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Simulation of Time and Temperature as a Public Health Control for Food Served during Field Trips

ABSTRACT

Field trips present a challenge to school nutrition programs to provide a nutritious meal that is stored and handled properly to ensure food safety. During a field trip, coolers containing sack lunches might remain on school buses for extended periods of time, without temperature regulation, resulting in a potential food safety hazard. This study was designed to investigate this concern by monitoring changes in *Salmonella* and *Listeria monocytogenes* populations on turkey sandwiches, sliced apples, and baby carrots subjected to simulated field trip conditions. Inoculated products were packaged into individual sack lunches and packed into two coolers: a cooler with no ice and a cooler with one layer of ice on the bottom. These coolers were subjected to conditions simulating those in a school bus on a day with elevated temperatures. Thermocouple data collected from both coolers indicate that temperatures were conducive to foodborne pathogen growth. However, *L. monocytogenes* and *Salmonella* populations did not significantly ($P > 0.05$) increase during the 5-hour simulation. These data suggest that establishing time (< 5 hours) as a public health control may reduce risk of *Salmonella* and *Listeria monocytogenes* growth in deli sandwiches, apple slices, and baby carrots stored in coolers during field trips.

INTRODUCTION

School nutrition programs are required to have a food safety program based on Hazard Analysis Critical Control Point (HACCP) principles wherever food is prepared, stored, or served. Meals that are provided by the school nutrition program and served on field trips are included in this requirement *(11)*. Serving meals outside the cafeteria poses specific temperature-control challenges. Once meals leave the school nutrition operation, equipment may not be available to maintain temperatures that ensure the safety of time/temperature control for safety (TCS) foods [defined as foods that require time/temperature control to limit pathogenic microorganism growth or toxin formation *(25)*], and high ambient air temperatures might pose a risk to food safety. Accordingly, the effective use of time itself—up to a maximum of four hours—as a public health control for

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food with an initial temperature of ≤ 5°C (41°F), must be considered in regard to growth of pathogens of concern, especially when using current practices for serving meals outside the school.

The Center for Food Safety in Child Nutrition Programs conducted a survey to gain information about foods served for field trip meals, including transportation and storage of those foods, and what standard operating procedures exist as guidance for teachers and school nutrition personnel for providing meals that are safe *(17)*. As reported by school nutrition managers (n = 192) and teachers (n = 800), TCS foods are frequently served for field trip meals, so using time and temperature control is important in keeping these foods safe *(17)*. Most school nutrition managers (71%) reported using insulated containers, typically made of plastic, to transport cold foods, while 69% of school nutrition managers also reported sending meals in individual sacks or boxes. Seventy-seven percent of managers reported using ice or ice packs. Teachers reported that 63% of meals were placed in a cardboard box, bag, or plastic tub, and 33% of meals were placed in coolers. School buses were almost always used for field trip transportation, and 50% of teachers stated that the school buses served as the storage site for meals *(17)*.

The 2017 Food Code *(25)* specifies that time can be used as a public health control for a maximum of four hours when foods have an internal temperature of 5°C or less when removed from cold storage. Bacteria grow readily in foods held between 5°C and 57.2°C (41°F–135°F), which is known as the Temperature Danger Zone (TDZ). Foodborne pathogens have varied infectious doses, which makes it difficult to establish a universal threshold of concern regarding growth within a food *(14)*. While temperature dramatically impacts microbial growth and lag periods, the interaction of time, intrinsic factors (e.g., pH), and extrinsic factors (e.g., humidity) must also be considered with regard to microbial generation times and lag periods *(25)*. To evaluate risk, it is important to assess the behavior of each pathogen of concern within a food matrix when subjected to specific extrinsic factors. Data collected for a particular food matrix cannot be assumed to be valid for other matrices.

Listeria monocytogenes outbreaks are traditionally associated with ready-to-eat meats and cheese, which are commonly used in preparing lunches served on field trips *(17)*. Increasingly, however, *L. monocytogenes* outbreaks are associated with fresh produce *(9, 27)*. Since the implementation of the Healthy, Hunger-Free Kids Act (HHFKA), U.S. children are consuming 23% more fruit and 16% more vegetables at lunch *(19)*. The success of the HHFKA has brought more fresh fruits and vegetables into the school lunch environment, and food safety concerns associated with fresh produce have subsequently increased. *Listeria monocytogenes* contamination has occurred in many vegetables, including celery *(10)*, tomatoes *(9)*, sprouts *(8)*, cabbage *(7)*, corn *(1)*, lettuce *(9)*, bagged salad *(5)*, parsley *(26)*, cucumber *(9)*, and carrots *(16)*. *Listeria moncytogenes* outbreaks have been less frequently associated with fresh fruits than other types of produce, but outbreaks associated with cantaloupes, stone fruits, and apples have occurred *(9)*.

Although *L. monocytogenes* outbreaks in U.S. schools are uncommon *(6)*, foods served in schools have been involved in recent recalls. In 2015, apple slices recalled for possible *L. monocytogenes* contamination were served in the Palm Beach County School District *(23)*. In 2015, 3 oz. single serving Blue Bell ice creams distributed to hospitals, nursing homes, and schools in 23 states were recalled *(22)*. In 2016, pre-prepared sandwiches sold to school foodservice distributors in 29 states were recalled because of possible *L. monocytogenes* contamination *(24)*.

Salmonella is a major pathogenic concern in the U.S., and since 1998, there have been 42 *Salmonella* outbreaks in schools, colleges, and universities, resulting in over 2400 illnesses and 180 hospitalizations *(6)*. According to the Centers for Disease Control and Prevention's (CDC) 2015 Annual Summaries of Foodborne Outbreaks *(6)*, *Salmonella* associated with seeded vegetables, pork, and vegetable row crops was responsible for three of the top five Food-Germ pairs causing outbreak-associated illness. School *Salmonella* outbreaks have been associated with many different kinds of foods, with 11 of the 42 reported outbreaks associated with fresh produce *(6)*. In summary, *L. monocytogenes* and *Salmonella* are two pathogens that represent a potential risk for schools.

Temperature control of food served away from a food preparation site is often challenging, especially during the summer season when many field trip and summer food service program meals are provided to children. Thus, there is a need to determine if the use of time as a public health control allows for minimal growth of pathogens of concern when using current practices in the service of meals outside of the school. Some programs may use time as a public health control for TCS foods, but little research has examined the microbiological growth that may occur under these types of temperature abuse situations. Therefore, this study was designed to determine the growth of foodborne pathogens in school lunch meals served off-site, packaged in insulated coolers, and exposed to extreme environmental temperatures. Population changes among *L. monocytogenes* and *Salmonella* were investigated on carrots, turkey sandwiches, and apple slices placed in coolers and held under conditions that simulate storage on a school bus.

MATERIALS AND METHODS

Food preparation

Products frequently consumed on school field trips were chosen for this study; turkey sandwiches, sliced apples, and baby carrots *(17)*. Individual sack lunches were packed to meet the requirements for National School Lunch Program meals *(18)*. Supplies to prepare the turkey

sandwiches, sliced apples, and baby carrots were purchased at a grocery store and used to make individual sack lunches. Sandwiches were prepared with two slices of oven roasted white turkey (approximately 45 g; Oscar Mayer, Madison, WI) and two slices of whole grain white bread (Sara Lee, Downers Grove, IL) and then stored in a re-sealable plastic sandwich bag. Riveridge Red Delicious apples were cored and sliced by hand. Approximately 50 g of sliced apples were packed in plastic, non-filtered stomacher bags and then sealed. Approximately 50 g of baby carrots (Grimmway Farms, Bakersfield, CA) were packed in plastic, non-filtered stomacher bags and then sealed. Brown paper bag (approximately 13 cm × 7.9 cm × 26.9 cm) sack lunches consisted of one deli turkey sandwich, one stomacher bag of sliced apples, and one stomacher bag of baby carrots.

School bus temperature profiling

This phase of the study was designed to represent a high-risk scenario with regard to temperatures on school buses that sack lunches and/or coolers may be exposed to during a field trip. Data were collected on the temperature profiles inside school buses and within sack lunches stored in coolers on school buses. These data were used to inform the design and execution of the subsequent inoculation study.

To effectively simulate cooler storage on a bus during a field trip, it was necessary to first determine the temperature profiles on a school bus from morning into early afternoon, as school lunch coolers are commonly stored for such times during a field trip. To obtain this data, temperature data loggers were distributed to school personnel in two districts in North Carolina (May 12–21 and June 2–4) and one district in Arkansas (May 27) during 2015. It was anticipated that these locations would represent warm climates and, therefore, high-risk scenarios. Four data loggers (TRIX-8 Temperature Data Recorder, LogTag, Auckland, NZ) recorded internal and external bus temperatures at two-minute intervals for a minimum of two days at each location. Interior data loggers were placed at random on two different locations of the bus. Exterior data loggers were placed on the exterior of the bus and out of direct sunlight. Magnetic clips and zip ties were utilized to ensure that data loggers were positioned with the temperature sensors facing away from walls to accurately record air temperatures.

Average, minimum, and maximum interior and exterior air temperatures were determined for the times most likely to correspond with packing, transporting, and serving sack lunches on field trips. These data were then used to create a commercial electronically controlled thermal processing unit (ECTPU) cycle to mimic high-risk scenario temperature changes on a school bus *(Table 1)*. To create a high-risk scenario, the ECTPU temperatures were higher than temperatures recorded by the school bus temperature data loggers. Specifically, the maximum temperature of the ECTPU was chosen to be 65.6°C (150°F), as this exceeded the highest temperature point obtained from the interior of the bus (42.7°C/108.9°F) by approximately 40°C/40°F. A starting ECTPU temperature of 23.9°C (75°F) reflects the starting interior bus temperatures recorded by the bus temperature data loggers. Throughout the 5-hour ECTPU program, the temperature increased by 5.5–5.6°C (10°F)

TABLE 1. Commercial ECTPU program used to subject sack lunches stored in coolers to temperature abuse conditions that simulate a high-risk school field trip scenario

Step	Program	Relative Humidity (%)	House Temperature (°C/°F)	Time (minutes)
1	Start			1
2	Dry Cycle	80	23.9 / 75	33
3	Dry Cycle	80	29.4 / 85	33
4	Dry Cycle	80	35 / 95	33
5	Dry Cycle	80	40.6 / 105	33
6	Dry Cycle	80	46.1 / 115	33
7	Dry Cycle	80	51.7 / 125	33
8	Dry Cycle	80	57.2 / 135	33
9	Dry Cycle	80	62.8 / 145	33
10	Dry Cycle	80	65.6 / 150	33
11	Stop			1

per hour until it reached 62.8°C (145°F), with an increase of 2.8°C (5°F) in the final 33 minutes to reach the target temperature of 65.6°C (150°F). An increase of 5.5–5.6°C (10°F) every 33 minutes was selected for the high-risk scenario, as the bus temperature data loggers indicated it often took 1.5–2 h or longer for the bus interior temperature to increase by 5.5–5.6°C (10°F).

Relative humidity was maintained at 80% throughout the ECTPU cycle; the combination of relative humidity and temperature was intended to simulate a high-risk scenario on a school bus during a field trip on a humid day with temperatures above 26.7°C (80.1°F). A 5-hour ECTPU cycle was chosen to represent the length of time that coolers would be stored on a bus before food was consumed (e.g., 8:00 a.m. departure with a 1:00 p.m. lunch), and to evaluate a period of time in excess of the maximum (4 hours) allowed for using time as a public health control in the 2017 Food Code *(25)*.

Cooler temperature profiling

Simulating a high-risk cooler-packing scenario required a preliminary evaluation of packing/storage methods to determine the scenarios most likely to favor pathogen growth. Temperature profiles were collected on non-inoculated turkey sandwiches, carrots, and apple slices (prepared as previously described) packed in coolers with various levels of ice. Coleman brand insulated coolers (approximately 62.2 cm × 31.8 cm × 35.6 cm) were prepared in the following ways:

1. Ice layered on bottom, middle, and top of interior of cooler
2. Ice layered on top of interior of cooler
3. Ice layered on bottom of interior of cooler
4. Ice layered on top and bottom of interior of cooler
5. No ice in cooler

Thermocouples (Measurement Computing USB-TC with MCC DAQ Software, Norton, MA; MultiPaq21 Data logger with Food Tracker® Software, Datapaq, Inc., Derry, NH) were placed in each product packed in the bottom, middle, and top of the coolers for the preliminary study. The coolers were then placed in a commercial ECTPU; once inside the ECTPU, coolers were subjected to a ECTPU cycle *(Table 1)* specifically designed to mimic high-risk temperature increases. All thermocouples recorded temperatures every 10 minutes throughout the 5-hour period. Resulting data were saved and analyzed using data logger software and Microsoft Excel. Temperature profile data were generated (data not shown) and used to identify the two packing methods that pose the greatest risk for pathogen growth (those with the highest product temperatures) for use in the subsequent inoculation study.

Inoculation study

The overall objective of this inoculation study was to study the behavior of *Salmonella* and *L. monocytogenes* on baby carrots, turkey sandwiches, and sliced apples under simulated field trip storage conditions. Temperature and cooler packing data obtained in the preliminary study were used to simulate high-risk sack lunch storage scenarios.

Bacterial cultures

Five-strain cocktails of *L. monocytogenes* [SLR 2249 (Cornell University laboratory-developed strain with the *actA* gene deleted), B-33043 (USDA-ARS isolate from turkey/ham luncheon meats, serotype 1/2a), B-33260 (USDA-ARS isolate from beef sausage, serotype not provided), B-33054 (USDA-ARS isolate from cucumbers, serotype 4b), B-33245 (USDA-ARS environmental isolate, serotype 1/2b complex)] and *Salmonella enterica*, subspecies *enterica* [serotypes Heidelberg (F5038BG1; CDC isolate from stuffed ham/chad slicer), Tennessee (ATCC 10722), Typhimurium (ATCC 14028), Newport (ATCC 6962), Senftenberg (ATCC 43845)] were prepared. Briefly, frozen stocks of the individual strains were streaked onto Tryptic Soy Agar (TSA; Difco™, Becton, Dickinson, and Company, Sparks, MD) and allowed to incubate at 37°C for 18–24 hours. One isolated colony was transferred to 45 ml of Tryptic Soy Broth (TSB; Difco™, Becton, Dickinson, and Company, Sparks, MD) and incubated (Fisher Scientific Isotemp 600 Series, Fisher Scientific, Hampton, NH) at 37°C for 18–24 hours. A separate cocktail was prepared for both pathogens by combining all five strains (total of 225 mL) and then centrifuging for 15 minutes at 5,520 × g at 4°C. The supernatant was decanted and the pellet re-suspended in 225 mL of 0.1% peptone water (Bacto™, Becton, Dickinson and Company, Sparks, MD). Following rehydration, 1 mL of each five-strain cocktail was combined with 0.1% peptone water for a total volume of 1 L at a target concentration of 1.0×10^6 CFU/mL.

Inoculation and preparation of food products

A target inoculation of 10^4 CFU/g of each pathogen was chosen in accordance with published inoculation recommendations for microbial challenge studies *(15)*. The 10^4 CFU/g inoculum concentration used in this study was higher than the recommended 10^2–10^3 CFU/g as a means to ensure that the *Salmonella* and *Listeria monocytogenes* populations would remain detectable throughout the study period. More specifically, the goal was to protect against decreases in pathogen populations that may occur (1) in response to the food matrix (as was observed with *Listeria monocytogenes* on carrots, which is discussed later), and (2) during the 4°C storage period.

Turkey lunchmeat, sliced apples, and baby carrots were mist inoculated with the prepared *L. monocytogenes* or *Salmonella* cocktail to achieve a target concentration of

approximately 1.0×10^4 CFU/g on the product. Food products were placed inside a biohazard bag and lightly misted with the inoculum using a calibrated hand-held misting bottle (~237 mLiGo spray bottle, The Bottle Crew, West Bloomfield, MI). The spray bottle was calibrated using a graduated cylinder before each inoculation to ensure consistent application of inoculum to each food product. Following inoculation, all products were allowed to rest at ambient temperature for 30 minutes to facilitate pathogen attachment. All inoculated food products were prepared and packaged as previously described and then stored for 12–15 h at 4°C. Non-inoculated sack lunches were also prepared to fill a single cooler with 30 sack lunches, which is the average number of meals packed per cooler for field trips *(17)*. On the day of the study, the sack lunches were removed from storage at 4°C and packed into coolers. Two Coleman brand insulated coolers (approximately 62.2 cm × 31.8 cm × 35.6 cm) were required for each replication of this study. One cooler was packed with a single layer of ice on the bottom (approximately 2 inches thick), while the second cooler was packed without ice. Each cooler contained 6 inoculated lunches and 24 non-inoculated lunches; these lunches were placed in the coolers in three layers, each layer containing 10 lunches. A total of seven inoculated lunches were prepared for each pathogen. One of these lunches served as the inoculated control, which was not packed in either cooler and was sampled immediately following the 12–15 hours of storage at 39.2°C (102.6°F). The inoculated control allowed for effective comparison with the experimental samples held within the coolers to determine if significant changes in pathogen populations occurred as a result of exposure to the simulated temperature abuse conditions. The remaining six lunches were stored in coolers in the following ways *(Fig. 1)*:

1. Top layer, cooler without ice
2. Middle layer, cooler without ice
3. Bottom layer, cooler without ice
4. Top layer, cooler with a layer of ice on the bottom
5. Middle layer, cooler with a layer of ice on the bottom
6. Bottom layer, cooler with a layer of ice on the bottom

Six thermocouples were placed in each cooler: one thermocouple in each food product of a lunch on the bottom layer, and one thermocouple in each food product of a lunch on the top layer.

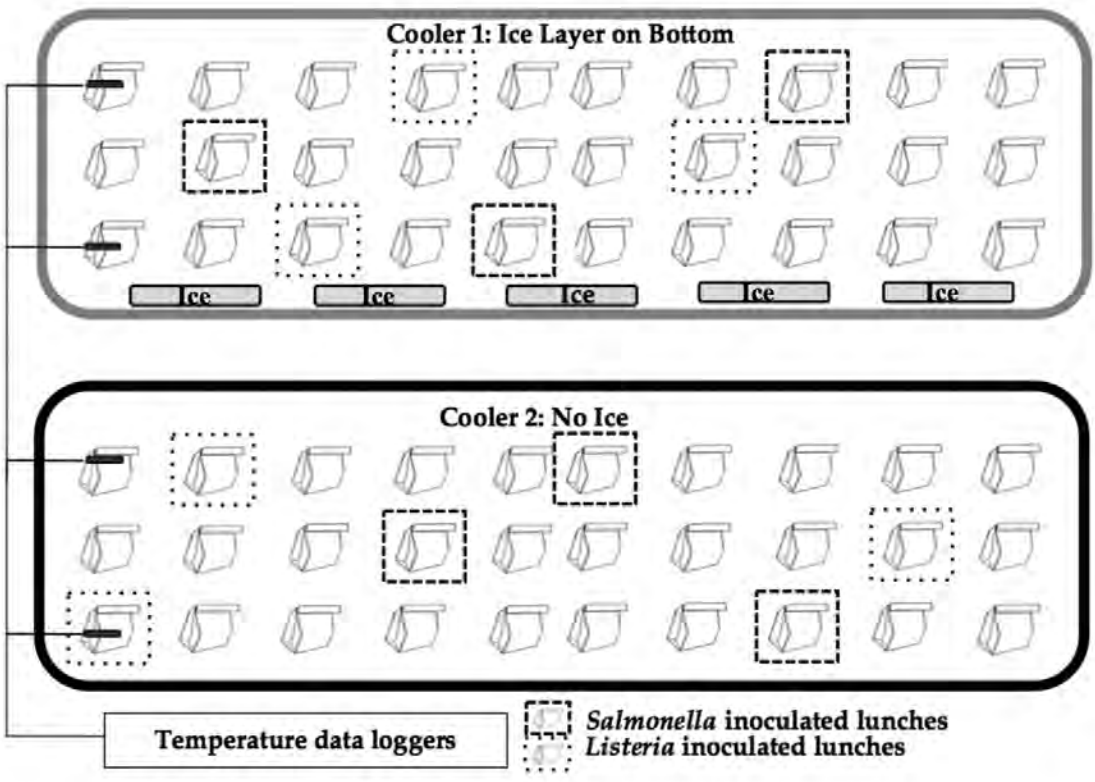

Figure 1. Cooler packing scenarios. Inoculated lunches were randomly assigned to a position within each layer of the cooler.

ECTPU simulation

The packed coolers were placed in a commercial ECTPU at the Biosecurity Research Institute and subjected to a 5-hour ECTPU cycle *(Table 1)*. Thermocouples recorded temperatures of the inoculated lunches and ECTPU ambient air temperature every 10 minutes throughout the 5-hour cycle, and these data were downloaded at the conclusion of data collection. Coolers were immediately removed from the ECTPU at the end of the 5-hour cycle and were transported (approximate transportation time: 10 minutes) to a nearby laboratory for sampling and microbiological analyses (approximate sampling time: 30 minutes).

Microbiological analyses

The inoculated lunches were removed from the coolers and 75 mL of 0.1% peptone water was added to the apple and carrot stomacher bags, which were then pulsated (Pulsifier Model PUL100; Microbiology International, Frederick, MD 21704) for 1 minute. For each inoculated sandwich, 50 g were aseptically excised from the middle, and these samples were combined with 75 mL of 0.1% peptone water in a stomacher bag and homogenized (Smasher™; AES CHEMUNEX, bioMérieux Inc., Hazelwood, MO) for 1 minute. Serial dilutions in 0.1% peptone water were prepared for all sample homogenates. The *L. monocytogenes* dilutions were spread plated on Modified Oxford agar (MOX; Difco™, Becton, Dickinson, and Company, Sparks, MD) containing Modified Listeria Selective Enrichment Supplement (Oxoid Ltd, Basingstoke, Hampshire, England). *Salmonella* dilutions were plated on Xylose Lysine Deoxycholate agar (Difco™, Becton, Dickinson, and Company, Sparks, MD) and then enumerated. MOX plates were incubated at 30°C for 24–48 hours; XLD plates were incubated at 35°C for 18–24 hours. Following incubation, *L. monocytogenes* and *Salmonella* populations were recorded.

Statistical analyses

All experimental procedures were replicated three times. Data collected from all three replications were tested for normality using the Shapiro-Wilk normality test in GraphPad Prism (GraphPad Prism 7; La Jolla, CA). Data were determined to be normal ($P > 0.05$) and were subsequently analyzed using the MIXED procedure of Statistical Analysis Software (SAS 9.4; Cary, NC). For each pathogen, the main factors (use of ice, and sack lunch location within the cooler) and interactions were evaluated at the product level for statistical significance at the $P \leq 0.05$ threshold.

RESULTS

Preliminary school bus temperature profiling

Two interior and two exterior data loggers recorded temperatures between 7:00 a.m. and 2:00 p.m. at ten-minute intervals on each sampling day for a total of 15 days. Average maximum temperature in the bus interior was 34.2°C (93.6°F) [SD = 4°C (7.2°F); Range: 25.2 – 40.1°C (77.4 –104.2°F)], and the average minimum temperature was 27°C (80.6°F) [SD = 4.6°C (8.3°F); Range: 18 – 33.1°C (64.4 – 91.6°F)]. Average maximum temperature in the bus exterior was 36.4°C (97.5°F) [SD = 4.8°C (8.6°F); Range: 23.3 – 41.6°C (73.9 – 106.9°F)], and average minimum temperature was 21.9°C (71.4°F) [SD = 3°C (5.4°F); Range: 17.1 – 26.3°C (62.8 –79.3°F)]. With regard to single temperature data points, the highest interior temperature recorded was 42.7°C (108.9°F), and the highest exterior temperature recorded was 42°C (108°F). Data were collected on 14 days in North Carolina and on one day in Arkansas. Participation from school districts in other geographic locations was solicited but not obtained; therefore, these data, which were used to inform the simulation temperature profile outlined in *Table 1*, are scanty.

Cooler temperature profiling

The highest single temperature data point recorded in the cooler packed with no ice was approximately 45°C (113°F) and was recorded in a bag of carrots at the top of the cooler. The highest single temperature data point in the cooler packed with a layer of ice on the bottom was approximately 28°C (82.4°F), which was recorded in a sandwich located in the top of the cooler. All products in the other cooler-packing scenarios remained below 20°C (68°F). These preliminary data indicate that packing a cooler with no ice, or with a single layer of ice on the bottom, presents the greatest risk for temperature abuse and foodborne illness. Therefore, these two packing methods were chosen for use in the subsequent inoculation study.

Inoculation study

All products packed in a cooler without ice *(Fig. 2)* reached temperatures above 20°C (68°F) at the end of five hours, with the highest average temperature recorded in apples at the top of the cooler being 35 ± 7.9°C (95 ± 14.2°F). In the cooler packed with a layer of ice on the bottom, a maximum temperature of 30 ± 1.9°C (86 ± 3.2°F) was observed in carrots at the top of the cooler *(Fig. 3)*. Those stored near the bottom of the cooler, in close proximity to the ice, maintained a temperature below 10°C (50°F) throughout the 5-hour simulation period.

Use of ice (no ice versus one layer of ice on the bottom), sack lunch location outside of the cooler (refrigerated, inoculated control) or within the cooler (top, middle, bottom), and use of ice × sack lunch location interaction were not statistically significant ($P > 0.05$) for sandwiches, apples, or carrots inoculated with *L. monocytogenes*.

Use of ice, sack lunch location within the cooler, and use of ice × sack lunch location interaction were not statistically significant ($P > 0.05$) for sandwiches and apples inoculated with *Salmonella*. Although the population difference between the two cooler types was marginal, at 0.18 $\log_{10}$ CFU/g, a use

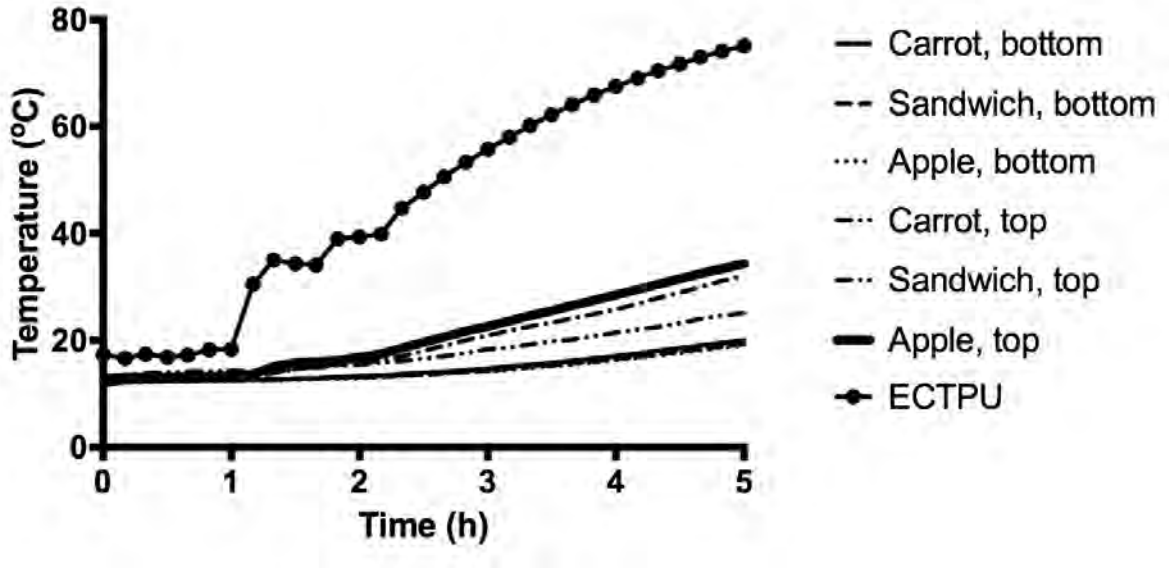

Figure 2. Exposure temperature for turkey sandwiches, sliced apples, and baby carrots packed in a cooler with no ice. Values represent the average temperature of three replications.

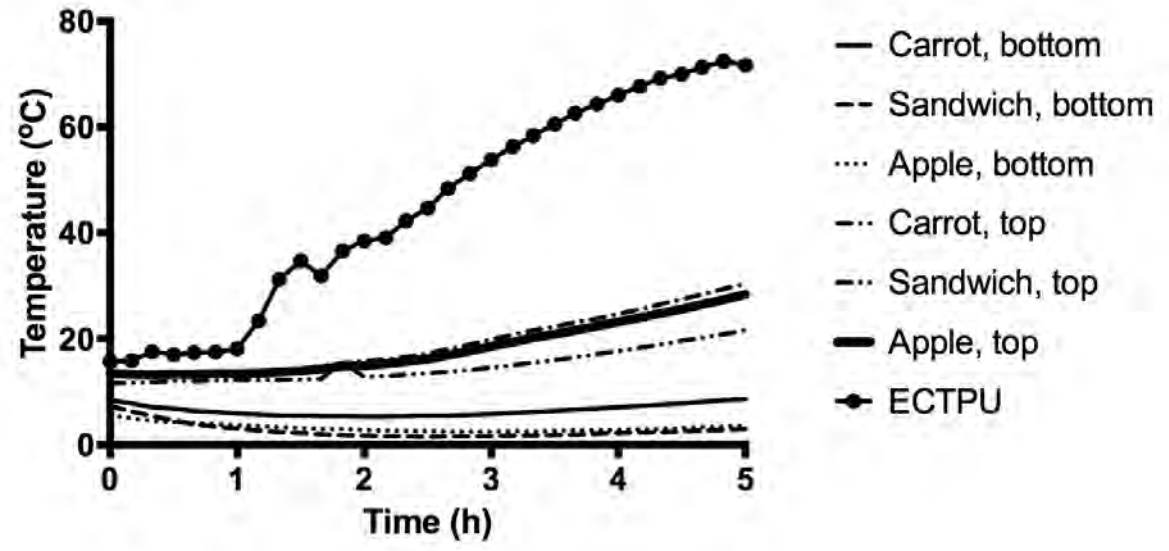

Figure 3. Exposure temperature for turkey sandwiches, sliced apples, and baby carrots packed in a cooler with a layer of ice on the bottom. Values represent the average temperature of three replications.

of ice effect approached statistical significance ($P = 0.0625$) for *Salmonella* on apples. When results for the cooler with no ice and for the cooler with a layer of ice were compared, *Salmonella* populations on carrots were not significantly different ($P = 0.0833$). The use of ice × sack lunch location interaction was not significant ($P = 0.5817$) for *Salmonella* on carrots. *Salmonella* populations on carrots did vary statistically ($P = 0.0022$) according to sack lunch location in the cooler *(Fig. 4)*.

DISCUSSION

Inoculation study

Intrinsic properties of foods (for instance, pH) greatly affect attachment, survival, and growth of microorganisms

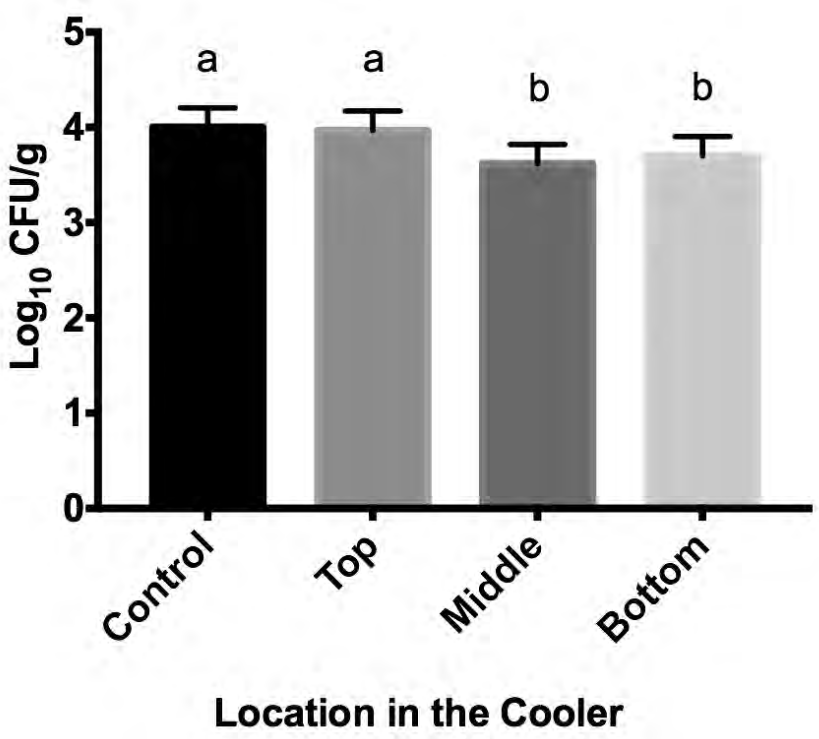

Figure 4. *Salmonella* populations on baby carrots according to their location within the cooler.
***Error bars represent the standard error of the mean.**
[a,b]Populations with different superscripts ($P \leq 0.05$) are significantly different.

(14), which is why data were analyzed at the product level and cannot be extrapolated to other food matrices. In general, *Salmonella* and *L. monocytogenes* populations were highest on turkey, followed by apples and then carrots (data not shown). Naturally occurring compounds in food products may also affect foodborne pathogens, which may explain why *L. monocytogenes* was not readily recovered from carrots in this study. Previous researchers have noted that carrots inhibit attachment and growth of *L. monocytogenes* *(2, 4)*. For example, Babic et al. *(2)* reported that purified ethanolic extracts obtained from shredded and peeled carrots demonstrate antimicrobial activity against *L. monocytogenes*.

Figures 2 and 3 illustrate that coolers packed with no ice, or with a layer of ice on the bottom, develop conditions of temperature abuse when exposed to simulated school bus conditions. According to thermocouple data collected from products stored at the top and the bottom of this cooler, all products were within the TDZ throughout the entire 5-hour period. Thermocouple data collected from the cooler containing a layer of ice on the bottom indicate that only apples and sandwiches packed on the bottom of the cooler remained out of the TDZ for the majority (approximately 4.5 hours) of the 5-hour test period.

Foodborne pathogens can grow under a variety of conditions, and temperature is a very important factor that must be considered when assessing risk for foodborne illness. *Listeria monocytogenes*, a psychrotroph, has optimal growth between 30–37°C (86–98.6°F) [range –1.5–45°C (29.3–113°F)] *(13)*. *Salmonella*, a mesophile, exhibits optimum growth at approximately 37°C (98.6°F) [range of 20–45°C (68–113°F)] *(14)*. The temperature profiles collected demonstrate a substantial risk for pathogenic growth. Based on temperature data alone, the risk for *L. monocytogenes* growth is particularly notable. During most of the 5-hour simulation period, product temperatures were within the optimum growth range for psychrotrophs.

Kim et al. *(12)* evaluated the temperature of milk, tofu, eggs, fresh meat, and frozen meat during a three-hour storage period in the trunk of a car. The trunk temperature reportedly increased from 32.3°C (90.1°F) to 41.5°C (106.7°F), and temperatures of all food products steadily increased to 30°C (86°F) within 90–130 minutes. Fresh meat reached the highest temperature, at 38.4°C (101.1°F), by the end of the evaluation period. One important difference between the Kim et al. study and this study was that the food products they evaluated were not stored in insulated coolers. However, this research provides additional evidence that storing food for extended periods in warm vehicles can result in optimal growth temperatures for *L. monocytogenes*, while also allowing for growth of mesophilic bacteria in food products.

Although accompanying temperature data suggest the risk for growth, statistical analysis of the microbiological data indicate that five hours is not sufficient time for *L. monocytogenes* to grow under these simulated conditions. Perhaps most notable is the lack of any significant difference ($P > 0.05$) in sack lunch location within coolers, which indicates that *L. monocytogenes* had the same population as the control products that were sampled immediately upon removal from cold storage at 4°C (39.2°F). Similarly, the lack of an interaction between use of ice × sack lunch location indicates that populations did not vary depending on where a sack lunch was packed within a specific cooler, whether the cooler contained ice or not. For example, *L. monocytogenes* populations recovered from apples packed at the top of the cooler with no ice were statistically the same as populations on apples at the bottom of the cooler packed with a layer of ice.

As *Figure 4* depicts, the inoculated control carrot sample and the carrot sample located in the top of the cooler each harbored the largest *Salmonella* population, at 4.0 $\log_{10}$ CFU/g, *Salmonella* populations recovered from carrots stored in the middle (3.6 $\log_{10}$ CFU/g) and bottom (3.7 $\log_{10}$ CFU/g) of the cooler were statistically the same ($P = 0.3942$), but significantly different ($P \leq 0.05$) from populations recovered from the inoculated control carrots and carrots stored at the top of the cooler. Therefore, the significant differences in *Salmonella* populations observed for sack lunch location within the cooler for carrots were not due to growth on samples stored in the coolers under simulated temperature abuse conditions.

The lack of use of ice × sack lunch location interaction for all products inoculated with *Salmonella* indicates that *Salmonella* populations did not vary by their location within a cooler, regardless of which use-of-ice method they were exposed to. Thus, the risk for *Salmonella* growth is the same throughout the cooler, whether it is packed with no ice or packed with one layer of ice on the bottom. As with *L. monocytogenes*, the temperature data in this study suggest that the simulated conditions present a risk for *Salmonella* growth on turkey sandwiches, sliced apples, and carrots. However, this anticipated risk is not substantiated by the microbiological data, as the lack of *Salmonella* growth on these products throughout the simulation period suggests that five hours is insufficient for the pathogen to grow on these food products under the simulated conditions.

Controlling time and temperature is critical in protecting food against foodborne pathogen growth during storage *(25)*. School nutrition programs have the challenge of preparing and packing lunches for field trips, maintaining the cold chain, and avoiding the TDZ, particularly on days with outdoor temperatures above 26.7°C. Controlling the amount of time that school lunches are held at elevated outdoor temperatures [32.2°C (90°F) *(20, 21, 26)* during a field trip is possible, but the ambient temperature at which the coolers are stored cannot be controlled. The situation is worsened when coolers are packed with little or no ice and are stored on a school bus with temperatures far exceeding already elevated outdoor temperatures. According to the 2017 Food Code *(25)*, time can be used effectively for a maximum of four hours as a public health control when foods have an internal temperature of 5°C or less when removed from cold storage.

This study was designed to simulate a high-risk scenario, and the recorded data generally suggest that *Salmonella* and *L. monocytogenes* populations do not significantly increase after five hours of exposure to extreme cooler storage conditions. Thus, the data presented herein provide evidence supporting the use of time as a public health control for storing sack lunches in portable coolers on field trips for a maximum of four hours. However, it is critical to note that the present study evaluated populations of *L. monocytogenes* and *Salmonella* on only turkey sandwiches, apple slices, and baby carrots. Accordingly, these data are limited in scope and can function only as an indication of how other pathogens (e.g., *Staphylococcus aureus* and *Escherichia coli*) might behave on sandwiches, sliced apples, carrots, and other food products under similar conditions. Additional studies are necessary to validate the behavior of other pathogens exposed to these products and conditions, and to other food products that might be used in such lunches. This study establishes an experimental design model that can be leveraged in future studies to generate a larger body of evidence regarding the use of time as a public health control for TCS foods.

Although the risk for foodborne illness appears to be low under these simulated conditions, it is recommended that sack lunches be packed in insulated coolers and one or more layers of ice should be used to maintain an appropriate temperature and reduce the amount of time the food products are exposed to the TDZ before the food is consumed. Avoiding cooler storage on school buses with elevated internal temperatures whenever possible is also recommended.

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REFERENCES

1. Aureli, P., G. C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes. N. Engl. J. Med.* 342:1236–1241.
2. Babic, I., C. Nguyen-The, M. J. Amiot, and S. Aubert. 1994. Antimicrobial activity of shredded carrot extracts on foodborne bacteria and yeast. *J. Appl. Bacteriol.* 76:135–141.
3. Beuchat, L. R., and R. E. Brackett. 1990. Inhibitory effects of raw carrots on *Listeria monocytogenes. Appl. Environ. Microbiol.* 56:1734–1742.
4. Beuchat, L. R., R. E. Brackett, and M. P. Doyle. 1994. Lethality of carrot juice to *Listeria monocytogenes* as affected by pH, sodium chloride and temperature. *J. Food Prot.* 57:470–474.
5. Centers for Disease Control and Prevention. 2016. Multistate outbreak of listeriosis linked to packaged salads produced at Springfield, Ohio Dole processing facility (final update). Available at: https://www.cdc.gov/listeria/outbreaks/bagged-salads-01-16/index.html. Accessed 21 December 2017.
6. Centers for Disease Control and Prevention. 2017. Surveillance for foodborne disease outbreaks–United States, 2015: Annual report. *In* U.S. Department of Health and Human Services, CDC., Atlanta, Georgia.
7. Conly, J., and B. Johnston. 2008. *Listeria*: A persistent foodborne pathogen. *Can. J. Infect. Dis. Med. Microbiol.* 19:327–328.
8. Dechet, A. M., K. M. Herman, C. Chen Parker, P. Taormina, J. Johanson, R. V. Tauxe, and B. E. Mahon. 2014. Outbreaks caused by sprouts, United States, 1998–2010: lessons learned and solutions needed. *Foodborne Pathog. Dis.* 11:635–644.
9. Garner, D., and S. Kathariou. 2016. Fresh produce-associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *J. Food Prot.* 79:337–344.
10. Gaul, L. K., N. H. Farag, T. Shim, M. A. Kingsley, B. J. Silk, and E. Hyytia-Trees. 2013. Hospital-acquired listeriosis outbreak caused by contaminated diced celery—Texas, 2010. *Clin. Infect. Dis.* 56:20–26.
11. Healthy, Hunger Free Kids Act of 2010. 2010. Session 3307, 111th Congress, Public Law 111–296.
12. Kim, S. A., S. J. Yun, S. H. Lee, I. G. Hwang, and M. S. Rhee. 2013. Temperature increase of foods in car trunk and the potential hazard for microbial growth. *Food Control* 29:66–70.
13. Lado, B., and A. E. Yousef. 2007. Characteristics of *Listeria monocytogenes* important to food processors. p. 157–213. *In* E.T. Ryser, and E.H. Marth (ed.), *Listeria,* listeriosis, and food safety. Third Edition. CRC Press Taylor & Francis Group, Boca Raton, FL.
14. Montville, T. J., K. R. Matthews, and K. E. Kniel. 2012. Food microbiology: an introduction. Third Edition. ASM Press, Washington, D.C.
15. National Advisory Committee on Microbiological Criteria for Foods. 2010. Parameters for determining inoculated pack/challenge study protocols. *J. Food Prot.* 73:140–202.
16. Ponniah, J., T. Robin, M. S. Paie, S. Radu, F. M. Ghazali, C. Y. Kqueen, M. Nishibuchi, Y. Nakaguchi, and P. K. Malakar. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. *Food Control* 21:774–778.
17. Sneed, J., and E. Patten. 2015. Current practices for providing school field trip meals: perspectives of school nutrition managers and teachers. *J. Child Nutr. Manag.* 39:1–13.
18. United States Department of Agriculture Food and Nutrition Service. 2012. Meal requirements for lunches and requirements for afterschool snacks. 7CFR§210.10.
19. United States Department of Agriculture Food and Nutrition Service. 2017. Fact sheet: Healthy, hunger-free kids act school meals implementation. Available at: https://www.fns.usda.gov/pressrelease/2014/009814 Accessed December 1, 2017.
20. United States Department of Agriculture Food Safety and Inspection Service. 2011. How temperatures affect food. Available at: https://www.fsis.usda.gov/shared/PDF/How_ Temperatures_Affect_Food.pdf. Accessed 21 December 2017.
21. United States Department of Health and Human Services. 2016. USDA urges consumers to keep clear of the danger zone this summer. Available at: https://www.foodsafety.gov/ blog/2016/05/memorial-day-danger-zone.html. Accessed 21 December 2017.
22. United States Food and Drug Administration. 2015. FDA investigates *Listeria monocytogenes* in ice cream products from Blue Bell Creameries. Available at: https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm438104.htm Accessed 1 December 2017.
23. United States Food and Drug Administration. 2015. Potential health hazard: recalled snack fresh brand red apple slices may have been distributed to Florida's Palm Beach County school district. Available at: https://www.fda.gov/safety/recalls/ucm462542.htm Accessed 1 December 2017.
24. United States Food and Drug Administration. 2016. Let's Do Lunch, Inc., dba Integrated Food Service, voluntarily recalls certain ready-to-eat sandwiches because of possible health risk. Available at: https://www.fda.gov/safety/recalls/ucm507583.htm. Accessed 13 December 2017.
25. United States Food and Drug Administration. 2017. Food Code 2017. Available at: https://www.fda.gov/downloads/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/UCM595140.pdf. Accessed 6 April 2018.
26. United States Food and Drug Administration. 2017. Refrigerator thermometers: cold facts about food safety. Available at: https://www.fda.gov/Food/ ResourcesForYou/Consumers/ucm253954.htm. Accessed 21 December 2017.
27. Zhu, Q., R. Gooneratne, and M. A. Hussain. 2017. *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. *Foods* 6–21.