Assessing the efficacy of within-animal control strategies against *E. coli* O157: A simulation study

J.C. Wood\textsuperscript{a}, I.J. McKendrick\textsuperscript{a,\*}, G. Gettinby\textsuperscript{b}

\textsuperscript{a} Biomathematics & Statistics Scotland, King’s Buildings, Edinburgh, EH9 3JZ, United Kingdom
\textsuperscript{b} Department of Statistics and Modelling Science, University of Strathclyde, Glasgow, G1 1XH, United Kingdom

Received 11 August 2004; received in revised form 3 November 2005; accepted 23 November 2005

Abstract

A stochastic simulation model was used to assess the efficacy of potential measures to control the levels of *Escherichia coli* O157 within the bovine host. The model described *E. coli* O157 population sizes at several sites along the bovine gut and therefore only interventions that operate at an individual animal level could be evaluated. In order to use the model to evaluate the control strategies, it was necessary to make assumptions about how each strategy affected *E. coli* O157 populations in vivo. The within-animal conditions under these control strategies were modelled by adjusting the growth rates of *E. coli* O157 at specific sites of interest in the gut, based on these assumptions. The model simulated the population dynamics of an initial dose of *E. coli* O157 inoculated into an animal in the presence of inhibitory probiotics or antibiotics, bactericidal antibiotics or probiotics, and following fasting. Of the control strategies considered, the use of inhibitory probiotics appeared most promising and continued development of a suitable product is to be encouraged.

Keywords: Control strategies; Gastrointestinal tract; Cattle; *Escherichia coli* O157
1. Introduction

The bovine gut has been identified as a source of *Escherichia coli* O157, which can cause severe illness in humans (Zhao et al., 1995). Therefore, there is a need for research into the infection dynamics of *E. coli* O157 in animals, and particularly cattle, with the ultimate aim of reducing the level of infection within the livestock reservoir (Hancock et al., 2001). Given the transient nature of the infection (Mechie et al., 1997), the short infection period, combined with the ability of the organism to survive in the environment means that a single test-and-cull programme is unlikely to be effective in completely eliminating infection. In addition, the fact that the organism is not host-specific and can possibly persist in compounds containing culture-negative livestock will enormously increase the risk of reinfection. Hence, the epidemiology of the infection restricts the choice of control measures that can be successfully implemented, and certainly at the moment, an eradication policy appears to be an unrealistic aspiration.

Efforts to reduce the volume of bacteria shed by animals can still generate considerable public health benefits, as dose–response models demonstrate that the greater the loading of an organism in food, for example, the greater the chance of illness (Jordan et al., 1999). At the moment, Hazard Analysis and Critical Control Point (HACCP) systems in place in abattoirs and along the food processing chain are the only methods in place to minimise the contamination levels of *E. coli* O157 on meat. These strategies aim to prevent direct and indirect faecal contamination of carcasses by reliance on good manufacturing practice (Buncic and Avery, 1997). However, Chapman et al. (1993) have shown that this strategy alone is not sufficient. In their study, they found that during the slaughter process, 30% of the carcasses originating from cattle whose faeces had tested positive for *E. coli* O157 had become contaminated, while the figure was 8% for carcasses from faeces-negative animals. Jordan et al. (1999) use a stochastic simulation model to investigate the effect of a range of measures designed to reduce the contamination of beef carcasses during the pre-slaughter period. They conclude that the most effective interventions are those associated with vaccination and the use of a microbial agent that suppresses proliferation of *E. coli* O157 in the bovine gut. Therefore, it can be concluded that a reduction in the levels of bacteria entering the human food chain could arise from either or both of two associated events: a reduction in the number of cattle shedding *E. coli* O157 or in the magnitude of this shedding.

These strategies must be implemented earlier in the food chain when cattle are still on the farm, although this approach is not trivial; Hancock et al. (2001) mention the “great potential for fecal-oral transmission” that exists on even the most clean of farms as a result of the non-hygienic behaviour generally displayed by cattle. In addition, there are difficulties associated with assessing the efficacy of control measures that have been proposed for implementation in the live-cattle population. For instance, the cost of setting up the necessary experimental studies may be financially prohibitive in many countries, with experiments involving *E. coli* O157 requiring high containment facilities. Some of the proposed control measures are still in the preliminary stages of development, not yet having been approved for use in animals intended for human consumption, while others remain purely hypothetical.
Therefore, this study used a simulation model to assess the efficacy of various intervention strategies. The model was previously described in Wood et al. (2006) and is outlined in Fig. 1. Briefly, a program was written using the C++ programming language to model the bacterial population sizes at key sites along the gut over time. For modelling purposes the gut was split into three compartments: the rumen, the abomasum and the faeces collecting in the colon. Bacterial growth, decay and migration rates were estimated separately for each site. Bacteria shed into the environment were modelled in a separate compartment, which allowed the effect of an environmental route of reinfection to be considered. Occasions where animals infected themselves through inadvertent ingestion of bacteria from the environment are referred to in this paper as ingestion events.

This simulation model is an appropriate vehicle for exploration of the effects of interventions that work by reducing the magnitude of shedding within individual animals, and thus indirectly reduce the number of infected animals. The remainder of this paper will assess the benefits of putative control measures, such as the use of probiotics, antibiotics and dietary intervention, by the examination of output from the simulation model.

2. Inhibitory probiotics and bacteriostatic antibiotics

The simulation study carried out by Jordan et al. (1999) identified the use of an agent that suppresses the shedding of *E. coli* O157 in cattle faeces as one of the pre-slaughter interventions with the greatest potential impact. Such agents include probiotic and prebiotic bacteria. Probiotic bacteria are bacteria which either compete with or are inhibitory to the target organism and by their introduction, the size of the population of microorganisms that are harmful to humans can be reduced (Fuller, 1989). Prebiotics are nutrients upon which specific bacteria thrive, providing these bacteria with a competitive advantage over pathogenic bacteria (Crittenden, 1999). However, the development of a suitable prebiotic product against *E. coli* O157 lags behind that of probiotics, and so this section concentrates on the use of probiotics.

Probiotic bacteria have successfully been used to reduce colonisation associated with salmonellae in poultry (Rantala and Nurmi, 1973) although the technique has not yet been used extensively in cattle. However, Zhao et al. (1998) have developed methods to produce a suitable probiotic by screening bacteria obtained from the bovine gastrointestinal tract for inhibitory or bactericidal activity against *E. coli* O157. The results, published in Zhao et al.
(1998), appear promising, as experiments showed that selected probiotic bacteria reduced the level of carriage and faecal shedding in cattle. A more successful study described in Ohya et al. (2001) found that after a probiotic product had been administered to calves exhibiting continuous faecal shedding of *E. coli* O157, not only was faecal shedding completely inhibited but re-shedding was not detected in any of the animals. These authors postulated that the activity of the probiotics was largely inhibitory. A recent large-scale study by Brashears et al. (2003) found that feeding cattle a *Lactobacillus*-based microbial decreased faecal shedding by more than 50% without having any negative effect on body weight gains.

Obviously, further studies are required with large populations of cattle, preferably involving natural infections of *E. coli* O157, in order to quantify the duration of carriage of the probiotic bacteria in cattle and gain more evidence on the effectiveness of this method in reducing pathogen shedding. However, even if the efficacy of probiotics is at the level defined by Zhao et al. (1998), a small reduction in shedding could have a marked effect on an entire herd, where the reduction in the associated environmental bacterial population size decreases the exposure levels for other animals. In addition, dosing cattle with probiotics, either via diet additives or oral inoculation, could have a further effect in that the probiotics may compete with *E. coli* O157 in environments outwith the animal, such as faecal pats on the pasture and feedlot manure, thus further decreasing the environmental levels (Harmon et al., 1999b). The use of probiotics appears to be a promising mode of intervention, and hence the simulation model is used to describe conditions in a gut with probiotic bacteria present, in order to investigate any resulting differences in the population dynamics of the infection.

Wood et al. (2006) detail the estimation process used to obtain parameter estimates for birth, death and migration of *E. coli* O157 at the sites of interest along the bovine gut for a non-intervention standard scenario. However, in order to run the simulation model to investigate the effect of probiotics, it was first necessary to derive a new set of parameters to describe the birth, death and migration rates of *E. coli* O157 in the presence of probiotic bacteria. In order to do this, the mechanisms by which probiotics reduce the levels of *E. coli* O157 had to be more closely examined. On the basis of the postulated ability of probiotic bacteria to inhibit the growth of *E. coli* O157 (Ohya et al., 2001), it was assumed that it was the growth, and hence, the birth rate in this model, that was suppressed. The evidence of very high levels of VFA observed in the study of Ohya et al. (2001) added further weight to this hypothesis, as high levels of VFA generally correspond to a strongly acidic pH level, with such conditions not being conducive to the growth of *E. coli* O157. Probiotics operating through bactericidal activity are discussed in Section 3. The responses of animals to treatment with bacteriostatic antibiotics are likely to be similar to those exposed to inhibitory probiotics, but throughout this section we will discuss the results in the context of probiotics.

It was therefore assumed that probiotic conditions could be modelled adequately by reducing the birth rate alone, and allowing the death and migration parameters to remain at the values derived for the standard model (see Wood et al., 2006). Zhao et al. (1998) were able to isolate probiotic bacteria from the same gastrointestinal tract sites as *E. coli* O157 at necropsy, which suggests that both populations inhabit the same areas. Therefore, it was necessary to adjust the birth rates in all within-animal compartments. Although it has been
surmised that probiotics could also have the potential to reduce the levels of *E. coli* O157 in the environment, in the absence of any experimental or observational data, it seemed sensible to choose the conservative modelling assumption of leaving the environmental pool parameters fixed at the values derived for them under the standard scenario.

The uncertainty in assessments of the likely impact of probiotics on *E. coli* O157 infections in vivo made it necessary to run the model and assess the effectiveness of the intervention under several possible probiotic scenarios. The situation in which probiotics proved to be 100% effective in inhibiting the growth of *E. coli* O157 was not considered as this would result in any infection becoming extinct almost immediately upon entry into an animal. Hence, the animal could be considered mathematically to act as an absorbing state for the infection, and thus the process would not be informative either mathematically or biologically, even if the associated outcome was highly desirable. More interesting, and probably more realistic, is the situation in which probiotics are not completely successful in inhibiting the growth of *E. coli* O157, although a reduction in the level of carriage is achieved. Therefore, the model was run with the assumptions that the probiotic bacteria present caused either a 50% or a 20% reduction in the growth rate of any *E. coli* O157 in the system. The assumption was made that the probiotics would have an identical proportional effect at all points along the gastrointestinal tract since there is currently no evidence to suggest otherwise. Thus, the parameters were altered by reducing the original values for each of the birth parameters in the within-animal compartments by 50% and 20% accordingly. The new values are listed in Table 1.

The simulation model was initialised assuming an initial dose of $10^4$ cfu of infection, which is reported as typical of the magnitudes of infection found in the pastural environment (Shere et al., 1998; Himathingkham et al., 1999). The model was then run under each of the probiotic scenarios and the results are plotted in Fig. 2. Fig. 2(b) shows the mean population size of *E. coli* O157 in each compartment following initial infection, averaged over 100 runs, obtained by running the model assuming that probiotic bacteria produced a 20% reduction in the growth of *E. coli* O157. The results from the standard model, assuming no probiotics were present, are shown in Fig. 2(a). Three clear differences can be observed when these two sets of results are compared. Firstly, the probiotics, even though they only caused a slight decrease in the growth of *E. coli* O157, appear to have had a considerable effect on the initial dose population that enters the animal, as the infection can be seen to die out almost immediately. Consequently, the environmental pool size was much lower than that in the standard model with no control measures, at all times. Throughout the realisation histories the environmental pool size must be lower than the level at which natural infection becomes likely as no ingestion events occurred.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Rumen (B)</th>
<th>Rumen (D)</th>
<th>Abomasum (B)</th>
<th>Abomasum (D)</th>
<th>Faeces (B)</th>
<th>Faeces (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No control</td>
<td>0.37</td>
<td>0.48</td>
<td>0</td>
<td>0.001</td>
<td>0.80</td>
<td>0.73</td>
</tr>
<tr>
<td>20% reduction</td>
<td>0.296</td>
<td>0.48</td>
<td>0</td>
<td>0.001</td>
<td>0.64</td>
<td>0.73</td>
</tr>
<tr>
<td>50% reduction</td>
<td>0.185</td>
<td>0.48</td>
<td>0</td>
<td>0.001</td>
<td>0.40</td>
<td>0.73</td>
</tr>
</tbody>
</table>
The results obtained from running the model assuming the presence of a probiotic that could reduce the growth rate of *E. coli* O157 by 50% can be seen in Fig. 2(c). The mean population size of *E. coli* O157 in each of the compartments over time, following the initial dose of infection, is plotted and reveals that similar behaviours are seen in the infection population dynamics for both probiotic scenarios, although the within-animal population appears to have died out even faster and the environmental pool size is even lower throughout where the probiotic had a stronger effect. The same graph is plotted over a

Fig. 2. Mean bacterial population sizes over time, after an initial dose of $10^4$ cfu (unless stated), using different inhibitory probiotic strategies. (a) No probiotics (>5 months), (b) 20% reduction (4 months), (c) 50% reduction (4 months), (d) 50% reduction (1 week), (e) no probiotics ($10^7$ cfu dose) and (f) 50% reduction ($10^7$ cfu dose).
shorter period of time in Fig. 2(d), which allows the infection dynamics to be observed in greater detail to establish the nature of the reductions. Comparisons with the standard case showed that the infection died out more quickly in the rumen when probiotics were present, which had a consequent effect in the following compartments. The differences in the abomasum populations were not substantial, although the infection levels were typically much lower in this region, but, critically, the differences in the faeces populations were considerable; the inability of the infection to reach high initial levels in this compartment before becoming extinct was the main factor contributing to the large reduction in the environmental pool size.

The simulation study in which probiotics with the potential to reduce the growth of *E. coli* O157 by 50% were used is repeated assuming an initial dose of $10^7$ cfu of infection in order to investigate whether the control measure could have a similarly dramatic effect at higher infection levels. However, the larger population sizes greatly increased the run time of the program and therefore, the mean bacterial levels plotted in Fig. 2(f) have been averaged over just 50 runs. An attempt has been made to provide a comparison with the standard case in which probiotics were not present, but the high population sizes and resulting prohibitive run time meant that the plot in Fig. 2(e) is of just a single realisation.

Examination of the plots reveals that the presence of probiotics had a large effect, reducing the population size of *E. coli* O157, even at higher infection levels. The population of *E. coli* O157 in the within-animal compartments died out more quickly, with this effect being particularly noticeable in the faeces compartment. Once again, the environmental pool was consistently lower throughout, although in this scenario the probiotics have not reduced the pool size to a sufficiently low level to prevent ingestion events. Nevertheless, the ingestion events that did occur did not appear to have much impact on the maintenance of the infection within the animal, as the short spikes that can be seen in the rumen compartment reflect a series of small doses of infection ($\sim 10^2, 10^3$ cfu) which died away quickly before the infection could build up in any of the subsequent compartments.

The mean results are not informative about the typical number of ingestion events that occurred to a single animal and so information from the individual realisations that contributed to the average population sizes plotted in Fig. 2(f) was considered from this perspective. This analysis revealed that when probiotics were used, the number of ingestion events per realisation ranged from 12 to 31, with a mean of 22 events, which compared favourably to the non-probiotic realisation plotted in Fig. 2(e) in which 107 events occurred. Therefore, even at these higher infection levels, the use of probiotics not only reduced the levels of *E. coli* O157 within animals and in the environment, but also reduced the frequency and severity of ingestion events.

The results obtained from the model simulating the conditions likely to face *E. coli* O157 in the presence of probiotics in vivo show that even if probiotic bacteria were only to inhibit the growth of *E. coli* O157 by 20%, the effects could be quite considerable; at low infection levels, within-animal infection dies out very quickly with the result that environmental levels remain sufficiently low to make ingestion events unlikely, while at higher infection levels, although the environmental pool size is not reduced enough to stop ingestion events occurring, they do occur more infrequently with a very low potential for
maintaining infection. Therefore, the output of the model adds further weight to the preliminary results in Zhao et al. (1998); Ohya et al. (2001) and Brashears et al. (2003), which indicate that continued development of a suitable probiotic product for livestock, followed by widespread application, would be highly advantageous in the control of \textit{E. coli} O157 infection. Similar considerations therefore apply to the potential effect of bacteriostatic antibiotics, unlikely as this control strategy may be. The results reported in this section have been based on the assumption that the sole mechanism of action for probiotics is growth inhibition. Probiotics which act by increasing the death rate of \textit{E. coli} O157 will be discussed in the next section.

3. Bactericidal antibiotics or probiotics

Probiotics and antibiotics can control \textit{E. coli} O157 either by decreasing the birth rate, or by increasing the death rate. Therefore, the model provides an interesting opportunity to establish whether there are likely to be any differences in effect between the two different mechanisms of control.

In practice, antibiotics are not used for the treatment of \textit{E. coli} O157 in humans and animals intended for human consumption. Retrospective analyses have suggested that in human infection these agents may be a risk factor for the clinical case progressing from diarrhoea to the much more serious disease of Haemolytic Uraemic Syndrome (Ryan et al., 1986; Bell et al., 1997). Antibiotics are not used in cattle to minimise the risk of bacteria developing antibiotic resistance. Although drug-resistant \textit{E. coli} O157 is not a problem per se since antibiotic treatment is contraindicated for human \textit{E. coli} O157 infection, it is thought that any such resistant \textit{E. coli} O157 could serve as a possible reservoir for the spread of resistant factors to other microorganisms (Galland et al., 2001).

Nevertheless, cattle still receive antibiotics both therapeutically to treat other infections, and to act as growth promoters. Therefore, since antibiotics are on occasion present in vivo, the drugs could potentially have an effect on the population dynamics of \textit{E. coli} O157. It is known that certain antimicrobials, such as ionophores, are more effective in inhibiting the growth of Gram-positive bacteria than Gram-negative bacteria. Therefore, it has been argued that this disturbance in the commensal microflora could actually cause \textit{E. coli} O157 and other Gram-negative bacteria to flourish (Rasmussen et al., 1999). However, recent experimental studies have found that the presence of ionophores has no effect on \textit{E. coli} O157 populations (Edrington et al., 2003).

However, certain antibiotics used in cattle to treat other infections do appear to be effective against \textit{E. coli} O157. For instance, Elder et al. (2002) have found that Neomycin Sulphate significantly reduces the volume of \textit{E. coli} O157 shed by cattle. Therefore, it is of interest to use the simulation model to investigate the potential magnitude of such an effect. The exercise is also relevant to inform the situation where an alternative control method might be proposed which has a similar effect to that of antibiotics on the death rate of \textit{E. coli} O157.

In order to simulate the effect that short-term use of a bactericidal antibiotic such as Neomycin has on the \textit{E. coli} O157 population, it was necessary to modify the model
parameters appropriately. These modifications involved an increase in the death rates since this most accurately reflected the way in which such antibiotics affect the growth of \textit{E. coli} O157. As before, the death rates in each of the within-animal compartments were affected since there was no indication to suggest that the efficacy of antibiotics is restricted to a particular area of the gut. The values of the remaining parameters were held at their standard values (Wood et al., 2006). The results in this section may equally be interpreted as applying to bactericidal probiotics, although the text throughout will refer to antibiotics.

As antibiotics are not used to treat \textit{E. coli} O157 infections, there is limited information regarding their efficacy for this purpose, so in order to make comparisons with the previous intervention method, it is decided to explore the scenarios in which the presence of antibiotics resulted in a 20% and 50% increase in the removal of any \textit{E. coli} O157 in the system. Therefore, the simulation model was run using the modified parameters outlined in Table 2 for the first 5 simulated days only (to reflect a 5-day course of antibiotics, for example), which were obtained by taking the original set of parameters and increasing the death rates by 20% and 50%, before reverting back to the original set of parameters, as used in the standard model in which no control measures were implemented, for the remainder of each run. The model assumed an initial dose of infection of $10^4$ cfu at the start of each run, coincidental with the start of the course of antibiotics in order to establish the maximum benefit which might be provided by this potential control method. This is a relatively plausible scenario, since when cattle are ill and hence have less effective immune-systems, there may be potential for other infections, such as \textit{E. coli} O157, to flourish in the disturbed microbial flora of the gastrointestinal tract during the (hopefully short) length of time until the appropriate treatment is administered.

The results from the simulation model as run under these two scenarios are plotted in Fig. 3. Fig. 3(a) shows the mean population size of \textit{E. coli} O157 in each compartment following infection by an initial dose of $10^4$ cfu, averaged over 100 runs, obtained by running the simulation model while assuming the presence of antibiotics, which increase the removal of \textit{E. coli} O157 by 20%. Fig. 3(b) shows the same results plotted over a shorter time-scale in order to reveal more detail. As with the previous control method, it can be clearly seen that the populations of \textit{E. coli} O157 died out very rapidly in all compartments following infection. As a result, there was a reduction in the amount of bacteria shed into the environment to such an extent that no ingestion events occurred throughout.

The exercise was repeated with the simulation model being driven by the parameters which had been modified in order to reproduce the effect of an antibiotic with the ability to increase the removal of \textit{E. coli} O157 by 50%. The mean bacterial population size in each of

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Rumen</th>
<th>Abomasum</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>D</td>
<td>B</td>
</tr>
<tr>
<td>No control</td>
<td>0.37</td>
<td>0.48</td>
<td>0</td>
</tr>
<tr>
<td>20% increase</td>
<td>0.37</td>
<td>0.576</td>
<td>0</td>
</tr>
<tr>
<td>50% increase</td>
<td>0.37</td>
<td>0.720</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2
Birth (B) and death (D) parameter values for different bactericidal antibiotic scenarios
the compartments following infection by an initial dose of $10^4$ cfu, averaged over 100 runs, are plotted in Fig. 3(c). The increased efficacy of the antibiotic is clearly illustrated; the within-animal infection died out even more quickly and the environmental pool size was considerably lower throughout when compared with that in the previous scenario. As previously, there were no ingestion events.

It has been shown experimentally that the short-term administration of certain bactericidal antibiotics can be effective against *E. coli* O157 (Elder et al., 2002), as confirmed by the results in Fig. 3. The model can also be used to investigate whether longer-term administration could generate greater benefits. This scenario was simulated by assuming that antibiotics with the ability to increase the removal rate of *E. coli* O157 by 50% were continually present in vivo, and therefore, the modified parameters were used throughout the run time for each realisation.

The mean bacterial population sizes for each of the compartments, averaged over 100 runs, can be seen in Fig. 3(d). The within-animal population sizes were almost identical for both short and long-term scenarios (Fig. 3(c and d), respectively). This is not surprising since in the majority of realisations the within-animal *E. coli* O157 population died out within the initial 5 days, during which time the same set of parameters were used in both scenarios. Although the short-term use did allow more bacteria to be shed into the

---

Fig. 3. Mean bacterial population sizes over time after an initial dose of $10^4$ cfu using different bactericidal antibiotic strategies. (a) 20% increase (5-day use), (b) 20% increase (5-day use), initial phase, (c) 50% increase (5-day use) and (d) 50% increase (continual use).
environment, and therefore the bacterial load in the environment was typically greater over the time considered, as seen above, the environmental pool remained sufficiently low to make it unlikely that any ingestion events would occur. This result would suggest that a control measure which inhibits the growth of *E. coli* O157 in this manner need not be implemented on a continual basis, which has important implications in terms of cost and sustainability. However, these results assume that implementation of short-term treatment is immediate upon infection. This is unlikely to be a realistic description of antibiotic treatment. In reality, the behaviour of the system will be intermediate between that seen in Figs. 3(a) and 2(a), depending on the length of time of shedding prior to treatment commencing. It is interesting to note the similarity of the results obtained in this section to those obtained in the previous section. This suggests that, whether through inhibition of growth or increased removal of *E. coli* O157, the different mechanisms of control, when assessed at equivalent levels of efficacy, are associated with similar levels of reduction in prevalence.

4. Dietary intervention

Unlike the previous control measures considered, dietary interventions have been the subject of extensive research into their potential to inhibit the growth of *E. coli* O157. In particular, changes of diet and feed deprivation have been examined, since it is biologically plausible that such interventions will have an effect on gut microbial populations. Nevertheless, despite the large volume of research undertaken in this area, there remains a lack of consensus on the effects, if any, of particular intervention methods on the *E. coli* O157 population. Therefore, the simulation model was used to explore two of these methods (fasting and the feeding of grain) to provide further insights into the dynamics of any changes arising from these strategies.

4.1. Fasting

When ruminants feed, the fermentation process results in the production of volatile fatty acids (VFA). The presence of these acids can inhibit the growth of *E. coli* O157, which suggests that when animals are well fed and hence, have high VFA levels, within-animal population levels of *E. coli* O157 will remain low (Rasmussen et al., 1993). However, most cattle are likely to have been starved for a period of time before being slaughtered. This occurs because cattle are generally not fed in transit to the abattoir, both for logistical reasons and because the associated reduction in the amount of intestinal waste aids the slaughter process. Obviously, it is important to establish whether temporary starvation may have the effect of causing the proliferation of *E. coli* O157 within the ruminant gut. Hence, there is a particular interest in the short-term effects of fasting, but to consider the effects of other types of fasting events, such as those associated with illness, and to facilitate comparison with the interventions described in other sections, long-term simulation results also will be presented in this section.

A series of experiments in the 1960s, which focused on other strains of *E. coli* and *Salmonella*, found that when animals were deprived of feed, the bacterial counts increased...
to an extent that they were shed in the faeces in detectable numbers, thereby supporting the argument that the lowered VFA levels induced by fasting allow enteric bacteria to flourish in the rumen (Brownlie and Grau, 1967; Grau et al., 1969). More recent work by Rasmussen et al. (1993) focused on \textit{E. coli} O157, and found that all strains showed unrestricted growth in ruminal fluid from fasted cattle. They suggested that, in addition, \textit{E. coli} O157 may have a competitive advantage over commensal rumen species under substrate-depleted conditions, which allows \textit{E. coli} O157 to flourish briefly in intermittently fed animals until the normal bacterial flora becomes re-established. In order to investigate the effects of dietary stress caused by transportation (to the abattoir, for instance), Cray et al. (1998) subjected calves to experimental conditions which imitated this situation. In contrast to the work cited above, this research failed to find a significant increase in the amount of faecal shedding, although this could be due to the fact that the infection was inoculated into the rumens of well-fed animals, which were then deprived of food, rather than inoculated into pre-fasted animals. However, this study did find that the \textit{E. coli} O157 inoculated into calves that had been fasted were more likely to survive in vivo. More recent studies have also found that fasting appears to have little effect on \textit{E. coli} O157 populations, although there is some evidence to suggest that cattle are most susceptible to \textit{E. coli} O157 infection while fasting (Harmon et al., 1999; Buchko et al., 2000).

Nevertheless, there is a general consensus that the rumen of fasted animals should provide favourable conditions for the growth of \textit{E. coli} O157, although the magnitude of such an effect remains unclear. Therefore, the simulation model was used to explore the effect of introducing the infection to fasted animals. Firstly, it was necessary to obtain a set of model parameters which were appropriate for the within-animal model under fasting conditions. When animals are deprived of food for 24–48 h, VFA levels decline to a concentration below 50 mmol/l (Harmon et al., 1999), and thus the pH value in the rumen can exceed 7.0. Utilising work by Rasmussen et al. (1993), these conditions correspond to an \textit{E. coli} O157 growth rate in the region of 0.72–0.89 h\(^{-1}\) depending on the strain involved. For this simulation study, a birth rate of 0.8 h\(^{-1}\) was chosen for the rumen compartment, which is more than twice the rate under normal conditions. The effects of dietary stress on the passage rate of transient bacteria populations from the rumen and on resident populations in the colon are unknown (Cray et al., 1998), but since the rumen is the main site of primary fermentation and hence the site most affected by fasting, it was assumed that any effects in other sites are minimal, and were therefore disregarded.

The simulation study assumed that an initial dose of infection entered a pre-fasted animal, for which all the parameters for the compartments were set at the values associated with the standard model, bar the ruminal birth rate, which took a higher value. Obviously, the animal would only be deprived of food on a temporary basis, so after the first 24 simulated hours, the rumen birth rate was returned to the standard value to model the effect of the animal being fed again. Full details of the parameter values used are given in Table 3.

The results from this study are illustrated in Fig. 4. The bacterial population sizes in each compartment for one realisation, following an initial dose of 10\(^3\) cfu administered during a period of fasting, are plotted over the short- and long-term in Fig. 4(c and d), respectively. It was not possible to apply an initial infection dose of 10\(^4\) cfu, as used in previous scenarios, since the fasting conditions caused the bacterial population size to
increase to such an extent that the running time for the model proved to be prohibitive. Although results could be obtained using the lower initial dose of $10^3$ cfu, the running time involved made it impossible to run the model sufficiently often to obtain meaningful mean values, and therefore, the results from a typical realisation are plotted. In order to facilitate comparisons, Fig. 4(a and b) show the equivalent plots obtained from running the standard model with an initial dose of $10^3$ cfu, although these figures present the mean population sizes, averaged over 100 runs.

Examining the within-animal population sizes for the fasted animal over the first week following infection (Fig. 4(c)), a clear effect of feed deprivation on the population dynamics of *E. coli* O157 can be seen: the population levels in all compartments increased

![Graphs showing bacterial population sizes over time after an initial dose of $10^3$ cfu for fasted and non-fasted animals.](image)

Fig. 4. Bacterial population sizes over time after an initial dose of $10^3$ cfu for fasted and non-fasted animals. (a) No fast (1 week), (b) no fast (4 months), (c) 24 h fast (1 week) and (d) 24 h fast (4 months).
sharply until the animal began feeding again at the 24 h point. At this time, the production of VFA caused the populations in the rumen and abomasum to start to decline steadily, although the infection present in the faeces decreased at a much slower rate, contributing to an increase in the environmental pool size. A comparison of Fig. 4(b and d) shows that this initial increase in the environmental pool had a considerable effect over time. Throughout the time-scale considered, the environmental pool originating from the fasted animal remained greater by 3–4 log cfu, with the consequence that while no ingestion events occurred in the original scenario, the fasted animal was subject to repeated ingestion events. In fact, these ingestion events occurred so frequently that the animal was virtually persistently infected.

The difference of up to 4 log cfu in the in vivo bacterial populations for an animal fasted for 24 h following infection and a well-fed animal which is observed here closely matches the results of Cray et al. (1998), who found that bacterial populations increased by 4–6 log cfu in both the rumen contents and in faecal samples obtained from animals which had feed withheld for 48 h. It is encouraging that the model predicts quantitatively similar behaviour to that demonstrated in experimental studies.

While a correlation between VFA concentration and the faecal levels of \( E. coli \) O157 has not always been observed (Harmon et al., 1999), the model has been used to explore the within-animal effect of introducing infection to diet-stressed cattle. The model has shown that the ruminal conditions induced by fasting have the potential to allow \( E. coli \) O157 to proliferate within the animal, the effect arising from the assumption that lower VFA concentrations lead to higher \( E. coli \) O157 growth rates. In practice, it is likely that any increase in growth will eventually be limited by ecological factors such as density-dependence and competition from commensal organisms (Cray et al., 1998). The temporary withholding of feed may provide a simple and economical method of increasing the likelihood of detecting the organism in infected animals, however, in general, the effects of fasting are not desirable (Armstrong et al., 1996). Hence, although feed deprivation is not a control measure in itself, an awareness that fasted animals are particularly susceptible to \( E. coli \) O157 proliferation should encourage the introduction of appropriate precautions if animals are likely to experience dietary stress. For example, probiotics, which, as shown previously, have the ability to inhibit the growth of \( E. coli \) O157, could be fed to cattle before they undergo a period of fasting. In this way, the use of probiotics before animals are transported to an abattoir could reduce the impact of \( E. coli \) O157 infection on the human food chain.

4.2. Grain-feeding

Another dietary intervention which has been the focus of much research is dietary change, and in particular, the effect of feeding grain to cattle. In the developed world, cattle are fed grain to promote more efficient weight gain. However, grain and a common alternative diet, fodder hay, have different effects on the pH and VFA levels in different parts of the bovine gastrointestinal tract. Diez-Gonzalez et al. (1998) found that feeding grain to cattle did not cause a significant difference in the amount of VFA in the rumen compared to the amount present when hay was fed, although the amounts in the colon increased approximately four-fold. This resulted in no change to ruminal pH level, but a
decrease to that of the colon. The effects of these changes on shedding are unclear. While some research workers have found no difference in levels of *E. coli* O157 in grain-fed and hay-fed cattle (Hancock et al., 1994; Jordan and McEwen, 1998), others have found that animals fed on forage produced more *E. coli* O157 (Hovde et al., 1999). Indeed, in Argentina, which has a high prevalence rate of *E. coli* O157, cattle are almost without exception fed on forage (Reilly, 1998; Rasmussen et al., 1999), although the high prevalence may be caused by other factors. However, these results do not concur with those of Diez-Gonzalez et al. (1998) who found that the manure of grain-fed animals contained 1000 times more *E. coli* O157 than that of hay-fed animals.

Further research is essential to clarify the effect of different diets on the bovine gastrointestinal system, and hence on the dynamics of the *E. coli* O157 population in vivo, where the use of a model such as that described in this paper would have considerable scope to inform the results of any such work.

5. Conclusions

This paper has used a simulation model of the bovine gastrointestinal tract to explore the efficacy of various methods in changing *E. coli* O157 population dynamics within the animal (Wood et al., 2006). The control methods considered each disturbed the normal microbial balance along the length of the animal digestive tract either by altering the environmental conditions or by adding competing microorganisms.

The simulation model was used to provide an alternative perspective to evaluate control strategies for which relevant experimental studies would be limited by financial or ethical constraints, or control strategies which are as yet hypothetical. As the model is being used to quantify information about intervention approaches whose mechanisms of control may be not fully understood or even unknown, it has been necessary to make assumptions about how each control method affects the within-animal *E. coli* O157 population dynamics.

While the model is no substitute for further experimental studies, it is anticipated that the information gained could supplement the results from such studies. As understanding of the control processes improves, it may be found that certain assumptions are inappropriate, but, in such an event, the design of the model allows appropriate modifications to be easily implemented. Nevertheless, it is encouraging to note that the model consistently predicted quantitatively similar behaviour to that demonstrated by relevant experimental studies.

A variety of control mechanisms have been evaluated using the simulation model. The conclusions about the dietary-based interventions were the least clear-cut. Having assumed an increased *E. coli* O157 growth rate arising from more favourable VFA conditions, the results from the model supported the view that fasted cattle are particularly susceptible to the infection, but this is at odds with recent studies that suggest that fasting has little effect on the *E. coli* O157 population.

The use of agents with antimicrobial properties were also examined. The model showed that bactericidal antibiotics or probiotics have the potential to reduce the level of *E. coli* O157 shed by cattle, and that such a strategy may not require anything beyond short-term
use of the medication. However, despite similar results having been obtained for a specific antibiotic in a recent experimental study, issues such as the risk of spread of antibiotic resistance and an uncertainty about whether the resultant disturbance to commensal microflora may actually cause bacterial proliferation appear to be hindering further developmental progress. Bactericidal probiotics may be a more viable option for development.

Of all the modes of control considered, the use of probiotic bacteria appears most promising. The model showed that probiotics need only be moderately successful at inhibiting the growth or increasing the death rate of \textit{E. coli} O157 to ensure that infection levels rapidly decrease. This reduction in shedding in turn reduced the pool of bacteria in the environment to such an extent that the frequency of animals reinfecting themselves through the inadvertent ingestion of environmental bacteria (and therefore maintaining the infection) was considerably reduced. As the results obtained from the model concur with those of recent experimental studies, it would appear that the continued development of a suitable probiotic product for livestock is well advised.

In summary, the bovine in vivo model has generated results broadly similar to those obtained from corresponding experimental studies. Unfortunately, the most promising methods for controlling \textit{E. coli} O157 populations in vivo, such as probiotic bacteria, require further development before they can be widely implemented. The use of a model such as that described in this study shows great promise as a tool to evaluate the efficacy of hypothetical control measures, and could ultimately be of use in optimising the operational details of a viable control strategy.

Acknowledgements

J.C.W. gratefully acknowledges the support of a BBSRC studentship. I.J.M. acknowledges support from the Scottish Executive Environment and Rural Affairs Department, project BSS/028/99 and the Wellcome Trust International Partnership Research Award for Veterinary Epidemiology. The authors would like to acknowledge the input of an anonymous referee in improving the structure of the paper.

References


