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Temporal and farm-management-associated variation in the faecal-pat prevalence of *Campylobacter jejuni* in ruminants

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SUMMARY

In a 2-year longitudinal study of adult animals on 15 dairy farms and four sheep farms in Lancashire, UK. *C. jejuni* was isolated from all farms, although not on every occasion. Faecal samples were collected and cultured using standard techniques for isolation of *Campylobacter*. Assignment to species was via PCR assays. Peak prevalence of *C. jejuni* in both cattle and sheep was observed during the summer and in cattle this apparent seasonality was associated with grazing pasture [odds ratio (OR) 2·14], while in sheep it was independent of grazing. Increased prevalence was associated with increased milk yield (OR 1·05) and herd size (OR 1·01) in dairy cattle, and with increased stocking density (OR 1·29) and pasture quality (OR 2·16) in sheep. There was considerable variation in prevalence between farms but no evidence of large-scale spatial variation. The association between *C. jejuni* prevalence and diet in dairy cattle deserves further investigation.

Key words: *Campylobacter*, cow, epidemiology, sheep.

INTRODUCTION

Campylobacter spp. are the foremost bacterial cause of gastroenteritis in the UK, with the estimated incidence being around 300 000 cases per annum [1]. While poultry products are well recognized sources of human infection, there is increasing evidence that ruminants also play a role [2, 3]. Routes of ruminant-derived human infection include consumption of raw milk [4] and contamination of water sources [5, 6], although the precise infection routes remain to be elucidated in most cases. Studies from the UK [7] and New Zealand [8] have suggested acquisition of infection via environmental exposures or contact with animals or their faeces may be important in rural settings, rather than just food sources. Seasonal trends in human cases are well recognized with peak cases occurring in the spring and/or summer months [9, 10]. Both environmental temperature [7] and the ecology of animal reservoirs of *Campylobacter* [11] have been suggested as seasonal drivers. Seasonality in thermophilic campylobacter excretion by dairy cattle has been demonstrated with peaks in the spring and autumn [12].

Campylobacter jejuni is a well recognized commensal of the gastrointestinal tract of ruminants worldwide [13–17]. A study based on sampling of freshly voided cattle faecal samples in the Wirral, Merseyside,

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UK reported a *C. jejuni* prevalence of 32·4% [18] in broad agreement with a study involving intensive environmental sampling of a 100 km² area of Cheshire which reported a bovine *C. jejuni* prevalence of 36% [19]. A number of studies [20, 21] have shown that the faecal prevalence of *Campylobacter* spp. is higher in young animals and larger numbers of campylobacters are excreted per gram of faeces by young animals compared to adults.

There is scant information regarding *Campylobacter* spp. in sheep although one study [22] investigated thermophilic *Campylobacter* spp. in sheep in Lancashire, UK and estimated a faecal-pat prevalence of 30%. No seasonal or grazing-associated variation in prevalence was observed, although at slaughter peak numbers of campylobacters were isolated in the spring.

The aim of the current study was to identify any temporal trends or farm-management practices associated with *C. jejuni* faecal-pat prevalence. Adult dairy cattle and sheep were sampled since these management groups represent the probable biggest contributors to the environmental burden of *C. jejuni* in rural Lancashire.

MATERIALS AND METHODS

The study design was a repeated cross-sectional study over a 2-year period starting in January 2006. Fourteen dairy and four sheep farms were recruited with the help of three spatially separated veterinary practices in Lancashire serving Southern Fylde (zone 1), North Lancashire (zone 2) and South East Lancashire (zone 3). Six dairy farms were recruited in zone 1 while four dairy and two sheep farms were recruited in each of the other zones. One farm in zone 2 ceased trading in December 2006 and was replaced with a neighbouring farm for the remainder of the study. Another farm in zone 2 ceased keeping cattle in June 2007, thus sampling on this farm was incomplete. Eligibility criteria for entry to the study were: dairy farms with >100 adult cows with or without a sheep enterprise; sheep farms with >150 breeding ewes and no other livestock enterprises.

Farms were visited at 8-week intervals when 20 freshly voided faecal samples were collected from the lactating cows on dairy farms or adult sheep on sheep farms. Samples were only collected from animals observed to defecate by the author. Each faecal pat was sampled from at least three sites within the pat and mixed thoroughly in a sterile sample pot. In the case of sheep faecal pellets, at least three were collected. Samples were transported to the laboratory on ice.

In the case of dairy cows, faecal consistency was scored using a score from 1 to 5 [23] and faecal fibre length and presence of partially digested grains assessed by sieving [24]. At each visit, current management and production details were obtained via a short questionnaire delivered by the investigator.

In the laboratory, 1 g faeces was placed in 9 ml *Campylobacter* enrichment broth (IDG Ltd, UK) with cefoperazone, vancomycin, trimethoprim and cycloheximide (CVTC supplement; IDG Ltd) and after homogenizing for 30 s in a Colworth 80 stomacher (A. J. Seward & Co. Ltd, UK) was incubated in a plastic universal bottle for 24 h at 37 °C in a variable atmosphere incubator (VAIN, Don Whitley Scientific Ltd, UK) maintaining a microaerobic atmosphere (12% CO_2, 3% H_2, 11% O_2, 74% N_2). After incubation, 50 μl of the enrichment broth was inoculated onto a *Campylobacter* blood-free selective agar (CSA) plate (IDG Ltd) enriched with cefoperazone and amphotericin (CA supplement; IDG Ltd). A second CSA plate was inoculated with a 5-μl loopful of enrichment broth. The CSA plates were incubated at 37 °C in a microaerobic atmosphere for 60–72 h after which plates were examined and up to four putative *Campylobacter* colonies (per faecal sample) were subcultured onto blood agar plates and incubated at 37 °C under microaerobic conditions as described previously. After 72 h incubation, single colonies were subcultured onto two blood agar plates. One plate was incubated for 48 h under microaerobic conditions and the other plate incubated for 48 h at 30 °C in air.

A crude DNA aqueous lysate was prepared by inoculating 200 μl distilled water with a small amount of the culture, heating at 100 °C for 15 min followed by centrifugation at 11 ***g*** for 10 min. All putative *Campylobacter* isolates were frozen in Microbank tubes (Pro-Lab Diagnostics, UK) and stored at −80 °C.

Assignment to species of putative campylobacters was by PCR using the following assays: 16S rRNA PCR for identification of the genus *Arcobacter* [25]; multiplex PCR for identification of *C. jejuni*, *C. coli* and *C. lari* [26]; duplex PCR for identification of *C. fetus* and *C. hyointestinalis* [27] and a monoplex PCR [28] for identification of any *C. jejuni* that failed to be identified by the colony multiplex PCR.

Data analysis was performed using Stata version 10 (StataCorp, USA). Covariates recorded at sampling

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Table 1. *Description of variables collected at sampling visits for initial inclusion in statistical analyses*

Variables	Species	Type	Description and coding of variable
Farm identity	Cattle/sheep	Categorical	
Purchase policy	Cattle	Categorical	0 = no purchased stock (closed herd) 1 = occasional purchase of cows 2 = frequent purchase
Group size	Cattle/sheep	Continuous	Number of animals in sampled group
Where sampled	Cattle/sheep	Categorical	Inside = 0 or Outside = 1
Zone	Cattle/sheep	Categorical	1 = Southern Fylde 2 = North Lancashire 3 = South East Lancashire
Date of sampling	Cattle/sheep	dd/mm/yy	
Average daily milk yield	Cattle	Continuous	Average daily milk yield (litres) on sampling day
Feeding system	Cattle	Categorical	Feeding system used as follows: 1 = TMR (total mixed ration) 2 = Hybrid TMR – TMR and parlour feed 3 = grazing and buffer feed and parlour feed 4 = grazing and parlour feed 5 = silage and parlour feed
Number of fresh cows	Cattle	Continuous	Number of cows calved within last month
Faecal score		Categorical	Score of 1–5 depending on consistency with score of 1 being very firm and score of 5 being liquid [23]
Sieve score		Categorical	Score of 1–3 being a composite score for presence of grains & long fibre (>1) with 1 = no grains or long fibre, 3 = large amounts of grains and presence of many long fibres [24]
Stocking density	Continuous	Number of sheep per hectare (transformed into quintiles)	
Pasture quality	Categorical	Quality of pasture scored 1–3 1 = poor 2 = mediocre 3 = lush	
Lambing season	Categorical	Were the flock lambing at the time of sampling?	1 = sampled during lambing season 0 = sampled out of lambing season

visits and considered for inclusion in statistical analyses are described in Table 1. The sampling period was split into 'summer' and 'winter' with the winter period defined as being from 1 October to 30 April. The term 'sampling event' is defined as 'a visit to a farm to collect samples'. *C. jejuni* faecal-pat prevalence estimates were calculated using Huber–White robust standard error estimates [29] to account for clustering at farm level.

Multivariable logistic regression models were fitted with the binary outcome variable being the *C. jejuni* test result (presence or absence) of the faecal-pat sample. Collinearity between covariates was investigated using Cramer's φ statistic and, if present, one of the covariates was discarded taking into account biological plausibility. All remaining covariates were included in the initial model. A backwards, stepwise model-building strategy [29] was employed whereby a full model was built and then each variable removed in turn, a likelihood ratio test performed and the resultant P value noted. The variable with the highest P value was then omitted and the process repeated. This process was repeated until only variables with $P<0{\cdot}2$ remained in the model. The omitted variables were then added back in turn, starting with the lowest P value, a likelihood ratio test performed after each addition, and the variable retained if $P<0{\cdot}2$. This process was continued until no further variables could be added, to produce the final model. Interactions between variables in the final model were considered

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Table 2. *Distribution of* Campylobacter *spp. and* Arcobacter *isolates by host species*

	Cattle	Sheep
Number of faecal-pat samples	3300	960
Number of potential isolates (4 per pat)	13 200	3840
Number of isolates grown (% of potential isolates)	7779 (58·9 %)	1720 (44·8 %)
Arcobacter spp. (% of actual isolates)	4299 (55·3 %)	236 (13·7 %)
Campylobacter jejuni (% of actual isolates)	1857 (23·9 %)	450 (26 %)
Campylobacter coli (% of actual isolates)	346 (4·4 %)	815 (47·4 %)
Campylobacter fetus (% of actual isolates)	871 (6·8 %)	211 (12·3 %)
Campylobacter hyointestinalis (% of actual isolates)	380 (4·9 %)	0
Campylobacter lari (% of actual isolates)	26 (0·33 %)	8 (0·05 %)

for inclusion and retained if they improved model fit as judged by the likelihood ratio test. No significant interactions were identified.

Time was offered to the model as a composite of four sine and cosine functions (harmonic regression) to allow modelling of seasonal periodicity if present [30]. Four time covariates (x_1, x_2, x_3, x_4) were generated as follows:

$$x_1 = \cos(2\pi t/52), \quad x_2 = \sin(2\pi t/52),$$
$$x_3 = \cos(4\pi t/52), \quad x_4 = \sin(4\pi t/52),$$

where t = week number with week 1 being the first week in January 2006 when sampling commenced.

Separate logistic regression models were fitted for cattle and sheep with the underlying *a priori* hypothesis being that time or season is a primary determinant of the probability of a faecal pat being colonized by *C. jejuni* with other more proximal covariates also having an effect. The data has a hierarchical structure in that each faecal pat is nested within a farm, with each farm being nested within a zone. A random-effects model and a fixed-effects model, with farm specified as either a random or fixed effect, were fitted for the cattle data while a fixed-effects model only was fitted for the sheep data in light of the small number of farms sampled. Model fit was assessed using the Hosmer–Lemeshow χ^2 statistic and by consideration of model deviance together with visual inspection of residuals.

RESULTS

The median herd size, defined as total number of lactating and dry cows, was 145 [inter-quartile range (IQR) 104–200 cows, range 71–280 cows]. The breed of cattle in 14 of the herds was Holstein Friesian while one herd comprised Ayrshire and Ayrshire × Friesian. Four herds were housed all the time during the study period while one herd was housed for the entire second year of the study. All other herds were housed during the winter months but grazed outside during the summer. Fourteen of the herds were housed in cubicle accommodation while the Ayrshire herd was housed in straw yards. Median annual milk yield was 8000 l (IQR 7200–9000 l, range 6000–9600 l per annum). Two of the sheep farms were lowland with one farm grazing on the salt marshes of the River Lune estuary while two were upland with one utilizing summer grazing on moorland. The predominant breeds of sheep kept were Swaledale and North Country Mules. The two upland farms kept 1000 and 700 ewes, respectively, while one lowland farm had 700 ewes and the other kept 150 ewes.

Twenty faecal samples were collected at each sampling visit, yielding a total of 4260 samples. Four potential isolates were taken from each sample yielding 17 040 potential bacterial isolates. In total, 9499 putative *Campylobacter* spp. isolates were grown and 2307 (24·3 %) were identified as *C. jejuni* (Table 2).

At pat level, this equated to a *C. jejuni* faecal-pat prevalence of 19·1 % (95 % CI 15·4–22·7) and 17·0 % (95 % CI 8·5–25·5) for cattle and sheep, respectively. There was no species difference in pat prevalence ($P = 0{\cdot}494$). Summary *C. jejuni* faecal-pat prevalence estimates are presented in Table 3.

Model 1. Random-effects logistic regression model for dairy cattle (Table 4)

Farm identity was considered as a random effect. The between-farm variance was estimated as 0·153

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Table 3. C. jejuni *faecal-pat prevalence estimates by measured covariates*

	Cattle prevalence, % (95% CI)	Sheep prevalence, % (95% CI)
	19·1 (15·4–22·7)	17·0 (8·5 to 25·5)
Geographical location		
Zone 1	16·7 (10·6–22·8)	n.a.
Zone 2	24·2 (21·4–27·0)	21·5 (20·9 to 22·0)
Zone 3	17·7 (11·8–23·6)	12·5 (8·2 to 16·8)
Sampling environment		
Housed	16·6 (12·0–21·3)	6·7 (−0·9 to 14·3)
Pasture	23·4 (18·5–28·3)	18·4 (7·0 to 30)
Season		
Winter	16·4 (12·0–20·8)	9·2 (2·1 to 16·4)
Summer	22·3 (17·1–27·4)	26·1 (17·4 to 34·9)
Feeding system (cattle)	Prevalence, % (95% CI)	No. of sampling events
TMR	21·4 (8·7–34·2)	7
Hybrid TMR	17·3 (12·0–22·6)	95
Grazing and buffer and parlour	22·9 (17·1–28·6)	47
Grazing and parlour	23·5 (21·8–25·2)	10
Silage and parlour	7·5*	6

CI, confidence interval; n.a., not available; TMR, total mixed ration.
* Confidence intervals could not be calculated since only one farm used this feeding method.

(95% CI 0·061–0·380). Sampling environment had a highly significant effect ($P<0·001$) (OR 2·11, 95% CI 1·57–2·84) suggesting that dairy cows kept outside have double the odds of excreting *C. jejuni* in their faeces after adjusting for other covariates including time of year, with which it is strongly associated since dairy cows are not kept outside during the winter months. There was a significant ($P<0·001$) although small (OR 1·05, 95% CI 1·02–1·08) marginal effect of increasing milk yield by 1 l on the odds of a cow excreting *C. jejuni*. As with milk yield there was a significant ($P=0·002$) although small (OR 1·01, 95% CI 1·00–1·01) effect of increasing group size by one cow. Group size at sampling was primarily a function of overall herd size although the calving pattern will also impact on this measure.

Removal of the time covariates from the model had no effect on model fit (likelihood ratio test: $\chi^2=4·57$, 4 D.F., $P=0·3343$) demonstrating an absence of any underlying seasonal periodicity to bovine *C. jejuni* faecal-pat prevalence. This was demonstrated visually by plotting the logit predictions using time covariates only (Fig. 1).

Model 2. Fixed-effects logistic regression model for dairy cattle

To improve our understanding of the between-farm variation in faecal-pat prevalence, a model was fitted with farm specified as a fixed effect. There was considerable variation in the effect of farm with odds ratios ranging from 0·4 (95% CI 0·20–0·81) (farm 14) to 2·24 (95% CI 1·39–3·61) (farm 18), suggesting that after adjusting for the recorded covariates in the model there remains considerable unexplained variation due to farm. When compared to the random-effects model, inclusion of herd as a fixed effect did not substantially alter the estimated coefficients for any of the measured covariates.

Model 3. Fixed-effects logistic regression model for sheep (Table 5)

Farm was considered as a fixed effect with farm 9 taken as baseline. The odds ratios ranged from 0·45 (95% CI 0·26–0·81) (farm 15) to 1·05 (95% CI 0·63–1·76) (farm 12), suggesting that after adjusting for the other covariates in the model there remains a considerable

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Table 4. *Random-effects multivariable logistic regression model including covariates associated with the probability of isolating* Campylobacter jejuni *from cattle faecal samples on Lancashire dairy farms*

Covariate	Estimate β	95% CI	OR	95% CI	Wald test P value
Baseline (housed)	−3·72	−4·57 to −2·87			<0·001
Pasture *vs*. housed	0·75	0·45 to 1·04	2·11	1·57–2·84	<0·001
Group size (cows)	0·005	0·002 to 0·01	1·01	1·00–1·01	0·002
Milk yield (litres)	0·052	0·02 to 0·08	1·05	1·02–1·08	<0·001

Time covariates were included in the final model.
CI, Confidence interval; OR, odds ratio.
Farm is considered as a random effect ($n=15$).
Between-farm variance 0·153 (95% CI 0·061–0·380).

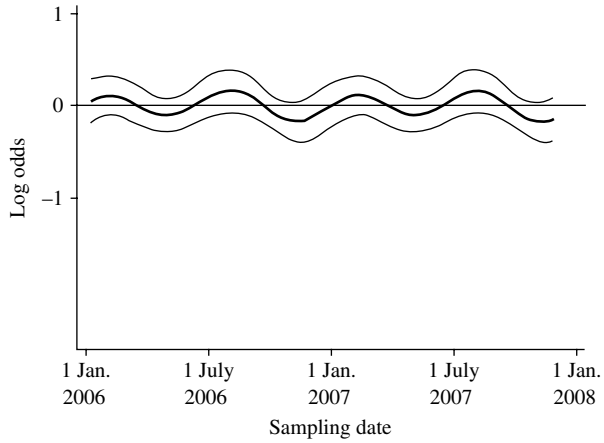

Fig. 1. The seasonal component to variation in *C. jejuni* faecal-pat prevalence on Lancashire dairy farms ($n=15$). The top and bottom lines represent the upper and lower 95% confidence limits.

amount of unexplained variation due to farm. It is of note that farms 15 and 16 are in zone 3 while farms 9 and 12 are in zone 2 suggesting that sheep farms in zone 2 (Lancaster) have about double the odds of an ovine faecal pat containing *C. jejuni* compared to sheep farms in the Clitheroe area. However the reliability of this finding must be questioned in light of the small number of farms sampled. Increased *C. jejuni* faecal-pat prevalence was associated with increased pasture quality (OR 2·16, 95% CI 1·39–3·35, $P=0·001$) and increased stocking density (quintiles) (OR 1·29, 95% CI 1·07–1·55, $P=0·004$). It is likely that stocking density (a management decision made by the farmer) will be a reflection of time of year, amount of grass growth (i.e. pasture type) and production targets being aimed for. Sampling during the lambing season was associated ($P=0·02$) with increased odds of a sheep excreting *C. jejuni* in its faeces (OR 4·68, 95% CI 1·28–17·17). The relative paucity of samples taken during this relatively short time period is reflected in the wide confidence intervals.

The seasonal component of the model, after adjusting for the other covariates in the model, was investigated by plotting logit predictions using time covariates (Fig. 2). This suggests that there are seasonal trends in the probability of a sheep faecal pat being colonized by *C. jejuni* with peaks during the summer months. This was confirmed by examining model fit with and without the time covariates using a likelihood ratio test. Inclusion of the time covariates significantly improved model fit (likelihood ratio test: $\chi^2=34·07$, 4 D.F., $P<0·001$).

DISCUSSION

The current study demonstrates that both dairy cattle and sheep act as significant reservoirs of *C. jejuni* with all herds and flocks in the study showing evidence of being colonized, although not at every sampling event.

Strong seasonality in *C. jejuni* faecal-pat prevalence was evident with highest prevalences in both cattle and sheep recorded during the summer months. In the case of dairy cattle, this apparent seasonality was a reflection of where the animals were sampled with higher prevalences recorded in cattle at pasture. Two alternative hypotheses may be generated. First, that dairy cattle are at a greater risk of exposure to, and thus colonization by *C. jejuni* when outside at pasture, due to presence of wildlife and drinking from natural watercourses [6]. However, the risk of a cow acquiring *C. jejuni* from a herd mate would probably be significantly reduced at pasture since faecal contamination and exposure to faeces is considerably less at pasture compared to when animals are housed. Cattle are known to avoid grazing grass which has faecal

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Table 5. *Multivariable logistic regression model including covariates associated with the probability of isolating* Campylobacter jejuni *from sheep faecal samples on Lancashire sheep farms*

Covariate	Estimate β	95% CI	OR	95% CI	Wald test *P* value
Baseline (farm 9)	−4·11	−5·43 to −2·79			<0·001
Farm					
Farm 12	0·048	−0·47 to 0·56	1·05	0·63–1·76	0·856
Farm 15	−0·79	−1·36 to −0·21	0·45	0·26–0·81	0·007
Farm 16	−0·43	−1·12 to 0·26	0·65	0·33–1·29	0·221
Other covariates					
Pasture quality	0·77	0·33 to 1·21	2·16	1·39–3·35	0·001
Stocking density (quintiles)	0·31	0·10 to 0·52	1·37	1·11–1·69	0·004
Lambing season	1·54	0·24 to 2·84	4·68	1·28–17·17	0·020

Time covariates were included in the final model.
CI, confidence interval; OR, odds ratio; n.a., not available.
Farm is considered as a fixed effect ($n=4$).
Deviance = 681, D.F. = 809.
Hosmer–Lemeshow χ^2 statistic = 7·01, $P=0·5361$ for 10 groups (D.F. = 8).

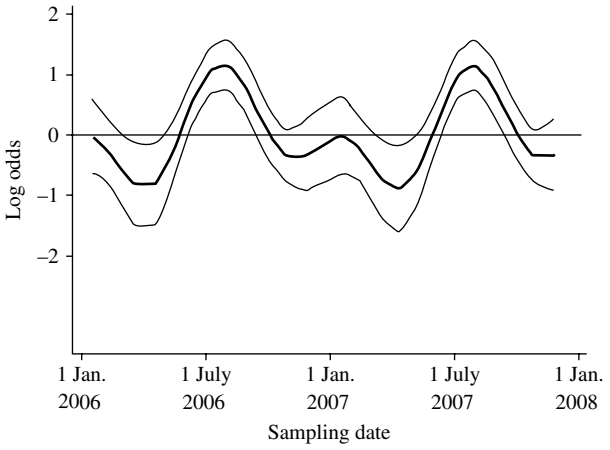

Fig. 2. The seasonal component to variation in *C. jejuni* faecal-pat prevalence on Lancashire sheep farms ($n=4$). The top and bottom lines represent the upper and lower 95% confidence limits.

contamination [31] and in the present study no slurry was spread on grazing pastures during the grazing season. The second hypothesis is that faecal-pat prevalence is a reflection of C. *jejuni* excretion rather than colonization *per se* and this is influenced by factors acting at an intestinal level in the animal. The diet received by housed cattle is markedly different from that received when grazing at pasture. Grazing cattle ingest high levels of soluble sugars but low levels of starches while housed animals on a diet of conserved forages and grain-based products ingest high levels of starches but minimal levels of sugars [32]. It may be hypothesized that the observed *C. jejuni* faecal-pat prevalence is a reflection of these very different diets which is likely to impact on the intestinal ecosystem in different ways.

Feeding system was not related to faecal-pat prevalence but this may be due to confounding by both season and sampling environment. No attempt was made to record actual feeds utilized due to the complex and dynamic nature of nutritional management in these herds. There was no association between faecal characteristics, i.e. consistency and sieve score, and faecal-pat prevalence.

There is little data regarding the influence of diet on faecal excretion of *Campylobacter* spp. A study in feedlot cattle suggested that high levels of grain feeding was associated with increased excretion of campylobacters [33] while Robinson *et al.* [18] found the presence of whole grain in the faeces of young cattle to be associated with an increased risk of isolating *Campylobacter* from faeces. The current findings, namely that faecal-pat prevalence increases in grass-fed animals would appear to contradict these findings. The findings of Robinson *et al.* refer to young animals in whom rumen development is incomplete and the finding of grain in faecal samples from these animals suggests a degree of rumen dysfunction. The Garcia *et al.* study [33] was carried out on feedlot cattle which by definition receive no grass, thus their findings can be interpreted as the effect of increased starch levels in animals already fed a

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high-starch, low-sugar diet. Thus neither study is comparable to the current study of adult dairy cattle.

There was a positive association between faecal-pat prevalence and increased group size. This may be due to increased exposure of individual animals to *C. jejuni* from their herd-mates. Increased prevalence of infectious agents is commonly associated with increased group size in cattle, e.g. paratuberculosis prevalence is strongly associated with increased herd size [34].

There was a positive association between increased milk yield and faecal-pat prevalence. Increased milk yield in a dairy cow is often interpreted as a proxy for increased 'metabolic stress' due to the increased metabolic demands placed on the animal. It has been demonstrated that stress, in its broadest terms, may increase susceptibility to bacterial infections such as *Salmonella* and *Campylobacter* [35] and, furthermore, may increase excretion of bacteria such as *Salmonella* spp. [36].

High intestinal carriage rates of thermophilic campylobacters (91%) have been demonstrated in lambs at slaughter in Lancashire by Stanley *et al*. [22] with higher counts being recorded than in cattle at slaughter. The same authors found faecal carriage in grazing sheep to be considerably lower (29·3%) which they attributed to intermittent excretion patterns. They found 87% of the campylobacters isolated from sheep faecal samples to be *C. jejuni* suggesting a *C. jejuni* faecal-pat prevalence of 25%. In their study, samples were collected during late spring and early autumn. Our *C. jejuni* prevalence estimates for sheep in summer (26·1%, 95% CI 22·0–30·3) are in close agreement with their findings, although in the current study, *C. jejuni* represented only 26% of total sheep isolates with *C. coli* accounting for 47% and *C. fetus* accounting for 12% (Table 3). In the current study, the prevalence of *C. jejuni* in sheep at grass was similar to that of dairy cattle although the prevalence of *C. coli* was considerably higher. There is scant data on the prevalence of *C. coli* in sheep although Brown *et al*. [19] reported isolating *C. coli* from 21% of sheep faecal samples. As with cattle, ovine *C. jejuni* faecal-pat prevalence was significantly lower during the winter months despite the animals not being housed, as is the case with dairy cattle. Multivariable modelling suggested that there is a true seasonal effect in sheep unlike in cattle, where the seasonal variation observed is driven primarily by changes in sampling environment.

Increased stocking density was positively associated with increased pat prevalence. This may be a reflection of increased exposure risk from other animals. There was a positive association between increased pasture quality and faecal-pat prevalence which may reflect a dietary effect. There was a strong positive effect of lambing, with sheep during the lambing season having an increased faecal-pat prevalence. However, cautious interpretation of these associations is required due to the small number of flocks sampled and the relatively infrequent sampling interval. With regards to lambing, only two flocks were sampled during the lambing season and they were both housed, with one flock being housed in poor, dirty conditions which are likely be conducive to both high transmission rates between animals and high excretion rates associated with stress as a result of the suboptimal housing conditions.

The geographical zone within Lancashire had no influence on bovine faecal-pat prevalence although it appeared that sheep farms in the Lancaster area had a higher pat prevalence. This finding was based on only two farms in each zone and is probably a reflection of the individual farms rather than true large-scale spatial variation. It should be borne in mind that since the study farms were recruited via their attending veterinary surgeons, there is total confounding of zone by veterinary practice. However, the veterinary practices were all multi-person mixed agricultural practices of similar size and client base.

In conclusion, our study has demonstrated that both cattle and sheep represent a significant reservoir of *C. jejuni* especially during the summer months when prevalence is highest in grazing cattle and sheep. While the variation observed in cattle faecal-pat prevalence is associated with sampling environment rather that season *per se*, in sheep there is an underlying seasonal periodicity.

Even after adjusting for the measured co-founders, considerable farm-level variation remained. Understanding the nature of this variation is likely to be crucial for possible future interventions to reduce ruminant *Campylobacter* prevalence.

The association between cattle faecal-pat prevalence and sampling environment deserves further investigation to elucidate the mechanisms involved with the possibility that it could lead to control strategies based on nutritional interventions.

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DECLARATION OF INTEREST

None.

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