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Increased colonization potential of *Campylobacter jejuni* strain 81116 after passage through chickens and its implication on the rate of transmission within flocks

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SUMMARY

The mechanisms by which *Campylobacter jejuni* rapidly spreads through large broiler flocks are unknown. Recent evidence suggests that the minimum infective oral dose for 100% caecal colonization is about 10^4 cfu, which, with such a 'fragile' organism, may limit transmissibility. However, the colonization potential of laboratory-adapted strains may be anomalous compared with fresh isolates or those passaged *in vivo*. The colonization potential of two chicken and one human *C. jejuni* isolates, which were minimally passaged, have been investigated using a quantitative model of chicken colonization. There was no detectable difference between these strains but all were more efficient colonizers than a laboratory-adapted strain 81116. In addition, the colonization potential of *C. jejuni* strain 81116 following a passage *in vivo* was also determined. The results indicated this increased *c.* 10000 fold following a single passage *in vivo*, such that a dose of only 40 cfu caused maximal colonization. Enhanced colonization potential may, therefore, account for the rapid rate of transmission within large flocks.

Although the sources and routes of transmission of *Campylobacter jejuni* infections to humans are not yet understood, there is strong circumstantial evidence to implicate poultry as a major source [1]. In England and Wales, most broiler flocks are infected by campylobacters by the time of slaughter [2]. An understanding of the epidemiology of campylobacters in these flocks is considered essential for the development of successful intervention regimens. To date, the sources of infection and routes of transmission within a poultry flock have been insufficiently investigated [3]. In broiler houses the birds usually remain uninfected until 2–3 weeks of age. Infection then spreads rapidly, so that up to 20000 birds become infected within 3 days [4].

Given the oral dose of *C. jejuni* strain 81116 required to infect 100% of birds is 10^4 cfu [5], its relatively slow rate of growth and fragility of the organisms *in vitro* and the apparent restricted movement of chickens within broiler houses, it is difficult to account for such a rapid spread via the faecal-oral route. However, because laboratory culture conditions for *C. jejuni* are sub-optimal, growth *in vitro* may not reflect that of the organism in a natural environment, i.e. the avian intestinal tract. For instance, there is evidence to suggest that adaptation to the avian intestinal tract enhances its virulence for susceptible hosts such as mice [6]. This is particularly a concern with *C. jejuni* strain 81116, which, because it originated from human faeces, has been stored a

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Table 1. *Geometric mean caecal colonization levels (cfu/g caecal content) of* C. jejuni *strain 81116P: groups 1 and 2, freshly isolated from 6-day-old chicks; groups 3–7, recovered from storage at −70 °C and subcultured five times*

Group	Dose (cfu)	No. colonized	Colonization level
1	4×10^1	9/9	$6{\cdot}4 \times 10^8$
2	$3{\cdot}5 \times 10^1$	8/8	$6{\cdot}9 \times 10^6$
3	4×10^0	0/10	$< 1 \times 10^2$
4	4×10^1	10/10	$4{\cdot}7 \times 10^9$
5	4×10^2	10/10	$5{\cdot}1 \times 10^9$
6	4×10^3	10/10	$4{\cdot}6 \times 10^9$
7	4×10^4	10/10	$4{\cdot}9 \times 10^9$

long time [7], and has been subcultured many times, may not be the optimal organism for such studies.

In order to investigate some of these factors, the effect of a single passage of *C. jejuni* strain 81116 through the avian intestinal tract on the colonization potential of the organism was determined using a quantitative chicken model of oral infection described previously [5]. Briefly, 1-day-old chicks were infected by oral gavage with *C. jejuni* strain 81116 and the passaged bacteria recovered from caeca after 5 days. The recovered bacteria, designated 81116P, were then used to colonize groups of 1 day-old chicks, either directly without further culture at doses of 40 cfu (9 chicks) and 35 cfu (8 chicks), or after being recovered from storage at −70 °C and subcultured 5 times at a dose range of $4-4 \times 10^4$ cfu (5 groups of 10 chicks). After 5 days the colonization levels were determined [5]. The detection limit was 100 cfu/g caecal contents.

The minimum infective dose of *C. jejuni* strain 81116 required to ensure maximum colonization after 5 days (10^8-10^{10} cfu/g of caecal contents) was 1×10^5 cfu [5]. By comparison, strain 81116P, which had not been frozen and which had been subcultured once only following isolation from the infected chicks, had a greatly enhanced colonization potential such that doses of 40 and 35 cfu colonized the majority of the 1-day-old chicks (9/9 and 4/8 respectively) at levels of between 10^8-10^9 cfu/g of caecal contents (Table 1). One of the four other chicks was colonized at a level of 10^7 cfu/g, while the remaining chicks were colonized just above the limit of detection. The storage and subculture of strain 81116P had no detectable effect on colonization potential (Table 1).

To ascertain whether the colonization potential of strain 81116 was anomalous, three other *C. jejuni* strains were investigated. These were two fresh chicken isolates (93/146 and 93/175), which were subcultured three times before storage, and a fresh human isolate (94/146), which was sub-cultured only once and not stored after initial isolation. With maximum colonization at doses of $2{\cdot}8 \times 10^2-10^3$ cfu each of these strains had a greater potential for colonization than strain 81116, but were less effective colonizers than strain 81116P.

Campylobacter jejuni strain 81116 has a low colonization potential compared to 'fresher' isolates. Strain 81116 showed a dose-responsiveness such that the larger the dose, the greater the level of colonization (up to the maximum level), whereas the other isolates tested showed an 'all-or-nothing' response whereby chicks were either colonized at the maximum level or below the detection limit, depending on the dose. However, the colonization potential of strain 81116 was greatly enhanced by a single *in vivo* passage through chickens, so that strain 81116P showed the 'all-or-nothing' response seen in the other strains.

Table 2. *Geometric mean colonization levels (cfu/g caecal content) for two chicken isolates, 93/146 and 93/175, and a human isolate, 94/146.* Where campylobacters were recovered, all birds in that group were colonized

93/146		93/175		94/146	
Dose (cfu)	Col. level	Dose (cfu)	Col. level	Dose (cfu)	Col. level
1×10^2	$< 1 \times 10^2$	$2{\cdot}8 \times 10^1$	$< 1 \times 10^2$	$1{\cdot}2 \times 10^1$	$< 1 \times 10^2$
1×10^3	$3{\cdot}0 \times 10^9$	$2{\cdot}8 \times 10^2$	$8{\cdot}3 \times 10^8$	$1{\cdot}2 \times 10^2$	$< 1 \times 10^2$
1×10^4	$1{\cdot}1 \times 10^9$	$2{\cdot}8 \times 10^3$	$2{\cdot}5 \times 10^8$	$1{\cdot}2 \times 10^3$	$8{\cdot}1 \times 10^8$
1×10^5	$2{\cdot}5 \times 10^9$	$2{\cdot}8 \times 10^4$	$6{\cdot}7 \times 10^8$	$1{\cdot}2 \times 10^4$	$4{\cdot}3 \times 10^8$
1×10^6	$9{\cdot}5 \times 10^8$	$2{\cdot}8 \times 10^5$	$2{\cdot}7 \times 10^8$	$1{\cdot}2 \times 10^5$	$1{\cdot}8 \times 10^9$

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The reduced colonization potential of strain 81116 compared with the other three strains tested presumably reflects the physiological status of the organism. *C. jejuni* as evolved to survive successfully in a wide range of environmental conditions. Storage and subculture presumably result in an organism more adapted to the *in vitro* environment than the avian intestinal tract. In this respect, *C. jejuni* strain 81116 may be analogous to those campylobacters found in and around poultry houses, which have adapted to the conditions of nutrient depletion and exposure to high oxygen tension. Such environmentally-stressed organisms might be poor colonizers. However, a single passage *in vivo* may result in organisms which are efficient colonizers. This would have a significant effect on both the rate and extent to which chickens become infected. This phenotypic switch induced *in vivo* leads to a phenotype with enhanced colonization potential, and could account for the lag phase of infection and the subsequent rapid rate of transmission observed in poultry houses [4]. The implications of such observations on the development of intervention procedures to reduce colonisation with campylobacters requires further investigation.

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