

## Advances in modeling microbial growth

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### SUMMARY

A mathematical model for bacterial growth, survival and death has been developed. This equation has been applied to a large set of data obtained with *Yersinia enterocolitica* to produce a predictive model. Favorable comparisons were obtained between predictions from the model, primarily as estimates of the population size as affected by local conditions, and data from an experiment with an inoculated food.

### INTRODUCTION

Populations of organisms vary as a consequence of birth and death [1–3,5,7–10]. Taking the human population as an example, if the birth rate of the population exceeds the death rate, the population will increase, and conversely, if the death rate exceeds the birth rate, the population size will decrease. This is true regardless of the nature of the population [28] (e.g. human, plant, microorganism).

The human population can be considered still further, in terms of the average per capita (per head of population) death rate of a human with respect to the age of the individual. It is well established that the first few hours of a child's life are critical—as with most biological populations, the 'new born' organism has to adjust to its new environment and during this period of adjustment, an increased number of the organisms will die. Then, assuming that the conditions are favorable for development, and as the organism adjusts to its surroundings, its development will continue. During this period, although the organism can die at any time, the per capita death rate may fall. However, as the organism reaches maturity the death rate can be seen to increase with the onset of old age. Thus, the general shape of the death rate curve described above follows that illustrated in Fig. 1.

The other factor controlling the size of the population is the birth rate [1–3,5,7–10]. Before an organism can multiply or give birth, it has to go through a period of maturation [2,3,5,8,9]. With some organisms this may involve a transition through a number of distinct stages, whereas with others the development process may be more continuous with the stages less well defined. However, all manifest a time period when the birth rate is zero. As the organism develops, its

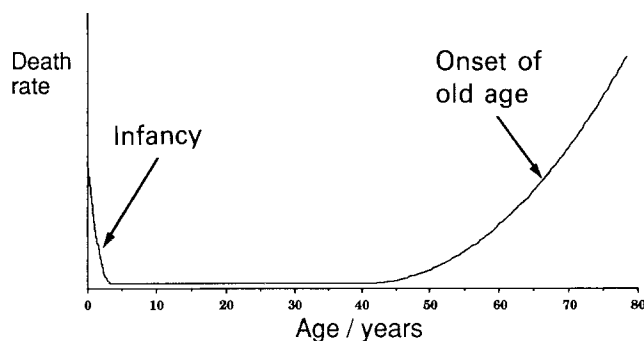


Fig. 1. Graphical representation of the suggested per capita death rate of the human population.

reproductive capabilities increase. Assuming that proliferation is proportional to the capacity to reproduce, the birth rate rises gradually from zero to a maximum, at which the organism reaches peak fertility, before falling again. In some organisms (e.g. humans), the birth rate will return to zero when reproduction is no longer possible. With others this birth rate is merely seen to decline slightly as old age sets in but does not return to zero before the organism dies. The shape of the birth rate curve described above is shown in Fig. 2.

These are general descriptions of the changes of biological populations. However, the theory could be applied more specifically and this paper is concerned with the specifics of populations of the bacterium *Yersinia enterocolitica*. When a microorganism is placed into a new environment, such as a food commodity or a culture medium, it undergoes a period of adjustment. The length of this period is partially dependent on the environment—if conditions are optimal, then the adaptation period may be brief, whereas in less favorable conditions the organism may take much longer to adapt. During this period a decrease may be observed in the size of the population. This could correspond to the

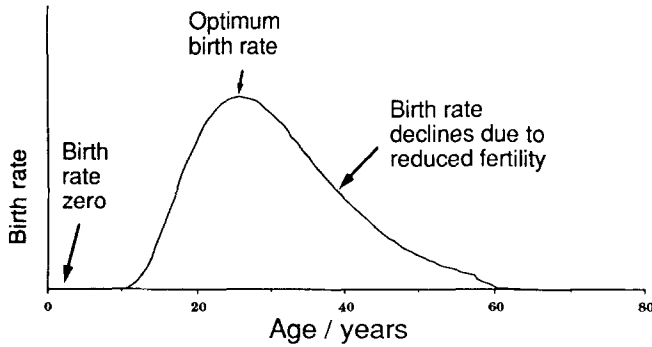


Fig. 2. Graphical representation of the suggested per capita birth rate of the human population.

initial increased 'death rate'. This may then be followed by a period of rapid growth in the population size. This could be the period where the 'death rate' is low. Then, as perhaps nutrients become a limiting factor, a decrease in the population size may be observed, which could correspond to the increase in the 'death rate' discussed above.

Similarly, the 'birth rate' or 'division rate' of the organism may follow the general scenario outlined above. Initially there may be a period when the population size is constant (i.e. the 'lag phase'). This could be the period where the organism is acclimatizing to its new surroundings and does not multiply. This may be followed by a period of rapid increase in the population size followed by a more gradual rise up to a maximum population size. This could correspond with a gradual increase in the 'birth rate' up to a maximum before falling again, as previously described and illustrated in Fig. 2.

#### Development of the equation

It is possible to consider the above assumptions in mathematical terms. The size of a biological population is controlled by both birth and death. So, it follows that the rate at which the population size changes, is dependent on both the 'birth' and 'death' rates. In mathematical terms, this can be represented by the following equation:

$$\frac{dN}{dt} = N \cdot \frac{dG}{dt} - N \cdot \frac{dM}{dt} \quad (1)$$

where  $N$  is the population size,  $t$  represents time,  $dG/dt$  is the per capita 'birth' or 'growth rate' and  $dM/dt$  is the per capita 'death' or 'mortality rate'.

Subsequently, due to the general shape they describe, the following two mathematical equations were then selected to represent the 'growth' and 'death rate' functions respectively:

$$\text{Growth rate} = \frac{A' \cdot t^3 \cdot e^{-t/B}}{6 \cdot B^4} \quad (2)$$

$$\text{Death rate} = \frac{\ln(2) \cdot (e^{(t-D)/C} + e^{-(t-D)/C})}{C} \quad (3)$$

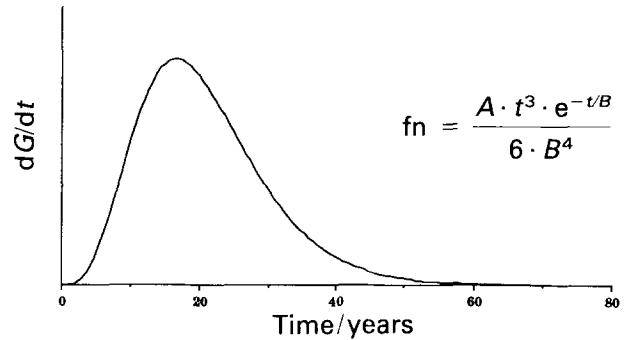


Fig. 3. Function selected to represent the per capita 'growth' or 'division' rate of a microorganism.

where  $A'$ ,  $B$ ,  $C$  and  $D$  are constants and  $t$  represents time. These functions are represented graphically in Figs 3 and 4.

These equations were then substituted into Eqn 1 and this differential equation was then integrated to give the following equation for population size:

$$N = N_0 \cdot 2^{(G(t)-M(t))} \quad t \geq 0 \quad (4)$$

where:

$$G(t) = A \cdot (1 - (1 + (t/B) + (t/B)^2/2 + (t/B)^3/6) \cdot e^{-t/B}) \quad (5)$$

$$M(t) = e^{(t-D)/C} - e^{-(t-D)/C} - e^{-D/C} + e^{D/C} \quad (6)$$

and  $A(A = A'/\ln(2))$ ,  $B$ ,  $C$  and  $D$  are constants,  $t$  represents time and  $N_0$ , the count at time 0, and  $N$  are actual counts *not* log counts.

#### Application of the equation

Data from a large, full factorial experiment investigating the effects of temperature, salt and pH on the population size of *Y. enterocolitica* were used. Data were recorded as a series of viable (plate) counts against time. A cocktail of strains of the organism were applied to a sterile growth medium (Trypticase Soy Broth) in which the salt and pH had been adjusted appropriately, and the medium then incubated at given constant temperatures. The eight tempera-

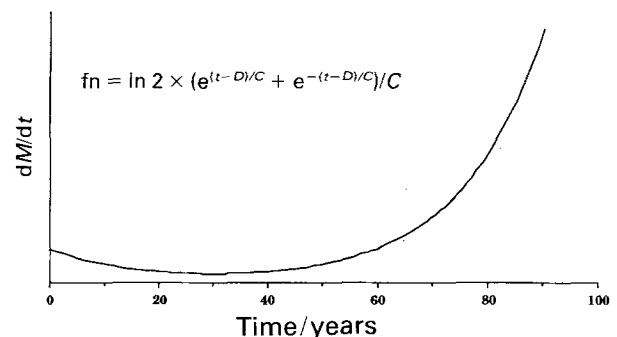


Fig. 4. Function selected to represent the per capita 'death' rate of a microorganism.

tures used in the design were 0, 2, 4, 7, 10, 15, 20, and 30 °C. The nine sodium chloride concentrations ranged from 0 to 8% at 1% intervals. The six pH levels used were in the range 4.5–7.0 at intervals of 0.5. The experiment therefore involved a total of 432 different combinations of factors with 4140 actual data points.

As a guide to the type of data to be fitted with the new equation, the individual curves were classified by types: growth was defined as at least a 10-fold increase in the number of colonies; death was defined as more than a 10-fold decrease in the number of colonies; and survival was defined as the remaining curves. From the results it was apparent that 42% of the curves could be classified as growth curves, 33% as survival curves and 25% as death curves.

The fitting was carried out using a quasi-Newton algorithm for finding the minimum of a function. The function minimized was:

$$\Sigma [\text{Log}(N) - \text{Log}(N_0) - \text{Log}(2) \cdot \{G(t) - M(t)\}]^2 \quad (7)$$

where  $G(t)$  and  $M(t)$  are defined in Eqns 5 and 6 respectively,  $t$  represents time,  $N_0$  is the (unlogged) count at time 0 and  $N$  the (unlogged) count at time  $t$ .

Initially, data from each curve were fitted to the new equation on an individual basis. It was immediately apparent that the *Yersinia* data did not exhibit an initial decrease in the death rate (i.e.  $D = 0$ ). Reviewing the data, this seemed to be reasonable as none of the growth curves showed a marked initial decrease. Consequently, parameter  $D$  was removed from the equation.

From plotting the values of each of the parameters against the respective factor levels (i.e. pH, temperature and salt), the resulting surface suggested the use of the following type of polynomial to represent each of the three parameters:

$$\ln(A) = A_0 + A_1T + A_2S + A_3P + A_4TS + A_5TP + A_6SP + A_7T^2 + A_8S^2 + A_9P^2 \quad (8)$$

where  $T$  represents temperature,  $S$  represents salt,  $P$  represents pH and  $A_0$ – $A_9$  are constants.

Consequently, polynomials were substituted for each of the parameters  $A$ ,  $B$  and  $C$  in Eqn 7, and the data were then used to find the minimum of this function (i.e. of the complete surface).

In order to compare the results with an existing modeling technique, a Gompertz-polynomial [4,6] was fitted to the subset of data originally classified as growth (i.e. the 42% described above). Polynomials were substituted for the parameters  $B$ ,  $C$  and  $M$  in the Gompertz equation and a quasi-Newton algorithm was used to minimize the sums of squares of the surface.

The new equation when fitted to all the data accounted for 96.4% of the variation and a mean squared error of 0.494. The Gompertz equation when fitted to just the growth data accounted for 89.4% of the variation giving a mean squared error of 0.849. In other words, the fit of the new

equation to all the data was better than the fit of the Gompertz equation to only the growth data for which it has been conventionally used.

As an assessment of individual fits, the mean squared errors were calculated for each growth curve as fitted by both the new equation and the Gompertz equation. Fig. 5 shows the mean squared error for an individual growth curve fitted using the Gompertz-polynomial against that for the same growth curve fitted using the new equation. The line through the middle is the equivalence line—it is formed when the fit of the data using either equation is equally good. If points are in the area above this equivalence line then the Gompertz equation has produced the better fit, and, conversely if points lie in the area below the line the new equation has produced a better fit. Each point represents a different level of temperature, pH and salt. In terms of percentages, 63% of the points lie in the area below the line, the area where the new equation fits better than the Gompertz. The graph indicates that the Gompertz curve fits poorly on a number of occasions. A review of the Gompertz model showed that these points were associated with low growth rates at the boundaries of growth and survival, areas where the data were not characteristic of a Gompertz equation. The exercise of plotting the mean square errors from the two types of equations was therefore repeated, but on this occasion the points representing a growth rate of  $<0.02$  ( $\log_{10}/h$ ) were omitted. Again, the results are represented graphically (Fig. 6). However, on this occasion many of the poorly fitting Gompertz type predictions were omitted resulting in an increase to 50% in the number of points above the line (cf. 37% as described above).

The results were encouraging in that the new equation performed as well as the Gompertz, despite the fact that the Gompertz equation was used with only those data most appropriate for it (the 42% of the data classified as growth), whereas the new curve used all of the data. In order to highlight the capabilities of the new equation, Fig. 7 shows

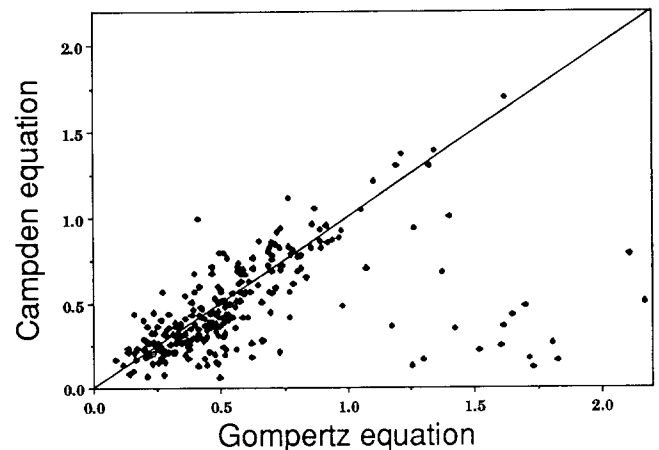


Fig. 5. Comparison of mean squared errors for all growth data fitted by a Gompertz-polynomial equation against the associated mean squared errors for data fitted using the new equation.

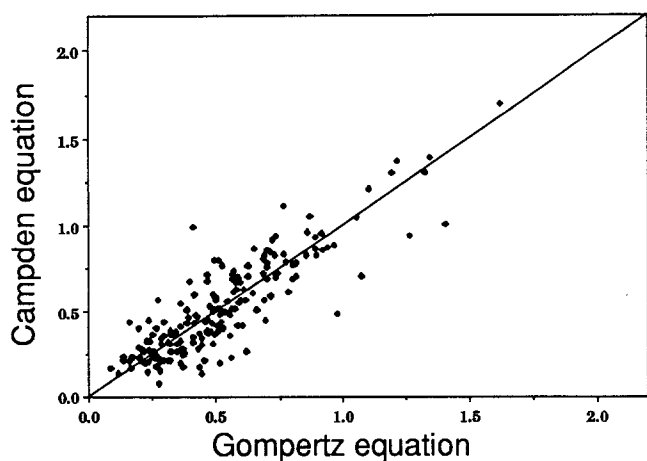


Fig. 6. Comparison of mean squared errors for growth data (high growth rate only) fitted by a Gompertz-polynomial equation against the associated mean squared errors for data fitted using the new equation.

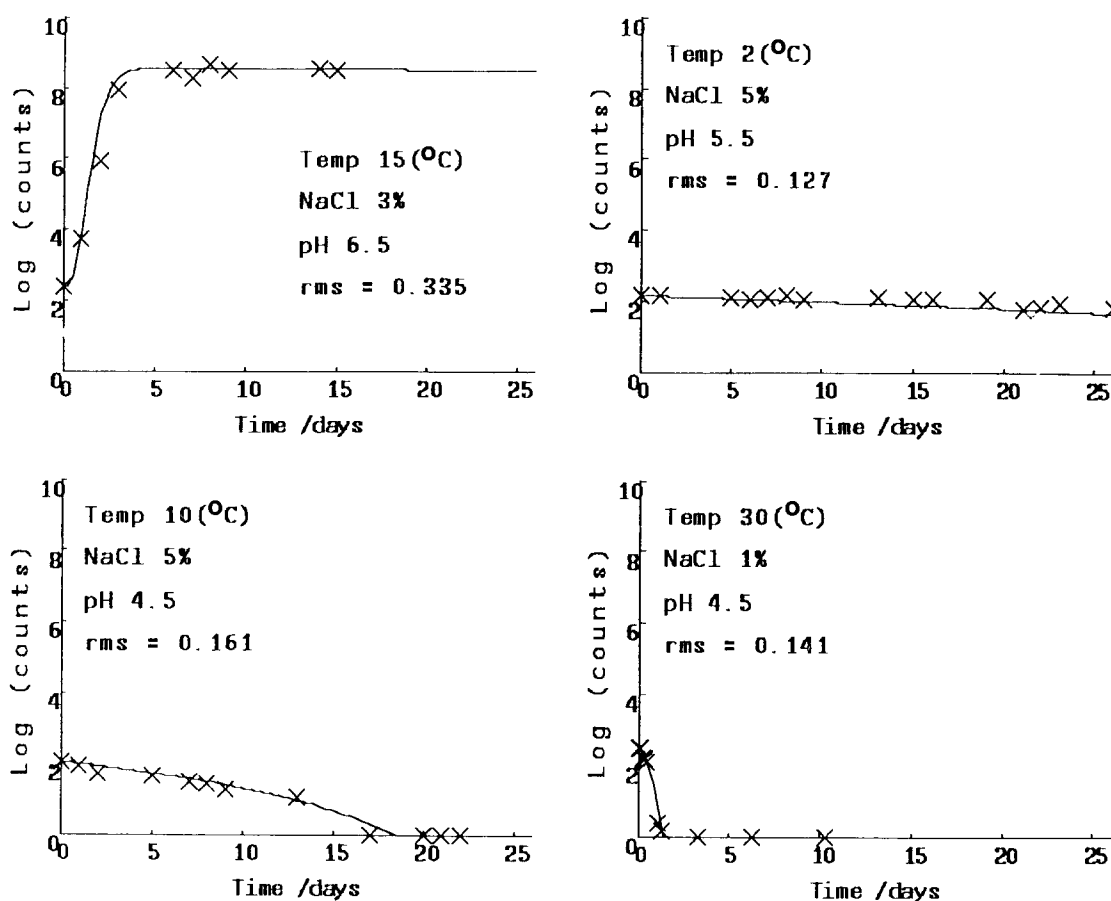


Fig. 7. Comparisons between actual data and log population sizes as calculated by the model with associated root mean squared errors (rms).

the data points recorded in the experiment (crosses) and the growth as calculated by the model (line).

An important part of the development of useful models is validation in foods. Consequently, for comparative purposes, and in order to assess the predictive capabilities of the

model from the new equation, a completely unrelated set of data was selected. These data were derived from a study of the growth of *Y. enterocolitica* in milk (UHT and pasteurized). To compare this growth data with the model, data points from the milk experiment were plotted against the growth as predicted by the model. Fig. 8 shows data at four different factor levels against the growth as predicted by the model. Clearly the graphs illustrate very good agreement between the growth as predicted by the model and that observed in the experimental study.

Currently, further comparative work, with both inoculated food studies and data from literature, on the growth, survival and death of *Y. enterocolitica* is under way in order to validate the model thoroughly. However, on the basis of evidence to date the model appears to have considerable potential for predicting changes in microbial population sizes (growth, survival and death) under given conditions.

## DISCUSSION

An equation has been developed to facilitate prediction of the growth, survival and death of bacterial species. The actual equation is more complicated than equations

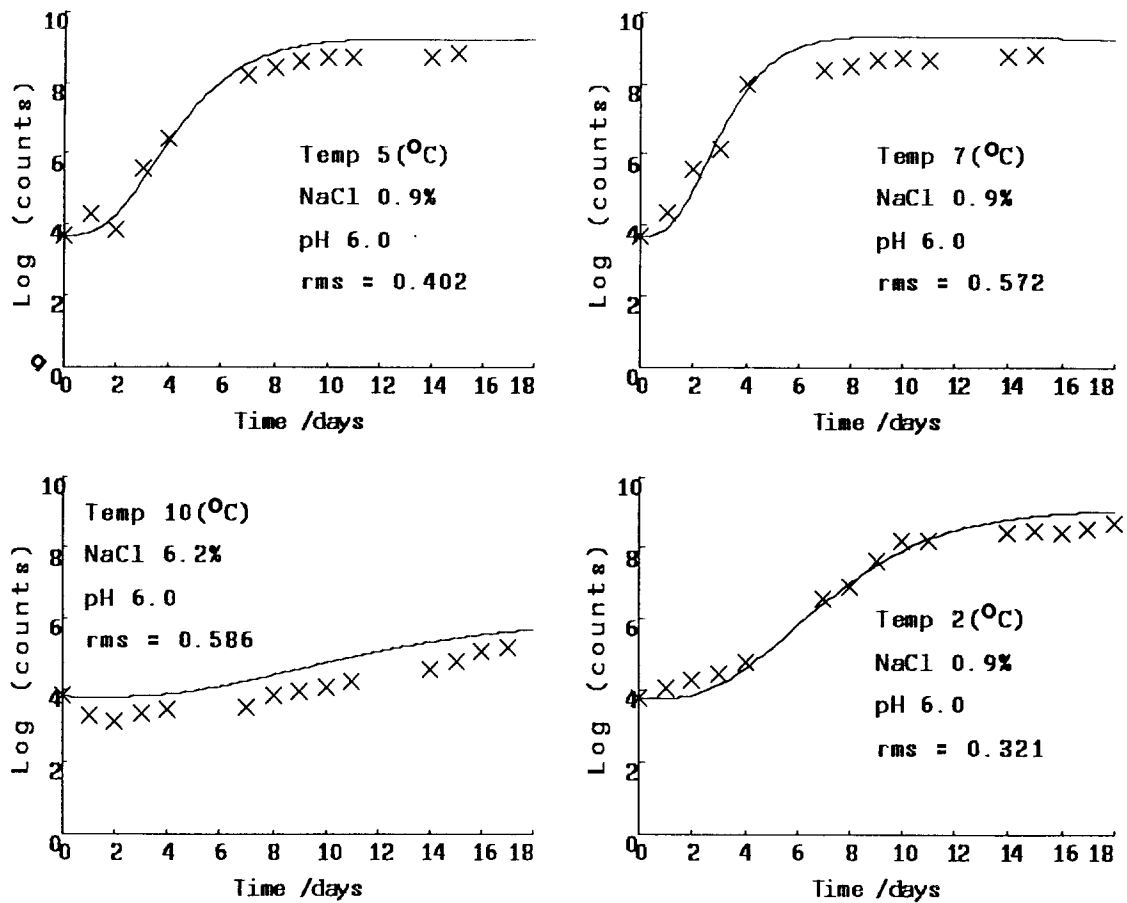


Fig. 8. Comparisons between data from a study on the growth of *Yersinia enterocolitica* in milk and log population sizes as predicted by the model, including associated root mean squared errors (rms).

conventionally used for such purposes, such as the Gompertz-polynomial. However, in contrast to equations currently in use it does have the capacity to handle growth, survival and death data, so that this one predictive equation can encompass all three aspects of population dynamics.

The theory behind the equation has been kept simple and consequently the model has a certain amount of flexibility. Further model development is already under way, considering, for example, different functions for the death rate of the microbial population, to improve yet further the model's performance.

Future work will see the merging of the data generated at Campden with that from the Institute of Food Research in Reading, UK, the Institute of Food Research in Norwich, UK and the University of Surrey, UK, in order to expand the scope of the model to a wider range of environmental conditions.

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with the views expressed in the report. The results of the research, are the property of the Ministry of Agriculture, Fisheries and Food and are Crown Copyright.

*Glossary.* ln is used to denote natural logs. Log is used to denote log to base 10.

#### REFERENCES

- 1 Anderson, R.M. 1982. The population dynamics and control of hookworm and roundworm infections. Population Dynamics of Infectious Diseases (Anderson, R.M., ed.), pp. 67-106, ISBN 0412216108, Chapman & Hall, NY.
- 2 Ashford, J.R., K.L.Q. Read and G.G. Vickers. 1970. A system of stochastic models applicable to studies of animal population dynamics. J. Anim. Ecol. 39: 29-50.
- 3 Gettinby, G., K. Bairden, J. Armour and C. Benitez-Usher. 1979. A prediction model for bovine ostertagiasis. Vet. Rec. 105: 57-59.
- 4 Gibson, A.M., N. Bratchell and T.A. Roberts. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6: 155-178.
- 5 Jones, J.E. 1988. A series of mathematical models of the life cycle of the nematode *Ostertagia ostertagi*. Ph.D. thesis, University of Exeter, UK.

- 6 Palumbo, S.A., A.C. Williams, R.L. Buchanan and J.G. Phillips. 1991. Model for the aerobic growth of *Aeromonas hydrophila* K144. *J. Food Prot.* 54: 429–435.
- 7 Paton, G. and G. Gettinby. 1983. The control of a parasitic nematode population in sheep represented by a discrete time network with stochastic inputs. *Proc. Royal Irish Acad. B* 83: 267–280.
- 8 Read, K.L.Q. and J.R. Ashford. 1968. A system of models for the life cycle of a biological organism. *Biometrika*: 55: 211–221.
- 9 Smith, G. 1982. An analysis of variations in the age structure of *Fasciola hepatica* populations in sheep. *Parasit.* 84: 49–61.
- 10 Thrusfield, M.V. and G. Gettinby. 1984. An introduction to techniques of veterinary modelling. *Pub. Soc. Vet Epidem. & Prev. Med.*, ISBN 0948073012.