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Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007

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ABSTRACT

The objective of this study was to examine incidences of *Campylobacter* in broilers and humans, and to describe seasonal variation and long-term trends by comparing longitudinal surveillance data in six Northern European countries (Denmark, Finland, Iceland, Norway, Sweden and the Netherlands). Due to high degree of seasonality and autocorrelation, seasonally adjusted (de-seasonalized) and trend adjusted data (de-trended) were used for comparing incidences within and between the six countries. De-seasonalized time series were obtained by fitting the incidence time series to mean monthly temperature and then removing this effect from the data. Long-term trends were fitted to the de-seasonalized time series. The incidence of *Campylobacter* colonization in broiler flocks and incidence of campylobacteriosis in humans showed a concordant seasonality for all the countries. There was a strong association between the incidence in both broilers and humans in a given month and the mean temperature of the northern hemisphere in the same month, as well as the preceding month, as shown by the cross-correlations and the chosen Generalized Additive Model. Denmark and Sweden showed a steadily decreasing trend for *Campylobacter* in broilers and human campylobacteriosis in the period 2001–2007. In Iceland, there was a decreasing trend for campylobacteriosis in humans from 1999 to 2007, whilst the broiler trend for *Campylobacter* was stable from 2001 to 2004, then falling thereafter. In Norway, the human campylobacteriosis trend showed a steady increase throughout the period. On the other hand, the Norwegian broiler trend for *Campylobacter* showed a decrease from 2001 until 2004, but was thereafter stable. There was no significant decrease or increase in incidence for human

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campylobacteriosis in the Netherlands, and the trend for *Campylobacter* in broilers was close to stable. The seasonality seen in broiler and human closely follows the temperature, and was probably caused, at least partly, by temperature related factors.

1. Introduction

Campylobacter are ubiquitous bacteria, frequently found in the alimentary tracts of animals, especially birds, and commonly contaminate the environment, including water. It is, however, less clear how the bacteria are distributed in the environment, and in what numbers. Since the 1970s, *Campylobacter* have been shown to be an important cause of enteritis in humans and regarded as the most common cause of reported bacterial gastroenteritis in the EU (Anonymous, 2007). Most *Campylobacter* infections occur as sporadic cases and consumption of undercooked broiler meat is regarded as a significant source of human infections (Reiersen et al., 2001; Vellinga and van Loock, 2002; Stern et al., 2003; Wingstrand et al., 2006; Anonymous, 2007). Possible sources of *Campylobacter* for broiler flocks are: low levels of biosecurity; presence of rodents on farms; age of broilers (Hansson et al., 2007; McDowell et al., 2008), drinking water that has not been disinfected (Kapperud et al., 1993); or other livestock on the farm/neighbourhood (van de Giessen et al., 1996; Bouwknegt et al., 2004). In the Netherlands, it has been estimated that 20–40% of all laboratory-confirmed cases are attributable to consumption of undercooked chicken. Nearly the same estimate (40%) was found in Belgium (Vellinga and van Loock, 2002; Janssen et al., 2008). Other identified sources are contaminated drinking water, raw milk and contact with pets (Kapperud et al., 2003; Ethelberg et al., 2005; Olson et al., 2008). Although these potential sources have been identified, the routes of transmission and their relative importance are still uncertain.

In wild birds and chickens the *Campylobacter* carriage rate (the number of birds carrying the bacteria), and the numbers of *Campylobacter* in the small intestine and caeca of broilers, have a seasonal pattern, with a distinct peak in prevalence in summer and a low prevalence during winter (Jacobs-Reitsma et al., 1994; Wallace et al., 1997; Wedderkopp et al., 2001; Hansson et al., 2004; Hofshagen and Kruse, 2005; Olson et al., 2008). This seasonal fluctuation in *Campylobacter* numbers is reflected in the increased risk of human infection from carcass contamination and environmental sources at certain times of the year. Similar seasonal pattern of raised summer incidence is also seen in the occurrence of human campylobacteriosis in Northern Europe (Nylen et al., 2002; Miller et al., 2004; Meldrum et al., 2005; Tam et al., 2006; Heier et al., 2006; van Hees et al., 2007; Olson et al., 2008). Several studies suggest that climate may play a role in colonization of both animals and humans (Patrick et al., 2004; Kovats et al., 2005; Louis et al., 2005; Valérie et al., 2005; Tam et al., 2006; Fleury et al., 2006). Seasonal variation in infection pressure may be dependent on climatic variables like temperature, length and intensity of daylight and precipitation, as well as changes in host social behaviour and in host immune system (Altizer et al., 2006). Seasonal variation in the incidence of infectious diseases is a well-known phenomenon worldwide, although the mechanisms behind are poorly understood in many instances (Grassly and Fraser, 2006).

One possible explanation for the seasonality of human campylobacteriosis, besides a documented seasonal variation in several food sources, include prevalence of *Campylobacter* in environmental reservoirs, which varies greatly with season (Jacobs-Reitsma et al., 1994; Stanley and Jones, 2003). The seasonal effectiveness of the human immune system response and the possible role acquired immunity plays, are also important factors in the epidemiology of campylobacteriosis (Mann et al., 2000; Skelly and Weinstein, 2003; Havelaar et al., 2009).

The objectives of this study were to compare whether the seasonal variation observed is similar in the six different countries, and to describe long-term trends in *Campylobacter* incidence for broilers and humans, as a way of generating hypotheses and to give a thorough description of the existing situation.

2. Materials and methods

2.1. Data collection

The incidence data for *Campylobacter* in broilers originated from the countries' surveillance data. The incidence estimation was based on the assumption that flocks are all expected to be negative at the beginning of the rearing period. The calculation of the annual/monthly incidence was based on date of slaughter, i.e. flock lifetime incidence. A slaughter group was regarded as positive if at least one of the cloacal or the caecal samples proved positive for *Campylobacter*. Details for each country are described in Table 1. The incidence data for campylobacteriosis in humans originated from the countries' surveillance data. Information regarding age, gender, country of infection and place of residency were available for all the countries. Annual/monthly incidence for domestically infected cases was calculated based on the total population by the end of the year/beginning of next year. Population data were derived from each country's national bureau of statistics. Details for each country are described in Table 2.

2.1.1. Temperature data

Data were provided by Climatic Research Unit, School of Environmental Sciences, University of East Anglia, Norwich, as the mean ambient temperature per month for the northern hemisphere.

Table 1
Broiler surveillance data for *Campylobacter* from Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands (NCFA: Nordic Committee on Food Analysis, mCCDA: Direct plating on Modified Charcoal-Cefazolin-sodium Deoxycholate Agar, Campy-Cefex: Semi-quantitive direct plate, PCR: Extracted DNA).

County	Starting	Sampling strategy	Sampling point	Sample type	Lab method/agar	Important changes over time
Denmark	1998	All flocks	At slaughter	Cloacal swabs 10 pooled to one	PCR	1998–2001 traditional cultivation method
Finland	2004	All flocks from June to October Random rest of the year	At slaughter	Caeca 10 pooled to one	mCCDA	No changes
Iceland	2000	All flocks	Before slaughter	Faecal droppings 10 pooled to one	Campy-Cefex	Until end of 2001: Caeca; 10 pooled to one
			At slaughter	Caeca 20 pooled to two	Campy-Cefex	Until primo 2001: Cloacal swabs; 10 pooled to one
Norway	2001	All flocks	Before slaughter	Swabs from faecal droppings 10 pooled to one	PCR	Until 2005: mCCDA
			At slaughter	Caeca 10 pooled to one	mCCDA	Until May 2004 Cloacal swabs. 10 pooled to one
Sweden	1991	All flocks	At slaughter	Caeca 10 pooled to one	mCCDA	1992–2004: Cloacal swabs analysed and cultured according to NCFA protocol
The Netherlands	1997	Every 3rd–4th flock	At slaughter	30 pooled to one	mCCDA	No changes

2.2. Data analysis

2.2.1. Incidence data

Monthly incidences of domestic human campylobacteriosis and of *Campylobacter*-positive broiler flocks were investigated by descriptive and explorative methods. Due to strong seasonality of the data, the time series of monthly incidences were plotted against mean ambient temperature per month for the northern hemisphere. The temperature mean of the northern hemisphere was selected due to the fact that incidence data of five countries were to be compared to one common climate series. Finland's surveillance data was only included in the presentation of annual incidences and not in the further analyses due to short time span of broiler data, i.e. from 2004 to 2007. Finland was still kept in the overall study because it adds information to the annual and monthly seasonal incidences.

2.2.2. De-seasonalized incidence data

Variations of Generalized Additive Models of the form:

$$Y_{i,t} = \alpha_i + f_i(T_t) + \varepsilon_{i,t} \quad (1)$$

were compared (GAMs; Hastie and Tibshirani, 1990) to achieve removal of seasonality (and a reduced level of autocorrelations) in the incidence data. Where $Y_{i,t}$ is the broiler or log-human *Campylobacter* incidence of a country i at month t, α_i is the intercept term, and f_i is a nonparametric function describing the effect of a factor such as temperature T at month t on the incidence $Y_{i,t}$. The degrees of freedom of this function were determined using cross-validation, library mgcv in *R* (Wood, 2006); ranging from a linear function to 4 degrees of freedom. The residuals, $\varepsilon_{i,t}$'s, were assumed Gaussian distributed. Based on the high cross-correlations between northern hemisphere and the incidence data at lag 0 and lag 1, different versions of T_t: (i) T_t = northern hemisphere temperature at lag 0 = T_NH_t, (ii) T_t = northern hemisphere at lag 1 = T_NH_{t-1} and (iii) T_t = the average of northern hemisphere temperature at lag 0 and lag 1, were used. Lag 0 is the mean ambient temperature for the sampling month. Lag 1 is the mean ambient temperature for the month preceding the sampling month. Also a version using a first-order autoregressive component of incidence instead of temperature was tested; i.e. T_t in Eq. (1) was exchanged by $Y_{i,t-1}$. The model with the highest deviance explained coupled with the lowest Akaike's Information Criteria (AIC) and a significant p-value was selected to be the best model for removing seasonality in the incidence data. Cross-validation gave 3 degrees of freedom for the nonparametric function. All the human incidence data were transformed using logarithm in order to

Table 2
Campylobacteriosis surveillance information from Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands (Notifiable disease: disease subject for registration/notifiable disease).

Country	Starting year	Notifiable since	Calculation basis for incidence	Country of infection
Denmark	1991	2000	Date of receival of sample in lab	Missing in 75% of cases
Finland	1995	1995	Date of sampling	Missing in 25% of cases
Iceland	1990	1997	Date of sampling	Missing in approx 7% of cases
Norway	1980	1980	Date of sampling	Missing in approx 8% of cases
Sweden	1990	1989	Date of reporting the case (lab/GP)	Missing in approx 9% of cases
The Netherlands	1993	No notifiable	End of week reporting by the lab	Travel relation known for 7–11% of cases

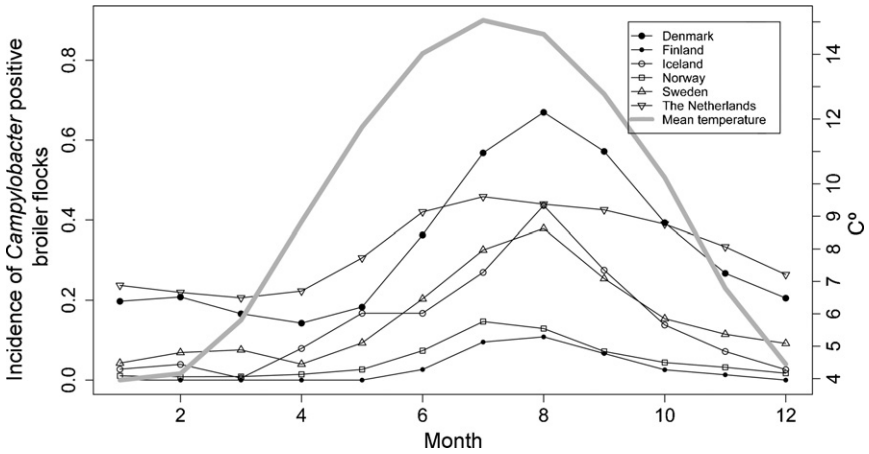

Fig. 1. Mean monthly incidences of broiler flocks positive for *Campylobacter* spp. in Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands during 2001–2007, compared with mean ambient temperature for the northern hemisphere. (The starting point for surveillance data: for Finland was 2004; for Norway was May 2001 and for Sweden July 2001.)

obtain approximately Gaussian distributed residuals. Transformation of the broiler incidence data was not necessary, as their residuals were approximately Gaussian distributed. All analyses were conducted in R version 2.6.2 (R development Core Team, 2008). The de-seasonalized incidence data which were obtained by removing the effect of the mean temperature (i.e. using residuals of the selected model), were evaluated by testing for autocorrelation, partial autocorrelation and by comparing the original time series with the de-seasonalized time series of *Campylobacter* for both human and broiler.

2.2.3. Long-term trends of the de-seasonalized incidence data

The long-term trends were extracted by using GAM to fit a nonparametric function (spline) of time to the de-seasonalized *Campylobacter* incidence data for broilers and humans. The degrees of freedom of the fitted trend were determined using cross-validation, ranging from a linear function to 3 degrees of freedom. A trend was considered significantly increasing or decreasing during time periods for which it was not possible to draw a straight horizontal line within the estimated (by GAM) confidence intervals.

2.2.4. De-trended incidence data

The de-trended data were evaluated by testing for autocorrelation and partial autocorrelation. In addition cross-correlations comparing the de-trended *Campylobacter* time series between broiler and humans and between countries were performed. The de-trended time series for both humans and broilers were evaluated by spectral analysis, plotting periodogram (by the R function **spectrum**) in order to identify any remaining periodicity (Venables and Ripley, 2002).

3. Results

Campylobacteriosis showed a gradual rise in spring, which peaked in late-summer (July–August), before the incidence returned to baseline level in late autumn. The seasonality seen for campylobacteriosis was most pronounced for Finland. The colonization of *Campylobacter*-positive broiler flocks had an equivalent spring-rise which cumulated in July–August before returning to baseline level late autumn (Figs. 1 and 2). For the Netherlands the peaks of incidence were less pronounced for both humans and poultry. The countries' monthly incidence for humans and broilers showed a strong temporal association (all cross-correlations at lag $0 > 0.65$). The variation in the annual incidence of campylobacteriosis and *Campylobacter*-positive broiler flocks can be seen in Figs. 3 and 4.

The concordance between seasonality in the incidence data and temperature of the northern hemisphere is visualized in Figs. 1 and 2. The cross-correlation between each of the countries' broiler/human data and the northern hemisphere temperature data calculations showed strong correlations at lag 0 (average temperature in the sampling month) and lag 1 (the month preceding the sampling month), were all cross-correlations > 0.7, except for human campylobacteriosis in Iceland. The time series were highly seasonal and autocorrelated. High degrees of autocorrelations are likely to make correlations spurious, and in order to improve any comparison between countries and between human and broiler incidences, de-seasonalized and de-trended data were used.

The model based on average temperature in the sampling month (lag 0) and the month preceding the sampling month (lag 1) gave the overall best fit for each country's human and broiler time series ($T_t = (T\_NH_t + T\_NH_{t-1})/2$), and this model was used to obtain de-seasonalized incidence data. With this model, the deviance explained for broilers ranged from 58.9 to 81.8% (Denmark 74.6%, Iceland 59.5%, Norway 74.1%, Sweden 81.8% and the Netherlands 58.9%). For humans, the deviance explained ranged from 23.1 to 82.7% (Denmark 54.8%, Iceland 23.1%, Norway 77.5%, Sweden 82.7%, and the Netherlands 70.6%). The autocorrelation coefficients for the de-seasonalized time series were low (<0.5) and the strong seasonality was removed. In the de-seasonalized time series, lag 0 showed the maximum correlation between human incidences and broiler incidences (Fig. 5). This indicates that

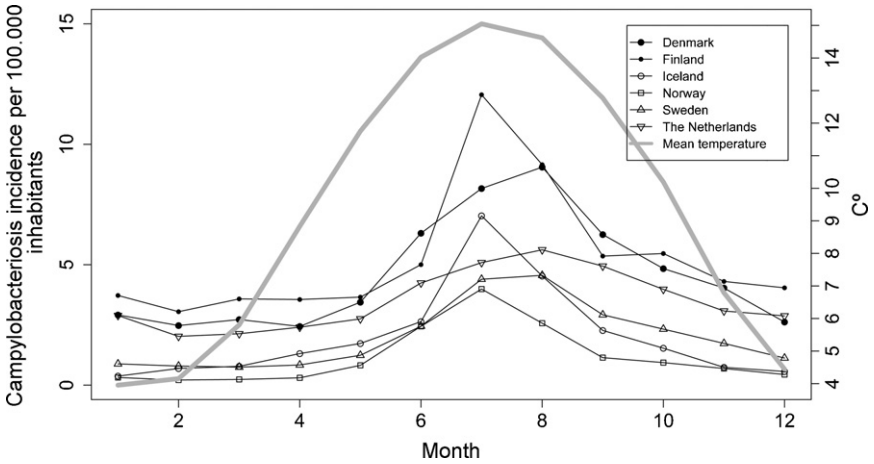

Fig. 2. Mean monthly incidences of domestic campylobacteriosis in humans in Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands during 1990–2007, compared with mean ambient temperature for the northern hemisphere. (In Iceland 1997–1999 excluded due to campylobacteriosis outbreak.)

the series tend to move together, rather than one series preceding the other.

In the de-seasonalized time series, Denmark and Sweden showed a steadily decreasing trend for *Campylobacter* in broilers and human campylobacteriosis, in the period 2001–2007 (Fig. 6). In Iceland, there was a decreasing trend for campylobacteriosis in humans from 1999 to 2007, whilst the broiler trend for *Campylobacter* was stable from 2001 to 2004, then falling thereafter. In Norway, the human campylobacteriosis trend showed a steady increase throughout the period. On the other hand, the Norwegian broiler trend for *Campylobacter* showed a decrease from 2001 until 2004, but was thereafter stable. There was no significant decrease or increase in incidence for human campylobacteriosis in the Netherlands, and the trend for *Campylobacter* in broilers was close to stable.

The de-trended time series for all the countries' broiler time series were normally distributed, had low autocorrelations (0.33 the highest value) and no remaining periodicity could be found by spectral analysis. The human de-trended time series appeared to be different amongst the countries. For Sweden, Iceland and the Netherlands the residuals were normally distributed, autocorrelations generally low, and no remaining periodicity were found. In the Danish and Norwegian time series the residuals showed remaining periodicity. By running a spectral analysis for the Norwegian data, a 6-month periodicity was found, the main frequency of the remaining periodicity was less clear for Denmark.

4. Discussion

This study analyses data from *Campylobacter* monitoring programs from six European countries. The data generated from the *Campylobacter* monitoring programs for broilers represents an active, "targeted surveillance", whilst the human data are collected from a passive surveillance system. The modes of data collection and

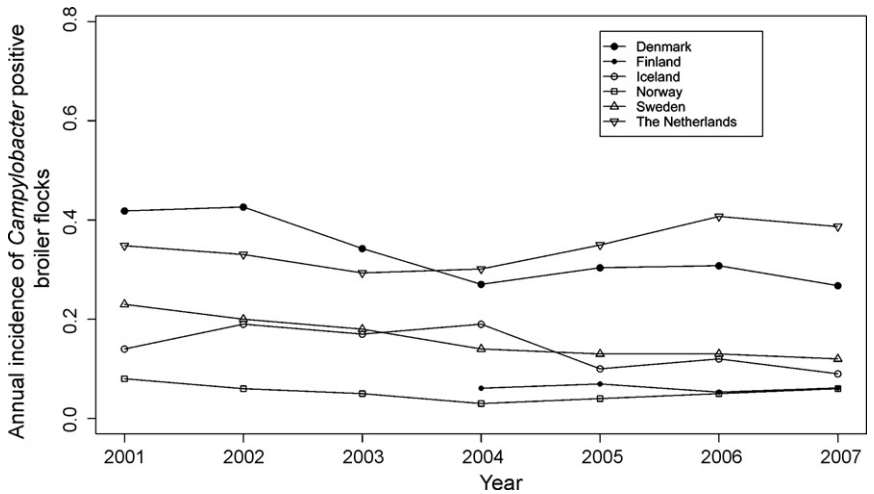

Fig. 3. Annual incidences of broiler flocks positive for *Campylobacter* spp. in Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands in the time period 2001–2007. (The starting point for surveillance data: for Finland was 2004; for Norway was May 2001 and for Sweden July 2001.)

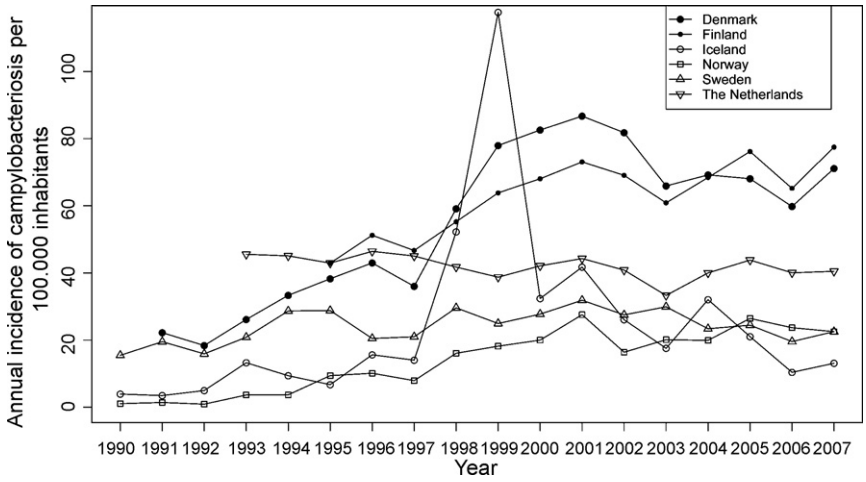

Fig. 4. Annual incidences of domestic campylobacteriosis in humans in Denmark, Finland, Iceland, Norway, Sweden, and the Netherlands in the time period 1990–2007. (Available data for Denmark from 1991, the Netherlands from 1993 and Finland from 1995.)

data sources are therefore different. This represents potential sources of bias in the study. The surveillance programs for broilers and humans in the six countries differ, and the sampling and laboratory methods used over time have varied during these years and between countries. These variations amongst the countries make the data not directly comparable, but still commensurable.

Enteric disease surveillance in humans is known to underestimate incidence considerably, so the aggregated disease incidences are probably conservative estimates. This aspect would again differ between the six countries'. Also improvement in notification systems, changes in diagnostic techniques, and general public awareness of the disease may play a role and can therefore explain some of the increase in the incidence of campylobacteriosis seen from 1990 to 2007.

This study demonstrates that the incidence of *Campylobacter* colonization in broiler flocks and the incidence of campylobacteriosis in humans show a concordant seasonality for all the six countries. This is consistent with findings in other studies in Europe (Nylen et al., 2002; Patrick et al., 2004; Kovats et al., 2005; Tam et al., 2006; Olson et al., 2008; Hartnack et al., 2009), suggesting that seasonal factors may play a role in infection. In contrast, no clear seasonal peak of campylobacteriosis has been detected in the tropics (Allos, 2001).

Several studies have demonstrated a higher level of *Campylobacter* in wild birds, broilers and domesticated animals during the warm season (Jones, 2001; Stanley and Jones, 2003; Newell and Fearnley, 2003; Hofshagen and Kruse, 2005). Though not all studies have found clear seasonal variations (Johnsen et al., 2006). Broiler carcasses are easily contaminated by *Campylobacter* during the slaughter process, and thus especially contribute during the seasonal peaks to the incidence of campylobacteriosis in humans. Consequently much effort is put into control strategies along the poultry meat production chain. Control strategies invented to reduce incidence of campylobacteriosis by reducing *Campylobacter* contamination on poultry meat have, however, only been partly successful.

Wild birds, sewage and faecal shedding by cattle, pigs and sheep can be source of *Campylobacter* contaminating surface waters, streams and agricultural run-off (Jones et al., 1990). Quantitative studies of surface waters show higher numbers of *Campylobacter* in winter than in summer (Jones et al., 1990). However, as *Campylobacter* may be viable, but not culturable, such data may be difficult to interpret. *Campylobacter* has a relatively low die-off rate in water and thus water may act as a reservoir for *Campylobacter* (Olson et al., 2008). Other factors which can contribute to the seasonal patterns seen in this study are human behaviour and life style-determined exposure

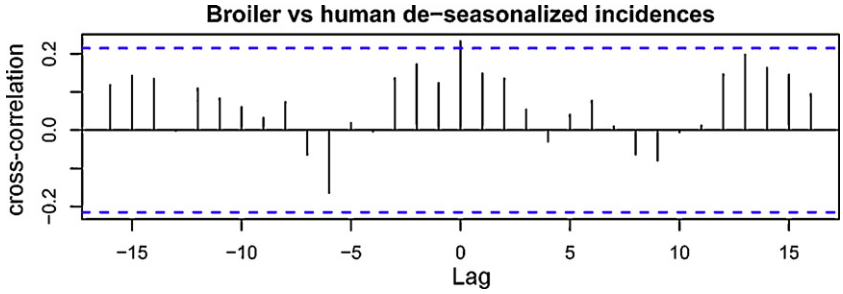

Fig. 5. Depiction of the cross-correlation between broiler and human time series incidences using the overall de-seasonalized mean incidence of Denmark, Iceland, Norway, Sweden and the Netherlands.

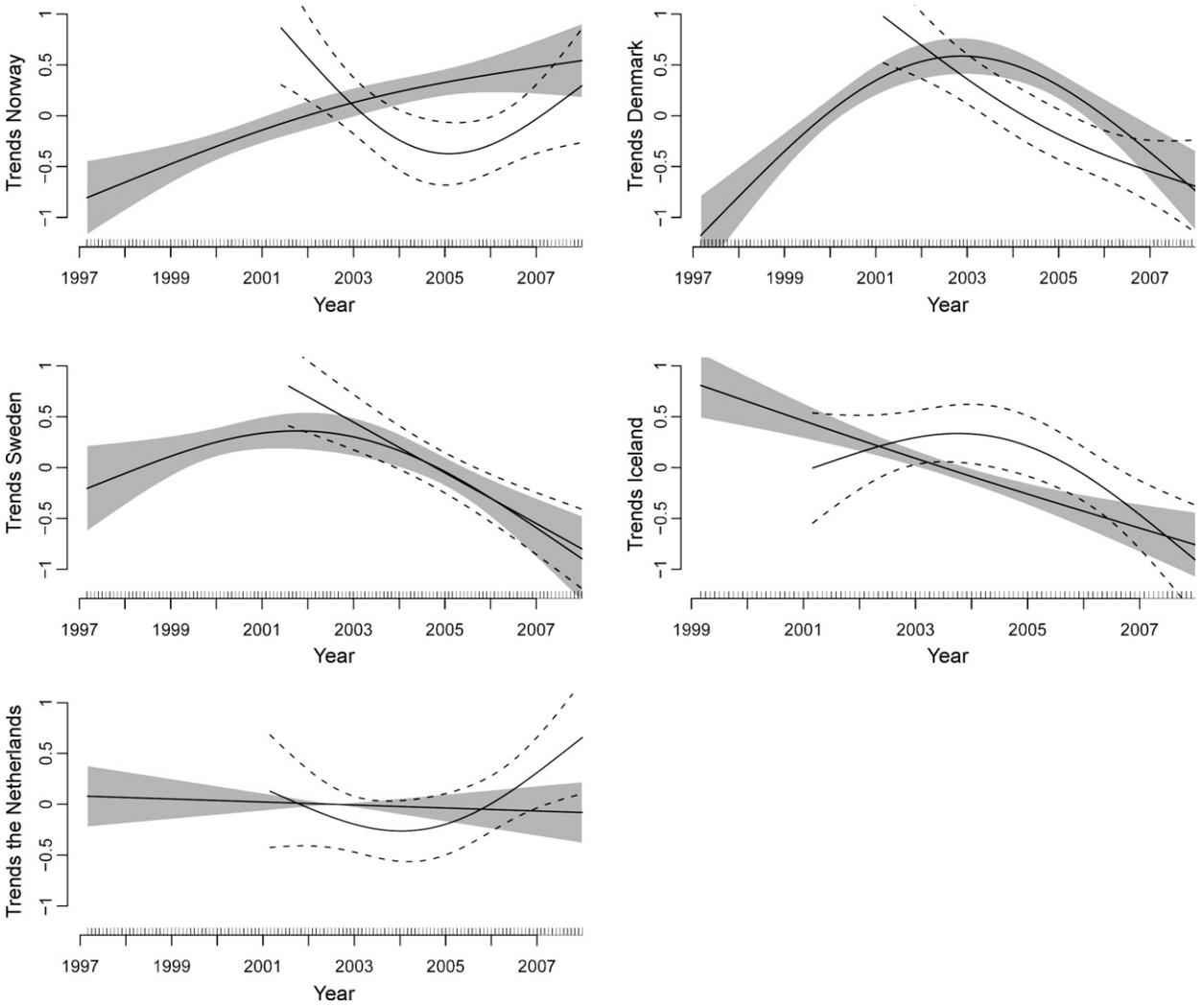

Fig. 6. Trends for campylobacteriosis in humans fitted to the de-seasonalized time series with confidence interval (black with grey hatched area), and trends for *Campylobacter* in broilers with confidence interval (black with black dotted lines). The time span for humans ranges from 1997 to 2007, and for broilers from 2001 to 2007. The time span for the human time series in Iceland is from 1999 to 2007 (1997–1999 excluded due to campylobacteriosis outbreak).

of people to *Campylobacter* both from broilers and from other sources. The inherent seasonality of the human immune system may also add to the seasonality seen for campylobacteriosis. The human immune system shows seasonal oscillation, being impaired with increased exposure to ultraviolet radiation (Mann et al., 2000; Lowell and Davis, 2008), coinciding with the time when exposure to *Campylobacter* from various sources is at the greatest.

Temperature was highly correlated with the incidence of *Campylobacter*-positive broilers as well as campylobacteriosis in humans. In this study, the mean ambient temperature for the sampling month (lag 0) and for the month preceding the sampling month (lag 1) had the highest correlation with the data (both human and broiler). The model combining the temperature of the sampling month, with that of the preceding month gave the overall best fit for both campylobacteriosis and broiler *Campylobacter* time series. For the Netherlands, a model including only the mean temperature for the month preceding the sampling month gave an even better fit for both the human campylobacteriosis and broiler incidence data. This might be a result of the Netherlands being the southernmost country in the study, where the *Campylobacter*-season starts earlier and lasts longer due to warmer climate. In this present study mean ambient temperature for the northern hemisphere was used. Adaptation to a sine curve could also have been used, since seasonal transmission often is assumed to be sinusoidal (Grassly and Fraser, 2006). However, in this study one reference series was chosen for comparing each country, and the temperature series is also close to sinusoidal. The finding of a strong relationship between temperature and incidence of human campylobacteriosis and broiler flocks positive for *Campylobacter* is in line with results from previous studies (Patrick et al., 2004; Louis et al., 2005; Tam et al., 2006; Fleury et al., 2006; Hartnack et al., 2009). Another study found that timing of the peaks was only weakly associated with high temperatures in the previous 3 months (Kovats

et al., 2005), but the study included countries such as Australia and New Zealand, which also show less consistent seasonality. They reasoned that intra-annual changes in the *Campylobacter* animal reservoirs could explain the seasonal patterns. Jacobs-Reitsma et al. (1994) suggested that the temperature effect on *Campylobacter* carriage state were indirect, and proposed that the *Campylobacter*-sources (e.g. wild birds, animals and livestock) were in fact temperature dependent. Seasonal peaks in incidence of diarrhoeal disease in Pakistan were found to be correlated with a high density of house flies (Chavasse et al., 1999). Flies have also been proposed as vectors in the transmission of campylobacteriosis, and suggested to account for the seasonality in both humans and poultry (Hald et al., 2004; Ekdahl et al., 2005). Meldrum et al. (2005) suggested that the seasonal increase in hours of daylight during spring reactivates latent *Campylobacter* cells. There are probably multiple environmental sources acting as seasonal drivers, influencing the seasonal rise in both humans and broilers by interacting in complex ways.

In the de-seasonalized incidence time series lag 0 showed the maximum correlation between human incidences and broiler incidences. In this study monthly data was used. Samples taken on a weekly basis would allow for a better evaluation of this correlation. This finding is however in line with previous studies (Pearson et al., 1993; Meldrum et al., 2005; Hartnack et al., 2009), and diminishes the relative importance of broiler flocks (either by contaminating the environment or by contaminating broiler meat) as a source of human infections.

For the model combining temperature of the sampling month and the month preceding the sampling month, the range of the deviance explained indicates a stronger seasonality of incidence in some countries compared to others. This may be explained by the temperature time series used in this study being a crude measure for the temperature in the individual countries, and the fact that temperature might not explain all of the seasonality. The long-term trends for human and broiler incidences differ between the countries. For Denmark, Sweden and Iceland one could hypothesize that the concomitant decreasing trend for both campylobacteriosis in humans, and *Campylobacter* in broilers, could be due to the incidence of *Campylobacter*-positive broiler flocks playing a more important role in these countries in determining the incidence of human campylobacteriosis. Variation in antigenic diversity of *Campylobacter* spp. encountered, previous history of repeated exposures or single exposures to the bacterium, age, and variations in host immunity might also explain differences between countries (Altizer et al., 2006). In Norway the steady increase in campylobacteriosis incidence despite the decreased/stable incidence of *Campylobacter*-positive broiler flocks shows that probably other sources than undercooked broiler meat are of greater importance. Non-disinfected drinking water has been identified as a major risk factor in Norway (Kapperud et al., 2003). Another possible factor contributing could be Norway's low prevalence of *Campylobacter*-positive broiler flocks (Hofshagen and Kruse, 2005), leading to fewer exposures in the general population and development of only partial immunity in the infected population (Havelaar et al., 2009). This results in a highly susceptible population when exposed to *Campylobacter*. Variability of immunity, total or partial, within the population can also lead to misinterpretation of trends, by creating oscillations in disease incidence. Acquired immunity is widely recognized in developing countries and thought to be due to multiple exposures with high genetic diversity (Havelaar et al., 2009). It could be hypothesized that this aspect of acquired immunity coupled with less variation in the temperature, is why seasonality is not seen in the tropics.

A better understanding of the seasonality of *Campylobacter* is vital for achieving more cost-effective and successful control strategies. Further studies should therefore aim to identify possible drivers of seasonality and to understand how these can alter the incidence of campylobacteriosis and *Campylobacter* colonization in broiler flocks, wild birds and domesticated animals.

5. Conclusion

When comparing incidence of human domestic campylobacteriosis and incidence of *Campylobacter* positive broilers at slaughter from six countries in Europe, distinct and similar seasonal variations were found both within and between countries. By seasonally adjusting and detrending the incidence time series, the strong and similar seasonal pattern seen between human and broiler incidences within and between countries were removed. A strong association between the mean temperature in the sampling month and the month preceding the sampling month with the incidence of *Campylobacter* spp. positive broiler flocks and human campylobacteriosis in a given month was found by cross-correlations and the chosen Generalized Additive Model. The long-term trends varied between Denmark, Iceland, Norway, Sweden and the Netherlands. The seasonality seen in broilers and humans closely followed the temperature, and was probably caused, at least partly, by temperature related factors.

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References

Allos, B.M., 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis. 32, 1201–1206.

Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M., Rohani, P., 2006. Seasonality and the dynamics of infectious diseases. Ecol. Lett. 9, 467–484.

Anonymous, 2007. The community summary report on trends and sources, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J. 130.

Bouwknegt, M., van de Giessen, A.W., Dam-Deisz, W.D., Havelaar, A.H., Nagelkerke, N.J., Henken, A.M., 2004. Risk factors for the presence of *Campylobacter* spp. in Dutch broiler flocks. Prev. Vet. Med. 62, 35–49.

Chavasse, D.C., Shler, R.P., Murphy, O.A., Huttly, S.R.A., Cousens, S.N., Akhtar, T., 1999. Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. Lancet 353, 22–25.

Ekdahl, K., Normann, B., Andersson, Y., 2005. Could flies explain the elusive epidemiology of campylobacteriosis? BMC Infect. Dis. 5, 11.

Ethelberg, S., Simonsen, J., Gerner-Smidt, P., Olsen, K.E., Molbak, K., 2005. Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991–2001. Am. J. Epidemiol. 1008–1015.

Fleury, M., Charron, D.F., Holt, J.D., Allen, O.B., Maarouf, A.R., 2006. A time series analysis of the relationship of ambient temperature and common bacterial enteric infections in two Canadian provinces. Int. J. Biometeorol. 385–391.

Grassly, N.C., Fraser, C., 2006. Seasonal infectious disease epidemiology. Proc. Biol. Sci. 273, 2541–2550.

Hald, B., Skovgard, H., Bang, D.D., Pedersen, K., Dybdahl, J., Jespersen, J.B., Madsen, M., 2004. Flies and *Campylobacter* infection of broiler flocks. Emerg. Infect. Dis. 10, 1490–1492.

Hansson, I., Engvall, E.O., Lindblad, J., Gunnarsson, A., Vagsholm, I., 2004. Surveillance programme for *Campylobacter* species in Swedish broilers, July 2001 to June 2002. Vet. Rec. 155, 193–196.

Hansson, I., Vagsholm, I., Svensson, L., Engvall, E.O., 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. J. Appl. Microbiol. 103, 640–649.

Hartnack, S., Doherr, M.G., Alter, T., Toutounian-Mashad, K., Greiner, M., 2009. *Campylobacter* monitoring in German broiler flocks: an explorative time series analysis. Zoonoses Public Health 56, 117–128.

Hastie, T.J., Tibshirani, R.J., 1990. Generalized Additive Models, Chapman & Hall.

Havelaar, A.H., Van, P.W., Ang, C.W., Wagenaar, J.A., van Putten, J.P., Gross, U., Newell, D.G., 2009. Immunity to *Campylobacter*: its role in risk assessment and epidemiology. Crit. Rev. Microbiol. 35, 1–22.

Heier, B.T., Nygård, K., Kapperud, G., 2006. Campylobacteriosis in Norway 2005. Norwegian Institute of Public Health, Norway.

Hofshagen, M., Kruse, H., 2005. Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. J. Food Prot. 68, 2220–2223.

Jacobs-Reitsma, W.F., Bolder, N.M., Mulder, R.W., 1994. Cecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: a one-year study. Poult. Sci. 73, 1260–1266.

Janssen, R., Krogfelt, K.A., Cawthraw, S.A., Van, P.W., Wagenaar, J.A., Owen, R.J., 2008. Host-pathogen interactions in Campylobacter infections: the host perspective. Clin. Microbiol. Rev. 21, 505–518.

Johnsen, G., Zimmerman, K., Lindstedt, B.A., Vardund, T., Herikstad, H., Kapperud, G., 2006. Intestinal carriage of *Campylobacter jejuni* and *Campylobacter* coli among cattle from south-western Norway and comparative genotyping of bovine and human isolates by amplified-fragment length polymorphism. Acta Vet. Scand. 48, 4.

Jones, K., 2001. Campylobacters in water, sewage and the environment. Symp. Ser. Soc. Appl. Microbiol. 68S–79S.

Jones, K., Betaieb, M., Telford, D.R., 1990. Thermophilic *Campylobacters* in surface waters around Lancaster, UK: negative correlation with Campylobacter infections in the community. J. Appl. Microbiol. 69, 758–764.

Kapperud, G., Espeland, G., Wahl, E., Walde, A., Herikstad, H., Gustavsen, S., Tveit, I., Natås, O., Bevanger, L., Digranes, A., 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case–control study in Norway. Am. J. Epidemiol. 158, 234–242.

Kapperud, G., Skjerve, E., Vik, L., Hauge, K., Lysaker, A., Aalmen, I., Ostroff, S.M., Potter, M., 1993. Epidemiological investigation of risk factors for *Campylobacter* colonization in Norwegian broiler flocks. Epidemiol. Infect. 111, 245–255.

Kovats, R.S., Edwards, S.J., Charron, D., Cowden, J., D'Souza, R.M., Ebi, K.L., Gauci, C., Gerner-Smidt, P., Hajat, S., Hales, S., Hernandez, P.G., Kriz, B., Kutsar, K., McKeown, P., Mellou, K., Menne, B., O'Brien, S., Van, P.W., Schmid, H., 2005. Climate variability and campylobacter infection: an international study. Int. J. Biometeorol. 49, 207–214.

Louis, V.R., Gillespie, I.A., O'Brien, S.J., Russek-Cohen, E., Pearson, A.D., Colwell, R.R., 2005. Temperature-driven *Campylobacter* seasonality in England and Wales. Appl. Environ. Microbiol. 71, 85–92.

Lowell, W.E., Davis Jr., G.E., 2008. The light of life: evidence that the sun modulates human lifespan. Med. Hypotheses 70, 501–507.

Mann, D.R., Akinbami, M.A., Gould, K.G., Ansari, A.A., 2000. Seasonal variations in cytokine expression and cell-mediated immunity in male rhesus monkeys. Cell Immunol. 200, 105–115.

McDowell, S.W., Menzies, F.D., McBride, S.H., Oza, A.N., McKenna, J.P., Gordon, A.W., Neill, S.D., 2008. *Campylobacter* spp. in conventional broiler flocks in Northern Ireland: epidemiology and risk factors. Prev. Vet. Med. 84, 261–276.

Meldrum, R.J., Griffiths, J.K., Smith, R.M., Evans, M.R., 2005. The seasonality of human *Campylobacter* infection and *Campylobacter* isolates from fresh, retail chicken in Wales. Epidemiol. Infect. 133, 49–52.

Miller, G., Dunn, G.M., Smith-Palmer, A., Ogden, I.D., Strachan, N.J., 2004. Human campylobacteriosis in Scotland: seasonality, regional trends and bursts of infection. Epidemiol. Infect. 132, 585–593.

Newell, D.G., Fearnley, C., 2003. Sources of *Campylobacter* colonization in broiler chickens. Appl. Environ. Microbiol. 69, 4343–4351.

Nylen, G., Dunstan, F., Palmer, S.R., Andersson, Y., Bager, F., Cowden, J., Feierl, G., Galloway, Y., Kapperud, G., Megraud, F., Molbak, K., Petersen, L.R., Ruutu, P., 2002. The seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand. Epidemiol. Infect. 128, 383–390.

Olson, C.K., Ethelberg, S., van Pelt, W., Tauxe, R.V., 2008. Epidemiology of *Campylobacter jejuni* Infections in Industralized Nations. In: Nachamkin, I., Szymanski, C.M., Blaser, M.J. (Eds.), Campylobacter. ASM Press, Washington, DC, pp. 163–190.

Patrick, M.E., Christiansen, L.E., Waino, M., Ethelberg, S., Madsen, H., Wegener, H.C., 2004. Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. Appl. Environ. Microbiol. 70, 7474–7480.

Pearson, A.D., Greenwood, M., Healing, T.D., Rollins, D., Shahamat, M., Donaldson, J., Colwell, R.R., 1993. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. Appl. Environ. Microbiol. 59, 987–996.

R development Core Team, 2008. R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing, URL http://www.R-project.org, Vienna.

Reiersen, J., Breim, H., Hardardottir, H., Gunnarsson, E., Georgsson, F., Kristinsson, K.G., 2001. Human campylobacteriosis epidemic in Iceland 1998–2000 and effect of interventions aimed at poultry and humans. Int. J. Med. Microbiol. 291 (Suppl. No. 31).

Skelly, C., Weinstein, P., 2003. Pathogen survival trajectories: an eco-environmental approach to the modeling of human campylobacteriosis ecology. Environ. Health Perspect. 111, 19–28.

Stanley, K., Jones, K., 2003. Cattle and sheep farms as reservoirs of *Campylobacter*. J. Appl. Microbiol. 94 (Suppl), 104S–113S.

Stern, N.J., Hiett, K.L., Alfredsson, G.A., Kristinsson, K.G., Reiersen, J., Hardardottir, H., Briem, H., Gunnarsson, E., Georgsson, F., Lowman, R., Berndtson, E., Lammerding, A.M., Paoli, G.M., Musgrove, M.T., 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. Epidemiol. Infect. 130, 23–32.

Tam, C.C., Rodrigues, L.C., O'Brien, S.J., Hajat, S., 2006. Temperature dependence of reported *Campylobacter* infection in England, 1989–1999. Epidemiol. Infect. 134, 119–125.

Valérie, R.L., Gillespie, I.A., O'Brien, S.J., Russek-Cohen, E., Pearson, A.D., Colwell, R.R., 2005. Temperature-driven *Campylobacter* seasonality in England and Wales. Appl. Environ. Microbiol. 71, 85–92.

van de Giessen, A.W., Bloemberg, B.P., Ritmeester, W.S., Tilburg, J.J., 1996. Epidemiological study on risk factors and risk reducing measures for *Campylobacter* infections in Dutch broiler flocks. Epidemiol. Infect. 117, 245–250.

van Hees, B.C., Veldman-Ariesen, M.J., de Jongh, B.M., Tersmette, M., Van, P.W., 2007. Regional and seasonal differences in incidence and antibiotic resistance of *Campylobacter* from a nationwide surveillance study in The Netherlands: an overview of 2000–2004. Clin. Microbiol. Infect. 13, 305–310.

Vellinga, A., van Loock, F., 2002. The dioxin crisis as experiment to determine poultry-related *Campylobacter* enteritis. Emerg. Infect. Dis. 8, 19–22.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Springer-Verlag, New York, NY.

Wallace, J.S., Stanley, K.N., Currie, J.E., Diggle, P.J., Jones, K., 1997. Seasonality of thermophilic *Campylobacter* populations in chickens. J. Appl. Microbiol. 82, 219–224.

Wedderkopp, A., Gradel, K.O., Jorgensen, J.C., Madsen, M., 2001. Preharvest surveillance of *Campylobacter* and *Salmonella* in Danish broiler flocks: a 2-year study. Int. J. Food Microbiol. 68, 53–59.

Wingstrand, A., Neimann, J., Engberg, J., Nielsen, E.M., Gerner-Smidt, P., Wegener, H.C., Molbak, K., 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis. 12, 280–285.

Wood, S.N., 2006. Generalized Additive Models: an introduction with R, Chapman & Hall/CRC.