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Impacts Of Ambient Temperature On Foodborne Salmonella Infection

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Impacts of Ambient Temperature on Foodborne *Salmonella* Infection

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Year Completed: 2019

Year Degree Awarded: 2019

Master of Public Health

Environmental Health Sciences

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Abstract:

Foodborne Disease (FBD) impacts individuals through the ingestion of foods contaminated with microbes and can lead to an array of adverse health consequences ranging from mild symptoms such as nausea to those that evolve to become life-threatening (World Health Organization, 2015a). The incidence of FBD is expected to increase in the presence of climate change due to an increase in ambient temperature creating an environment where microbes can rapidly multiply and thrive (Gregory, Johnson, Newton, & Ingram, 2009; Kovats et al., 2004). Foodborne cases of *Salmonella* make up the second largest cause of gastrointestinal infection in the United States (Scallan et al., 2011). In the United States, the Centers for Disease Control and Prevention's National Outbreak Response System database documents nation-wide occurrences of FBD outbreaks. In state-level analyses for Florida, Illinois, Maryland, Michigan, Minnesota, New York, Ohio, and Washington, negative binomial regression models were used to examine the association between monthly average maximum daily temperature and monthly incidence of foodborne *Salmonella* between 1998 and 2017, using models with the same month's average maximum daily temperature as the predictor (zero-month lag) and models with the previous month's average maximum daily temperature as the predictor (one-month lag). The zero-month lag analysis yielded significant results for Illinois, Maryland, Minnesota, New York, Ohio, and Washington, and the one-month lag analysis yielded significant results for Maryland, Minnesota, New York, and Washington. A single model with state as an additional covariate, was also run and found summary statistics with the middle and highest temperature categories having 1.52 (95% CI: 1.05, 2.21; $p = 0.03$) and 3.46 (95% CI: 2.40, 5.01; $p < 0.001$) times greater risk of illness due to *Salmonella* than in the lowest temperature category. This study illustrates a connection between higher temperatures and increased incidence of *Salmonella* infection, both at the individual state level and across all analyzed states.

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Background:

Introduction

This paper investigates the relationship between meteorological variables and foodborne disease (FBD) outbreaks in the United States by analyzing the relationship between outbreaks of *Salmonella* and temperature in selected areas. The analysis of temperature and precipitation impacts can illuminate potential consequences associated with climate change, as climate change will impact these meteorological variables. Researchers have already seen an impact of climate change on aspects of agriculture, such as through decreasing nutrient contents in foods, and, in turn, nutrition and health (Zhu et al., 2018). Food security and health can be impacted through increases in droughts, decreases in water access, increases in extreme weather events, along with an increase in FBD. While there have been many initiatives to curb the number of outbreaks of FBD in the developed world, few exist in developing countries, which face the highest burden (World Health Organization, 2015b). Through focusing on the relationship between temperature and FBD outbreak cases of *Salmonella*, this paper aims to better understand the role ambient temperature play in FBD outbreaks.

What is FBD?

FBD occurs around the world mainly from the ingestion of foods contaminated with microbes, chemical residues, and other toxic substances (World Health Organization, 2015a). However, in addition to food sources, the disease can be transmitted through environmental and human sources. FBD cases are frequently characterized by milder symptoms such as nausea, vomiting, and diarrhea. However, if not treated properly these cases can evolve to be life-threatening. Severe cases have led to kidney and liver failure, brain and neurological disorders,

paralysis, and death, and are a suspected contributor to the future development of cancers (World Health Organization, 2015b).

While FBD is caused by both chemical and microbial sources, this paper focuses on bacterial cases, specifically those attributed to *Salmonella*, as cases of *Salmonella* transmitted by solely food make up the second largest cause of intestinal infection in the United States (Centers for Disease Control and Prevention, 2018c, 2019b; Scallan et al., 2011).

Transmission

Animals raised for food serve as a major reservoir for FBD. With bacteria being shed through animal fecal matter, infection can be transmitted via the contamination of animal products, particularly during the slaughtering and processing stages (Tauxe, 1997). From here, humans are presented with animal products such as meat and milk that, if not properly treated with high enough heat to kill bacteria, will infect humans (Food and Agriculture Organization, 2017; World Health Organization, 2008). Another mechanism of transmission via food occurs with fresh produce and unpasteurized juices (Bennett et al., 2017; Berger et al., 2010; Jain et al., 2009). Produce can cause infections following exposure to manure that hosts bacteria. Contamination occurs when manure is improperly used on crops or, more frequently, when large farms have too much manure to properly contain, causing the manure to flow into local water sources that are then used to water crops. An additional contamination risk is when large storms drop heavy volumes of rainwater, resulting in flooding wherein the rainwater comes in contact with manure, which flows downstream contacting crops (Tauxe, 1997). These contaminated crops may then be sold as fresh produce or can be turned into juices that are sold to distributors or the public.

The human-to-human pathway is also a common way that FBD is transmitted where transmission occurs via the fecal-oral route (Centers for Disease Control and Prevention, 2013a). Typically, humans are exposed to the fecal matter of other humans through eating food that has contacted human feces. However, direct contact with fecal matter will also facilitate disease transmission. This also frequently happens in restaurant and home settings where there are poor hygiene standards, particularly with improper handwashing prior to food preparation (Washington State Department of Health, 2017).

Transmission to humans also occurs from the environment through exposure to animals and their fecal matter in the environment. While this is not a food source of FBD cases, these infected humans can then spread the infection further through human-to-human transmission pathways.

Prevention

Prevention is the first and biggest line of defense when dealing with FBD cases. Due to a strong trend in changing dietary patterns, many individuals are increasingly eating foods produced from outside of their homes (Cheng, Olsen, Southerton, & Warde, 2007; Warde, Cheng, Olsen, & Southerton, 2016). From this change it can be deduced that there is an increasing risk of developing FBD as there are more steps involved in producing the food served. There are multiple levels in which prevention techniques can be implemented. From a public perspective, prevention strategies include proper education regarding techniques to minimize FBD cases as well as prompt communication when there are outbreaks reported. Prevention strategies can also be implemented through recalling food products if they have been identified as the source of outbreaks. Some of these educational techniques can be implemented at the

individual level, including the “Five Keys to Safer Food” that were developed by the World Health Organization, seen as follows (World Health Organization, 2006):

1. Keeping clean
2. Separating raw and cooked foods
3. Cooking foods thoroughly
4. Keeping foods at safe temperatures
5. Using safe water and raw materials

Furthermore, individual prevention actions include using antibiotics properly, avoiding contact with wild animals, exercising caution with pets, and choosing to consume products that are pasteurized (Centers for Disease Control and Prevention, N. D.). Additionally, the WHO has published guidelines on safe food handling as a training guide to prevent occurrences of foodborne disease (Jacobs, 1989). Many states and countries also mandate food safety training for individuals as part of food safety programs.

The Infectious Agent: Salmonella

Salmonella is a bacterium in the family Enterobacteriaceae that consists of two species, *Salmonella enterica* and *Salmonella bongori*, with 2,463 serotypes (Brenner, Villar, Angulo, Tauxe, & Swaminathan, 2000). This illness typically lasts between four to seven days and does not require further treatment; however in severe cases patients may develop sepsis and can lead to death (Centers for Disease Control and Prevention, 2019b). Certain populations are at an elevated risk of severe consequences from *Salmonella* infection, specifically children under the age of 5, the elderly, and those with weakened immune systems (Centers for Disease Control and Prevention, 2014b). In some cases, those infected with *Salmonella* develop reactive arthritis lasting from months to years and potentially developing into chronic arthritis (Centers for

Disease Control and Prevention, 2014b). Infection by certain *Salmonella* serotypes can also lead to the development of enteric fevers, such as those caused by *S. typhi*, however this infection has been deemed rare (Baron, 1996).

In the United States, the CDC estimates 1 million illnesses, 19,000 hospitalizations, and 380 deaths attributed to food transmitted cases of *Salmonella* infection per year (Centers for Disease Control and Prevention, 2019b). The following food sources have been connected to *Salmonella* outbreaks in the United States: vegetables, sprouts, eggs, chicken, pork, fruits, and nut butters (Centers for Disease Control and Prevention, 2019c).

Diagnosis

Diagnosis of *Salmonella* relies heavily on the patients' history in addition to the epidemiologic features and objective findings of the case (Guerrant et al., 2001). Early symptoms and their timing are identified to aid the diagnosis process. Telling signs of a case of FBD due to *Salmonella* are the presence of diarrhea (particularly within 24-48 hours of ingestion and potentially bloody), dehydration, fever, abdominal pain, and weakness; in some instances sepsis may develop (Centers for Disease Control and Prevention, 2015b).

In addition to looking at early symptoms, a physical examination should be conducted where one takes general physical assessments (such as mental status, skin turgor to assess dehydration, and abdomen pain), orthostatic pulse, and blood pressure (Guerrant et al., 2001). Further tests can be conducted if deemed necessary, primarily collecting a stool culture and in severe cases collecting blood cultures. Collection of a stool culture is necessary to identify the bacterial causes of FBD cases, making this one of the reasons for underreporting as not all individuals provide samples. If a sample is collected, it can be useful for identifying outbreaks (Guerrant et al., 2001).

When an outbreak is suspected, a laboratory analysis of bacterial samples is conducted for confirmation. Typically, health departments utilize Pulsed-field Gel Electrophoresis (PFGE) in identifying FBD cases including those caused by *Salmonella*. PFGE is a technique where a unique “DNA fingerprint” is created for the bacteria sampled. PFGE separates DNA fragments according to size through an agarose gel with an electrical field (MacCannell, 2013).

Laboratories have the potential capacity to produce gel patterns in about 24 hours (Ribot et al., 2006).

Following the running of the gel the unique “DNA fingerprint” is produced, which can then be analyzed, typically with a software program such as BioNumerics (Centers for Disease Control and Prevention, 2016). The pattern is then uploaded to the database where an investigation can occur to determine if the case is part of an outbreak. It is critical to interpret PFGE data in the context of the epidemiology in the region of interest. This is because the pattern itself does not offer sufficient information to determine an outbreak as some PFGE patterns may be common or similar strains may have infected individuals through different pathways (Barrett, Gerner-Smidt, & Swaminathan, 2006). Additionally, studies have shown that patterns may be different but still related epidemiologically, such as when four different patterns were found from *E coli* O157 isolates with the same source (Jackson et al., 2000).

There are issues associated with utilizing PFGE in outbreak investigations as some observed differences may not be real (i.e. could be artifacts due to a multitude of factors such as utilizing the incorrect primers or contamination of samples), organism diversity may impact PFGE patterns, and differences in timing of outbreaks can impact PFGE patterns (longer outbreak periods may lead to increased diversity in patterns as it provides time for mutations to occur) (Barrett et al., 2006).

Climate Change and Salmonella

It is predicted that climate change will increase the number of cases of FBD due to an increase in ambient temperature creating an environment where microbes can rapidly multiply and thrive (Gregory et al., 2009; Kovats et al., 2004). Additionally, increases in the number of extreme weather events will impact food access and safety (National Institute of Environmental Health Sciences, 2010).

Many studies have looked to establish a relationship between seasonality and FBD outbreaks, forming the basis of hypotheses that climate change will impact outbreaks. Most of these studies assess the impact of temperature through a proxy of seasonality, as well as the impact of relative humidity. These studies have typically found a relationship between seasonality and the appearance of outbreaks (Kim, Park, Chun, Choi, & Bahk, 2015; Lee, Lee, Kim, & Park, 2009; Wang et al., 2016).

Research has also shown a connection between high precipitation levels and increases in cases of FBD, especially caused by *Campylobacter*, *Salmonella*, *Vibrio*, and *E.Coli* (Park, Park, & Bahk, 2018; Semenza et al., 2011; Soneja et al., 2016). These studies have indicated that heavy precipitation may lead to flooding that brings bacterial agents (from sewage or manure) in contact with food products. One study found that *Salmonella* may be transmitted between from an airborne source to produce through a medium of rain, then surviving for up to 3 days on plants, indicating a potential relationship between elevated precipitation levels and *Salmonella* cases (Cevallos-Cevallos, Danyluk, Gu, Vallad, & van Bruggen, 2012). Through studying the impact of climatic variables on FBD outbreaks, one may determine if there is cause for concern given a changing climate with projected higher temperatures and precipitation levels.

There have been a number of studies specific to addressing the seasonality impacts on FBD cases attributed to *Salmonella*. General caution to consumers has been suggested by the CDC, warning that *Salmonella* cases are higher in the summer and a 2014 FoodNet report stating that the highest number of cases for *Salmonella* in the United States was found in August, due to the warm weather promoting *Salmonella* growth (Centers for Disease Control and Prevention, 2014a, 2019c). Research has looked at the impact of temperature and precipitation on *Salmonella* both in the United States and abroad. Studies in Europe have found a relationship between increasing temperature and increases in cases of Salmonellosis, another term for FBD cases caused by *Salmonella*, with one in Macedonia finding ambient temperature to be a predictor for cases (Kendrovski & Gjorgjev, 2012; Kovats et al., 2004; Yun et al., 2016). Studies in Canada have found a relationship with ambient temperature and cases of *Salmonella*, however one study did not find a relationship with precipitation (Ravel et al., 2010; Varga et al., 2013). An Australian study in Queensland also found a correlation between ambient temperature and Salmonellosis (Stephen & Barnett, 2016). Specific to the United States, a study in Georgia found *Salmonella* cases to be more associated with food festivals occurring during the summer and fall, and a study focusing on Mississippi, Alabama, and Tennessee, found a strong relationship between high temperature and cases of *Salmonella* infection (Akil, Ahmad, & Reddy, 2014; Wilson, 2015).

With a possible rise in Salmonellosis due to climate change, the United States may be faced with a number of adverse consequences. One of these is the potential economic burden from not only higher numbers of individuals becoming ill leading to lost productivity, but also higher costs in healthcare expenditure. Increases in outbreaks may also impact agricultural practices, with consumers exercising caution with certain food items associated with outbreaks,

leading to profit losses. A 2013 publication by the United States Department of Agriculture (USDA) found that the economic burden associated with *Salmonella* cases in the United States was over \$3.6 billion (Hoffmann, 2015). Additionally, increases in case numbers may lead to elevated mortality rates, particularly among vulnerable populations.

Materials and Methods:

National Outbreak Response System / Foodborne Disease Outbreak Surveillance System

Data regarding cases of foodborne *Salmonella* infection was obtained from the CDC's National Outbreak Response System (NORS) (Centers for Disease Control and Prevention, 2019a). NORS receives data on FBD outbreaks from the CDC's Foodborne Disease Outbreak Surveillance System (FDOSS). In addition to *Salmonella* infections, NORS contains information on cases caused by Astrovirus, *Bacillus*, Brucella, *Campylobacter*, Ciguatoxin, *Clostridium*, *Cryptosporidium*, *Cyclospora*, *Escherichia coli*, *Giardia*, Hepatitis A, Histamine, *Listeria*, mycotoxins, Norovirus, Rotavirus, Scombroid, *Shigella*, *Staphylococcus*, *Streptococcus*, *Trichinella*, *Vibrio*, and *Yersinia*. State, local, and territorial public health agencies report outbreaks of FBD (defined as having two or more cases) and include details on the cases involved to FDOSS; however reporting is not mandatory (Centers for Disease Control and Prevention, 2015a, 2019d). The NORS dashboard contains an open-access dataset spanning from January 1998 to December 2017 (Centers for Disease Control and Prevention, 2018b). The information reported includes the month and year of outbreak, the number of cases involved in each outbreak, the state, food or drink implicated, where the food or drink was prepared, and the agent involved (Centers for Disease Control and Prevention, 2018a). Data on case numbers was collected for *Salmonella* infections from 1998 to 2017 where every case belonged to an outbreak,

such that all cases were part of an outbreak and were not individually reported cases. As data was restricted to the month level, the analysis could not be performed using the daily case numbers.

National Oceanic and Atmospheric Administration / National Centers for Environmental Information

Temperature and precipitation data was gathered from the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Environmental Information (NCEI), specifically the Statewide Time Series in their Climate at a Glance program (National Oceanic and Atmospheric Administration, 2019c). Climate at a Glance provides monthly temperature and precipitation data for states in the contiguous United States from January 1895 to the present gathered from the U.S. Climate Divisional Database (National Oceanic and Atmospheric Administration, 2019b). This data has been adjusted to account for artificial effects such as instrument changes (National Oceanic and Atmospheric Administration, 2019a). Monthly data for Florida, Illinois, Maryland, Michigan, Minnesota, New York, Ohio, and Washington were utilized, specifically monthly average mean daily temperature, monthly average maximum daily temperature, monthly average minimum daily temperature, and monthly average precipitation. Temperature data are reported in degrees Fahrenheit while precipitation data are reported in inches.

Statistical methods

An analysis of the relationship between temperature and incidence of outbreak-associated foodborne *Salmonella* case incidence was conducted. This analysis focused on *Salmonella* because, among bacterial causes of FBD reported to FDOSS, the highest number of cases were reported for *Salmonella*. This opportunity to study *Salmonella* was exploited to add to the growing body of research looking into the impacts of seasonality and ambient temperature on

Salmonella incidence. This study focuses on the states of Florida, Illinois, Maryland, Michigan, Minnesota, New York, Ohio, and Washington due to the high *Salmonella* case numbers in these states. California, the state with the highest number of *Salmonella* cases, was excluded due to the large variability in temperature across the state (the *Salmonella* case data was restricted to the state level). In our analyses, the outcome variable was state-level monthly number of outbreak-associated cases from 1998 to 2017. Comparably, state-level temperature (°F) and precipitation (inches) data were averaged for every month from 1998 to 2017.

Because the relationship between temperature and *Salmonella* incidence is most likely non-linear, temperature was analyzed as a categorical rather than continuous variable. To account for differences in temperature distributions across states, a standardization function was applied to the temperature data using the scale function in R. This function centers the data by calculating the state-specific mean and standard deviation, then scales the temperature according to the number of standard deviations from the mean. Temperature categories were set from -2.5 to -1, -0.99 to 0.5, and 0.51 to 2. The lowest temperature category was set as the reference category for analysis. Analysis was conducted using the monthly averages of average daily temperature, maximum daily temperature, and minimum daily temperature, with preliminary findings yielding similar results. For simplicity, the results are presented looking at the impact of maximum temperature only.

Data were collected on the yearly population in each state as well through Statista from the US Census Bureau, with this yearly value being applied to each month in that respective year as a population offset (Statista, 2019). To control for long-term trends in case reporting, such that these effects can be separated from short-term associations between temperature and *Salmonella*

incidence, we created a continuous monthly time variable (Bhaskaran, Gasparrini, Hajat, Smeeth, & Armstrong, 2013).

Poisson regressions were conducted for each state looking at the outcome of case numbers, with a primary predictor of maximum temperature (categorical as explained above), a co-variate of precipitation (continuous), a long-term time trend indicator, and a population offset. Models were run with zero-month and one-month lag. In the zero-month models, the predictor was the temperature in the same month in which the cases occurred; in the one-month lag models, the predictor was the prior month. Over-dispersion was accounted for by using a quasipoisson model. Utilizing a Poisson regression, however, led to a poor fit for the data yielding p-values for the deviance goodness of fit test of less than 0.05.

The data was then modeled using a negative binomial regression model, with an outcome of case numbers, and included a primary predictor of maximum temperature (categorical), a co-variate of precipitation (continuous), a long-term time trend indicator, and a population offset. Models were again run with zero-month and one-month lag. A model including all of the states, with state as an additional covariate, was also run to calculate a summary result. All statistical analyses were conducted using R, an open-source statistical software.

Results and Discussion:

All states analyzed had at least 1,000 outbreak cases of *Salmonella* during the period of interest from 1998 to 2017. As illustrated in Table 1, these case numbers range from 1,035 in Washington to 3,116 in Illinois, totaling 13,664 cases and 850 outbreaks occurring during the study period. Cases originated from a range of sources, including restaurants, private homes, and grocery stores. Figure 1 showcases the trend over time in case reporting, with a seemingly

downward trend during the study period. Long-term time trends in reporting were controlled for through a continuous monthly time variable, as explained above.

State	<i>Salmonella</i> Cases	Number of Outbreaks
Florida	1,747	160
Illinois	3,116	129
Maryland	1,173	87
Michigan	1,214	59
Minnesota	1,368	98
New York	2,100	122
Ohio	1,911	114
Washington	1,035	81
Total	13,664	850

Table 1. Number of *Salmonella* Case and Outbreaks in the States Analyzed from 1998 to 2017. Total number of *Salmonella* Cases reported for the states of interest from 1998 to 2017. All cases are from outbreaks of *Salmonella*.

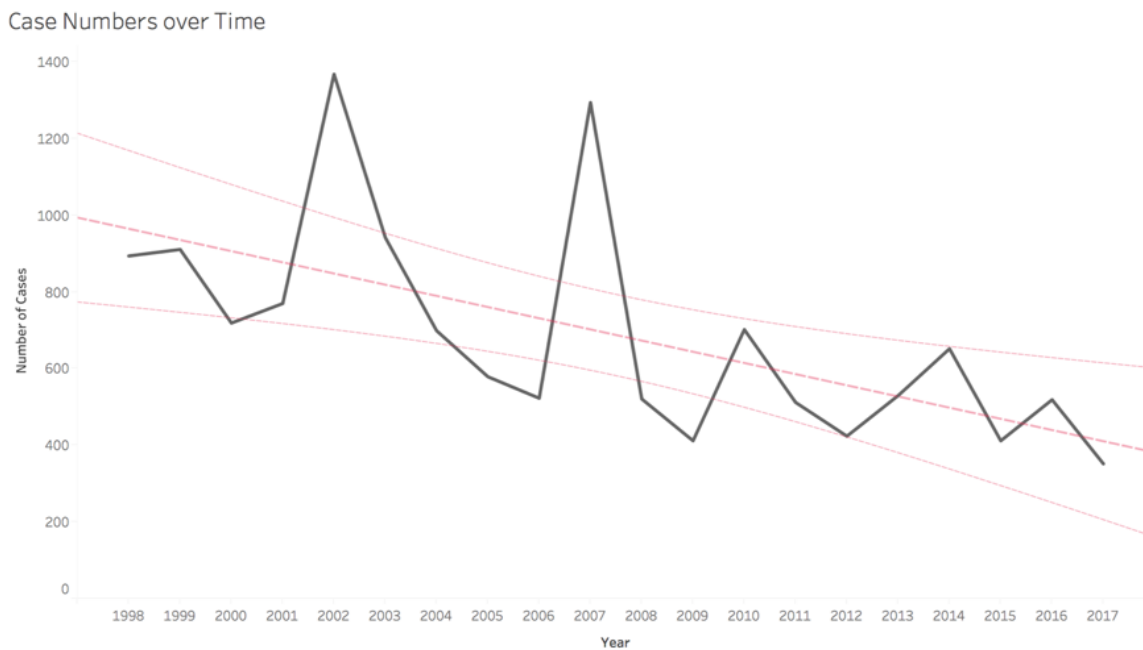
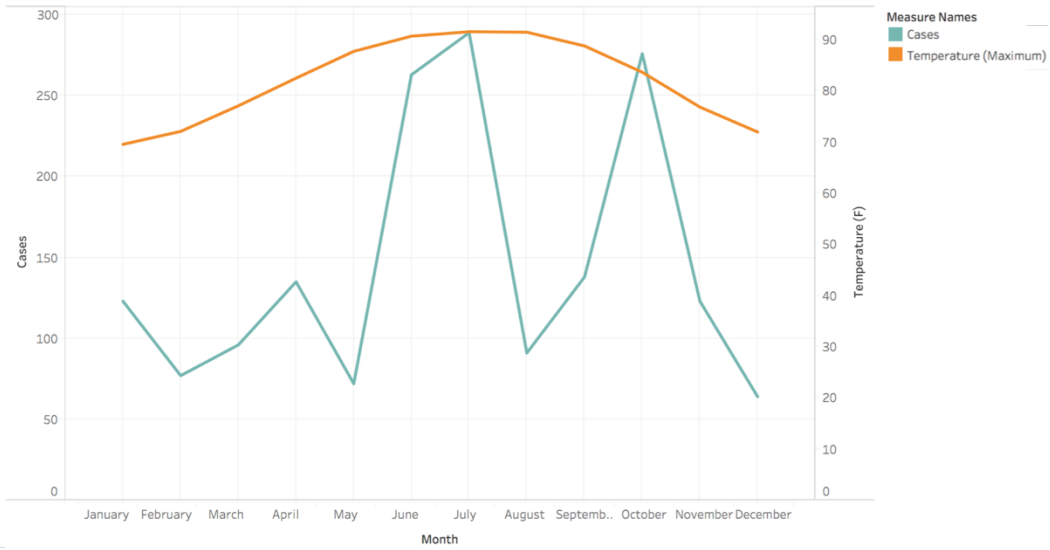


Figure 1. Trends in Reported *Salmonella* Cases. Number of *Salmonella* cases reported to the CDC from 1998 to 2017. These numbers are summed from the cases in Florida, Illinois, Maryland, Michigan, Minnesota, New York, Ohio, and Washington. This figure includes a trend line and bands representing a 95% confidence interval.

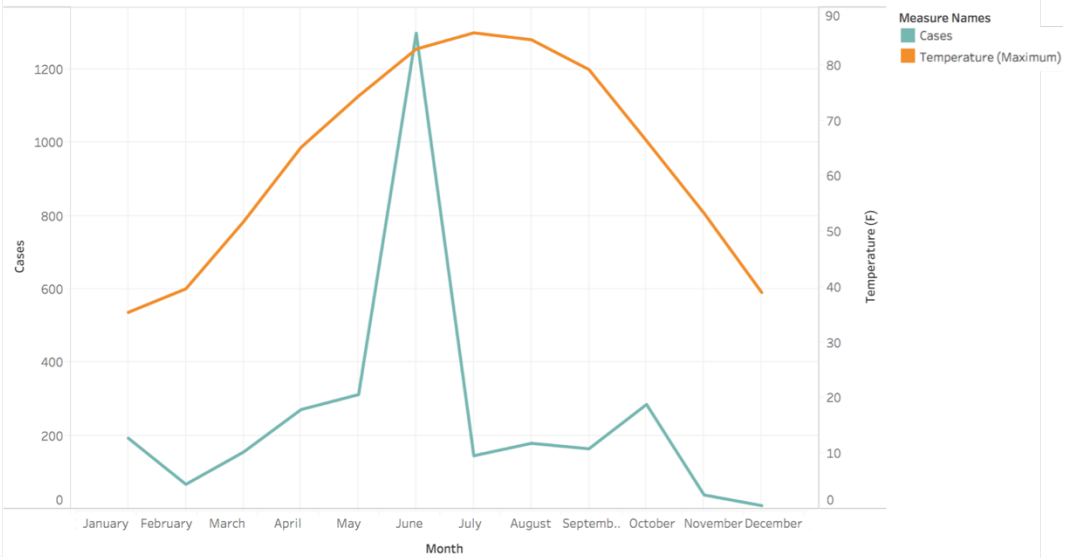
Reported case numbers were visualized in conjunction with monthly average maximum daily temperature for each state (Figures 2a-h). These figures show a typically normal distribution for average, maximum, and minimum temperature and a generally normal trend in *Salmonella* cases for all states except for Michigan which has a multimodal distribution. Temperatures for states varied dramatically, such as the monthly averages of daily maximum temperature in Minnesota ranging from 11.4°F-86°F (74.6°F difference) and the average temperature in Florida ranging from 63.1°F-95.7°F (32.6°F difference). This variation led to the use of a standardized temperature in determining temperature categories for analysis, as described in Methods. As seen in Figure 2, all states exhibited peaks in case numbers during the warmest months of the year (June, July, and August); however, in Michigan, this peak was not as large as the peaks in November or February.

Tables 2 and 3 detail the results of the negative binomial models for each state, with Table 2 presenting results for the zero-month lag and Table 3 presenting results for the one-month lag. The Akaike Information Criterion (AIC) estimates between models looking at no lag and a one-month lag were similar, with differences ranging from 0.15 to 5, well below the standard difference of 10 used to distinguish models of better fit, indicating that both sets of models fit the data well.

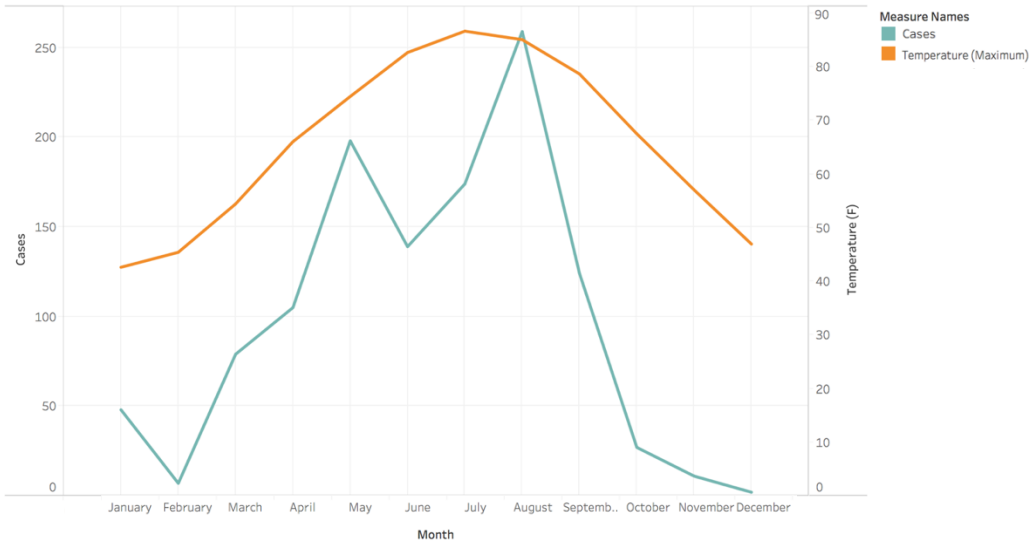
Florida



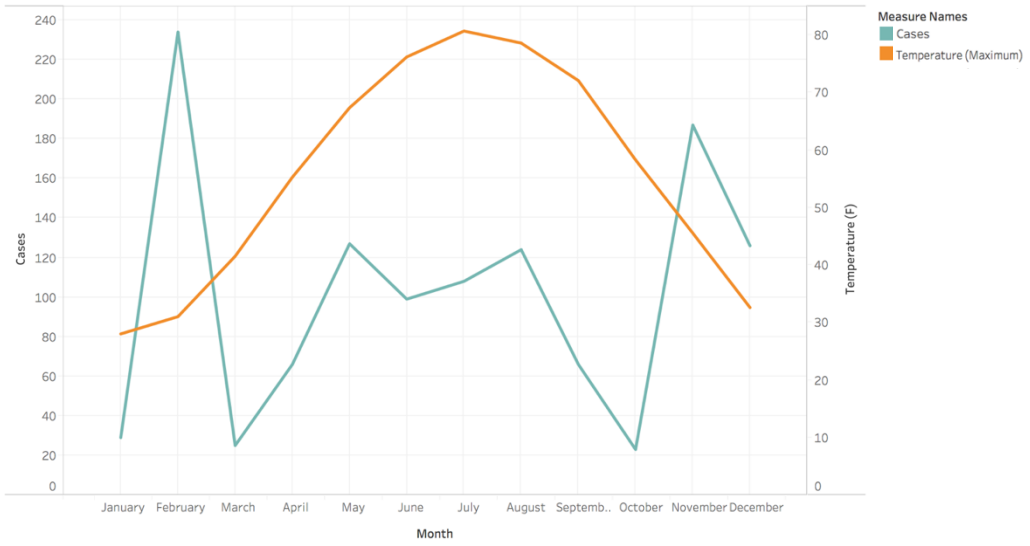
Illinois



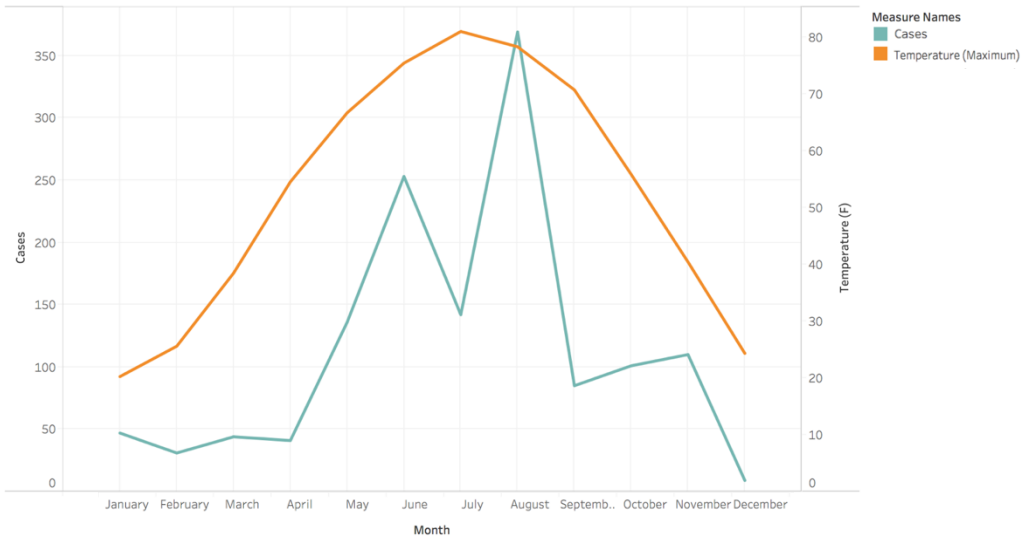
Maryland



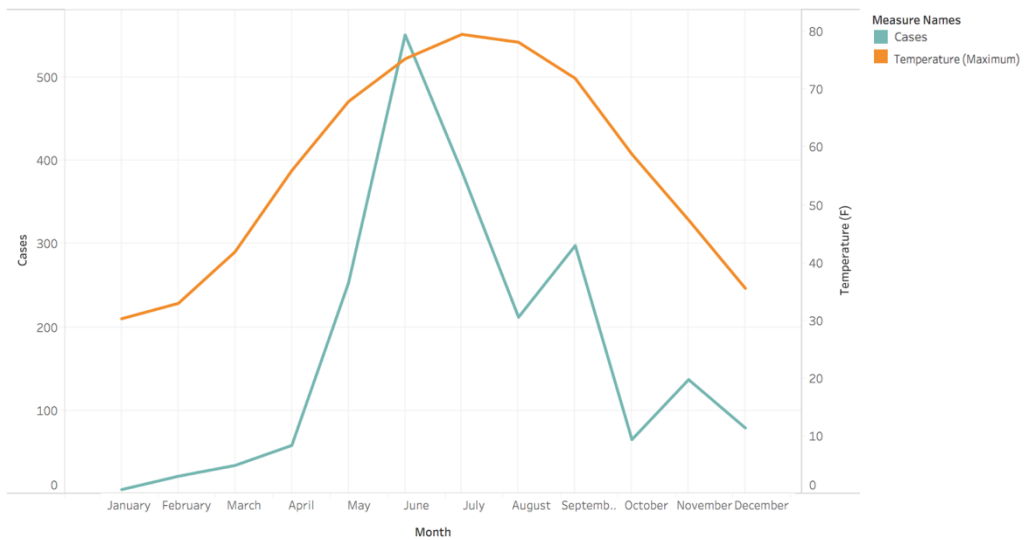
Michigan

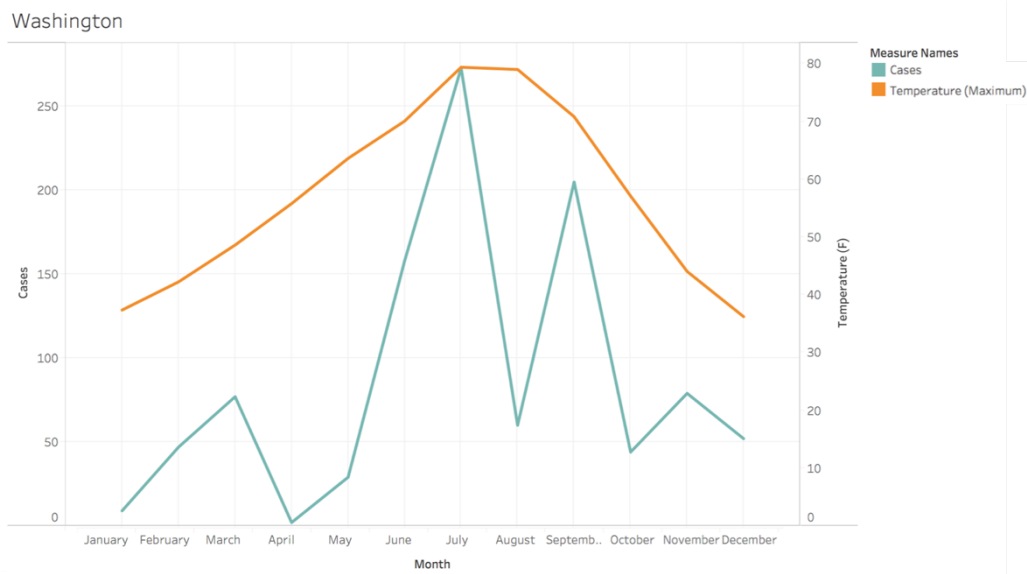
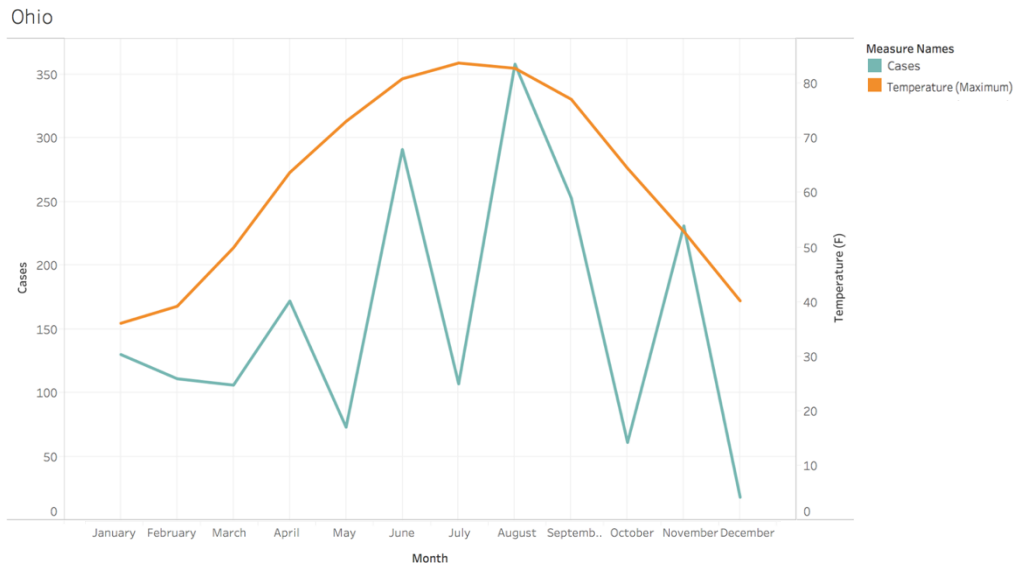


Minnesota



New York





Figures 2a-h. State Specific Case Numbers and Meteorological Variables. Monthly number of *Salmonella* cases (orange) and maximum monthly temperature (red) for Florida, Illinois, Maryland, Michigan, Minnesota, New York, Ohio, and Washington from 1998 to 2017.

State		Corresponding Temperature	IRR	95% CI	P-Value
Florida	Temp. Cat. 1	63.1°F – 73.7°F	1		
	Temp. Cat. 2	73.8°F – 85.7°F	1.33	(0.54, 3.26)	0.53
	Temp. Cat. 3	86.3°F – 95.7°F	1.05	(0.37, 3.04)	0.92
	Precipitation		1.08	(0.92, 1.27)	0.36
Illinois	Temp. Cat. 1	25.5°F – 44.9°F	1		
	Temp. Cat. 2	45°F – 72.4°F	2.19	(0.82, 5.85)	0.12
	Temp. Cat. 3	72.5°F – 95°F	4.64	(1.77, 12.14)	0.0018*
	Precipitation		0.90	(0.72, 1.12)	0.33
Maryland	Temp. Cat. 1	35°F – 49.9°F	1		
	Temp. Cat. 2	50°F – 73.9°F	3.26	(1.07, 9.94)	0.038*
	Temp. Cat. 3	74°F – 91°F	11.18	(3.64, 34.33)	<0.001*
	Precipitation		1.05	(0.83, 1.33)	0.66
Michigan	Temp. Cat. 1	18.6°F – 36.5°F	1		
	Temp. Cat. 2	36.6°F – 65.6°F	0.62	(0.15, 2.46)	0.49
	Temp. Cat. 3	65.7°F – 86°F	0.96	(0.24, 3.77)	0.95
	Precipitation		0.89	(0.56, 1.42)	0.64
Minnesota	Temp. Cat. 1	11.4°F – 30.8°F	1		
	Temp. Cat. 2	30.9°F – 63.4°F	2.73	(1.01, 7.41)	0.048*
	Temp. Cat. 3	63.5°F – 86°F	8.18	(3.07, 21.79)	<0.001*
	Precipitation		1.17	(0.93, 1.48)	0.17
New York	Temp. Cat. 1	20°F – 38.4°F	1		
	Temp. Cat. 2	38.5°F – 65°F	2.07	(0.81, 5.29)	0.13
	Temp. Cat. 3	65.1°F – 84°F	7.61	(2.91, 19.90)	<0.001*
	Precipitation		1.04	(0.80, 1.36)	0.75
Ohio	Temp. Cat. 1	27°F – 44.4°F	1		
	Temp. Cat. 2	44.5°F – 70.9°F	2.57	(0.95, 6.93)	0.062
	Temp. Cat. 3	71°F – 89.4°F	4.60	(1.64, 12.88)	0.0037*
	Precipitation		0.83	(0.63, 1.10)	0.19
Washington	Temp. Cat. 1	30.5°F – 41.9°F	1		
	Temp. Cat. 2	42°F – 64.4°F	1.36	(0.42, 4.44)	0.61
	Temp. Cat. 3	64.5°F – 83.9°F	10.75	(2.28, 50.77)	0.023*
	Precipitation		1.17	(0.93, 1.48)	0.17
Summary	Temp. Cat. 1	11.4°F – 42.5°F	1		
	Temp. Cat. 2	42.6°F – 71.3°F	1.52	(1.05, 2.21)	0.03*
	Temp. Cat. 3	71.4°F – 95.7°F	3.46	(2.40, 5.01)	<0.001*
	Precipitation		0.96	(0.89, 1.03)	0.27

Table 2. Results from Negative Binomial Models with Zero-Month Lag. Results from the negative binomial regressions analyzing the impact of the primary predictor of temperature on case numbers, while accounting for precipitation, population, and long-term trends in reporting at the state level. Resultant Incidence Rate Ratios (IRR), Confidence Intervals (CI), and p-values are reported.

State		Corresponding Temperature	IRR	95% CI	P-Value
Florida	Temp. Cat. 1	63.1°F – 73.7°F	1		
	Temp. Cat. 2	73.8°F – 85.7°F	0.89	(0.37, 2.18)	0.81
	Temp. Cat. 3	86.3°F – 95.7°F	2.27	(0.81, 6.37)	0.12
	Precipitation		0.97	(0.83, 1.12)	0.66
Illinois	Temp. Cat. 1	25.5°F – 44.9°F	1		
	Temp. Cat. 2	45°F – 72.4°F	1.29	(0.48, 3.49)	0.62
	Temp. Cat. 3	72.5°F – 95°F	2.63	(0.99, 6.98)	0.053
	Precipitation		0.82	(0.65, 1.02)	0.073
Maryland	Temp. Cat. 1	35°F – 49.9°F	1		
	Temp. Cat. 2	50°F – 73.9°F	2.17	(0.68, 6.89)	0.19
	Temp. Cat. 3	74°F – 91°F	4.01	(1.24, 12.94)	0.020*
	Precipitation		1.11	(0.86, 1.42)	0.43
Michigan	Temp. Cat. 1	18.6°F – 36.5°F	1		
	Temp. Cat. 2	36.6°F – 65.6°F	1.50	(0.37, 6.00)	0.57
	Temp. Cat. 3	65.7°F – 86°F	1.19	(0.30, 4.74)	0.81
	Precipitation		0.84	(0.53, 1.33)	0.45
Minnesota	Temp. Cat. 1	11.4°F – 30.8°F	1		
	Temp. Cat. 2	30.9°F – 63.4°F	1.76	(0.64, 4.82)	0.27
	Temp. Cat. 3	63.5°F – 86°F	4.91	(1.83, 13.17)	0.0016*
	Precipitation		1.08	(0.86, 1.37)	0.51
New York	Temp. Cat. 1	20°F – 38.4°F	1		
	Temp. Cat. 2	38.5°F – 65°F	5.78	(2.16, 15.46)	<0.001*
	Temp. Cat. 3	65.1°F – 84°F	7.85	(2.80, 22.05)	<0.001*
	Precipitation		1.08	(0.81, 1.43)	0.60
Ohio	Temp. Cat. 1	27°F – 44.4°F	1		
	Temp. Cat. 2	44.5°F – 70.9°F	0.90	(0.33, 2.45)	0.83
	Temp. Cat. 3	71°F – 89.4°F	1.64	(0.59, 4.56)	0.34
	Precipitation		0.90	(0.68, 1.19)	0.45
Washington	Temp. Cat. 1	30.5°F – 41.9°F	1		
	Temp. Cat. 2	42°F – 64.4°F	1.77	(0.56, 5.59)	0.33
	Temp. Cat. 3	64.5°F – 83.9°F	7.43	(1.96, 28.21)	0.0032*
	Precipitation		1.05	(0.86, 1.27)	0.65
Summary	Temp. Cat. 1	11.4°F – 42.5°F	1		
	Temp. Cat. 2	42.6°F – 71.3°F	1.41	(0.91, 2.05)	0.073
	Temp. Cat. 3	71.4°F – 95.7°F	2.83	(1.95, 4.11)	<0.001*
	Precipitation		0.95	(0.89, 1.02)	0.19

Table 3. Results from Negative Binomial Models with a One-Month Lag. Results from the negative binomial regressions analyzing the impact of the primary predictor of temperature on case numbers, while accounting for precipitation, population, and long-term trends in reporting at the state level. The one-month time lag results in analyzing the impact of the temperature from the prior month on the current month's case numbers. Resultant Incidence Rate Ratios (IRR), Confidence Intervals (CI), and p-values are reported.

For models with no time lag, Illinois, Maryland, Minnesota, New York, Ohio, and Washington had significant results, with no significant results in Florida and Michigan. In Illinois, there was a 4.64 (95% CI: 1.77, 12.14) greater risk for *Salmonella* cases in the highest temperature category (72.5-95°F) than in lowest temperature category (25.5-44.9°F). In Maryland, there was a 3.26 (95% CI: 1.07, 9.94) times greater risk and an 11.18 (95% CI: 3.64, 34.33) greater risk for *Salmonella* cases in the middle (50-73.9°F) and highest (74- 91°F) temperature categories, respectively, than in the lowest temperature category (35-49.9°F). In Minnesota there was a 2.73 (95% CI: 1.01, 7.41) times greater risk and an 8.18 (95% CI: 3.07, 21.79) times greater risk for *Salmonella* cases in the middle (30.9-63.4°F) and highest (63.5-86°F) temperature categories, respectively, than in the lowest temperature category (11-30.8°F). In New York, there was a 7.61 (95% CI: 2.91, 19.90) times greater risk for *Salmonella* cases in the highest temperature category (65.1-84°F) than in the lowest temperature category (20-38.4°F). In Ohio, there was a 4.60 (95% CI: 1.64, 12.88) times greater risk for *Salmonella* cases in the highest temperature category (71-89.4°F) than in the lowest temperature category (27-44.4°F). In Washington there was a 10.75 (95% CI: 2.28, 50.77) times greater relative risk for *Salmonella* cases in the highest temperature category (64.5-83.9°F) than in the lowest temperature category (30.5-41.9°F).

For models with a one-month time lag, there were significant results in the states of Maryland, Minnesota, New York, and Washington, with no significant results in Florida, Illinois, Michigan, and Ohio. In Maryland, there was a 4.01 (95%CI: 1.24, 12.94) greater risk for *Salmonella* cases in the highest temperature category (74- 91°F) than in the lowest temperature category (35-49.9°F). In Minnesota, there was a 4.91 (95% CI: 1.83, 13.17) greater risk for *Salmonella* cases in the highest temperature category (63.5-86°F) than in the lowest temperature

category (11-30.8°F). In New York, there was a 5.78 (95% CI: 2.16, 15.46) greater risk and a 7.85 (95% CI: 2.80, 22.05) greater risk for *Salmonella* cases in the middle (38.5-65°F) and highest (65.1-84°F) temperature categories, respectively, than in the lowest temperature category (20-38.4°F). In Washington, there was a 7.43 (95% CI: 1.96, 28.21) greater risk for *Salmonella* cases in the highest temperature category (64.5-83.9°F) than in the lowest temperature category (30.5-41.9°F). The significance associated with lag time could be interpreted to mean that high temperatures in one month influences the occurrence of *Salmonella* cases in the following month. The temperatures between adjacent months, however, are highly correlated, thus this may be an inaccurate interpretation. To account for this, models were run that included both the zero-month lag and one-month lag variables and assessed for significance. For all states, except for New York, significance only remained for the zero-lag variables. Additionally, the IRRs are generally greater for zero-month lag models, suggesting that the zero-month lag models are a better predictor. Kendrovski and Gjorgev have found significance associated with a one-month lag and the potential for sustained higher temperatures to lead to elevated case numbers in Macedonia, highlighting the potential influence of a one-month lag and the need for further research in the United States (2012).

Both the no time lag and one-month time lag models had similar states with significant results, with Ohio and Illinois being the only states different between the two as there were no significant results when accounting for a time lag. Additionally, when looking at both sets of models, the highest incidence rate ratios were observed for the highest temperature category compared with middle category, showing that a dose response relationship exists with the most impact at the highest temperatures. The 95% confidence intervals for these categories, however, are fairly wide, particularly for Washington. Wide confidence intervals are indicative of small

sample sizes, thus it is not surprising that Washington, as seen in Table 1, had the lowest number of *Salmonella* cases.

Florida and Michigan yielded models with no significant results. The lack of significant results for Florida may be explained by the relatively small amount of temperature variation throughout the year, with a low in the maximum temperature of 63.1°F and a high of 95.7°F, only a 32.6°F variation. This may not have been enough variation in temperature to make a significant difference in the resultant number of *Salmonella* cases, which in itself affirms the role that elevated temperatures may play in disease outcomes. Looking at Michigan, Figure 2 illustrates how cases in the state followed a multimodal, rather than a normal distribution. In this analysis, the multimodal case distribution was unique to Michigan and may have influenced the relationship between temperature and *Salmonella* cases. Finally, both Florida and Michigan may have appreciable north-south temperature gradients, such that analyzing their data on the state level resulted in substantial exposure misclassification and therefore bias toward the null.

In creating a single model adjusting for state, a similar result was found, with the middle and highest temperature categories having 1.52 (95% CI: 1.05, 2.21; $p= 0.03$) and 3.46 (95% CI: 2.40, 5.01; $p <0.001$) times greater risk of illness due to *Salmonella*, than in the lowest temperature category. The elevated IRR's indicate the higher risk of illness when the temperature is warmer, especially when the temperature is in the highest range as the greatest risk is associated with the third temperature category. When accounting for a one-month time lag, the middle and highest temperature categories had 1.41 (95% CI: 0.91, 2.05; $p= 0.073$) and 2.83 (95% CI: 1.95, 4.11; $p <0.001$) times greater risk of illness due to *Salmonella*, than in the lowest temperature category. In this instance, however, there was a lack of significance associated with the IRR comparing the lowest and middle categories, indicating that overall, the one-month lag is

most significant when dealing with the highest temperatures, and suggesting a stronger zero-month lag effect than a one-month lag effect.

Limitations:

Inherent to the topic of FBD, this analysis is limited by the large amounts of underreporting that is expected to occur as the majority of individuals afflicted by FBD may not experience symptoms that they believe warrant medical attention or the need to alert health officials. Additionally, stool samples are not collected for most people seeking medical attention for a gastrointestinal infection, another cause of underreporting. One study suggests only collecting samples when symptoms persist for longer than one day, the individual is immunocompromised, if there is bloody stool, fever, symptoms of sepsis, or if they work in a role that may lead to infecting others (such as food services) to optimize cost-effectiveness (Hatchette & Farina, 2011). Research in Canada has approximated that for every reported case of gastrointestinal illness there are hundreds of unreported cases, with studies reporting a median of 285 to 347 unreported cases for every reported case (MacDougall et al., 2008; Majowicz et al., 2005). While a limitation, this analysis assumed that underreporting trends would be consistent throughout the period of interest. The lack of *Salmonella* case information at lower administration levels, such as counties or cities, limited the analysis to solely the state level. In order to account for the temperature variation within states, states with high case numbers and large variation in temperature, such as California, were excluded. Even though this was implemented, the inability to analyze data beyond the state level proved a limitation within the selected states. Similarly, the restriction of cases to the month of occurrence limited this analysis, as data at the daily level would have supported a more detailed analysis.

Conclusions:

This study illustrates an association between seasonality, particularly high temperatures, and cases of *Salmonella* connected with outbreaks in the states of Illinois, Maryland, Minnesota, New York, Ohio, and Washington. While there may be influence from a one-month lag, the zero-month lag models were a better fit for the data. Further research can explore the impact of time lags with larger datasets, as well as the causality of this relationship.

Underreporting may have impacted results, limiting the number of cases included in the analysis compared to the actual number of cases that occur leading to a bias if there were a trend in underreporting that was not linear. Improving surveillance and monitoring programs may be useful in improving data quality, as well as aiding individuals impacted by FBD. Given the significant findings, if underreporting is reduced in the future, it may be possible to glean more precise results. More research can also be done into the apparent downward trend in case numbers, whether this is general underreporting, potentially an increase in less severe cases but a decline in severe cases leading to a lack of reporting, or a true decline.

Climate change is already having a pronounced and deleterious impact around the world, from an increase in extreme weather events to shifting ranges of infectious disease vectors. Given the results of this analysis, there exists the likelihood for rising case numbers of FBD, potentially beyond *Salmonella*, as temperature increases and climate change advances. Additionally, FBD cases can spread from the occurrence of extreme weather events leading to the flooding of manure systems, thereby potentially introducing bacteria to food stuffs. The confluence of these variables may exacerbate and compound the number of *Salmonella* outbreaks, with the introduction of greater numbers of bacteria along with elevated temperatures supporting growth. Therefore, it is imperative to implement appropriate interventions to address *Salmonella* outbreaks as temperature increases continue. In order to properly address this

concern, additional research is needed to further analyze the relationship between seasonality and *Salmonella* outbreaks in the United States, rather than just eight states. Improving surveillance systems and expanding open-source data to beyond the state and monthly levels may facilitate this process. By better understanding the relationship between seasonality, ambient temperature, and cases of FBD caused by *Salmonella* and other agents, health officials will be better able to prepare and support vulnerable populations in the face of rising outbreaks.

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