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The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves

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K.N. STANLEY, J.S. WALLACE, J.E. CURRIE, P.J. DIGGLE AND K. JONES. 1998. The epidemiology of clinical cases of campylobacter in temperate climates shows a striking seasonality. In the search for a seasonal environmental reservoir changes in the carriage rate and population size of campylobacters in bovine hosts with time have been measured. Most probable number (MPN) methodology was used to enumerate thermophilic campylobacters in samples taken from the small intestines of beef cattle at slaughter and the fresh faeces of four dairy herds and new-born calves. Statistical analyses revealed significant evidence for seasonal periodicity in the data from dairy herds ($P = 0{\cdot}044$). Not only was there a departure from constancy within a 12-month interval but these data revealed a true seasonality, that is, the same periodicity in numbers from one year to the next. Each herd had two peaks per year, in approximately spring and autumn. Peaks coincided in herds on neighbouring farms but those on farms in the north preceded those on farms in the south by 2 and 1 months, respectively ($P = 0{\cdot}0057$). Intestinal carriage by beef cattle at slaughter was 89·4% ($n = 360$) with an average MPN campylobacters per gram fresh weight (MPN gfw^{-1}) of $6{\cdot}1 \times 10^2$. Average MPN gfw^{-1} in faeces from the dairy herds and calves were 69·9 (S.D. 3) and $3{\cdot}3 \times 10^4$ (S.D. $1{\cdot}7 \times 10^2$). There was no evidence of seasonal periodicity in the size of the campylobacter population in beef cattle at slaughter. Calves were campylobacter free at birth but became colonized within a few days.

INTRODUCTION

Campylobacter jejuni and related thermophilic species are important zoonoses causing the highest frequency of diarrhoea in the UK (Skirrow 1991) and other industrialized countries (Tauxe 1992). The major environmental reservoirs of thermophilic campylobacters are the intestines of birds and warm-blooded mammals, where it is thought they are commensal with the gut flora rather than pathogenic, at least in older animals (Park *et al.* 1991). They are readily isolated from the faeces of domestic farm animals: beef cattle (Garcia *et al.* 1985) and dairy cows (Humphrey and Beckett 1987), sheep (Grau 1991), pigs and poultry (Stern 1992; Wallace *et al.* 1997). The growth of *C. jejuni* is restricted by its microaerophilic nature and inability to grow outside the temperature range 32–44 °C and it is difficult to envisage where these requirements would be met in the environment. Therefore, the intestines of host animals are a critical site of amplification in the contamination cycle of this organism.

In temperate climates the disease in humans has a striking seasonality. In the UK the number of clinical infections peaks sharply in the early summer months of May and June with a lower secondary peak in the autumn (Skirrow 1991). Summer peaks also occur in the USA (Tauxe 1992) and a number of other European countries including Italy (Stampi *et al.* 1992) and Spain (De Matteo 1997), although in Northern Europe the peak occurs in mid–late summer (Walder and Forsgren 1982; Kaijser and Svedhem 1982). Spring and summer peaks have also been reported for countries with temperate climates in the southern hemisphere, including New Zealand (Brieseman 1985, 1990), Australia (Grau 1991) and South Africa

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(Franco 1988). Unlike the summer peak in clinical cases of salmonella, the late spring and summer peaks of campylobacter infections cannot be attributed to temperature abuse of contaminated food products.

Although common source outbreaks of campylobacter are rare they also show a marked seasonality, with contaminated water and milk the most commonly implicated vehicles or risk factors. Such outbreaks have a bimodal distribution with peaks in May and October (Tauxe 1992). Epidemiological data suggest that the vehicles and circumstances of sporadic cases are quite different from those of outbreaks. Sporadic cases, which account for the majority of laboratory reported cases, are believed to arise largely from the handling and consumption of poultry. Tauxe (1992) has suggested that the seasonality of human infections may reflect important differences in the ecology of the poultry and bovine reservoirs of campylobacters.

In the search for seasonal reservoirs to explain the late spring/summer peak in clinical infections, several studies carried out in the UK and Northern Europe have found evidence of late spring or summer peaks in the carriage rates (Kaperrud *et al.* 1993; Jacobs-Reitsma *et al.* 1994; Wallace *et al.* 1997) and numbers of thermophilic campylobacters in the intestines and caeca (Wallace *et al.* 1997) in commercial poultry flocks. There are few reports in the literature concerning the seasonality of the bovine reservoir of campylobacters over extended intervals. Jones *et al.* (1990) showed that samples from sewers in the Lancaster area draining a local abattoir yielded much high numbers of thermophilic campylobacters than sewers draining residential areas and also showed high numbers coincident with the early summer peak, suggesting that wastes from domestic livestock also constitute a similar significant seasonal environmental reservoir.

The significance of campylobacter colonization of cattle relates not only to the potential for contamination of milk at the farm and the carcass at slaughter, but also environmental and water contamination during disposal of abattoir effluents and slurries to land. The prevalence of campylobacters among cattle herds is commonly measured by examining the rate of carriage but this provides little insight into the variation in the risk of contamination when the size of the campylobacter population changes. In an attempt to investigate the seasonal variation in bovine hosts further, the number of thermophilic campylobacters in the small intestines of beef cattle at slaughter and the fresh faeces of four dairy herds has been measured using the most probable number (MPN) method. In order to examine both the periodicity (the departure from constancy within a 12-month interval) and the true seasonality (the same periodicity in numbers from one year to the next), monthly samples were taken over a 2-year period. Counts were also made along the intestinal tract of slaughtered cattle to determine the principal sites of colonization and amplification. Fresh faecal samples were taken from three batches of new calves born to one of the dairy herds in order to assess the age and rate at which campylobacter colonization occurs among young calves reared for beef.

MATERIALS AND METHODS

Sampling

Beef cattle at slaughter were sampled for enumeration twice per month in 1993 and once per month in 1994. The carriage rate was assessed once per month in 1994. The dairy herds were sampled once per month during 1993 and 1994 and calves were sampled once or twice per month between June 1994 and July 1995.

Beef cattle at slaughter. A large abattoir in Preston (Lancashire, UK) received daily between 70 and 130 beef cattle from markets and farms from all over the northwest of England, north Wales and southwest Scotland. On each sample date, samples were taken from 30 animals chosen at random. Immediately after evisceration, approximately 40 ml of pre-faecal material was collected from the lumen of the small intestine from which subsamples were taken with sterile cotton swabs in order to determine the number of animals carrying campylobacters (carriage rate). They were transported in 0·1% buffered peptone water. The samples of pre-faecal material from random groups of 10 animals were amalgamated, homogenized and transported to the laboratory in sterile glass bottles in an insulated cool box for enumeration. Laboratory analysis was carried out within 2 h.

Location of campylobacters along the digestive tract. The prevalence of campylobacters along the intestinal tract was estimated from the intestines of 10 individual cattle at slaughter taken on two sampling occasions in February and May. Samples of the gut contents were taken from the rumen, true stomach and the lumen of the small intestine, large intestine and caeca and each sample was enumerated individually.

Enumeration of thermophilic campylobacters in the fresh faeces of four Lancashire dairy herds. Four average-sized Holstein Friesian dairy herds were sampled. Farms 1 and 2 were situated approximately 20 miles north of Lancaster at the mouth of the Kent estuary, Morecambe Bay, within 3 miles of each other. Farms 3 and 4 were situated approximately 7 miles southeast of Lancaster, 5 miles inland and within 1 mile of each other. The dairy herds were gathered in the collecting yard before morning milking where freshly voided faeces from the whole herd were mechanically homo-

genized by the farmer. Samples of 100 ml were collected for enumeration.

New-born calves. Surplus calves born to the dairy herd on farm 4 between May 1994 and June 1995 were reared in four batches. Monthly samples of fresh calf faeces were taken from the calf-rearing pens.

Media and growth conditions

Campylobacters were enriched in Preston selective enrichment broth (Bolton *et al.* 1982a) containing Nutrient broth No.2 (lab M), 5% lysed horse blood (TSC Biologicals), campylobacter growth supplement (SR084E; Oxoid) and Preston campylobacter selective supplement (SR117E; Oxoid). Bijou bottles were filled to leave a minimal headspace to prevent anaerobiosis. Enrichment cultures were incubated initially at 37 °C for 4 h, to allow resuscitation of injured cells (Humphrey and Muscat 1989), and then at 42 °C for a further 44 h, before inoculation onto campylobacter blood-free selective agar (m-CCDA-Preston; Oxoid) containing Preston selective supplements amphotericin B (10 mg l^{-1}) and cefoperazone (32 mg l^{-1}) (Prolab Diagnostics, Neston, Cheshire, UK). Plates were incubated at 42 °C for 48 h in anaerobic gas canisters (Don Whitley Scientific, Leeds, UK). Microaerobic conditions were created by partial evacuation of canisters to 20 mmHg and refilling with a mixture of tank gases containing 10% CO_2, 10% H_2 and 80% N_2.

Carriage rate. The carriage rate was determined by inoculating the swabbed sample onto m-CCDA and then by enrichment of the swab in Preston broth. The presence of thermophilic campylobacters was confirmed by colony formation, positive oxidase reaction, Gram stain with carbol fuchsin counterstain and cell morphology.

Enumeration of thermophilic campylobacters. All amalgamated samples of pre-faecal material were enumerated by the MPN method as described by Wallace *et al.* (1997).

Statistical analysis

Triplicate MPN measurements were recorded every month for a period of 2 years. These measurements were transformed to ln (1 + MPN) to stabilize the variance and then averages of triplicates were taken. The resulting transformed MPN estimates were then assumed to be independent from one occasion to the next. To assess possible association between the transformed MPN and environmental variables linear models consistent with the analysis described by Wallace *et al.* (1997) were used. The environmental variables considered were minimum temperature, maximum temperature, average rainfall and number of sunshine hours recorded, in each case calculated over the month preceding each sampling occasion (data collected from Hazelrigg site, Lancaster University, UK). Seasonality was also investigated by fitting a superposition of sine and cosine functions with annual and 6-monthly periods. This model is used not because the data are believed to exhibit 6-monthly cycles but because the resulting curves that are achievable with this method are more flexible than if only sines and cosines with annual periods are used. Differences in cycles between the four geographical locations of the dairy herds were assessed by allowing the regression parameters to vary between locations.

RESULTS

Carriage rate and MPN for beef cattle at slaughter and MPN from dairy herds

Thermophilic campylobacters were isolated from 89·4% ($n = 360$) of beef cattle at slaughter using a combination of direct and enrichment methods (Table 1). When measured by the enrichment method, carriage rates were always higher than by direct isolation on agar. During each month in 1994 between 97 and 100% of animals were found to be carrying thermophilic campylobacters, except in January (80%), April (53%), September (70%) and November (80%) (Table 2).

The average MPN of thermophilic campylobacters per gram fresh weight (MPN gfw^{-1}) of sample from the small intestine of beef cattle at slaughter was $6{\cdot}1 \times 10^2$ (S.E. 0·12) over the sampling period (Table 1). Samples taken in June 1994 yielded the highest average MPN gfw^{-1} ($3{\cdot}6 \times 10^5$, S.D. $1{\cdot}4 \times 10^2$) while the minimum average MPN gfw^{-1} in 1994 was 69 (S.D. 3) in February. In 1993 the maximum and minimum MPN gfw^{-1} of thermophilic campylobacters were found in August ($4{\cdot}5 \times 10^3$, S.D. 15) and September (53, S.D. 11), respectively. However, the MPN gfw^{-1} from amalgamated groups of 10 animals, before averaging of data, ranged between 0 in October 1993 and $2{\cdot}4 \times 10^7$ in June 1994. The average MPN gfw^{-1} of thermophilic campylobacters in fresh dairy faeces was 69, nearly 10 times lower than the average from intestinal samples from beef cattle at slaughter (Table 1).

When samples from the whole length of the intestinal tract of a number of individual animals at slaughter were examined, campylobacters were isolated from 30% ($n = 10$) of rumen samples and 60% ($n = 30$) of small intestine samples but not from the true stomach, large intestine or caeca (Table 3). The average MPN of campylobacters found in the rumen was almost 10 times lower than the number found in the small intestine. These results support the choice of the small intestine as the sample site for the seasonal enumerations.

Table 1 Overall carriage rate, average most probable number (MPN) values and *P* values for analysis of seasonal variation of thermophilic campylobacters in beef cattle at slaughter, dairy herd and calves

	Carriage rate (%) (*n*)	Average MPN g^{-1} fresh weight (S.E., *n*)	*P* value*
Beef cattle at slaughter	89·4 (360)	$6{\cdot}1 \times 10^2$ (2, 1080)	0·19
Dairy herd	ND	69 (2, 1080)	0·044
Calves	ND	$3{\cdot}3 \times 10^4$ (180, 32)	ND

**P* value from the F distribution comparing the models including sine and cosine functions with annual and 6-monthly periods with the null model excluding these terms. ND, Not done.

Table 2 Monthly percentage carriage rates of thermophilic campylobacters in beef cattle at slaughter in 1994

Month (1994)	Carriage rate (%) ($n = 30$ $month^{-1}$)
January	80
February	97
March	97
April	53
May	100
June	100
July	97
August	100
September	70
October	100
November	80
December	100

Table 3 Percentage isolation rate and most probable number (MPN) of thermophilic campylobacters at different sites in the intestinal tract of 10 individual beef cattle at slaughter

Sample site	Isolation rate (%)	Average MPN g^{-1} fresh weight (S.D.)
Rumen	30	1·7 (2·4)
True stomach	0	<0
Small intestine	60*	17 (14)*
Large intestine	0	<0
Caeca	0	<0

* $n = 30$.

Seasonal variation of thermophilic campylobacters in beef cattle and in the fresh faeces of dairy herds. Statistical analysis of the monthly MPN data (*P*-value from the F-distribution comparing models with sine and cosine functions with annual and 6-monthly periods with the null model excluding these terms) suggested that there was no significant seasonal periodicity in the number of thermophilic campylobacters in the small intestines of beef cattle at slaughter ($P = 0{\cdot}19$). Figure 1 shows the transformed data (average ln (1 + MPN)) and the fitted model. However, there is significant evidence ($P = 0{\cdot}044$) for seasonal periodicity in the data from the dairy herds. There was significant disparity in seasonal patterns between the four farms ($P = 0{\cdot}039$) arising from differences between northerly and southerly farms ($P = 0{\cdot}006$). Once this had been accounted for there was no residual difference between seasonal patterns ($P = 0{\cdot}449$). Fitted models for the northerly (Fig. 2a) and southerly farms (Fig. 2b) suggest that the number of thermophilic campylobacters peaks twice per year in each herd. These peaks roughly occur in spring and autumn but the peaks of farms 1 and 2 precede those of farms 3 and 4 by approximately 2 months in spring and by 1 month in autumn.

Relationship with environmental parameters. There is no evidence at the 5% significance level of any relationship between the MPN data from the beef cattle at slaughter or from the dairy herds and environmental parameters such as maximum and minimum temperature, rainfall or number of hours of sunshine. Table 4 shows the *P*-values from the F-distribution from testing alternative models including environmental explanatory variables against the null model.

Calves. The average numbers of thermophilic campylobacters in fresh calf faeces were nearly 100 times higher than the number in intestinal samples from beef cattle at slaughter (Table 1). During the period between May 1994 and July 1995 three different batches of calves were reared on farm 4. All calves were free of campylobacters at birth but most began shedding from the age of 4 d. High numbers could be found in faecal samples by 1–2 months of age (Fig. 3). Calves born in May and June of both 1994 and 1995 began shedding very high numbers of campylobacters by 1 month of age.

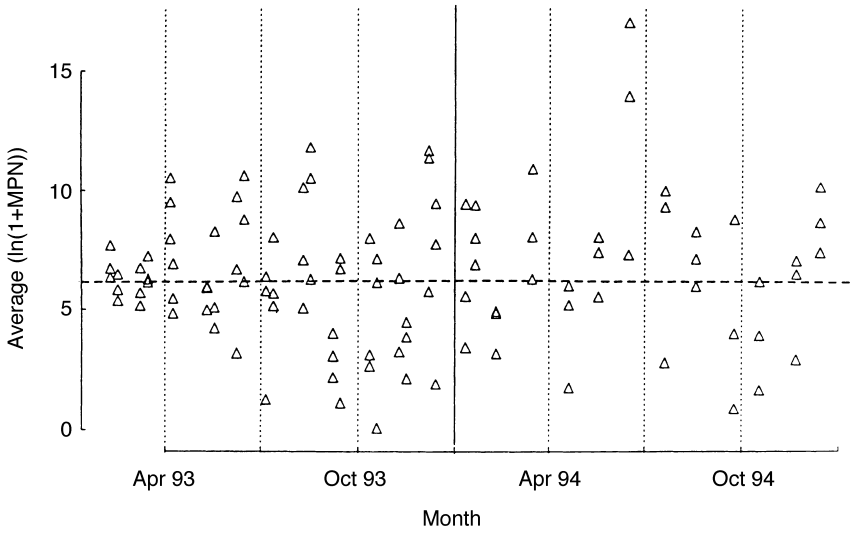

Fig. 1 Transformed most probable number (MPN) (average ln (1 + MPN)) of thermophilic campylobacters in the small intestines of beef cattle at slaughter (△); fitted model (---)

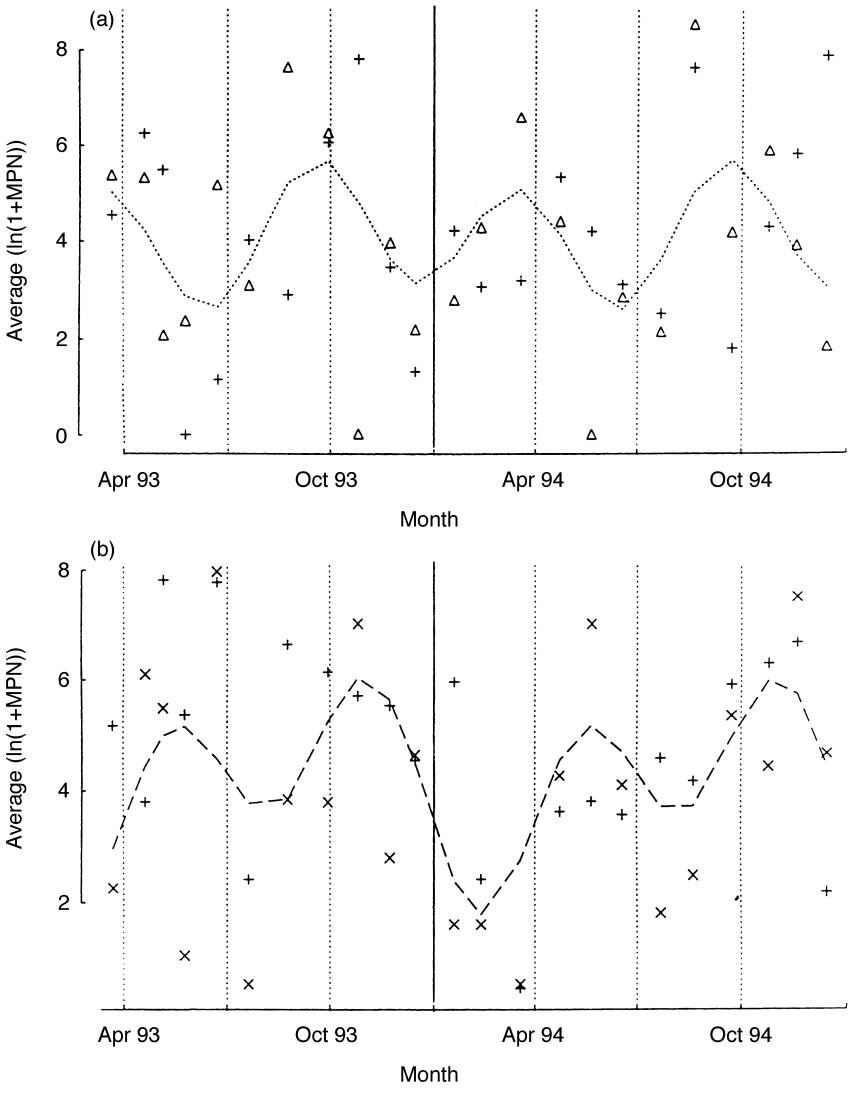

Fig. 2 (a) Transformed most probable number (MPN) (average ln (1 + MPN)) of thermophilic campylobacters in fresh faeces of dairy herds on northerly farms. Farm 1 (△); farm 2 (+); fitted model (···). (b) Transformed MPN (average ln (1 + MPN)) of thermophilic campylobacters in fresh faeces of dairy herds on southerly farms. Farm 3 (+); farm 4 (×) and fitted model (---)

Table 4 Regression of most probable number of thermophilic campylobacters in beef cattle and dairy herds on environmental parameters

	P values*	
Environmental variable	Beef cattle	Dairy herds
Maximum temperature	0·4501	0·3316
Minimum temperature	0·2579	0·283
Rainfall	0·1913	0·8182
Sun hours	0·6061	0·9265

P values from the F-distribution from testing for alternative models including an environmental explanatory variable against the null model.

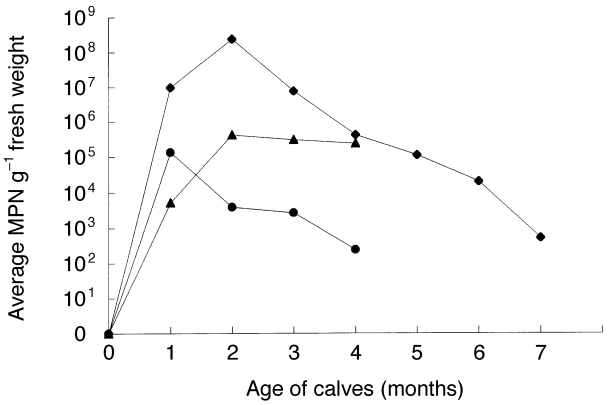

Fig. 3 Average most probable number (MPN) of thermophilic campylobacters in calf faeces. Calves born in June (◆), December (●) and March (▲)

Individual calves born in June and early July 1994 yielded more than $2{\cdot}4 \times 10^8$ MPN campylobacters gfw^{-1} of faeces before they were 1 month old but the number declined thereafter. Calves born in winter months, between December and March, also acquired campylobacters within a few days of birth but peak numbers were lower than in the summer. The numbers of campylobacters shed in the faeces of calves born during December peaked at 1 month with a maximum number of $2{\cdot}4 \times 10^5$ MPN gfw^{-1}. Calves born in late February and March, negative for campylobacters when first sampled at 4 d, shed peak numbers of $4{\cdot}8 \times 10^5$ MPN gfw^{-1} campylobacters when they were 2 months old.

DISCUSSION

The present study has revealed a significant seasonal variation in the numbers of thermophilic campylobacters in the fresh faeces of dairy herds. Not only is there a departure from constancy within a 12-month interval, but these data revealed true seasonality, that is, the same periodicity in numbers from one year to the next. Although these data do not epidemiologically link the seasonal variation of campylobacters in dairy cattle with the late spring peak in clinical cases in the UK, the patterns roughly match those of the bimodal distribution of outbreaks in the USA commonly caused by milk and water observed by Tauxe (1992), and the spring peak on farms 3 and 4 roughly coincide with the peak in general outbreaks and clinical cases reported through the Communicable Disease Surveillance Centre in the UK (Pebody *et al.* 1997). In contrast, seasonality was not revealed in the number of campylobacters in the small intestines of beef cattle at slaughter.

Robinson (1982) concluded that faecal shedding of campylobacters within dairy herds is intermittent. The data presented in this study further suggest that faecal shedding shows a seasonal periodicity with peaks in spring and autumn. It is not clear whether periodicity in the campylobacter populations is due to recrudescence, that is fluctuations in population levels of indigenous campylobacters, or indicates seasonal re-infection. The two peaks in the data from dairy cow faeces roughly correlate with traditional milk flushes and periods of calving which suggest that reproductive hormones and/or stress may exert a seasonal regulatory effect, particularly as these peaks are absent in those animals reared for beef. However, UK farmers (including those involved in this study) are nowadays encouraged to calf their dairy herds all year round to meet the constant consumer demand for milk and dairy products. The peaks roughly coincide with the spring transition from winter housing to summer grazing and the autumn return to winter housing, and may reflect a change in diet or water source.

Other studies have reported seasonal periodicity in carriage rates within dairy herds in temperate areas of both the northern and southern hemispheres. During a longitudinal study of two dairy herds in the northwest of England, *C. jejuni* was isolated from approximately 10% of each herd during the summer and from neither during the winter, but it re-emerged during spring (Robinson 1982). In New Zealand, Meanger and Marshall (1988) measured the isolation rate during three seasons and found high isolation rates in both summer (24%) and autumn (31%) but low rates in winter (12%). The authors suggested that climatic conditions in autumn closely matched those of an English summer and implied that these observations were directly related to climate. The consistency of the peaks in our data over 2 years further implies that there is a temporal regulatory factor.

Using regression analysis, however, we found no evidence to suggest that the seasonal peaks in dairy herds are directly related to minimum or maximum air temperatures, hours of sunshine or rainfall. The similarity in the data obtained from

farms situated adjacent to one another yet varying from farms situated only 20 miles away suggests that there may be sources of thermophilic campylobacters more local to farms which are responsible for the seasonal variation. Similar analysis of poultry data showed a correlation with minimum temperature and a negative correlation with maximum temperature and sunlight hours (Wallace *et al.* 1997) and direct factors, such as elevated temperatures and high humidity, have previously been correlated with increase in carriage (Doyle 1984) and colonization (Willis *et al.* 1991) in broiler chickens. In contrast, the seasonal variation of thermophilic campylobacters in dairy cattle could not be explained directly by the environmental variables tested and, as suggested by Jacobs-Reitsma *et al.* (1994), indirect temperature-dependent factors, for example migratory birds, rodents or insects, may be important.

The average numbers of thermophilic campylobacters in slaughter cattle are similar to those previously observed by Grau (1988), who found an average of 2·6 $\log_{10}$ cfu *C. jejuni* g^{-1} in the intestines of adult cattle. However, in the present study, a number of amalgamated samples yielded greater than 10^7 MPN campylobacters gfw^{-1} of sample. Given the very low infectious dose of around 10–100 *C. jejuni* cells (Robinson 1981) this represents a considerable risk of infection if the meat should become contaminated with the visceral contents during the slaughter process. Stern *et al.* (1985) observed that the highest incidence of contamination of retail red meat was during June and September (8·5%) and lowest in December (4%). Molecular subtyping of a number of strains of *C. jejuni* isolated from beef and dairy cattle during this study has shown that they share identical 16S ribotypes and pulse field gel electrophoresis groupings to clinical isolates from the Lancaster area (Owen *et al.* 1995). Therefore these strains can be considered truly zoonotic and their presence in the food chain represents a risk to public health.

European legislation requires that all animals going to slaughter are apparently healthy (Berends *et al.* 1993). The seasonal peaks in the dairy herds and high numbers of campylobacters observed in slaughter animals and calves could not be accounted for by apparent clinical disease. It is a widely held view that campylobacters are commensal, apathogenic colonizers of the intestinal tract of adult ruminants (Stern 1992) and these studies support that view. The presence of campylobacters in the rumen of individual animals at slaughter suggests recent ingestion (Grau 1991). It is unlikely that campylobacters are able to grow in the rumen, but it has been suggested that an undeveloped rumen may increase the ease of infection of the lower intestinal tract in younger animals (Grau 1991).

New-born calves, which were initially free of campylobacters, rapidly acquired infection from the farm environment via horizontal transmission. The average numbers of campylobacters in calf faecal samples on farm 4 were approximately 100 times higher than those found at the abattoir in visceral samples of beef cattle which are, on average, 18 months old by the time they come to slaughter. In contrast with dairy farming, it is common practice in the rearing of beef cattle for farmers to simply throw down fresh straw on top of old litter to soak up liquid waste. Calves have physical contact with their bedding at all times and it is unlikely that they could avoid re-ingestion of material of faecal origin either via their own contaminated hides or from food or water troughs. It is likely that animals housed in this way are constantly re-infected from their own litter as well as from other sources and this may be reflected in the high carriage rates of the beef cattle at slaughter.

The consistently high frequency of carriage observed in the viscera of cattle presented for slaughter during the 12-month period between January 1994 and December 1994 vastly exceeds that observed in most previous studies. Over the last two decades a wide variation in carriage rates in adult cattle has been found, 0·8% in Norway (Rosef *et al.* 1983) and 21% and 23·5% in the UK (Bolton *et al.* 1982b; Manzer and Dalziel 1985). More recent studies have reported rates of 19·5% carriage in adult cattle in Portugal (Cabrita *et al.* 1992) and 46·7% in Japan (Giacoboni *et al.* 1993). A number of factors may have contributed to these elevated results, including a combination of direct and enrichment isolation methods, the strict use of fresh agar and choice of sample site.

The sampling procedure was designed to include a number of variables on each date, such as geography, herd size and type, age of animal and husbandry practices, some of which have been suggested to account for significant differences in isolation rates (Stern 1981). For example, a higher incidence of *C. jejuni* has been observed in cattle raised in feedlots compared with cattle raised on pasture (Garcia *et al.* 1985; Grau 1988). Garcia *et al.* (1985) concluded that statistically the gall bladder, large intestine and small intestine are equally suitable sampling sites for recovering campylobacters from slaughter cattle. The gall bladder was not sampled during this study, but the small intestine was found to be a superior sampling site to the large intestine in terms of both isolation rate and population size. Other workers have sampled faeces or rectal contents and, while samples taken from faeces or the rectum give an indication of recent shedding (Humphrey and Beckett 1987), they may underestimate the true rate of infection. Our choice of the small intestine as the sampling site may account for the large difference between carriage rates observed in this study and those reported by previous workers. The most recent studies in sheep (Stanley *et al.* 1998) and poultry (Wallace *et al.* 1997) have also revealed higher carriage rates than previous studies and, when considered alongside the increasing trend in clinical infections, may reflect an increasingly endemic problem in the livestock in the UK.

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