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Growth of *L. monocytogenes* strain F2365 on ready-to-eat turkey meat does not enhance gastrointestinal listeriosis in intragastrically inoculated A/J mice

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Abstract

There have been significant outbreaks of listeriosis associated with consumption of contaminated ready-to-eat (RTE) turkey meat products. In this study, we investigated whether growth on RTE deli turkey meat sends environmental signals to listerial cells that makes them more virulent in the gastrointestinal tract of mice. *L. Listeria monocytogenes* strain F2365 grew from a starting inoculum of 10³ CFU/mL to final numbers of 10⁸–10⁹ CFU/mL (within 12 days at 10 °C) when inoculated onto sliced processed, or whole muscle, turkey breast, or into emulsified whole turkey breast. We did not observe any difference in the numbers of CFU recovered from the spleens and livers of A/J mice inoculated intragastrically with *L. monocytogenes* grown on sliced turkey meat, in emulsified turkey meat, or in brain heart infusion broth. These results suggest that growth on RTE sliced deli turkey, or in RTE emulsified deli turkey, does not enhance the ability of *L. monocytogenes* F2365 to cause gastrointestinal listeriosis in intragastrically challenged A/J mice.

Keywords

Listeria monocytogenes; Virulence; Meat; Mice

1. Introduction

Listeria monocytogenes is a ubiquitous gram-positive bacterium that is often found in food processing plants (Kathariou, 2002; Notermans et al., 1998) from which it can contaminate ready-to-eat (RTE) food products (Tompkin, 2002; Wong, 1998). Consumption of these contaminated RTE foods by susceptible individuals poses a risk for contracting foodborne listeriosis that can result in meningitis, septicemia and abortion in susceptible individuals. In 2000 and 2002, listeriosis outbreaks associated with consumption of RTE turkey meat resulted in a combined 76 cases with 11 adult deaths and six fetal miscarriages/stillbirths (CDC, 2000, 2002; Gottlieb et al., 2006; Olsen et al., 2005).

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A joint effort by the USDA Food Safety Inspection Service and the Food and Drug Administration in 2003 was performed to assess the relative risk to the public of foodborne listeriosis from RTE foods. Out of twenty-three different RTE food categories, the report ranked RTE deli meats as the highest relative risk food category for contracting listeriosis on both a per serving basis and per annum basis. This ranking was given based on data that deli turkey meats constitute a high number of servings per year. Although deli meats have a low level of contamination at retail, those products are sometimes stored at refrigeration temperatures long enough to allow the listerial cells to multiply to a significant level become quite high (CFSAN 2003). This report also identified a need for infection studies to model the relative risk of individual foods for the transmission of foodborne listeriosis.

An unanswered question regarding the 2000 and 2002 outbreaks and the risk assessment report is whether *L. monocytogenes* encountered environmental stimuli, while growing on RTE turkey meat, which influenced its virulence. *L. monocytogenes* is very responsive to environmental conditions such as pH, temperature, and salinity (Jaradat and Bhunia, 2002; Koutsoumanis et al., 2003; O'Driscoll et al., 1996; Ripio et al., 1996; Sue et al., 2004) and produces regulatory molecules that alter expression of virulence determinants in response to such stimuli (Cotter et al., 1999; Sue et al., 2004). Perhaps these changes enable the listerial cells to survive in the harsh gastrointestinal environment, and translocate more efficiently across the intestinal mucosa, from where they can further disseminate and cause systemic disease. Our laboratory recently found that listerial cells grown on sliced turkey meat at refrigeration temperatures become more resistant to inactivation by synthetic gastric fluid compared to listerial cells grown in brain heart infusion broth (Peterson et al., 2007). The present study evaluated whether growth of *L. monocytogenes* on sliced RTE turkey meat, or in emulsified turkey meat, influenced the severity of gastrointestinal listeriosis using inoculation of genetically susceptible A/J mice (Czuprynski et al., 2003a,b).

2. Materials and methods

2.1. Inoculation of deli turkey meat with *L. monocytogenes*

Two deli turkey meat products were used for this study. The label for the whole breast product stated it contained: turkey meat, turkey broth, less than 2% salt, and sodium phosphates. The processed turkey meat product contained: turkey breast, water, corn syrup, modified corn starch, 2% or less salt, sodium phosphate, sodium erythrobate, sodium nitrite and flavoring. This study used *L. monocytogenes* strain F2365 (Linnan et al., 1988), that was previously demonstrated to be virulent in the A/J mouse model (Faith et al., 2005a). For all experiments, *L. monocytogenes* strain F2365 was thawed, inoculated into BHI, and incubated overnight at 37 °C with shaking until stationary phase growth was reached. The bacterial suspension was then serially diluted in sterile phosphate buffered saline (PBS) to the desired concentration. To inoculate sliced turkey meat, two slices of whole muscle (approximately 35 g per two slices) or processed turkey (approximately 28 g per two slices) meat were aseptically placed in commercial grade food packaging plastic bags and inoculated with 3 mL of the *L. monocytogenes* inoculum (final conc. 10^3 CFU/mL). Inoculated bags were then vacuum packed, heat sealed, and incubated at 10 °C for up to 12 days. For emulsified turkey meat experiments, 25 g of sliced whole muscle turkey meat was

homogenized with PBS at a 1:4 ratio (w/v). The supernatant was decanted and the remaining slurry of emulsified turkey meat was poured into two 50 mL polycarbonate tubes and inoculated with 3 mL of the prepared *L. monocytogenes* inoculum (final conc. 10^3 CFU/mL), and incubated at 10 °C for up to 12 days. As a control, 1 mL of the *L. monocytogenes* inoculum was placed into two 15 mL conical tubes containing 9 mL BHI broth (final concentration 10^3 CFU/mL) and incubated at 10 °C for up to 12 days. Growth of *L. monocytogenes* was monitored by daily removal of 0.5 mL fluid from the inoculated meat or BHI broth and subsequent dilution and plating on blood agar (trypticase soy agar with 2% sheep's blood agar; BBL, Becton Dickinson, Franklin Lakes, N.J.) (BAP).

2.2. Inoculation of mice

Female A/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 5–6 weeks of age and housed under microisolator caps at the School of Veterinary Medicine animal care facility at the University of Wisconsin, Madison. Mice were acclimated to these conditions for at least 1 week before use in experiments. Food was removed 5 h prior to intragastric inoculation to ensure the stomachs of the mice were not engorged with feed that could interfere with delivery of the listerial inoculum. Mice were anesthetized by i.p. injection with sodium pentobarbital (Abbott, Abbott Park, IL) at 1 mg per 25 g mouse and then inoculated i.g. with stationary phase *L. monocytogenes* cells (10^6 CFU suspended in a total volume of 0.1 mL PBS per mouse) as described previously (Czuprynski et al., 2003a,b). For those inocula where the listerial cells were grown in a slurry of turkey meat, the suspension was shaken with a vortex-mixer, but not centrifuged, before dilution in PBS. Mice were euthanized at the peak of the bacterial burden (day 3) by asphyxiation with CO₂, followed by exsanguination. Blood was collected into a syringe containing 0.4% sodium citrate (final concentration) as anticoagulant. The blood was then serially diluted in sterile PBS, plated in duplicate on BAP and the plates were incubated for up to 48 h at 37 °C. The number of colonies were counted and used to estimate the CFU/mL of viable *L. monocytogenes* during bacteremia. The abdominal cavity was opened aseptically and portions of the liver and spleen were removed. These were weighed and placed into 15 mL homogenizing tubes (Wheaton Science Products, Millville, NJ) containing 1 mL sterile PBS. The gallbladder and cecum were removed and placed into separate tubes containing 1 mL sterile PBS. Tissues were homogenized, diluted in sterile PBS and plated in duplicate on BAP, except for the cecal homogenates which were plated on modified Oxford agar plates consisting of Oxford Listeria Agar (Alpha Biosciences, Baltimore, MD), 1% w/v colistin, and 1% w/v moxalactam. Plates were allowed to dry and then incubated at 37 °C for up to 48 h. Colonies were enumerated and expressed as the mean $\pm$ S.E.M. $\log_{10}$ CFU *L. monocytogenes* per gram tissue.

3. Results and discussion

L. monocytogenes grown on processed sliced turkey grew at a similar rate as listerial cells in BHI, reaching final bacterial concentrations of approximately 10^9 CFU/mL (Fig. 1). *L. monocytogenes* cells grew nearly as well when inoculated onto sliced whole muscle turkey meat (not shown), or in emulsified whole muscle turkey meat, reaching final bacterial concentrations of approximately $8.5 \log_{10}$ CFU/mL (Fig. 1). When A/J mice were inoculated

i.g. with listerial cells grown to stationary phase on whole muscle sliced turkey meat (Fig. 2), or in emulsified turkey meat (Fig. 3), the CFU of *L. monocytogenes* recovered from tissues of the mice were not significantly different than those recovered from mice inoculated i.g. with *L. monocytogenes* cells grown in BHI broth ($p>0.05$). Likewise, the CFU of *L. monocytogenes* recovered from the tissues of mice inoculated i.g. with stationary phase listerial cells grown on processed turkey slices did not differ ($p>0.05$) from mice inoculated with listerial cells grown in BHI broth (Fig. 4). We also examined whether logarithmic phase listerial cells (8 days of growth at 10 °C) grown on turkey meat or in BHI broth would differ in virulence for i.g. inoculated A/J mice. Here too we found similar results (i.e. no difference between listerial cells grown on turkey or in BHI broth) to those obtained with mice inoculated i.g. with stationary phase listerial cells (data not shown).

In the experiments described above, the *L. monocytogenes* cells were delivered i.g. in PBS, which might buffer the acidity of the murine stomach. We were concerned whether this buffering effect might reduce the barrier imposed by the acidic conditions of the stomach. Therefore, we replicated these experiments using a listerial inoculum delivered in unbuffered isotonic saline. However, the results obtained were similar to those using PBS, and did not demonstrate a difference between listerial cells grown on RTE turkey meat or in BHI broth (data not shown).

The data presented here indicate that *L. monocytogenes* F2365 did not become more virulent for i.g. inoculated A/J mice when grown on turkey meat than when grown in BHI broth. It should be noted that these experiments were conducted with two specific commercially prepared turkey products. These results should not be extrapolated to other commercial products without additional testing. It is also possible that other *L. monocytogenes* strains might respond differently. Alternatively, perhaps our mouse model did not provide a full assessment of virulence mechanisms of *L. monocytogenes* F2365. However, a study similar to the work presented here found that growth of meat were more resistant to inactivation in synthetic gastric fluid in vitro than were listerial cells grown in BHI broth (Peterson et al., 2007). Exposure of *L. monocytogenes* to the osmotic stress of increased NaCl concentrations induced expression of several genes related to virulence (Garner et al., 2006). It is also reported that growth of *L. monocytogenes* in the presence of increased NaCl at 37 °C, but not 7 °C, increased the ability of the listerial cells to invade Caco-2 human intestinal epithelial cells in vitro (Sue et al., 2004). Finally, it should be noted that the results of the present study reflect events that occur in the murine gastrointestinal tract and may differ to some extent from those that occur in the human gastrointestinal tract.

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Fig. 1.

Growth curves of *L. monocytogenes* F2365 inoculated (10^3 CFU/ml) in BHI broth, onto RTE sliced processed deli turkey meat, or into emulsified whole muscle turkey breast meat and then incubated at 10 °C for 12 days. Samples were aseptically removed at daily intervals and plated on blood agar or modified Oxford agar. Results are presented as the mean $\pm$ S.E.M. from one representative experiment of 3 separate experiments performed (two replicates for each growth condition).

Fig. 2.

L. monocytogenes F2365 cells grown in BHI broth (closed bars) or on sliced whole muscle turkey breast (open bars) for 12 days at 10 °C do not differ in their ability to infect A/J mice when given intragastrically. Results are the mean $\pm$ S.E.M. of two independent experiments ($n = 12$ per experimental group). The limit of detection for the gallbladder was 10^3 CFU per gram and for all other tissues was 10^1 CFU per g tissue. Tissues having no detectable *L. monocytogenes* colonies were given a value of 2.95 or 0.95 log¹⁰ CFU per g tissue, respectively.

Fig. 3.

L. monocytogenes F2365 cells grown in BHI broth (closed bars) or in emulsified whole muscle turkey (open bars) for 12 days at 10 °C do not differ in their ability to infect A/J mice when given intragastrically. Results are the mean $\pm$ S.E.M. of three independent experiments ($n = 18$ per experimental group). The limit of detection for the gallbladder was 10^3 CFU per gram and for all other tissues was 10^1 CFU per g tissue. Tissues having no detectable *L. monocytogenes* colonies were given a value of 2.95 or 0.95 $\log_{10}$ CFU per g tissue, respectively.

Fig. 4.

L. monocytogenes F2365 cells grown in BHI (closed bars) or on sliced processed turkey meat (open bars) for 12 days at 10 °C do not differ in their ability to infect A/J mice when given intragastrically. Results are the mean $\pm$ S.E.M. of two independent experiments (n = 12 per experimental group). The limit of detection for the gallbladder was 10³ CFU per gram and for all other tissues was 10¹ CFU per g tissue. Tissues having no detectable *L. monocytogenes* colonies were given a value of 2.95 or 0.95 log₁₀ CFU per g tissue, respectively.