrence and genotypes of *Campylobacter* in broiler flocks, other farm animals, and the environment during several rearing periods on selected poultry farms. *International Journal of Food Microbiology* **125**: 182-187.