Received: 3 March 2021 | Revised: 9 August 2021 | Accepted: 4 September 2021

DOI: 10.1111/jam.15294

Journal of Applied Microbiology

ORIGINAL ARTICLE

Competitive growth kinetics of *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* with enteric microflora in a small-intestine model

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Funding information
Food Safety Commission, Cabinet Office, Government of Japan, Grant/Award Number: 1802 and 2004

Abstract

Aims: The biological events occurring during human digestion help to understand the mechanisms underlying the dose–response relationships of enteric bacterial pathogens. To better understand these events, we investigated the growth and reduction behaviour of bacterial pathogens in an *in vitro* model simulating the environment of the small intestine.

Methods and Results: The foodborne pathogens *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 were cultured with multiple competing enteric bacteria. Differences in the pathogen's growth kinetics due to the relative amount of competing enteric bacteria were investigated. These growth differences were described using a mathematical model based on Bayesian inference. When pathogenic and enteric bacteria were inoculated at 1 log CFU per ml and 9 log CFU per ml, respectively, *L. monocytogenes* was inactivated over time, while *C. jejuni* and *E. coli* O157:H7 survived without multiplying. However, as pathogen inocula were increased, its inhibition by enteric bacteria also decreased.

Conclusions: Although the growth of pathogenic species was inhibited by enteric bacteria, the pathogens still survived.

Significance and Impact of the Study: Competition experiments in a small-intestine model have enhanced understanding of the infection risk in the intestine and provide insights for evaluating dose–response relationships.

KEYWORDS

bacterial competition, *Campylobacter jejuni*, dose–response, enteric bacteria, *Escherichia coli* O157:H7, *in vitro* intestinal model, *Listeria monocytogenes*, quantitative microbial risk assessment

INTRODUCTION

Quantitative microbial risk assessment (QMRA) plays a major role in ensuring microbial food safety. The dose–response model, an important element in QMRA, helps to characterize the risk of illness linked to the presence of foodborne pathogens (Buchanan et al., 2000; Codex Alimentarius Commission, 1999; FAO/WHO, 2003, 2021). However, the data available for the development of such dose–response models are limited. For example during an

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outbreak, it is difficult to find the food of the outbreak origin and accurately estimate the ingested bacterial count/concentration, owing to the quick digestion of the contaminated food and postincident bacterial count fluctuations. Although the dose–response relationship data obtained from human volunteer feeding trials seem to be realistic, only high-dose responses are usually visible in healthy young subjects. These high-dose responses need to be extrapolated to estimate low-dose–response relationships. However, a precise and realistic QMRA requires a more accurate measure of low-dose–response relationships.

An analytical approach that provides insight into low-dose–response relationships based on the biological events occurring due to exposure to bioactive substances that cause adverse health effects has gained attention (Abe, Koyama, & Koseki, 2021; Koseki, Mizuno, et al., 2011; Miszczycha et al., 2014). The key events dose–response framework (KEDRF) has been proposed (Buchanan et al., 2009; Julien et al., 2009) to examine the major events occurring in the body from the entry of a bioactive substance until the first appearance of symptoms; at each step of digestion, factors including dose, pathogen's characteristic and protective host mechanisms are examined. One recent study has indicated the validity of the KEDRF concept based on the epidemic data of *C. jejuni* (Abe, Takeoka, et al., 2021). The use of the KEDRF fills the gap in our knowledge of digestive phenomena that providing insight into the low-dose–response relationships.

Gaining knowledge of these internal mechanisms will help reduce the reliance on extrapolation. For example Buchanan et al. (2009) presented five key events occurring during *Listeria* infection: (1) survival in the upper gastrointestinal tract, (2) establishment in the intestine, attachment to and uptake into the epithelial cells, (3) survival and escape from phagosomes in enterocytes and transfer of to phagocytes, (4) transmission across the placenta and (5) pathogen growth leading to fetal morbidity and mortality. Developing and combining the mathematical prediction model for these infection processes will improve the accuracy of the dose–response relationship data.

The establishment of foodborne pathogens in the intestine plays an important role in disease pathogenesis. Pathogens ingested via meals pass through the stomach, reach the intestinal tract and invade epithelial cells. The first step of infection in the intestine is the attachment of foodborne pathogens to epithelial cells (Boyle & Finlay, 2003). However, the human intestinal tract contains more than 500 different indigenous microbial flora, which constitute a heterogeneous microbial ecosystem (Berg, 1996). Experiments in murine models have shown that resident microflora compete with the growth of invading *Salmonella* in intestinal tracts (Stecher et al., 2007). Therefore, bacterial pathogens that reach the small intestine must survive and compete with indigenous bacteria. The behaviour of foodborne pathogens in the intestinal tract increases their ability to invade epithelial cells. Shedding light on the changes in bacterial pathogen growth during their competition with small-intestinal microflora for behaviour will contribute to understanding the infectious mechanisms of bacterial pathogens in the context of the KEDRF concept.

The purpose of this study was to model the competitive effects of intestinal microflora on the growth of foodborne pathogenic bacteria invading the human small intestine. We co-cultured small-intestine microflora along with pathogenic bacteria in an *in vitro* small-intestine environment. This experimental system enabled the evaluation of different ratios of the concentration of the target pathogenic bacteria divided by the concentration of the competing microflora at initial stage, as well as quantification of their effects on the competitive behaviour of pathogen and microflora. *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* O157:H7—three of the major foodborne infectious pathogenic bacteria worldwide (WHO, 2020)—were examined in an environment with competing microflora. Since the growth kinetics include numerical variance such as microbiological variations and uncertainties, the parameters of the primary growth model were assessed using Bayesian inference. Bayesian inference has been used to achieve the evaluation of a probability distribution (Delignette-Muller et al., 2006; Pouillot et al., 2003; Quinto et al., 2018). Therefore, the predictive model in this study used a parameter estimation based on the Bayesian statistics.

MATERIALS AND METHODS

Simulation of small-intestine fluid

As a model to reproduce the competition in the small intestine, simulated intestinal fluid was prepared as described in a previous study (Suzuki et al., 2013) which indicates no significant influence on the growth of *Lactobacillus brevis* due to the presence of enzymes (pepsin, trypsin and pancreatin). Furthermore, preliminary experiments of this study showed that there was no significant difference in all the bacteria tested in this study in terms of growth due to the presence of the enzymes (data not shown). The composition of the Simulated Intestine Fluid: SIF was: 5.9% (w/v) Gifu anaerobic medium broth powder (Nissui Pharmaceutical Corporation) and 0.3% (w/v) bile salts (Oxoid) diluted by pure water.

Enteric bacteria

For all the pathogenic bacteria tested except *E. coli* O157:H7, nine species of enteric bacteria were used:

Bacillus cereus ATCC 10987, *Deinococcus radiodurans* ATCC BAA-816, *Enterococcus faecalis* ATCC 47077, *E. coli* ATCC 700926, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus mutans* ATCC 700610, *Lactobacillus acidophilus* JCM 1132, *L. brevis* JCM 1059 and *Lactobacillus gasseri* ATCC 33323. For *E. coli* O157:H7, six bacteria species were used: *B. cereus* ATCC 10987, *D. radiodurans* ATCC BAA-816, *S. epidermidis* ATCC 12228, *L. acidophilus* JCM 1132, *L. brevis* JCM 1059 and *L. gasseri* ATCC 33323, because the growth of these three strains was not inhibited on selective agar for *E. coli* O157:H7 (CT-SMAC agar). Human faecal isolates of *L. acidophilus* and *L. brevis* were acquired from the Japan Collection of Microorganisms (Tsukuba, Japan). The remaining seven species, suitable for the intestinal environment, were selected from the human microbiome MSA-2003 provided by the American Type Culture Collection (Manassas, Virginia). *Lactobacillus* spp. were stored in de Man-Rogosa-Sharpe broth (MRS broth; Merck) containing 10% glycerol, while the other strains were stored in tryptic soy broth (TSB; Merck) containing 10% glycerol at −80°C.

Lactobacillus spp. were activated by incubating for 48 h on de Man-Rogosa-Sharpe agar (MRS agar; Merck) and the other strains for 24 h on tryptic soy agar (TSB; Merck), at 37°C. This was followed by two incubations in simulated intestinal fluid for the respective time periods and at the same temperature indicated above. After washing by SIF thrice and centrifuging at 3000 *g* for 10 min, the bacteria were diluted in SIF to different concentrations (Power et al., 2014): 4 and 9 log CFU per ml for the competition experiments in jejunum and ileum respectively.

Foodborne pathogens

We used 11 strains of *C. jejuni* (RIMD 0366026, RIMD 0366027, RIMD 0366028, RIMD 0366029, RIMD 0366042, RIMD 0366043, RIMD 0366044, RIMD 0366048, RIMD 0366049, RIMD 0366050 and RIMD 0366051), six strains of *L. monocytogenes* (ATCC 19111, ATCC 19117, ATCC 19118, ATCC 13932, ATCC 15313 and ATCC 35152), and four strains of *E. coli* O157:H7 (HIPH 12361, RIMD 0509939, RIMD 05091896 and RIMD 05091897). *Campylobacter jejuni* was stored in Bolton broth (Oxoid) containing 10% glycerol and the other strains were stored in TSB containing 10% glycerol at −80°C. *Campylobacter jejuni* was activated by incubating at 42°C for 48 h on Preston agar (Oxoid) under microaerophilic conditions (6%–12% O_2, 5–8% CO_2) with Anaero Pack MicroAero (Mitsubishi) followed by two incubations in Bolton broth under the same conditions. *Listeria monocytogenes* and *E. coli* O157:H7 were activated by incubating at 37°C for 24 h on TSA, followed by two incubations in TSB under the same conditions. After incubation, the bacterial cells were washed using SIF as described above for the intestinal bacteria. The bacteria were diluted in SIF to different concentrations (1, 2 and 4 log CFU per ml) during the competition experiments.

Competition assays

Concentration levels of 1, 2 and 4 log CFU per ml for foodborne pathogens and 4 and 9 log CFU per ml for enteric bacteria were used in competition assays. To determine the bacterial counts of pathogens and microflora, each combination of regulated bacterial solution was inoculated separately into SIF. This suspension was incubated at 37°C under microaerophilic conditions. Incubation times were 0, 6, 12, 24, 36, 48, 60 and 72 h. Bacteria at each incubation time were counted to calculate the number of colonies counting on plates as the colony-forming units: CFU. The experiment in each condition was conducted in triplicate.

Selective agar media were used to distinguish between the colonies of the food-poisoning bacteria and intestinal bacteria. These media were Preston agar for *C. jejuni*, CHROMagar Listeria base (CHROMagar, Paris, France) for *L. monocytogenes* and CT-SMAC agar (Merck) for *E. coli* O157:H7. *C. jejuni* was incubated in Preston agar at 42°C for 48 h under microaerophilic conditions with Anaero Pack MicroAero, while *L. monocytogenes* and *E. coli* were incubated in CHROMagar Listeria base and CT-SMAC agar, respectively, at 37°C for 24 h. To calculate concentrations for the enteric bacteria, diluted cultures were plated on TSA and incubated for 24 h at 37°C. After incubation, the concentrations for the food poisoning bacteria were subtracted from the total numbers of colonies counted on TSA to quantify the concentrations of the enteric bacteria.

Modeling competitive growth kinetics using Bayesian inference

To describe mathematically the behaviour of both pathogenic and competing intestinal bacteria, this study employed the Baranyi model (Baranyi & Roberts, 1994) using the following equation (Baty & Delignette-Muller, 2015):

$$\log_{10}N = \log_{10}N_{\max} + \log_{10}\frac{-1 + e^{\mu_{max}\times\lambda} + e^{\mu_{max}\times t}}{e^{\mu_{max}\times t} - 1 + e^{\mu_{max}\times\lambda}\times 10^{(\log_{10}N_{max} - \log_{10}N_0)}}$$

where $\log_{10}N_{\max}$ (log CFU per ml), $\log_{10}N_0$ (log CFU per ml), μ_{max} (h^{-1}) and λ (h) represent maximum population density (MPD), number of initial bacteria, maximum growth rate

and lag time respectively. In addition, Bayesian inference was used to describe the growth behaviour that showed variability and uncertainty. Bayesian analysis has been incorporated in previous studies as an effective method for handling data with disparate behaviour (Powell et al., 2006; Quinto et al., 2018) because it can estimate parameters as distributions which is not possible in classical parameter estimation. To derive posterior parameter distributions, Markov chain Monte Carlo (MCMC) algorithm sampling was conducted using the No-U-Turn sampler. Inferences were conducted using 3.5×10^3 iterations with four independent chains after a warmup step of 1.0×10^3 iterations. We adopted a normal distribution with the standard deviation σ for describing the observed bacterial count: $\log_{10} N_{(t,i)}$ where t denotes the cultured time duration and i the index for experimental iterations ($i = 1, 2, 3$). The following predictive model equation was used for describing each strain's growth behaviour:

$$\log_{10} N_{(t,i)} \sim \text{Normal}\left(\log_{10} N_{\max} + \log_{10} \frac{-1 + e^{\mu_{\max} \times \lambda} + e^{\mu_{\max} \times t}}{e^{\mu_{\max} \times t} - 1 + e^{\mu_{\max} \times \lambda} \times 10^{(\log_{10} N_{\max} - \log_{10} N_0)}}, \sigma\right)$$

The parameters: $\log_{10} N_0$, $\log_{10} N_{\max}$, $\mu_{\max}$, λ and σ were estimated in each condition. The above calculations were performed using Python package: Pystan (version 2.19) under the Anaconda distribution (Python 3.7.5).

RESULTS

Growth of *C. jejuni* with enteric bacteria

The growth kinetics of *C. jejuni* in competition with enteric bacteria with stochastic variation estimated by Bayesian inference are shown in Figure 1. The Gelman–Rubin convergence statistic (R-hat value), which is an indicator of parameter convergence, converged within 1.1 for all parameters. In other words, the Bayesian estimation with the input of the Baranyi model successfully converged with the posterior distribution of the data in this study (Table 1). Figure 1 depicts the changes in bacterial numbers over time in competitive environments, based on the estimated parameters and experimental results. The solid line, the dark colour band and the light colour band indicate the median, the 50th percentile interval of the MCMC sample and the 80th percentile interval respectively. The variations in our growth kinetic data were successfully described using the probability distributions of these parameters.

The pH in the culture medium during culturing was approximately 6.8 and there were no significant changes during the incubation period. We found that the MPD of *C. jejuni* decreased owing to the competition with enteric bacteria. The MPD decreased with increasing differences in the initial population of *C. jejuni* and enteric bacteria. For example when the initial cell number of enteric bacteria was 4 log CFU per ml (Figure 1a–c), and the initial competing ratio of bacterial count of *C. jejuni* to enteric bacteria was 1, 10^{-2} and 10^{-3}, the MPD decreased to 6 log CFU per ml, 4 log CFU per ml and 3 log CFU per ml, respectively, after 72 h. In contrast, the MPD of a monoculture of *C. jejuni* reached 8 log CFU per ml. Growth inhibition was more pronounced when the initial enteric bacteria inoculum was 9 log CFU per ml. When the initial ratio of *C. jejuni* to enteric bacteria was low (10^{-5}, 10^{-7} and 10^{-8}) (Figure 1d–f), the growth of *C. jejuni* was suppressed after 72 h of incubation, with a maximum increase of 10^2-fold from initial cell numbers. In other words, the growth of *C. jejuni* was greatly affected by the initial number of enteric bacteria.

Growth of *L. monocytogenes* with enteric bacteria

Figure 2 shows competition between *L. monocytogenes* and enteric bacteria with stochastic variation estimated by Bayesian inference. The Bayesian estimation with the input of the Baranyi model successfully converged with the posterior distribution of the data of *L. monocytogenes* as summarized in Table 2. The variations in the growth kinetic data were successfully described using the probability distributions of these parameters.

Although the growth of *L. monocytogenes* was delayed by competing enteric bacteria, a reduction in MPD was not observed at any ratio (Figure 2a–e) except when the initial competing ratio was 10^{-8} (Figure 2f). When the initial ratio of *L. monocytogenes* to enteric bacteria was 10^{-7} or higher, *L. monocytogenes* exhibited growth within 72 h without undergoing cell death. The limit of detection was 0.33 log CFU per ml. In all monoculture experiments, *L. monocytogenes* grew by 8 log CFU per ml within 24 h and then declined rapidly. The pH of the environment was approximately 6.8 during culturing period as well as in the case of *C. jejuni* culturing.

Growth of *E. coli* o157:H7 with enteric bacteria

Figure 3 shows the results of competition between *E. coli* O157:H7 and enteric bacteria with stochastic variation estimated by Bayesian inference. The Bayesian estimation with the input of the Baranyi model successfully

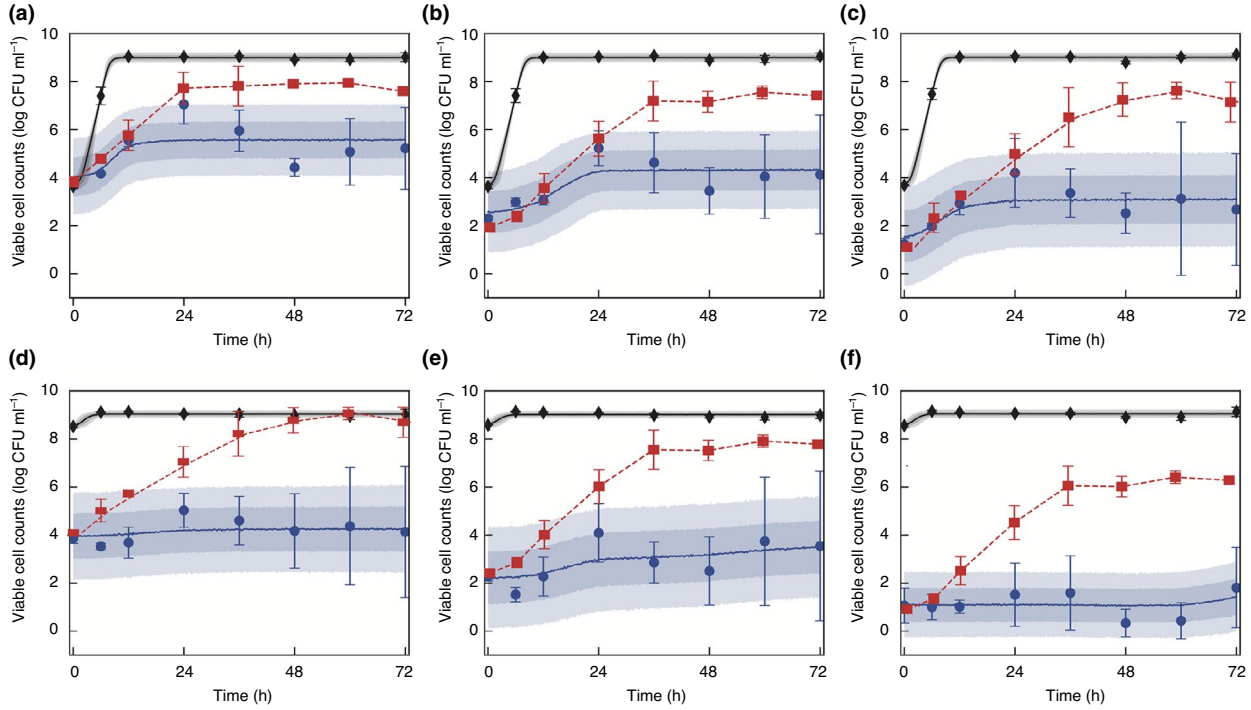

FIGURE 1 Estimated (curves and ranges) and observed variation in growth of *Campylobacter jejuni* (●) and intestinal bacteria (◆) co-cultured in artificial small-intestine fluid at 37°C under micro-aerophilic conditions. The solid line represents the median, the dark blue range represents 50% prediction intervals, and the light blue range represents 80% prediction intervals. Median and prediction intervals were obtained by Bayesian estimation using 1.0×10^4 Monte Carlo simulation. *Campylobacter jejuni* monoculture (■) was used as control. The initial bacterial count were set as followings, (a) *C. jejuni*: 4 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (b) *C. jejuni*: 2 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (c) *C. jejuni*: 1 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (d) *C. jejuni*: 4 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (e) *C. jejuni*: 2 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (f) *C. jejuni*: 1 log CFU per ml; intestinal bacteria: 9 log CFU per ml. The error bars indicate the standard errors of measurement in triplicate trials

converged with the posterior distribution of the *E. coli* O157:H7 data as summarized in Table 3.

The pH in the culture medium was approximately 6.8 as well as other two pathogens' culturing. The growth of *E. coli* O157:H7 was not affected by competing enteric bacteria in most cases. As the initial ratio of *E. coli* O157:H7 to enteric bacteria increased, the growth of *E. coli* O157:H7 slowed. In particular, when the initial ratio of *E. coli* O157:H7 to enteric bacteria was 10^{-7} and 10^{-8}, *E. coli* O157:H7 did not grow in 72 h of incubation and did not undergo cell death. A small amount of *E. coli* O157:H7 always survived after coculture for 72 h despite the competition (Figure 3a–f).

DISCUSSION

The competitive behaviour of each foodborne pathogen with enteric bacteria, as derived from our *in vitro* model, showed their own growth characteristics of each pathogen (Figures 1–3). The reason for the difference in growth behaviour could be due to the differences in the bacterial growth rate, although physiological characteristics of bacteria and interactions between bacteria are also possible causes. Looking at the growth of the monoculture, *E. coli* O157:H7 was the fastest and *C. jejuni* was the slowest. When the concentration of enteric bacteria was 4 log CFU per ml, the pathogens appear to grow strongly in the order of their growth rate in the monoculture. Previously, Buchanan and Bagi (1997, 1999) reported that the degree of inhibition depends on the relative growth ratio of the competing bacteria. As shown in Tables 1–3, the μ_{max} of *E. coli* O157:H7, *L. monocytogenes and C. jeuni* in the case of a monoculture was 2.05, 1.65 and 0.6 h^{-1} respectively. Therefore, the differences in growth rates observed in our model may influence the behaviours in human intestinal tracts.

The incubation period for campylobacteriosis is relatively long (2–7 days) (Evans et al., 1996), which can be attributed to the late onset of growth of *C. jejuni*. After passing through the stomach, foodborne pathogens enter the jejunum, where the enteric bacterial count is 10^3–5 log CFU per ml (Power et al., 2014). Our study showed that, when 2 log CFU per ml of *C. jejuni C. jejuni*

TABLE 1 Estimated parameters of the Baranyi model for the growth of *Campylobacter jejuni* competition with enteric bacteria by Bayesian inference

Number of initial bacteria (Enteric bacteria: *C. jejuni*)	$\log_{10}N_{max}$ (log CFU per ml)			$\log_{10}N_0$ (log CFU per ml)			μ_{max} (h^{-1})			λ (h)		
	Mean[a]	SD[b]	Rhat	Mean	SD	Rhat	Mean	SD	Rhat	Mean	SD	Rhat
10^4: 10^4	5.60	0.40	1.0	4.04	0.54	1.0	1.57	1.00	1.0	10.17	11.40	1.0
10^4: 10^2	4.33	0.33	1.0	2.57	0.49	1.0	1.54	1.01	1.0	13.69	8.85	1.0
10^4: 10^1	3.10	0.38	1.0	1.55	0.67	1.0	1.55	1.01	1.0	12.13	14.07	1.0
10^9: 10^4	4.32	0.62	1.0	3.96	0.45	1.0	1.47	1.05	1.0	32.77	21.77	1.0
10^9: 10^2	3.60	0.70	1.0	2.22	0.50	1.0	1.48	1.07	1.0	29.40	19.95	1.0
10^9: 10^1	1.72	1.16	1.0	1.11	0.34	1.0	1.43	1.06	1.0	47.62	22.27	1.0
C. jejuni in monoculture of 10^4	8.08	0.12	1.0	4.03	0.26	1.0	0.60	0.27	1.0	3.48	2.43	1.0
C. jejuni in monoculture of 10^2	7.91	0.15	1.0	2.32	0.25	1.0	0.45	0.06	1.0	3.89	2.13	1.0
C. jejuni in monoculture of 10^1	7.79	0.24	1.0	1.60	0.32	1.0	0.40	0.07	1.0	2.34	2.23	1.0

[a]Mean value of estimated by 1.0×10^4 Monte Carlo simulation.
[b]Standard deviation of the estimated by 1.0×10^4 Monte Carlo simulation.

was co-cultured with 4 log CFU per ml of enteric bacteria, the exponential growth of *C. jejuni* began after an average lag phase of 13 h (Table 1), and after 24 h, the growth was stopped. However, the enteric bacteria did not have any bactericidal effect on *C. jejuni*. Therefore, campylobacteriosis is likely to be caused by surviving *C. jejuni* that establishes in the intestinal tract and slowly proliferates.

The ability of *L. monocytogenes* to grow to 8 log CFU per ml in a competitive environment suggests a high risk of infection in the intestinal tract. Monocultures of *L. monocytogenes* grew rapidly and then entered a decline phase after about 36 h. The growth rate of *L. monocytogenes* in co-culture was slower than that in monoculture, but *L. monocytogenes* in co-culture survived longer in the medium. Interactions with other bacteria may be delaying the onset of the decline phase. Listeriosis is often caused by a large intake of *L. monocytogenes* for normal adults. Aureli et al. (2000) reported disease onset at 24 h after ingestion of 10^6 CFU per g of *L. monocytogenes* in food containing corn. Although the risk of infection is considered to be low at low doses, Carrique-Mas et al. (2003) reported listeriosis following the consumption of raw milk cheese containing 10^1–10^7 CFU per g of *L. monocytogenes* after an incubation period of 1–15 days. In this study, we observed significant growth in 72 h, when the difference in bacterial counts of *L. monocytogenes* and enteric bacteria was less than 8 log CFU per ml. Even when the number of invading *L. monocytogenes* were few, if *L. monocytogenes* survived in a gut for a certain period of time, the survival *L. monocytogenes* might grow within a few days. Therefore, it may be necessary to consider the increased risk of infection associated with the long-term establishment of *L. monocytogenes* in the intestine.

The ability of *E. coli* O157:H7 to grow to high numbers in the intestinal environment in a short time period may be linked to its low infectious dose. Strachan et al. (2005) reported *E. coli* O157: H7 infection following the ingestion of only 10–100 cells, a tiny number relative to the infectious doses of other food-poisoning bacteria. Proliferation in the small-intestine environment is a key event that occurs following survival in the gastric acid environment. Koseki, Takizawa, et al. (2011) reported that *E. coli* O157:H7 has more tolerance for gastric juices than *L. monocytogenes*, allowing living *E. coli* O157:H7 to pass into intestines. In our study, in competition with 4 log CFU per ml of enteric bacteria (such as the jejunum), *E. coli* O157:H7 grew immediately to approximately 6 log CFU per ml within 12 h, even if its number of invasions was approximately 1 log CFU per ml (Figure 3c). The growth of *E. coli* O157:H7 was faster than that of *C. jejuni* and *L. monocytogenes*, suggesting higher risk of infection at low doses compared to other foodborne pathogens.

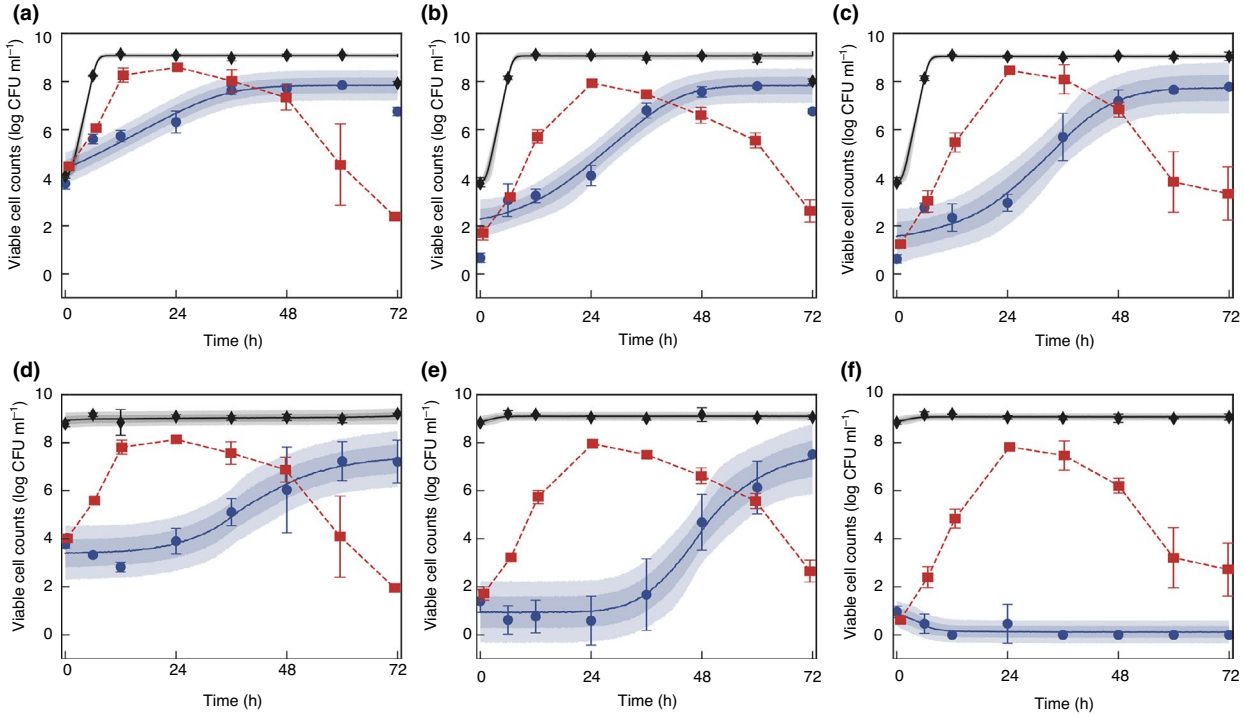

FIGURE 2 Estimated (curves and ranges) and observed variation in growth of *Listeria monocytogenes* (●) and intestinal bacteria (◆) co-cultured in artificial small-intestine fluid at 37°C under micro-aerophilic conditions. The solid line represents the median, the dark blue range represents 50% prediction intervals, and the light blue range represents 80% prediction intervals. Median and prediction intervals were obtained by Bayesian estimation using 1.0×10^4 Monte Carlo simulation. *Listeria monocytogenes* monoculture (■) was used as control. The initial bacterial count were set as followings, (a) *L. monocytogenes*: 4 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (b) *L. monocytogenes*: 2 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (c) *L. monocytogenes*: 1 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (d) *L. monocytogenes*: 4 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (e) *Listeria monocytogenes*: 2 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (f) *L. monocytogenes*: 1 log CFU per ml; intestinal bacteria: 9 log CFU per ml. The error bars indicate the standard errors of measurement in triplicate trials

The results of our competition experiments revealed that differences between the numbers of pathogens and enteric bacteria had an inhibitory effect on the foodborne pathogens. Previous studies have reported similar growth inhibition. Mellefont et al. (2008) reported that when the competition was initiated by a difference in the initial number of bacteria, in most cases the numerically dominant population inhibited the growth of competitors. Similarly, Al-Zeyara et al. (2011) reported that when *L. monocytogenes* competed for growth with the aerobic plate count (APC) of food, the higher initial APC resulted in the lower *L. monocytogenes* counts at 24 h, which is a significant correlation.

The Jameson effect, which is the inhibitory effect of a dominant bacterium on a small number of competing bacteria in a co-culture environment, has been reported in multiple studies (Buchanan & Bagi, 1997, 1999; Carlin et al., 1996; Devlieghere et al., 2001; Jameson, 1962; Komitopoulou et al., 2004). We observed the Jameson effect in *C. jejuni* (Figure 1) and *E. coli* O157:H7 only under certain conditions (Figure 3e,f). However, the MPD did not decrease, and in most cases, growth resumption was observed on prolonged incubation, as observed in some previous studies (Koseki, Mizuno, et al., 2011; Koseki, Takizawa, et al., 2011; Mellefont et al., 2008; Ongeng et al., 2007). Accordingly, the Jameson effect might appear in rare cases in which factors such as differences in the numbers of bacteria, bacterial growth rate, competition for nutrients, oxidative stress and bacterial gene induction are involved.

Several studies on bacterial competition have reported a reduction in the number of pathogenic bacteria due to lower pH induced by the production of bacteriolysins by *Lactobacillus* (Gálvez et al., 2007; Quinto et al., 2016). In contrast, in this study, although three strains of human-derived lactic acid bacteria (*L. acidophilus*, *L. brevis* and *L. gasseri*) were used as enteric bacteria, the pH of the experimental environment was maintained at approximately 6.8. The pooled duodenal pH of elderly people during meal ingestion was reported as pH 6.2–7.0 (Russell et al., 1993) and that of young people was reported as pH 6.0–6.7 (Dressman et al., 1990). The reason for this could be attributed to the fact that the growth rate of the *Lactobacillus* spp. used in this study was lower than that of other competing enteric bacteria in our model

TABLE 2 Estimated parameters of the Baranyi model for the growth of *Listeria monocytogenes* competition with enteric bacteria by Bayesian inference. In conditions where no proliferation was observed, lag was assumed to be blank

Number of initial bacteria (Enteric bacteria: *L. monocytogenes*)	$\log_{10}N_{max}$ (log CFU per ml)			$\log_{10}N_0$ (log CFU per ml)			μ_{max} (h^{-1})			λ (h)		
	Mean[a]	SD[b]	Rhat	Mean	SD	Rhat	Mean	SD	Rhat	Mean	SD	Rhat
10^4: 10^4	7.85	0.16	1.0	4.4	0.2	1.0	0.24	0.04	1.0	1.59	0.99	1.0
10^4: 10^2	7.84	0.2	1.0	2.3	0.34	1.0	0.41	0.25	1.0	8.67	6.69	1.0
10^4: 10^1	7.74	0.32	1.0	1.58	0.44	1.0	0.49	0.33	1.0	12.8	8.92	1.0
10^9: 10^4	7.48	0.68	1.0	3.41	0.28	1.0	0.45	0.49	1.0	23.91	7.73	1.0
10^9: 10^2	7.54	0.91	1.0	0.95	0.28	1.0	0.7	0.56	1.0	33.39	5.59	1.0
10^9: 10^1	0.12	0.08	1.0	0.91	0.22	1.0	1.26	0.95	1.0			
L. monocytogenes in monoculture of 10^4	7.78	0.13	1.0	3.76	0.20	1.0	1.65	0.75	1.0	3.31	1.12	1.0
L. monocytogenes in monoculture of 10^2	8.12	0.13	1.0	1.82	0.17	1.0	1.02	0.10	1.0	2.40	0.80	1.0
L. monocytogenes in monoculture of 10^1	8.21	0.18	1.0	0.86	0.23	1.0	1.01	0.12	1.0	1.85	0.98	1.0

[a]Mean value of estimated by 1.0×10^4 Monte Carlo simulation.
[b]Standard deviation of the estimated by 1.0×10^4 Monte Carlo simulation.

environment. Therefore, the production of bacteriolysins associated with the growth of *Lactobacillus* spp. was suppressed. Accordingly, these *Lactobacillus* spp. had no effect on the growth inhibition of the inoculated pathogenic bacterial species. Since a large variety of enteric bacteria exist in the intestinal environment, a more comprehensive view of bacterial populations will be necessary to provide a more lifelike intestinal environment in the future.

Previous animal experiments have presented different results than this study with regard to the behaviour of *L. monocytogenes*. Becattini et al. (2017) reported a reduction in the cell count of *L. monocytogenes* amongst intestinal bacteria in *ex vivo* mouse experiments. Inoculation of *L. monocytogenes* (2–6 log CFU·100 μl^{-1}) into the small intestine of mice resulted in a decrease in the viable *L. monocytogenes* count after incubation. In contrast, we observed that *L. monocytogenes* showed gradual growth in the presence of competing intestinal bacteria (Figure 2). This difference could be attributed to different bacterial flora and nutrient conditions in the culture environment. Regarding the effect of nutritional environment on competition, Vital et al. (2012) reported that the competitiveness of *E. coli* O157:H7 decreased when the culture medium was diluted and nutrient concentration decreased. In our study, the culture environment was nutrient-rich owing to the simulating of postprandial environments, in order to identify the effect of the difference in cell counts on pathogen inhibition. In other words, the nutrient concentration in this study may have been higher than that *in vivo* environment.

Quantitative analysis of biological variables is required for obtaining a valid QMRA (den Besten et al., 2017). Previous studies suggest the use of Bayesian inference to quantify uncertainty in the field of microbial risk assessment, such as bacterial inactivation. Additionally, they also suggest that it can be implemented in a wide range of predictive microbiology (Garre et al., 2020; Koyama et al., 2019; Powell et al., 2006; Quinto et al., 2018; Teunis et al., 2005). In this study, the behaviour of foodborne pathogens showed large variance, which included variability due to differences in behaviour and uncertainty due to unintentional experimental manipulation errors. Therefore, the variance of the growth behaviour was quantified and represented probabilistically using Bayesian estimation (Figures 1–3). We found that the variance of the competing foodborne bacterial behaviours was greater than that of the monoculture. Bayesian fitting showed that this variance could be represented by a probabilistic range. Abe et al. (2021) proposed a model for predicting the invasion of epithelial cells in the small intestine by *C. jejuni* using Bayesian inference. Key event models, including this study, which are expected to be used to develop dose–response models important for risk assessment, should be developed using Bayesian estimation.

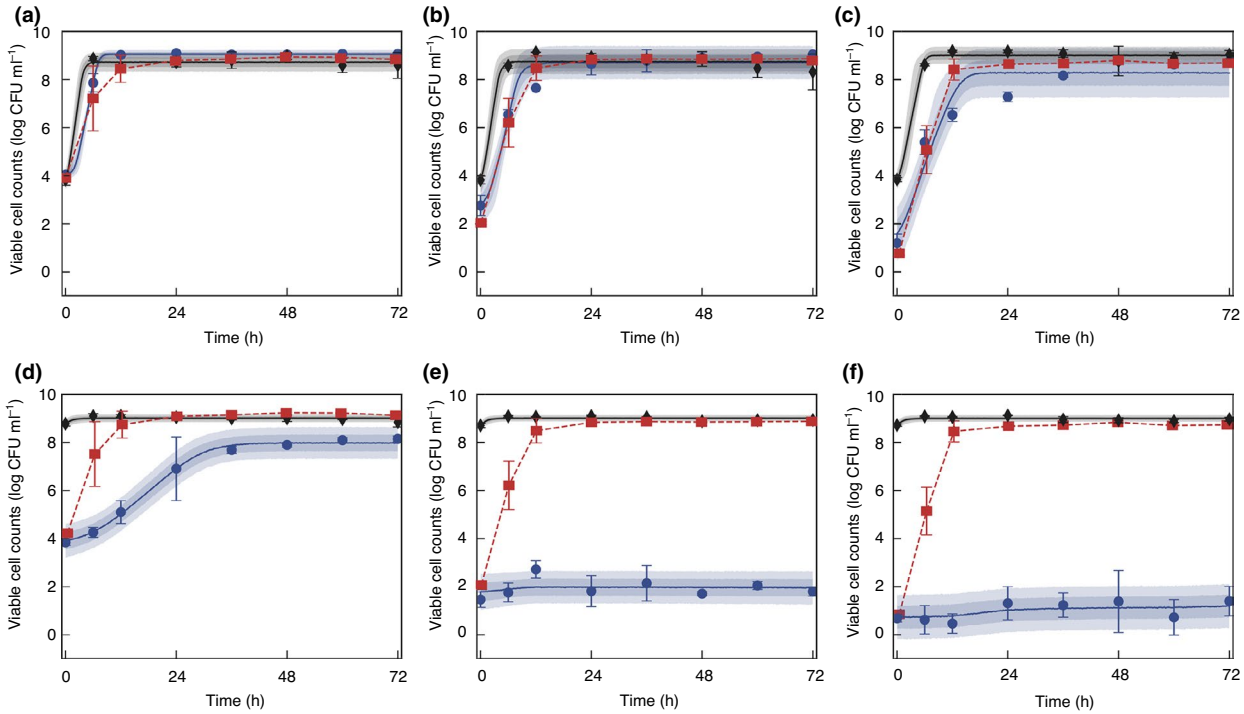

FIGURE 3 Estimated (curves and ranges) and observed variation in growth of *Escherichia coli* O157:H7 (●) and intestinal bacteria (◆) co-cultured in artificial small-intestine fluid at 37°C under micro-aerophilic conditions. The solid line represents the median, the dark blue range represents 50% prediction intervals, and the light blue range represents 80% prediction intervals. Median and prediction intervals were obtained by Bayesian estimation using 1.0×10^4 Monte Carlo simulation. *Escherichia coli* O157:H7 monoculture (■) was used as control. The initial bacterial count were set as followings, (a) *E. coli* O157:H7: 4 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (b) *E. coli* O157:H7: 2 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (c) *E. coli* O157:H7: 1 log CFU per ml; intestinal bacteria: 4 log CFU per ml, (d) *E. coli* O157:H7: 4 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (e) *E. coli* O157:H7: 2 log CFU per ml; intestinal bacteria: 9 log CFU per ml, (f) *E. coli* O157:H7: 1 log CFU per ml; intestinal bacteria: 9 log CFU per ml. The error bars indicate the standard errors of measurement in triplicate trials

The growth prediction of foodborne pathogens in human intestines can be an important factor in estimating dose–response relationships. There already have been some examples for application of a simple growth prediction in intestines for mechanistic dose–response model (Rahman et al., 2016, 2018), although they used simple monocultured behaviour for *L. monocytogenes* dose–response model. Recently, Abe, Koyama, et al. (2021) reported that the cell-invasion rate of *C. jejuni* into human intestinal cells depends on the pathogen concentration around intestinal cells, and the prediction of cell-invading behaviour has the potential to estimate dose–response relationship (Abe, Koyama, et al., 2021; Abe, Takeoka, 2021). Therefore, an accurate estimation of the pathogen concentration in human intestinal tracts will be necessary for estimating the dose–response model for pathogens such as *C. jejuni*, *L. monocytogenes* and *Salmonella* spp., which invade body tissues and cause adverse health effects. Combing the key event models including the prediction of pathogen growth in the intestine competing with gut microflora could contribute to realize a more realistic prediction of the dose–response relationship. However, more detailed studies on each key event during the digestion process are needed for validation of potentials of the dose–response model based on the KEDRF.

This study investigated and developed a predictive model for the growth behaviour of *C. jejuni*, *L. monocytogenes* and *E. coli* O157:H7 competing with cocktail of nine or six enteric bacterial species as intestinal microbiota under simulated small intestinal condition, aiming to be a key event model for mechanistic dose–response model. Competing with an initial bacterial concentration in duodenums (the first part of the small intestine), all three pathogens demonstrate growths in all conditions of the initial pathogen concentration of this study. The three pathogens have grown in each different trend, and the result of competing did not always indicate the Jameson effect. As no Jameson effect was apparent, the pathogen growth behaviours were described by simple Baranyi models using Bayesian inference. In order to validate the potential of KEDRF, further research will be needed to develop a mathematical model to describe the pathogen dynamics in a human body at all important events.

TABLE 3 Estimated parameters of the Baranyi model for the growth of *Escherichia coli* O157:H7 competition with enteric bacteria by Bayesian inference

Number of initial bacteria (enteric bacteria: *E. coli* O157:H7)	$\log_{10}N_{max}$ (log CFU per ml)			$\log_{10}N_0$ (log CFU per ml)			μ_{max} (h^{-1})			Λ (h)		
	Mean[a]	SD[b]	Rhat	Mean	SD	Rhat	Mean	SD	Rhat	Mean	SD	Rhat
10^4: 10^4	8.99	0.03	1.0	3.98	0.08	1.0	2.29	0.52	1.0	1.96	0.8	1.0
10^4: 10^2	8.64	0.14	1.0	2.73	0.33	1.0	2.01	0.82	1.0	1.49	1.15	1.0
10^4: 10^1	8.21	0.23	1.0	1.51	0.43	1.0	1.36	0.58	1.0	0.81	0.8	1.0
10^9: 10^4	7.98	0.16	1.0	3.91	0.25	1.0	0.4	0.17	1.0	5.32	3.32	1.0
10^9: 10^2	1.96	0.26	1.0	1.79	0.27	1.0	1.54	1.05	1.0	27.42	23.93	1.0
10^9: 10^1	1.29	0.65	1.0	0.74	0.24	1.0	1.49	1.1	1.0	29.37	19.74	1.0
E. coli O157:H7 in monoculture of 10^4	9.06	0.12	1.0	3.99	0.29	1.0	2.05	0.79	1.0	1.83	1.2	1.0
E. coli O157:H7 in monoculture of 10^2	9.08	0.10	1.0	2.02	0.23	1.0	2.41	0.77	1.0	1.54	1.09	1.0
E. coli O157:H7 in monoculture of 10^1	9.09	0.09	1.0	0.86	0.22	1.0	2.41	0.73	1.0	1.46	1.06	1.0

[a]Mean value of estimated by 1.0×10^4 Monte Carlo simulation.
[b]Standard deviation of the estimated by 1.0×10^4 Monte Carlo simulation.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Food Safety Commission, Cabinet Office, Government of Japan (Research Program for Risk Assessment Study on Food Safety, nos 1802 and 2004). We thank Editage (www.editage.com) for English language editing.

CONFLICT OF INTEREST

No conflict of interest declared.

DATA AVAILABILITY STATEMENT

All experimental data (.csv) and source codes (Python) for the analisis are available at: https://github.com/Hiroki-Abe/Competition2021.

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How to cite this article: Fuchisawa, Y., Abe, H., Koyama, K. & Koseki, S. (2022) Competitive growth kinetics of *Campylobacter jejuni*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* with enteric microflora in a small-intestine model. *Journal of Applied Microbiology*, 132, 1467–1478. https://doi.org/10.1111/jam.15294