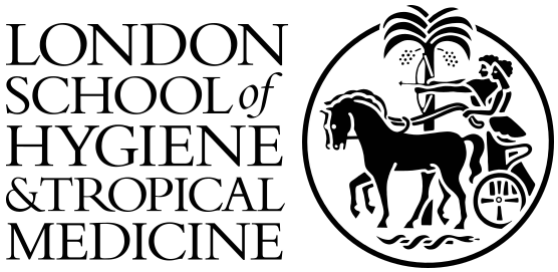

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Temperature dependence of reported *Campylobacter* infection in England, 1989–1999

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SUMMARY

Campylobacter is the most common bacterial cause of gastroenteritis in England and Wales, with 45 000 cases reported annually. *Campylobacter* incidence is highly seasonal; the consistent peak in late spring suggests a role for meteorological factors in the epidemiology of this organism. We investigated the relationship between ambient temperature and *Campylobacter* enteritis using time-series analysis to study short-term associations between temperature and number of *Campylobacter* reports adjusted for longer-term trend and seasonal patterns. We found a linear relationship between mean weekly temperature and reported *Campylobacter* enteritis, with a 1 °C rise corresponding to a 5% increase in the number of reports up to a threshold of 14 °C. There was no relationship outside this temperature range. Our findings provide evidence that ambient temperature influences *Campylobacter* incidence, and suggest that its effect is likely to be indirect, acting through other intermediate pathways.

INTRODUCTION

Over 45 000 laboratory-confirmed cases of *Campylobacter* enteritis are reported in England and Wales each year and an estimated half a million community cases occur annually in England alone [1, 2], making this the most common bacterial cause of gastrointestinal illness in this setting. The risk factors for *Campylobacter* enteritis, as identified by case-control studies and outbreak reports, are numerous and varied. They include consumption of inadequately cooked poultry, both in the home [3, 4] and in restaurants [5–7], barbecued meats [3, 4, 8], untreated water [7, 8], unpasteurized milk [3, 4], contact with pets, particularly puppies [4, 7, 9, 10], and occupational and recreational exposure to farm animals [3, 7, 8, 11]. A large proportion of sporadic cases remain unexplained by commonly recognized risk factors [5, 10].

Campylobacter enteritis in temperate countries exhibits a distinctive seasonal pattern. A spring/summer peak in reported cases is typical. The exact timing of the peak varies between countries but displays remarkable consistency from year to year [12]; in England and Wales, it occurs in late May/early June. The factors underlying this seasonality are unknown. Several hypotheses have been suggested,

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including seasonal changes in the prevalence of *Campylobacter* in animal hosts [13–15] and sewage sludge [16, 17] and changes in human behaviour leading to greater exposure, such as increased recreational water contact in the summer. However, none of these has been definitively linked to human disease and the many risk factors for infection indicate that a number of different factors may be involved. The consistency of the seasonal pattern suggests that meteorological factors could play a role, either directly or as a more distal, indirect influence driving other intermediate pathways. In this study, we investigated the relationship between short-term variations in climate, particularly ambient temperature, and incidence of *Campylobacter* enteritis using time-series methodology.

METHODS

Laboratory reports

We obtained weekly numbers of *Campylobacter* spp. infections reported in England between 1989 and 1999 from the national database of laboratory-confirmed infections [18]. A total of 623 817 cases were reported during this period. Since laboratory reports rarely include information on foreign travel, we used an indirect method to exclude travel-related cases. The England and Wales *Campylobacter* Sentinel Surveillance Scheme (CSSS) [19] collected self-reported exposure information – including foreign travel in the 2 weeks prior to illness – for laboratory-confirmed *Campylobacter* enteritis cases from collaborating health authorities from May 2000 to April 2003. Overall, $\sim$20% of cases report foreign travel in the 2 weeks prior to illness [20]. The proportion of travel-associated cases in English health authorities per week was determined from this dataset, and this proportion of cases was subtracted from the weekly time-series of laboratory reports used in the analysis.

We used the nearest available date to patients' date of onset for all analyses, usually the specimen date. Data from the CSSS indicate that the median delay between patients' date of onset and the specimen date is 4 days (interquartile range 3–7 days) and that in 90% of cases the delay is less than 14 days.

Meteorological variables

Daily time-series of meteorological variables were obtained from the Met Office (UK) and converted into mean weekly values. The climate variables included relative humidity, sunlight hours and mean central England temperature (CET), an aggregate variable representative of ambient temperature in the Midlands region of England [21]. Regional temperature variations display a very high degree of correlation, as determined by a correlation matrix of the mean weekly temperature series between the nine English Government Office regions (r values exceeding 0·95, data not shown). We thus used mean CET as a sensitive indicator of temporal variations in temperature in the country as a whole. Rainfall data were available for the North East and South West Government Office Regions of England. Preliminary analyses showed that mean weekly rainfall was not associated with *Campylobacter* reporting in these regions, and rainfall was not used in further analyses.

Statistical analysis

We used time-series-adapted regression techniques to study short-term associations between mean weekly ambient temperature and *Campylobacter* reports, adjusting for trend and seasonal patterns and other relevant climatic factors. We also adjusted for public holidays to account for the artifactual drop in reporting during these weeks. Negative binomial regression was used in all analyses to account for over-dispersion in the data (the variance being greater than the mean) [22].

Confounding by temporal factors

The question of interest in this study was the following: 'Is a change in ambient temperature in a given week associated with a change in the number of *Campylobacter* reports x weeks later?' Temporal associations between climate and disease are confounded by trend and seasonal patterns. In particular, any association between temperature and *Campylobacter* enteritis could be explained partly by the fact that ambient temperature and reported *Campylobacter* infection have similar seasonalities. We adjusted for trend and seasonality respectively by including in the model indicator variables for year and Fourier terms up to the 6th harmonic. Fourier terms can be used to re-create any periodic signal (such as a consistent seasonal pattern) using a linear combination of sine and cosine waves of varying wavelength [23]. The number of harmonics defines the lowest wavelength reproduced (i.e. the level of

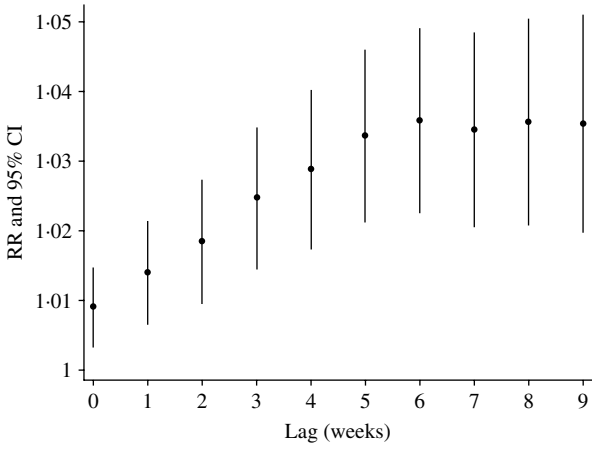

Fig. 1. Linear effect of temperature on *Campylobacter* spp. reports over lags 0–9. Each point indicates the combined effect of temperature in the corresponding lag and all preceding lags and represents the relative increase in number of reports per 1 °C increase in temperature, assuming a linear relationship between temperature and *Campylobacter* reports. Models are adjusted for trend, seasonality (up to the 6th harmonic), public holidays and relative humidity.

seasonal adjustment), with six harmonics corresponding to a wavelength of 9 weeks (one sixth of a year). Having adjusted for these longer-term trend and seasonal patterns, the short-term effects of climate variables on *Campylobacter* incidence can be investigated.

Lag effects

To account for delays in the effect of temperature on the number of reported cases, a lagged temperature variable was incorporated in the model. To identify the optimum lag period, we first assumed a linear relationship between temperature and *Campylobacter* reports. We performed sequential regressions of temperature on *Campylobacter* reports adjusted for trend, seasonality and public holidays, adding one lag at a time to determine the linear contribution of each additional lag. Temperature was included in these regressions as the combined effect of all lags up to the lag of interest (Fig. 1), e.g. in Figure 1, the effect shown at lag 1 is the combined effect of temperature in the current and previous weeks. A lag effect of up to 6 weeks was determined to be the optimum (Fig. 1). The effect of each additional lag was approximately linear, i.e. each additional lag resulted in a similar increase in the relative risk. In such a case, the average temperature over the 7-week period between any given week and the 6 weeks preceding it gives an unbiased estimate of the temperature effect over this lag period, and this was used in subsequent analyses. Adjustment for seasonal effects beyond this 6-week lag period was achieved in further analyses by including Fourier terms up to the 8th harmonic.

Regression analysis

Initially, natural cubic splines of the temperature series were used to obtain a smooth nonlinear function and determine the shape of the temperature–disease relationship (Fig. 2) [24]. This strategy involves dividing the temperature series into equal intervals. Within each temperature interval, the relationship with *Campylobacter* reports is defined using a cubic function. The cubic functions are constrained to join at the break-points of each interval so that a smooth function is obtained over the whole temperature range. This technique enables complex relationships to be modelled making better use of the available information and without making assumptions about the shape of the temperature–disease association. The number of break-points, or knots, used determines the level of smoothing of the data: the smaller the number of intervals, the smoother the function. A smooth function incorporating two knots was deemed to be the most appropriate using Akaike's Information Criterion (AIC) [23]. The choice of two knots was not crucial to the model, as regressions with up to five knots yielded similar (though less parsimonious) relationships (data not shown). Similarly, the effect of relative humidity was adjusted for by including natural cubic splines of the humidity series in the model. The full spline model thus included indicator variables for each year, Fourier terms up to the 8th harmonic, an indicator variable for weeks in which public holidays occurred and splines of the mean temperature and relative humidity series with two and five knots respectively.

Having obtained a smooth function for the temperature–*Campylobacter* relationship, the model was then simplified by reducing this smooth function to linear terms. Based on the adjusted spline model (Fig. 2, dashed line), a linear model was assumed with a threshold at a certain temperature, beyond which temperature has no effect on the number of *Campylobacter* reports. Repeated regressions were carried out varying the threshold by 1 °C each time to find the break-point providing the best fit (as determined by AIC). The residuals of the best-fitting model were checked for serial correlation using the partial

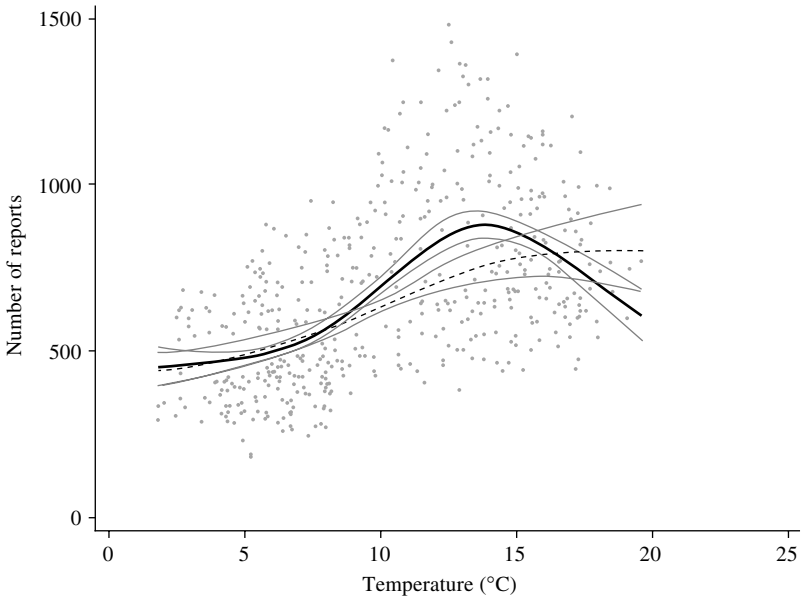

Fig. 2. Relationship between temperature and *Campylobacter* spp. reports, natural cubic spline models with two knots for the temperature series. —, Unadjusted model; - - -, the effect of temperature adjusted for trend, seasonality (up to the 8th harmonic), public holidays and relative humidity.

autocorrelation function. Three autoregressive terms were included in the model, as graphical inspection indicated that, after full adjustment, some residual correlation between the number cases on any given week and those in the previous 3-week period still remained. The final model gave an estimate of the relative increase in the number of *Campylobacter* reports for every 1 °C rise in temperature up to a certain temperature threshold. All analyses were performed using STATA version 8 (StataCorp, College Station, TX, USA).

In order to investigate whether the results were sensitive to the level of seasonal adjustment, the analysis was repeated using Fourier terms up to the 4th harmonic (one quarter year), Fourier terms up to the 16th harmonic (3·25 weeks), and indicator variables for month.

RESULTS

After adjusting for trend, seasonality, public holidays and relative humidity, we found a linear relationship between mean ambient temperature in the previous 6-week period and reported *Campylobacter* enteritis up to a threshold of 14 °C, with a 1 °C rise corresponding to a 5% increase in the number of reports [relative risk (RR) 1·045, 95% confidence interval (CI) 1·032–1·059]. No association was seen with mean sunlight hours.

Varying the level of seasonal adjustment had little effect on the results: model with four harmonics (RR 1·048, 95% CI 1·033–1·063, $P<0·001$); model with 16 harmonics (RR 1·047, 95% CI 1·033–1·060, $P<0·001$); model with month indicators (RR 1·056, 95% CI 1·044–1·074, $P<0·001$).

DISCUSSION

In England, there appears to be a significant association between temperature and *Campylobacter* incidence up to a threshold of 14 °C. This association persisted even after adjustment for yearly trend effects, seasonal patterns and public holidays. Important alternative explanations for this apparent association include insufficient seasonal adjustment and inadequate control for meteorological or other time-varying variables. However, repeating the analysis with varying degrees of seasonal adjustment yielded similar results and of the meteorological variables investigated, only relative humidity showed a weak relationship with *Campylobacter* reporting. The nature of this relationship was complex and nonlinear, and has not been characterized further.

Using the CSSS data to indirectly exclude travel-related cases could mean that some residual confounding due to cases who acquired infection abroad might remain. However, the sentinel surveillance scheme on which our travel data were based collected

information on ~15% of all laboratory-reported cases from health authorities with a wide geographical range; it is unlikely that the proportion and seasonality of travel-related cases in this dataset differs substantially from those of all reported cases.

A potential limitation of the analysis is the high variability of the data, apparent from the considerable scatter of the data points above and below the fitted curve. The reason for this high variability is unclear, but it might be dependent on a number of factors. First, the use of specimen date introduces a variable lag (in most cases no more than 2 weeks) that is unrelated to temperature. Second, the use of mean CET as an aggregate temperature measure for the whole of England could have diluted the temperature effect. More detailed region-specific analyses might address this problem, albeit at the cost of statistical power. Third, the high variability may reflect the many sources and routes of transmission for *Campylobacter*, some of which might not be temperature-dependent. Even where temperature is involved, the effect is likely to be mediated through complex pathways. *Campylobacter* exhibits limited growth below 30 °C, so it is unlikely that temperature will have a direct effect on *Campylobacter* incidence over the temperature range that we have described. Our model suggests an effect of temperature up to a threshold of 14 °C. The biological significance of this threshold is unclear, and this value is likely to be subject to statistical variation. However, our results suggest that the effect of temperature may be exerted through more complex temperature-dependent mechanisms. One such mechanism could involve survival of *Campylobacter* in environmental water sources. There is considerable evidence that survival of campylobacters in aquatic environments is inversely related to water temperature and that reversion to a 'viable but non-culturable' (VNC) form can extend survival to several months [25–28]. In some cases, infectivity of these VNC strains in rats and chicks has been demonstrated [29, 30]. In addition, extended survival of *Campylobacter jejuni* within *Acanthamoeba polyphaga* vacuoles at low water temperatures has been demonstrated under experimental conditions, suggesting a role for this waterborne protozoan in the ecology of *Campylobacter* [31].

Despite the numerous outbreaks of waterborne *Campylobacter* enteritis described in the literature, case-control studies have not found water contact to be a major transmission route for sporadic cases. This may partly be due to the difficulty in quantifying these exposures, which, in addition, may only be harmful intermittently; if water contact is a risk factor only under certain temperature conditions, studies that do not take into account the interaction between water contact and season might not detect this. Alternatively, water sources could be an important factor in the environmental spread of *Campylobacter*, while not necessarily being a direct source of infection. Under this model, aquatic survival of *Campylobacter* at lower temperatures would result in spread to wildlife and farm animals, leading to an amplification cycle involving multiplication in animal hosts, epizootic transmission and faecal re-contamination of water sources. The fact that ambient temperature does not accurately reflect surface water temperature may partly explain the variability of the data. Such an ecological model would also explain the relatively long lag effect of temperature on *Campylobacter* incidence (up to 6 weeks), as some time would be required for an amplification stage to result in human infection.

Our study has demonstrated an association between ambient temperature and incidence of *Campylobacter* enteritis in humans. It should be noted that our study was not aimed at explaining the seasonal pattern of *Campylobacter* gastroenteritis; such analyses have recently been carried out with inconclusive results [32]. The consistency of the seasonal pattern strongly suggests a role for climatic factors in the epidemiology of *Campylobacter*, and our results support this. Although several hypotheses for the seasonal peak have been suggested, including seasonal changes in the prevalence of *Campylobacter* in animals [13–15] and sewage [16, 17] and changes in human behaviour, none of these has been definitively linked to human disease. The numerous risk factors for infection indicate that a number of different factors may be involved. Our findings provide evidence for ambient temperature being one of these factors, probably acting as a more distal, indirect influence driving other intermediate pathways.

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DECLARATION OF INTEREST

None.

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