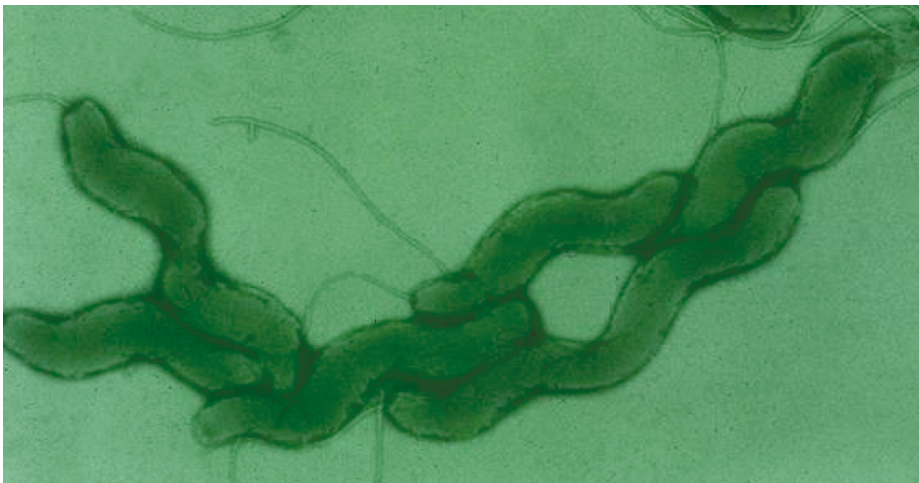


Campylobacter spp: not quite the tender flowers we thought they were?

Tom Humphrey



to cope with conditions outside the host. There is much dispute about:

- the relationship between *Campylobacter* physiology and infectivity
- whether these bacteria enter a 'viable but non-culturable (VBNC) state'
- the place of coccoid cells in the epidemiology of human and animal infection.

One thing is certain, however, *Campylobacter* spp. did not evolve to grow on laboratory media. We

In England and Wales, as elsewhere in the developed world, *Campylobacter* spp, principally *Campylobacter jejuni* (Fig. 1), continue to be highly important food- and water-borne pathogens. The Public Health Laboratory Service (PHLS) estimated that there would be almost 60,000 reported cases in England and Wales in 2001 (J. Frost, personal communication). Given the data from the study on Infectious Intestinal Disease in the UK, where cases of *Campylobacter* infection have been shown to be under-reported, the true incidence of infection is likely to approach 500,000 cases. *Campylobacter* spp. have been said to be sensitive to the extra-intestinal environment. Thus laboratory-derived data show that *C. jejuni* survives poorly at high temperature, at low pH, under dry conditions and at low temperature and would seem to be markedly more sensitive to hostile environments than *Salmonella* spp. (Fig. 2). Such sensitivity is, perhaps, surprising in a human pathogen, which is so successful and which will be exposed to many harsh conditions on the path from farm to fork. It is also pertinent to remember that the thermophilic *Campylobacter* species have a minimum growth temperature of 30 °C and cannot accumulate in foods in the manner of most other food-borne pathogens.

One possible explanation for the success of *C. jejuni* as a human pathogen is that contamination levels in key foods such as chicken can be very high and although many *Campylobacter* cells can die, an infectious dose may still remain. For example, work has shown that a single rinse of a chicken carcass can recover over 1 million cells of *C. jejuni*. This is clearly of significance when the infectious dose has been reported to be as low as 500 cells in a healthy adult. *C. jejuni* has also been isolated from chicken muscle, which may explain why under-cooked chicken is often identified as a vehicle for human infection.

Clearly we have yet to fully understand the behaviour of these bacteria, especially with regard to their ability

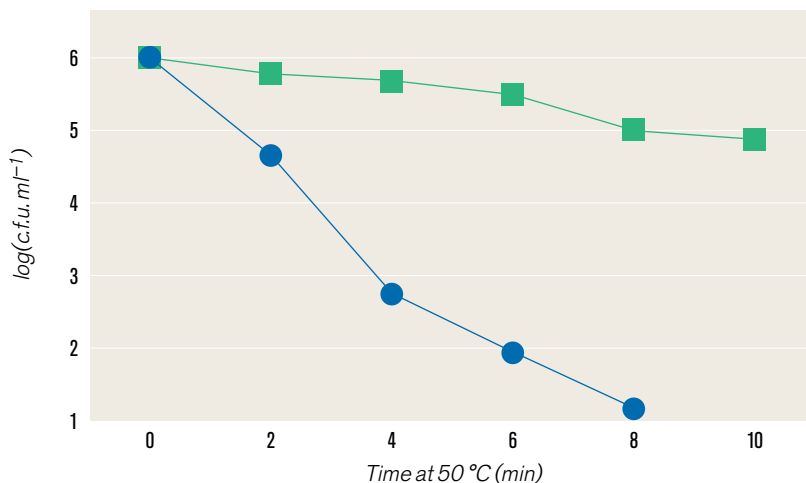
often choose to culture these bacteria under conditions which may be entirely alien to them. The data on survival mentioned above were generated using traditional culture techniques where bacterial cells were exposed to damaging environments and then recovered by either plating on agar or by broth culture. It is perhaps unfortunate that many studies have used media and/or incubation regimens which would not have maximized the recovery of damaged cells and which may have over-estimated sensitivity.

● Sub-lethal injury in *Campylobacter* spp.

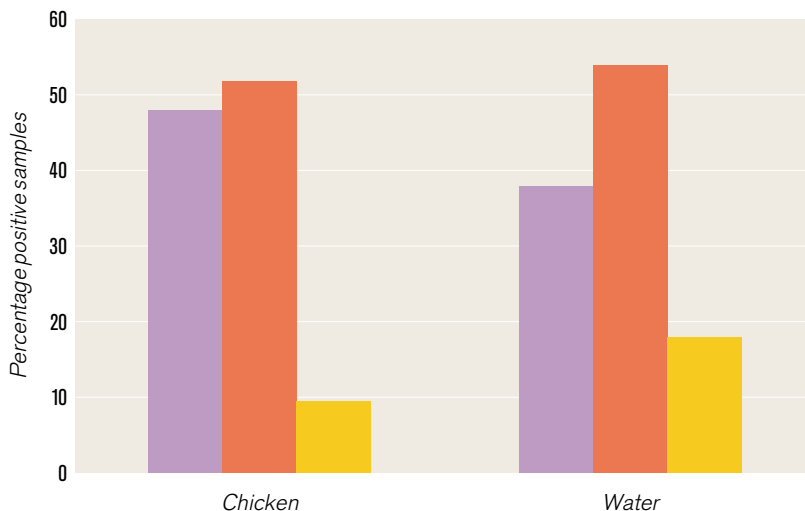
Campylobacter spp. are rarely, if ever, present in naturally contaminated samples in pure culture. Their isolation from food and environmental samples requires that competing microbial floras be suppressed. Thus selective media, which can contain up to five different antibiotics, are used. In common with other Gram-negative bacteria, *Campylobacter* spp. will experience physiological damage as a consequence of exposure to hostile environments (which are common in food production). This is often termed 'sub-lethal injury'. Its principal manifestation

TOP LEFT:
Fig. 1. Cells of *C. jejuni* isolated from an infected human case.
PHOTO TOM HUMPHREY

BELOW:
Fig. 2. Survival of *C. jejuni* (●) and *Salmonella* Typhimurium (■) at high temperature.
COURTESY REBECCA HUGHES, UNIVERSITY OF BRISTOL



RIGHT:
Fig. 3. The influence of the selective regimes used on the isolation of *Campylobacter* spp. from water or chicken. Samples were placed directly into Exeter broth (■), base broth, which was incubated for 8 h, after which selective agents were added (■), or broth containing cefoperazone, amphotericin and trimethoprim with rifampicin and polymixin being added after 8 h (■).
 COURTESY MARK MASON



is a compromised ability to grow in selective media. This is because damage to the bacterial outer membrane will allow the ingress of antibiotics, such as rifampicin, which will be excluded by undamaged cells. Injured *Campylobacter* cells will show a range of increased sensitivities and these are outlined in Table 1.

The stresses on injured *Campylobacter* will often act in tandem. For example, uninjured cells of *C. jejuni* have been shown to become extremely sensitive to rifampicin in the presence of low levels of peroxide. The effects with damaged populations are even more extreme. Peroxides accumulate in media during storage, even when plates or broths are stored at refrigeration temperatures and in the dark. If accurate data on the behaviour of *Campylobacter* spp. are to be obtained, it is vital that studies are designed in such a way that the challenged populations at least have a fighting chance of survival and growth, post-exposure. Research using pure cultures is relatively simple and principally requires that account be taken of the various manifestations of sub-lethal injury. Thus the media used should ideally be less than 7 days old and should contain blood and agents which quench peroxides and other potentially injurious compounds. The latter usually comprise a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP). Where *Campylobacter* populations have been exposed to very harsh conditions, such as drying, it may be necessary to incubate the recovery media for extended periods (see below). An advantage of pure culture studies is that it is not necessary to include selective agents.

The examination of foods and environmental samples is rather more difficult. These may contain only small numbers of highly damaged *Campylobacter* cells as part of a large, mixed bacterial population. The sensitivity of *Campylobacter* to the presence and actions of other bacteria (Fig. 3) means that it is not possible to adopt the approach used for the isolation of other food-borne pathogens, where samples are first cultured in non-selective media. Different strategies are required. PHLS uses 'Exeter' selective medium in its surveillance studies on foods. Work has shown that three of the antibiotics in

this medium, cefoperazone, trimethoprim and amphotericin are well tolerated by damaged *Campylobacter* cells. The addition of these three antibiotics to the primary broth culture helps to suppress competing flora and allows the recovery of damaged *Campylobacter* cells. Rifampicin and polymixin can then be added after this pre-enrichment period. This regimen has been shown to improve recovery rates from naturally contaminated water (Fig. 3) and dry surfaces. This approach, coupled with an increase in the length of broth incubation to 4–5 days, has allowed *Campylobacter* spp. to be recovered from dry surfaces 24 h after contamination, albeit in low numbers.

● **Does the presence of low numbers of damaged *Campylobacter* cells pose a risk to public health?**

Improvements in the isolation methods for *Campylobacter* spp. mean that it is now possible to recover viable cells from environments previously thought not to harbour these bacteria and after treatments previously thought to render cells non-culturable. Thus it is now relatively easy to isolate *Campylobacter* from kitchen and farm surfaces, kitchen items such as dishcloths and the natural environment. In many instances, these bacteria will be severely damaged. Work, largely using the young chicken model, has demonstrated that sub-lethally injured *Campylobacter* spp. are compromised in their ability to cause infection. Presumably, this may also mean that they will be less likely to be able to infect humans. Cross-contamination has been shown to be important in outbreaks of *Campylobacter* infection. It is quite clear, from the explosion in kitchen products containing anti-bacterial agents that there is a belief that contaminated surfaces pose a risk to human health. Does this extend to *Campylobacter*, given its inability to grow on food and its apparent sensitivity to the extra-intestinal environment? There is need for properly constructed studies where sensitive culture techniques are combined with work examining gene and protein expression to determine the infectivity of *Campylobacter* populations showing different levels of injury.

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Further reading

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Table 1. Manifestations of sub-lethal injury in *Campylobacter* spp. exposed to conditions common in food production

■ Increased sensitivity to rifampicin and polymixin
■ Reduced ability to grow at elevated incubation temperatures
■ Greatly enhanced sensitivity to peroxides
■ Extreme lag phases in recovery broth
■ Reduced ability to grow in mixed culture