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# A search for the source of *Campylobacter jejuni* in milk

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## SUMMARY

Samples of milk from 1501 cows with mastitis were negative for *Campylobacter jejuni*. The faeces of 74 healthy Friesian cows were screened for *C. jejuni*: 13% of the samples were positive during the summer when the cows were on pasture, and 51% were positive in the winter when the cows were housed. Positive samples contained on average $1 \times 10^4$ campylobacters per g faeces.

It is concluded that faecal contamination rather than udder infection is the means by which campylobacters enter milk and thereby infect man.

## INTRODUCTION

Since 1977 *Campylobacter jejuni* has been recognized as a major cause of human enteritis (Skirrow, 1977). In the UK raw milk has been implicated in the largest campylobacter enteritis outbreaks (Robinson & Jones, 1981). The route by which *C. jejuni* enters milk has been much debated and two hypotheses have been presented: milk may be contaminated by means of a naturally occurring campylobacter mastitis, or it may be contaminated with bovine faeces during or after milking. Both are theoretically possible because *C. jejuni* is commonly present in cattle faeces (Robinson & Jones, 1981) and a campylobacter mastitis has been produced experimentally by Lander & Gill (1980).

We have attempted to establish which of these hypotheses is more probable by conducting two surveys. The first was a search for campylobacters in foremilk samples from cows with mastitis. The second determined the number of cows in a dairy herd that were excreting campylobacters and the numbers of campylobacters present in positive faeces samples.

## MATERIALS AND METHODS

### *Milk*

Foremilk collected by farmers or veterinarians from cows with clinical mastitis was obtained from the Ministry of Agriculture, Fisheries & Food, Veterinary

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Investigational Centre (VIC) at Reading, or was sent by first-class post from VICs at Shrewsbury, Lincoln, Liverpool or Leeds. Samples from Reading had been refrigerated for up to 4 days but the history of other samples was not known.

Information on the use of antibiotics before sample collection was not available. The 1214 milk samples from Reading VIC represented all samples received by them during one year. These were submitted for diagnosis either because of clinical mastitis or because of abnormal milk appearance or reduced yield. The remaining samples came from herds which suffered from bacterial mastitis, mainly caused by *Escherichia coli* and *Streptococcus uberis*.

*Faeces*

Faeces was obtained rectally from 74 lactating Friesian cows once during the period from August to October 1981 and again between January and February 1982. In the summer the cows were on pasture, but in the winter they were housed and fed mainly on silage. No cow appeared to have diarrhoea at the time of sampling.

*Cultivation*

*Milk.* Direct and enrichment culture methods were used to screen samples. Milk was streaked on plates of well-dried selective agar consisting of Oxoid Blood Agar Base No. 2 (CM271), containing 5% defibrinated horse blood, 10 mg/l rifampicin, 2500 i.u./l polymyxin B, 5 mg/l trimethoprim and 100 mg/l cycloheximide (antimicrobial agents from Sigma London Chemical Company Ltd). Plates were incubated at 42 °C for 40 h in anaerobic jars from which two-thirds of the air had been removed (500 mmHg below atmospheric pressure) and replaced with a 5% $CO_2$/95% $N_2$ mixture.

In addition, 1–3 ml portions of each sample were added to about 15 ml selective broth in 28 oz McCartney bottles. The broth consisted of thioglycollate broth (Lab M), 5% defibrinated horse blood and the same selective agents as used in the agar with increased concentrations of polymycin B (10000 i.u./l) and trimethoprim (20 mg/l). Bottles were incubated with the caps screwed down in air at 42 °C for 2–4 days and then subcultured to selective agar.

*Faeces.* On the day of collection 10 g of faeces were suspended in 90 ml quarter-strength Ringer's solution and then treated in the same way as milk samples. For the enumeration of campylobacters 0·1 ml amounts of decimal dilutions of each suspension were spread in duplicate on well-dried plates of nutrient agar (Oxoid Nutrient Agar No. 3) containing 3 g/l yeast extract (Difco), 2 g/l potassium L-aspartate, 10 mg/l haematin (both Sigma) and selective agents as described for blood agar cultures. Representative colonies were smeared and stained by Gram's method to confirm identity before counting.

All cultures were checked for growth at 30·5 °C, sensitivity to 40 mg/l nalidixic acid, and the ability to produce $H_2S$ in a medium containing iron, metabisulphite and pyruvate (Skirrow & Benjamin, 1980*a*). The isolates were not further speciated, and should properly be termed *C. jejuni/coli*. For brevity, they will here be termed *C. jejuni*.

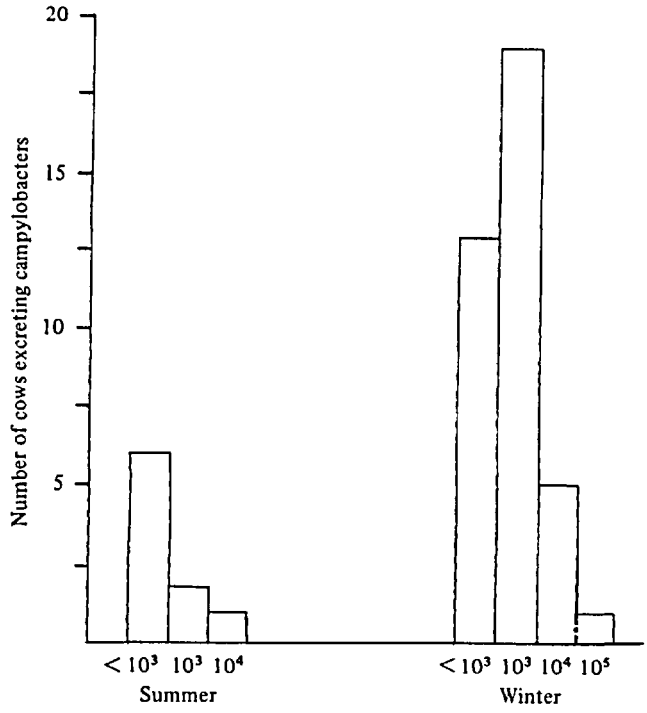


Fig. 1. Excretion of *C. jejuni* by a herd of 74 cows in summer and winter.

## RESULTS

### *Milk*

Campylobacters were not isolated from any of the 1501 samples of foremilk from cows with mastitis. In at least 191 of 872 milk samples, no recognized pathogens such as streptococci, staphylococci or *E. coli* were isolated; the remaining samples were reported by the investigating centre to contain significant bacteria.

As a form of quality control four samples were resubmitted after they had been deliberately inoculated with small numbers of campylobacters by another member of the laboratory without the knowledge of the authors. Three of these samples were positive. *C. jejuni* was also isolated from enrichment broth inoculated with raw milk containing less than 10 *C. jejuni*/ml and from enrichment broth inoculated with milk from cows with artificially produced campylobacter mastitis.

### *Faeces*

Campylobacters were detected in the faeces of 13% (8/74) of the herd during the summer and in 51% (38/74) of the herd in the winter. Campylobacter counts were higher in the winter than in the summer (average $1{\cdot}6 \times 10^4$/g and $6{\cdot}1 \times 10^3$/g respectively; see also Fig. 1). The maximum number of campylobacters detected was $3 \times 10^5$/g faeces. Three of the cows excreting campylobacters in the summer were found not to be excreting them in the winter.

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*Identification*

All 48 campylobacter strains isolated were identified as *C. jejuni/coli* (Skirrow & Benjamin, 1980*a*). Only one strain was resistant to nalidixic acid. This strain, unlike the other strains tested, grew at 30·5 °C, but not at 45·5 °C. All strains tested were sensitive to TTC (Skirrow & Benjamin, 1980*b*). The strain resistant to nalidixic acid was more sensitive to TTC and was the only strain to grow anaerobically with nitrate or fumarate (Razi, Parke & Skirrow, 1981).

## DISCUSSION

The ease with which large numbers of campylobacters have been isolated from cattle faeces contrasts strikingly with the failure to isolate the organism from 1501 samples of milk from mastitic cows. The use of a different detection method, such as that developed by Lovett, Francis & Hunt (1983), may have facilitated the isolation of *C. jejuni* but is unlikely to have altered our conclusion. Although this survey does not exclude the possibility that a campylobacter mastitis occurs naturally, if it does occur it must be uncommon. The high prevalence of *C. jejuni* in cattle in faeces in the winter, compared with the summer, parallels the greater frequency of milk-borne outbreaks during the first four months of the year (Robinson & Jones, 1981). Moreover, all the milk-borne outbreaks of campylobacter enteritis in the U.K. have been caused by *C. jejuni/coli*, the type exclusively isolated from faeces in the survey.

As campylobacters have been found in healthy cows at counts of about $10^5$ per g of faeces (far higher counts may be present in scouring cows) it follows that only a few grams of faeces need contaminate a bulk tank to produce a potentially infective dose in a glass of milk. Human infection can result from the ingestion of a few hundred campylobacters (Robinson, 1981; Black *et al.* 1983). Faecal contamination too small to be detected visually could account for many of the recorded outbreaks of milk-borne campylobacter enteritis.

We conclude that if a naturally occurring campylobacter mastitis exists, it is rare. Thus, contamination of milk with bovine faeces containing campylobacters is the most probable reason for their presence in milk. The practical difficulty of ensuring absence of faecal contamination emphasizes the need to pasteurize all milk for human consumption.

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