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Original article

Neural network model for growth of *Salmonella* Typhimurium in brain heart infusion broth

Thomas P. Oscar*,†

United States Department of Agriculture, Agricultural Research Service, Residue Chemistry and Predictive Microbiology Research Unit, Center for Food Science and Technology, University of Maryland Eastern Shore, Room 2111, Princess Anne, MD 21853, USA

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Summary Models that predict growth of *Salmonella* as a function of variables in the current and previous environment are valuable tools for assessing the safety of food. Therefore, this study was undertaken to develop a model for growth of *Salmonella* Typhimurium in brain heart infusion broth as a function of previous pH (5.7–8.6), temperature (15–40 °C), pH (5.2–7.4) and time. Viable count data (log CFU mL^{-1}) were modelled using a neural network approach. The variable impacts were 2.4% for previous pH, 29.0% for temperature, 4.9% for pH and 63.7% for time. The proportion of residuals in an acceptable prediction zone (pAPZ) from -1 (fail-safe) to 0.5 log CFU mL^{-1} (fail-dangerous) was 0.965 (1061/1100) for dependent data and 0.939 (386/411) for independent data for interpolation. A pAPZ ≥ 0.7 indicated that the model provided predictions with acceptable accuracy and bias. Thus, the model was successfully validated.

Keywords Growth, modelling, neural network, *Salmonella*, validation.

Introduction

Salmonella are a leading cause of foodborne illness in the United States (Scallan *et al*., 2011; Painter *et al*., 2013) and throughout the world (D'Aoust, 1994; Van Cauteren *et al*., 2017). Many kinds of food, such as poultry meat and eggs, beef, pork, seafood, milk products, fresh produce, and nuts and tree fruits, are contaminated with *Salmonella* (Carrasco *et al*., 2012; Besser, 2018). Although the level of contamination may be low initially (Oscar, 2014, 2016), *Salmonella* is able to grow when it is exposed to the proper environmental conditions (Li *et al*., 2017; Shakeri *et al*., 2017). Thus, models that predict growth of *Salmonella* as a function of environmental and food variables like temperature and pH are valuable tools for assessing food safety (Soboleva *et al*., 2000; Santillana Farakos *et al*., 2016).

Physiological state is an important variable that affects growth of *Salmonella* when it is shifted to a new environment (Oscar, 1999b; Ribaudo *et al*., 2017). Physiological state is an important variable for growth of other human bacterial pathogens as well (Skandamis *et al*., 2007; Hereu *et al*., 2014). In fact, physiological state is an important variable in models that predict the growth of human bacterial pathogens over time (Baranyi *et al*., 1993; McKellar & Lu, 2005). Thus, the previous environment, which can alter the physiological state of a human bacterial pathogen, is also an important variable to include in a predictive model for growth of a human bacterial pathogen in food (Beuchat & Mann, 2008; Kataoka *et al*., 2016). These statements also apply to food spoilage bacteria.

Models that predict growth of a human bacterial pathogen, like *Salmonella*, as a function of variables (e.g. temperature and pH) in the current and previous environment are valuable tools for assessing the safety of food (Oscar, 1999a,b). Typically these models are developed using a three-step, nonlinear regression approach that involves primary, secondary and tertiary modelling (Oscar, 2005a). This process can be cumbersome, complicated and time-consuming. An alternative approach that involves just one step and that is not cumbersome, complicated, or time-consuming is neural networks (Oscar, 2009). With the advent of commercial software applications that can train and test neural networks and produce a user-friendly model, it is now easy for this technology to be applied in the field of

*Correspondent: Fax: +1 410 651 8498;
e-mail: thomas.oscar@ars.usda.gov
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predictive microbiology as it is being applied in other fields, such as robotics and self-driving cars. Therefore, this study was undertaken to develop a neural network model for growth of *Salmonella* Typhimurium in brain heart infusion broth as a function of previous pH (5.7–8.6), temperature (15–40 °C) and pH (5.2–7.4). The model was actually an expanded version (addition of stationary phase) of a previously developed model (Oscar, 1999c) that predicted lag and exponential phases of growth of *Salmonella* Typhimurium in brain heart infusion broth as a function of previous pH (5.7–8.6), temperature (15–40 °C) and pH (5.2–7.4).

Materials and methods

Data collection

Data for model development and validation were collected as previously described (Oscar, 1999c). In brief, a single strain (American Type Culture Collection 14028, Manassas, VA, USA) of *Salmonella* Typhimurium was grown to stationary phase at 30 °C in 5 mL of brain heart infusion broth (Difco, Becton Dickinson, Sparks, MD, USA) with pH of 5.7, 6.7, 7.8 or 8.6 for model development and with pH of 6.3, 7.4 or 8.3 for model validation for interpolation. A single strain rather than a cocktail of strains was used so that the model could be expanded in the future to include strain variation as an independent variable.

After growth at various pH (i.e. previous pH), the 5-mL cultures of *Salmonella* Typhimurium were serially diluted and then inoculated into 50 mL of brain heart infusion broth adjusted to pH of 5.2, 6.3 or 7.4 for model development and to pH of 5.7 or 6.7 for model validation for interpolation. The 50-mL cultures of brain heart infusion broth with an initial concentration of 4 log CFU of *Salmonella* Typhimurium per mL were incubated at 15, 20, 25, 30, 35 or 40 °C for model development and at 17.5, 22.5, 27.5, 32.5 or 37.5 °C for model validation for interpolation. Thus, the experimental design was a 4 (previous pH) by 3 (pH) by 6 (temperature) full factorial for model development and a 3 (previous pH) by 2 (pH) by 5 (temperature) full factorial for model validation for interpolation. A single replicate of each combination of independent variables was conducted with a few exceptions where two replicates were conducted. The concentration of *Salmonella* Typhimurium in the 50-mL brain heart infusion broth cultures over time was determined by viable counts on brain heart infusion agar. Undiluted and serially diluted samples of the 50-mL brain heart infusion broth culture were spiral-plated onto brain heart infusion agar at selected sampling times and colonies that formed after incubation at 30 °C for 24 h were counted using an automated colony counter.

Model development

A multiple-layer feed-forward neural network model with two hidden layers of two nodes each was developed as previously described (Oscar, 2017b) except that it had four rather than two input variables. The structure of the neural network was determined by previous experience and by trial and error. In brief, a data set was created that had 1100 combinations of the four independent numerical variables (previous pH, temperature, pH and time) and one dependent variable (log CFU mL^{-1}). Seventy per cent of the dependent data (n = 770) were randomly selected and used to train the neural network, whereas 30% of the dependent data (n = 330) were randomly selected and used to test the neural network for generalisation. The data set was created in a computer spreadsheet (Excel, MicroSoft Corporation, Redmond, WA, USA), and the neural network was trained and tested with a spreadsheet add-in program (NeuralTools, Palisade Corporation, Ithaca, NY, USA). An independent set of data (n = 411) was used to test the model for interpolation. These data were collected with the same methods as the data used to develop the model. In addition, they were collected at intermediate values of the independent variables used in model development; this was done to provide a complete and unbiased evaluation of model performance per the test data criteria of the acceptable prediction zone method (Oscar, 2005b).

Model performance and validation

The test data and model performance criteria of the acceptable prediction zone (APZ) method were used to evaluate model performance as previously described (Oscar, 2005a,b). These criteria were based on established statistical concepts and principles, established performance standards in the U.S. educational system (Oscar, 2005b) and an analysis of the distribution of absolute relative errors associated with the enumeration method (Oscar, 2005a). In brief, a residual (observed–predicted) was considered acceptable when it was in an APZ from -1 (fail-safe) to 0.5 log CFU mL^{-1} (fail-dangerous). The model was considered to provide predictions with acceptable accuracy and bias for the test data when the proportion of residuals in the APZ (pAPZ) was $\geq$0.7. The model was considered validated when pAPZ for dependent data and independent data for interpolation were acceptable and there were no local prediction problems.

Independent variable impacts

One of the outputs of the neural network software was the relative impact of the independent variables

(previous pH, temperature, pH and time) on the dependent variable (log CFU mL^{-1}). These values summed to 100% and the independent variable with the largest value was the one that had the largest effect on the dependent variable. They were calculated as follows:

$$Y_n = \frac{\overline{\Delta}_n}{\overline{\Delta}_{ppH} + \overline{\Delta}_T + \overline{\Delta}_{pH} + \overline{\Delta}_t},$$

where Y_n was the relative impact (%) of independent variable n (previous pH, temperature, pH or time), $\overline{\Delta}_n$was the mean delta for independent variable n, $\overline{\Delta}_{ppH}$ was the mean delta for previous pH, $\overline{\Delta}_T$ was the mean delta for temperature, $\overline{\Delta}_{pH}$ was the mean delta for pH, $\overline{\Delta}_t$ was the mean delta for time, and delta was the difference between maximum and minimum dependent values from the training set and was obtained by stepping the through the values of independent variable n while the other independent variables were fixed.

Results

Neural network model

Figure 1 shows a screenshot of the user-friendly form of the neural network model that was developed. This form of the model is similar in format to those in the U.S. Department of Agriculture, Pathogen Modeling Program. To use the model, values for previous pH, temperature and pH are entered. These values should be within the indicated ranges because predictions of the model that are outside these ranges might not be reliable. After the values for the independent variables are entered, the model predicts the complete growth curve. The model is programmed to limit the growth curve to times used in model development and validation.

Growth curves

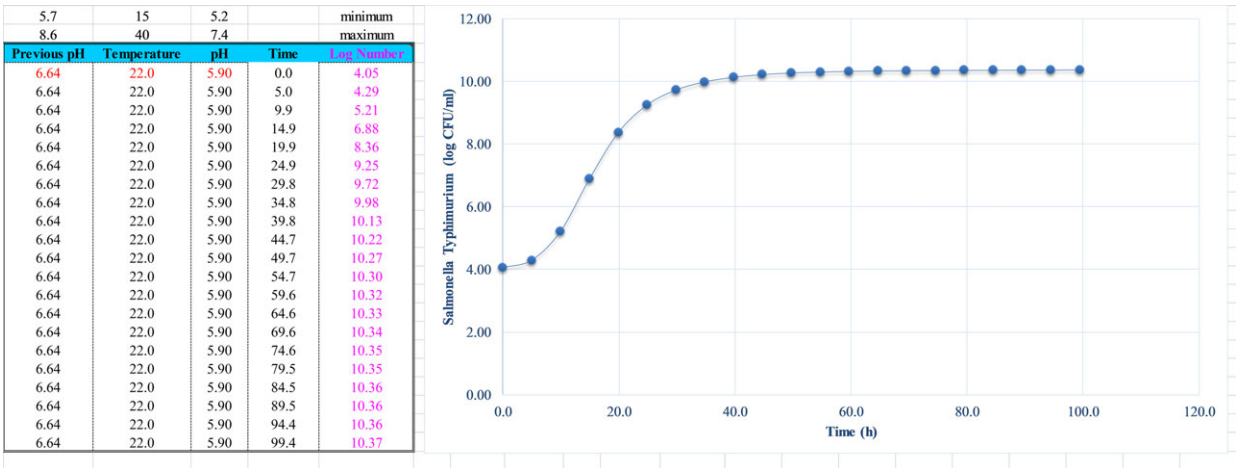
Examples of growth curves are shown in Figure 2 for dependent data (panels a–d) and for independent data for interpolation (panels e and f). Symbols are observed values whereas solid lines are growth curves predicted by the model.

Independent variable impacts

Independent variable impacts were 2.4% for previous pH, 29.0% for temperature, 4.9% for pH and 63.7% for time. Predicted growth curves in Figure 3 show that time had the largest effect on growth (all panels), previous pH had a very small effect on growth (panel a), temperature had a large effect on growth (panel b), and pH had a small effect on growth (panel c). These results agree with the independent variable impact results.

Model performance

Residual plots for dependent data (panels a and b) and independent data for interpolation (panel c) are shown in Figure 4. Values of pAPZ for dependent data were 0.964 (742/770) for training data, 0.967 (319/330) for testing data and 0.965 (1061/1100) for training data plus testing data, whereas the pAPZ for independent data for interpolation was 0.939 (386/411). There were no signs of local prediction problems.



5.7	15	5.2		minimum
8.6	40	7.4		maximum
Previous pH	Temperature	pH	Time	Log Number
6.64	22.0	5.90	0.0	4.05
6.64	22.0	5.90	5.0	4.29
6.64	22.0	5.90	9.9	5.21
6.64	22.0	5.90	14.9	6.88
6.64	22.0	5.90	19.9	8.36
6.64	22.0	5.90	24.9	9.25
6.64	22.0	5.90	29.8	9.72
6.64	22.0	5.90	34.8	9.98
6.64	22.0	5.90	39.8	10.13
6.64	22.0	5.90	44.7	10.22
6.64	22.0	5.90	49.7	10.27
6.64	22.0	5.90	54.7	10.30
6.64	22.0	5.90	59.6	10.32
6.64	22.0	5.90	64.6	10.33
6.64	22.0	5.90	69.6	10.34
6.64	22.0	5.90	74.6	10.35
6.64	22.0	5.90	79.5	10.35
6.64	22.0	5.90	84.5	10.36
6.64	22.0	5.90	89.5	10.36
6.64	22.0	5.90	94.4	10.36
6.64	22.0	5.90	99.4	10.37

Figure 1 Screenshot of the user-friendly version of the neural network model for growth of *Salmonella* Typhimurium in brain heart infusion broth as a function of previous pH, temperature, pH and time. [Colour figure can be viewed at wileyonlinelibrary.com]

Thus, the model was found to provide predictions with acceptable accuracy and bias as the proportion of residuals in the acceptable prediction zone (pAPZ) was ≥ 0.7 for both dependent data and independent data for interpolation. Therefore, the model was successfully validated per the test data and model performance criteria of the acceptable prediction zone method (Oscar, 2005a,b) meaning that users of the model can be confident that its predictions are reliable.

Discussion

One common application of predictive models in the food industry is to verify critical control points in Hazard Analysis and Critical Control Point programs for red meat and poultry processing (Elliott, 1996). For example, predictive models are often used to assess the effect of a process deviation on outgrowth of a spore-forming pathogen like *Clostridium perfringens* during cooking and cooling of meat products like roast beef and turkey (Juneja *et al*., 2011; Huang & Vinyard, 2016). Here, a 1-log cycle increase in *Clostridium perfringens* is used as an indicator that the critical control point is out-of-control and that corrective action is needed to ensure food safety.

Another common application of predictive models for human bacterial pathogens is in the exposure assessment component of a risk assessment to predict the change in pathogen number in a unit operation of a food production chain (Hildebrandt & Kleer, 2004; Oscar, 2004). Here, a model that can consider the previous environment provides a more accurate prediction of the change in pathogen number within the unit operation being simulated. For example, in a risk assessment for *Salmonella* and fresh leafy greens the following unit operation scenario could be relevant for the model developed in this study: a food handler who is shedding *Salmonella* Typhimurium from a recent episode of salmonellosis is working at a restaurant with a salad bar and forgets to wash his hands after

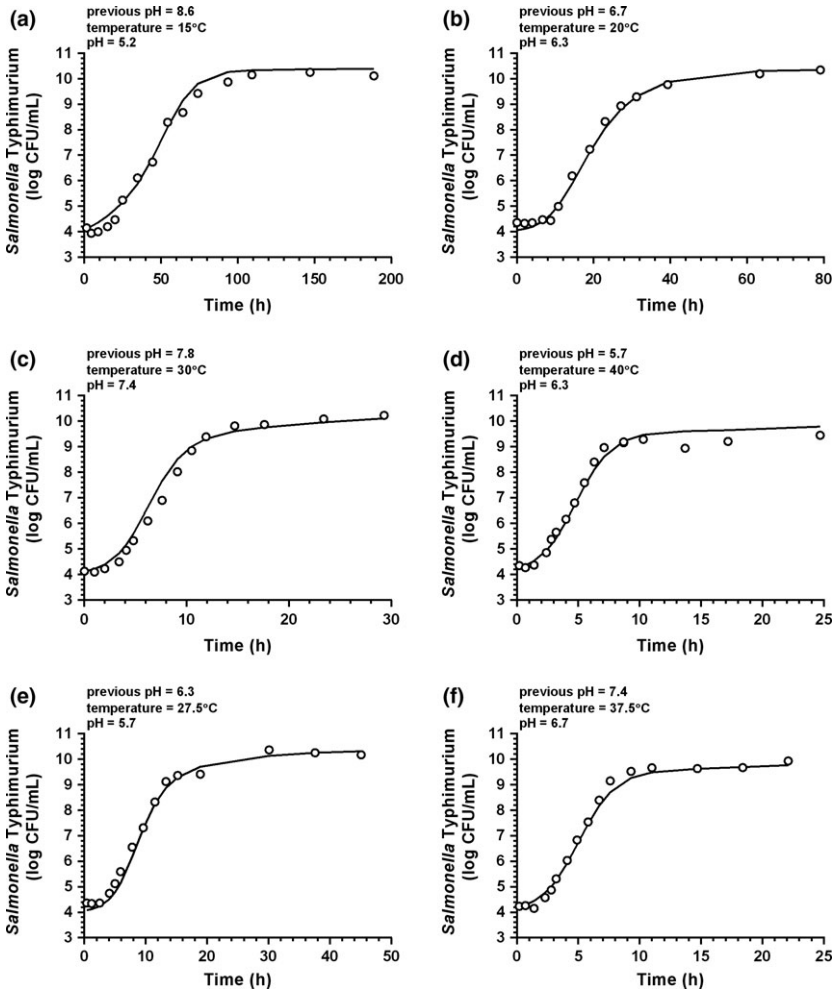


Figure 2 Examples of growth curves for *Salmonella* Typhimurium in brain heart infusion broth as a function of previous pH, temperature, pH and time for data used in model development (panels a–d) and data used in model validation for interpolation (panels e and f).

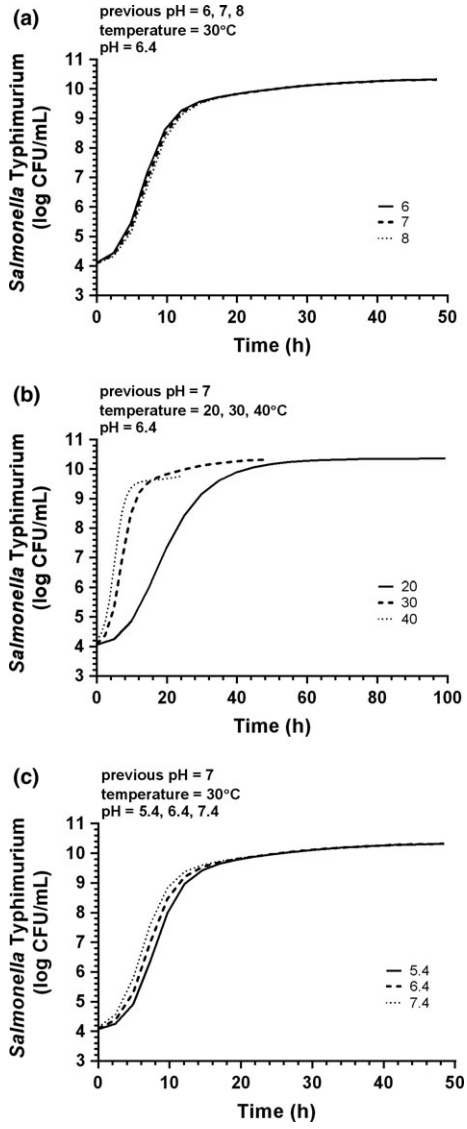


Figure 3 Impact of (a) previous pH, (b) temperature and (c) pH on predicted growth curves for *Salmonella* Typhimurium in brain heart infusion broth.

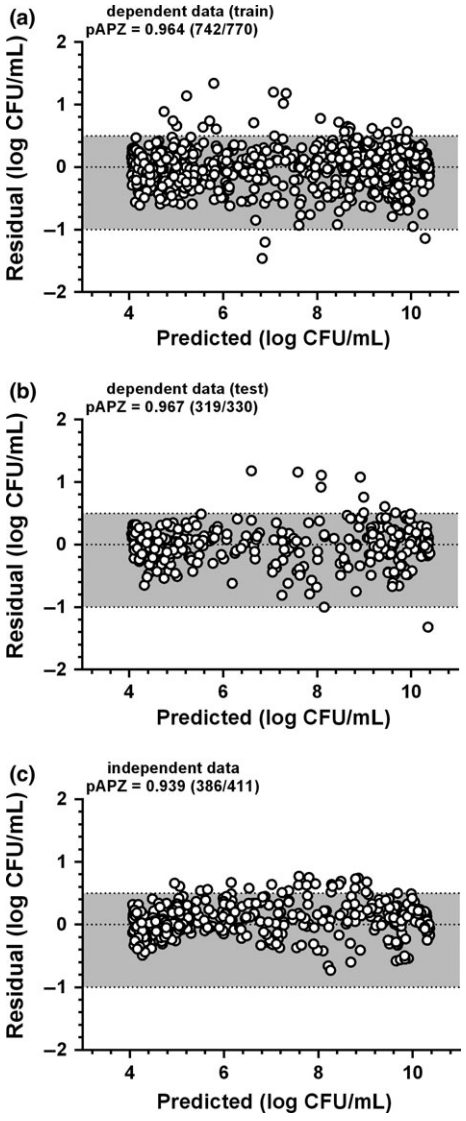


Figure 4 Residual plots and acceptable prediction zones for evaluating performance of the neural network model for growth of *Salmonella* Typhimurium in brain heart infusion broth: (a) dependent data used to train the model, (b) dependent data used to test the model for generalisation and (c) independent data used to test the model for interpolation.

visiting the rest room. He then cross-contaminates fresh-cut lettuce (pH = 5.90) with *Salmonella* Typhimurium (10^4 CFU) from faecal material (i.e. previous environment) on his hands. The lettuce is then added to the salad bar and held at room temperature (22 °C) for various times before being consumed. The food safety question that could be answered with the model is: what impact will this scenario have on consumer exposure over time? Here, the pH of human faeces (i.e. previous pH) was assumed to be 6.64, which is the median value reported by Rose *et al.* (2015). This information (previous pH of human faeces = 6.64, pH of lettuce = 5.90 and temperature of holding for the lettuce = 22 °C) was entered into the model to generate a growth curve for this scenario, see Figure 1. The growth curve could then be used to answer the posed food safety question.

Nonlinear regression is the most common method of modelling data for growth of human bacterial pathogens in laboratory broth (McClure *et al.*, 1994; Whiting & Buchanan, 1997). This approach usually involves three sequential steps: primary modelling,

secondary modelling and tertiary modelling (Whiting & Buchanan, 1993; Whiting, 1995). The tertiary model, which is created by incorporating the secondary models into the primary model, predicts the growth curve as a function of the independent variables (Oscar, 2005a). An alternative approach for modelling growth of human bacterial pathogens in laboratory broth, which was used in the current study and previous studies (Jeyamkondan *et al*., 2001; Garcia-Gimeno *et al*., 2002), is artificial neural networks. An advantage of the artificial neural network approach (Hajmeer *et al*., 1997; Schepers *et al*., 2000; Jeyamkondan *et al*., 2001) and the global regression approach (Martino & Marks, 2007) over the nonlinear regression approach is that the model is developed in one rather than three steps, which results in better model performance. Like tertiary models, the neural network model predicts the growth curve as a function of the independent variables. Similar to previous studies (Oscar, 2017a,b) in which artificial neural networks were used for modelling thermal inactivation of *Salmonella* Typhimurium in ground chicken thigh meat with native microflora, the neural network model developed in the present study was found to provide highly accurate and unbiased predictions (pAPZ > 0.93) of sigmoid-shaped curves.

Conclusions

Similar to the original study (Oscar, 1999c), previous pH was found to have only a very small effect on the subsequent growth of *Salmonella* Typhimurium in brain heart infusion broth even after inclusion of the stationary phase of growth. Thus, in the future, as the model is expanded to include other independent variables, previous pH could be excluded without any appreciable loss of model performance, which would have the added benefit of reducing the cost of model expansion and improvement. More specifically, the next steps are to expand the model to include other independent variables, such as inoculum size, strain variation and water activity that will allow it to be applied to a wider range of prediction scenarios. Once these steps are accomplished, it will be possible to evaluate the expanded model for its ability to predict the growth of *Salmonella* in a wider range of food products. Validation of the model in food will be an important step that will make the model an even more valuable tool for the food industry.

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