Weather Conditions and Campylobacteriosis In Germany

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List of abbreviations

BfR	German Federal Institute for Risk Assessment (‘Bundesinstitut für Risikobewertung’)
BKG	Federal Agency of Cartography and Geodesy (‘Bundesamt für Kartographie und Geodäsie)
Campylobacter spp.	*Campylobacter* species
ECA&D	European Climate Assessment & Dataset project
EFSA	European Food Safety Authority
EU	European Union
HACCP	Hazard Analysis and Critical Control Points
NOAA	National Oceanic and Atmospheric Administration
NUTS	Nomenclature of Territorial Units for Statistics
R^2	Coefficient of determination
RA	Reporting area
RKI	Robert Koch Institute
RR	Total daily precipitation
SSD	Sum squared deviation
Tavg	Average daily temperature
Tmax	Maximum daily temperature
Tmin	Minimum daily temperature
VBNC	Viable but non-culturable state
WHO	World Health Organization

1. Introduction

Campylobacter spp. is the leading bacterial cause for human gastroenteritis in the industrialised countries and the most common cause of bacterial foodborne illness in Germany (RKI, 2016). Among several known pathogenic subspecies, *Campylobacter jejuni* and *Campylobacter coli* are the most commonly associated subspecies for human campylobacteriosis (RKI, 2015a). While interventions to reduce zoonosis in the food chain, e.g. *Salmonella* succeeded in Germany; campylobacteriosis stays on an elevated level with 76,483 cases in 2018. Although outbreaks can occur, campylobacteriosis cases are mostly sporadic (Park, 2002).

Clinical symptoms can range from mild gastro-enteric symptoms with abdominal cramps to diarrhoea, and fever. The illness is mostly self-limiting; however severe chronical neurologic symptoms or arthritis sometimes occur (RKI, 2015a). Campylobacteriosis shows a bimodal age distribution, with high incidences in infants under the age of five and young, primarily male adults between the age of 20 to 29 (RKI, 2015a). In immunocompromised humans campylobacteriosis can be fatal (WHO, 2016).

The main transmission route of *Campylobacter* spp. is foodborne, via the consumption of undercooked poultry products, unpasteurised milk or contaminated drinking water, but also via contact with pets, working or living on a farm and traveling (Mattila et al., 1992; Saeed et al., 1993; Hawker, 2001; Evans et al., 2003; EFSA, 2014b; RKI, 2015a). Not least because campylobacteriosis is a foodborne disease, hygienic interventions at all stages of the food chain play a vital role in prevention and control.

Campylobacter is thermophilic, replicating at unusually high temperatures of 30 to 47 °C mirroring the conditions of its major host, the gut of warm-blooded avians (Doyle and Roman, 1981; Ketley, 1997; Park, 2002). Although replication is restricted to specific conditions, *Campylobacter* spp. is able to survive over a wide range of temperatures and climatic conditions. In wet, cold environments such as water currents survival is especially prolonged (Thomas et al., 1999).

In temperate climates, campylobacteriosis incidence shows strong seasonality peaking in the summer months (Allos and Taylor, 1998; Weisent et al., 2014). For Germany, a second

peak occurs in early January (Bless et al., 2017). It is well documented that increases in campylobacteriosis infection are attributed to weather, especially to temperature, and could explain its seasonality (Kovats et al., 2005; Louis et al., 2005; Weisent et al., 2014). Evidence for the association between temperature and campylobacteriosis exists for Canada, Australia and New Zealand (Kovats et al., 2005), Great Britain (Louis et al., 2005), the United States of America (Friedman et al., 2000) and Germany (Hartnack et al., 2009; Yun et al., 2016). The evidence for association with precipitation is inconsistent (Patrick et al., 2004; Kovats et al., 2005; Louis et al., 2005; Bi et al., 2008; Nichols et al., 2009; Lal et al., 2013).

For Germany, the association of campylobacteriosis and weather parameters has previously been studied on a low resolution and for selected regions (Hartnack et al., 2009; Yun et al., 2016). However, the mechanism of weather dependency in *Campylobacter* infections is not well understood.

So far, campylobacteriosis and weather parameters have not been studied on a dense, nationwide resolution. Therefore, the purpose of this thesis is to examine the association and possible dependency between campylobacteriosis incidence and local weather conditions on a dense temporal and spatial scale for Germany. Significant weather parameters and their timing with increased incidences on different weekly lag times will be identified.

With a changing global climate system, local weather is supposed to change at an unprecedented rate. By the end of this century, the global surface temperature is likely to exceed an increase of 2 °C (IPCC, 2013). Extreme weather events such as heat waves are very likely to take place more often and last longer, and extreme precipitation events will become more frequent and intense (IPCC, 2013).

In Germany, mean temperatures are predicted to rise, annual total precipitation to increase and extreme weather events such as hot days or heavy rain events to occur more frequently (Schönthaler et al., 2015). In this context of changing weather conditions, it will be discussed what the results of this study can contribute to predicting future trends of campylobacteriosis.

An understanding of the exact weather dependencies and lag times of campylobacteriosis towards specific weather conditions can inform health authorities and epidemiologists and ultimately help to enhance local forecasts, prevention, and control of campylobacteriosis in Germany.

The first chapter will introduce the clinical features of campylobacteriosis and the pathogen *Campylobacter*, its public health significance and climate-health implications. It also sets the framework for this study. In the ‘material and methods’ section, the data background and the novel analytical approach will be introduced; the third section presents the results which will be discussed in section 4. Chapter 5 draws conclusions and discusses the limitations of this study.

1.1 Campylobacteriosis

1.1.1 Clinical features of campylobacteriosis

While campylobacteriosis remains asymptomatic in most cases, immunocompromised patients may experience severe symptoms of acute enteritis, which cannot be distinguished from other aetiologies. The mild form of campylobacteriosis is self-limiting, with relapses of 5 to 10 % of all cases (RKI, 2015a).

Prodromal symptoms are developed approximately three days after infection and consist of fever (38 to 40 °C), headache with myalgia and fatigue. Common enteric symptoms include watery, sometimes bloody diarrhoea, abdominal pain, and cramps (RKI 2015). Acute diarrhoea itself lasts between two to three days on average (Ketley, 1997). The intestinal mucosa reveals oedema, hyperemia with petechiae and brittleness (Ketley, 1997). Rarely, post-infectious and chronical complications occur such as the Guillain-Barré syndrome, irritable bowel syndrome or reactive arthritis (RKI 2015, Altekruse et al. 1999). Guillain-Barré is a post-inflammatory polio-like form of paralysis, in some cases leading to respiratory failure, arrhythmia, and death (Masuhr und Neumann 2005). Deaths from *C. jejuni* are rare and occur mainly in infants, the elderly and patients with pre-existing illnesses (Altekruse et al. 1999). Occasionally, *C. jejuni* becomes invasive and may manifest as meningitis, pneumonia or miscarriage (Hunt et al., 2001).

The minimal infective dose is usually small and varies between 5 to 800 pathogenic germs on average (Black et al., 1988). However, depending on the *Campylobacter* strain, the

susceptibility of the host and environmental stressors, the infective dose can alter with up to 10,000 cells (Hunt et al., 2001). Since the average infectious dose is relatively low, survival of only a few organisms is relevant for transmission (Ketley, 1997). Still, the rate of infection increases with dose (Black et al., 1988).

Incubation time ranges between two to five days, with a variance of one to ten days (RKI, 2015a). Contagiousness lasts as long as *Campylobacter* is secreted with the stool which lasts approximately two to four weeks (Cha and Lehman, 2016).

The usual way of diagnosis is by detecting *Campylobacter* in culture from stool samples (Allos 2001). Furthermore, antigen analysis via ELISA as well as DNA detection with PCR technique is increasingly applied. In the case of epidemiological issues, such as revealing infection chains in outbreaks, refined typing via pulsed-field gel electrophoresis can be useful (RKI, 2015a).

Since the disease is often self-limited, a symptomatic therapy suffices typically including volume and electrolyte substitution. For more complicated infections in patients with high fever, suspected sepsis or chronical courses, antibiotic treatment is indicated. Preferably macrolides such as Erythromycin or second line quinolones should be applied then (RKI 2015). Unfortunately, increasing resistance of *C. jejuni* strains against antibiotic drugs has been observed. Especially fluoroquinolone resistance (e.g., Ciprofloxacin) is high in humans (Altekruse et al., 1999; EFSA, 2014b). The EFSA (2014b) reported that over half of *Campylobacter jejuni* and two-thirds of *C. coli* isolates in humans are resistant. However, co-resistances are still low in humans, with 1.7 % in *C. jejuni* and 4.1 % in *C. coli* (EFSA, 2014b).

1.1.2 *Campylobacter* spp.

To find out how weather conditions affect campylobacteriosis, it is compulsory to gain an understanding of what is known about campylobacters morphology, sources and transmission routes, ecology, genetics and life cycle. However, knowledge about its microbiology is still incomplete.

1.1.2.1 Morphology and bacterial characteristics

Campylobacter spp. are Gram-negative, non-spore forming bacteria. From 20 currently identified species, three among them are pathogenic for human beings: *C. jejuni*, *C. coli,* and *C. fetus*. *C. jejuni* is the most frequently designated causative agent in human campylobacteriosis and stands for more than 99 % of all isolates (Hunt et al., 2001; Neumeister, 2009; Semenza et al., 2012). The morphology of *Campylobacter jejuni* is comma and corkscrew-like with flagella on each end providing motility (Fig. 1). As a result of stress, such as increased temperature or oxygen, *Campylobacter* transforms into a coccoid form (Ikeda and Karlyshev, 2012).

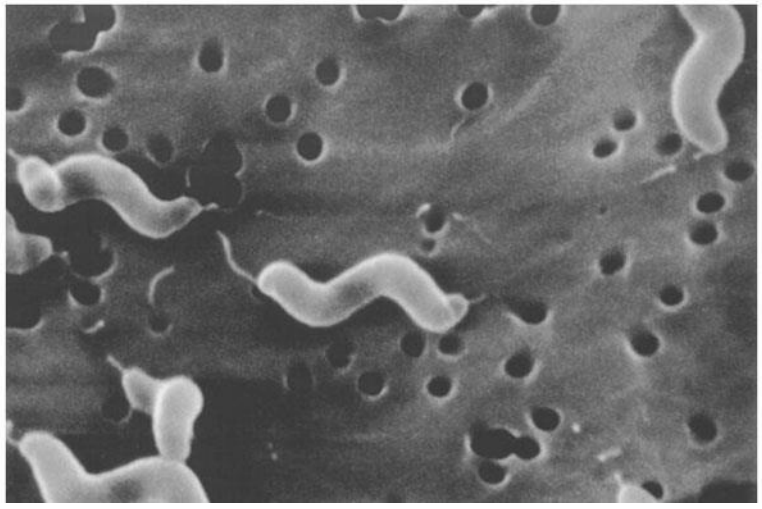

Fig. 1: Electron microscope image of *Campylobacter* jejuni showing its corkscrew shape (adapted from: Altekruse et al., 1999).

The work of Elizabeth O. King was the first to provide a detailed description of *Campylobacter* spp., called ‘related vibrios’ back then (King, 1962). Later it was found that they represented the two species *Campylobacter jejuni* and *C. coli* (Skirrow, 1977). In the 1970s, *Campylobacter* was firstly isolated from human stool samples in patients with acute enterocolitis (Dekeyser et al., 1972; Skirrow, 1977).

1.1.2.2 Sources and transmission routes

Campylobacter occurs ubiquitary. As natural enteral commensals, major reservoirs are the guts of wild and domestic animals, such as birds and mammals. Although *Campylobacter* is widespread, favoured hosts are wild and urbanised avian populations, e.g. ducks, geese or gulls (Fallacara et al., 2001; Newell and Fearnley, 2003; Kinzelman et al., 2008). An important source of *Campylobacter* is productive livestock, mainly poultry, in rare cases also pigs, dairy cattle or veal (Ghafir et al. 2007). Among poultry, high quantities of *Campylobacter* spp. are found in the gut, cloacae, and ceca, but here they are restricted to the mucosa of intestinal crypts (Beery et al., 1988).

Besides animal hosts, the aquatic environment is an important reservoir with excellent survival conditions for campylobacters. Water bodies, such as rivers, surface waters, and streams have been identified as potential sources of infection (Bolton et al., 1987; EFSA, 2014b). Especially, streams running through grazed farmland are substantially contaminated with campylobacters (Jones et al., 1996; Baker et al., 2002; McBride et al., 2005). In this context, private well waters in rural areas used as water supply were found responsible for campylobacteriosis cases (Lévesque et al., 2013). Moreover, bathing in natural lakes with ingestion of contaminated water poses a risk (Schonberg-Norio et al., 2004; RKI, 2015a).

Campylobacter transmission is mainly believed to happen through various foodstuffs, encompassing the whole chain from farm to fork. Fig. 2 summarises potential transmission trajectories of *Campylobacter*.

Among sporadic cases, key sources are raw and undercooked poultry meat and related products, though not eggs (Friedman et al., 2000; EFSA, 2014b; RKI, 2015a). Especially carcasses of broiler chickens are highly contaminated due to contact with faeces during slaughter (BfR, 2013). The higher prevalence of *Campylobacter* in neck skin samples compared to caecum skin samples supports the assumption that relevant contamination of carcass bodies with digestive tract content takes place during slaughter (Stingl et al., 2012; BVL, 2016). In Germany, 52.3 % of all carcasses were found *Campylobacter*-positive in 2013 (BfR, 2013). Compared to numbers in 2011 this is an increase of 11.5 %. Less than at slaughterhouse level, broiler meat is still substantially contaminated at retail with a share of 37.5 % (BfR, 2013). At the farm level, cross-contamination plays a vital

role in the bacterial transfer, for example when persons take care of several houses, surface water is non-disinfected (Kapperud et al., 1993), other animals live on the farm, or hygiene measures are inadequately carried out (van de Giessen et al., 1998). Apart from poultry, other food sources of infection include non-pasteurised milk, contaminated drinking water, bottled water (Evans et al., 2003), shellfish and ostriches (WHO, 2017), raw mince, contaminated fruit, such as grapes (Neimann et al., 2003) and vegetable e.g. salad vegetable (Evans et al., 2003). Especially in outbreaks, raw milk and contaminated drinking water have been identified as a significant source of infection (Hänninen et al., 2003; EFSA, 2014b). Moreover, the habit of eating out, e.g., in a restaurant, canteen, etc. has been identified as a crucial risk factor (RKI, 2017).

Apart from foodstuffs, transmission routes occur through infected domestic animals, mainly cat, and dog puppies but also farm animals (Saeed et al., 1993; Hawker, 2001; Evans et al., 2003; EFSA, 2014b; RKI, 2015a). Direct human-to-human transmission is rare but especially present in children (RKI, 2015a). Further along the infection chain, essential contamination areas are kitchens where raw poultry meat is prepared, sometimes leading to cross-contamination (Cogan et al., 1999). Other important trajectories are vectors, such as flies (Nichols, 2005) or litter beetles (Refrégier-Petton et al., 2001) which have been discussed as transmission vehicles at the farm as well as at food preparation level.

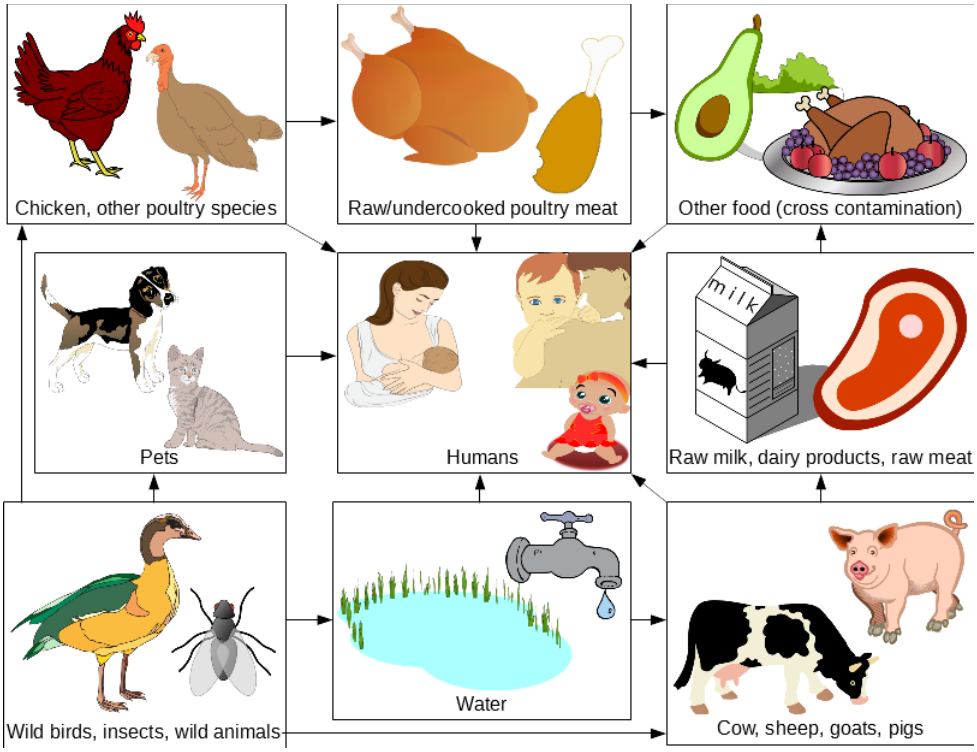

Fig. 2: Transmission trajectories of *Campylobacter* (adapted from: Friedman et al., 2000).

1.1.2.3 Ecology and life cycle

Genetics and life cycle

During its life campylobacters cope with multiple stress conditions in the environment, survival in vectors such as flies, passing through the gastrointestinal system of animal and human hosts, during food processing and storage. Therefore, the successful colonisation and infection of the host is a complex 'multifactorial process with key roles for swimming motility, chemotaxis, interaction with gut epithelial cells, toxin production, and oxidative and metabolic stress adaptation' (Vries et al., 2017).

The genome of C. *jejuni* consists of a relatively small genome of approximately 1.7 megabases (Taylor, 1992). Vries et al. (2017) found 486 genes essential for C. *jejuni* survival and growth, making up roughly a third of the genome. Unlike other enteric agents, campylobacters lack common key stress regulators (Parkhill et al., 2000). However, the genome comprises genes that are vital for *Campylobacter* adaptation in various parts of the life cycle, such as genes of the flagella system (e.g., fliW and fliD), as well as surface structures like lipooligosaccharides and capsular polysaccharides. These structures are

needed for colonisation of the human and animal host (e.g., pstABS and phoR), chemotaxis (e.g., livM), adhesion and invasion of human gut epithelial cells (e.g., engD) (Vries et al., 2017). Some genes are found to be crucial in the invasion of several hosts (e.g., chicken, pigs, and humans). Others exclusively work for distinct environmental conditions. For example, the TrxC gene is required for survival at low temperatures and plays a role in oxidative stress responses (Vries et al., 2017). For the growth of *C. jejuni*, elements of the gluconeogenesis pathway used for biosynthesis are required (Vries et al., 2017). These identified genes responsible for *Campylobacter* fitness in the host and environment might provide targets in future control interventions.

Oxidative stress

Campylobacter jejuni is microaerophilic, requiring an O_2 concentration of 5 to 10 % and a CO_2 concentration of 3 to 5 % (Ketley, 1997; Newell, 2002; Neumeister, 2009). For protection against adverse atmospheric conditions, *Campylobacter* spp. forms a variety of biofilms to increase its resistance (Joshua et al., 2006). Additionally, several anti-oxidant enzymes are known in campylobacters as oxidative defence systems, such as superoxide dismutase (SOD) and catalase (KatA) playing an essential role for survival, especially in foodstuffs (Purdy and Park, 1994; Purdy et al., 1999). In an environment of oxidative stress combined with high temperatures (42 °C), *Campylobacter* declines more rapidly than at low temperatures (4 °C) (Garénaux et al., 2008). Hence, increased temperature decreases the resistance of campylobacters against oxidative stress.

Temperature and humidity

Campylobacter spp. is thermophilic only growing at temperatures between 30 °C and 47 °C (Doyle and Roman, 1981; Hazeleger et al., 1998; Chan et al., 2001). The optimum growth temperature of 42 °C is unusually high (King, 1962; Doyle and Roman, 1981; Hunt et al., 2001). This characteristic is interpreted as an adaptation to its natural habitat, the gut of warm-blooded birds (Ketley, 1997; Koenraad et al., 1997; Park, 2002; Cha and Lehman, 2016).

The thermophilic character of *Campylobacter* spp. has several implications. *Campylobacter* is unable to replicate at temperatures used to store food or at room

temperature (Lawley, 2013). Genome sequencing revealed that, unlike other bacteria*, C. jejuni* does not produce cold shock proteins (Parkhill et al., 2000) which allow other bacteria to grow below their optimum growth temperature (Phadtare et al., 1999). This characteristic might, in part, explain the inability of campylobacters to grow below 30 °C. In contrast, *C. jejuni* produces a number of heat shock proteins as a response to heat stress, which could explain its thermophilicity (Parkhill et al., 2000).

While replication is unlikely outside the host microcosm and below temperatures of 30 °C, *Campylobacter* can survive over a wide range of environmental conditions for an extended period (Blaser et al., 1980; RKI, 2015a; Cha and Lehman, 2016).

Especially at low ambient temperatures of around 5 °C and in aquatic niches *Campylobacter* survives best (Thomas et al., 1999). Several authors found prolonged survival at 4 °C under wet and microaerophilic conditions in vitro where *C. jejuni* persisted for four weeks up to four months (Rollins and Colwell, 1986; Hazeleger et al., 1998; Reezal et al., 1998; Hunt et al., 2001). The viability for months in stream water makes it possible for the bacteria to overwinter in slow currents and be uptaken by animal hosts in spring when temperatures rise (Rollins and Colwell, 1986). Against drought stress, *Campylobacter* is very sensitive though, and its resistance varies only between two to ten hours before they decay (Fernández et al., 1985).

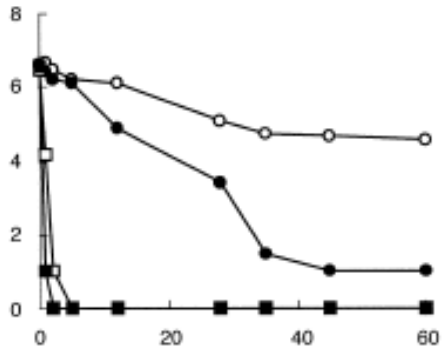

Fig. 3: Survival *of Campylobacter jejuni* in sterile de-ionized water at 5 °C (o), 15 °C (•), 25 °C (□) and 37 °C (■) (adapted from: Thomas et al, 1999).

At freezing temperatures of -20 °C, *C. jejuni* is able to survive two to five months and can still be isolated from frozen meat (Fernández and Pisón, 1996; Hunt et al., 2001). However, viability is significantly reduced at these temperatures with a marked decline within the first 10 to 30 days (Humphrey and Cruickshank, 1985; Chan et al., 2001; Hunt et al., 2001). Reduced viability at -20 °C has been explained by cell death due to freezing, thawing and contributive oxidative stress (Stead and Park, 2000; Chan et al., 2001).

With increasing temperature, inactivation of campylobacters is more rapid (Fig. 3 (Rollins and Colwell, 1986; Thomas et al., 1999; Membré et al., 2013). Although many studies exist on the survival and growth of *Campylobacter* at different temperatures, not the complete thermal spectrum is covered.

Especially the survival of *Campylobacter* at a range from 5 to 25 °C needs further consideration to find out which ambient temperature defines the tipping point of survival.

Strains of *C. jejuni* which are presumably of paramount relevance for infections in consumers are those adapted to survive in refrigerated alimentation stored at approximately 4 °C (Chan et al., 2001). Survival of *Campylobacter* was found 15 times longer at 2 °C than at 20 °C. At room temperature of 20 °C survival was only restricted to a few days (Chan et al., 2001; Lawley, 2013). Terzieva and McFeters (1991) also found better survival at 6 °C than at 16 °C in surface water. Above temperatures of 48 °C, *Campylobacter* cells are ultimately destroyed and therefore do not survive pasteurisation (Lawley, 2013). In sum, at temperatures of 4 °C under humid conditions *Campylobacter* spp. shows maximal survival.

Besides mere survival, temperature also influences motility in campylobacters. Motility is realised by a flagellum, which is an important virulence factor since it regulates the ability to colonise the host. Motility is supposed to be particularly high in campylobacters due to the combination of flagellum and cell shape (Ketley, 1997). Flagella production has been found to be influenced by the activity of the temperature-dependent σ^{54} promoter which is highest around the optimum growth temperature of 42 °C (Ketley, 1997). As a result, colonisation in the avian host can be most effective (Alm and Guerry, 1993).

Viable but non-culturable state

In adverse environments, *Campylobacter* tends to transform from a culturable rod shape into a non-culturable coccoid form, the so-called ‘viable but non-culturable state’ (VBNC). This non-culturable form occurs when stored at 4 °C for example (Rollins and Colwell, 1986). There is reason to suspect that VBNC can colonise poultry, although evidence about transmission relevance is inconsistent (Medema et al., 1992; Stern et al., 1994; Cappelier et al., 1999). This ability of ‘degraded’ campylobacters to colonise could explain inoculation of chicken from the environment and could bridge the gap between survival in an adverse environment until a new host is found (Stern et al., 1994).

pH

Optimal *Campylobacter* growth occurs at a pH of 6.5 to 7.5 (Lawley, 2013). Below pH 4.9 *Campylobacter* does not grow in culture and a pH <3.6 is lethal (Blaser et al. 1980). Consequently, refrigerated milk is an example of a perfect milieu where a suitable acid buffer is provided for campylobacters to survive for several weeks (Blaser et al., 1980). Other milieus with favourable pH conditions and thus a high potential for transmission are contaminated urine from animals, stool from infected patients and contaminated cold (4 °C) groundwater. Gastric acid altering physiologically between a pH of 1 to 7, is an essential determinant of the infectious dose of an individual (Blaser et al., 1980).

Biofilm and water movement

Stationary media provide favourable growth conditions for campylobacters since the production of viscous biofilm layers is facilitated. Those microcosms, produced by the bacteria itself, offer controlled conditions concerning oxygen and nutrient concentration. In contrast, moving systems like stream waters are aerated with oxygen which results in a decline of campylobacters or transformation into a VBNC state (Rollins and Colwell, 1986).

Metabolism and nutrients

Campylobacter spp. are not able to ferment carbohydrates nor to degrade other complex substances, so culture media have to meet special requirements (Skirrow, 1977; Bolton

and Robertson, 1982; Griffiths and Park, 1990). The composition of media has to be adapted to the *Campylobacter* strain, antibiotic resistance patterns and the sample type in question (Griffiths and Park, 1990). *Campylobacter* spp. is longer culturable in nutrient-poor environments, probably due to lower metabolic activity (Hazeleger et al., 1995). Here again, temperature determines transition into the VBNC state, that is faster at 25 °C than at 4 °C. This is explained by higher metabolism at higher temperatures and therefore quicker transition from spiral to coccoid forms. In contrast, nutrient-rich environments seem to accelerate the process of coccus formation (Rollins and Colwell, 1986; Hazeleger et al., 1995).

UV radiation

It has been examined that UV radiation can be toxic to campylobacters (Hoffman et al., 1979). While campylobacters are present all year in rivers, they decline with coinciding high sunshine hours and higher temperatures (Bolton et al., 1987; Obiri-Danso et al., 2001). Thus, elevated temperatures and high UV radiation have been used to explain low *Campylobacter* numbers in coastal waters during summer (Obiri-Danso et al., 2001).

1.1.2.4 Intermediate summary

Infection trajectories of *Campylobacter* are complex due to its numerous animal and environmental reservoirs, multiple risk factors, and vehicles. At the same time, campylobacters genetics reveal a high survival fitness and ability to adapt to various environmental conditions.

Weather (e.g., ambient temperature) does not have a direct effect on *Campylobacter* replication since it is unlikely outside the host. However, the pathogen can survive in various environmental conditions, especially in aquatic niches. While moderate to cold temperatures with low oxygenation are optimal for an extended survival, warm temperatures are more limiting. Additionally, intact biofilms prolong survival of campylobacters, and their high motility is the foundation for colonisation success. For the microbiology of the pathogen, especially temperature but also humidity are essential drivers of motility, metabolism, resistance towards stressors such as oxygen,

transformation into the VBNC state, growth and survival of *Campylobacter*. It needs consideration in how far these associations can be conferred to the relationship between campylobacteriosis and weather conditions.

1.1.3 Public health significance of *Campylobacter* in Germany

1.1.3.1 Epidemiology

Campylobacteriosis occurs worldwide, and *Campylobacter* spp. is considered the most common cause of human bacterial gastroenteritis in the world (WHO 2016). The socio-economic burden is substantial due to its high prevalence, duration, and complications. Per year one in ten people contract an infection and 33 million healthy life years are lost worldwide (WHO 2016). In the European Union (EU), *Campylobacter* is the most common gastrointestinal bacterial pathogen, and notification rates show a significant increase of 13 % from 2013 to 2014 (ECDC 2016). 97 % of all infections are identified as being sporadic cases. Less frequent are cases in the context of detected outbreaks (Pebody et al., 1997; Park, 2002). EU-wide, 47.7 % of all campylobacteriosis cases were hospitalised, and 31 deaths were counted in 2012 (EFSA, 2014b).

Since 2011, Germany was listed among the EU countries with the highest annual number of cases, together with the United Kingdom and Czech Republic (ECDC 2016). In 2014, Germany even topped the EU-wide list with the highest total case numbers, reaching 70,190 cases (RKI, 2015c; ECDC, 2016a). At present, *Campylobacter* enteritis is the most commonly reported bacterial gastrointestinal infection and, after Norovirus, the second most common diarrheal infection in Germany (EFSA, 2014b; RKI, 2015c). In comparison, the widely perceived salmonellosis is solely ranked number 4 among the most frequent enteropathogenic diseases in Germany (RKI, 2011).

During this study’s observation period between 2001 and 2014, the overall trend of campylobacteriosis incidences was rising in Germany. The study data can reproduce this trend. Fig. 4 displays the cumulated Germany-wide annual incidence rate between 2001 and 2014. The graph demonstrates the peaks and falls of incidence over time and shows a cyclic pattern. In this period, the yearly incidence increased from 70.6 per 100,000 in 2001 to 95.6 in 2014 where it also reached its overall maximum. Drops in incidence

occurred in 2003 and 2006. The total minimum incidence was reached in 2003 with 61.3. Whereas incidences increase up to 78.4 in 2005, fall steeply in 2006 to 66.0 to rise again steeply by 26.5 % to 83.5 in 2007. This steep rise from 2006 to 2007 is congruent with data from Stark et al. (2009). The rising trend of campylobacteriosis highlights the relevance of the disease and must be taken into account when interpreting results.

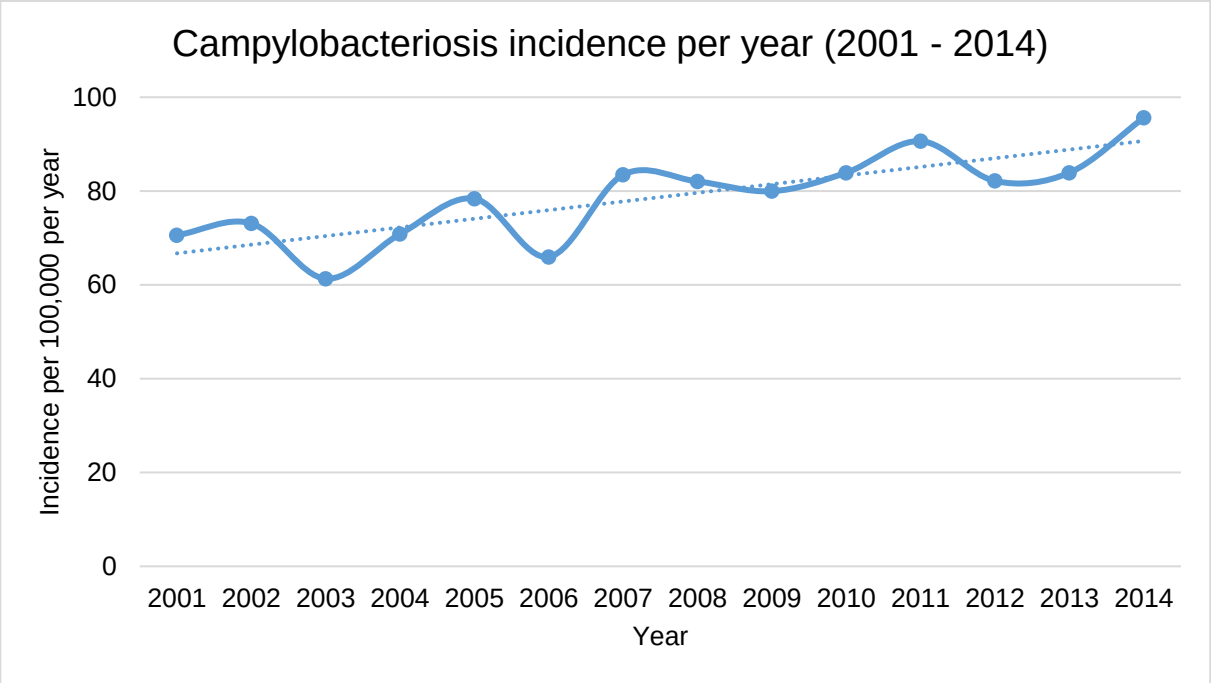

Fig. 4: Reported annual campylobacteriosis rates per 100,000 inhabitants and trend line(dotted), 2001 – 2014 (Data source: SurvStat@RKI, last reviewed: 21.08.2016).

For campylobacteriosis, several risk groups have been identified. Highest incidences experience infants under the age of five with equal distribution among the genders (Louis et al., 2005; ECDC, 2016a). In Germany and other industrialised countries, a second peak is observed in young, mainly male adults between the age of 20 to 29 (Louis et al., 2005; RKI, 2015a). Not only do young infants represent the largest age group among campylobacteriosis cases, but they are also at higher risk of developing severe complications (Louis et al., 2005; Fitzenberger et al., 2010; ECDC, 2016a). Deaths from campylobacteriosis are rare, but they occur more likely in infants and immunocompromised patients, such as the elderly and patients with pre-existing diseases

(Altekruse et al. 1999). For immunocompromised patients with HIV/AIDS, bacterial infections like campylobacteriosis occur more frequently and are more severe due to bacteraemia as a potentially lethal complication (Angulo and Swerdlow, 1995; Manfredi et al., 1999). Furthermore, medical conditions like reduced gastric acid due to use of proton pump inhibitors (Neal et al., 1996; RKI, 2017) or diabetes disease (Neal and Slack, 1997) are associated with increased risk of campylobacteriosis. Also, people living on a farm or in rural areas with agricultural activities nearby are at higher campylobacteriosis risk than people living in urban areas (Louis et al., 2005; Lévesque et al., 2013). Other risk factors are the use of household water coming from private wells and the occupation in a pet shop, zoo or veterinary clinic for instance (Lévesque et al., 2013).

1.1.3.2 Reporting obligations and surveillance

If a person with a suspected *Campylobacter* infection is identified, there are certain reporting obligations to consider. According to Article 1 § 7, German Infection Protection Act, campylobacteriosis is a mandatory reportable disease in Germany (RKI, 2015a). Direct and indirect detection of *Campylobacter* spp. in humans is to be reported by name to the local health authority where the patient resides. Furthermore, a suspected acute infectious gastroenteritis must be reported in case of one of the following conditions: Either the person concerned is handling with food or is working in an institution of communal catering (Art. 42, Sect. 1), or two or more similar diseases occur with a suspected epidemiological connection (RKI, 2015a). Additionally, to avoid outbreaks directors of community bodies (nurseries, e.g.) have to report to the local health authority if children under the age of 6 fall ill of an infectious gastroenteritis or are suspected to do so (RKI, 2015a).

In the reporting algorithm, the first reporting step is carried out by the attending doctor or diagnostic laboratory. Reports are made to the local health authorities within 24 hours maximum after notice. Via postal code of the patient’s principal residence, the report is allocated to the local health authority in charge (RKI, 2015b). Although the patient’s residence is not necessarily equal to the place of infection, most reported diseases (92 %) in Germany are acquired in Germany, as well (RKI, 2017). The notification data will be anonymised and forwarded from local to state health authority within one working day and

then collected nationwide by the Robert Koch Institute (RKI), the federal public health institute of Germany (RKI, 2015b). The nationwide data collection is transferred to the EU commission and the World Health Organisation (WHO).

The RKI counts four conditions as cases in their statistics (RKI, 2015c): (i) Clinical epidemiological cases, (ii) clinical laboratory-diagnosed cases, (iii) laboratory-diagnosed cases with unfulfilled clinical presentation and (iv) laboratory-diagnosed cases with unknown clinical presentation.

Fig. 5 shows a flow-chart of the reporting system for Saxony-Anhalt which is comparable to other federal states in the case of campylobacteriosis.

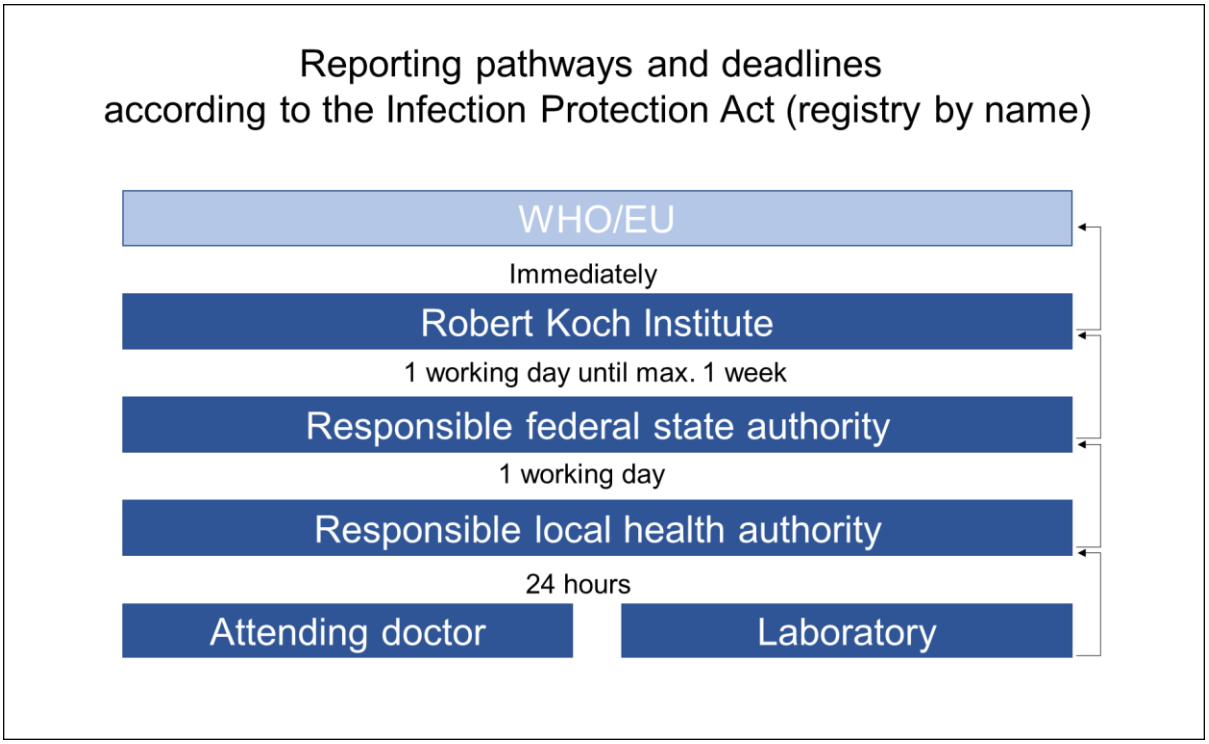

Fig. 5: Reporting system of namely notifiable diseases (adapted for Saxony-Anhalt from SA-LAV, 2017).

1.1.3.3 Prevention

At several levels of the known transmission routes, preventive measures can be applied. At slaughterhouse level, adequate slaughter hygiene should be considered especially in

poultry and pigs. Thereby, the overall *Campylobacter* spp. infestation in the slaughtered animal population should be minimised (RKI, 2015a). On the consumer side, preventive measures primarily consist of adequate handling of potentially contaminated foods. Meat, especially poultry should be well cooked and should reach an internal temperature of at least 73.9 °C as measured with a food thermometer. Freezers should register at least -17.8 °C and refrigerators 4.4 °C. Freezing significantly reduces bacterial load while refrigerating will not (Christensen et al., 2001). However, freezing is not reliable to entirely inactivate campylobacters. Raw milk must be pasteurised and drinking water treated with water filters (FSIS, 2013). Moreover, utensils and surfaces in the kitchen should be washed with soapy water between different food items, especially between raw food and meat. Towels must be washed in the hot washing programme (FSIS, 2013).

In general, cross-contamination is minimised if different food items prone to *Campylobacter* contamination are separated at all times from retail to refrigerator (FSIS, 2013). To minimise human-to-human-transmission, hands should be washed with soap for at least 20 seconds before and after handling foods, after using the bathroom and after contact with potentially contagious people, animals or objects (FSIS, 2013).

An individual suffering from campylobacteriosis is supposed to stay at home carrying out hygiene measures and only re-enter community bodies after full remission of diarrhoeal symptoms (RKI, 2015a). A person diseased with campylobacteriosis must not work in a food processing facility or other related facilities handling food, according to paragraph 42, German Protection against Infection Act (RKI, 2015a). Prophylaxis in the form of vaccination against *Campylobacter* infections is unsatisfying to date (RKI, 2015a).

In the case of an outbreak, the source of infection must be identified to prevent further infections. Therefore, medical and veterinary institutions have to cooperate, and in case of a suspected epidemiological connection to foods or infectious animals, the local health authority informs the Veterinary and Food Control Authority and vice versa (RKI, 2015a).

1.2 Climate change and health

Environmental and social determinants of human health are affected by a changing climate. The following chapter gives an overview of present and future climate trends from global to local scale, its effects on health and implications for infectious diseases such as campylobacteriosis.

1.2.1 General aspects

The climate system is undergoing unprecedented changes. On a global scale, the averaged surface temperature has increased by 0.85 °C between 1880 and 2012. Warm days and nights have increased whereas cold days and nights have decreased. In addition, the ocean has warmed, and the sea level has risen 18 mm per year since 1961. At the same time, snow and ice have diminished and greenhouse gases within the atmosphere have increased (IPCC, 2013). Moreover, extreme weather events such as heat waves and heavy rain events have tripled worldwide since the 1960s until present (WHO, 2017).

In Germany, climatic changes have also been observed. Average yearly air temperature has increased by 1.2 °C between 1881 and 2013 (Schönthaler et al., 2015). The most pronounced warming with 1.3 °C has been registered in the spring season. Furthermore, hot days defined as days with maximal daily temperatures of 30 °C or higher, have increased from three to eight days per year between 1951 and 2013. In the same period, ice days have decreased from 27 to 21 days (Schönthaler et al., 2015). Apart from changes in temperature, alterations in precipitation amounts and patterns have been registered in Germany. Annual total precipitation rose on average by 10.6 % since 1881. Seasonally, most changes occurred in winter where the total amount of precipitation has tripled in the same period. Droughts have not significantly changed in their frequency (Schönthaler et al., 2015).

Prospected climate trends taper off what has been observed until present. On the global scale, the surface temperature is likely to exceed an increase of 1.5 °C with a variance of up to 4.0 °C by the end of this century (IPCC, 2013). Extreme precipitation events are

projected to become more frequent and intense in some regions (IPCC, 2013). Other regions will experience an increase in desiccation. Mean sea level will continue to rise and oceans will acidify and continue to warm (IPCC, 2013).

In Germany, the mean air temperature is projected to increase between 2.5 to 3.5 °C in the period from 1950 to 2100 (Jacob et al., 2008). Warming is expected to be more pronounced in winter than in summer. Winter temperatures are likely to increase by 2.0 to 4.5 °C and summer temperatures by 1.5 to 5.0 °C by the end of this century (Jacob et al., 2012). Germany-wide there is not a pronounced trend regarding annual average of precipitation (Jacob et al., 2008). However, seasonal precipitation will underlie significant alterations. Projections estimate wetter winters with an increase in precipitation of up to + 20 % by the end of this century. Summers are projected to be drier with a decrease of total precipitation within a range of -25 % to +5 % (Jacob et al., 2012). These changes are expected to underlie regional variabilities. For example, in eastern continental regions of Germany, heat extremes are likely to increase whereas summer precipitation is projected to decrease (EEA, 2017). The Atlantic regions in the north-west of Germany are likely to be more prone to heavy rain events and climatic hazards such as storms (EEA, 2017). In sum, the climate in Germany is projected to become wetter in winter, drier in summer, and on average warmer and more extreme.

1.2.2 Climate change effects on health

There is a broad consensus that climate change represents a substantial challenge for human health (Schönthaler et al., 2015) and is expected to cause about 250,000 deaths per year worldwide between 2030 and 2050 (WHO, 2017). The WHO (2017) stated in its report on climate change and health:

> ‘Climate change affects the social and environmental determinants of health – clean air, safe drinking water, sufficient food and secure shelter.’

Thus, the effects of climate change on human health are multidimensional. While extreme heat contributes directly to deaths from cardiovascular and respiratory disease, it also indirectly contributes by increasing levels of ozone and other pollutants. With increasing temperature, pollen levels rise and exacerbate asthmatic diseases (WHO, 2017).

Moreover, rising sea levels diminish the livelihood of many people since more than half of the world's population lives within 60 km distance from the sea. Extreme weather events like floods can destroy homes, medical facilities and other services, and water supplies can be perturbed through flooding. In turn, a lack of safe drinking water increases the risk of diarrheal diseases, vector- and waterborne diseases. In poor regions, instability of food production due to variability in precipitation can lead to mal- or undernutrition which will decrease human resilience against diseases (WHO, 2017).

In Germany, the increased burden of heat has led to approximately 52,000 deaths in the hot summer of 2003. Especially the elderly, chronically sick people, as well as small children, are affected (Schönthaler et al., 2015). These groups are at special risk since their ability to adapt to heat stress is constitutionally reduced. Causes of health impairments are a loss of fluid and electrolytes, and excessive stress for the cardiovascular system (Schönthaler et al., 2015).

Apart from direct consequences from heat stress, a warmer climate in Germany has also indirect health implications. With a milder climate and prolonged vegetation periods, pollen-flight times are extended. This increases the burden of allergic symptoms in patients with atopic predispositions. Moreover, with increased ambient temperature thermophilic plants find more suitable conditions. This is the case for the introduced *Ambrosia artemisiifolia*, originating from North America. This mugwort plant is becoming more common in Germany since the 1990s. It is highly allergenic and relatively low pollen concentrations can already trigger allergic symptoms in sensitised patients. Other health impairing phenomena which seize ground with warmer temperatures are the oak processionary (*Thaumetopoea processionea*) in oak forests, as well as increased blue algae/cyano-bacteria appearance in swimming lakes, both causing toxic-irritative skin lesions (Schönthaler et al., 2015). Moreover, especially in the Rhine valley, the Asian tiger mosquito (*Aedes albopictus*) has been observed since 2007. Originating from Southeast Asia, this mosquito is a potential vector for infectious diseases such as Chikungunya fever or Dengue (Schönthaler et al., 2015). In sum, climate change raises public health issues in Germany and worldwide and might impair human health in the future.

1.2.3 Climate and infectious diseases

The WHO considers climate as an important determinant in the epidemiology of infectious diseases (Stark et al., 2009). 63 % of infectious pathogens in European human and domestic animals are known to be climate sensitive (McIntyre et al., 2017). Among those, 82 % are sensitive to major drivers such as rainfall and temperature (McIntyre et al., 2017). With climate change, especially vector-borne diseases are projected to shift their distribution patterns, and their transmission probability is raised (Semenza and Menne, 2009; Scholte and Schaffner, 2007). The second largest group among climate sensitive pathogens constitute those causing waterborne and foodborne diseases such as campylobacteriosis (McIntyre et al., 2017). Climatic factors determine growth and survival of pathogens as well as their transmission routes. Higher ambient temperature can result in more efficient proliferation cycles of pathogens or in changes of climate-related migration and reservoir hosts (Lafferty, 2009). Further along the transmission route, prolonged warm periods may promote ‘temperature misuse’ such as discontinuities in the food cooling chain and thus promote infections (ECDC, 2016b). In addition to the direct effects of climatic conditions, warmer temperatures might indirectly influence human behavior concerning diet or recreational activities.

In the case of campylobacteriosis and heat stress, identical risk groups can be identified such as small children and the elderly. Hence, it is likely that climate change will increase the susceptibility of infectious diseases like campylobacteriosis, worsen symptoms and complications in these patients. For example, loss of fluid and electrolytes from campylobacteriosis might add up to fluid loss from heat stress. Since these two risk factors aggravate in the same season, it is even more probable that they coincide. Thus, a focused concern for these risk groups in terms of surveillance and interventions is needed.

In summary, a changing climate system has implications for human health from global to local scale. Therefore, it will be mandatory to carefully examine the relationship between local weather parameters and responses of climate-sensitive pathogens in order to shed light on the drivers of infection. This understanding can help to tune adaptation strategies for infectious diseases such as campylobacteriosis in Germany.

1.3 Scope of the study

1.3.1 Academic discourse

The gastro-enteric disease campylobacteriosis is the leading cause of human bacterial diarrheal infections in Germany (Silva et al., 2011; Allos and Taylor, 1998; RKI, 2015c; RKI, 2015a). The incidences of campylobacteriosis continues to increase over the last decades and its socio-economic burden is substantial (RKI, 2015a).

1.3.1.1 Seasonality of campylobacteriosis

It is known that campylobacteriosis similar to numerous other infectious diseases, both bacterial and viral, underlies seasonal patterns (Kovats et al., 2004; Louis et al., 2005; Freeman et al., 2009; Rohayem, 2009; Fuhrmann, 2010). However, the drivers of the seasonal dependence often remain unresolved (Dowell and Ho, 2004). Seasonality in infectious diseases describes ‘a periodic surge in disease incidence corresponding to seasons or other calendar periods’ (Fisman, 2007).

In line with other infectious diseases, seasonality in campylobacteriosis has been the subject of many studies (Refrégier-Petton et al., 2001; Wedderkopp et al., 2001; Kovats et al., 2005; Louis et al., 2005; Naumova et al., 2007; Hartnack et al., 2009; Andreoletti et al., 2011; Spencer et al., 2012; EFSA, 2014b). In European temperate climates, campylobacteriosis incidence peaks in the summer months between June and August (Allos and Taylor, 1998; Semenza et al., 2012; Weisent et al., 2014). This incidence peak in northern Europe is mirrored in temperate climates of the southern hemisphere e.g. in New Zealand (Brieseman, 1990) or South Africa (Franco, 1988).

Seasonality of campylobacteriosis in Germany can also be reproduced by the study data. Fig. 6 shows weekly campylobacteriosis incidences in Germany between 2001 and 2014. The peak between June and August is congruent with the findings in the literature and is perpetuated over the whole observation period. Incidences show an increase of 30 % in the observation period from 1.96 in July 2001 to 2.54 in July 2014.

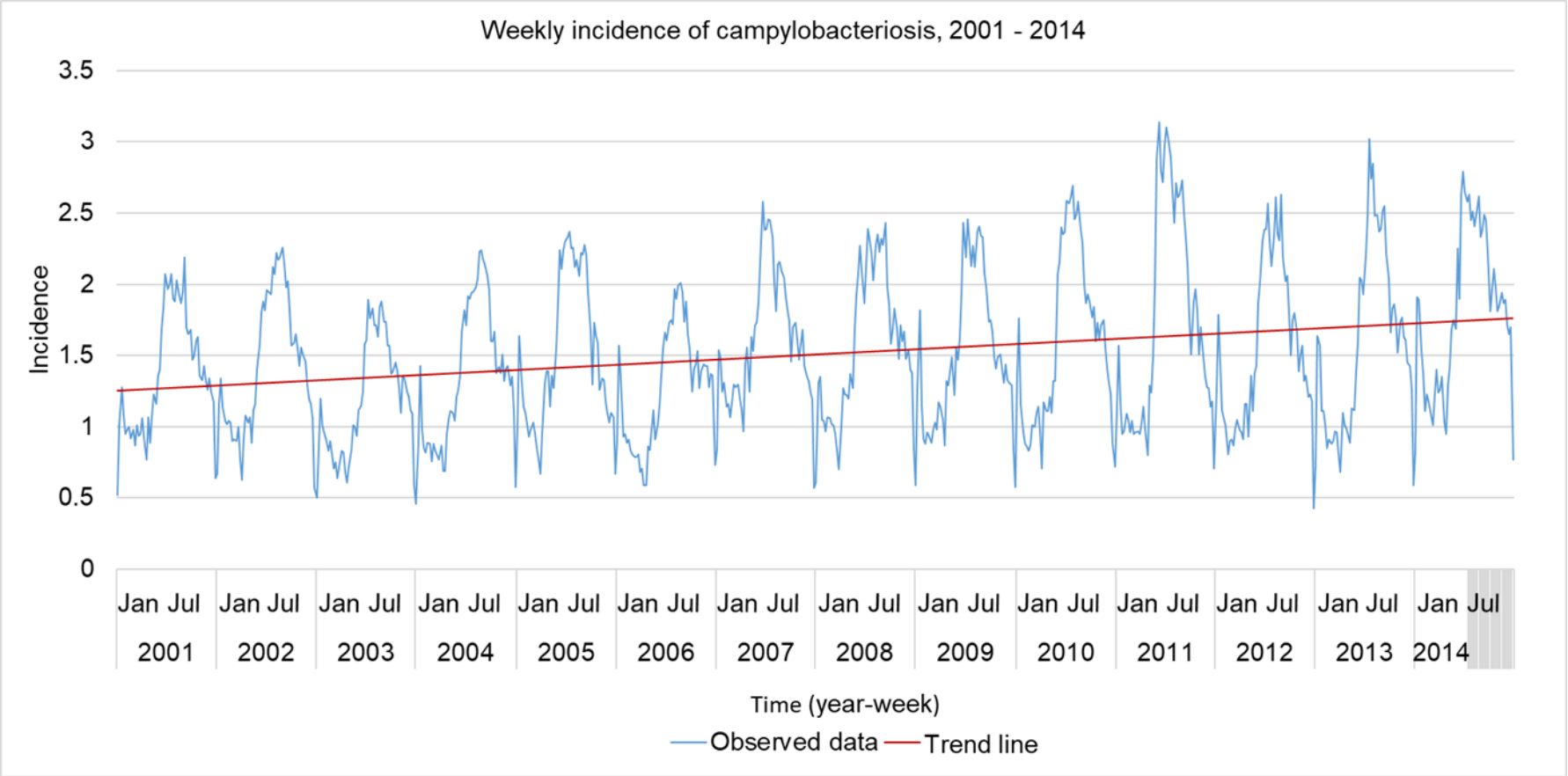

Fig. 6: Weekly incidence (weekly cases per 100.000 inhabitants) of campylobacteriosis from 2001 to 2014 in Germany (Data source: Survstat@RKI, last reviewed: 21.08.2016).

The exact drivers of the seasonality of campylobacteriosis are still poorly understood. Several drivers or a combination of them might contribute to the pattern, for instance, pathogen responses to climatic factors, animal host interactions or human responses to higher temperatures (ACMSF, 2005). Several hypotheses have been put forward to explain the seasonal peak, which will be introduced in the following section.

Bird-pecked milk consumption

Exposure to bird-pecked milk has been associated with a higher risk of obtaining campylobacteriosis in the UK (Southern et al., 1990; Lighton et al., 1991; Neal and Slack, 1997). Southern et al. (1990) explain the rise in *Campylobacter* isolates in the UK during May with bird attacks on doorstep-delivered milk bottles. However, the practice of doorstep milk delivery is uncommon in German households and therefore a profound impact unlikely.

Buying puppies in summer

Several authors found an increased risk for campylobacteriosis by puppies (Salfield and Pugh, 1987; Wolfs et al., 2001; Friedman et al., 2004). Evans (1993) could attribute a contribution to the seasonal rise of human campylobacteriosis cases to the higher acquirement of puppies in the summer months.

Migrating wild birds

Pacha et al. (1988) and Broman et al. (2004) suggested migrating wild birds to be a source of human campylobacteriosis. Especially wild birds with closer contact to human settlements, such as starlings (*Sturnus vulgaris*) and blackbirds (*Turdus merula*) were found to be associated with human campylobacteriosis (Broman et al., 2004). Wild bird-attributed infection was higher in warmer months, which could have been contributed to the seasonal pattern of *Campylobacter* infection (Cody et al., 2015).

Seasonality of campylobacters in bovine hosts

Stanley et al. (1998b) found a seasonal variation of *Campylobacter* isolates from fresh faeces in dairy cattle from the UK with a bimodal peak in spring and autumn. Meanger and Marshall (1989) found corresponding peaks in summer and autumn in New Zealand. However, other authors found the risk from drinking unpasteurized milk in the UK to be more pronounced in winter which has been associated with seasonal carriage rates of campylobacters in dairy cattle (Waterman et al., 1984). These conflicting results question the part that bovine hosts play in seasonality of human campylobacteriosis.

Seasonality of chicken contamination

There is wide evidence that *Campylobacter* occurrence in broiler flocks is seasonal (Refrégier-Petton et al., 2001; Ellerbroek et al., 2010). However, a seasonal increase of campylobacters in chicken is not always preceding increase of human campylobacteriosis (Nylen et al., 2002). For Germany, Hartnack et al. (2009) found the human campylobacteriosis peak preceding the peak in broiler prevalence. The same pattern was found in a Norwegian study by Kapperud et al. (1993). In Denmark, the seasonal peak in chicken and humans occurred simultaneously in the year 1998. A year later, human cases peaked before the increase in broiler contamination (Brøndsted et al., 2000). For the UK, Meldrum et al. (2005) could not explain seasonal human campylobacteriosis with a rise of *Campylobacter* isolates in poultry and suggest environmental sources to be probable. In sum, seasonal colonisation in broiler flocks is not able to explain the seasonality of human campylobacteriosis. It is possible though, that external environmental factors or practises influence both, seasonality in broiler flocks and in human campylobacteriosis.

Presence of rodents around broiler flocks

An increased presence of rodents (e.g. rats or mice) on chicken farms in the summer months has been suggested to increase the risk of *Campylobacter* colonisation in broiler flocks (Kapperud et al., 1993; Berndtson et al., 1996; Jorgensen et al., 2011). Higher temperature may alter the survival of vectors such as rodents, change their population growth, forage supply and behaviour (Kapperud et al., 1993; Hunter, 2003). Thus, climatic

factors can foster broiler flock contamination through vectors and in turn human campylobacteriosis.

Seasonality of fly transmission

Several authors have suggested transmission of flies as a probable explanation for the seasonal increase in campylobacteriosis (Shane et al., 1985; Hald et al., 2004; Ekdahl et al., 2005; Nichols, 2005; Guerin et al., 2008). Flies are expected to function like vectors carrying infected material from chicken faeces for example to foods and expose humans to it (Hald et al., 2004; Nichols, 2005). The seasonal appearance of large fly populations is suggested to relate to seasonal patterns of temperature and precipitation (Nichols, 2005).

Seasonality of agricultural activity

Agricultural activity has been associated with campylobacteriosis and shows a seasonal pattern in Germany. *Campylobacter* rates have been found to be positively correlated with the total agricultural area, agricultural labour force, and the total amount of cattle, pigs, sheep and poultry (Louis et al., 2005). Moreover, manure spread and occupational bovine exposure, but also high water contamination show similar seasonal peaks to those of campylobacteriosis (Lévesque et al., 2013). Another factor in the context of agricultural activity is husbandry practices of cattle, which also occurs seasonally and might be a source of *Campylobacter* transmission for broiler flocks (Jorgensen et al., 2011).

Seasonality of Campylobacter concentration in sewage effluent

Campylobacter load peak in sewage effluent has been found to coincide with the campylobacteriosis peak in summer. Though, earlier studies showed the lowest bacterial concentration in sewage effluent in summer (Jones et al., 1990a; Jones et al., 1990b). Thus, depending on the tested sewage effluent, results contradict an association with seasonal campylobacteriosis. The authors suggest that an association of seasonal human campylobacteriosis might be an indirect effect of seasonal alterations in animal populations and in turn faeces intake into water bodies (Jones, 2001).

Seasonality of diet and human behaviour

Human (diet) behaviour shows seasonal patterns. For instance, the significantly higher consumption of barbecued meat in the summer months has been associated with higher campylobacteriosis incidences due to undercooking and cross-contamination, with the largest infection risk found between June and October (Deming et al., 1987; Kapperud et al., 1992; Neimann et al., 2003). Moreover, salad vegetables and bottled mineral water are identified as risk factors for *Campylobacter* infections and might contribute to its seasonality (Evans et al., 2003).

Besides the summer peak, another incidence peak is found in wintertime in several European countries (Bless et al., 2017). A study from Switzerland links the campylobacteriosis peak in winter to the Swiss festive tradition of meat fondue, commonly called ‘Fondue Chinoise’ (Bless et al., 2014). In Germany, a meat dish different to the one in Switzerland is preferably eaten around New Year’s Eve and is called ‘Raclette’. Both dishes are associated with increased exposure to undercooked meat and have been correlated with the winter incidence peak in Germany (Bless et al., 2017). Thus, human eating behaviour is a relevant factor for campylobacteriosis incidence and could in part explain its seasonality.

Furthermore, certain leisure activities become more relevant during the summer months. These include swimming in contaminated lakes and other natural water sources. Campylobacteriosis outbreaks in Finland have been reported as a result of such activities (Schonberg-Norio et al., 2004). Moreover, agrotourism is more frequent in the summer and often includes contact with animals or the consumption of raw milk. Contaminated raw milk, in turn, has been retraced as being responsible for several campylobacteriosis outbreaks in the past, especially among children (Wood et al., 1992; Thurm et al., 2000).

1.3.1.2 Weather conditions and seasonality of campylobacteriosis

Several studies have associated seasonality of campylobacteriosis to weather conditions. The NOAA (2009) defines weather as ‘the state of the atmosphere with respect to wind, temperature, cloudiness, moisture, pressure, etc. Weather refers to these conditions at a given point in time (e.g., today's high temperature), whereas climate refers to the ‘average’

weather conditions for an area over a long period of time'. Thus, weather describes short-term variations of selected climatic variables such as precipitation or temperature.

Precipitation

There is inconsistent evidence about the association between precipitation and campylobacteriosis incidence. An association between extreme rain events and increased risk of campylobacteriosis has been found (Soneja et al., 2016). Campylobacteriosis outbreaks were found to be triggered especially by heavy rain events (McQuigge, 2000; Clark et al., 2003). In addition to this, Nichols et al. (2009) were able to attribute very high rainfall (> 40 mm), but also very low rainfall to *Campylobacter* outbreaks in the UK, relating it in particular to contaminated drinking water. It seems straightforward that during low rainfall conditions, the proportion of contaminated groundwater from sewage or surface water run-off increases in a water body, while the filter function of cracked soil is reduced (Nichols et al., 2009). On the other hand, very high rainfall may lead to increased total surface run-off and manure wash-out from fields. This subsequently increases the bacterial contamination of streams and rivers, particularly in agricultural and rural areas (Bolton et al., 1987).

In Germany, Rechenburg and Kistemann (2009) could show that elevated river contamination coincides with the discharge of combined sewage overflow from river spillway basins after heavy rain events. Especially in summer after short and heavy thunderstorms, the discharge of combined sewage overflow was associated with increased bacterial river contamination. Thus, the highest risk of infection from surface water was found in summer after heavy rainfalls. These contaminated surface waters may become a health risk when used for recreational bathing or for irrigation of cultivated vegetables (Rechenburg and Kistemann, 2009). A similar association between total rainfall and seasonal *Campylobacter* prevalence in streams has been described in the United States (Vereen et al., 2007; Vereen et al., 2013). Conflicting with the latter results, Jones et al. (1990c) and Jones et al. (1996) found the highest *Campylobacter* numbers in rivers during winter months and lowest between May and July, at the time of peak campylobacteriosis infections in the UK. This observation has been explained with

elevated hours of sunshine and higher temperatures in summer and campylobacters susceptibility to toxic photo-illumination and high temperatures (Bolton et al., 1987).

At the farm level, rainfall in the month of chicken flock placement has been associated with increased *Campylobacter* prevalence. This association is comprehensible because rainfall may provide surface water reservoirs for *Campylobacter* survival and as a result increases the risk of broiler contamination (Jorgensen et al., 2011).

While the named studies found associations to explain campylobacteriosis seasonality with precipitation, others found conflicting results or no significant association (Jones et al., 1996; Patrick et al., 2004; Kovats et al., 2005; Louis et al., 2005; Bi et al., 2008; Lal et al., 2013).

Seasonality of incidences and precipitation can be reproduced by the study data. Fig. 7 shows the Germany-wide incidence rate of campylobacteriosis and the median of daily maximum precipitation. Incidences are averaged by calendar week for the year 2001 to 2014. The graph demonstrates the strong seasonal trend of campylobacteriosis incidence which peaks in the summer months. The summer peak has been described by other authors (Stark et al., 2009). Although precipitation shows a high inter-weekly variability, a seasonal pattern becomes apparent over the course of the year. While spring (calendar week 9 - 22) is the driest season, summer weeks (week 23 - 34) show the highest maximum precipitation. Autumn (week 35 - 48) on average is drier than summer but more humid than spring. With rising median precipitation from week 17 onwards, campylobacteriosis incidences are steadily increasing. In calendar week 29 during the summer season, precipitation reaches its overall maximum with 10 mm of rain on average. While incidences decrease after this peak, the decline of precipitation is less profound. Due to the high inter-weekly variability, a clear time offset is not visible here.

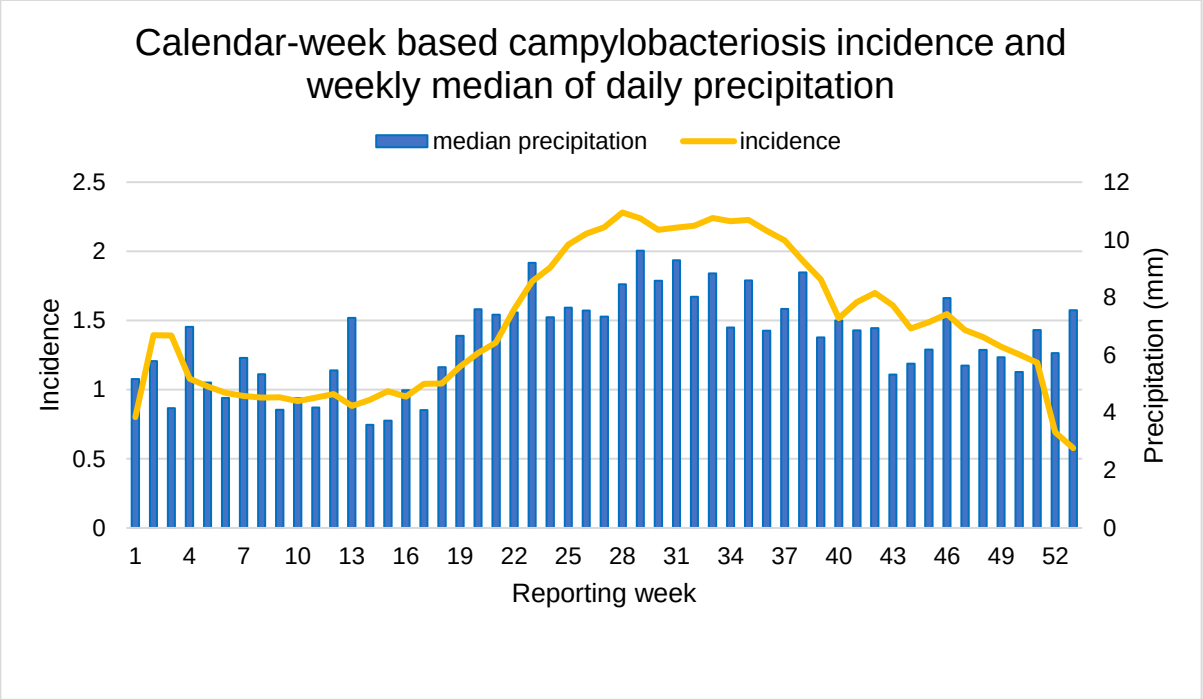

Fig. 7: Calendar week-based incidence of campylobacteriosis and median of daily maximum precipitation, averaged over the period between 2001 and 2014 (Data source: Survstat@RKI, last reviewed: 21.08.2016).

Temperature

It is documented that campylobacteriosis infection is associated with temperature, which can in part explain its seasonality in temperate climates. However, there are inconsistencies in the literature. Temperature-associated seasonality of campylobacteriosis is documented for Iceland (Steingrimsson et al., 1985), the United States (Friedman et al., 2000; Naumova et al., 2007; Weisent et al., 2014), Canada (Fleury et al., 2006), Denmark (Patrick et al., 2004) and the United Kingdom (Louis et al., 2005; Tam et al., 2006; Nichols et al., 2012). In addition, Nylen et al. (2002) noticed similar associations in Europe and New Zealand which varied with longitude. No association between temperature and campylobacteriosis was found by Spencer et al. (2012) and Lal et al. (2013) for New Zealand.

For Germany, the relationship between weather parameters and human campylobacteriosis incidence has been studied on a low resolution and for selected regions. In the work of Hartnack et al. (2009), incidence and weather data has been

examined on a monthly scale over a short investigation period of three years. However, using low resolution bears the risk that small-scaled associations remain undetected.

The work of Yun et al. (2016) used small-scale data. However, only a reduced number of German regions have been selected for analysis using a relatively small ‘n’. Selecting individual regions can lead to regional biases by over- or underestimating external drivers. Hence, an overall nationwide statement cannot be made from this study. Moreover, Yun et al. (2016) used absolute case counts instead of incidences harbouring the risk of poor comparability between unequally populated areas.

In the data used for the present study, campylobacteriosis seasonality can be reproduced on a weekly scale. Fig. 8 illustrates the Germany-wide incidence rate of campylobacteriosis and median temperature over the course of a year, averaging the incidences calendar week-based for the year 2001 to 2014. Campylobacteriosis incidence is visualised again to compare it with the annual temperature profile. Compared to precipitation, the seasonal pattern of the temperature profile is more pronounced here. The median temperature increases steadily in spring, peaks in summer and decreases with the same steadiness in autumn. The incidence rate shows some variation from this pattern by steeply increasing in spring around calendar week 16. The absolute incidence peak of 2.26 is reached in calendar week 29, which falls into the second half of July. Between calendar week 29 and 35 (i.e. from mid-July to the end of August), the incidence rate stays on an elevated level forming a plateau. Then incidence decreases again in autumn but not as steep as it has risen in spring. This difference in the incidence rate in spring and autumn is remarkable since the temperature profile is comparable in the two seasons.

A second annual incidence peak occurs in week 2 and 3 in mid-January with an incidence of 1.39. This winter peak is caused by a high number of cases in that period and not as a result of late registration (RKI, 2015c). In general, a lag between the incidence increase and temperature increase of approximately three weeks can be noticed in Fig. 8.

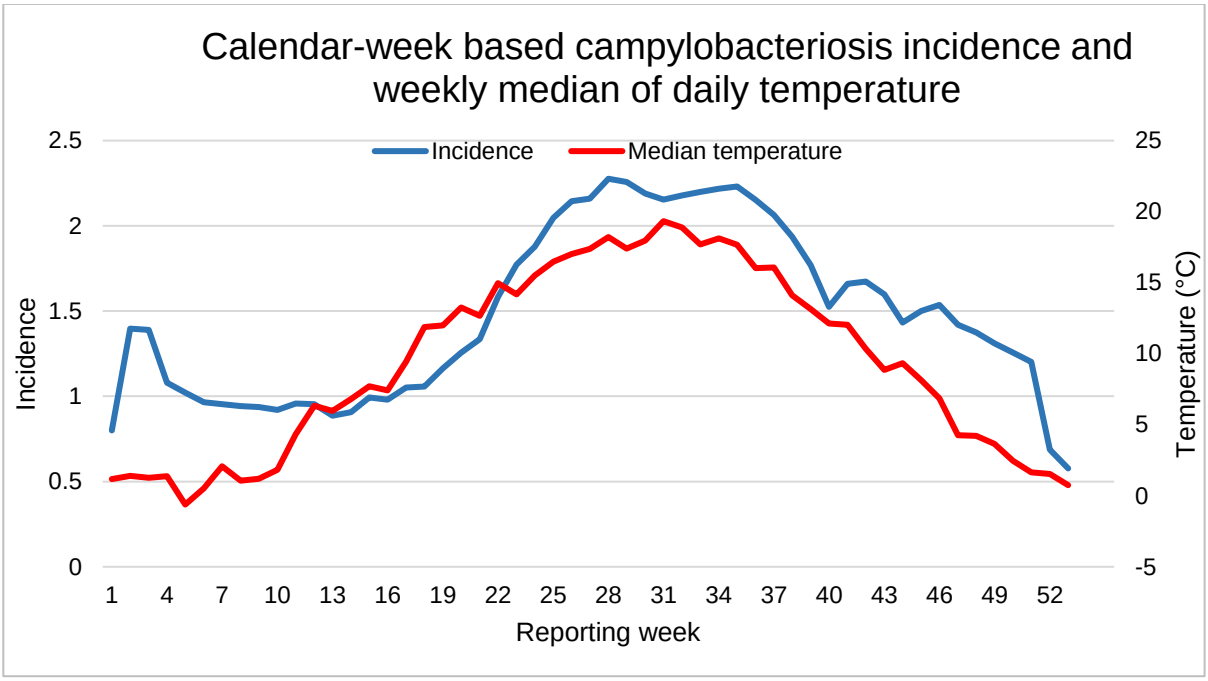

Fig. 8: Campylobacteriosis incidence per reporting week and median temperature, averaged over the period of 2001 until 2014 in Germany (Data source: Survstat@RKI, last reviewed: 21.08.2016).

1.3.2 The concept of lagtypes

Lag times between weather parameters and campylobacteriosis have already been considered by several authors (Kovats et al., 2005; Louis et al., 2005; Fleury et al., 2006; Hartnack et al., 2009; Koch, 2013; Yun et al., 2016). In 1965, Sir Austin Bradford Hill developed the „Bradford-Hill criteria', a catalogue of criteria to establish epidemiologic evidence and now widely used in public health research (Hill, 1965; Mohr et al., 2012; Grant, 2014). Among them, one criterion is to test different lag times between expected cause and observed effect.

This study aims to identify effective lag times, here defined as 'lagtypes', on a dense spatiotemporal perspective. The identification of a special time lag between local weather conditions and campylobacteriosis can strengthen the assumed association, highlight crucial stages of the infection chain and furthermore help epidemiologists and health authorities to improve forecasts and local early warning systems.

1.3.3 Objectives of the study

As described above, the correlation between seasonal patterns of weather and seasonality of campylobacteriosis incidence has been examined at length. If the weather is the main driver of campylobacteriosis outcome and causes its seasonality, this association should be reproducible for small-scaled weather and incidence. This would give evidence to the influence of weather on campylobacteriosis.

The objective of this study is to examine the correlation between selected local weather parameters, temperature and precipitation in particular, and human campylobacteriosis outcome in Germany. Since Germany-wide, local weather conditions and campylobacteriosis incidence have not been studied on a dense temporal and spatial resolution over a long observation period, this study aims to close this gap. Compared to other studies, this research does not stay on a national level assuming a homogeneous climate but examines local weather conditions and locally reported campylobacteriosis incidences using long-term data. The strength and quality of correlations are examined, and most significant weather parameters are identified. Moreover, specific temperature ranges may be identified that correlate with the highest incidences.

If time lags occur on a broader scale between weather patterns and incidences, there should be lagtypes, which show a maximum association between small-scale temporal and spatial weather parameters and incidences. If temperature ranges that are strongly associated with high incidences can be identified, it can be estimated how campylobacteriosis incidence will develop under the conditions of climate change.

The selection of weather parameters is based on relevance in scientific literature in addition to the availability of data on a high temporal and spatial resolution covering the complete time span where incidence data is available. The source of weather data covers all member states of the EU. Thus, with the successful realisation of the method, it could be applied to further EU member states and further pathogens.

The approach finds a solution for the small number of reports in extreme weather regions and handles the problem of varying sensitivity of external drivers on incidence in unequally populated reporting areas. The solution was to classify data by weather parameter, to filter it by parameter outcome and to build classes of 5,000 reports. Equally large classes with

variable widths resulted from this calculation. This method has been applied with different temporal lags.

1.3.4 Hypotheses

From these research objectives, the following hypotheses arise which will be addressed by this study:

H1. Spatially and temporally small-scaled weather conditions are associated with the outcome of local campylobacteriosis incidence in Germany.

H2. The selected weather parameters temperature and precipitation differ in their strength of association to campylobacteriosis incidence on a temporal and spatial small-scale perspective.

H3. There exist definable temperature ranges, which show a strong correlation with increasing incidences of campylobacteriosis.

H4. A maximum correlation between weather conditions and incidences can be observed on a special lag.

H5. Different weather parameters show a significant difference in temporal lag patterns.

H6. Temperature ranges can be identified which are expected to play a key role in increasing incidences under the conditions of climate change.

2. Materials and methods

2.1 Methodology

2.1.1 Preface

This study avoids comparing incidences of reporting areas (RA) on a one-to-one approach. By comparing each incidence week of each RA and the linked weather week with the incidence-weather week combination of all other 413 RAs, would have produced 301,490 combinations ($= 413\ RAs * 730\ incidence\ weeks$; Tab. 1) of a single incidence of a single RA in a certain weather week, for each weather parameter and lagtype. Instead, a novel approach has been applied. Before introducing the new methodology, two common problems in epidemiological studies, the varying numbers of reported weather parameter values and the small numbers problem, will be discussed to make further steps comprehensible.

2.1.1.1 Varying numbers of reported weather parameter values

A problem of weather data is the varying number of reported parameter values. Along the temperature axis, certain temperature values are being reported more frequently than others, similar to a Gaussian curve. If the reported incidences are grouped by a set temperature width, e.g. a temperature width of 5 K, this would result in very unequal class sizes and would lessen statistical significance.

2.1.1.2 Small numbers problem

Data of population distribution often exhibits the difficulty of being unequally distributed in space when rural and urban areas are included. The chosen spatial resolution in this study is reporting areas on the NUTS-3 level. Although the NUTS-3 level reporting areas should provide comparability, this not the case (Destatis, 2017). The population of RAs range between 33,807 and 1,798,836 inhabitants which corresponds to a ratio of 1:53 (Fig. 14). This alters the sensitivity for external drivers by this factor. In a low populated RA for example, one case has a relatively high impact on the incidence whereas in densely

populated RAs one single case might not change the incidence value at all. Moreover, certain incidence values cannot be reached by low populated RAs while high populated RAs report risk more sensitively. This is due to the fact, that cases can only produce integer numbers. Moreover, the variance of incidence depends substantially on the size of the denominator, i.e. the population at risk. The variance of incidence is high when the denominator is small and vice versa (Jacquez, 2010).

An example may illustrate the small numbers problem. For instance, if reporting area A reports one case in 33,000 inhabitants this results in an incidence of 3 per 100,000 = 0.00003. In contrast, reporting area B which hosts e.g. 1 Mio. inhabitants would need to report 30 cases to reach the same incidence. The two reporting areas have the same health risk. However, a single case has a higher impact on incidence values in a less populated RA like reporting area A, whereas a few cases in a densely populated RA like reporting area B might not affect the incidence value at all.

To solve the problem of varying numbers of reported weather parameter values and the small numbers problem, a novel approach has been adopted, which will be described in the following chapter.

2.1.2 Study design

2.1.2.1 A novel approach to varying numbers of reported weather parameter values

The proposed solution to the problem of varying numbers of reported weather parameter values is to create equal classes of 5,000 reports populating each class. A report describes the outcome of campylobacteriosis of one week in one reporting area and represents the smallest spatiotemporal reporting unit available. It consists of the number of cases, incidences and an identification number of the reporting area. Each report is combined with the associated weather conditions. While the number of reports per class is equal, the class width, e.g. temperature range varies. In extreme classes the class width is large, so uncertainty will shift towards extreme temperature classes. As a result, a high statistical certainty can be reached for non-extreme classes.

To combine weather conditions with reported incidence, time lags, called ‘lagtypes’ in the following, were set. Weekly weather conditions with a time lag of one week up to eight

weeks preceding the reported incidence were established. Thus, eight different lagtypes could be analysed separately for each weather parameter.

After defining the spatiotemporal unit and the temporal frame, every reported incidence was combined with the selected weather parameter for the selected lagtype (Fig. 9, Fig. 10). For example, the temperature parameter ‘Weekly average of daily maximum temperature in lagtype 4’ combines the current weekly incidence with the weekly average of daily maximum temperature four weeks ago. This procedure produces 301,490 combinations (= reporting areas × incidence weeks) for each temperature parameter and each lagtype. Subsequently, this list of combinations is sorted by weather parameter value (e.g. ‘Weekly average of daily maximum temperature’) in ascending order. Defined by the study design, classes of 5,000 reports are created. The classification results in 61 classes for each temperature parameter. 60 classes contain 5,000 reports, the last class (Nr. 61) contains 1,490 reports. Note, that for precipitation parameters class numbers vary. This is due to a methodological adjustment for this parameter in the study design, which will be described at a later stage.

The selected class size is large enough to be statistically robust for analysis. Moreover, the number of classes allows a dense resolution and examination of each weather parameter along the scale. This is possible due to the vast data background containing 413 reporting areas and 730 reporting weeks. It is important to notice that the summary of each class is solely defined by weather parameter value, regardless of the spatial or temporal location.

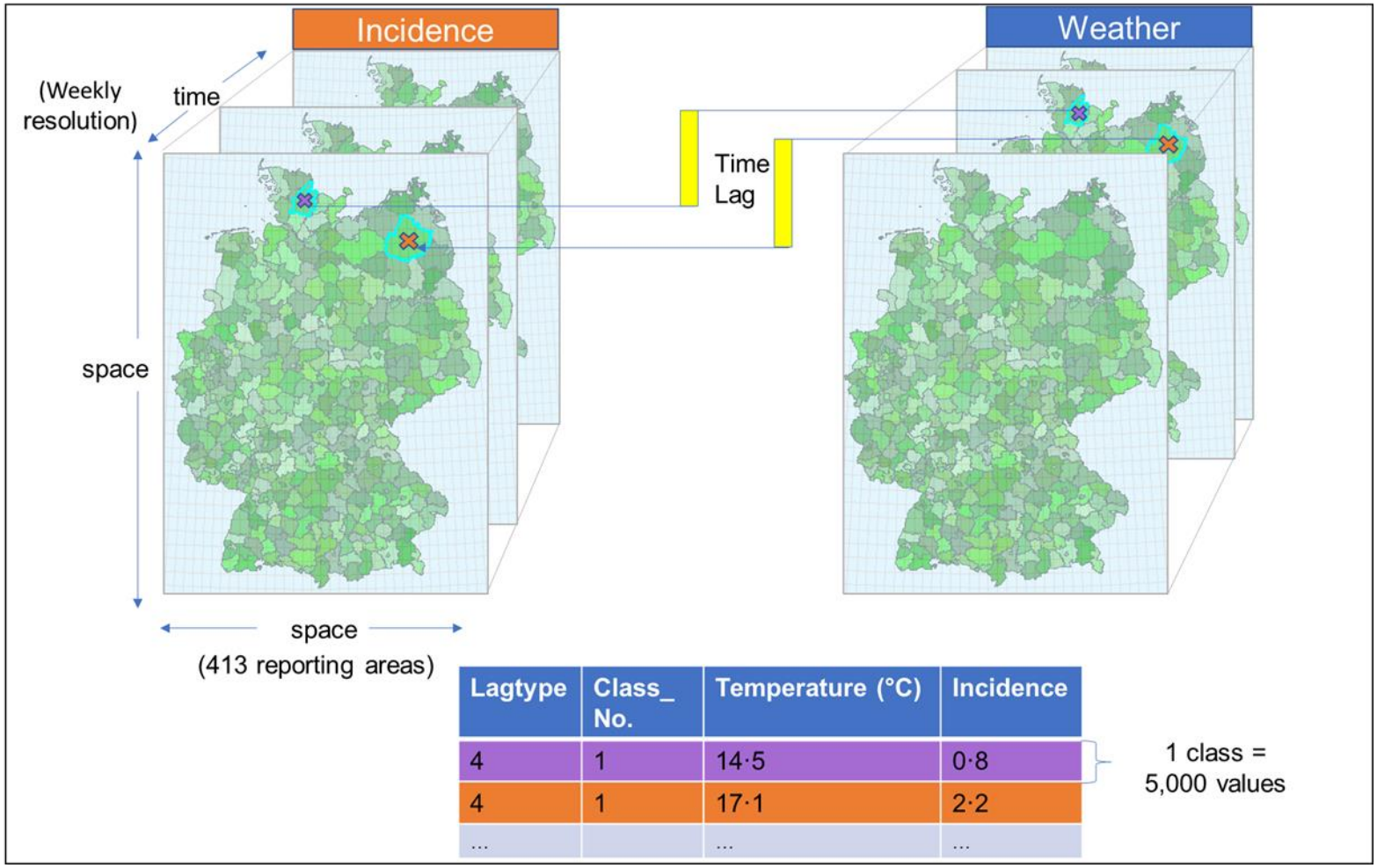

Fig. 9: Explanation of study design: E.g. for lagtype 4, the weather week four weeks prior to the incidence week is examined. According to weather conditions, values are sorted in ascending order and classified, one class containing 5,000 reports (Data sources are refered to in chapter 2.2).

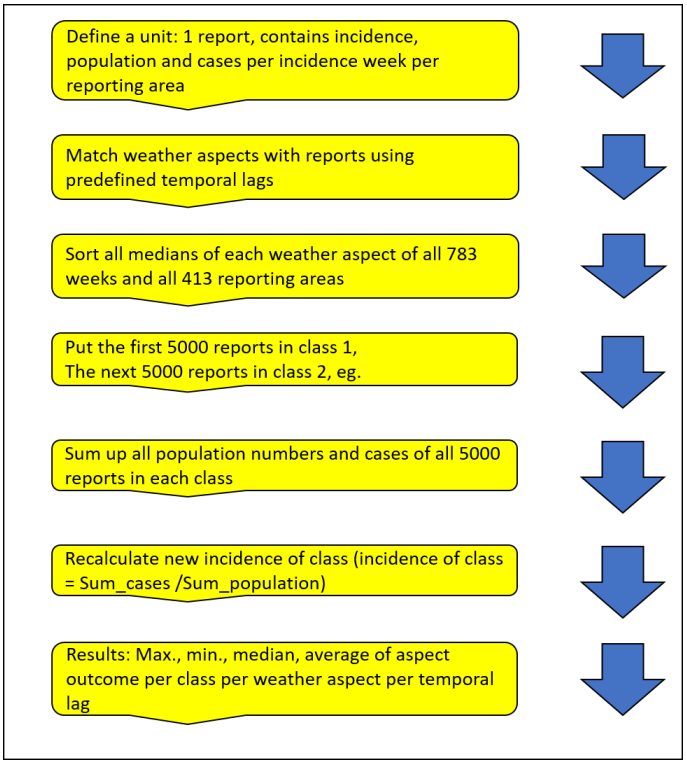

Fig. 10: Flowchart of the study design in single steps.

2.1.2.2 A novel approach to the small numbers problem

The applied method creates classes of 5,000 reports. These reports from one class might be related to reporting areas from all over Germany. Thus, the participating reporting areas in each class may differ largely in their population density. This, in turn, alters the incidence value sensitivity, described as the small numbers problem in the methodology preface.

The solution is to create a new incidence value per class. For each report, the cases and total population per reporting area are known. Thus, the sum of cases and the sum of populations could be calculated per class of 5,000 reports, and a new representative class incidence was created. To use incidence as a measure establishes comparable ratios between reporting areas. This calculation solved the small numbers problem and was carried out with SQL stored procedures.

2.2 Data background

Detailed information on the underlying data can be found in Tab. 1. The cited files in this document can be found in the supporting documents.

Tab. 1: Underlying data.

Descriptor	**Value**	**Comments**
sum of cases	901,623	
spatial coverage	413 RA[1]	
temporal coverage	783 weather weeks	1999 - 2014
	730 incidence weeks	2001 - 2014
report count	301,490	incidence weeks * RA
total German population	83,209,409 inhabitants	averaged for all years
population per reporting week	83,209,409 inhabitants	60,742,868,740 inhabitants /730 incidence weeks
population per RA	201,476 inhabitants	total German population/ RA count
population of all reporting weeks and all RA	60,742,868,740 inhabitants	total population * week count
population per single report	201,476 inhabitants	population of all reporting weeks and all RA/total report count
class size (1-60)	5,000 reports	defined by method
class size (61)	< 5,000 reports	defined by method
expected population per class of 5,000	1,007,377,836	population per single report * 5,000
expected sum of cases per class of 5,000	14,952.78	sum of cases/sum of classes
expected incidence per class of 5,000	432,122.98/301,490 = 1.433 cases/100,000 inhabitants	sum of all incidence values/ number of all incidence entries

[1] RA = Reporting area

2.2.1 Campylobacteriosis data

The epidemiological data is derived from the free online database of the Robert Koch Institute (RKI) (RKI, 2020). On this platform, the RKI collects and provides epidemiological data since 2001 of all reportable infectious diseases in Germany. Thus, the data has national coverage, and the reporting system has remained constant from 2001 until present. Data before 2001 was not used because of a newly established reporting system at RKI in 2001 which is in accordance with the current German Protection against Infection Act.

Cases and incidences were used which were namely reported to the local health authorities and federal state authorities. The week, campylobacteriosis has been reported defines the reporting week. Here, the incidence is defined as new cases per 100,000 inhabitants per week per county. To access campylobacteriosis data on SurvStat@RKI, the following query was carried out (Fig. 11):

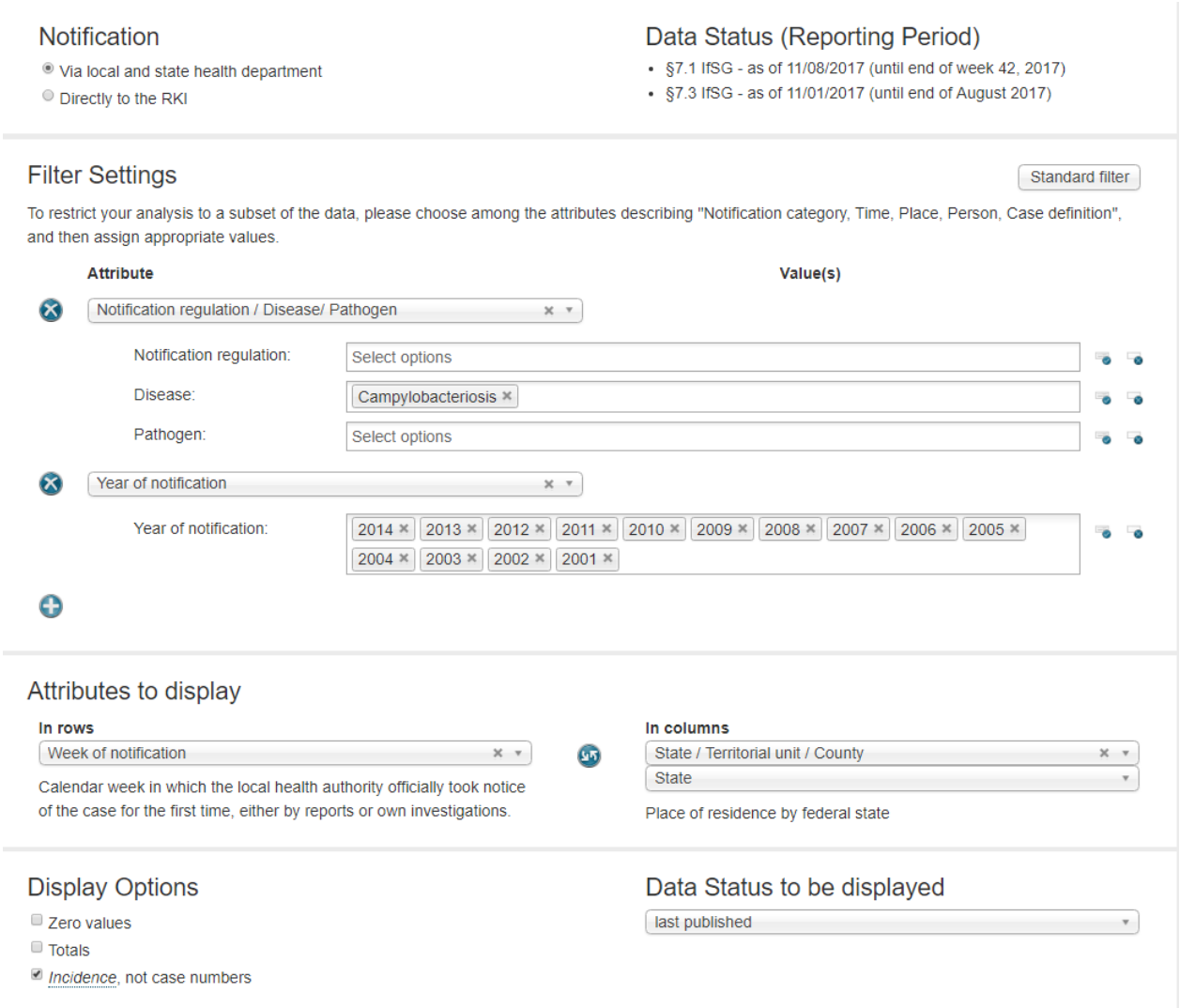

Fig. 11: Query settings at SurvStat@RKI 2.0.

Cases and incidences of the selected disease ‘Campylobacteriosis’ were selected and downloaded separately providing one Excel sheet for cases and one for incidences[2]. To filter incidences, ‘Incidence, not case numbers’ were selected. The selection of the pathogen included all recorded subspecies of *Campylobacter*. The incidence data covers the years 2001 to 2014, and the examined incidences and cases have a weekly resolution. Overall, 730 weeks were covered for incidence reporting. The case definition does not differentiate between clinical cases and laboratory-reported cases.

Campylobacteriosis data covers the whole of Germany and is grouped by 413 counties, in the following called ‘reporting areas’. They are represented on NUTS-3 level. NUTS-3 stands for ‘Nomenclature of Territorial Units for Statistics’ and is a geocode standard to divide countries into units. The reporting areas on this level represent the highest possible territorial level resolution provided by RKI and represent administrative districts, district-free cities, or city regions (Fig. 12). Cases and incidences relate to the reporting area where the case person resides, not necessarily where the infection took place. Note that year 2004 and 2009 contain 53 weeks, whereas all other years hold 52 weeks[3].

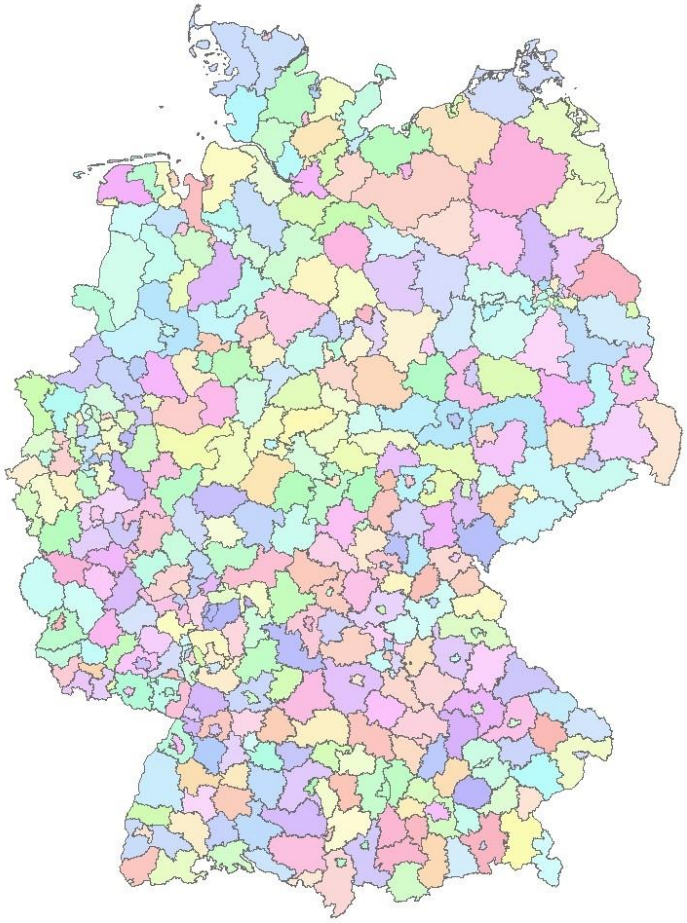

Fig. 12: Spatial distribution of reporting areas (Data sources are refered to in chapter 2.2).

[2] Original RKI Excel file „RKI_Campylobacter.ods’, Downloaded 07/01/2015.
[3] For explanations, see excel file ‘calendar weeks.xlsx’ in the supporting documents.

The downloaded cases and incidence tables from RKI have been reformatted in Excel in a way that each reporting area received a distinct identification number. The reformatted tables ('Campylobacter_for_export.xlsx') were then exported to an SQL database[4] and named 'Campylobacter_import'[5].

Overview of raw incidence data

The following description gives an overview of the raw incidence data. It is shown that requirements on NUTS-3 regions of comparability, with regard to area and number of inhabitants, are not fulfilled. Incomparability of the reporting areas backs the decision to refrain from a one-to-one approach and opt for a novel methodology.

The frequency of areal sizes among the reporting areas

Reporting areas on the NUTS-3 level are the smallest spatial units in Germany and originate from historical administrative units.

Area sizes of each reporting area are provided by the Federal Agency of Cartography and Geodesy, in the following called 'BKG' (BKG, 2015) and for more differentiated area data in the Berlin region by the Senate Department for Urban Development and Housing (Senatsverwaltung für Stadtentwicklung und Umwelt Berlin, 2015). The frequency of areal sizes of reporting areas is represented in Fig. 13. Reporting areas vary between 21 km² and 5,496 km² in size. The median size of a reporting area is 777 km².

[4] to reproduce the SQL tables, use a MySQL-interface (e.g. HeidiSQL© or phpMyadmin© or mysql workbench©) and connect to the project's database. Subsequently, use the sql code 'show create table + tablename' to receive a detailed description of the selected table.

[5] reformat the Excelsheet as follows: row 1 =field names, add column 3 „Monday' (which holds number of day for Monday in the respective week, day 1 = 1.1.2000, Monday of week 1 in year 2001 is = 367), add column 4 which holds an indicator for the data type (i=incidence, c=cases), save as excel file, save as csv file, create an empty MySQL table 'Campylobacter_import', import created csv file to 'Campylobacter_import' using MySQL function Load Data INFILE and import number of cases and afterwards import incidence values into the same database table, create table 'NEWconvertedcampylobacter', transfer data from 'Campylobacter_import' to 'NEWconvertedcampylobacter' using MySQL stored routine 'convert-tonewconvertedcampylobacter'.

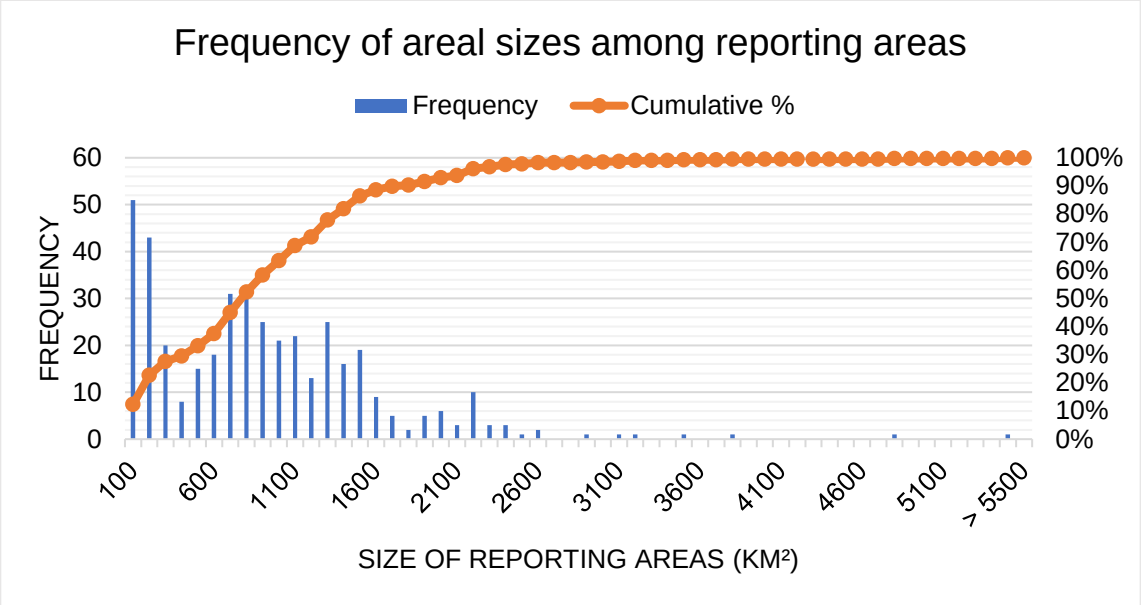

Fig. 13: Frequency of areal sizes in the 413 reporting areas of Germany (BKG, 2015; Senatsverwaltung für Stadtentwicklung und Umwelt Berlin, 2015).

Frequency of number of inhabitants per reporting area

The number of inhabitants per reporting area varies between 33,807 and 1,798,836 inhabitants, a relation of 1:53 (Fig. 14). The average population for each reporting area is 201,476 inhabitants. Data from the 'BKG' and 'Senatsverwaltung Berlin' was used to calculate the number of inhabitants and was then overlapped with the reporting areas in GIS (see also chapter 'GIS data').

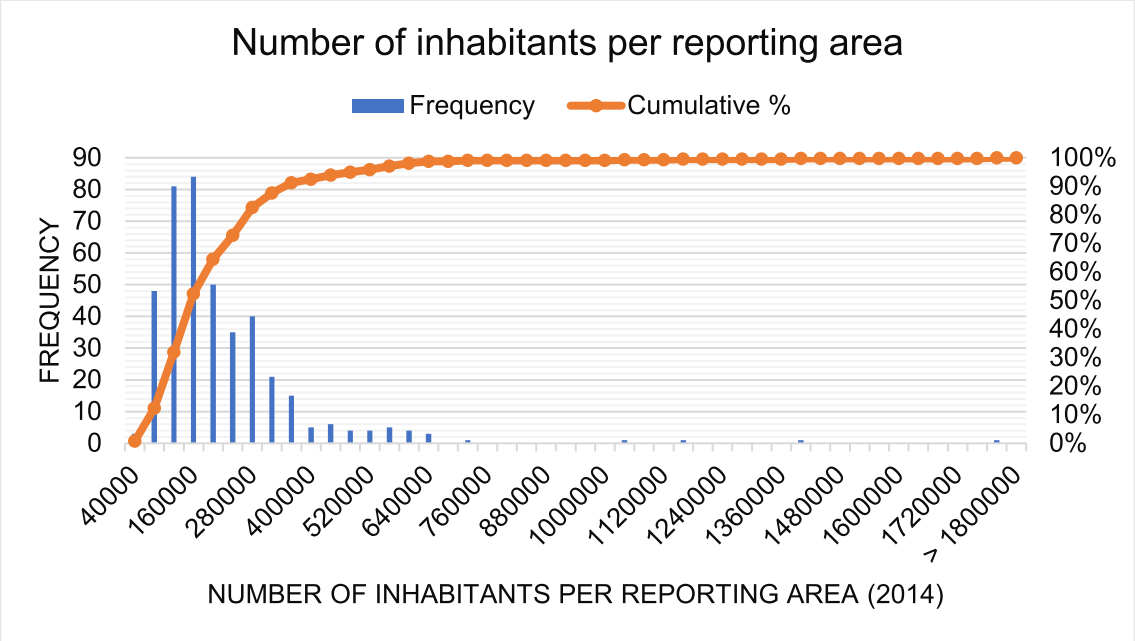

Fig. 14: Distribution of the number of inhabitants in the 413 reporting areas (BKG, 2015; Senatsverwaltung für Stadtentwicklung und Umwelt Berlin, 2015).

The frequency of incidence values

The raw data set holds 301,490 records of incidences in total over 14 years with a weekly resolution and is distributed over 413 reporting areas. Around 23 % (68,322) of all records include 0 cases, whereas the maximum incidence is 41.58. Thus, a wide range of incidence values is covered. The frequency of incidence values is displayed in Fig. 15.

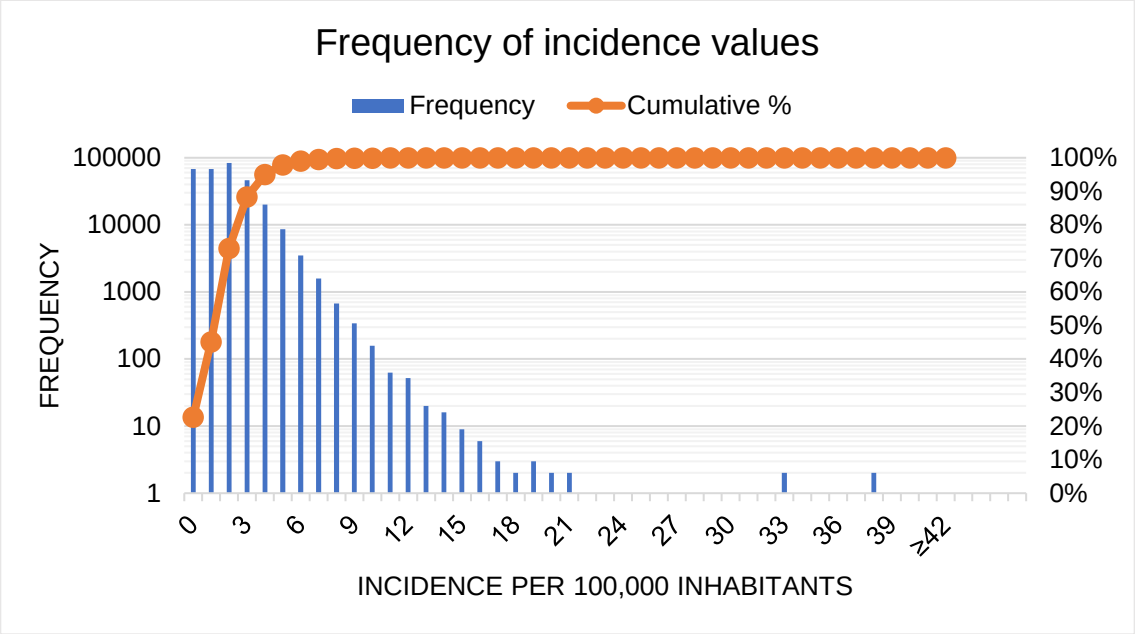

Fig. 15: Incidence values. Histogram for 301,490 reports (RKI, 2020).

Incidence outcome in rural and urban areas

Incidence outcome between rural and urban areas has been examined. Rural areas can be hot spots for infections due to a higher risk of exposure to environmental sources of *Campylobacter* spp. (Fitzenberger et al., 2010). Moreover, sparsely populated areas might be overrepresented in certain weather regions, for instance, regions with more extreme weather conditions. To uncover a potential rural-urban bias, the population density of all reporting areas and averaged incidence over the whole observation period have been visualised in a scatter plot (Fig. 16). No significant bias can be observed between population density and incidence outcome, e.g. incidence is not increasing with low population densities in this case.

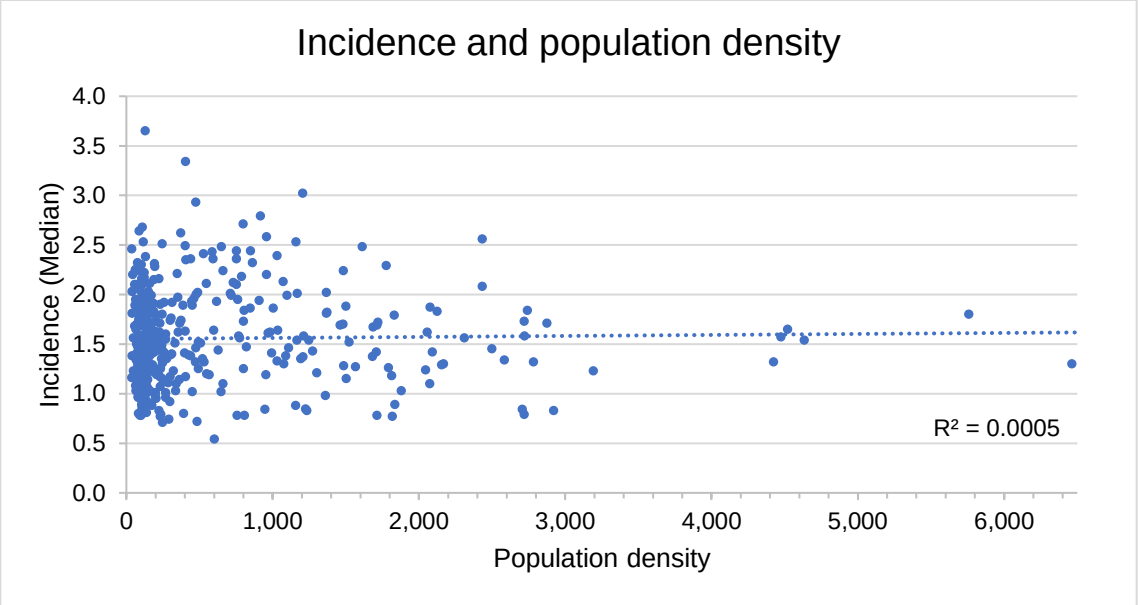

Fig. 16: Rural-urban bias. Incidence is plotted against population density of all 413 reporting areas. The dotted line represents the trendline.

Spatial distribution of incidence outcome

The distribution of incidence in Germany has been examined to account for possible regional incidence bias in the raw data. Fig. 17 shows that incidence outcome is on average higher in the East related to the West and higher in the North compared to the South. Due to the applied method of classifying incidence along local weather conditions solely and not selecting specific regions, a regional bias can be avoided.

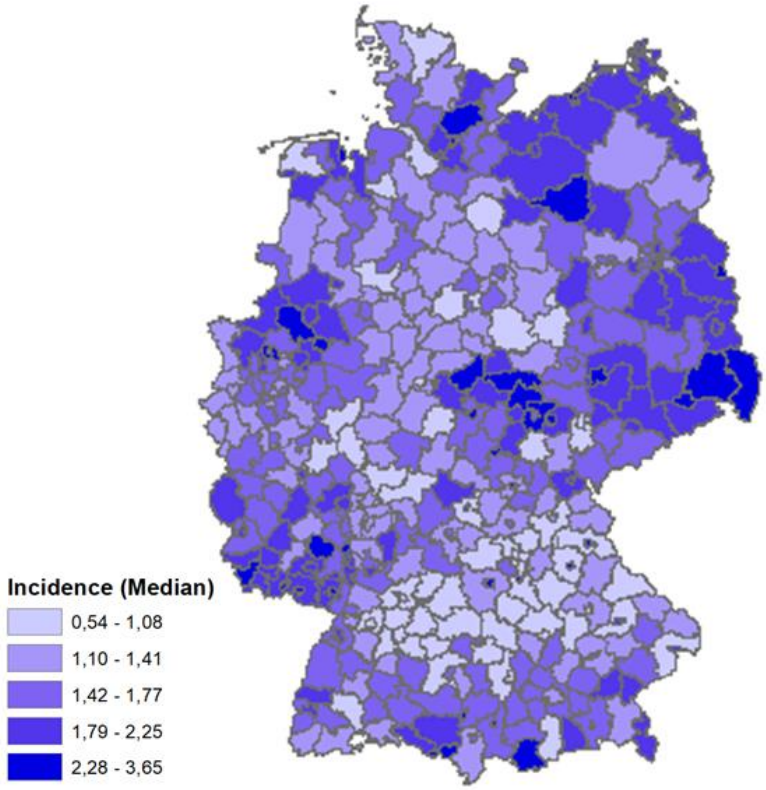

Fig. 17: Spatial distribution of incidence outcome in Germany. The median incidence over the whole investigation period has been calculated for every reporting area (Data sources are refered to in chapter 2.2).

2.2.2 Weather data

The meteorological data is obtained from the European Climate Assessment & Dataset project (ECAD, 2015). The ECA is a research project initiated by the European Climate Support Network of GIE_EUMETNET, a cooperation of 31 European national meteorological services (EUMETNET, 2020). It observes changes in climate extremes in Europe and provides free and online available weather data[6].

Raw data contains five different weather parameters, among them air pressure was excluded. Please note the change of terms from the original dataset to the terms used in the following:

[6] downloaded: 08/06/2015, latest version: 04/11/2015, available at http://eca.knmi.nl/dailydata/predefinedseries.php).

Tab. 2: Raw weather data terms and descriptions.

Original dataset term	Description	Study term
TG	daily mean temperature	‘tmean’ in °C
TN	daily minimum temperature	‘tmin’ in °C
TX	daily maximum temperature	‘tmax’ in °C
RR	daily precipitation sum	‘rr’ in mm (1 mm= 1 L/ 1 m²)

The data is made available on 0.25 * 0.25-degree regular grid, has a daily resolution and covers the whole of Europe.

To obtain suitable meteorological data for the examination region Germany, the following steps were carried out:

1. All five weather parameters were downloaded and unzipped.
2. The netcdf file format is displayed with Open GrADS© software[7].
3. The downloaded file is reduced using the Open GrADS© script ‘reduceSize.gs’ to the appropriate spatial and temporal coverage with a longitude of 5 – 16°E and a latitude of 47– 56°N. The examined meteorological time span covers 5479 days in total, from 01/01/2000 to 12/31/2014.
4. Reformatted weather data with Open GrADS© is converted from the Open GrADS© net file format into csv file format with the script „exportcsv.gs’ (new csv-file: „DE_weather.csv’). This is done to import the data into a SQL database. With the script ‘exportcsv.gs’ a numerator ‘t’ was added representing a day-count and starting with „1’ on date 01.01.2000. This makes it possible to combine weather and epidemiological data temporarily at a later stage.
5. Import file „DE_weather.csv’ into a SQL database:
   To import weather data into a SQL database, see the file ‘from netcdf to mysql table.txt’ for detailed information. The imported table is called ‘NEWgridweather’ (see a subset in Tab. 3). This table contains x and y values for the 36 x 44 grid cells covering

[7] Open GrADS© Version 2.0.2oga.2-win32_superpack was used. Download at: http://sourceforge.net/projects/opengrads/files/grads2/2.0.2.oga.2/Windows/grads-2.0.2.oga.2-win32_superpack.exe/download).

Germany. The starting point of the counting is on the bottom left corner. Grid cells covering the sea do not contain weather data because the ECA only samples data on land. Therefore, the table ‘NEWgridweather’ contains 7,062,431 records and not 44 [x] x 36 [y] x 5479 [t] = 8,678,736 rows. The grid cells have an area size of 435 to 527 km² depending on the respective latitude. An average grid cell is 28 km (North-South) to 17 km (West-East) in size (Fig. 18).

Tab. 3: SQL table NEWgridweather (subset). A t-value and x and y values are added to the weather data.

gridweatherID	x	y	t	rr	tmax	tmean	tmin
1	1	1	1	1.6	5.04	3.32	1.48
2	1	1	2	0	5.47	4.57	3.3
3	1	1	3	0	6.86	4.24	1.5
4	1	1	4	1.4	8.14	3.5	-0.95
5	1	1	5	0	10.5	7.47	4.32
6	1	1	6	0	8.89	4.81	0.83
7	1	1	7	0	6.49	4.62	2.45
8	1	1	8	1.5	5.27	2.57	-0.08
9	1	1	9	6.9	5.75	4.73	3.63
10	1	1	10	0	3.26	2.8	2.16
11	1	1	11	0	3.31	2.76	1.98
12	1	1	12	0	3.9	2.49	0.84
13	1	1	13	0	4.58	1.61	-1.33
14	1	1	14	0	5.71	0.83	-3.47

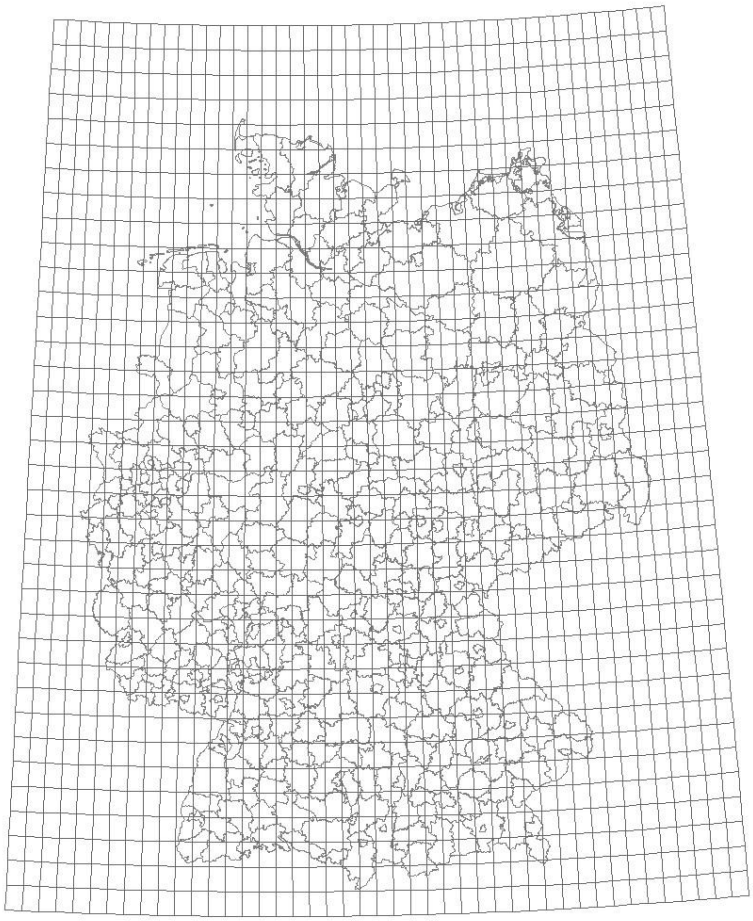

Fig. 18: Gridded weather data, overlapped with incidence reporting areas.

The temporal coverage of meteorological data contains an additional year, the year 2000, compared to the epidemiological data. This is to relate precedent weather parameters to incidences that occur with a specified time offset (lagtypes).

Seasonal bias

Calendar week-based incidence and median of daily mean temperature were plotted over the course of the year to examine whether the season is a confounding factor. There is an apparent seasonal variation in incidences with a peak in the summer months (Fig. 8, page 39). However, similar temperatures do not result in an equivalent pattern of incidence outcome over time. For example, the increase of incidence is steeper in spring and less dramatic in autumn while temperatures are similar during both seasons. Even

though temperatures constantly decrease in autumn, incidences stay on a higher level compared to equal temperatures in spring.

Moreover, the rise and fall of incidence outcome are lagging temperature by three to four weeks (lagtype 1 includes one week of time lag already). While a seasonal bias is implicated, this study avoids the latter due to the applied methodological approach. Here, local weather conditions are correlated with campylobacteriosis outcome selected solely along weather parameter values. This provides a clear signal minimising spatiotemporal bias.

2.2.3 GIS data

GIS shapefiles were used to provide spatial information for reporting area-based incidence values. The data contains reporting areas as well as NUTS-3 level IDs and is also provided by the BKG[8].

The BKG data set had to be enhanced for the Berlin area because the RKI incidence dataset has a higher spatial resolution than the BKG data set in the Berlin region. More detailed data for the reporting areas of Berlin was provided by 'Senatsverwaltung für Stadtentwicklung und Wohnen Berlin'[9].

In ArcGIS©[10], the shapefile with the districts of Berlin were intersected with the BKG dataset to provide a complete map congruent with RKI data. UTM32 was selected as the georeferencing system. To conduct area calculations, equal areas were needed. Therefore, 'Europe_Albers_Equal_Area_Conic' was chosen for projection. The GIS data background is included in the Personal Geodatabase 'CampylobacterGeodatabase.mdb' and can be visualised in the ArcMap document 'CampylobacterGISdata.mxd'.

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[8] Download BKG data at:
http://www.geodatenzentrum.de/geodaten/gdz_rahmen.gdz_div?gdz_spr=deu&gdz_akt_zeile=5&gdz_anz_zeile=1&gdz_unt_zeile=16&gdz_user_id=0.
The file 'Verwaltungsgebiete 1:1.000.000 – Stand 31.12.2014' has been used.
For more information on the data see the full documentation online at:
http://www.geodatenzentrum.de/geodaten/gdz_rahmen.gdz_div?gdz_spr=deu&gdz_akt_zeile=5&gdz_anz_zeile=1&gdz_unt_zeile=16&gdz_user_id=0#dok.

[9] Data for Berlin is available as WebFeatureService (WFS) at:
http://www.stadtentwicklung.berlin.de/geoinformation/geodateninfrastruktur/de/geodienste/wfs.shtml. „Bezirke von Berlin' (districts of Berlin) were selected, checked on 09/11/2015.

[10] In this study, ArcGIS version 10.4 for desktop was used for spatial data processing.

2.3 Data processing

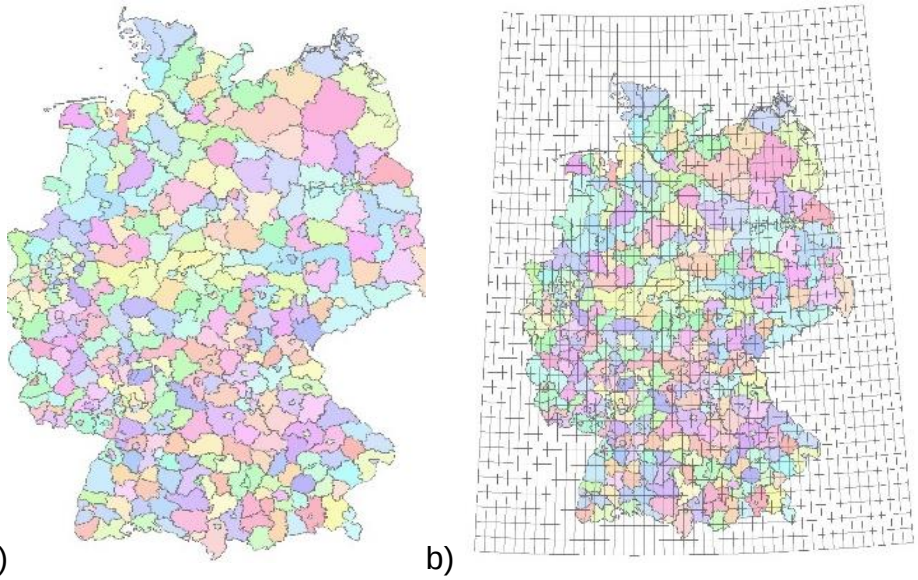
2.3.1 Calculating population

Based on incidences and cases, the population per reporting area was calculated with the SQL stored procedure ‘calculate_pop.sql’. For those reports where the population could not be calculated from incidences and cases because cases are zero, another step had to be carried out. For a specific reporting area, the same stored procedure (‘update_pop.sql’) searches up (coming week) and down (past week) the time range until a population value was found where cases are not equal to zero. If two values before and after an empty cell were found, the average of those two population values was calculated and replaced the missing value. Furthermore, this procedure deleted the 53rd week[11].

[11] Resulting table „newconvertedcampylobacter_updatepop.xlsx’

2.3.2 Spatial compatibility

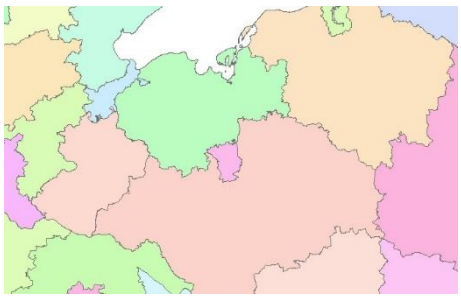
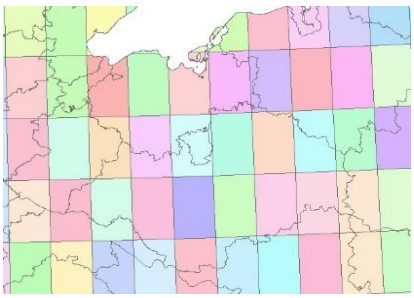
Vector formatted incidence-reporting areas and gridded weather data had to be processed and were made compatible to form one complete dataset (Fig. 19).



a) b)

c)

Fig. 19: GIS Data: a): Spatial coverage of incidence reporting area. b): Resolution of gridded weather data. c): Merged incidence reporting areas and weather data.

a)  b) 

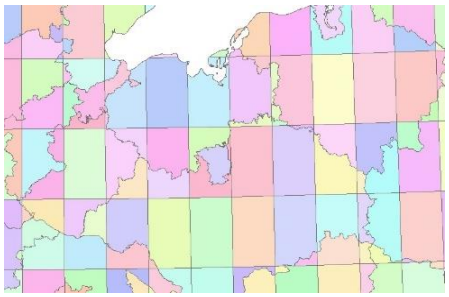
c) 

Fig. 20: Assigning gridded weather data to reporting areas. To the reporting-area shape of incidences (a), a fishnet congruent to the weather raster layer (b) was added. Subsequently, the created fishnet was intersected with the shapefile containing the reporting areas (c).

It was necessary to recalculate gridded weather data to reporting areas to obtain spatial compatibility (Fig. 20). The calculation was carried out with ArcGIS© and MySQL© stored procedures. To manage the obtained databases, the free client HeidiSQL© was used.

The following steps were carried out to create spatial compatibility:

1. A new column ‘RKI_to_GIS’ was created in the reporting-area shapefile with ArcGIS©. Through this, weather data could be joined with epidemiological data at a later stage.
2. Using ArcGIS©, a fishnet congruent to the weather raster layer (0.25 reg deg) was created and added to the reporting-area shape of incidences. The outline of the net in decimal degrees are: Upper extreme = 56; Lower extreme = 47; Right = 16; Left = 5. Width and length of every raster are 0.25 decimal degrees.

3. Under the ArcGIS© function, ‘Environment’, x and y values were changed to decimal degrees and the coordinate system to WGS 1984.
4. To obtain x and y coordinates for each fish-net grid cell provided by the exported weather data, the function ‘Calculate Geometry’ and ‘X Coordinate of Centroid’ was carried out. The following formula was used in the ‘Field Calculator’ of ArcGIS© to obtain integer values:

((X_coord. – 5) + 0.125)/0.25 = 1

((Y_coord. – 47) + 0.125)/0.25 = 1

5. The fishnet points that received an x and y value were fixed by a Join between the fishnet ID (‘FID’) and the ID of the fishnet grid cells, so ultimately every grid cell received x and y values.
6. The areal size of each fishnet grid cell was then calculated by ‘Calculating Geometry’ in km² which is different for each latitude (field: ‘FISHNET_Area_EAEA’). The file was then exported via ‘data export’ as a shapefile to fix the Join permanently (file: ‘fishnet5extended.shp’).
7. The areal size of each reporting area was calculated in the shapefile ‘RKI_newconvertedcampylobacter_updatepop’ (field: ‘RKI_area_EAEA’).
8. Subsequently, the created fishnet was intersected with the shapefile containing the reporting areas (new file: ‘rki_intersect’). The Join was carried out by ‘FID_copy’ being congruent with the ‘RKI_to_GIS’ column created earlier on. The fishnet can be described as the mould cutting the reporting areas into smaller subareas (Fig. 20).
9. A new column, called ‘Split_area_EAEA’, was added to the shapefile ‘newrkisplit’ containing the calculated areal size of the new subareas.
10. Every reporting area is covered by several weather grid cells reporting different weather outcomes. In the example depicted in Fig. 21, the RA ‘Dresden’ is covered by three different weather grid cells. To calculate a total reporting area weather, every subarea of a reporting area was weighted by dividing each reporting subarea by the whole areal size of the respective reporting area to know its proportion. This proportion or weighting factor (stored in field: ‘Split_RKI_weight’ = ‘Split_area_EAEA’/’RKI_area_EAEA’) was used at a later stage to calculate a total reporting area weather for each reporting area.

Fig. 21: Example of areal weight: The reporting area ‘Dresden’ is covered by three weather grid cells. The areal proportion of each grid cell to the reporting area is calculated based on the overall areal size of the latter.

11. The resulting ArcGIS© attribute table ‘newrkisplit’ was exported as dbf format into a SQL database[12] and was updated. Updating was carried out by the stored procedure ‘updatenewrkisplit’ (Resulting table: ‘newrkisplit’, see a subset in Tab. 4). This update adjusted weighting for non-complete covered reporting areas. For example, some reporting areas might be placed along a shore where the grid cell mostly covers the sea. Thus, valid weather data is not available here, as determined by the data source from ECA.

At this stage, reporting areas were fully processed to be joined with weather data in the following steps.

[12] For detailed information on how to export the ArcGIS© attribute table „newrkisplit’ into MySQL see text file ‘arcgis2mysql.txt’.
Dbf files may be opened using OpenOffice© since the latest version of Microsoft© Excel does not support dbf format.

Tab. 4: Subset of SQL table ‘newrkisplit’. See Tab. 5 for variable descriptions.

Split_ID	Fishnet_ID	Fishnet_area_EAEA	x	y	GIS_ID	RKI_area_EAEA	Split_RKI_weight	Split_RKI_weight4Grid
1	63	524.817	20	2	426	1,528.64	0.00069201	0.000692008
2	64	524.817	21	2	426	1,528.64	0.146578	0.146577
3	65	524.817	22	2	426	1,528.64	0.186272	0.186272
4	67	524.817	24	2	416	1,011.83	0.046522	0.046522
5	68	524.817	25	2	416	1,011.83	0.189168	0.189168

Tab. 5: Variable description of Tab. 4 ‘newrkisplit’.

Variable	Description
Split_ID	Split area identification number
Fishnet_ID	Fishnet area identification number
Fishnet_area_EAEA	Areal size of the fishnet area in km²
x	X coordinate for the fishnet grid cell
y	Y coordinate for the fishnet grid cell
GIS_ID	Identification number of the RKI reporting areas
RKI_area_EAEA	Areal size of the reporting area in km²
Split_area_EAEA	Areal size of the split area in km²
Split_RKI_weight	Share of the split area on the respective reporting area
Split_RKI_weight4Grid	Share of the split area of the respective weather grid

2.3.3 Calculating weather for incidence-reporting areas

To join weather data and reporting areas in the SQL database, a unique identifier was needed, here called ‘GIS_ID’, compatible with RKI reporting area identification numbers (‘FID_copy’). The join was carried out by the stored procedure ‘cal_ra_weather’, combining table ‘NEWRKI_split’ (containing reporting areas split by fishnet, as described

above) and ‘newgridweather’. The new table ‘newrkiweather1’ contains a summary of weather in split reporting areas weighted by the areal portion of the respective reporting area. The suffix ‘1’ stands for the daily resolution of the weather data in the created table (Tab. 6). Additionally, the table contains ISO-year, ISO-week and the day value of the week’s Monday (Tab. 7) (see glossary for further explanation).

Tab. 6: Subset of SQL table ‘newrkiweather1’.

RKIweather_id	GIS_ID	RKI_area_EAEA	year	week	Monday	day	RKI_rr	RKI_tmin
1,970,576	28	1,018.2000	1999	52	0	1	1.5829	2.1517
1,970,577	28	1,018.2000	1999	52	0	2	0.0000	1.7047
1,970,578	28	1,018.2000	2000	1	3	3	7.4823	5.6677
1,970,579	28	1,018.2000	2000	1	3	4	4.3193	2.8983
1,970,580	28	1,018.2000	2000	1	3	5	0.0000	2.3742
1,970,581	28	1,018.2000	2000	1	3	6	3.8775	1.8316
1,970,582	28	1,018.2000	2000	1	3	7	0.0000	4.5031
1,970,583	28	1,018.2000	2000	1	3	8	0.6646	3.8726
1,970,584	28	1,018.2000	2000	1	3	9	0.0000	1.1671
1,970,585	28	1,018.2000	2000	2	10	10	0.0000	0.8726
1,970,586	28	1,018.2000	2000	2	10	11	0.0368	0.4559
1,970,587	28	1,018.2000	2000	2	10	12	0.0000	0.3825
1,970,588	28	1,018.2000	2000	2	10	13	0.0000	0.7231
1,970,589	28	1,018.2000	2000	2	10	14	0.0000	0.2610
1,970,590	28	1,018.2000	2000	2	10	15	0.7903	0.8846
1,970,591	28	1,018.2000	2000	2	10	16	0.6180	1.5860

Tab. 7: Variable description of Tab. 6 ‘newrkiweather1’.

Variable	Description
RKIweather_id	Row ID
GIS_ID	Identification number of RKI reporting areas
RKI_area_EAEA	Areal size of reporting area in km²
Year	ISO-Year of reporting
Week	ISO-Week of reporting
Monday	Day value of the monday of reporting week
Day	Day of reporting
RKI_rr	Sum of precipitation
RKI_tmin	Minimum temperature on this day
RKI_tmean	Average temperature on this day
RKI_tmax	Maximum temperature on this day

The table ‘newrkiweather1’ contains 2,261,588 rows, resulting from the following parameters:

- Temporal coverage of 5,476 days with a daily resolution
- Spatial coverage of 413 Reporting areas
- The first Monday of the year 2000 is the third day of the year, in this case, the 3rd of January 2000 (Monday = 3).

In a next step, the table ‘newrkiweather1’ was summarised from a daily to a weekly resolution by the stored procedure ‘createweather7’ to harmonise weather with weekly epidemiological data. This operation resulted in 323,379 rows (created table: ‘newrkiweather7’) (Tab. 8).

Tab. 8: SQL table structure of ‘newrkiweather7’.

#	Name	Datatype
1	**ID**	**INT**
2	GIS_ID	INT
3	RKI_area_EAEA	DOUBLE
4	year	INT
5	week	INT
6	monday	INT
7	RKI_rr_avg	DOUBLE
8	RKI_rr_sum1	DOUBLE
9	RKI_rr_sum2	DOUBLE
10	RKI_rr_sum3	DOUBLE
11	RKI_rr_sum4	DOUBLE
12	RKI_tmin_avg	DOUBLE
13	RKI_tmean_avg	DOUBLE
14	RKI_tmax_avg	DOUBLE
15	RKI_rr_min	DOUBLE
16	RKI_tmin_min	DOUBLE
17	RKI_tmean_min	DOUBLE
18	RKI_tmax_min	DOUBLE
19	RKI_rr_max	DOUBLE
20	RKI_tmin_max	DOUBLE
21	RKI_tmean_max	DOUBLE
22	RKI_tmax_max	DOUBLE

To calculate the weekly weather out of daily data caused the loss of extreme values. Maximum and minimum values of each weather parameter and each reporting week were added to keep this information. In addition to this, in the case of precipitation totals were created of the weekly precipitation sum (RKI_rr_sum1), two-week precipitation sum including the total precipitation of the respective week plus the total precipitation of the precedent week (RKI_rr_sum2), etc.. Precipitation sums were calculated for up to four weeks.

At this stage of data processing, weekly weather data consisted of 16 different weather parameters (listed in the following). No lag times were applied yet. Incidence data and weather data refer to the same day, and both data types were still stored in two different tables. However, the two data types have incidence-reporting areas as a common spatial

reference at this stage. Incidences and cases have a weekly resolution; weather parameters provide daily and weekly resolution. These tables were prepared for joining in the following, applying eight different temporal lags with the use of SQL stored procedures.

2.3.4 Implementing the new methodological approach

The methodological approach described above was then implemented in the processed data. The MySQL© stored routine ‘create_results_all’ created a new table called ‘results_all’ (see subset in Tab. 9).

Tab. 9: Subset of SQL table ‘results_all’ created with a SQL routine.

aspect_name	**pp_min**	**pp_min**	**pp_min**	**pp_min**	**pp_min**
Lagtype	1	1	1	1	1
classNo	1	2	3	4	5
classN	5000	5000	5000	5000	5000
aspect_min	973.948999	989.337973	992.496515	994.5424157	995.975405
aspect_max	989.337858	992.495549	994.542389	995.9746924	997.178634
aspect_avg	985.549906	990.981086	993.621874	995.2884522	996.587567
aspect_median	986.441684	991.000306	993.69983	995.3048516	996.6
sum_cases	12323	12115	12077	12318	11477
sum_pop	1088663488	1078589070	1062259873	1077175166	1006675483
incidence	1.1319	1.1232	1.1369	1.1435	1.1401

Firstly, this routine sorted all values from smallest to largest by weather conditions, for each weather parameter separately. Secondly, classes of 5,000 reports were created, adding up cases and population for all 5,000 reports to create a single incidence for each class. Every class is inhomogeneous regarding temporal and spatial linkage, so it is very likely that one class contains reports from different reporting areas and different weeks. The class size on the basis of 5,000 reports provides a robust statistical background for interpretation. The created table ‘results_all’ contains the following columns:

- All weather parameters e.g., Tmax_min, Tmax_avg, rr_min (‘aspect_name’)
- Lagtypes 1 to 8 (‘Lagtype’)
- Reports grouped into classes of 5,000 reports (‘classN’) which are numbered (‘classNo’)
- For each class, minimum, maximum, average and median of the weather parameter value of this class is calculated (‘aspect_max’, ‘aspect_median’, etc.)
- Sum of class cases (‘sum_cases’)
- Sum of the class population (‘sum_pop’)
- Incidence per class from 5,000 reports per class (‘incidence’)

Note, that for precipitation data an additional class was introduced called ‘classNo 0’. This class contains all reports with a precipitation sum of 0 mm. Due to this, the number of classes for precipitation parameters varies and is different from the number of classes of temperature parameters.

At the end of data processing, 16 weather parameters have been created from the raw data consisting of temperature and precipitation parameters:

- Weekly maximum of daily maximum temperature (‘Tmax_max’)
- Weekly average of daily maximum temperature (‘Tmax_avg’)
- Weekly minimum of daily maximum temperature (‘Tmax_min’)
- Weekly maximum of daily mean temperature (‘Tmean_max’)
- Weekly average of daily mean temperature (‘Tmean_avg’)
- Weekly minimum of daily mean temperature (‘Tmean_min’)
- Weekly maximum of daily minimum temperature (‘Tmin_max’)
- Weekly average of daily minimum temperature (‘Tmin_avg’)
- Weekly minimum of daily minimum temperature (‘Tmin_min’)
- Weekly maximum of daily precipitation (‘rr_max’)
- Weekly average of daily precipitation (‘rr_avg’)
- Weekly minimum of daily precipitation (‘rr_min’)
- One-week total of (daily) precipitation (‘rr_sum1’)
- Two-week total of (daily) precipitation (‘rr_sum2’)
- Three-week total of (daily) precipitation (‘rr_sum3’)

- Four-week total of (daily) precipitation (‘rr_sum4’)

Weather parameters and related reporting weeks were tested for different time lags. Weather parameters were examined directly before the reporting week (lagtype 1) with a time offset of up to eight weeks (lagtype 8) before an incidence reporting week. For example, in lagtype 6 the respective incidence week correlates with the weather conditions six weeks ago not considering the five intermediate weeks.

2.4 Statistical analysis

The analysis is based on the final SQL table ‘results all’. To investigate the association of weather and health outcome, campylobacteriosis incidence was compared with predating weather parameters with lag times of one to eight weeks. To identify the most effective weather parameter and lagtype, the sum of squared deviation from the expected incidence and the R^2 value for a fitting regression model was calculated for each variant.

2.4.1 Sum of squared deviation of incidence vs expected incidence

The sum of squared deviation of incidence outcome (‘SSD’) describes the deviation of incidence outcome in the respective week from the overall averaged incidence, called ‘expected incidence’ per weather parameter and lagtype. The expected incidence is calculated by summing up all reported incidence values and dividing it by the total number of incidence entries (Equation 1). It is assumed that the expected incidence is the incidence value which is reached if there is no association between campylobacteriosis incidence and weather conditions, also called the null hypothesis.

Equation 1 $\boldsymbol{expected\ incidence\ =\ \Sigma\_(k=1)\hat{}N\ i(k)\ /\ N}$

The deviation from the expected incidence is considered to quantify the influence of the tested weather parameter on the incidence outcome. Thus, the higher the SSD value, the better the incidence outcome is explained by the tested weather parameter. The calculated expected incidence is 1.433. Note that the SSD referred to here is not synonymous with the traditional SSD term. Here, SSD should increase rather than decrease to explain incidence outcome.

2.4.2 Regression analysis

A four-parameter sigmoid logistic model (Equation 2) was used to describe the association between campylobacteriosis incidence and temperature and incidence and precipitation.

All estimated parameters were statistically significant (all p<0.001) and robust against single values validated with the bootstrap procedure[13].

Statistical analysis was carried out using STATA© (Version IC 13.1) and Mathematica (Version 11.3). For all regression analysis, the median of each class of 5,000 reports was used because of its robustness against extreme values.

Since polynomial models have been used to describe campylobacteriosis and climatic factors (Patrick et al., 2004) third, fourth-degree polynomials and sigmoid regression were bootstrapped for comparison. Third-degree polynomials turned out not to be significant, and fourth-degree polynomials returned very wide confidence intervals when being bootstrapped. The sigmoid regression turned out to be the best model, in which all parameters were robust against single values, significant and the confidence intervals stayed narrow after bootstrapping. If the incidence, as suggested by the polynomial model, would fall or rise in extreme temperatures, the confidence intervals of the sigmoid regression would not exclude this possibility. In any case, only conclusions within the range of tested data will be made. The formula of the sigmoid regression with four parameters is:

Equation 2 $\boldsymbol{log4}$: $\boldsymbol{y = b0 + b1/(1 + \exp(-b2 * (x - b3)))}$

R-squared (R^2), the coefficient of determination, was calculated to determine how well the observed incidence outcome could be explained by the chosen regression model. It describes the quality of the regression model within a range of 0 to 1. The rest of the variance cannot be explained by the weather parameter and might be influenced by other factors. In order to estimate the steepest incidence increase as a function of temperature, the slope in the inflexion point (b3), has been calculated for each sigmoid function as:[14]

Equation 3 $\boldsymbol{slope = (b1 * b2)/4}$

[13] The bootstrap procedure can be found in the corresponding folder in the supporting documents.
[14] Slope analysis can be found in the corresponding folder in the supporting documents.

2.4.3 Extreme classes

For regression analysis, the two extreme classes were omitted. This was carried out for several reasons. Firstly, the upper extreme class of each weather parameter contains a much smaller n of mostly 1,490 reports, compared to all other classes that contain 5,000 reports as predefined by the method (Fig. 22). Unequal classes may lead to skewed results. Secondly, the class widths of class 1 and 61 are much wider than those of all other classes, which causes difficulties when comparing classes. In addition, extreme classes defined by the weather conditions might represent regions of extreme, hostile weather conditions unsuitable to host dwellers. This fact may over-represent rural regions in high precipitation conditions and underrepresent those in mild temperature regions.

Tmean_max	week	1	55	5000	21.58021851	22.0624094
Tmean_max	week	1	56	5000	22.06246065	22.5797754
Tmean_max	week	1	57	5000	22.57986006	23.2459414
Tmean_max	week	1	58	5000	23.24614189	24.0518926
Tmean_max	week	1	59	5000	24.05194986	25.1130342
Tmean_max	week	1	60	5000	25.11332168	26.9763761
Tmean_max	week	1	61	1490	26.97765873	30.1877433

Tmean_max	week	1	61	301,490	-12.91433509	30.1877433
			[max]	[sum]	[min]	[max]
aspect	**temptype**	**lagtype**	**ClassNo**	**n**	**aspectMIN**	**aspectMAX**

Fig. 22: Subset of the table ‘evaluation.xlsx’ which contains all results. The upper extreme class, here class ‘61’ for temperature, only contains 1,490 reports compared to all other classes filled with 5,000 n.

Weather conditions are not equally distributed over Germany. So, it is expected that extreme weather conditions cannot be produced in every region. Therefore, there is reason to suspect that not every reporting area in Germany is represented in the extreme classes. Consequently, the participation of reporting areas in these classes is unequal and results in a few selected reporting areas dominating them. This phenomenon of unequally large classes has been exemplarily checked for the parameter ‘weekly minimum

of daily mean temperature' (Fig. 23) and is exemplified by reporting areas on the edge of a mountain chain, e.g. the Alpine foreland in the South or the Ore Mountain Range ('Erzgebirge') in the East of Germany (Fig. 24).

In class 1 to 58 (and class 60) each reporting area is included in each class. On the other hand, the maximum number of reports from a single reporting area is very high in the lower extreme class and very low in the upper extreme class. Furthermore, the upper extreme class only includes 406 out of 413 reporting areas. Thus, extreme classes are not representative for all reporting areas as expected.

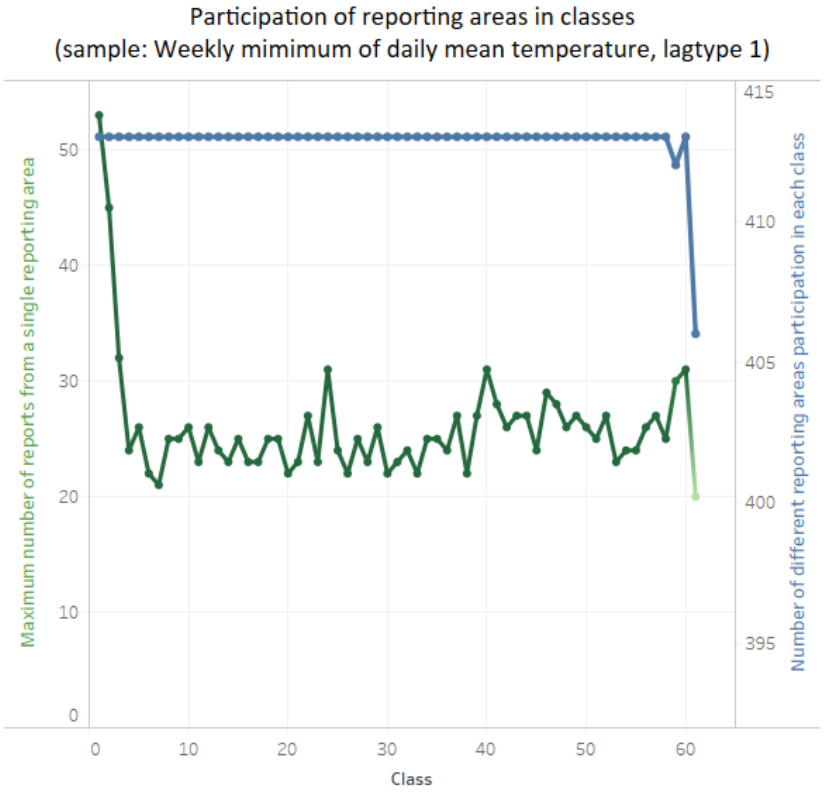


Fig. 23: Participation of reporting areas in classes.

By excluding extreme classes, rural-urban bias, bias regarding plain versus mountainous regions, east-west bias and skewed outcomes can be reduced, and results are more representative for the whole of Germany. Consequently, interpretation is based on stable results with equally large classes of 5,000 reports with narrow class widths. In sum, two

out of 61 classes (for temperature parameters) have been omitted. Omitting class 1 excludes 5,000 reports of a total of 301,490 reports while omitting class 61 excludes 1,490 reports of a total of 301,490 reports. Altogether, 6,490 reports are excluded (2.15 %) from raw data for each temperature parameter.

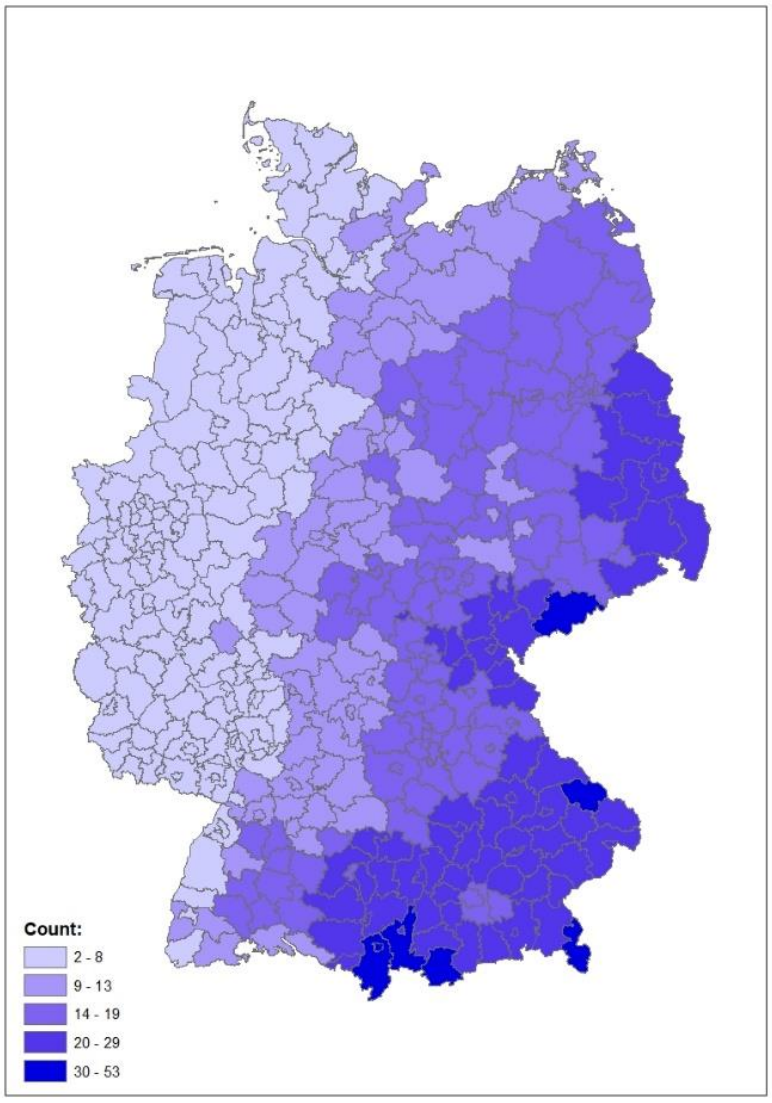


Fig. 24: Participation of reporting areas in extreme classes for the weekly minimum of daily mean temperature. Reporting areas on the edge of mountain chains are overrepresented in extreme classes with up to 53 counts.

2.4.4 Selecting significant weather parameters and lagtypes

This study aims at identifying the most significant combinations of 16 weather parameters and 8 lagtypes. Therefore, the following operation was carried out:

For each of the ($16 * 8 =$) 128 evaluations, R-squared for sigmoid regression and the sum squared deviation (SSD) have been calculated. Subsequently, for each climate variable (precipitation and temperature) the 5 % best outcomes of R^2 and SSD were selected to identify the most significant combination of weather parameter and lagtype. To examine temporal patterns in the association between weather conditions and incidence, the results of R^2 and SSD were visualised as a scatter plot and lagtypes per weather parameter were linked using RStudio© (Version 1.0.153)[15].

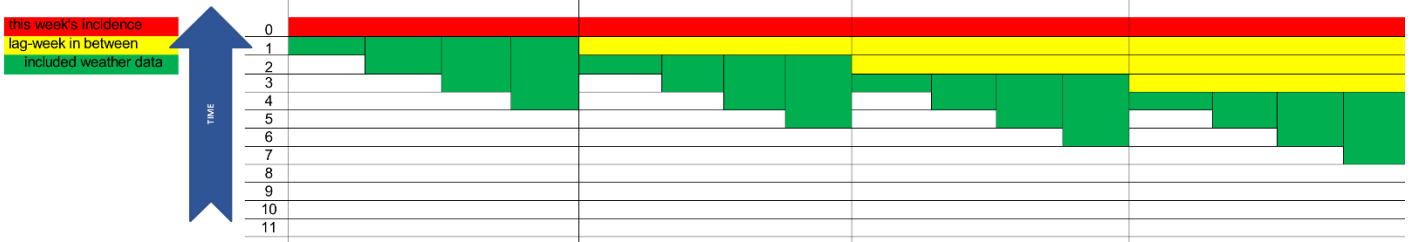
[15] The code to reproduce the scatterplot can be found in the corresponding folder in the supporting documents.

3. Results

All results for 16 weather parameters and eight lagtypes are summarised in Tab. 10 and Tab. 11[16]. Tab. 10 shows all results of the sum squared deviation (SSD) for each parameter-lag-combination, including all classes. Tab. 11 shows the calculated coefficient of determination (R^2) for each parameter-lag-combination while two extreme classes were omitted. To determine the weather parameters with the strongest association to incidence, the 5 % best SSD and R^2 results for each weather parameter and lagtype were identified which will be presented in detail in the following chapter[17]. The most effective lagtypes were identified by analysing the highest R^2 and SSD values. To visualise a temporal evolution along the lagtypes, R^2 and SSD values were plotted and the lagtypes linked for each weather parameter.

[16] Excel table „values_SSD_R².xlsx' is available in the supporting documents.
[17] The complete evaluation is available in the supporting documents in Excel table 'evaluation.xlsx'.

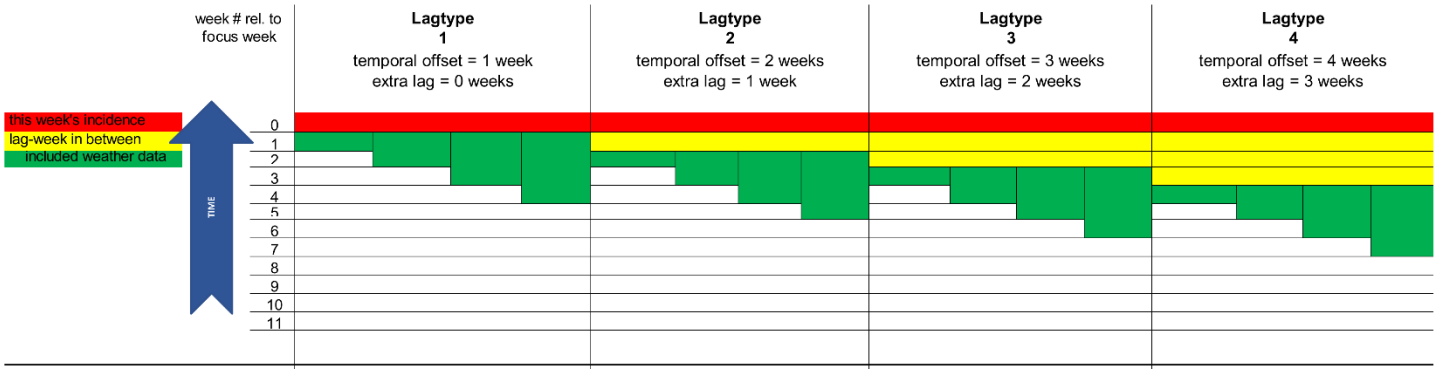
Tab. 10: Results. Sum of squared deviation (SSD) of incidence vs expected incidence of all classes.



	Lagtype 1 temporal offset = 1 week extra lag = 0 weeks	Lagtype 2 temporal offset = 2 weeks extra lag = 1 week	Lagtype 3 temporal offset = 3 weeks extra lag = 2 weeks	Lagtype 4 temporal offset = 4 weeks extra lag = 3 weeks
Precipitation				
this week's daily maximum	0.7709	0.7698	0.8615	0.8634
this week's daily average	0.5535	0.5767	0.6271	0.6300
this week's daily minimum				
Precipitation	1.3244	1.3465	1.4885	1.4934
one-week sum	0.5535	0.5767	0.6271	0.6300
two-week sum	0.8940	0.9531	0.9763	0.9363
three-week sum	1.5637	1.5833	1.6152	1.3794
four-week sum	1.6856	1.6545	1.5656	1.3568
Precipitation week sum	4.6967	4.7677	4.7842	4.3024
daily max. Temperature				
this week's maximum daily maximum	9.1025	10.0939	10.3203	10.6527
this week's average daily maximum	10.1050	11.0032	11.0378	11.2806
this week's lowest daily maximum	10.5995	11.2769	11.2007	11.2499
daily mean Temperature				
this week's maximum daily mean	10.5476	11.4433	11.5553	11.8246
this week's average daily mean	11.3017	12.1615	11.9977	12.0409
this week's lowest daily mean	11.5280	12.2628	12.0876	11.9426
daily min. Temperature				
this week's maximum daily minimum	11.5840	12.0460	11.6275	11.5054
this week's average daily minimum	11.9380	12.4871	12.1030	11.8212
this week's lowest daily minimum	11.6833	12.2021	11.9418	11.6724
Temperature	98.3898	104.9768	103.8718	103.9903

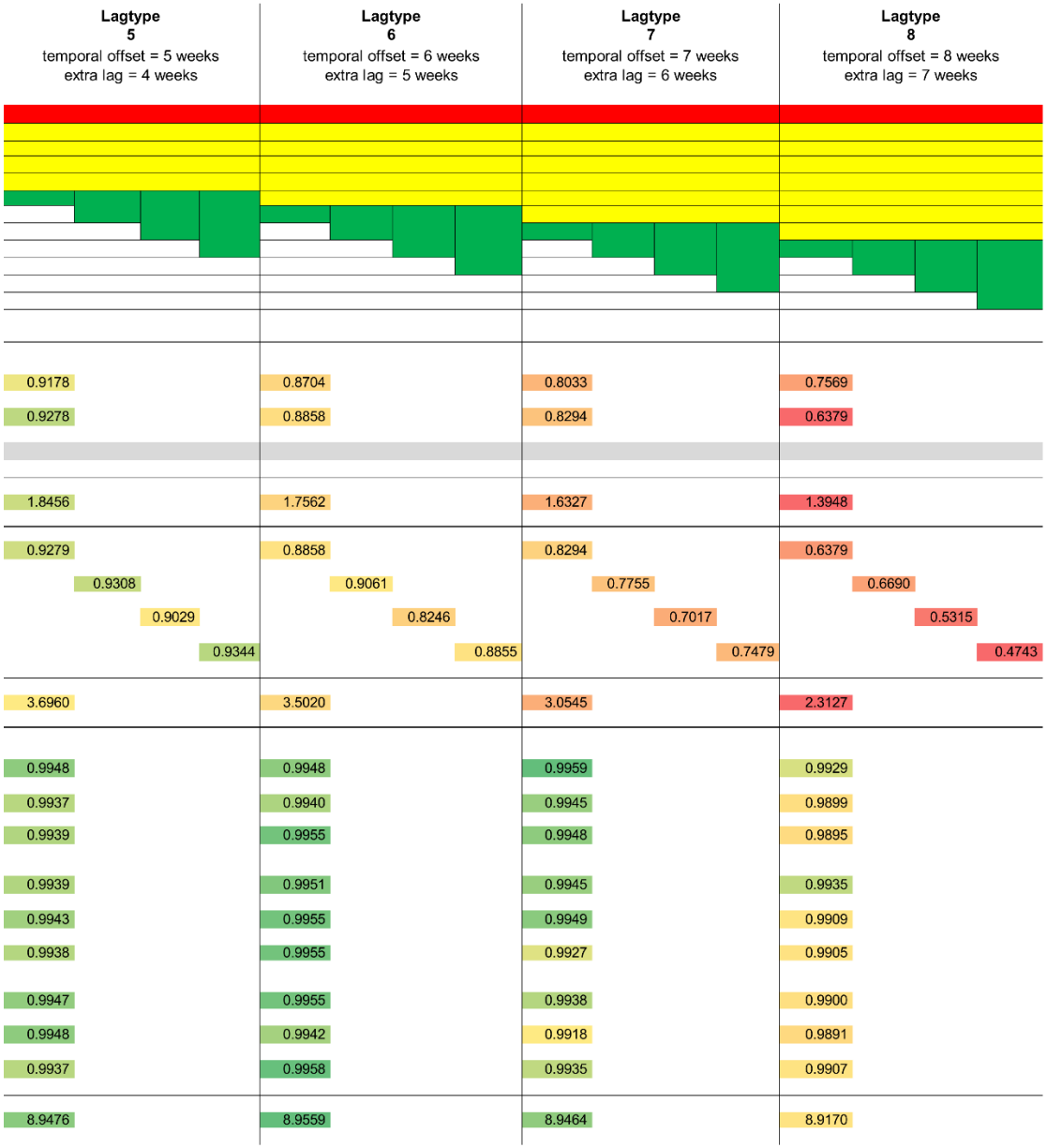
Tab. 10: Results. Sum of squared deviation (SSD) of incidence vs expected incidence of all classes, continued.

Tab. 11: Results. Coefficient of determination (R^2) for sigmoid regression (two extreme classes omitted).



	Lagtype 1 temporal offset = 1 week extra lag = 0 weeks	Lagtype 2 temporal offset = 2 weeks extra lag = 1 week	Lagtype 3 temporal offset = 3 weeks extra lag = 2 weeks	Lagtype 4 temporal offset = 4 weeks extra lag = 3 weeks
Precipitation				
this week's daily maximum	0.9396	0.9324	0.9249	0.9214
this week's daily average	0.9124	0.9153	0.8977	0.9467
this week's daily minimum				
Precipitation	1.8520	1.8477	1.8226	1.8681
one-week sum	0.9124	0.9153	0.8974	0.9467
two-week sum	0.9523	0.9517	0.9550	0.9402
three-week sum	0.9250	0.9361	0.9256	0.8897
four-week sum	0.9477	0.9587	0.9356	0.9391
Precipitation week sum	3.7374	3.7618	3.7136	3.7157
daily max. Temperature				
this week's maximum daily maximum	0.9832	0.9875	0.9878	0.9921
this week's average daily maximum	0.9727	0.9794	0.9838	0.9896
this week's lowest daily maximum	0.9715	0.9822	0.9859	0.9910
daily mean Temperature				
this week's maximum daily mean	0.9873	0.9922	0.9902	0.9931
this week's average daily mean	0.9727	0.9792	0.9827	0.9910
this week's lowest daily mean	0.9728	0.9799	0.9825	0.9901
daily min. Temperature				
this week's maximum daily minimum	0.9896	0.9947	0.9919	0.9948
this week's average daily minimum	0.9857	0.9917	0.9910	0.9942
this week's lowest daily minimum	0.9883	0.9909	0.9904	0.9923
Temperature	8.8238	8.8777	8.8862	8.9282

Tab. 11: Results. Coefficient of determination (R^2) for sigmoid regression (two extreme classes omitted), continued.



3.1 Temperature

Fig. 25 gives an overview of all temperature parameters, which were plotted against incidence, for lagtype 1. Among all temperature parameters, within a temperature corridor of -5 to 28 °C, incidence increased with rising ambient temperature. Below and above this corridor, the incidence is not significantly altered with changing temperatures. Among all temperature parameters, the absolute maximum incidence is reached at the weekly average of daily minimum temperature ('Tmin_avg') in lagtype 1 with an incidence of 2.25 and a temperature median of 13.91 °C. Very close to the absolute incidence maximum is the maximum incidence at the weekly average of daily mean temperature ('Tmean_avg') in lagtype 1 and at the weekly minimum of daily minimum ('Tmin_min') in lagtype 1 (Tab. 12).

Tab. 12: Absolute maximum incidence among all temperature parameters.

temperature parameter	**lagtype**	**incidence^{max}**	**temperature^{median} (°C)**	**temperature range (°C)**
tmin_avg	1	2.25	13.91	- 7.2 - 15.9
tmean_avg	1	2.24	18.15	- 4.0 - 22.2
tmin_min	1	2.24	10.52	-12.5 - 13.7

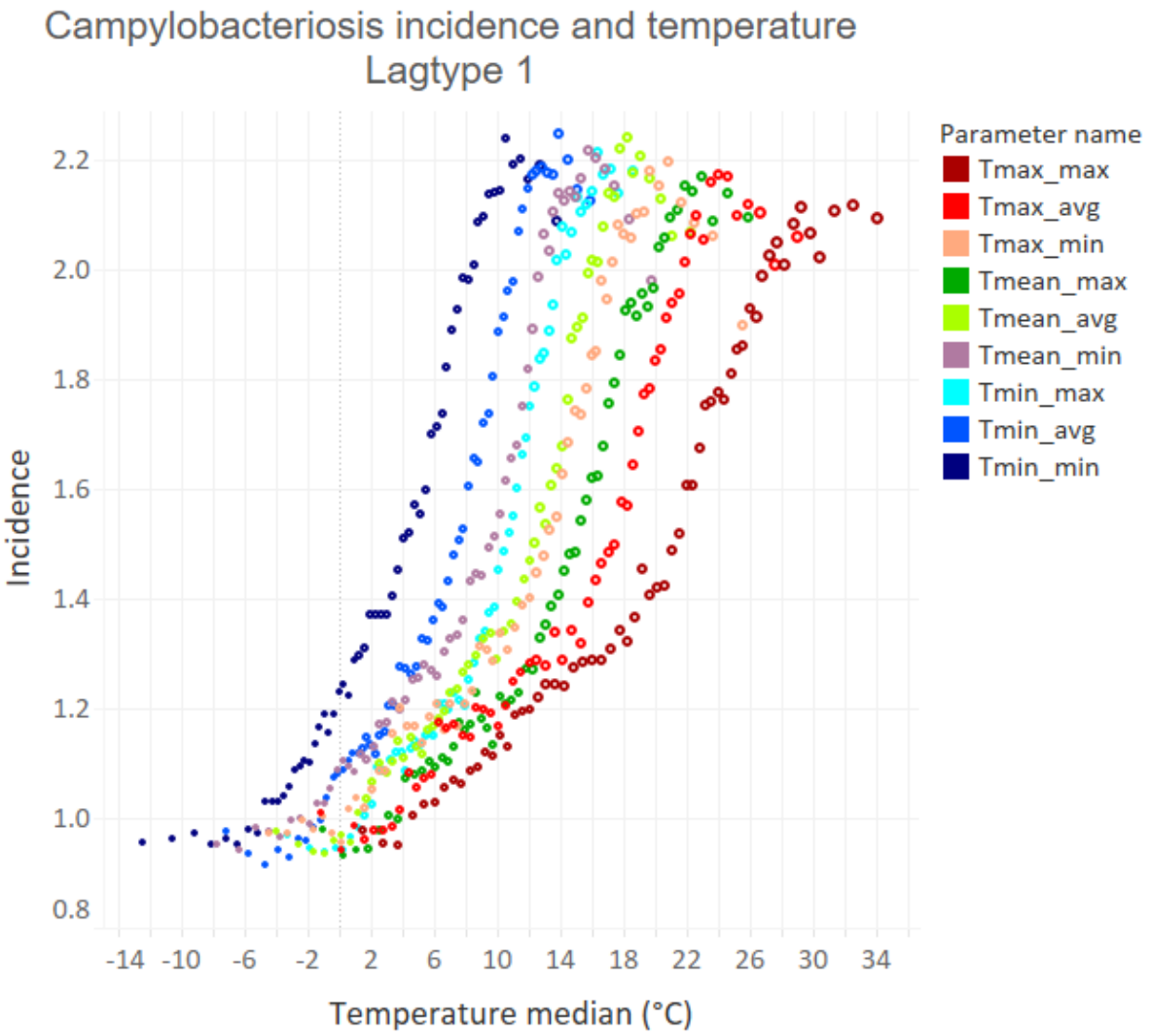


Fig. 25: Scatterplot of all temperature parameters, lagtype 1.

A sigmoid regression model was fitted to all temperature parameters[18]. A significant association between local ambient temperature and local campylobacteriosis incidence in Germany was found. In total, all sigmoid regressions for each temperature parameter and lagtype had a very good fit and showed comparable results (R^2: 0.9715 – 0.9959; $\bar{x} = 0.99$). Each temperature parameter in each lagtype explained more than 97 % of the health outcome while two extreme classes were omitted. Moreover, SSD ranged from 7.9291 to 12.4871 ($\bar{x} = 10.8995$) for all temperature parameters and each lagtype.

[18] All regression results for every lagtype are available in the Excel file 'evaluation.xlsx' in the supporting documents.

The gradient for all temperature parameters averaged 0.1 ($SD(m) = 0.01$). Thus, the sigmoid regression estimated that a 5 °C increase in temperature is associated with an incidence increase of 0.52 in the observation period.

In the following, the most significant temperature parameters and lagtypes will be examined.

3.1.1 Temperature parameters

In lagtype 2, incidence rose with increasing temperature in a temperature corridor of -5 to 17 °C. Below and above this temperature corridor, incidence stayed stable (Fig. 26). Temperature parameters with maximum SSD values in lagtype 2 are displayed in Tab. 13.

Tab. 13: Temperature parameters with maximum SSD values in lagtype 2. 'Tmin_avg' represents the weekly average of daily minimum temperature, 'Tmean_min' the weekly minimum of daily mean temperature, 'Tmin_min' the weekly minimum of daily minimum temperature and 'Tmean_avg' the weekly average of daily mean temperature.

temperature parameter	**incidence^{max}**	**temperature^{median} (°C)**	**temperature range (°C)**	**SSD**
Tmin_avg	2.20	13.91	-7.23 – 15.88	12.4871
Tmean_min	2.21	16.24	-7.73 – 19.77	12.2628
Tmin_min	2.19	10.95	-12.49 – 13.73	12.2021
Tmean_avg	2.24	17.74	-4.00 – 22.23	12.1615

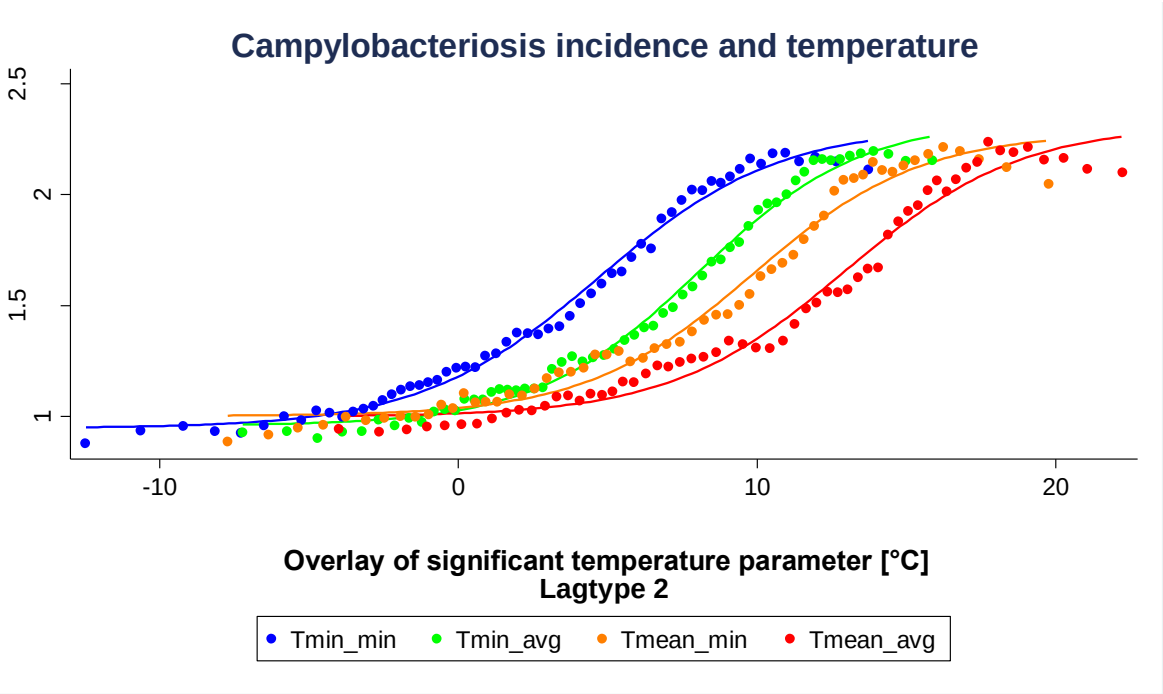


Fig. 26: Sigmoid regression envelope of significant temperature parameters selected by maximum SSD values in lagtype 2.

The most significant weather parameter in lagtype 2, the weekly average of daily minimum temperature ('Tmin_avg'), with the highest SSD value and highest incidences shows a temperature range of -7.2 to 15.9 °C. Most changes in incidence occurred between a temperature corridor of approximately 0 to 12 °C (Fig. 26).

In lagtype 6, incidence rose with increasing temperature in a corridor of -5 to 17 °C. Below and above this temperature corridor, the incidence was not altered with changing temperature (Fig. 27). Temperature parameters with maximum R^2 values in lagtype 6 are displayed in Tab. 14.

Tab. 14: Temperature parameters with maximum R^2 values in lagtype 6. 'Tmin_min' represents the weekly minimum of daily minimum temperature, 'Tmin_max' the weekly maximum of daily minimum temperature, 'Tmean_min' the weekly minimum of daily mean temperature, 'Tmean_avg' the weekly average of daily mean temperature and 'Tmax_min' the weekly minimum of daily maximum temperature.

temperature parameter	**incidence^{max}**	**temperature^{median} (°C)**	**temperature range (°C)**	**R^2**
Tmin_min	2.05	10.52	-12.49 – 13.73	0.9958
Tmin_max	2.14	18.55	-3.28 – 18.55	0.9955
Tmean_min	2.04	16.24	-7.73 – 19.77	0.9955
Tmean_avg	2.10	22.23	-4.00 – 22.23	0.9955
Tmax_min	2.03	23.67	-4.49 – 25.52	0.9955

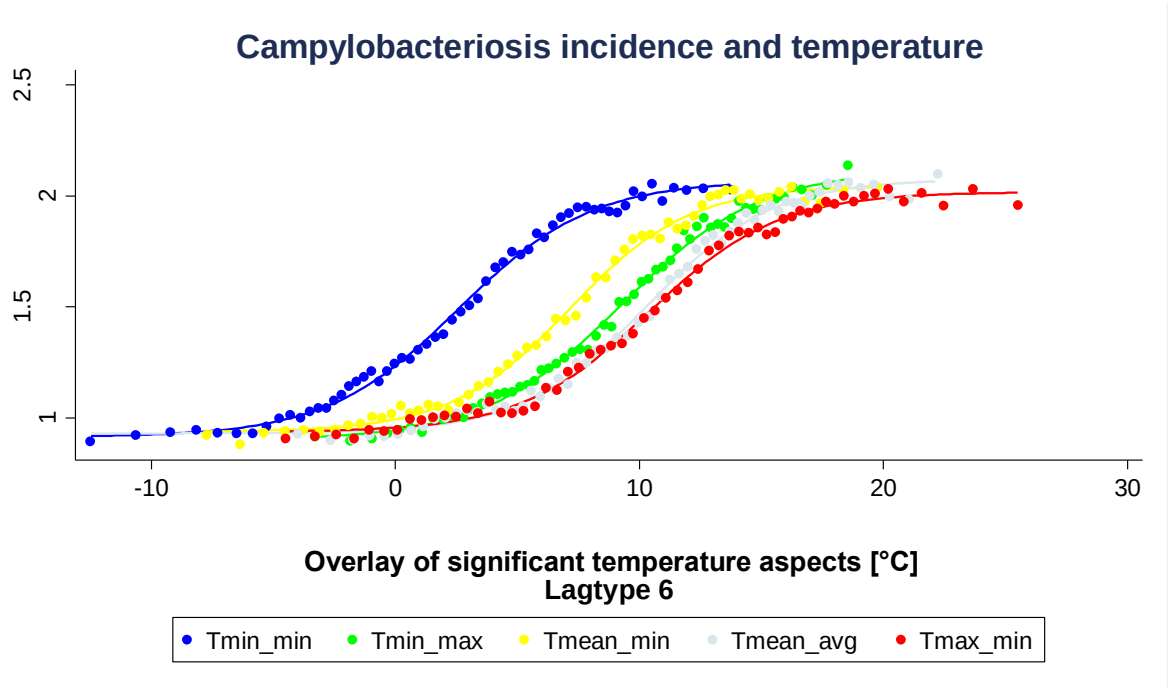


Fig. 27: Sigmoid regression of significant temperature parameters selected by maximum R^2 values in lagtype 6.

Maximum R^2 values in lagtype 7 were reached for the weekly maximum of daily maximum temperature ('Tmax_max'; R^2: 0.9959). For this temperature parameter, most changes in incidence occurred between 10 to 28 °C (Fig. 28).

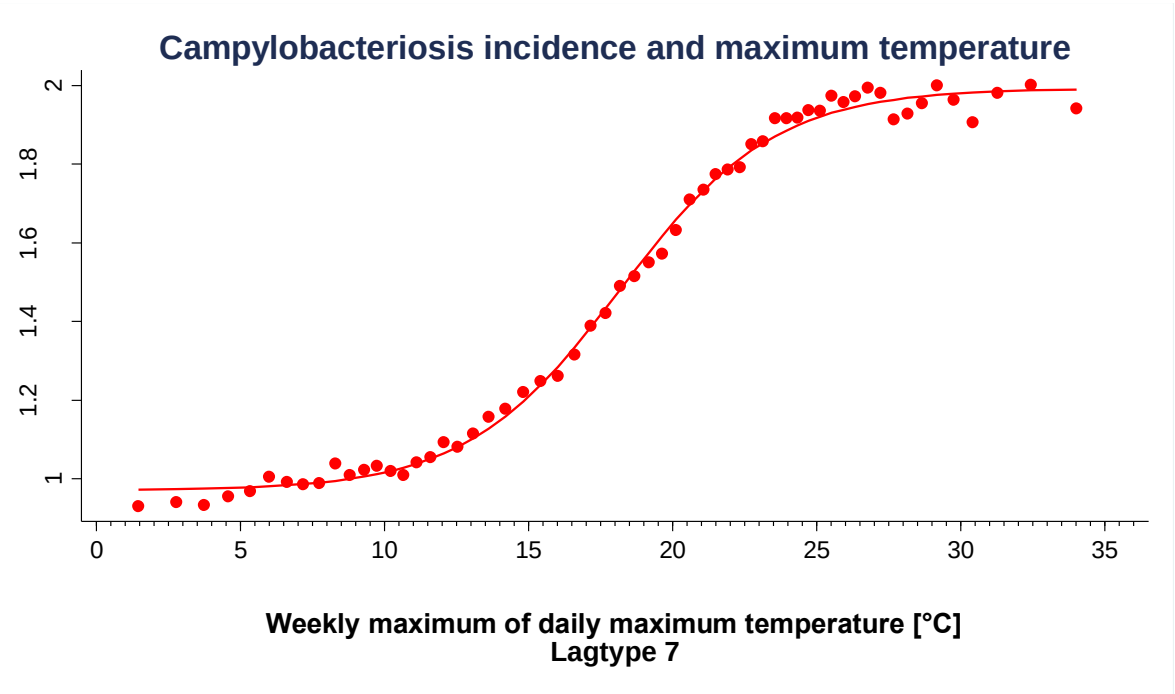


Fig. 28: Significant temperature parameter 'weekly maximum of daily maximum temperature' selected by maximum R^2 values in lagtype 7.

Overall, maximum SSD and R^2 values were reached for minimum and mean temperatures, especially in the weekly minimum of daily minimum temperature, the weekly average of daily minimum temperature, the weekly minimum of daily mean temperature, and the weekly average of daily mean temperature with maximal changes of incidence within a corridor of -5 to 17 °C.

3.1.2 Lagtypes

R^2 and SSD values of all temperature parameters and linked lagtypes are plotted in Fig. 29 and Fig. 30. R^2 and SSD values of all temperature parameters showed a temporal evolution along lagtype 1 to 8. Low and average weekly mean temperatures showed high correlations over a wide range of lagtypes (Fig. 29, lower chart). However, a temporal evolution was visible. R^2 values increased steadily up to lagtype 4 where the correlation became maximal (weather week four weeks before incidence-week). Between lag times 4 to 6, R^2 continued to increase while SSD values already decreased again. Between an

offset of six to eight weeks, both indicators decreased steeply. Thus, low and average weekly mean temperatures four weeks before incidence outcome resulted in a maximum correlation (lagtype 4).

Low and average weekly maximum temperatures (‘Tmax_avg’; ‘Tmax_min’) showed a similar temporal pattern. Here, R^2 reached its maximum at a five-week offset and then decreased again with increasing lags (Fig. 29, lower chart). Thus, an offset of five weeks between this temperature range and incidence (lagtype 5) was identified as being most influential. In contrast, the weekly minimum temperatures were most influential on incidence with a much shorter delay of two weeks (lagtype 2) (Fig. 29, upper chart). After the two-week offset, the association decreased sharply with increasing time intervals. Besides low temperatures, also high weekly maximum temperatures showed a particular temporal dynamic (Fig. 30). Here again, lagtype 5 was found most potent but R^2 and SSD values from lagtype 1 to 5 showed a steep upward trend of whereas from lagtype 5 to 8 a steep downward trend became apparent. Thus, the larger the time interval from lagtype 5 the less incidence could be explained by high weekly maximum temperatures.

In sum, for weekly mean and maximum temperature parameters, lagtype 4 and 5 were most effective. For weekly minimum temperature parameters, lagtype 2 was most effective

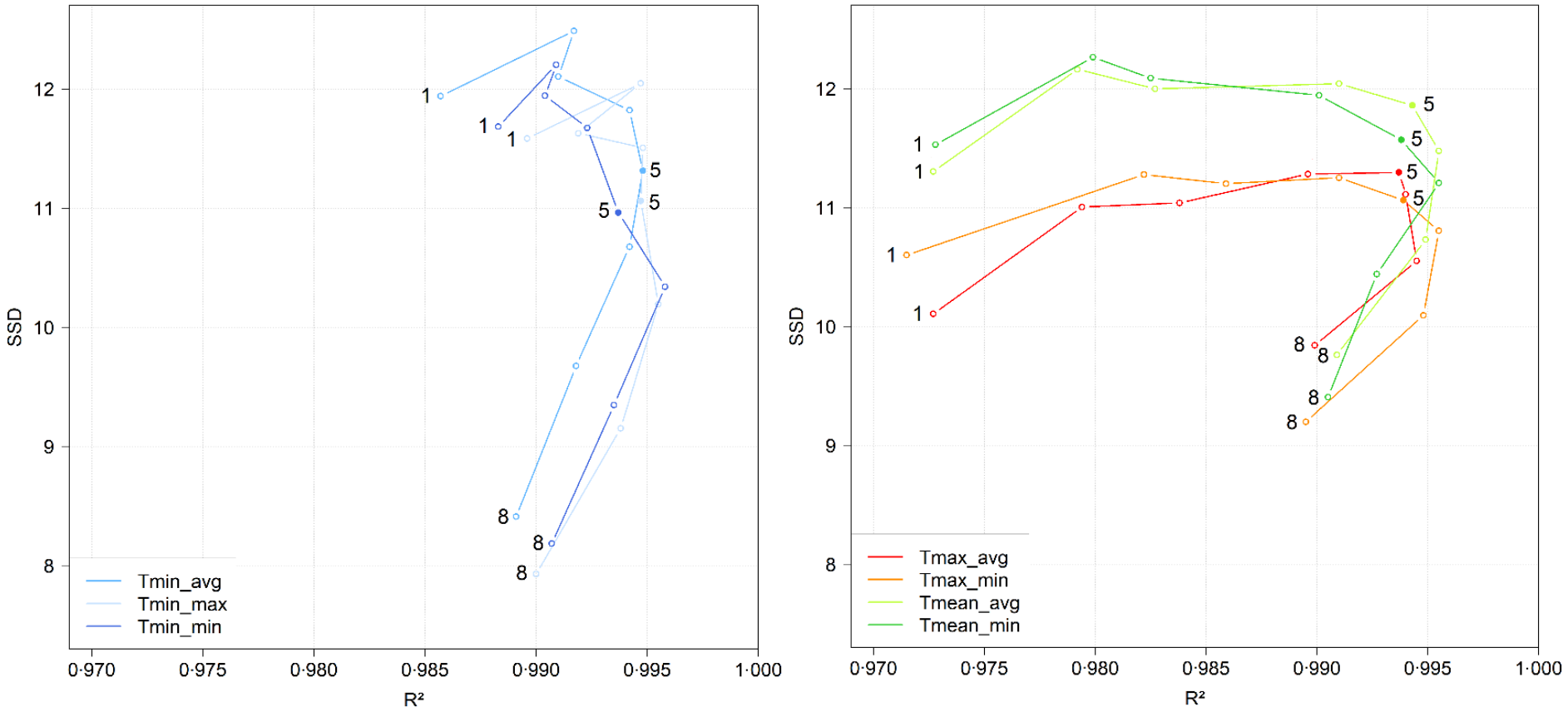


Fig. 29: Lagtype pattern and temporal evolution. Upper chart: Weekly minimum temperature parameters. Lower chart: Lower weekly mean and maximum temperature parameters.

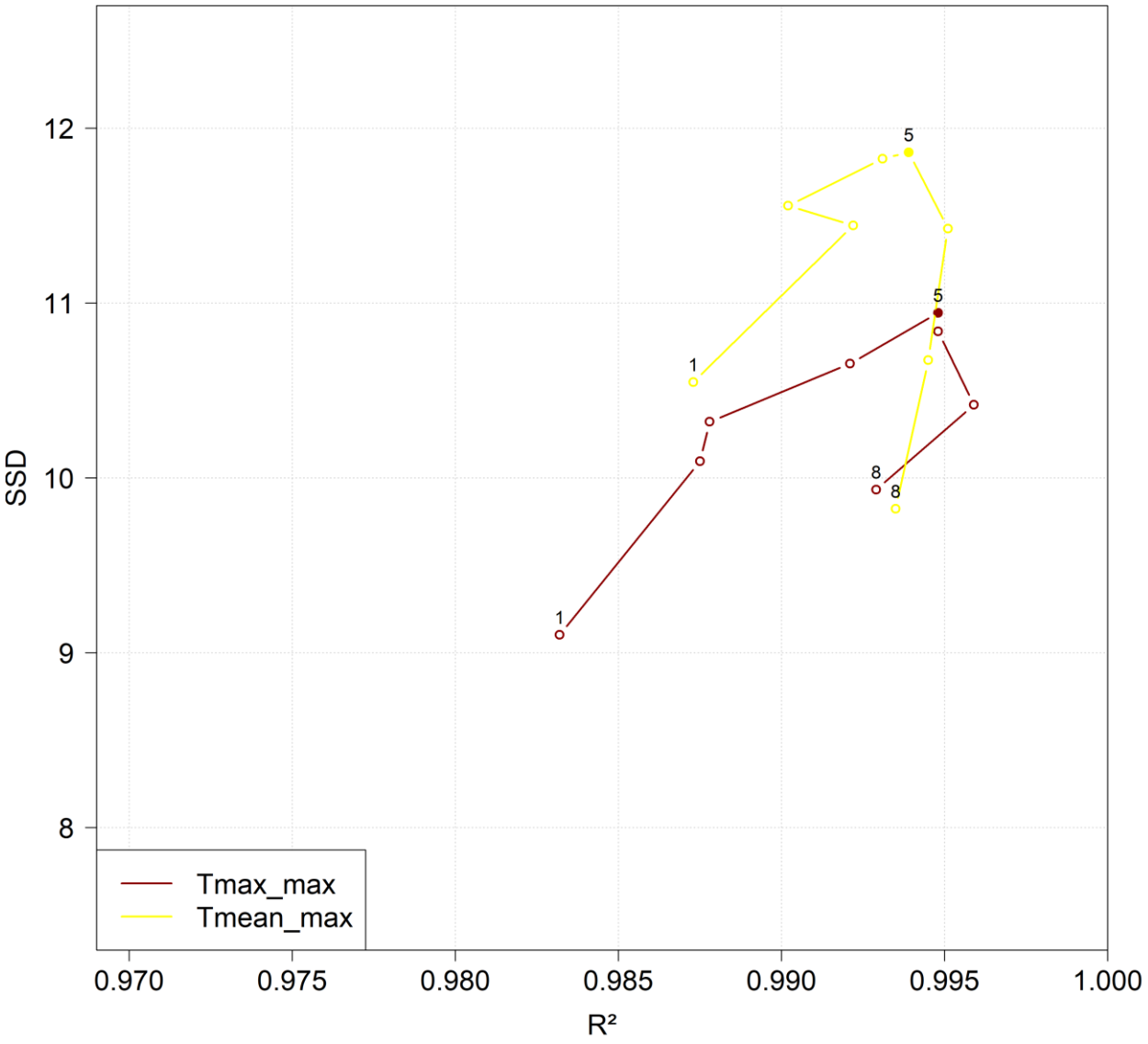


Fig. 30: Lagtype pattern and temporal evolution. Number 1 equals Lagtype 1, number 8 equals Lagtype 8, etc.. Maximum temperature parameters.

3.2 Precipitation

Fig. 31 gives an overview of all precipitation parameters, which are plotted against incidence, here for lagtype 2. All precipitation parameters showed an increase in incidence with rising precipitation. Although no definitive lower or upper threshold could be observed, incidence plateaued at different precipitation levels depending on the precipitation parameter in question. Exceptions were the three- and four-week total precipitation parameters, where the plateau was less profound.

Among all precipitation parameters, the absolute maximum incidence of 1.85 is reached in lagtype 2 of the four-week total precipitation at 162.18 mm. Very similar results reached the maximum incidence of the four-week total of precipitation in lagtype 1 (incidence: 1.83; median: 162.18 mm) and lagtype 3 (incidence: 1.82; median: 162.18 mm).

A sigmoid regression model was fitted to all precipitation parameters (Fig. 31)[19]. A significant association between local precipitation values and local campylobacteriosis incidence was found for Germany. Overall, all regressions for each precipitation parameter and lagtype had a good to very good fit (R^2: 0.4743 – 0.9587; $\bar{x} = 0.8649$). Each precipitation parameter in each lagtype explained more than 47 % of the health outcome while two extreme classes were omitted. The SSD ranged from 0.2897 to 1.6856 ($\bar{x} = 0.8137$) for all precipitation parameters and lagtypes. The gradient for all precipitation parameters averaged 0.08 ($SD(m) = 0.23$). Thus, the sigmoid regression estimated that a 10 mm increase in rainfall is associated with increases in campylobacteriosis incidence of 0.78.

In the following, the most significant precipitation parameters and lagtypes will be examined.

[19] All regressions for every lagtype are available in the Excel file ‘evaluation.xlsx’ in the supporting documents.

3.2.1 Precipitation parameters

Maximum SSD values and maximum R^2 values among all precipitation parameters were found for the following parameters listed in Tab. 15 and Tab. 16.

Tab. 15: Maximum SSD values among all precipitation parameters.

Precipitation parameter	**Lagtype**	**SSD**
Four-week total precipitation (‘rr_sum4’)	1	1.6856
Four-week total precipitation (‘rr_sum4’)	2	1.6545
Three-week total precipitation (‘rr_sum3’)	3	1.6152

Tab. 16: Maximum R^2 values among all precipitation parameters.

Precipitation parameter	**Lagtype**	**R^2**
Four-week total precipitation (‘rr_sum4’)	2	0.9587
Two-week total precipitation (‘rr_sum2’)	3	0.955
Two-week total precipitation (‘rr_sum2’)	1	0.9523

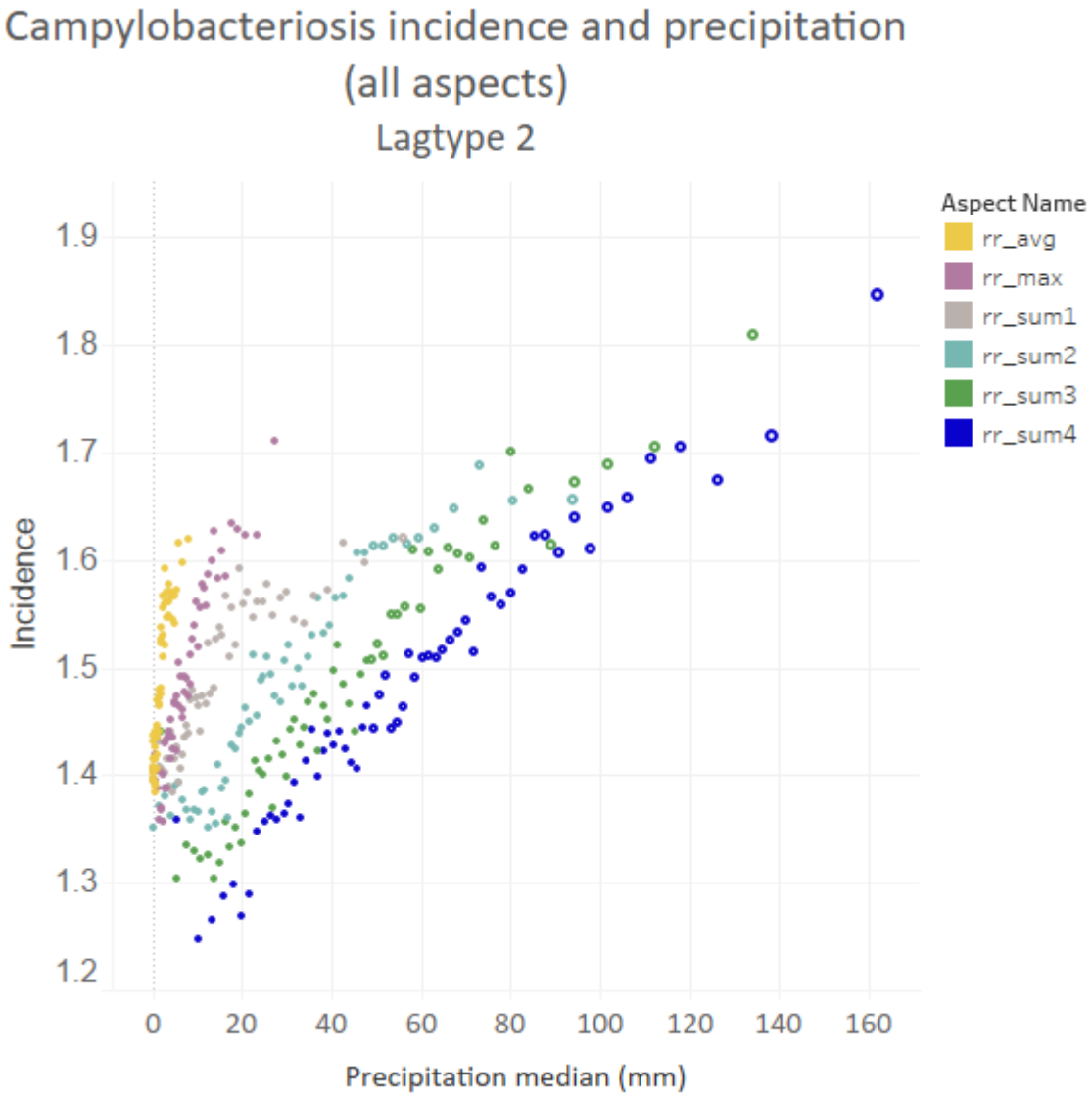


Fig. 31: Scatterplot of all precipitation parameters in lagtype 2.

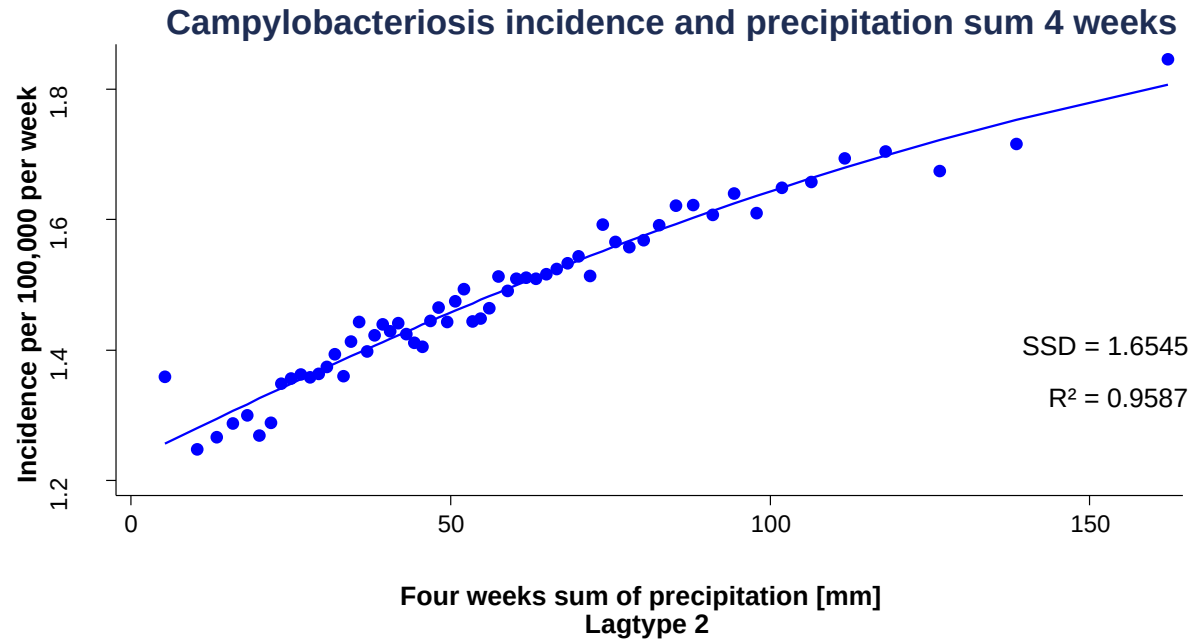


Fig. 32: Sigmoid regression of most significant precipitation parameter 'four-week total precipitation' selected by maximum SSD and R^2 values in lagtype 2.

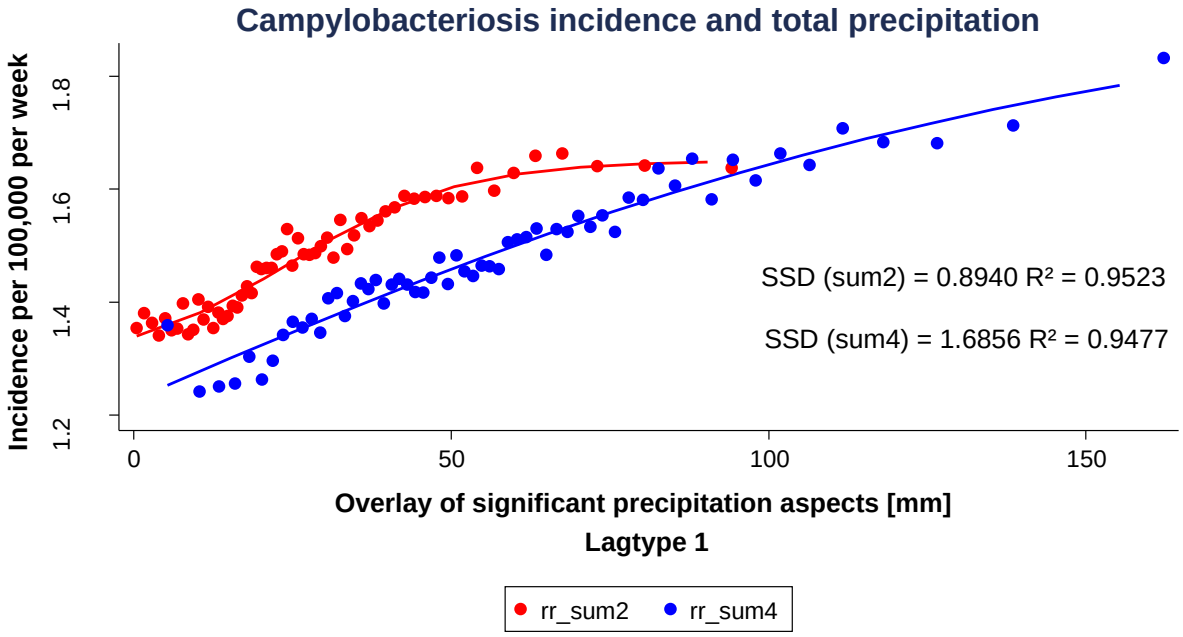


Fig. 33: Sigmoid regression of significant precipitation parameters in lagtype 1.

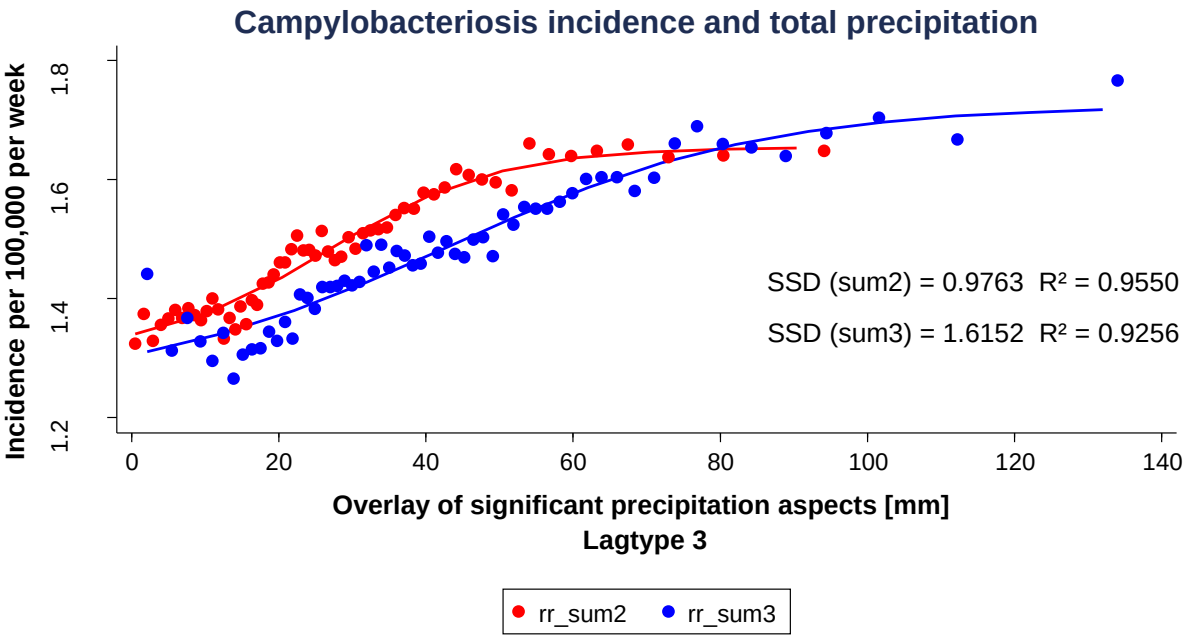


Fig. 34: Sigmoid regression of significant precipitation parameters in lagtype 3.

The combination of maximum SSD and R^2 values was reached at the four-week total precipitation in lagtype 2. Between 5.32 and 162.18 mm of precipitation, incidence increased from 1.36 to 1.83 (Fig. 32). Furthermore, in lagtype 1 at the four-week total precipitation and in lagtype 3 at the three-week total precipitation, incidence rose with increasing precipitation (Fig. 33, Fig. 34). At the two-week total precipitation in lagtype 1 and 3, incidences plateaued at approximately 50 mm of precipitation at an incidence of 1.6 (Fig. 33, Fig. 34).

Thus, most changes in incidence occurred between 0 and 50 mm of precipitation. Plateauing incidences can be found in other precipitation parameters at various precipitation levels. For example, a plateau was apparent at the one-week total precipitation in lagtype 1. Here, incidence (1.6) plateaued at lower amounts of precipitation, of approximately 30 mm of total weekly rainfall (Fig. 35). At the weekly average of the daily sum of precipitation in lagtype 1 (Fig. 36), the plateau is already reached at 4 mm on average (incidence: 1.6).

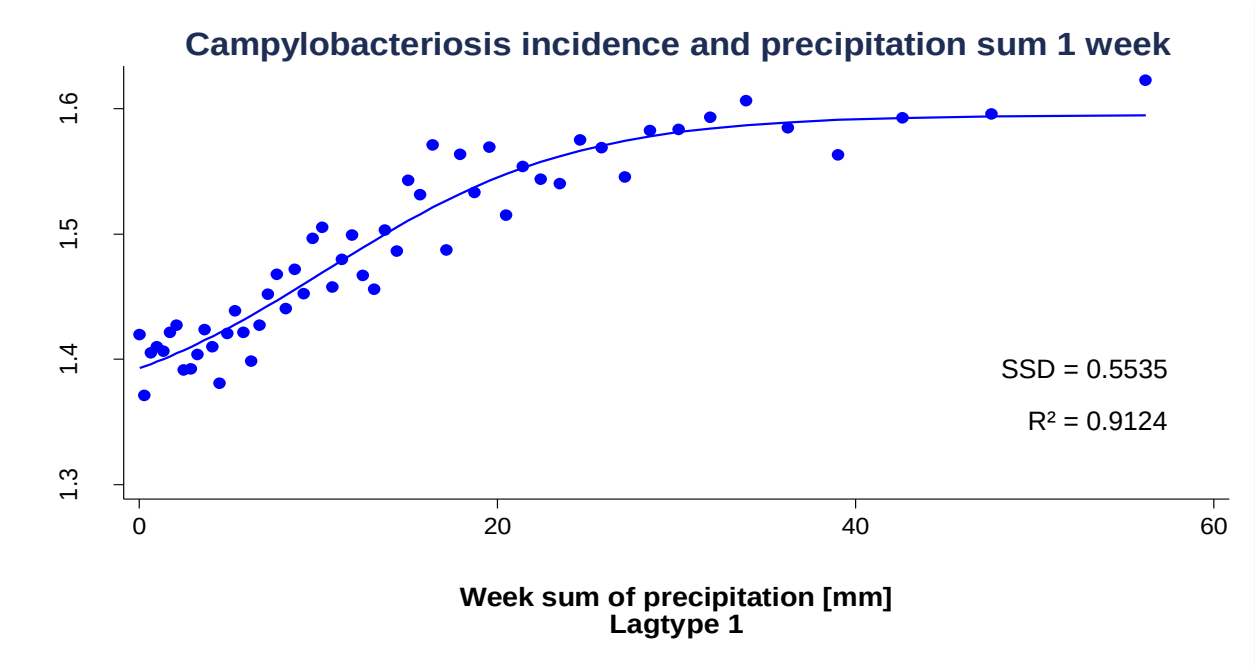


Fig. 35: Sigmoid regression of the parameter ‘one-week total precipitation’ in lagtype 1.

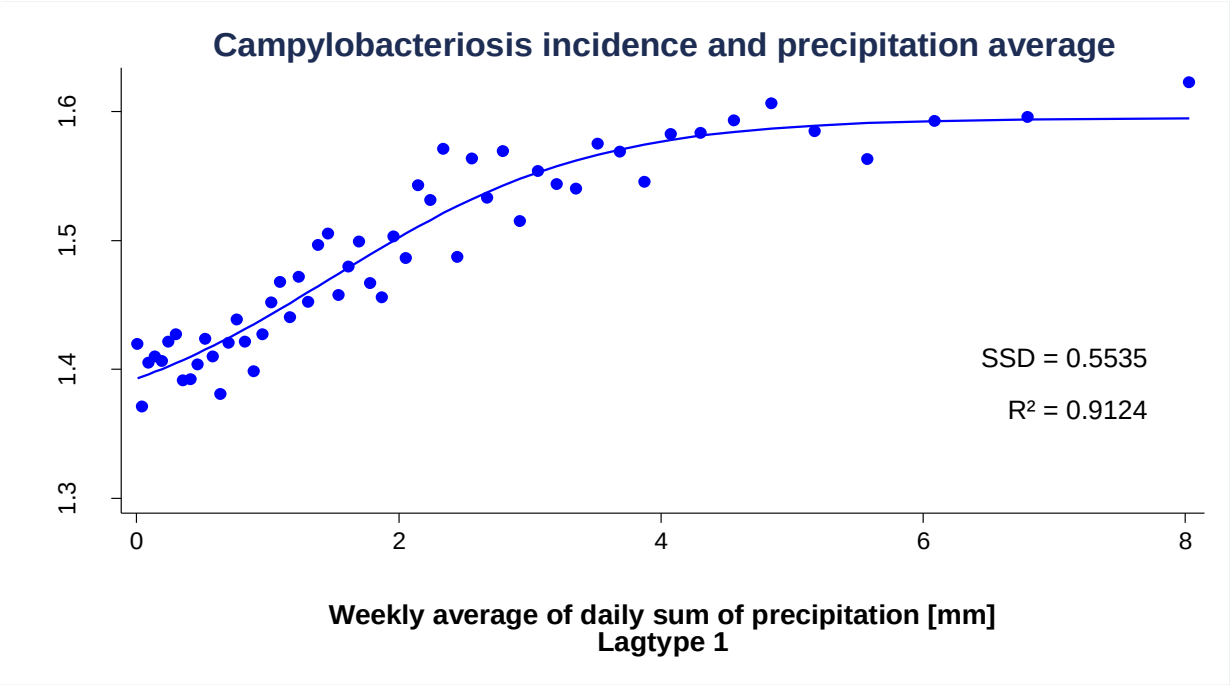


Fig. 36: Sigmoid regression of the parameter ‘weekly average of daily sum of precipitation’ in lagtype 1.

In addition to the rising incidences at high amounts of precipitation, it is remarkable that also a total rainfall of approximately 0 mm produced high incidences (incidence: 1.4; median: 1.6 mm at the two-week total precipitation in lagtype 1, Fig. 33).

Note, that the sigmoid regression of the precipitation parameter ‘weekly minimum precipitation’ could not be modelled due to a missing number of classes.

3.2.2 Lagtypes

R^2 and SSD values of all precipitation parameters and linked lagtypes are plotted in Fig. 37 and Fig. 38. The correlation between precipitation and incidence showed a temporal evolution from lagtype 1 to 8 for all parameters. The one-week total precipitation and the weekly average total precipitation showed very similar R^2 and SSD values resulting in overlapping graphs. Therefore, two different figures have been created.

In all precipitation parameters, the correlation between parameter and incidence was maximal in lagtype 1 and 2 (one and two weeks offset to incidence) and decreased with increasing lagtype. An offset of six to eight weeks between precipitation week and incidence week showed close to no correlation. Thus, the larger the time interval gets between precipitation conditions and incidence, the less incidence can be explained by precipitation.

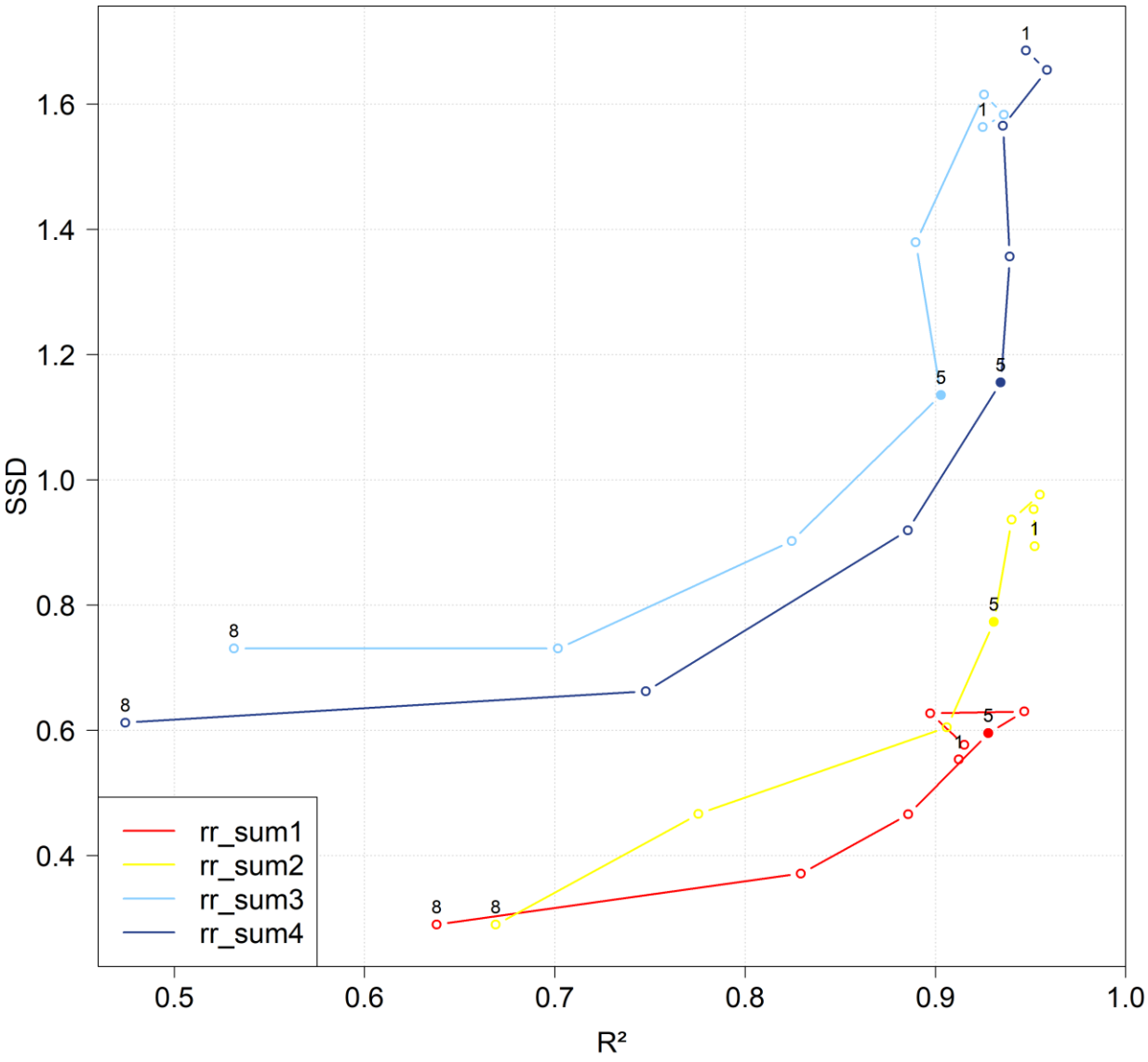


Fig. 37: Scatterplot of R^2 and SSD values. Identifying effective lagtypes for one to four-week total precipitation.

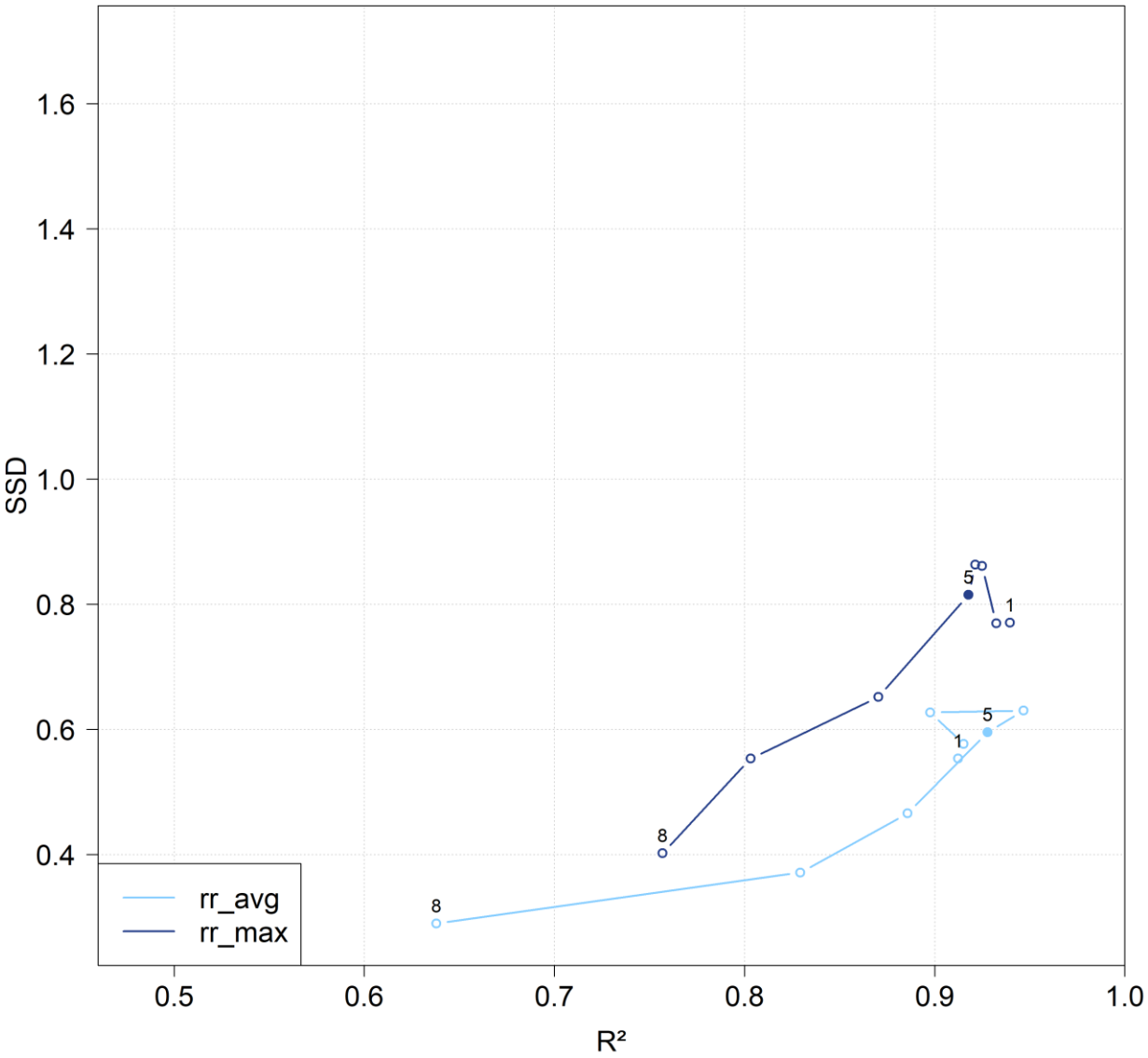


Fig. 38: Scatterplot of R^2 and SSD values: Identifying effective lagtypes for weekly average and maximum of daily total precipitation.

3.3 Summary of results

In brief, temperature and precipitation differed in their strength of association to campylobacteriosis incidence. The temperature had a better fit to the sigmoid regression model and explained more of the observed incidence outcome than precipitation.

All temperature parameters in each lagtype explained more than 97 % of the variation in incidence outcome while two extreme classes were omitted. Within a temperature corridor of -5 to 28 °C incidence outcome increased with rising temperature. Below and above this corridor, incidence was not altered with changing temperature. The weekly average of daily minimum temperature showed the highest incidences and explained most of the incidence outcome by SSD value. Regarding this parameter, most incidence changes occurred within temperatures of 0 to 12 °C. Maximum correlation between temperature and incidence occurred with an offset of two weeks at minimum temperatures, and with an offset of four to five weeks at weekly mean and maximum temperatures.

When referring to all small-scale precipitation parameters, incidence increased with rising precipitation. All precipitation parameters in every lagtype showed an upper plateau where incidence stabilised with increasing precipitation. However, this plateau was less profound at the three- and four-week total precipitation. At the two-week total precipitation, a plateau of incidence was reached at 50 mm. However, the plateauing incidences at approximately 1.6 were also observed at lower total rainfall and varied between precipitation parameters. High incidences were also found in conditions of close to 0 mm rainfall. Overall, the precipitation parameter with the strongest association to incidence and the maximum incidence was the four-week total precipitation in lagtype 2. The lagtypes with maximum correlation occurred with an offset of one to two weeks for all precipitation parameters.

4. Discussion

4.1 Effects of weather on campylobacteriosis

4.1.1 Temperature

This study found robust evidence that local incidence of campylobacteriosis is positively correlated with local temperature in Germany. The examined small-scale local weather covering the whole of Germany can reproduce the correlation found at low resolutions in previous studies (Louis et al., 2005; Fleury et al., 2006; Hartnack et al., 2009; Lake et al., 2009; Yun et al., 2016). The strength of the association gives evidence for the influence of temperature on campylobacteriosis. Averaged over all temperature parameters and lagtypes, the study's sigmoid regression model estimated an incidence increase of 0.52 per 5 °C rise. Estimations solely refer to the observation period, and to the best of my knowledge, have not been made for temperature and campylobacteriosis incidence yet.

Among all tested temperature parameters, the correlations were comparable. This observation is supported by Yun et al. (2016) who found comparable associations for mean and maximum temperatures. Since the current study found a maximal association to incidence at minimum and mean temperatures, it is suggested that these temperature ranges are causative for higher campylobacteriosis incidence. Among all weather parameters, the weekly average of the daily minimum temperature with a temperature range of -7.2 to 15.9 °C and most incidence changes between 0 to 12 °C, could explain most of the incidence outcome and be associated with maximum incidences. This outcome is consistent with the findings of Ailes (2010) who also found a maximal association with minimum temperatures.

As the temperature increases, there is a corresponding incidence increase over a wide temperature corridor of -5 to 28 °C among all temperature parameters. Below -5 °C, temperature does not seem to affect incidence. At 28 °C incidence plateaus, and above 28 °C incidence stagnates with increasing temperature. A similar corridor effect has been observed in previous studies but was narrower, ranging from 5 °C to 18 °C in Yun et al. (2016) and from 8 to 20 °C in Patrick et al. (2004). This could, in part, be explained by different methodological approaches. Patrick et al. (2004) averaged weather parameters for the whole of Denmark. Thus extreme values could have been lost or underrepresented.

In this study, moderate and extreme weather parameters are equally represented by equally large classes.

The reasons for the identified upper and lower threshold that frame the wide temperature corridor remain unknown. It is generally accepted that *Campylobacter* is highly thermotolerant and can survive in the identified corridor temperature and beyond (Thomas et al., 1999; Hunt et al., 2001; Chan et al., 2001; Lawley, 2013). This characteristic of campylobacters enables the campylobacteriosis incidence increase in the first place.

However, the mere microbiology of campylobacters cannot explain the correlation between increased temperature and incidence outcome. As already underlined, replication of *Campylobacter* is unlikely outside the host (Park, 2002; EEA, 2017). Furthermore, increasing temperatures have adverse effects on inactivation of *Campylobacter* (Buswell et al., 1998; Thomas et al., 1999; Obiri-Danso et al., 2001; Hunter, 2003). A meta-analysis by Membré et al. (2013) found that *Campylobacter*, regardless of medium or species, survives better at cold temperatures than at warm ambient temperatures. This is consistent with previous studies which found that the survival rate of *Campylobacter* is highest at temperatures of around 5 °C (Thomas et al., 1999) and survival is still possible even at freezing temperatures of -20 °C (Abram and Potter, 1984; Lee et al., 1998; Chan et al., 2001). Thus, while survival is reduced at freezing temperatures, minimum temperatures found at chilled storage, for instance, provide good conditions for prolonged *Campylobacter* survival. In contrast, warmer temperatures are limiting for *Campylobacter* survival. In conditions of increasing temperatures between 5 and 25 °C, the inactivation of *Campylobacter* isolates was found to be particularly rapid (Thomas et al., 1999; Chan et al., 2001; Solow et al., 2003; Membré et al., 2013). These findings are contradictory to the observed relationship of the current study. However, it might explain the higher incidences at average minimum temperatures where survival of campylobacters is prolonged.

Nonetheless, the discrepancy between *Campylobacter* microbiology and campylobacteriosis patterns in increasing temperature conditions remains and leads to the hypothesis that the weather-sensitive mechanism increasing campylobacteriosis risk must lie outside the direct weather-pathogen relationship. These factors, themselves temperature-sensitive, are likely to act through intermediate trajectories, as already

concluded by other authors (Tam et al., 2006). These intermediate variables are the driving momentum in human campylobacteriosis at increasing temperature and outweigh the direct relationship between pathogen and temperature when the whole chain of infection is considered.

Various weather-sensible drivers of human campylobacteriosis have been suggested. *Campylobacter* spp. has been found to be more prevalent from spring to autumn in food-producing animals such as poultry, when temperatures show a seasonal increase (Fig. 8) (Kovats et al., 2005; EFSA, 2015). This seasonal rise has been linked with a higher prevalence of vectors, such as flies that contaminate broiler flocks (Hald et al., 2004; Hald et al., 2008; Andreoletti et al., 2011). Nichols (2005) found that fly numbers increase from spring through autumn. The increase in fly population was steeper in spring than the autumn decline. In addition to that, *Campylobacter*-positive broilers were more prevalent in autumn than in spring (Patrick et al., 2004). This is remarkable because the seasonal campylobacteriosis incidence pattern echoes the annual fly population of a steep increase in spring and a slow decline in autumn, while the temperature profile in spring is the mirrored profile of autumn (Fig. 8). An explanation for this phenomenon is provided by Guerin et al. (2008), who suggests that fly activity and breeding require several sequential warm days to complete a life cycle. Thus, sustained high temperatures in summer could explain higher flocks contamination in autumn compared to spring and as a consequence higher human campylobacteriosis incidences. Patrick et al. (2004) back this hypothesis by stating that rather than intermittent heat days, a consistency of warm temperatures is key for predicting infection in broiler flocks and humans.

Apart from vector prevalence, human behaviour is assumed to be highly weather-sensitive (Patrick et al., 2004). Weather-dependent human behaviour includes outdoor recreational activity, food culture or agricultural activity. Leisure habits such as swimming in natural lakes, picnicking or increased animal contact at warmer temperature may increase exposure to *Campylobacter*. Furthermore, eating habits such as barbecuing or eating raw fruits and vegetables increase the risk of *Campylobacter* infections. These human behaviour-led risk factors altogether could explain part of the incidence summer peak (Evans et al., 2003; Schonberg-Norio et al., 2004; Lawley, 2013).

The high relevance of risky human diet behaviour on campylobacteriosis is highlighted by the observed incidence peak in Germany from late December until early January (Fig. 8). This peak cannot be explained by warm temperatures but has been attributed to the traditional consumption of meat tabletop grilling called ‘Raclette’ around Christmas and New Year by previous studies (Bless et al., 2017). Similar phenomena have been observed in Switzerland for the meat dish ‘Fondue Chinoise’ (Bless et al., 2014). Both dishes have in common that raw meat is cooked at the table on the respective grill or fondue pot and the risk of undercooking is of relevance.

Besides leisure and eating habits, agricultural activities also show temperature dependencies. Grazing farm animals, manure spread and exposure to animals increase at warmer temperatures (Jorgensen et al., 2011; Lévesque et al., 2013). Manure contains a high amount of zoonotic pathogens, including *Campylobacter* (Schijven et al., 2013). The spread of manure is subject to strict regulations in Germany. Between the harvest of the main crop until the end of January, manure spreading on cropland is prohibited (LWK, 2017). Thus, the seasonal increase of manure spreading from spring throughout autumn when temperatures increase may contribute to a higher risk of *Campylobacter* infection in this period.

In the light of the weather dependencies of human behaviour, the identified temperature parameters are assumed to act as prognostic indicators for high-risk human behaviour, which is followed by an increased incidence. Although the role of human behaviour is complex and underlies fluctuations, it might be in part explanatory for this study’s findings.

However, the driving weather-sensitive mechanism is yet to be identified. Elucidation is needed at what stages of the chain of infection temperature is directly linked to campylobacteriosis. As a first order approximation, the lengths of significant lagtypes can help to highlight stages in the infection pathway where the temperature may be most effective. It was demonstrated that incidence highly associates with the average minimum temperatures two weeks before. This indicates that average minimum temperatures are especially relevant in the recent part of the infection chain. In the case of a foodborne infection, this period of the food chain includes transport, food storage, processing and time of consumption. A short delay provides evidence for a possible causal relationship between local ambient temperature and campylobacteriosis outcome (Hill, 1965). The

apparent temporal evolution of the correlation backs this hypothesis. A similar temporal evolution showed the association between incidence and the low mean and low maximum temperatures. However, the highest association with these temperatures was found at a larger time offset of four to five weeks and decreased again as the interval between temperature week and incidence week further increased. These findings are consistent with results from Yun et al. (2016) who found best fitting lags of four weeks (for Munich) and six weeks (for Berlin) with mean temperatures. Results of the current study indicate that at high maximum temperatures, an offset of five weeks provided a strong correlation, whereas all other lagtypes were much less relevant. Comparable are the findings of a previous study from Denmark, which found maximum temperatures lagged at four weeks to be the best predictor (Patrick et al., 2004). This result indicates that high maximum temperatures increase the risk of campylobacteriosis around the time when food-producing animals are still exposed to the farm environment involving risk factors such as vector exposition, transmission between other agricultural animals and contaminated surface water (Kapperud et al., 1993; Hald et al., 2004).

It remains ambiguous why the delay in minimum temperatures is shorter than that of higher temperatures as well as the underlying mechanisms, which result in the temporal evolution. However, the temporal evolution found in this study demonstrates that temperature is likely to be influential at several stages of the infection chain between pathogen and its pathogenic manifestation in humans. The identified lagtypes highlight stages of the infection chain which are associated with ambient temperatures. Furthermore, they offer a time window for campylobacteriosis forecast.

4.1.2 Precipitation

This study gives evidence for the positive correlation between local precipitation and local campylobacteriosis incidence in Germany. Previous studies with data of lower resolution showed ambiguous associations. While some authors found a weak association between precipitation and incidence (Patrick et al., 2004; Kovats et al., 2005), others found no significant correlation (Louis et al., 2005; Bi et al., 2008; Lal et al., 2013). The strength of association identified in the current study gives evidence for the influence of precipitation on campylobacteriosis, although weaker than the correlation with temperature. Averaged

over all precipitation parameters and lagtypes, the study's sigmoid regression model estimates an incidence increase of 0.78 per 10 mm precipitation. Estimations solely refer to the observation period, and to the best of my knowledge, have not been made for precipitation and campylobacteriosis incidence yet.

The strength of correlation was distinctive between the precipitation parameters. The precipitation parameter with the strongest correlation to incidence was the four-week total precipitation one to two weeks prior to incidence. It represents the precipitation parameter with the maximum incidence (1.85) and the highest absolute total rainfall (162.18 mm). Among all sums of precipitation (e.g. 'rr_sum1', 'rr_sum2'), the parameters with higher weekly total rainfall showed a stronger correlation with incidences than lower weekly rainfalls. Furthermore, the weekly maximum rainfall was stronger correlated with incidence than the weekly average rainfall in all lagtypes. Altogether, these results suggest that a rise in total rainfall increases the risk of *Campylobacter* infections. Since campylobacters are sensitive to dry conditions (Fernández et al., 1985; Patrick et al., 2004), it is straightforward that wet weather conditions are advantageous for campylobacters' survival and therefore the microbiology of *Campylobacter* is able to explain the incidence increase with rising rainfall. Apart from the pathogen ecology, a high amount of rain alters the distribution of campylobacters out of its reservoirs into the environment. With a certain amount of precipitation, the filtration capacity of the soil is exceeded leading to substantially increased surface run-off. This, in turn, facilitates manure wash-out from fields and subsequently increases the bacterial contamination of streams and rivers (Bolton et al., 1987; Giddens and Barnett, 1980). Moreover, increased rainfall provides additional reservoirs for *Campylobacter*. At the farm level, rainfall in the month of chicken flock placement was associated with increased *Campylobacter* prevalence (Jorgensen et al., 2011). Furthermore, heavy rainfall of 11 to 30 days before slaughter increased broiler flock infection with campylobacters (Jonsson et al., 2012b). This association is comprehensible because rainfall may provide additional surface water reservoirs for campylobacters and as a result increases the risk of broiler contamination. In addition, broiler contamination could increase since the responsible vectors encounter more suitable conditions with increasing rainfall. This hypothesis is supported by the findings that an increase in rainfall results in an increase in fly populations (Nichols, 2005).

Inconsistent with the current study were the results of Patrick et al. (2004), who describe precipitation to be a weak predictor of human *Campylobacter* infections. Since precipitation is highly variable regarding its magnitude as well as temporal and spatial patterns, the resolution applied in Patrick's study could have been too low since measurements were summarised nationally and thus precipitation-incidence patterns could have remained undetected.

The results of this study show, that incidence increases with rising precipitation over the entire range of measured precipitation (0 to 162 mm) and ultimately reaches a plateau. The plateau can be interpreted to that effect that at a certain amount of rainfall a further increase does not alter the incidence. However, the precipitation level where incidence begins to stabilise varies between the precipitation parameters. For instance, at the one-week total precipitation incidence plateaued at approximately 30 mm, at the two-week total precipitation at around 50 mm. Especially in short-term observation periods such as the weekly or the two-week total precipitation, incidence saturated at approximately 1.6. Over a period of four weeks of cumulative rainfall though, where the total precipitation is higher, the plateau was less profound.

In summary, campylobacteriosis incidence increases with rising precipitation and is mostly altered during the first 50 mm of total rainfall. When precipitation reaches a prominent level, the increase in incidence is less profound, reaches a plateau and is expected to be less influenced by precipitation. To the best of my knowledge, this study is the first to describe a plateau phenomenon in the relation between precipitation and campylobacteriosis. Since the plateau occurs at various precipitation levels, a certain precipitation cut-off level cannot be identified. Although the mechanism leading to the plateauing incidence remains uncertain, several explanations can be put forward. It is conceivable that an intolerable rainfall level will trigger a behavioural change of susceptible individuals in a way that activities decline which would normally increase the risk of campylobacteriosis (e.g. outdoor activities, barbecuing). This, in turn, would stabilise the risk of further infections.

Furthermore, certain levels of rainfall might initiate early warning systems at water bodies and thus decrease human exposure to contaminated waters. Such an early warning system is applied at lake Baldeney in North Rhine-Westphalia, for example. When a water

gauge of 5 mm per day is exceeded in the catchment area of the Ruhr river, after a heavy rain event, for instance, a bathing prohibition is automatically called out by the public health authorities (Lenz, 2017). Furthermore, since outbreaks have often been related to contaminated water, several cases of epidemic concern might be responded with preventive measures by public health authorities. This crowding effect might also lead to saturating incidences at certain precipitation levels.

In previous studies, high rainfall events were associated with increased risk of campylobacteriosis infections (Soneja et al., 2016). Rechenburg and Kistemann, (2009) found that the river contamination with campylobacters profoundly increased during and after heavy rainfalls. With increased precipitation, sewage treatment plants and combined sewer overflows may discharge (Rechenburg and Kistemann, 2009). This, in turn, contaminates river water with campylobacters. Several authors have related heavy rainfall before a campylobacteriosis outbreak to congestion of contaminated drinking water caused by a backflow of sewage water into the drinking water reservoirs (Clark et al., 2003; Kuhn et al., 2017). Thus, an increased risk of human campylobacteriosis can be deduced from high precipitation events.

The examination of heavy rain events was not within the scope of this study. Among the weekly precipitation parameters, it was not possible to determine if the associated amount of precipitation was the result of a heavy rain event or a sequence of rainy weather days. Heavy rain events are singular events that occur temporally and spatially restricted. To detect these events, an even higher temporal data resolution is required. However, the observed stronger association to maximum rainfall than to average rainfall indicates that heavy rain events might be more influential on incidence than continuous wet weather conditions.

While heavy rain conditions were found to be an important risk factor for campylobacteriosis by other authors, most studies examined heavy rain conditions in the context of outbreak situations (McQuigge, 2000; Clark et al., 2003; Thomas et al., 2006; Nichols et al., 2009; Kuhn et al., 2018). However, 97 % of all campylobacteriosis cases are sporadic (Park, 2002; RKI, 2017). Since this study examined a 100 % of the reported incidences in the observation period in Germany, the current research may be more

representative in risk factors and strengthens the evidence, that sporadic cases could also be associated to high rainfall conditions.

The demonstrated intervals between heavy rain events and outbreaks in previous studies were usually short, approximately between one to two weeks (McQuigge, 2000; Clark et al., 2003). The optimal lag to predict *Campylobacter* incidence with precipitation was found at a lag of three weeks (Patrick et al., 2004). This is consistent with the current study which also found the highest associations in the shortest examined intervals available. Among all precipitation parameters, an offset of one to two weeks between incidence week and precipitation week provided the best fit. This is straightforward since the effect of a heavy rain event can be immediate with the impacts described above. A rise in rainfall is a predictor for increased campylobacteriosis risk and the short lag time emphasises the need to start prevention measures immediately, as demonstrated by the bathing prohibition at lake Baldeney which report water gauges within hours (Lenz, 2017). All in all, maximum campylobacteriosis incidence at highly cumulated rainfall at a short lag time and the consistency of campylobacters microbiology of prolonged survival in wet conditions suggest that the effect of increased precipitation on campylobacteriosis might be more direct than those of temperature.

Apart from increasing incidence with high rainfall conditions, the current study also found an incidence increase in dry conditions of close to 0 mm total rainfall. This is consistent with the work of Nichols et al. (2009) who suggest outbreaks to be correlated both, with high rainfall (> 40 mm) but even more so with low rainfall (< 20 mm rainfall per week) in the previous seven days before the outbreak date. While campylobacters microbiology cannot explain the high incidences in dry weather conditions, human behaviour might play a key role. Rainless (and sunny) weather conditions could increase risky diet behaviour, like barbecuing or eating out. Another factor was suggested by Nichols et al. (2009) who reasoned that during low rainfall conditions, the proportion of contaminated groundwater from sewage or surface water run-off increases in a water body, while the filter function of cracked soil is reduced.

In summary, the wetter the weather conditions are, the higher is the risk of *Campylobacter* infections. The current small-scale approach avoided a one-to-one approach to account for the high spatiotemporal variability of precipitation. It examined incidences from all

cases, sporadic and outbreak-related, and thus may be more sensitive in registering precipitation-incidence patterns than previous studies on outbreaks and at lower resolutions. After all, the incidence-dependencies on precipitation are complex since the whole chain of infection from pathogen to its disease-causing manifestation is likely to be influenced by multiple pathways. However, its effect on campylobacteriosis incidence might be more direct than those of temperature. More research with small-scale data and sporadic cases is needed to support the current findings to be able to set a cut-off level for high-risk total rainfall.

4.2 Future campylobacteriosis trend and intervention strategies

This study presents evidence that campylobacteriosis is associated with climate parameters. Especially mild minimum temperatures and wet weather conditions are strongly associated with high incidences. Since the global climate is undergoing unprecedented changes, including global warming and increased numbers of extreme weather phenomena, the development of campylobacteriosis incidence under these conditions needs consideration. Therefore, implications that can be drawn from this study on campylobacteriosis risk in Germany under predicted future climate as well as mitigation strategies will be discussed in the following chapter.

4.2.1 Which climatic factors may drive or curb campylobacteriosis risk?

As for the German climate, a likely temperature increase between 2.5 to 3.5 °C is predicted by the end of this century (Jacob et al., 2008). This study showed that increasing local campylobacteriosis incidence is strongly correlated with rising temperatures. Thus, the weather-campylobacteriosis associations found in this study indicate that campylobacteriosis is expected to increase with a warming climate in Germany.

There exist several pathways how temperature might drive campylobacteriosis incidence. At an early stage of the food chain at farm level, there is evidence that the increase in campylobacteriosis commensurates with contamination rates of chicken flocks, itself driven by elevated temperature-sensitive fly activity (Jonsson et al., 2012a). In contrast,

increased temperatures at the stage of food processing were found to curb the survival of *Campylobacter* significantly and subsequently the bacterial burden at the far end of the food chain (Hänel and Atanassova, 2007). This occurs, for example, when the cooling chain is interrupted. However, with regard to the food chain, the curbing effects do not seem to outweigh the driving mechanisms that increase the overall disease burden with warmer temperature exposure. Therefore, it can be assumed that the curbing effects are presumably negligible and that a temperature association in the early part of the food chain is more likely to increase the disease burden.

In addition to that, the growing season is projected to extend in Northern Europe with a warmer climate, due to an earlier onset in spring and belated senescence in autumn (EEA, 2017). This could result in an increased agricultural activity where water availability is not restrictive to the growth of crops (Elsgaard et al., 2012). Higher agricultural activity increases the risk of manure wash-out, surface runoff and contamination of water bodies. However, a climate-runoff model by Sterk et al. (2016) projected a limited impact of climate change on *Campylobacter* runoff and consequently associated infection risk. Despite this, a more extended season of sustained warm temperatures may also increase suitable habitats for vectors such as flies. Moreover, the season of barbecuing could be prolonged, and thus the risk rises to consume contaminated undercooked poultry.

Despite the temperature, this study has presented evidence of the driving effects of high total precipitation on campylobacteriosis and is consistent with the microbiology of campylobacters (Fernández et al., 1985; Patrick et al., 2004). While climate models project the overall total precipitation to remain constant in Germany, precipitation patterns are predicted to alter regionally (Jacob et al., 2008). For example, the north-west of Germany is likely to have an increase in heavy rain events. Although this study didn’t examine heavy rain events, the results indicate that rain peaks are more influential regarding an incidence increase than frequent low dose rain. Rain peaks bear the risk of subsequent flooding and surface run-off and thus higher contamination rates in water sources (EEA, 2017). In contrast, continental eastern regions are more likely to experience decreased summer precipitation and increased heat extremes (EEA, 2017).

Thus, there is reason to believe that the campylobacteriosis trend in Germany, with regard to precipitation and temperature, could show regional differences; an increased risk of

campylobacteriosis in the north-west and a decreased risk in the continental region can be assumed. Regional trends should be monitored, and regional prioritisation can help policymakers to focus disease control in the context of limited public-health resources. For example, regions prone to increased heavy rain events and thus contamination of water bodies could adapt their wastewater management. Therefore, the health risk by contaminated water sources is likely to increase, but the magnitude largely depends on the efficiency of mitigation strategies.

4.2.2 Which non-climatic factors will play a role in future campylobacteriosis trend?

Beside climatic factors, also non-climatic factors may influence the future disease burden of campylobacteriosis. Since it is a foodborne disease mainly caused by contaminated poultry (RKI, 2015c), alteration of diet habits, food safety interventions and campylobacteriosis risk management will play a crucial role in the magnitude of future campylobacteriosis incidence. Currently, the consumption of poultry in Germany is rising at an elevated level. From 2001 to 2006 the poultry consumption rose from 10.9 kg to 12.5 kg per capita, and popularity is still expected to increase (BLE, 2017).

On the other hand, alternative diet trends are thriving in Europe. Especially in Germany, vegetarianism and veganism are becoming increasingly popular. Around ten per cent of Germans lived meat-free in 2016 with an ascending trend (BMEL, 2008; Skopos, 2016).

While warmer temperatures may increase the risk of campylobacteriosis via foodstuffs, reduced consumption of animal products might implicate a curbing of this trend. When extrapolated, a mostly plant-based diet can even be an adaptation strategy to diminish the most severe impacts of climate change, since it requires fewer resources and causes fewer emissions than animal products (Hertwich, 2010). Therefore, future changes in eating patterns may have vast implications for the development of campylobacteriosis.

Apart from diet habits, food safety interventions will play a vital role in the mitigation of foodborne diseases. The occurrence of campylobacters in broiler meat remains high (BfR, 2014). Interventions in the food chain have been largely discussed for different control points, for instance at the farm level, the slaughterhouse, meat processing, packaging,

storage, purchase and food preparation (Wassenaar, 2011; EFSA, 2016). It has been found that most contamination occurs early in the food chain before processing and retail (EFSA, 2014b). Chicks become contaminated in the chicken stand through flies or treatment at an early stage (Evans and Sayers, 2000; Nichols, 2005). Also, poultry slaughter is still a major trajectory through which broilers get contaminated (BfR, 2014). Thus, biosecurity interventions by manufacturers in the primary production are estimated to have higher public health benefits than measures at a later stage of the food production (Andreoletti et al., 2011; EFSA, 2015). The European Food Safety Authority (EFSA) published a scientific report on possible control points and targets in broiler meat production at different stages of the food chain (Andreoletti et al., 2011). If these targets are translated into measures, the EFSA (2014a) predicts that interventions before poultry slaughter could reduce campylobacteriosis by 50%. Interventions in the meat production which reduce the concentration of *Campylobacter* to a maximum of 500 to 1000 CFU/g carcass skin could lead to a reduction of human campylobacteriosis by up to 90 % (Andreoletti et al., 2011). Currently, these limits would not be met by German broiler carcasses (Stingl et al., 2012). It is likely that not one but multiple food safety interventions need to be implemented to reduce bacterial load (Wassenaar, 2011). The wide thermotolerance of *Campylobacter* (EFSA, 2014b) has to be taken into account when establishing interventions.

Safety measures can be key to influencing campylobacteriosis. Several countries have already adopted the recommended systematic Hazard Analysis and Critical Control Points (HACCP) by the EFSA to control campylobacteriosis and ensure food quality. The UK, for instance, launched a risk management campaign in 2014, combining expertise from scientists and industrial and policy representatives to reduce *Campylobacter* levels in the food chain, from farm to plate (FSA, 2017). New Zealand already puts interventions successfully into practice. A study from New Zealand showed that the introduction of industry-led interventions to reduce contamination in poultry meat (e.g. freezing of fresh poultry meat) coincided with a significant decline in incidence by 50 % since 2007 (Sears et al., 2011; Lee et al., 2017).

In 2012, the German Federal Institute for Risk Assessment had launched a three-year innovation program called ‘InnoStep’ that aims at developing and validating a

microbiological control point system in the meat production to reduce *Campylobacter* spp. in poultry (BMEL, 2014; BfR, 2015). It identified critical production stages to identify possible sources of infection. When infection sources are found technological changes in the process flow can be established. This knowledge, together with the expertise from other countries can help to implement a systematic HACCP concept in Germany to reduce *Campylobacter* contamination in the food chain and thus prevent and control campylobacteriosis. The establishment of food safety measures will have a crucial impact on the future campylobacteriosis trend.

Apart from safety measures in the food industry, the enhancement of public awareness of *Campylobacter*-related food safety risk, infection trajectories and knowledge on preventive hygienic measures will have a vital impact on the campylobacteriosis incidence trend since campylobacteriosis, despite its relevance, is not well known in the German public to date (BfR, 2014). Furthermore, in the mitigation of *Campylobacter*-related illness, it is crucial to what extent risk management will focus on high-risk groups, such as young infants in nurseries, the elderly in rest homes or populations with pre-existing illnesses. For example, should patients with medical treatment of proton pump inhibitors be informed on an increased risk of bacterial infections under treatment and therefore asked to avoid certain foods. With an expected increase in campylobacteriosis incidence under a warmer climate, especially the very young and the elderly are disproportionately at risk because they equally suffer more severely from heat stress and dehydration (Vardoulakis and Heaviside, 2012).

In a nutshell, a systematic strategy comprising every stage of the infection chain should be put in place aiming to reduce *Campylobacter* infections. This can be realised by reducing the level of contamination throughout the food chain, in farm-animal hosts, in processing and at the consumer level.

This study discusses the need to adopt surveillance and prevention attempts to the seasonality of campylobacteriosis by taking into account the identified lag times to prevent and control campylobacteriosis in Germany. It emphasises the role of human behaviour and hygienic measures in primary food production as major factors in the future campylobacteriosis trend. Public awareness programs combating food handling mistakes

should be intensified especially at the beginning of summer (before the barbecue season) and should have a focus again around the festive winter season.

Further investigation of the role of vectors should be carried out and subsequently vector management be introduced, as is already the case for other vector-borne diseases. Promising were findings from Denmark where the introduction of fly nets coincided with reduced bacterial contamination in broiler houses (Hald et al., 2007). Chicken vaccination programmes, promising for *Salmonella* and so far an object of research for *Campylobacter*, could be introduced to prevent colonisation in the first place (Wassenaar, 2011; Meunier et al., 2017).

Moreover, further studies should cover the whole range of ambient temperatures under standardised conditions and examine a possible survival tipping point of *Campylobacter*, especially temperatures between 5 and 25 °C since this range has not been covered in laboratory experiments on a high resolution yet.

This study has pointed out the multiple pathways through which illness with *Campylobacter* acts, identified associated climatic and non-climatic factors in *Campylobacter* propagation and the uncertainty it bears to project future trends. Although a general increase in campylobacteriosis risk with a warming climate can be derived from the research results, this study refrained to name specific temperature ranges which will play a key role under future climate conditions nor to predict to what extent incidences will develop under the conditions of climate change. These predictions would be highly uncertain. To predict the definite extent of incidence change requires, that all contributing factors, climatic and non-climatic and their interdependences are known and remain constant and their dynamic can be projected. This study aimed to describe the influence of weather parameters on campylobacteriosis incidence. Among the complex web of factors influencing it, climatic factors are only a few. Predicting would involve to estimate changes in the supply chain, technologies in food production, legislation, interventions, trade, migration (itself in part induced by climate change), travel-related cases, human behaviour etc.. So far, many pathways through which climate factors may affect *Campylobacter*-related food-borne diseases are known, but only a few among these have been systematically examined. This provides a high uncertainty as to what the impacts of climate change will be. A complex model is needed to meet these requirements. A first

approach could be the climate change tool by Schijven et al. (2013). The authors estimated campylobacteriosis to decrease with climate change though, due to increased inactivation with higher temperatures. This shows that especially the adverse effect of warm temperatures on *Campylobacter* survival makes it difficult to make exact predictions. As those requirements are not fulfilled, it has been refrained to name temperature ranges which will play a key role in climate change nor to do predictions to what extent incidences will develop under the conditions of climate change. Under a warmer climate, incidence might change, some experts state (Vardoulakis and Heaviside, 2012), but to what extent remains elusive at present. Complex system analysis models will be needed for exact predictions.

4.3 Limitations of the study

The following chapter discusses the limitations of the study regarding the data background and the methodology.

Underreporting

Underreporting can occur in the following situations: cases without medical consultation, wrong medical diagnosis, correct diagnosis but failed reporting or incorrect reporting. Overreporting is possible for cases with a travel background where the local weather is not influential for infection. Moreover, imported food and interrupted freezer chains on the way cannot be identified. In the case of a common foodborne disease like campylobacteriosis, in Germany, underreporting is more likely a source of error (Wheeler et al., 1999).

Temporal lag in reporting

When identifying a special lagtype between weather conditions and incidence, it should be considered that the underlying incidence data represents the reporting week not necessarily the week of infection.

Reporting times between European countries may have a wide variance between 0 and 30 days (Kovats et al., 2004). While reporting procedures within German borders are more homogenous for campylobacteriosis, variabilities can still occur due to infrastructure or rural-urban differences, a shift due to the varying numbers and distribution of public holidays, individual practices in seeking a doctor’s help, individual susceptibility, or varying notification practices by health professionals. Moreover, incubation time, the period between infection and the first clinical symptoms, varies between two to ten days (RKI, 2015a). The period between prodromal until specific gastroenteric symptoms lasts approximately 24 hours and rarely up to two or three days (Allos, 2017). At the point in time when *Campylobacter* enters the human host, weather parameters are affecting incidence by interacting with the homoeostasis of the human individual. Since the exact time of infection cannot be determined, it remains unclear how long *Campylobacter* is exposed to weather in the environment and how long it lives in the homogenous

environment of the human host (Fig. 39). Yet, the weather around the time of infection might play a crucial role in the success of developing the illness and be counted as a case. For example, may cold wet weather conditions around the time when *Campylobacter* is still exposed to the environment, be advantageous for a successful infection. In contrast, hot and dry weather at the point in time when *Campylobacter* enters the human host may increase the success of developing campylobacteriosis as it aggravates symptoms in the human host and alters individual susceptibility to falling ill. Thus, the sensitivity of lag times could be variable between regions and individuals. However, the unavoidable delay between clinical manifestation and diagnosis and reporting is expected to be systematic and included in the lagtype.

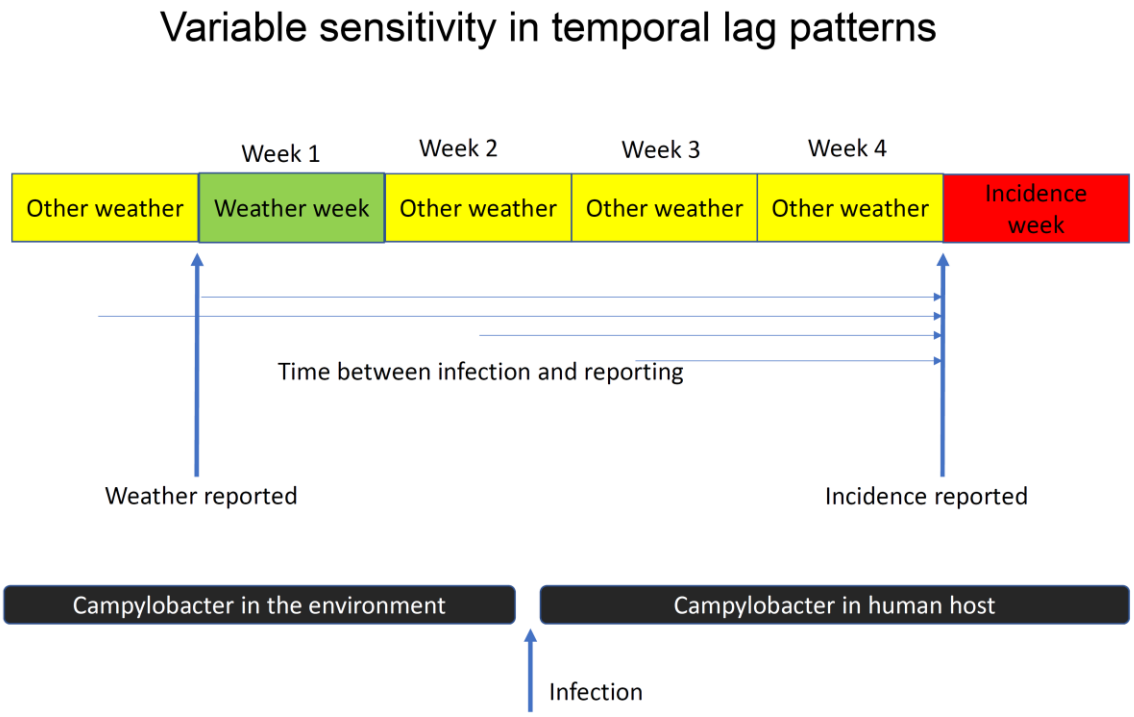


Fig. 39: Variable sensitivity in temporal lag patterns: Example for lagtype 4.

Uncertainty due to spatial harmonisation

A spatial harmonisation of reporting areas and weather grid cells had to be realised. For this study it had to be chosen between: Either 1.) recalculating weather data to reporting

areas, or recalculating 2.) incidence cases to weather grid cells. It was opted for option 1.).

Weather data has been allocated to a reporting area by weighing its areal proportion of the reporting area. These weather subareas were then summarised to a new reporting area weather. Since a weighted weather average has been allocated, extreme values are lost. Moreover, the weather subareas were only recalculated regarding their surface ratio. It has not been considered if a subarea with extreme weather values was lowly populated, maybe as a result of a hostile climate. It had to be assumed that all reporting areas were equally populated. Furthermore, it has been considered that weather conditions within a grid cell were equal at each point, which was most likely not the case.

Besides the methodological considerations, uncertainties regarding the assumed local association between campylobacteriosis and weather conditions must be discussed. *Campylobacter* is a dominantly foodborne pathogen. Its sources of transmission, mainly poultry meat, are embedded in a food chain that spans national and international wide. At every stage between pathogen and its pathogenic outcome in human beings, the weather is influential. This is unlikely to occur in a constant spatial unit. While this study examines local weather influence, it does not consider these dynamic effects.

Uncertainty due to temporal harmonisation

Daily weather data had to be recalculated to incidence weeks. Calculating weekly weather from daily data caused the loss of extreme values. To minimise information loss, weekly maximum, mean and minimum values of each weather parameter were stored.

The increase of yearly campylobacteriosis cases in Germany

Underlying campylobacteriosis case and incidence data have been analysed. In the investigation period, 979,005 campylobacteriosis cases were reported. Average annual cases were 65,267 and varied between 50,612 (2003) and 77,224 (2014) cases (Fig. 40). Annual cases increased by 33 % from 2001 to 2014. This increase coincides with some of the warmest years between 1881 and 2014, for example 2014, 2011, 2007 and 2002 suggesting a temperature effect. Thus, the case increase might be temperature-related

but may also reflect changes in exposure or improvements in surveillance and reporting (EFSA, 2016). However, the overlying increasing trend does not cause a temporal bias because the incidences are not compared between different time spans but classified solely along weather parameter values. Thus, each year can be represented in each class, and high incidence-years will not skew results.

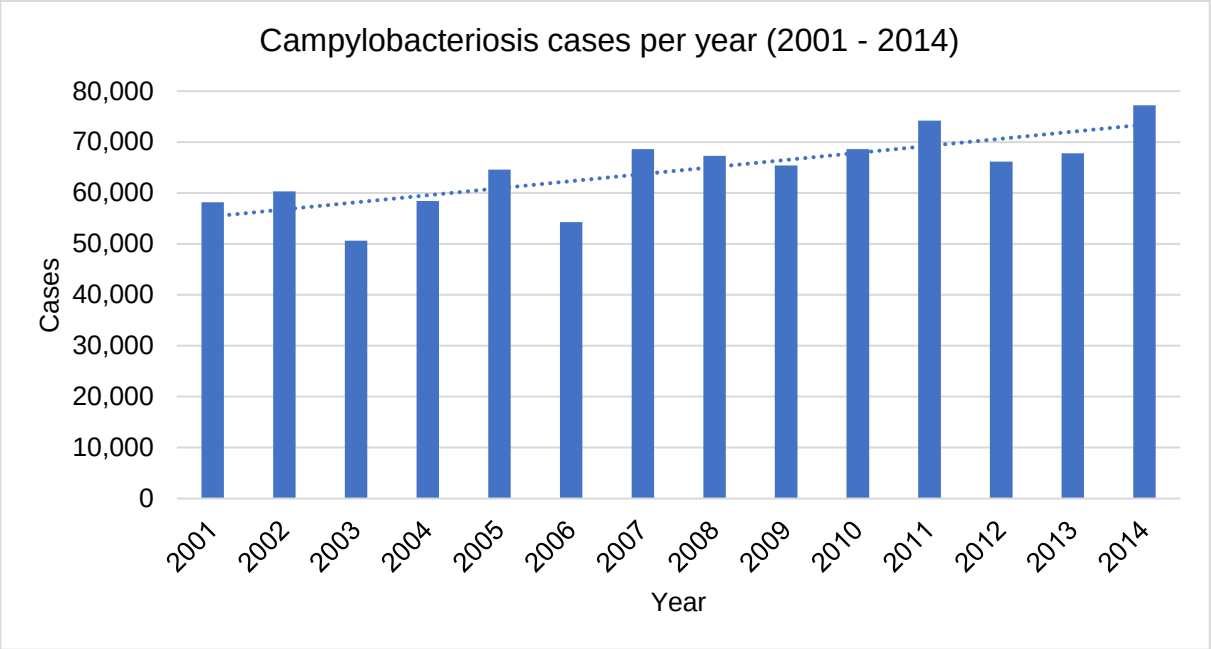


Fig. 40: Reported campylobacteriosis cases per year and trend line (dotted), 2001 – 2014 (SurvStat@RKI, last reviewed: 15.03.2017).

Range overlap of temperature parameters

All temperature parameters showed very similar correlations to incidence. A reason for comparable results might be an overlap in temperature parameter values. Fig. 41 shows that the created weather parameters are not distinct but overlap on a wide temperature range. Moreover, the difference in temperature width of each parameter, spanning from 24 K (Kelvin) in ‘Tmin_max’ to 36 K in ‘Tmax_max’ are outlined. This could, in part, explain similar correlation results among overlapping weather parameters.

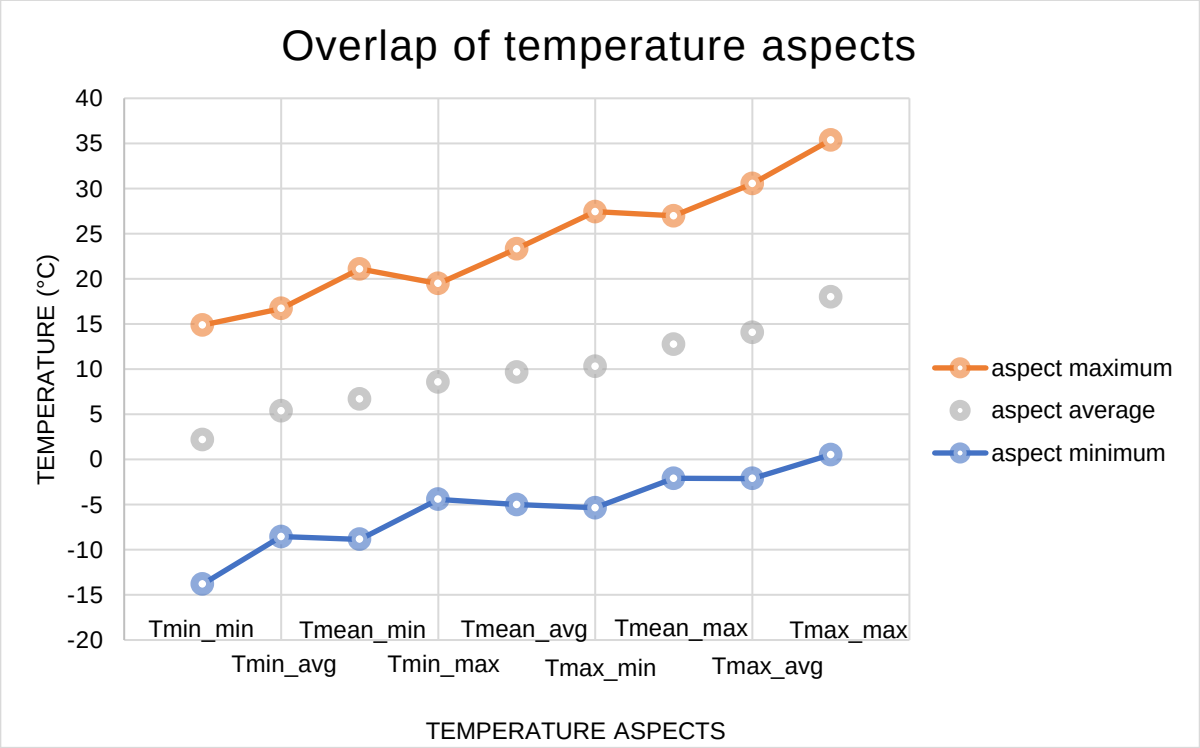


Fig. 41: Overlap of temperature parameters. The parameter width with minimum, average and maximum is figured.

5. Methodology-relevant definitions

Classes are defined by a weather parameter. The data is sorted based on weather condition and grouped in equally large classes. The classes are filled along the values of the selected weather parameter until 5,000 reports populate a class (see methodology for details). A class, e.g., class number 22, defined by the values of one weather parameter is not congruent to the class 22 defined by another weather parameter, neither by spatial nor temporal attribution and is very likely to include a differing collection of reporting areas in time and space. Due to the comparable underlying number of reports, the results do not lose quality along the range of classes for a single weather parameter. However, classes have a varying width to allow a collection of 5,000 reports.

Climate variable refers to the term used by NOAA to describe variables such as temperature or precipitation (NOAA, 2017).

Day-values are calculated with day numbers from 1 = Saturday, 1.1.2000 (in week 52/1999) to day 5470 = Monday 22.12.2014 = week 52/2014. Days are serially numbered.

Incidence is defined as the number of new cases of a health outcome per 100,000 inhabitants in a week.

Inhabitants refer to the number of citizens within a reporting area.

Lagtype: A lag is a temporal offset between reported incidence (or number of cases) and weather conditions. In this analysis, weekly data has been compared. The shortest lag compares the immediately preceding week. The shortest lag, called ‘lagtype 1’, has no intermediate weeks between the week of health outcome and weather conditions.

Mondays identify a week and have day-values 3, 10 17, 24, …, 5463, 5470. The weather data covers a complete year in advance to provide a lag prior to incidence values. Therefore, the smallest Monday# identifying the first incidence week is 367 = 1.1.2001.

NUTS-3 is an abbreviation for ‘Nomenclature of Territorial Units for Statistics’ and defines the classification system which divides the territory of the European Union into smaller units. NUTS-3 regions represent the smallest territorial unit, here called ‘reporting areas’.

The **Population** is used to describe the number of data participating e.g. reports within a class.

A **Report** describes the outcome of campylobacteriosis for one week in one reporting area and represents the smallest spatiotemporal reporting outline available. It consists of the number of cases, the incidence, and the identification of the reporting area. The inhabitants can be calculated, and the spatial location is available as well.

Reporting areas or counties are the smallest defined spatial units, which report the number of cases, number of inhabitants and incidence values. In Germany, this is equivalent to the NUTS-3 level and is called ‘Kreis’.

Weather parameters refer to the created differentiated climate variables of temperature and precipitation such as ‘Tmin_min’, ‘Tmax_min’, etc.

A **Week** is the smallest temporal reporting unit for campylobacteriosis. Weeks can be identified by the Monday-day-value of this week. Within a year, weeks are additionally numbered from 1 to 52 or 53 for each year to provide access to a seasonal evaluation. Weeks within a year are calculated referring to ISO standard (International Organization for Standardization).

6.1 Abstract

Foodborne *Campylobacter* species are the most common causes of bacterial gastrointestinal infections in Germany. Campylobacteriosis incidence in temperate climates shows a seasonal pattern which previous studies have attributed to climatic and non-climatic factors. In Germany, the association of campylobacteriosis and weather condition has already been examined on a low resolution and for selected regions. However, to define ranges of weather parameters that strongly correlate with incidences, a dense spatial and temporal data resolution is required.

This study applies a novel approach, examining the correlation between weather parameters, such as temperature and precipitation, and human campylobacteriosis in Germany from 2001 to 2014. Therefore, daily weather data and weekly reported incidences on the NUTS-3 level were examined, which provided a dense temporal and spatial resolution and a statistically robust data background for analysis. The data were sorted based on weather condition and grouped in equally large classes to solve the problem of shrinking numbers of reports in extreme weather conditions. The applied method manages the small numbers problem in lowly populated areas by recalculating incidences from class cases and class populations. Lag times between weather condition and incidence for up to eight weeks were tested. A sigmoid regression was fitted to the data.

The regression analysis showed a strong positive correlation between all tested temperature and precipitation parameters and local human campylobacteriosis incidence in Germany. A rise in temperature correlated stronger with increasing incidence than a rise in precipitation. Among all temperature aspects, within a wide temperature corridor of -5 °C to 28 °C incidence increased with rising ambient temperature. Among the tested temperature parameters, the weekly average of daily minimum temperature was identified as most significant with the most pronounced incidence increase within a temperature corridor of approximately 0 °C to 12 °C. The correlation for the minimum temperature occurred with an offset of two weeks and for the weekly mean and maximum temperature with an offset of four to five weeks. The strongest correlation among all precipitation parameters occurred at the four-week total precipitation with a time offset of approximately two weeks to incidence.

Overall, the present small-scale data analysis gives evidence of the crucial role increasing mild minimum temperatures play in campylobacteriosis incidence. However, since increasing ambient temperatures have diametrical effects on *Campylobacter* survival and furthermore replication is unlikely outside the host, *Campylobacter*'s microbiology cannot explain the study results. It is more likely that other variables such as vectors or human behaviour are more directly linked with temperature parameters than the pathogen itself. These other variables outweigh the direct relationship between temperature and pathogen when the whole chain of infection is considered. Therefore, it was assumed that campylobacteriosis incidence is the integral of multiple interdependent factors. Among them, ambient temperature is one contributing factor and could act as an indicator of elevated human exposure towards *Campylobacter*. Regarding precipitation parameters, the results show that high precipitation is strongly associated with campylobacteriosis incidence which can be explained with the pathogen's microbiology. Rain peaks are likely to be more influential than frequent low dose rain.

The study results demonstrate that campylobacteriosis is strongly associated with ambient temperature and precipitation. It gives evidence of an increased incidence with projected warmer temperatures and an increase in heavy rain events in the context of climate change. However, the driving weather-susceptible mechanism is likely to act beyond the direct weather-pathogen relationship and is yet to be identified.

6.2 Zusammenfassung

Die nahrungsmittelassoziierte Pathogenfamilie *Campylobacter* spp. gehört zu den häufigsten Ursachen bakterieller Gastroenteritiden in Deutschland. Campylobacterioseinzidenzen zeigen in den gemäßigten Breiten ein saisonales Muster, welches bisherige Studien auf klimatische und nicht klimatische Faktoren zurückgeführt haben. Für den Raum Deutschland wurde der Zusammenhang zwischen Campylobacteriose und Wetter bereits für gering aufgelöste Skalen und selektierte Räume untersucht. Um jedoch Wetterparameterbereiche zu identifizieren, welche mit Inzidenzen assoziiert sind, wird eine räumlich und zeitlich hohe Datenauflösung benötigt.

Diese Studie wählt einen neuen Ansatz, um die Korrelation zwischen Wetterparametern, am Beispiel von Temperatur und Niederschlag, und Campylobacteriose in Deutschland im Zeitraum von 2001 bis 2014 zu analysieren. Dazu wurden täglich aufgelöste Wetterdaten und wöchentlich gemeldete Inzidenzen auf NUTS-3 Kreisebene untersucht. So wurde einerseits eine angemessene zeitlich und räumlich hohe Auflösung erzielt und andererseits ein statistisch robuster Datenhintergrund gewährleistet. Die Daten wurden basierend auf der Wetterausprägung sortiert und in gleich große Klassen gruppiert, um das Problem der schrumpfenden Meldungszahlen in extremen Wetterlagen zu lösen. Die verwendete Methodik findet eine Lösung für das Problem der kleinen Zahlen in bevölkerungsarmen Kreisen, indem auf der Basis von Klassenfall- und Klassenbevölkerungszahlen die Inzidenzen pro Klasse neu berechnet wurden. Zeitversatze von bis zu acht Wochen wurden zwischen Wetterausprägung und Inzidenz getestet. Eine sigmoide Regression wurde an die Daten angepasst.

Die Regressionsanalyse ergab, dass alle getesteten Temperatur- und Niederschlagsparameter stark positiv mit Campylobacterioseinzidenzen in Deutschland korrelieren. Steigende Inzidenzen korrelierten stärker mit einem Temperatur- als Niederschlagsanstieg. Bezogen auf alle Temperaturparameter stieg die Inzidenz in einem weiten Temperaturkorridor von -5 °C bis 28 °C mit zunehmender Umgebungstemperatur an. Darunter konnte das wöchentliche Mittel der täglichen Minimumtemperatur als signifikantester Temperaturparameter identifiziert werden, welcher zwischen 0 °C bis 12 °C den ausgeprägtesten Inzidenzanstieg aufwies. Die stärkste Korrelation zwischen Inzidenz und Minimumtemperaturen trat mit einem Zeitversatz von zwei Wochen auf, für

Mittel- und Maximumtemperaturen mit einem Versatz von vier bis fünf Wochen. Die höchste Korrelation unter allen Niederschlagsparametern wurde für die Vier-Wochen-Niederschlagssumme mit einem Zweiwochenversatz zur Inzidenz beobachtet.

Insgesamt ergibt die vorliegende kleinskalige Datenanalyse den Nachweis über die wichtige Rolle ansteigender milder Minimumtemperaturen in Bezug auf Campylobacterioseinzidenzen. Da ansteigende Umgebungstemperaturen jedoch diametrale Effekte auf das Überleben von *Campylobacter* zeigen und zudem eine Vermehrung außerhalb des Wirtes nicht möglich ist, können die Resultate nicht durch die Biologie von *Campylobacter* spp. erklärt werden. Daher ist es wahrscheinlich, dass andere Einflussgrößen wie Vektoren oder menschliches Verhalten einen direkteren Zusammenhang zu Temperaturparametern haben als das Pathogen selbst. Diese anderen Variablen dominieren gegenüber dem direkten Zusammenhang zwischen Temperatur und Pathogen bei der hiesigen Betrachtung der gesamten Infektionskette. Die Studie kommt deshalb zu dem Schluss, dass Campylobacterioseinzidenzen das Integral multipler interdependenter Faktoren sind. Unter diesen kann die Umgebungstemperatur als Indikator erhöhter menschlicher Exposition gegenüber *Campylobacter* fungieren. Die Ergebnisse der Niederschlagsparameteruntersuchung zeigen, dass hoher Niederschlag stark mit Campylobacterioseinzidenzen assoziiert ist, was mit der Biologie des Pathogens erklärt werden kann. Niederschlagspitzen sind wahrscheinlich einflussreicher als beständiger geringfügiger Regen.

Die Studienergebnisse zeigen, dass Temperatur und Niederschlag stark mit Campylobacteriose assoziiert sind und erbringen Hinweise auf eine zunehmende Inzidenz unter projizierten wärmeren Temperaturen und gehäuften Starkregenereignissen im Kontext des Klimawandels. Jedoch agiert der treibende wettersensible Mechanismus wahrscheinlich außerhalb der direkten Wetter-Pathogen-Beziehung und muss noch identifiziert werden.

7. List of figures

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9. References

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