

## Growth and survival characteristics of *Campylobacter jejuni* in liquid egg

By M. L. HÄNNINEN,\* H. KORKEALA\* AND P. PAKKALA†

\* *College of Veterinary Medicine, Department of Food Hygiene, Hämeent. 57, 00550, Helsinki 55, Finland*, † *National Board of Health, Finland*

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### SUMMARY

Growth and survival of four *Campylobacter jejuni* strains in yolk, in liquid whole egg and in white during aerobic storage at 37, 20 and 4 °C was followed. In 48 h at 37 °C the cell counts of *C. jejuni* increased by about 3 log<sub>10</sub> units in yolk and 1.60–3.35 log units in liquid whole egg. The growth of *C. jejuni* was slightly better in yolk than in liquid whole egg. At 20 °C during 48 h the cell counts decreased by about 0.5–1.5 log<sub>10</sub> units in yolk and in liquid whole egg. At 4 °C the decrease in cell counts after 21 days ranged from 1 to 2 log<sub>10</sub> units, except for one strain, KH 3, which could not be detected after 14 days storage in yolk. In liquid whole egg the cell counts of this strain also decreased considerably during storage.

In white the number of inoculated *C. jejuni* cells decreased rapidly. The killing effect of white was shown to be temperature-dependent; at 37 and 20 °C no positive samples were detected after 24 h and at 4 °C no positive samples were found after 48 h.

### INTRODUCTION

Human enteric disease caused by *Campylobacter jejuni* has a world-wide distribution (Skirrow, 1982). This infection has many characteristics in common with human salmonellosis; foods of animal origin, for example, are suspected to be important vehicles for the transmission of campylobacters to humans. Although there have not been many food-borne campylobacter epidemics reported, the importance of foods of animal origin in the epidemiology of this disease is based on the fact that many mammalian and avian species carry campylobacters as part of their intestinal flora. In particular, wild birds and poultry have been shown to form an important reservoir of *C. jejuni* (Grant, Richardson & Bokkenheuser, 1980; Hänninen & Raevuori, 1981; Kapperud & Rosef, 1983), although it will be easier to evaluate the importance of these species in the epidemiology of human campylobacter infections after the distinctive characteristics of human pathogenic *C. jejuni* strains are known.

Liquid egg is widely used as a food ingredient at home, in food service establishments and in the food industry. Up to now salmonellas have been the only human pathogens transmitted via eggs to humans (Elliott & Hobbs, 1980). Nowadays we can add to the list another pathogen, *C. jejuni*, which probably can also be transmitted via faecally contaminated eggshells to liquid egg. In most

countries liquid egg products are pasteurized and thereafter stored either refrigerated or frozen. The pasteurization process probably destroys campylobacters as well as salmonellas, since the heat resistance of these organisms is roughly similar (Doyle & Roman, 1981).

The purpose of the present study was evaluate the safety of liquid egg with respect to *C. jejuni* by following the growth of *C. jejuni* at 37 °C and the survival of the organism at 20 °C and at 4 °C in yolk, in whole liquid egg and in white.

## MATERIALS AND METHODS

### *Bacterial strains and cultivation methods*

Four *C. jejuni* strains were used: NCTC 11168, KH 3, N 104 and L 41A, of human, bovine, and ovine origin, respectively. All strains were classified as *C. jejuni* according to Skirrow & Benjamin (1980).

The strains were grown on plates of brucella agar (Difco) containing 5% blood and incubated at 42 °C for 48 h. One loopful of the growth on the blood agar plates was transferred into brucella broth supplemented with 0.05% each of sodium pyruvate, ferrous sulphate and sodium metabisulphite as recommended by Hoffman, Krieg & Smibert (1979), and incubated at 42 °C for 24 h. This growth was used as the inoculum in the experiments. The number of *C. jejuni* cells in the inoculum was counted by 10-fold dilutions in 0.1% sterile peptone on brucella blood agar plates. The number of *C. jejuni* cells in the liquid egg during storage was counted on a Skirrow-type medium (Hänninen & Raevouri, 1981). All incubations were done microaerophilically (5% O<sub>2</sub> + 10% CO<sub>2</sub> + 85% N<sub>2</sub>) at 42 °C.

### *Survival of C. jejuni in liquid egg*

Fresh eggs were purchased, the shells were washed with soap and disinfected with ethanol, and the eggs were broken aseptically into sterile glass-jars. Three types of liquid egg samples were prepared: (1) whites; (2) yolks; (3) liquid whole eggs. For each treatment, 600 g of each type of liquid egg sample was inoculated with 6 ml of 1:10 (vol./vol.) diluted campylobacter culture. The inoculum was mixed carefully into the liquid egg samples. The inoculated liquid egg samples were divided into 200 g portions, which were stored either at 37, at 20 or at 4 °C in air in sterile glass bottles with tightly closed caps for 48 h, 48 h or 21 days, respectively. At the intervals desired, 10 g samples were taken from each product and serially diluted in sterile 0.1% peptone water; the colony-forming units of *C. jejuni* were then counted. Other microbiological analyses of the samples were counting of aerobic mesophilic bacteria (Plate-count Agar, Difco, incubated at 30 °C for 72 h) and coliform bacteria (Violet red bile agar, Difco, incubated at 35 °C for 24 h). The pH of the samples was also measured. Each campylobacter strain was examined twice under each condition.

## RESULTS

At the beginning of the study, the pH of the yolk was 6.55 and the aerobic plate count ranged from  $< \log_{10} 1$  to  $\log_{10} 3.1$ . The corresponding values for liquid whole egg were 7.70 and  $< \log_{10} 1 - \log_{10} 4.0$ . The egg white was sterile and its pH was 9.25. No coliform bacteria were detected in any samples during the whole study period.

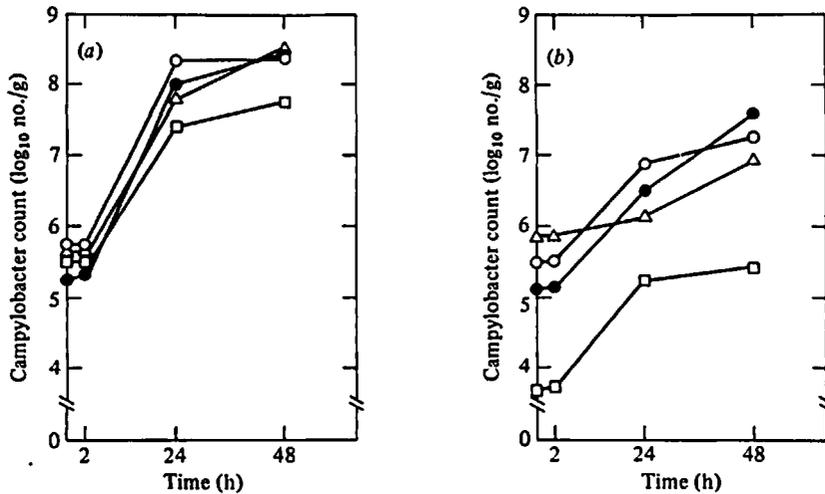


Fig. 1. Growth of *C. jejuni* in yolk (a) and in liquid whole egg (b) at 37 °C. Strains and symbols used: NCTC 11168 (○), N 104 (●), L 41A (△), and KH 3 (□).

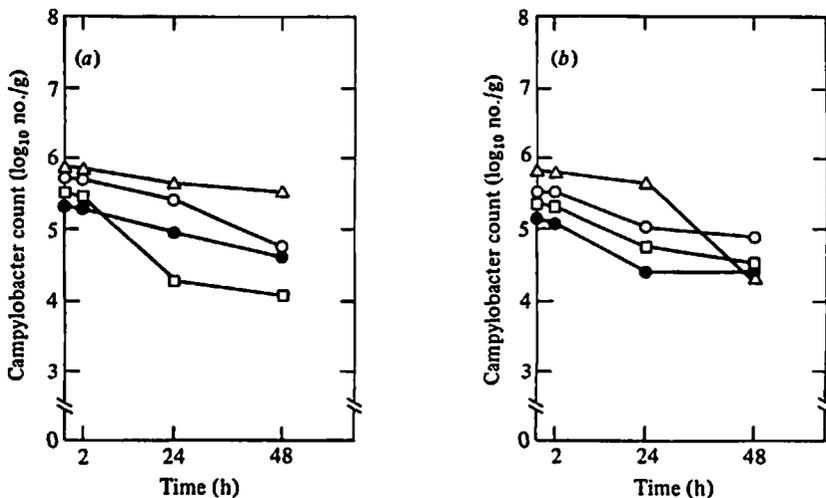


Fig. 2. Survival of *C. jejuni* in yolk (a) and in liquid whole egg (b) at 20 °C. Strains and symbols as in Fig. 1.

At 37 °C the cell counts of all campylobacter strains increased during 48 h storage in air both in yolk and in liquid whole egg (Fig. 1). The increase in the campylobacter cell counts was approximately 3 log<sub>10</sub> units in yolk and from 1.60 to 3.35 log<sub>10</sub> units in liquid whole egg. No great differences in the growth rate of campylobacter strains were noted at this temperature. In the yolk samples taken after 48 h, the pH value ranged from 6.30 to 6.65 and the aerobic plate count from < log<sub>10</sub> 1 to log<sub>10</sub> 8.5. A high or low aerobic plate count in the samples did not seem to have any effect on campylobacter counts. The corresponding values in liquid whole egg ranged from 7.70 to 7.85 and from < log<sub>10</sub> 1 to log<sub>10</sub> 8.0. Egg white was inhibitory to all *C. jejuni* strains. The counts of *C. jejuni* decreased considerably during 4 h incubation at 37 °C and in the samples taken after 24 h incubation no *C. jejuni* cells could be detected.

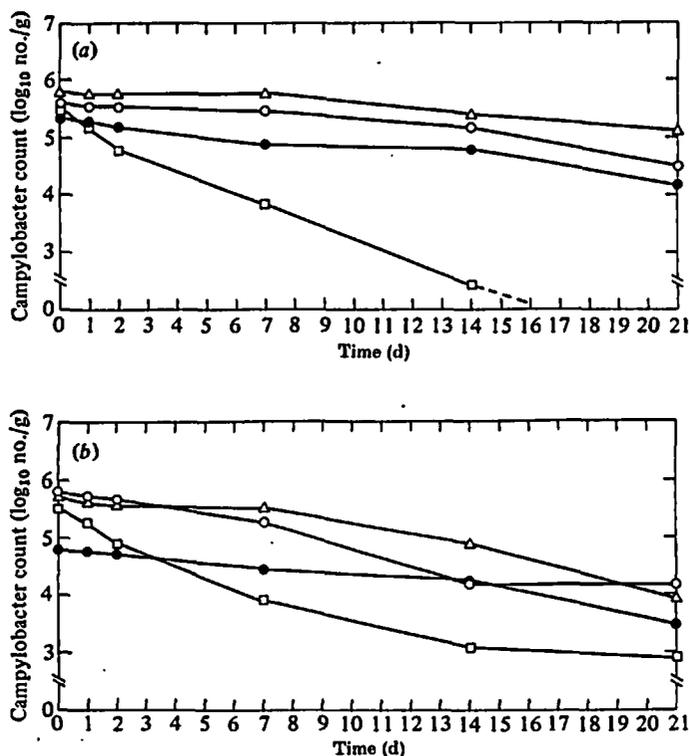


Fig. 3. Survival of *C. jejuni* in yolk (a) and in liquid whole egg (b) at 4°C. Strains and symbols as in Fig. 1.

At 20 °C the counts of inoculated *C. jejuni* cells decreased by about 0.5–1.5 log<sub>10</sub> units both in yolk and in liquid whole egg during 48 h (Fig. 2). After 48 h, no noteworthy differences in the cell counts between strains were noted. After incubation, the aerobic plate counts ranged from < log<sub>10</sub> 1 to log<sub>10</sub> 8.0, the pH value of the yolk was 6.5 and that of the liquid whole egg 7.75. In white a rapid decrease in campylobacter cell counts occurred during 4 h storage and no campylobacter cells were detectable after 24 h. The pH value of the white after incubation was 9.30.

At 4 °C the campylobacter cell counts decreased during 21 days storage 1–2 log<sub>10</sub> units in yolk and by approximately 1 log<sub>10</sub> unit in whole liquid egg (Fig. 3). Strain KH 3 was different from the other strains studied. After 14 days storage it was not detectable in yolk, and also in liquid whole egg its counts decreased during the total storage period by about 2.5 log<sub>10</sub> units. After storage, the aerobic plate counts ranged in both samples from < log<sub>10</sub> 1 to log<sub>10</sub> 7.0. In white the counts of *C. jejuni* decreased rapidly during 24 h storage and after 48 h storage no positive samples were detected.

#### DISCUSSION

The present study showed that in foods rich in nutrients, which have a suitable environment, such as egg yolk and liquid whole egg, *C. jejuni* can grow in air.

Aerobic growth of *C. jejuni* has formerly been obtained in a cultivation medium by adding superoxide anion quenching chemicals to the medium (Hoffman *et al.* 1979), on red meat (Gill & Harris, 1982), and in sterile ground chicken meat (Blankenship & Craven, 1981), but not, for example, in milk (Christopher, Smith & Vanderzant, 1982). Egg yolk contains proteins and lipids, and its iron content is relatively high (Board, 1969). Yolk has been shown to improve the recovery of bacterial cells after various environmental stresses by destroying toxic compounds in the recovery medium (Baird-Parker, 1965). The semifluid consistency of yolk and liquid whole egg and the protective capacity of yolk against toxic forms of oxygen are probably important factors in controlling the growth of *C. jejuni* in these foodstuffs stored in air. The pH values of yolk and whole liquid egg are favourable for the growth of *C. jejuni*.

Egg white contains numerous antimicrobial systems, such as lysozyme and conalbumin, which chelates iron, copper and zinc; it also has an alkaline pH and a low nutrient content (Boad, 1969). In the present study *C. jejuni* was shown to be rapidly killed in white. The phenomenon was temperature-dependent; at 37 and 20 °C the cells were destroyed more rapidly than at refrigeration temperature. Other gram-negative organisms, such as salmonellas, have also been shown to be effectively destroyed in white, although when the pH of white was adjusted to 7–8 they grew well (Banwart & Ayres, 1957). The relative importance of the various antimicrobial factors present in egg white was not analysed in the present study. The high pH value of white was probably not the most important factor in the inhibition of growth or survival of the organism, since certain *C. jejuni* strains have been shown to grow in a cultivation medium at pH 9.5 (Doyle & Roman, 1981). When the white and yolk are mixed the antimicrobial properties of white are lost (Elliott & Hobbs, 1980). This was also found in the present study at all temperatures used. However, the growth of *C. jejuni* was slightly better in yolk than in the whole liquid egg.

In many countries, liquid egg products are pasteurized and thereafter stored either frozen or refrigerated. Pasteurized liquid egg has a remarkably long refrigerated shelf-life, 20–22 days (Kraft *et al.* 1967). The present study showed that *C. jejuni* will survive in liquid egg at least as long as the shelf-life of these products. The survival of *C. jejuni* in yolk and in liquid whole egg, both at 20 and at 4 °C, was roughly the same as that observed earlier in meat (Christopher *et al.* 1982), or on meat (Gill & Harris, 1982). With respect to the survival of *C. jejuni* at refrigerated temperature, there are probably differences between strains; in the present study one *C. jejuni* strain was shown to be more sensitive to refrigerated storage than the other three strains. Survival at 20 or at 4 °C in the present test conditions in yolk and in liquid whole egg was not shown to be temperature-dependent, as has been found to be the case in many other milieus, including milk, water, urine and faeces (Blaser *et al.* 1980) or on meat (Gill & Harris, 1982); the decrease in the cell counts during two first days of storage was almost the same at 4 and at 20 °C.

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