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Campylobacter jejuni in dairy cows and raw milk

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SUMMARY

Twelve herds of dairy cows were examined by rectal swabbing for the presence of *Campylobacter jejuni*. Ten herds were positive with the incidence of colonized animals ranging from 10 to 72% of those tested. With the exception of the two negative herds where mains water only was consumed, all animals drank from rivers or streams when grazing. There was no relationship between total and coliform counts and the presence of *C. jejuni* in raw milk. However, milk from one farm that consistently gave positive results had significantly higher *Escherichia coli* counts than other samples.

INTRODUCTION

Numerous outbreaks of enteritis caused by *Campylobacter jejuni* have been associated with the consumption of unpasteurized cows' milk (Potter *et al.* 1984; Sharp, Paterson & Barrett, 1985; Barrett, 1986). This organism can be isolated readily from bovine faeces (Svedhem & Kaijser, 1981; Elegbe, 1983; Manser & Dalziel, 1985) and has been shown to cause bovine mastitis (Hutchinson *et al.* 1985). However, many surveys have found the incidence of contaminated milk samples to be very low (Doyle & Roman, 1982; Oosteram *et al.* 1982; Lovett, Francis & Hunt, 1983; de Boer, Hartog & Borst, 1984; Waterman, Park & Bramley, 1984).

In common with other bacteria, *C. jejuni* can be sub-lethally damaged by exposure to low temperature (Humphrey & Cruickshank, 1983; Humphrey, 1984; Ray & Johnson, 1984a, b; Humphrey & Cruickshank, 1985). This renders the organism both sensitive to certain selective agents commonly used in isolation and enrichment media, and less able to grow at routinely used high (42–43 °C) incubation temperatures (Humphrey, 1986*a*). Thus, investigations that use techniques that do not take account of the above phenomena are likely to underestimate the incidence and numbers of *C. jejuni* in refrigerated milk and food. It is possible, therefore, that some of the previous work may not have truly reflected the potential hazards of unpasteurized cows' milk.

In Devon, UK, over 200 farmers are licensed to sell raw milk. As this represents a potentially serious public health problem, it was decided to try and establish the incidence of *C. jejuni* in both dairy cows and bulk tank milk using techniques

designed to optimize the isolation of *C. jejuni* likely to be sub-lethally damaged (Humphrey, 1986*b*).

MATERIALS AND METHODS

Selective media for the isolation of campylobacter

For the isolation and enumeration of *C. jejuni* from samples of milk, 'EV' broth (Humphrey, 1986*b*) was used as the initial medium. It contained the following per litre; nutrient broth No. 2 (Oxoid), 25 g; lysed blood, 50 ml; sodium pyruvate, 200 mg; sodium metabisulphite, 200 mg; ferrous sulphate, 500 mg; trimethoprim, 10 mg; vancomycin, 10 mg; cefoperazone, 15 mg; amphotericin, 2 mg; colistin, 4 mg. For the examination of faeces or for plating from the above medium, when used in broth form, EX-1 medium (Humphrey, 1986*b*) was used. This differed from EV medium in that rifampicin, 10 mg/l replaced vancomycin.

Campylobacter jejuni *in dairy cows*

Cows in 12 herds were included in the study. The animals were sampled on up to five occasions and, in order to disturb their routine as little as possible, rectal swabs were taken as soon as each one had been milked. The swabs were placed immediately in a universal bottle containing 24 ml of EX-1 broth and stored at ambient temperature for a maximum of 2 h before incubation at 43 °C for 48 h. A loopful of each broth was then streaked on an EX-1 agar plate which was incubated at 43 °C for a further 48 h. Colonies typical of campylobacter were identified using standard laboratory techniques and biotyped using the scheme of Lior (1984).

Examination of milk samples

One hundred and fifty seven samples, each of 500 ml, were examined. They were taken from farm bulk tanks immediately after morning milking. Most were transported to the laboratory in refrigerated, insulated containers and tested within 1 h of collection; the rest were submitted by local Environmental Health Departments and were usually received in the laboratory within 6 h of collection.

Milking hygiene was assessed by performing Total Viable Counts (TVC), using the techniques of Miles & Misra (1938), on 5% blood agar incubated at $30 \pm 0{\cdot}2$ °C for 48 h and *Escherichia coli* and coliform counts, using Violet Red Bile Agar (Oxoid CM 107) pour plates incubated at 44 and 37 °C respectively, for 48 h. In addition, the remaining milk was cultured for *C. jejuni*. The Most Probable Number (MPN) of *C. jejuni* per 100 ml milk in the first group of samples was determined by adding equal volumes of milk to 1×100 ml, 1×50 ml, 5×10 ml and 5×1 ml of double strength 'EV' broth (Humphrey, 1986*b*). Where appropriate, the culture bottles were topped up with single strength medium so that an air space of less than 1 cm remained. The broths were incubated for 2 h at 37 °C followed by 46 h at 43 °C. A sample, 0·2 ml, from each broth was inoculated over the surface of an EX-1 agar plate which was then incubated for 48 h at 43 °C. Suspect colonies were confirmed as described above and the number of bottles positive for *C. jejuni* were used to determine the MPN in the manner recommended for water (DHSS, 1982).

The milk submitted by local authorities, as part of their routine monitoring

programmes, was tested for the presence of *C. jejuni* by filtering through a gauze or cotton wool pad (Sandys, personal communication) under low vacuum. This was placed in a disposable plastic pot which was then filled with 60–70 ml of single strength 'EV' broth. Incubation and culture was then carried out as above. In these samples, examination for campylobacter was confined to presence or absence as they were tested as part of the normal routine work of the laboratory.

Examination of water samples

Natural and mains water supplies to which the cows had access were examined for total coliforms/*E. coli* using Formate Lactose Glutamate medium (DHSS, 1982) and for *C. jejuni* using previously published techniques (Humphrey, 1986*b*).

Influence of low temperature on viability of C. jejuni

Samples of milk from one farm were consistently positive for *C. jejuni*. To establish whether this was due to the presence of organisms with increased cold resistance, the effects of refrigerated storage on viability and antibiotic sensitivity in these and other strains were measured using reported methods (Humphrey & Cruickshank, 1985).

Statistical analysis of data

Differences in the levels of microbial contamination were compared using an unpaired *t* test.

RESULTS

Intestinal carriage of C. jejuni *in dairy cows*

Cows in 10 of the 12 herds sampled were found to be carrying *C. jejuni* in their faeces (Table 1). The incidence of colonized animals ranged from 10–72 % of those examined (Table 1); of 120 cows that were tested more than once, six were positive twice and two positive 3 times. In two herds none of the animals were shown to be carrying the organism despite being tested on five separate occasions. Although the management regimens and feedstuffs used on these two farms were similar to the others in the study, they differed in having mains water as their only supply.

Campylobacter jejuni *in farm water supplies*

With the exception of those on the two farms described above, all the animals had access to rivers and streams when grazing. All such sources tested in this context were positive for *C. jejuni*. During the winter months when the cows were housed, water was supplied from wells, boreholes or the mains. *E. coli* or campylobacter were not detected in any of these supplies.

Indicators of milk hygiene and presence of Campylobacter jejuni

One hundred and eleven milk samples from five farms (SH, S, G, L and PT) with cows positive for *C. jejuni* and 30 from the two negative farms (Table 1) were examined at regular intervals for microbiological indicators of milking hygiene and numbers of *C. jejuni*. Milk from two farms (S and SH) in this group were found to contain *C. jejuni* and in both cases samples were positive on a number of occasions. Data from farm SH are presented in Table 2. Nine of the 111 samples

Table 1. *The incidence of* Campylobacter jejuni *in dairy cows*

Date sampled	Farm	No. of cows tested	No. of cows positive for *C. jejuni*	Percentage positive	Biotype
Oct. 1984	P	44*	0	0	—
Oct. 1984	L	35*	0	0	—
Oct. 1984	SH	61†	6	10	I
Oct. 1984	S	41†	7	17	I
Mar. 1985	PT	64†	17	27	I
Mar. 1985	G	35†	8	23	I
Apr. 1985	MW	152	20	13	I
June 1985	LA	49	9	18	I
June 1985	LE	37	20	54	I
Jan. 1986	GR	50	36	72	I
Mar. 1986	D	50	19	38	I
June 1986	C	50	22	44	I

* Cows from these herds tested on five separate occasions.
† Cows from these herds tested on three separate occasions.
All other cows were tested once.

Table 2. *Examination of a series of bulk tank samples* for indicators of milking hygiene and* C. jejuni

Date	Number per ml of milk of: Coliforms	*E. coli*	Total count	MPN/100 ml *C. jejuni*
30. *x.* 84	6	6	5000	4
31. *x.* 84	28	12	40000	*Nil*
5. *xi.* 84	26	4	1860	*Nil*
14. *xi.* 84	4	1	20000	*Nil*
19. *xi.* 84	614	578	25000	100
21. *xi.* 84	14	6	1660	*Nil*
26. *xi.* 84	2	2	4166	*Nil*
28. *xi.* 84	4	1	4700	*Nil*
3. *xii.* 84	2	2	1250	1
12. *xii.* 84	2	2	500	2
14. *i.* 85	37	28	3030	5

* *Taken from farm SH.*

from the five positive farms contained *C. jejuni*, an incidence of 8·1 %. The mean level of contamination was 16±30 organisms/100 ml milk.

The total, *E. coli* and coliform counts of the samples from the other farms mentioned above did not differ significantly from those from farms S and SH. However, all were negative for *C. jejuni* despite being examined at the same time using identical techniques and media.

When data from the above investigations were analysed no significant differences could be demonstrated in any of the microbiological parameters between *C. jejuni* positive and negative samples or farms. However, the one time when high numbers (100/100 ml) of *C. jejuni* were present was associated with a high *E. coli* count and thus gross faecal contamination (Table 2).

Milk from another farm (MW), where there had been an outbreak of campylo-

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bacter enteritis in people staying at the farm, was examined for the presence of *C. jejuni* over a period of a few weeks using the gauze filtration technique. Eleven (69%) of the 16 samples tested were positive. The mean *E. coli* count was 192·3 ± 326·0/ml, whereas that of the milk from the seven farms described above was 17·6 ± 93·5/ml ($P = 0·0036$). This farm differed from the others in having an automated tandem parlour with automatic udder washing systems. During visits by MAFF staff it was noticed that udders were not dried before milking and it was considered that this had led to the unacceptable levels of faecal contamination in the milk.

Survival of C. jejuni *in raw milk held at refrigeration temperatures*

The various strains of *C. jejuni* isolated from the milk samples from farms S, SH and MW did not differ significantly ($P > 0·1$) in cold resistance. The mean D(1 °C) value of the various strains was 16 ± 2·0 h. It is unlikely that the repeated presence of this organism in samples from farm MW was due to increased cold tolerance.

DISCUSSION

The data on the incidence of intestinal carriage of *C. jejuni* in dairy cows presented in this paper is similar to that in previous studies. It is, however, likely to be an underestimate as most of the animals were tested only once and as many had defaecated before they entered the milking parlour their rectums were almost empty of faeces. Few cows were positive on more than one occasion supporting the view (Robinson, 1982) that excretion of campylobacter is intermittent. The 10 (83%) of 12 herds that had campylobacter positive animals all drank from rivers or streams when grazing, unlike the two negative herds where only mains water was consumed. *C. jejuni* is a frequent contaminant of natural water courses (Knill, Suckling & Pearson, 1981). Its isolation from those rivers and streams tested and its absence from all supplies that had received treatment, whether in the form of natural filtration as with deep wells and boreholes or chlorination as with mains water, is not surprising. The apparent relationship between water supply and campylobacter carriage (Table 1) is, however, of interest and may go a little way towards unravelling the epidemiology of this organism in dairy cattle. All the farms in the study followed approximately the same management and feeding regimens and there were close similarities in the distribution of wild birds and animals. Lactating cows may consume in the order of 45–70 l of water per day and those drinking from the contaminated supplies would also have imbibed considerable numbers of *C. jejuni*. The data presented here suggest that drinking water may be the main source of campylobacter in cows. Unfortunately, the theory could not be tested further as the high rainfall and abundance of natural water supplies in Devon, made it impossible to find more farms with mains water only. It is hoped that the results presented here will stimulate further work on this subject.

Two points of interest arose from the above data. All the strains of *C. jejuni* isolated from the cows were biotype I. Also, that some cows were still positive for this organism, even during the winter when they were on mains water, suggests that colonization persisted from the summer or autumn.

The high incidence of milk samples contaminated with *C. jejuni* compared with

previous studies may be due to sampling bias because the work, in the main, was concentrated on herds in which there were carrier animals. It is also possible that the use of a technique specifically designed to allow the enumeration of cold-injured *C. jejuni* served to reduce the number of false negative results. However, as our data indicate that most herds are likely to be positive, the results presented here may be a truer reflection of the potential hazard of unpasteurized milk. Other studies on this topic have reported either presence or absence of campylobacter only. This paper is the first to attempt to measure the degree of risk to consumers of raw milk by measuring numbers of *C. jejuni*. Generally the degrees of contamination were low but on one occasion, when a milking cluster dropped onto the parlour floor and became badly soiled, the numbers of *C. jejuni* reached 100/100 ml milk, a number close to an infective dose for this organism (Robinson, 1981).

By applying good hygiene during milking it is possible to produce milk which has both low total counts and an absence of intestinal pathogens. Despite this, many milk-borne campylobacter outbreaks occur each year and we have observed that even on the best run farms accidents happen which can result in potentially dangerous levels of contamination. Dairy herd sizes continue to increase and it will become more and more difficult for dairy farmers to produce milk consistently free from faecal contamination, the principal source of campylobacter and salmonella in raw milk.

It is the duty of the regulatory authorities to ensure that milk which is to be sold unpasteurized is safe. Microbiological tests can play an important part in this, but it is vital that they are designed to detect potential and real hazards. Neither the methylene blue test nor the total viable count, which are currently being used in the United Kingdom, are particularly useful in this respect. The ratio of the numbers of *E. coli* to those of campylobacter in cows' faeces is always likely to be high. One would expect, therefore, many more of the former in milk contaminated with faeces. Campylobacter was isolated repeatedly from the milk from farm MW and the mean *E. coli* count was significantly higher ($P = 0{\cdot}0036$) than that of milk from other producers. This observation, coupled with the fact that the one sample to yield high numbers of campylobacter had an *E. coli* count of 578/ml, lends support to the view put forward by Humphrey & Hart (1986) that an *E. coli* count should be included in the routine monitoring of raw milk.

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