The Journal of Veterinary Medical Science

FULL PAPER

Public Health

Effects of climatic elements on *Salmonella* contamination in broiler chicken meat in Japan

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ABSTRACT. The effects of climatic elements on *Salmonella* contamination of chicken meat were investigated. Logistic regression analysis was performed to evaluate the association between *Salmonella* isolation, for 240 chicken samples purchased from March 2015 to February 2017, and climatic elements, over 65 days of chicken rearing. *Salmonella* was isolated from 143 samples (59.6%), and the most dominant serovars identified were Infantis (77/240, 32.1%) and Schwarzengrund (56/240, 23.3%). Previous studies have reported *S.* Schwarzengrund contamination of broiler chickens only in western Japan; however, in the present study, *S.* Schwarzengrund was also isolated from meat produced in eastern Japan—20% (12/60) in the C prefecture to 36.4% (8/22) in the Y prefecture—suggesting that *S.* Schwarzengrund-contaminated areas have expanded towards eastern Japan. Air temperature showed a significant negative association with *S.* Schwarzengrund isolation for chicken meat produced during periods with rising temperature (spring and summer) [odds ratio (OR), 0.894 to 0.935; $P<0.01$]. Moreover, the risk of *S.* Schwarzengrund contamination of chicken meat was higher during spring (OR, 3.951; $P=0.008$) and winter (OR, 4.071; $P=0.006$) than during summer. Effects of climatic elements and differences in contamination risk across seasons were not observed for any *Salmonella* serovars and only *S.* Infantis, which could be attributed to differences in transmission patterns and vehicles among *Salmonella* serovars. These findings are valuable for understanding the dynamics of *S.* Schwarzengrund dissemination in broiler farms.

KEY WORDS: broiler, climate, *Salmonella*, Schwarzengrund, temperature

J. Vet. Med. Sci.
82(5): 646–652, 2020
doi: 10.1292/jvms.19-0677

Received: 18 December 2019
Accepted: 18 March 2020
Advanced Epub:
26 March 2020

Salmonella is the most common causative agent of human foodborne enteritis worldwide [2] including in Japan [8]. Human salmonellosis is often attributed to exposure to contaminated food products, such as meat from broiler chickens and eggs [12].

In Japan, egg grading and packaging (GP) centers wash eggshells according to government guidelines for hygienic practice in GP centers. *Salmonella* was isolated from five out of 2,030 eggshell samples (ten eggs/sample; 0.25%), but not from the contents of the eggs [15]. In contrast, higher rates of *Salmonella* isolation from chicken meat were reported, which ranged from 20.0% (164/821) [6] to 54% (54/100) [1]. Moreover, *Salmonella* was isolated at a rate of 86.1% (248/288) from broiler flocks in Japan [14]. Previously, it was shown that the slaughter of *Salmonella* positive chickens led to contamination of the processing line and the final products [11]. Therefore, contamination of chicken carcasses and colonization of broiler chickens by *Salmonella* should be reduced to prevent the spread of human salmonellosis. Although the distribution and dynamics of *Salmonella* in broiler chickens in farms have previously been reported [21], the details remain unknown and effective precautionary measures to reduce *Salmonella* contamination of broiler chickens remain to be established.

Almost half of all salmonellosis cases (173/360; 48.1%) were reported in summer (June to August) from 2010 to 2018, according to food poisoning statistics of the Japanese Ministry of Health, Labour and Welfare (https://www.mhlw.go.jp/toukei/list/112-1.html). However, seasonal influence on food contamination with *Salmonella* in Japan remains unknown [1, 6].

Both *Campylobacter* and *Salmonella* colonize chickens and contaminate chicken carcass during processing. Both pathogens

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

show high rates of chicken contamination [1, 5, 6]. It is necessary to prevent colonization of both pathogens on broiler farms, but the strategy is not clear. Previous studies have shown seasonal variations in the incidence of *Campylobacter* contamination of chicken products available in the market [5], which could be due to the effect of climatic elements during the chicken-rearing period in Japan [4]. Identification of the effect of climatic elements on *Salmonella* contamination of chicken meat will help understand *Salmonella* dynamics in broiler flocks and serve as the basis for developing precautionary strategies to reduce the incidence of *Salmonella* colonization in broiler chickens. In the present study, the effect of climatic elements on *Salmonella* contamination of chicken meat was investigated by analyzing the association between climatic elements during the chicken-rearing period and *Salmonella* isolation from chicken mince purchased over a period of two years.

MATERIALS AND METHODS

Sample collection

A total of 240 chicken mince samples were purchased from 12 retail stores in Tokyo from March 2015 to February 2017 and analyzed for *Salmonella* contamination. Supermarkets and butcher shops selling chicken mince were chosen. Ten samples were collected once a month or five samples were collected twice a month. In all cases, ten samples a month were tested for a total of 24 months. In general, one sample of chicken meat was purchased from one store on the sample collection day. If two or more chicken meat samples were purchased from the same store on the same day, meat products from different brands or from different production centers were selected. Defrost meat was not included in this study.

Each sample was analyzed for *Salmonella* contamination before its expiration date. If the expiration date was not indicated on the label, the sample was analyzed on or one day after the date of purchase.

Salmonella isolation, identification, and serotyping

Approximately 25 g of minced chicken was added to 225 m*l* of buffered peptone water (BPW, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), pre-warmed at 35°C, in a sterile stomacher bag, mixed using the Pro-media SH-IIM Stomacher (Elmex Co., Ltd., Tokyo, Japan) for 15 sec, and incubated at 35°C for 24 hr for pre-enrichment. Thereafter, 0.1 m*l* of pre-enrichment culture was inoculated into 10 m*l* of Rappaport-Vassiliadis broth (Eiken Chemical Co., Ltd., Tokyo, Japan) and incubated at 42°C for 24 hr. The selective enrichment cultures were streaked on DHL agar (Nissui Pharmaceutical Co., Ltd.) and ES *Salmonella* agar II (Eiken Chemical Co., Ltd.), and the isolation media were incubated at 35°C for 24 hr. Two presumptive *Salmonella* colonies, from each isolation medium, were picked for each sample and sub-cultured on Mueller-Hinton agar (Oxoid Ltd., Hampshire, UK). If presumptive *Salmonella* colonies showed different characteristics [height, diameter, color strength (bright/dark pink), and/or proportion of black colonies], one colony per characteristic was selected. Isolates were analyzed by Gram staining and oxidase tests using a cytochrome oxidase test strip (Nissui Pharmaceutical Co., Ltd.) and subsequently inoculated in LIM medium (Nissui Pharmaceutical Co., Ltd.), TSI agar (Nissui Pharmaceutical Co., Ltd.), and Simmons citrate agar (Nissui Pharmaceutical Co., Ltd.) for biochemical identification. Moreover, the biochemical characteristics of the isolates were confirmed using API 10S (bioMérieux, Japan Ltd., Tokyo, Japan). Isolates identified as *Salmonella* spp. were serotyped using *Salmonella* antisera (Denka Seiken Co., Ltd., Tokyo, Japan), as per the manufacturer's instructions, with minor modifications.

Climate data

To evaluate the effects of climatic elements on *Salmonella* contamination during the feeding period of broiler chickens, climatic data from capitals of prefectures (where the chicken samples were produced) were collected from the Japan Meteorological Agency (http://www.data.jma.go.jp/obd/stats/etrn/index.php). The districts for some samples could not be identified; these samples were labeled as 'domestic'. Given that these samples were purchased from Tokyo (Kanto region), the climatic data of Chiba City, the capital of Chiba prefecture, where most broiler chickens were reared in the Kanto region (https://www.maff.go.jp/j/tokei/kouhyou/tikusan/index.html), was used. Climatic elements analyzed in the present study included air temperature (°C), relative humidity (%), sunshine duration (hr), and rainfall (mm).

The effects of climatic elements on *Salmonella* contamination of chicken meat, during 65 days, counting backwards from the processing date, were evaluated. Sixty-five days were divided into 13 five-day terms. The first term (0-term lag) included the processing date mentioned on the label plus four days before the processing date. Purchase dates were used for 50 samples, where the processing date was not shown on the label. The terms were numbered backwards, beginning from the 0-term lag; they included the processing date and were expressed as -term lag. In other words, 1-term lag represented the term before the 0-term lag. Values from the 0-term lag to the 12-term lag, which corresponds to 65 days before the processing date, were analyzed.

The mean values of air temperature, humidity, sunshine duration, and rainfall for each of the 13 terms were calculated. Climate data obtained from the 13 terms before the processing date (i.e., data for the lag terms) were used to determine potential effects of lag. In Japan, the average age of a broiler flock, at the time of shipment, is 54 days (range, 46–73 days) [16]. Approximately one week is required for transporting raw chicken from the slaughterhouse to the retail shop. Therefore, the 0-term lag corresponds to the period of transport from the farm to the slaughterhouse, chicken-processing plants, and retail store. The 55-day period, ranging from the 1-term lag to the 11-term lag and the 12-term lag corresponded to the feeding period of chickens and the interval between two production cycles (where chicken houses were empty), respectively.

To evaluate the effects of climatic elements, the chickens were divided into two groups, namely, those reared during periods of rising temperature (spring and summer) and those reared during periods of decreasing temperature (autumn and winter), according

Table 1. Percentage isolation of *Salmonella* serovars for each production region

Production region[a)]		No. of samples	Number of positive samples (%)							
Area	Prefecture		*S.* Infantis	*S.* Schwarzengrund	*S.* Typhimurium	*S.* Manhattan	*S.* Agona	*S.* Yovokome	*S.* Kedougou	O:-, H:r,1,5
Eastern Japan										
	I	72	21 (29.2%)	21 (29.2%)	1 (1.4%)	0	0	1 (1.4%)	0	0
	C	60	20 (33.3%)	12 (20%)	6 (10%)	0	0	0	1 (1.7%)	1 (1.7%)
	Y	22	9 (40.9%)	8 (36.4%)	2 (9.1%)	0	2 (9.1%)	0	0	0
	H	1	1 (100%)	0	0	0	0	0	0	0
	A	1	1 (100%)	0	0	0	0	0	0	0
Western Japan										
	M	12	2 (16.7%)	3 (25%)	0	0	0	0	0	0
	S	2	0	1 (50%)	0	0	0	0	0	0
Domestic[b)]		70	23 (32.9%)	11 (15.7%)	1 (1.4%)	7 (10%)	1 (1.4%)	1 (1.4%)	0	0
Total		240	77 (32.1%)	56 (23.3%)	10 (4.2%)	7 (2.9%)	3 (1.3%)	2 (0.8%)	1 (0.4%)	1 (0.4%)

a) The production regions were identified from the product's retail label. b) The disstrict was unknown.

to our previous study on *Campylobacter* contamination of chicken products [4] and *Campylobacter* colonization of broiler chicken flocks [3]. Periods of rising and decreasing temperatures were determined based on differences (positive or negative) in air temperature between the 0- and 10-term lags.

Statistical analysis

A sample was considered *Salmonella*-positive if *Salmonella* was detected in a mince sample, using at least one of the two isolation media. For univariate analysis, the effect of climate on *Salmonella* isolation (positive or negative) during the 0- to 12-term lags was analyzed by logistic regression using the forced entry method. *Salmonella* isolation was used as the dependent variable. Each climate data set for the 0- to 12-term lags was considered as an independent variable.

For multivariate analysis, independent variables with P values <0.1 in the univariate analysis were selected. The correlation between the two climate data sets selected for each term lag was confirmed by all possible regressions. A variable with absolute value of Pearson's correlation coefficient (r) of less than 0.3 was considered an appropriate variable for multivariate analysis. Moreover, variance inflation factors were calculated by linear regression to confirm the multicollinearity among three climatic data sets. Variance inflation factors with value of more than 10 were excluded as variables for multivariate analysis. 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.

Logistic regression analyses were performed using the SPSS statistics 25 software (IBM Japan Co., Tokyo, Japan). The isolation percentages were compared among production sites, using the chi-square test. When at least one expected frequency was less than five, Fisher's exact test was used for comparison between two groups. Statistical analyses were performed using R version 3.1.2.

RESULTS

Salmonella isolation and serotyping

Salmonella was isolated, using two isolation media, from 88 samples (36.7%). Additionally, 53 samples (22.1%) and two samples (0.8%) tested positive for *Salmonella* using ES *Salmonella* agar II and DHL agar alone, respectively. Thus, a total of 143 out of 240 chicken meat samples tested positive for *Salmonella* (59.6%).

For each sample, one isolate obtained using ES *Salmonella* agar II was selected for serotyping. For the two samples from which *Salmonella* was isolated using only DHL agar but not ES *Salmonella* agar II, the corresponding isolate, obtained using DHL agar, was also selected. Presumptive *Salmonella* colonies on the isolation medium showed different characteristics for the 27 samples; therefore, one colony per characteristic was selected for serotyping.

Salmonella isolates obtained from 143 samples were classified into seven serovars (Table 1). Isolates of different serovars from each sample were confirmed for 14 out of the 27 samples for which two isolates showed different characteristics on the isolation agar medium. The O serotype of one isolate could not be determined (Table 1). Infantis was the most prevalent serovar and was detected in 77 out of 240 samples (32.1%). The rate of *S.* Schwarzengrund isolation was also high (56/240, 23.3%). One hundred and nine chicken samples were labeled with seven brand names, and the remaining 131 samples did not have a brand name. Given that the number of tested chicken samples produced in prefectures I, C, Y, and M was greater than 10, the rates of *S.* Infantis and *S.* Schwarzengrund isolation were compared. However, four prefectures showed no significant difference in the rate of isolation for both serovars (*S.* Infantis, P=0.527; *S.* Schwarzengrund, P=0.423) by Fisher's exact test.

Effect of climatic elements on Salmonella contamination

Table 2 shows the climatic elements at each term lag that showed significant associations with *Salmonella* isolation (all

Table 2. Univariable analyses of the effects of climatic elements on *Salmonella* isolation from chicken meat

Period	Isolation	Lag term[a)]	Variable	OR	(95% CI)	*P*
Rising temperature						
	Salmonella	3	Rainfall	0.883	(0.8075–0.9662)	0.007
		3	Sunshine duration	1.221	(1.0434–1.4289)	0.013
		6	Humidity	0.964	(0.9318–0.9964)	0.030
		6	Sunshine duration	1.201	(1.0154–1.4199)	0.032
	S. Infantis	9	Sunshine duration	1.166	(1.0039–1.3550)	0.044
	S. Schwarzengrund	0	Temperature	0.930	(0.8823–0.9793)	0.006
		0	Humidity	0.941	(0.8937–0.9902)	0.019
		1	Temperature	0.924	(0.8747–0.9769)	0.005
		2	Temperature	0.935	(0.8899–0.9832)	0.009
		3	Temperature	0.931	(0.8849–0.9793)	0.006
		3	Rainfall	0.833	(0.7125–0.9731)	0.021
		3	Humidity	0.941	(0.9031–0.9810)	0.004
		4	Temperature	0.919	(0.8735–0.9671)	0.001
		5	Temperature	0.916	(0.8660–0.9687)	0.002
		6	Temperature	0.918	(0.8694–0.9698)	0.002
		6	Humidity	0.954	(0.9175–0.9918)	0.017
		7	Temperature	0.921	(0.8705–0.9739)	0.004
		8	Temperature	0.922	(0.8720–0.9750)	0.004
		9	Temperature	0.913	(0.8611–0.9672)	0.002
		10	Temperature	0.897	(0.8437–0.9544)	0.001
		11	Temperature	0.907	(0.8524–0.9654)	0.002
		12	Temperature	0.902	(0.8452–0.9624)	0.002
Decreasing temperature						
	Salmonella	1	Humidity	0.967	(0.9355–0.9996)	0.047
		5	Humidity	0.970	(0.9402–0.9997)	0.048
		6	Humidity	0.954	(0.9122–0.9969)	0.036
		12	Rainfall	1.096	(1.0077–1.1919)	0.032
	S. Schwarzengrund	7	Rainfall	1.156	(1.0262–1.3011)	0.017

Temperature, mean air temperature; humidity, mean relative humidity; sunshine duration, mean sunshine duration; rainfall, mean rainfall; OR, odds ratio; CI, confidence interval. a) We analysed the effect of climatic elements on *Salmonella* contamination, considering the 65 days before the processing day. A 5-day period was defined as one term. The 0-term lag constitutes the processing day and 4 days before processing; 1-term lag represents the term (5-day period) before the 0-term lag; and so on until the 12-term lag, which corresponds to 65 days before the processing day.

serovars) by univariate analysis. Meat samples from chickens reared during periods of rising and decreasing temperatures were evaluated separately. In chickens reared during the period of rising temperatures, only rainfall at the 3-term lag and humidity at the 6-term lag showed a negative association with *Salmonella* isolation. Sunshine duration at the 3- and 6-term lags showed positive associations with *Salmonella* isolation. In chickens reared during periods of decreasing temperature, negative associations were observed between humidity at the 1-, 5-, and 6-term lags and *Salmonella* isolation, while rainfall at the 12-term lag showed a positive association with *Salmonella* isolation. The effect of climatic elements on *Salmonella* isolation was observed intermittently. Therefore, the associations between *S.* Infantis or *S.* Schwarzengrund isolation in each sample and climatic data were additionally analyzed using logistic regression.

Although *S.* Infantis was isolated from most samples (32.1%), only sunshine duration at the 9-term lag during periods of rising temperatures, showed a significant positive association with *S.* Infantis isolation (Table 2, P=0.044). In contrast, air temperature showed a significant negative association with *S.* Schwarzengrund isolation from the 0-term lag to the 12-term lag during periods of rising temperature (Table 2, P<0.01). Moreover, humidity values at the 0-, 3-, and 6-term lags and rainfall at the 3-term lag showed a negative association with *S.* Schwarzengrund isolation; however, significant associations of *S.* Schwarzengrund isolation with humidity and rainfall were intermittently observed. During periods of decreasing temperature, rainfall alone, at the 7-term lag, showed a positive association with *S.* Schwarzengrund isolation.

Among the independent variables of the same term lag with P value less than 0.1 according to univariate analysis, the following two pairs of two variables had an absolute value of Pearson's correlation coefficient (r) that was less than 0.3: temperature and rainfall at the 7- or 12-term lag for *S.* Schwarzengrund isolation during periods of rising temperatures. Since only two variables were selected, confirmation of multicollinearity was not needed. According to the results of stepwise backward logistic regression analysis, temperature was the only variable independently associated with *S.* Schwarzengrund isolation at the 7-term lag (adjusted odds ratio (OR), 0.921: 95% confidence interval (CI), 0.871 to 0.974; P=0.004) and 12-term lag (adjusted OR, 0.902; 95% CI, 0.845 to 0.962; P=0.002). The area under the curve plotted on a receiver operating characteristic curve was 0.686 ± 0.054 (95% CI,

Table 3. The risk involved in *Salmonella* Schwarzengrund isolation from chicken meats during winter, spring, and autum compared to that during summer

Variable	OR	(95% CI)	*P*
Four seasons (vs. Summer)			
Winter	4.071	(1.494–11.095)	0.006
Spring	3.951	(1.440–10.840)	0.008
Autumn	2.489	(0.877–7.067)	0.087

OR, odds ratio; CI, confidence interval.

0.581 to 0.792; P=0.002) and 0.723 ± 0.057 (95% CI, 0.611 to 0.834; P=0.0003) at the 7- and 12-term lags, respectively. Therefore, the data were suggested to fit these final models.

A significant negative association between *S.* Schwarzengrund isolation from meat samples of chickens reared during a period of rising temperatures (spring and summer) and air temperature indicated that low temperatures during the chicken-rearing period increased the likelihood of *S.* Schwarzengrund contamination of chicken meat. Therefore, ORs for four seasons [spring (March to May), summer (June to August), autumn (September to November), and winter (December to February)] were also calculated by logistic regression analyses (Table 3). The ORs of *S.* Schwarzengrund isolation during spring (3.951; 95% CI, 1.440 to 10.840) and winter (4.071; 95% CI, 1.494 to 11.095) were higher than one (P<0.01; reference, summer). Therefore, *S.* Schwarzengrund contamination of chicken meat was more frequently detected during spring and winter than during summer. Although the ORs of *Salmonella* or *S.* Infantis isolation were also calculated, seasonal differences in either was not observed (data not shown).

The production districts for the 70 samples labeled as 'domestic' could not be identified. Since these samples were purchased from the Kanto region, the climatic data for Chiba, where the majority of broiler chickens of the Kanto region were reared, was used. To confirm that the analysis using climatic data of Chiba, instead of those of the actual unidentified production districts, did not lead to erroneous results, logistic regression analysis was performed by excluding the 70 samples labeled as 'domestic'. A significant negative association between *S.* Schwarzengrund isolation and air temperature from 0- to 12-term lags (OR, 0.911 to 0.941; P, 0.006 to 0.042), except for the 2-term lag (OR, 0.946; P=0.051), during periods of rising temperature, was observed for all samples.

Some of the significant but intermittent associations observed upon data analyses for all samples were not observed in the analysis that excluded samples labeled as 'domestic' (data not shown).

DISCUSSION

Salmonellosis is the most common foodborne enteritis worldwide [2]. According to food poisoning data from the Japanese Ministry of Health, Labour and Welfare (https://www.mhlw.go.jp/toukei/list/112-1.html), 35 salmonellosis outbreaks affecting 1,183 individuals were reported to the administrative agency as food poisoning cases in 2017. Although the infection sources in most of these cases has not been identified, 26 outbreaks of these (74.3%) according to the list of food poisoning cases, affecting 922 individuals (77.9%), occurred from July to September (summer and early autumn). A higher frequency of human salmonellosis in summer than in winter has also been reported in France [13]. However, the consecutive effects of climatic elements on the isolation of *Salmonella* (all serovars) and *S.* Infantis from chicken meat was not observed in this study. In contrast, lower air temperatures increased the frequency of *S.* Schwarzengrund isolation from chicken meat reared during periods of rising temperatures. Moreover, the risk of *S.* Schwarzengrund isolation from chicken meat during spring (OR, 3.951; P=0.008) and winter (OR, 4.071; P=0.006) was higher than that observed during summer. However, it has been reported that under isothermal experimental conditions, *Salmonella* grows better at 35°C than at 10°C, 15°C, or 25°C [20]. Moreover, the selective enrichment for *Salmonella* was performed at 42°C in this study. As a result, *S.* Schwarzengrund were also isolated (56/240, 23.3%). For that reason, the optimal temperature of *S.* Schwarzengrund is not considered to be much lower than those of *S.* Infantis and others. Therefore, in a farm environment, *Salmonella* including *S.* Schwarzengrund should grow better in summer at a higher temperature than in spring and winter. Moreover, the air temperature in broiler houses must be strictly controlled depending on the age of chickens. In other words, the increased risk of *S.* Schwarzengrund isolation from chicken meat in spring and winter may not be due to *Salmonella* growth in the environment inside or outside of broiler houses. Conversely, management policies, such as those affecting ventilator volumes or period for opening the covers of the windows in chicken houses, should be changed depending on the season. The kinds and number of vehicles, such as sanitary insects or wild animals that carry *Salmonella* into broiler flocks, would also change depending on the air temperature outside the broiler houses. *Salmonella* was isolated from wild birds in Japan in a previous study [7], although it did not include *S.* Schwarzengrund. Winter birds, which fly from northern countries to Japan in autumn and spend in Japan during winter, may carry *S.* Schwarzengrund, as wild waterfowls brought the highly pathogenic avian influenza (HPAI) virus to the zoo, causing some HPAI outbreaks in winter of 2016 [18]. As this study did not identify a vehicle for *S.* Schwarzengrund whose number increases at low temperatures, further research for identifying the vehicle is needed to establish preventive measures.

A previous study revealed that the rate of *Salmonella* contamination in chicken carcasses in summer (23.3%, 14/60) was higher than that in winter (6.7%, 4/60; P<0.05) in South Korea [9]. In the same study, *S.* Typhimurium (11/18, 61.1%), *S.* Hadar (2/18),

and *S.* Rissen (2/18), but not *S.* Schwarzengrund were detected. In the Netherlands, *Salmonella* contamination in broiler farms was reported to peak from July to December (the third and fourth quarters of the year) [19]. Sivaramalingam *et al.* [17] reported the effects of season on *Salmonella* detection in environmental samples from poultry breeder flocks in Canada between 1998 and 2008, and analysis of all samples showed that the risk of *Salmonella* contamination was higher during fall than during winter [OR, 2.11 (95% CI, 1.11 to 4.00), P=0.023]. To the best of our knowledge, the present study is the first to confirm that the risk of *S.* Schwarzengrund contamination of chicken meat is higher during spring and winter than during summer.

Independent analysis of the effects of climatic elements on *S.* Schwarzengrund isolation from *Salmonella* (all serovars) or only *S.* Infantis isolation could reveal the effect of air temperature during the chicken-rearing period on *S.* Schwarzengrund-contaminated chicken meat and the risk for *S.* Schwarzengrund isolation across the four seasons. Climatic elements exerted different effects on different *Salmonella* serovars, with differences being especially observed between *S.* Schwarzengrund and *S.* Infantis.

Previous studies have reported that *S.* Schwarzengrund was frequently detected in broiler chicken flocks and chicken meat only in the western part of Japan [1, 6, 14]. However, in the present study, *S.* Schwarzengrund was isolated from chicken meat produced in the I (21/72, 29.2%), C (12/60, 20%), and Y (8/22, 36.4%) prefectures of eastern Japan. The above findings suggested that the *S.* Schwarzengrund-contaminated area of broiler farms was not limited to western Japan, but also expanded to eastern Japan. Given that *S.* Schwarzengrund is likely to have spread across broiler farms during the period of our investigation, the effect of air temperature during the chicken-rearing period on *S.* Schwarzengrund isolation can additionally be identified. Eastern Japan has lower temperatures than Western Japan (Supplementary Table 1). Therefore, the risk of spread of *S.* Schwarzengrund contamintoation is higher in eastern Japan than that in western Japan.

In 1995, Infantis was reported to be the dominant serovar contaminating broiler chickens in Japan [10]. A previous study indicated that Infantis remained the dominant serovar between 2007 and 2010 [14]. To our knowledge, this is the first study to reveal that *S.* Infantis isolation from chicken meat did not vary across seasons.

Our findings suggest that the *S.* Schwarzengrund-contaminated area of broiler farms expanded to eastern Japan. The risk of *S.* Schwarzengrund contamination of chicken meat was higher in the spring and winter than in summer. Lower air temperature was associated with higher rates of *S.* Schwarzengrund isolation from meat produced from chickens reared during periods of rising temperature. The effects of climatic elements and the differences in contamination risk across seasons were observed only for *S.* Schwarzengrund but not for *Salmonella* (all serovars) and *S.* Infantis. Chickens shipped in winter and spring were at high risk of *S.* Schwarzengrund contamination, and hygiene measures during the rearing of these chickens would need to be strengthened. These observations could be attributed to differences in dissemination patterns and vehicles among *Salmonella* serovars. These findings will be useful for understanding the dynamics of *Salmonella* transmission in broiler farms, especially those of the Schwarzengrund serovar and could serve as the basis for the development of efficient strategies for preventing *Salmonella* colonization in broiler farms.

ACKNOWLEDGMENTS. We are grateful to Ms. Sakiko UEDA of the Women's Future Development Organization, Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, for her assistance in data analyses.

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