Effect of climatic elements on *Campylobacter* colonization in broiler flocks reared in southern Japan from 2008 to 2012

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ABSTRACT To demonstrate the effect of climatic elements on *Campylobacter* colonization in broiler chickens reared in Japan, the correlation between *Campylobacter* isolated from chickens (191 of 236 flocks, 80.9%) between 2008 and 2012 and climatic elements was analyzed by logistic regression. We divided the rearing process into 13 terms of 5 d each (total: 65 d). Terms were numbered backwards, wherein a 0-term lag was considered as the sampling day plus 4 d before sampling; 1-term lag was the 5-d term before the 0-term lag, and so on, until the 12-term lag. We obtained climatic data tracing back from the 0-term to the 12-term lags. For evaluation in each season, we divided chickens reared during periods of rising temperature (spring, summer) and decreasing temperature (autumn, winter). Air temperature showed a positive correlation with *Campylobacter* colonization from the 0- to 12-term lags in chickens reared during the period of rising temperature (odds ratio [OR], 1.069 to 1.104), and from the 0- to 4- and 6-term lags (OR, 1.079 to 1.105) in chickens reared during the period of decreasing temperature. The strong positive effect of air temperature on *Campylobacter* colonization, particularly during the period of rising temperature, may be associated with the effect on the *Campylobacter* environmental sources and/or vectors. A positive correlation was observed between *Campylobacter* colonization and humidity when chicken houses were empty and new chicks were introduced (from the 9- to 12-term lags) during the period of decreasing temperature (OR, 1.076 to 1.141). Thus, high humidity would be an important factor causing carry-over of *Campylobacter* infection during the period of decreasing temperature. We also found that solar radiation increased *Campylobacter* colonization during the period of decreasing temperature, from the 2- to 8-term lags, except for the 4- and 5-term lags, in Japan. The results of this study demonstrate the effects of air temperature, humidity, and solar radiation on *Campylobacter* colonization in broiler chickens, and are potentially important for developing strategies to reduce the risk of *Campylobacter* contamination in broiler chickens.

Key words: broiler chicken, *Campylobacter*, climate, temperature, humidity

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Highlights

- High temperature was suggested to increase *Campylobacter* colonization, especially in warmer seasons.
- Higher humidity would increase carry-over of *Campylobacter* infection in autumn.

INTRODUCTION

Campylobacter spp. are the major causative agents of bacterial diarrheal infection in humans. The high number of campylobacteriosis cases in Japan makes this pathogen one of the 2 major causes of foodborne illness every year since 2000 (Infectious Agents Surveillance Report, 2010). Most cases of campylobacteriosis are sporadic, and the consumption of poultry products has been identified as a risk factor (Vellinga and Loock, 2002; Domingues et al., 2012). One approach for preventing campylobacteriosis is to reduce the risk of contamination of chicken carcasses with enterobacteria, including *Campylobacter* and other pathogens, during processing. Another strategy is to rear broiler chickens in such a way as to prevent colonization by *Campylobacter*. Therefore, it is crucial to identify both the risk factors and the protective factors involved in colonization of broiler chickens by *Campylobacter*.

A previous study reported that broiler chicken production in western Japan faced a higher risk (odds ratio [**OR**], 2.68) of *Campylobacter* colonization than that

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in eastern Japan (Sasaki et al., 2011). Approximately 300 million broiler chickens were reared in the Kyushu region (western Japan) in 2013, constituting almost 50% of all broiler chickens reared in Japan that year (E-Stat, 2013). Variations among regions, with respect to the percentage of *Campylobacter*-contaminated retail broiler meat products, have also been reported in the United States (Williams and Oyarzabal, 2012).

In our previous study, the extent of contamination of chicken products (meat, skin, and liver) by *Campylobacter* was found to be influenced by air temperature, humidity, and sunshine duration during chicken rearing, suggesting that the environmental conditions observed in broiler farms are correlated with the rate of *Campylobacter* colonization in broiler flocks (Ishihara et al., 2012). Other studies have corroborated these findings, highlighting air temperature, humidity, and sunlight as the main factors affecting *Campylobacter* colonization in broiler flocks (Patrick et al., 2004; Jore et al., 2010).

In the present study, we decided to investigate the effect of climatic elements on *Campylobacter* colonization in chickens. For the purpose, we isolated *Campylobacter* from chicken cecal samples at a slaughterhouse.

MATERIAL AND METHODS

Sample Collection and Farm Information

From January 2008 to December 2012, samples of cecal contents were collected from broiler chickens (∼50 d old) at a slaughterhouse in Kagoshima prefecture. Kagoshima is a leading producer of broiler chickens. Sample collection was performed systematically at 14-d intervals throughout the study period. We collected 3,776 cecal samples from 236 broiler flocks that were reared on 121 farms over 5 years. At each sampling day, we selected 2 flocks with the largest number of chickens (∼10,000 birds). These 2 flocks were reared on different farms. From each flock, we collected 16 cecal samples. Ninety-one farms were sampled once or twice, 25 farms were sampled 3 or 4 times, and in 5 farms, samples were collected 5 to 7 times (average 1.9 flocks per farm during the 5 yr of investigation).

All broiler flocks were reared in open-sided chicken houses under standard hygiene control procedures. All farms included in the study were located within the Kagoshima prefecture. Although the management of rearing broiler chickens was under the direction of each farm, consistency in practices was confirmed. The size of chicken houses, ventilation system, rearing of chicks, feed, vaccination programs, and hygiene control procedures were generally consistent between farms.

Campylobacter *Isolation and Identification*

Cecal samples were inoculated into Preston enrichment broth (Oxoid Ltd., Hampshire, UK) supplemented with 5% lysed horse blood, and were incubated in a micro-aerobic atmosphere at 42°C for 48 hours. Enrichment cultures were streaked onto Butzler agar plates (Oxoid Ltd.). Suspected *Campylobacter* colonies were picked and sub-cultured on blood agar medium. These isolates were tested using Gram staining and catalase and oxidase production tests. One isolate from each cecal sample was identified by PCR (Chuma et al., 1997), using the strains *Campylobacter jejuni* ATCC 33560^{T} and *Campylobacter coli* ATCC 33559^{T} as positive controls.

Climate Data

Climatic data at Kagoshima city (N31° 33.3′; E130° 32.8′), which is the prefectural capital, were obtained from the Japan Meteorological Agency (http://www.data.jma.go.jp/obd/stats/etrn/index.php), who has published climatic data in terms of daily values and as normal values for 10-d periods, from 1981 to 2010. Climatic data included air temperature (°C), relative humidity (%), solar radiation (MJ/m^2), and rainfall (mm).

A 5-d period was defined as one term. The first term includes the day when each sample was collected; however, as we were exploring the potential factors of climate influencing the sample infections, we required information on periods before our sampling. Thus, the terms were numbered backwards beginning from the sampling term, expressed as -term lag. In other words, the 0-term lag is constituted by the sampling day plus 4 d before sampling; 1-term lag represents the term (5-d period) before the 0-term lag, and so on until the 12-term lag, which corresponds to 65 d before sampling.

We analyzed the effect of climatic elements on *Campylobacter* colonization, considering the 65 d before cecal sampling. We calculated mean values of air temperature, humidity, and solar radiation, and the total amount of rainfall for each of the 13 terms from the daily climatic data published by the Japan Meteorological Agency. These climatic data obtained from periods before sampling (i.e., data for the lag terms) were used to analyze any potential lagged effects. In Japan, the average age of a broiler flock at shipment time is 54 d (range 46 to 73 d) (Sasaki et al., 2011); therefore, these 13 terms (from 0- to 12-term lags; 65 d) corresponded to the feeding period (0- to 10-term lags), and the interval between the 2 production cycles, when chicken houses were empty (11- and 12-term lags).

To evaluate climatic effect in each season, chickens reared during periods of rising and decreasing temperature were divided for analysis according to our previous report (Ishihara et al., 2012). Periods of rising and decreasing temperature were determined by differences (positive or negative) in air temperature between the 0- and 12-term lags.

Statistical Analysis

If *C. jejuni* or *C. coli* was isolated from at least one chicken from a flock, the flock was determined as *Campylobacter*-positive. For univariate analysis, the correlation between *Campylobacter* colonization from each flock (*Campylobacter* flock status; positive or negative) and climatic data for the 0- to 12-term lags was analyzed by logistic regression analysis, using the forced entry method.

To select the variable for multivariate analyses, the correlation between 2 climatic sets of data at 0-term lag was confirmed by all possible regression. An absolute value of Pearson's correlation coefficient (**r**) of less than 0.3 was considered an appropriate variable for multivariate analyses. Moreover, variance inflation factors were calculated by linear regression to confirm the multicollinearity among climatic data. Variance inflation factors of more than 10 were excluded as a variable for multivariate analyses. A stepwise backward logistic regression analysis (likelihood ratio) was used for the multivariable analysis. Variables with a *P*-value of 0.05 or less in the final model were considered significant. Statistical analyses were performed using the SPSS statistics 23.0 software (IBM Japan Co., Tokyo, Japan).

RESULTS

Climatic Characteristics of Sampling Place

Climatic data of normal values for 10-d periods at Kagoshima city are shown in Figure 1. The climate in Kagoshima is influenced by seasonal winds; therefore, the total amount of rainfall and humidity increases from June to July. In winter, rainfall and humidity decrease. As Kagoshima prefecture is surrounded by the sea, the humidity does not decrease severely.

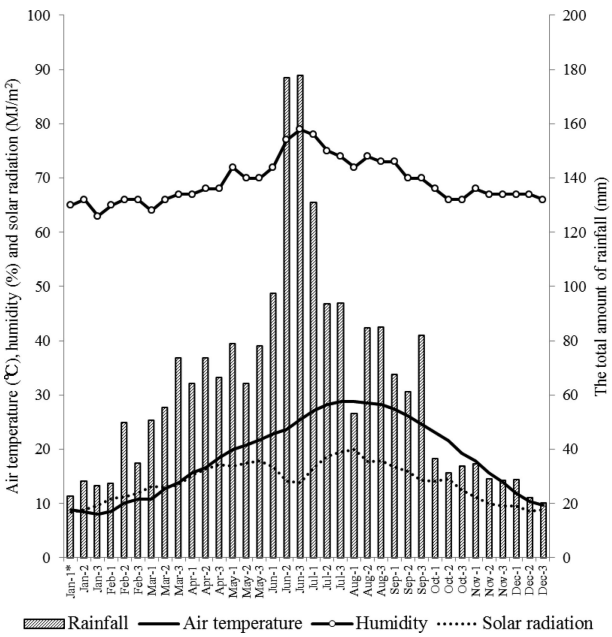

Figure 1. The normal values of climatic data for Kagoshima city, expressed in means (%, °C, MJ/m^2) and total amount (mm), per 10-d periods (1980 to 2010). The climatic data were obtained from the Japan Meteorological Agency (http://www.data.jma.go.jp/obd/stats/etrn/index.php). *-1, 1st to 10th; -2, 11th to 20th; -3, 21st to the last day of each month.

Isolation of Campylobacter spp.

In total, 191 of 236 flocks (80.9%) and 1,987 of 3,776 cecal samples (52.6%) were positive for *Campylobacter* spp. *C. jejuni* and *C. coli* isolates were obtained from 1,761 cecal samples (46.6%; 180 flocks [76.3%]) and 226 cecal samples (6.0%; 40 flocks [16.9%]), respectively. Both *C. jejuni* and *C. coli* were isolated from 29 flocks (12.3%). Eleven flocks (4.7%) were positive for only *C. coli.*

Univariate Analyses

Table 1 shows significantly correlated climatic elements at each term lag. Results also indicate seasonality

Table 1. Univariable analyses of the effect of climatic elements on *Campylobacter* isolation.

Lag term	Variable	Coef.	SE	*P*	OR	(95% CI)
	During the period of rising temperature					
0	Temperature	0.079	0.035	0.024	1.083	(1.011 to 1.160)
1	Temperature	0.080	0.033	0.015	1.083	(1.015 to 1.155)
	Humidity	0.052	0.026	0.043	1.054	(1.002 to 1.108)
2	Temperature	0.099	0.035	0.005	1.104	(1.031 to 1.183)
	Humidity	0.060	0.023	0.010	1.062	(1.015 to 1.112)
3	Temperature	0.093	0.032	0.004	1.098	(1.030 to 1.170)
	Humidity	0.054	0.025	0.031	1.055	(1.005 to 1.108)
	Rainfall	0.024	0.009	0.007	1.024	(1.007 to 1.042)
4	Temperature	0.099	0.033	0.003	1.104	(1.035 to 1.177)
	Humidity	0.084	0.028	0.002	1.087	(1.030 to 1.148)
5	Temperature	0.080	0.033	0.015	1.083	(1.015 to 1.155)
6	Temperature	0.078	0.031	0.013	1.081	(1.017 to 1.150)
7	Temperature	0.088	0.034	0.009	1.092	(1.022 to 1.166)
	Humidity	0.074	0.030	0.014	1.077	(1.015 to 1.143)
8	Temperature	0.067	0.032	0.037	1.069	(1.004 to 1.138)
9	Temperature	0.066	0.032	0.040	1.069	(1.003 to 1.138)
10	Temperature	0.069	0.033	0.040	1.071	(1.003 to 1.144)
	Rainfall	0.020	0.010	0.044	1.020	(1.001 to 1.040)
11	Temperature	0.085	0.035	0.016	1.089	(1.016 to 1.167)
12	Temperature	0.085	0.036	0.019	1.089	(1.014 to 1.169)
	Solar radiation	0.096	0.047	0.043	1.100	(1.003 to 1.207)
	During the period of decreasing temperature					
0	Temperature	0.090	0.044	0.041	1.094	(1.004 to 1.193)
1	Temperature	0.099	0.041	0.016	1.105	(1.019 to 1.198)
2	Temperature	0.076	0.038	0.046	1.079	(1.002 to 1.162)
	Solar radiation	0.226	0.099	0.022	1.253	(1.032 to 1.521)
3	Temperature	0.087	0.037	0.020	1.091	(1.014 to 1.173)
	Solar radiation	0.259	0.095	0.007	1.295	(1.075 to 1.562)
4	Temperature	0.077	0.036	0.034	1.080	(1.006 to 1.159)
6	Temperature	0.086	0.035	0.014	1.090	(1.018 to 1.167)
	Solar radiation	0.195	0.078	0.012	1.216	(1.044 to 1.416)
	Rainfall	−0.024	0.010	0.020	0.976	(0.956 to 0.996)
7	Solar radiation	0.196	0.074	0.008	1.217	(1.052 to 1.408)
8	Solar radiation	0.127	0.061	0.036	1.136	(1.008 to 1.280)
9	Humidity	0.132	0.047	0.005	1.141	(1.040 to 1.251)
10	Humidity	0.125	0.041	0.002	1.133	(1.045 to 1.228)
11	Humidity	0.111	0.043	0.010	1.118	(1.027 to 1.216)
12	Humidity	0.073	0.036	0.043	1.076	(1.002 to 1.154)

Temperature, mean of air temperature; Humidity, mean of humidity; Solar radiation, mean of solar radiation; Rainfall, total amount of rainfall; Coef., coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

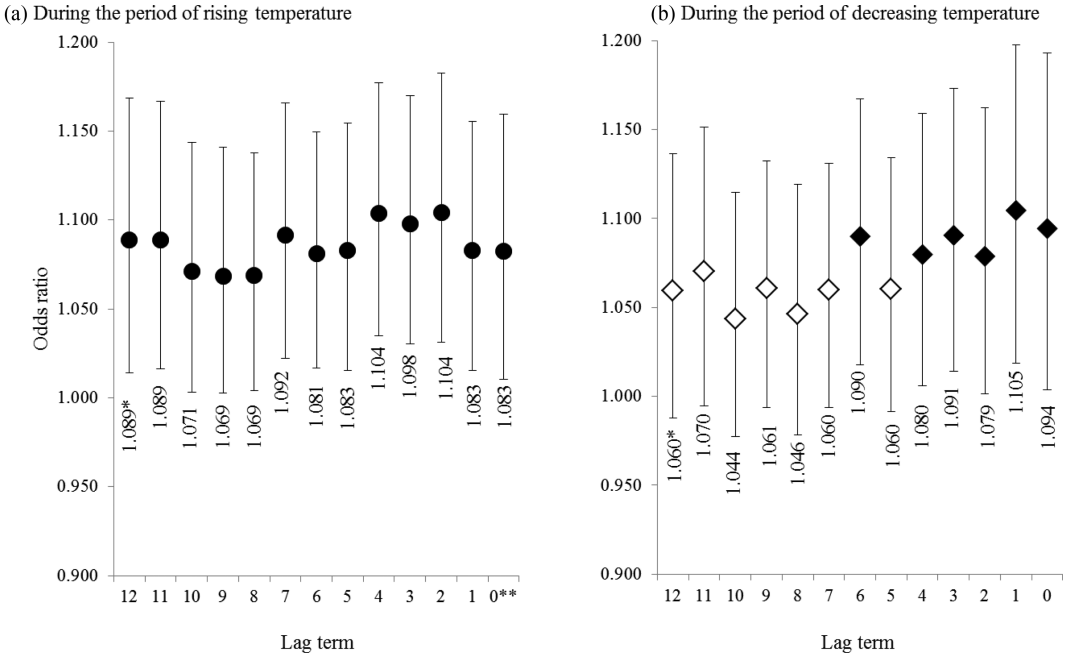

Figure 2. The effects of air temperature (°C) on *Campylobacter* colonization. 95% confidence interval of odds ratio is shown by error bar; *odds ratio; **it represents the period just before sampling; filled circle and filled diamond shape, $P < 0.05$.

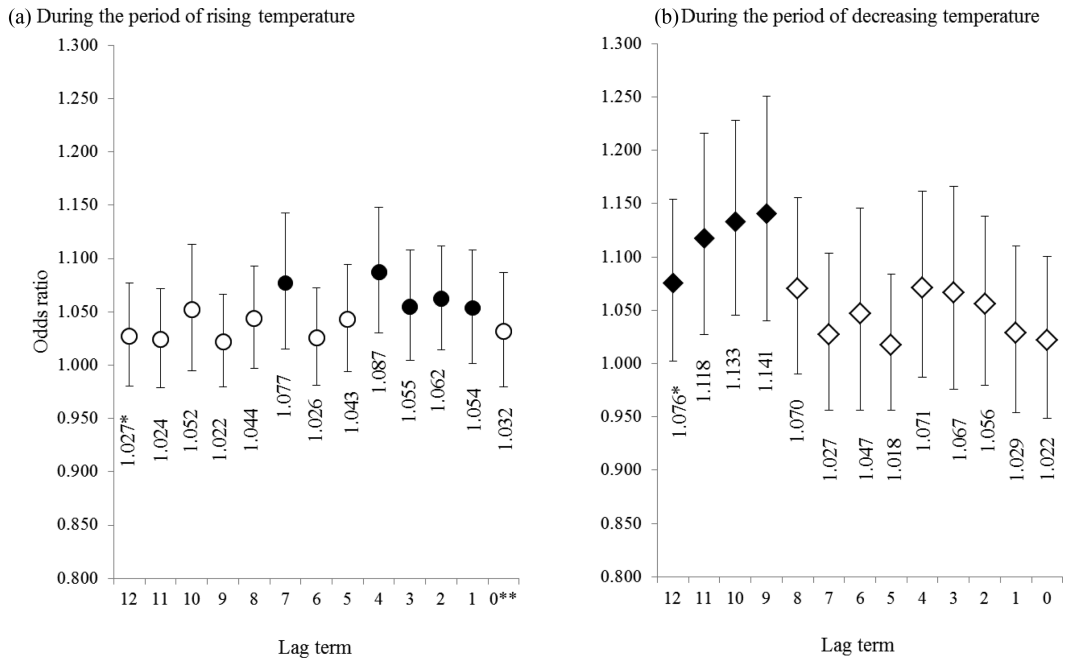

Figure 3. The effects of relative humidity (%) on *Campylobacter* colonization. 95% confidence interval of odds ratio is shown by error bar; *odds ratio; **it represents the period just before sampling; filled circle and filled diamond shape, $P < 0.05$.

(i.e., if reared during periods of rising or decreasing temperatures). Air temperature showed a positive correlation with *Campylobacter* colonization from the 0- to 12-term lags, without observable influence of the periods of rising temperature (OR, 1.069 to 1.104). In chickens reared during the period of decreasing temperature, a positive correlation of air temperature with *Campylobacter* colonization was observed from the 0- to 4- and 6-term lags (OR, 1.079 to 1.105) (Table 1 and Figure 2). A positive correlation was observed between *Campylobacter* colonization and humidity during periods of rising temperature from the 1- to 4-term lags, and in the 7-term lag (OR, 1.054 to 1.087), and from the 9- to 12-term lags for decreasing temperature periods (OR, 1.076 to 1.141) (Table 1 and Figure 3). During the period of decreasing temperature, solar radiation showed a positive correlation with *Campylobacter* colonization from the 2- to 8-term lags (OR, 1.136 to

1.295), except in the 4- and 5-term lags. However, solar radiation showed a positive correlation only at the 12-term lag for rising temperature conditions (OR, 1.100) (Table 1). During periods of rising temperature, the correlation between total amount of rainfall and *Campylobacter* colonization at the 3- and 10-term lags was positive (OR, 1.024 and 1.020, respectively); however, a significant negative correlation with the amount of rainfall (OR, 0.976) was observed only at the 6-term lag during periods of decreasing temperature (Table 1). In summary, air temperature and humidity showed a positive correlation with *Campylobacter* colonization at the same terms (1- to 4- term lags, and 7-term lag), during periods of rising temperature, but not during periods of decreasing temperature. Air temperature and solar radiation showed a positive correlation with *Campylobacter* colonization at the 2-, 3-, and 6-term lags, during periods of decreasing temperature.

Multivariate Analyses

To select the variables for multivariate analyses, the potential correlation between each pair of climatic data variables was assessed. Mean air temperature and total amount of rainfall were the only paired variables that showed independency (i.e., were not correlated), regardless of season ($r < 0.3$). Even without multicollinearity, we carried out the multivariate analyses. In the multivariate analysis, we included the variables that presented P-values < 0.1 in the univariate analysis. However, the adjusted ORs obtained in the multivariate analysis were equivalent to the ORs obtained in the univariate analysis. Therefore, the results of the multivariate analysis are not shown.

DISCUSSION

Based on univariable logistic regression analyses, air temperature, humidity, and solar radiation were found to have a significant effect on *Campylobacter* colonization in broiler chickens. In particular, air temperature was consistently correlated with *Campylobacter* colonization in chickens reared during the period of rising temperature. In European countries, a strong positive correlation has been reported between mean air temperature and the incidence of *Campylobacter* in broilers (Jore et al., 2010). In the present study, we analyzed the effects of climatic elements on *Campylobacter* colonization in broiler chickens. Strict temperature control must be performed in broiler houses, depending on the age of chickens. Therefore, air temperature outside broiler houses might affect the environmental sources and/or vectors of *Campylobacter.* Broiler chicken houses are more ventilated during warmer seasons, increasing the chances of contact between susceptible chickens and external or environmental sources of the pathogen (Newell and Fearnley, 2003). A previous study reported that proximity to cattle increased the risk of *Campylobacter* colonization in broiler chickens (Ellis-Iversen et al., 2009). Another study revealed that *Campylobacter* increased during spring and autumn in dairy cattle (Stanley et al., 1998); in broilers, *Campylobacter* also tended to increase in spring. Thus, broilers also would increase their infection in spring, because of an increase in the infection source. In addition, flies are known to act as vectors in the transmission of *Campylobacter* (Hald et al., 2007). In this context, Guerin et al. (2008) reported that temperature-related variables, which were very important determinants of flies' activity, might be valuable in predicting the risk of *Campylobacter* colonization of broiler chickens.

In chickens reared during periods of rising temperature, a significant correlation between air temperature and *Campylobacter* colonization was observed for the 13 consecutive terms (65 d). The same correlation was detected in chickens reared during periods of decreasing temperature, but only in 6 terms, not during the 13 terms detected during rising temperature terms. In a previous study, air temperature was suggested to have a lesser effect on *Campylobacter* prevalence among broiler chickens during colder months than during warmer months (Patrick et al., 2004). Moreover, air temperature was thought to be associated with the production of *Campylobacter*-contaminated chickens only during the period of rising temperature, but not during the period of decreasing temperature in Japan (Ishihara et al., 2012). Therefore, *Campylobacter* colonization in broiler chickens was thought to increase along with air temperature during warmer seasons, which correspond to the periods of rising temperature in the present study.

From the 9- to 12-term lags, the effect of air temperature on *Campylobacter* colonization was not observed in broiler chickens reared during the period of decreasing temperature; thus, humidity was identified as a risk factor. Moreover, high humidity increased *Campylobacter* colonization between the 1- and 4-term lags, and in the 7-term lag in broiler chickens reared during the period of rising temperature. Since *Campylobacter* cannot survive in dry conditions, higher humidity would increase its prevalence near broiler houses. Depending on the season, the lag term when humidity was associated with *Campylobacter* colonization differed. Air temperature was relatively high during the period in which there was a significant correlation between humidity and *Campylobacter* colonization, independent of the season.

Previous studies have highlighted the previous presence of a *Campylobacter*-positive flock on the farm as a risk factor (Chowdhury et al., 2012; Sandberg et al., 2015). Our previous study also reported that carry-over of *Campylobacter* infection is thought to occur among chicken flocks on the same farm (Ishihara et al., 2006). In summer (the period of rising temperature in the present study), *Campylobacter* colonized more broiler chicken flocks, owing to the higher air temperature.

Thus, carry-over of *Campylobacter* infection would be a more relevant factor in autumn (period of decreasing temperature). Therefore, high humidity is potentially an important factor causing carry-over of *Campylobacter* infection during decreasing temperature periods, when the chicken houses are empty (corresponding to the 11- and 12-term lags), and rearing of chicks has begun (corresponding to the 10- and 9-term lags). Previous studies have demonstrated that humidity is important for the transmission of *Campylobacter* from spent litter to chickens; in fact, a delay in *Campylobacter* colonization was observed under conditions of low humidity (approximately 30%) (Line, 2006).

We found that high levels of solar radiation increased *Campylobacter* colonization in chickens reared during the period of decreasing temperature, although the effect was observed intermittently. Air temperature is determined mainly by solar radiation; therefore, solar radiation is highly correlated with air temperature, which was a risk factor for the *Campylobacter* colonization of chickens. However, the number of terms in which the effect of solar radiation was observed was smaller than those in which the effect of air temperature was observed. Since sunlight was reported to inactivate *Campylobacter* in surface water (Rodriguez and Araujo, 2012), solar radiation was thought to reduce *Campylobacter* colonization through a decrease in the infection source. However, we did not observe this reducing effect of solar radiation. We could not conduct the multivariate analyses, because we detected a correlation between air temperature and solar radiation. Therefore, we could not confirm an independent effect of solar radiation on *Campylobacter* colonization. In fact, the inhibitory effect of solar radiation on *Campylobacter* might be reversed by high air temperature, which was found to increase *Campylobacter* colonization.

In summary, the results of our study suggest that high air temperature and humidity promote *Campylobacter* colonization in broiler chickens in Japan. In chickens reared during periods of rising temperature, high air temperature increased *Campylobacter* colonization, irrespective of the age of the chickens. In chickens reared during the period of decreasing temperature, air temperature was a significant risk factor for older chickens, which were approaching the time of shipping and processing. The high humidity at the time when chicken houses were empty and chicks were younger also increased *Campylobacter* colonization in chickens reared during periods of decreasing temperature. In fact, humidity was suggested to be an important factor causing the carry-over of *Campylobacter* infection.

In conclusion, the present study, carried out in Kagoshima, which is a major producer of broiler chickens of Japan, demonstrated that the effects of climatic elements on *Campylobacter* colonization in chickens depend on the season and the age of chickens. To develop efficient strategies for preventing *Campylobacter* colonization, further studies are required to identify the source of infection, potential reservoirs, and vectors for transmission of *Campylobacter*, which in turn could be associated with climatic elements.

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