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The heat-sensitivity of *Campylobacter jejuni* in milk

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SUMMARY

It is now established that milk can be a vehicle for the spread of enteritis due to *Campylobacter jejuni*. By determing the lethal effect of heat on six isolates it has been shown that *C. jejuni* is unlikely to survive pasteurization.

INTRODUCTION

Human enteritis due to *C. jejuni* and *C. coli* has been widely recognized only in the last 5 years (Skirrow, 1977). Raw milk is a major cause of campylobacter enteritis outbreaks involving transfer from animals to man (Robinson & Jones, 1981). The size and pattern of these outbreaks suggests that there is heavy but brief contamination, which may explain why attempts to isolate the organism from suspect milk appear to have failed. However, on two occasions (Porter & Reid, 1980; Robinson *et al.* 1979) *C. jejuni* was isolated from milk socks (filters in the milking equipment).

As the sale of raw milk is still permitted in the U.K. (Davidson, 1980) and U.S.A. it is important to know whether pasteurization would kill campylobacters and make such milk safe.

MATERIALS AND METHODS

Test organisms. Six campylobacter strains were tested. Five were obtained from faeces of patients suffering from diarrhoea, and one originated from a suspected milk-borne outbreak of enteritis (kindly supplied by Dr M. B. Skirrow of Worcester Royal Infirmary).

Strains were stored at 42 °C in nutrient broth containing 0·2% agar (Oxoid Technical No 3), 0·2% potassium L-aspartate and 0·3% yeast extract (Difco) (YNAB) adjusted to pH 7·2 and dispensed in 15 ml volumes in McCartney bottles. For each experiment the six strains were streaked over the surface of well dried blood agar (BA) plates (Oxoid B.A. No 2 with 5% defibrinated horse blood) and incubated at 42 °C for 24 h in anaerobic jars from which two-thirds of the air had been removed (500 mmHg below atmospheric pressure) and replaced with a 5% carbon dioxide/95% nitrogen mixture. Bacteria from these cultures were suspended

Table 1. *D values (min) of 6 strains of* C. jejuni

Temp (°C)	Strains: 24791 *C. jejuni* Biotype 2†	16000 *C. jejuni* Untyped	21033 *C. jejuni* Untyped	17259 Untyped	16509* *C. jejuni* Biotype 1	5388 *C. jejuni* Biotype 1
49·50	—	15·8	—	—	—	—
50·00	5·7	—	7·2	—	7·3	36·0
51·50	3·9	4·7	1·8	—	—	—
51·75	—	—	—	—	5·6	—
52·00	—	—	—	5·2	—	12·5
53·00	—	—	—	—	1·3	—
53·50	1·0	2·2	—	—	—	—
54·00	—	—	—	—	—	0·7
54·50	—	—	—	0·8	—	—
55·00	—	—	—	—	1·1	—
55·50	—	—	—	—	—	0·6
56·00	—	—	0·3	0·9	—	—

* Suspected milk-borne outbreak strain.
† Skirrow & Benjamin, 1980.

in 1/4 strength Ringer's solution to a final concentration of *c.* 10^9 colony forming units per ml and mixed using a rotamixer (SN604, Hook and Tucker Ltd) in order to break up clumps of organisms.

Thermal treatment. For each test 1 ml of bacterial suspension was thoroughly mixed with 9 ml of pasteurized cow's milk in test-tubes (150 × 15 mm) fitted with cotton wool plugs. These were placed in a water bath (Grant SB2), with the water level to the base of the tube plug. Temperatures were measured with a standardized thermometer. Three min were allowed for equilibration before the first tube (0 min) was removed.

Recovery. After cooling the tubes in water at *c* 20 °C appropriate decimal dilutions were made in 1/4-strength Ringer's solution. Duplicate 0·1 ml volumes were surface plated on YNAB medium with 1·2% agar containing 0·05 gl^{-1} haematin (Razi & Park, 1979) and selective agents (Skirrow, 1977).

Inclusion of selective agents had previously been shown not to inhibit recovery of heat-stressed cells. An unheated milk sample was similarly plated to confirm viability of the test organism. After microaerobic incubation for 40 h at 42 °C the number of colonies on the test and control plates were compared.

Diagnostic thioglycollate broth (Lab M) containing 5% defibrinated horse blood, 10 i.u. m^{-1} polymyxin B, 20 $\mu g\ ml^{-1}$ trimethoprim, 40 $\mu g\ ml^{-1}$ vancomycin and 100 $\mu g\ ml^{-1}$ cycloheximide dispensed in 20 ml volumes in 1 oz McCartney bottles was used as a selective enrichment medium.

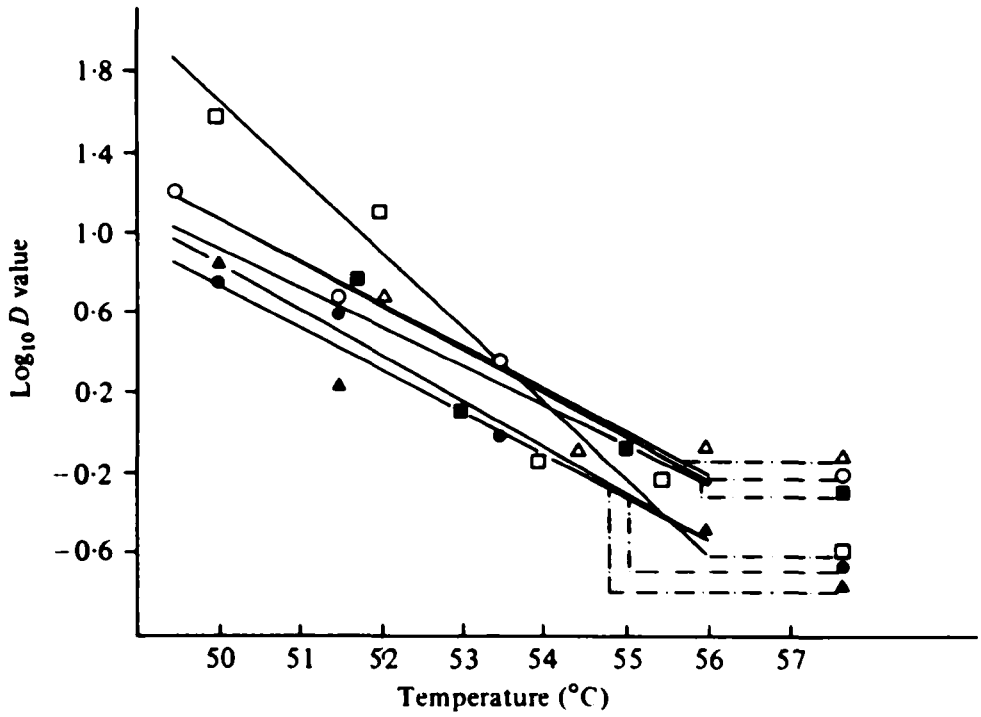

Fig. 1. Thermal death time as a function of temperature. *z* values for six isolates: ●, 24791 (4·5); ○, 16000 (4·7); ▲, 21033 (4·8); △, 17259 (4·8); ■, 16509 (5·3); □, 5388 (2·8).

RESULTS

Calculation of thermal destruction. Death rates were assumed to be exponential. The logarithm of survivors was plotted against time and a regression line fitted (regression coefficient $\geqslant -0{\cdot}95$). The time (min) required to destroy 90% of the cells at a given temperature was called the *D* value. Table 1 shows the *D* values of the six strains tested.

The logarithm of *D* values were plotted against temperature and regression lines were fitted (regression coefficient $\geqslant -0{\cdot}92$) (Fig. 1). The change in temperature necessary to reduce the *D* values by 90% was called the *z* value.

A computer program was used to compare the regression lines for each *z* value by comparing the residual sum of squares. It was evident that strain 5388 has a lower *z* value than the other five isolates. A single regression line was fitted to the data from the five other strains and extrapolated to obtain a single *D* value prediction for pasteurization temperature (Katzin, Sanholzer & Strong, 1943) (Fig. 2). Isolate 5388 was more sensitive to higher temperatures than the other strains tested. The *D* value 0·7 min was omitted from *z* value calculations for 5388.

Laboratory pasteurization. Strains 24791 and 5388 were laboratory pasteurized ($63{\cdot}5 \pm 0{\cdot}5$ °C for 30 min) using 10 replicate tubes. One ml of milk from each tube was incubated in enrichment broth at 42 °C and subcultured to BA after 2 d. There was no growth in any bottle.

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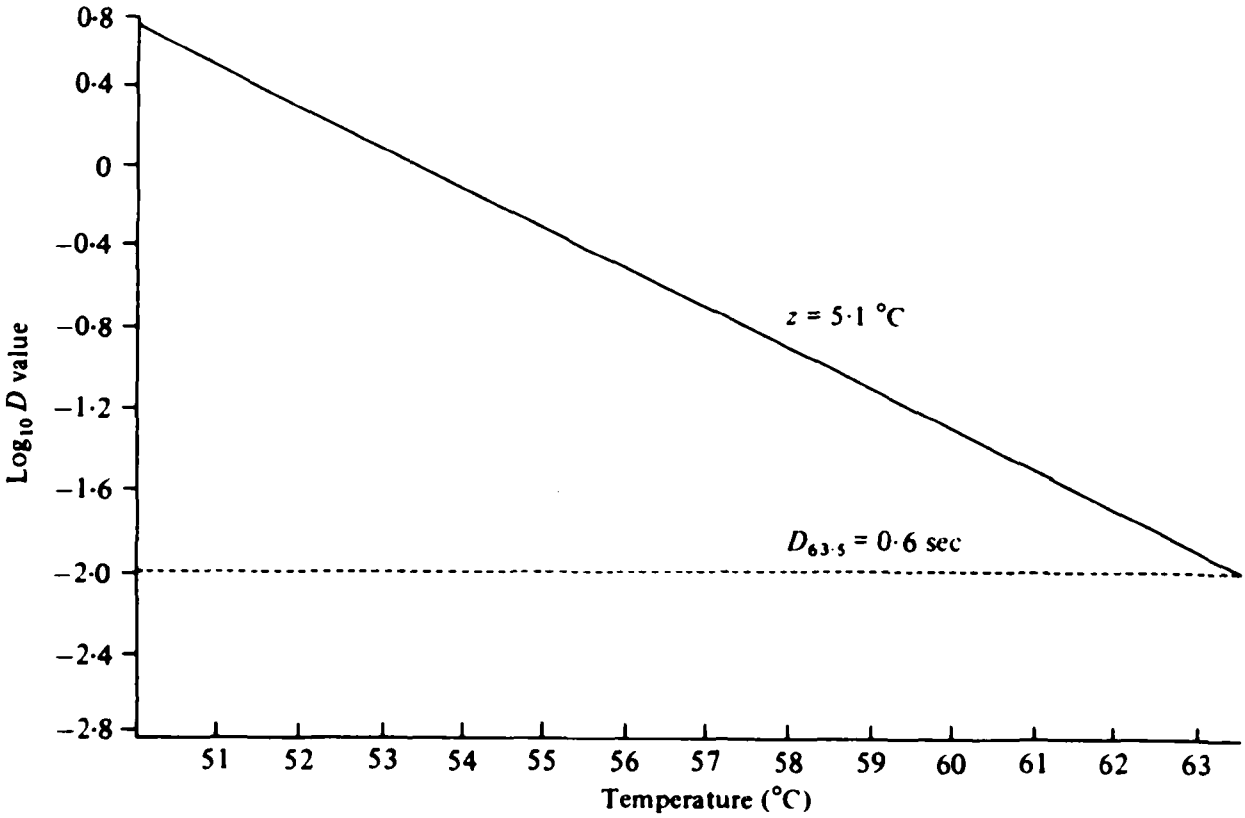

Fig. 2. Joint regression line for thermal death time as a function of temperature extrapolated to pasteurization temperature (63·5 °C). Isolate 5388 omitted.

DISCUSSION

Various methods, each with advantages and disadvantages can be used to determine the heat sensitivity of organisms. The tube method used in this study did not enable a uniform population to be studied at each temperature. However, slight variations would not alter the conclusions. A flask method (Stumbo, 1973) was used in an attempt to overcome this problem, but consistent results were not obtained possibly due to evaporation from the large surface area of milk involved.

Despite these difficulties it is clear from this study that pasteurization should give complete protection against the spread of campylobacter enteritis by milk even when large numbers of organisms are present. A similar conclusion, but using skimmed milk has just been reported by Doyle & Roman (1981).

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