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Modeling On-Farm *Escherichia coli* O157:H7 Population Dynamics

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Abstract

Escherichia coli O157:H7 is a potentially fatal foodborne pathogen with a putative reservoir for human infection in feedlot cattle. In order to more effectively identify targets for intervention strategies, we aimed to (1) assess the role of various feedlot habitats in *E. coli* O157:H7 propagation and (2) provide a framework for examining the relative contributions of animals and the surrounding environment to observed pathogen dynamics. To meet these goals we developed a mathematical model based on an ecological metapopulation framework to track bacterial population dynamics inside and outside the host. We used *E. coli* O157:H7 microbiological and epidemiological literature to characterize *E. coli* O157:H7 habitats at the pen level and account for *E. coli* O157:H7 population processes in water troughs, feedbunks, cattle hosts, and pen floors in the model. Simulations indicated that *E. coli* O157:H7 was capable of maintaining viable populations in the feedlot without net growth in the cattle gastrointestinal tract, suggesting *E. coli* O157:H7 may not always act as an obligate parasite. Water troughs and contaminated pen floors appeared to be particularly influential sources driving *E. coli* O157:H7 population dynamics and thus would serve as prime environmental targets for interventions to effectively reduce the *E. coli* O157:H7 load at the pen level.

Introduction

Enterohemorrhagic *Escherichia coli* is a serious and potentially fatal pathogen of humans across the globe. *E. coli* O157:H7 infection can result in hemolytic uremic syndrome and accounts for thousands of cases of severe foodborne illness in the United States each year (Mead *et al*., 1999). *E. coli* O157:H7 can infect humans from a wide variety of sources; however, the most common source of exposure and subsequent infection is contaminated food. Poorly cooked, tainted ground beef and other bovine food products have often been implicated as the primary source of infection in outbreaks (e.g., Chapman *et al*., 1992; Morgan *et al*., 1993; Tarr 1995; Armstrong *et al*., 1996). Cattle are frequently test-positive and actively shed *E. coli* O157:H7 intermittently after challenge infection but rarely show clinical signs of disease (Besser *et al*., 1997; Hancock *et al*., 1997; Shere *et al*., 1998; Khaitsa *et al*., 2003). Cattle are putatively considered the primary reservoir for *E. coli* O157:H7 infecting humans (as reviewed by Hussein and Sakuma, 2005). It has been shown that a reduction in *E. coli* O157:H7 shedding in feedlot cattle is correlated with a reduction in carcass contamination at slaughter, indicating that preharvest interventions may be an effective means of controlling human foodborne infection (Chapman *et al*., 1992; Elder *et al*., 2000).

A number of interventions have been proposed to reduce *E. coli* O157:H7 load or prevalence in cattle that follow one of two general strategies: to reduce cattle's exposure to *E. coli* O157:H7 or to increase the resistance of cattle to the bacteria (Sargeant *et al*., 2007). Despite extensive testing including randomized clinical trials, no single intervention appears ready for extensive implementation that would result in the control of O157:H7 in bovine populations (Callaway *et al*., 2004; Ahmadi *et al*., 2007; LeJeune and Wetzel 2007). This suggests further work is needed to identify intervention measures that are both efficacious in reducing the load of *E. coli* O157:H7 at harvest and economically and logistically feasible to administer to large cattle holdings. However, it is not always clear if the failures of previous control strategies stem from an inefficient mechanism of action in the intervention when applied at the population level or are the result of inadequately targeting the vulnerable habitats of *E. coli* on the farm and feedlot. Currently, this question is difficult to answer as the relative importance of factors leading to propagation and maintenance of *E. coli* O157:H7 on the farm are poorly understood.

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Fecal–oral contamination is presumed to be an important mechanism for transmission of *E. coli* O157:H7 between cattle in close proximity. The potential for direct transmission between cattle has been demonstrated in proof of concept studies; however, given the relatively high load and potential for reproduction of *E. coli* O157:H7 in water troughs (LeJeune *et al*., 2001a, 2001b; Van Donkersgoed *et al*., 2001), feed bunks (Lynn *et al*., 1998), slurry (Maule, 2000), feces (Wang *et al*., 1996; Fukushima *et al*., 1999), pen floors (Berry and Miller, 2005), and surrounding environment (Trevena *et al*., 1996; Beuchat, 1999; Taormina and Beuchat, 1999; Maule, 2000) and the high level of contact between these habitats and feedlot cattle, it seems unlikely that cow-to-cow transmission accounts for the majority of *E. coli* O157:H7 propagation and maintenance. Further support is found in the work of LeJeune *et al*. (2004), who reported that restriction endonuclease digestion patterns observed in *E. coli* O157:H7 remained consistent within feedlot from year to year despite massive cattle turnover, which the authors concluded implicated the farm environment, rather than incoming cattle, as a source for *E. coli* O157:H7.

Mathematical models have generally provided a framework in which the effect of interventions is able to be tested under a wide range of management conditions, thus allowing researchers to identify areas and processes vulnerable to disruption and offering specific management guidance. Previous mathematical modeling work has attempted to parse the contribution of environmental contamination and direct and indirect cattle to cattle transmission to observed *E. coli* O157:H7 dynamics (Turner *et al*., 2003, 2006, 2008; Liu *et al*., 2005, 2007; Ahmadi *et al*., 2007; Wood *et al*., 2007). However, the role of environment in on-farm *E. coli* O157:H7 propagation remains unclear.

We aim to address two central objectives through this study. Firstly, we examine the importance of various *E. coli* habitats (water troughs, feed bunks, cattle gastrointestinal tracts, and surrounding pen environment) to the propagation of the *E. coli* O157:H7 strain within the context of an ecologically realistic cattle feedlot. Secondly, we aim to identify vulnerable points for control of *E. coli* O157:H7 propagation and maintenance in cattle and the surrounding pen environment through various interventions in the absence of an explicitly modeled contact-based pathogen transmission process. To robustly examine these questions, we depart from the infectious disease compartmental framework (i.e., Suspectible Infected Susceptible models) used in previous studies and develop pathogen-level metapopulation models capable of reflecting widespread bacterial growth and population dynamics outside an animal host. This will allow us to focus current and future efforts to develop intervention strategies aimed at lowering the load of *E. coli* O157:H7 presented to the human food chain.

Methods

Model description

To meet these aims we follow methods based in an ecological metapopulation framework (Hanski, 1998) modified to represent a free-living pathogen capable of extra-host replication. The metapopulation framework allows for the study of populations consisting of organisms capable of moving between and growing in spatially segregated habitats. Briefly, a system of coupled ordinary differential equations is parameterized to represent potential replication habitats (i.e., patches) and population dynamics for *E. coli* O157:H7 while allowing for transfer between patches.

The dynamic equations in this model system represent *E. coli* O157:H7 organisms migrating between water troughs (*W*), feedbunks (*F*), cattle (*C*), and surrounding pen environment (*E*) compartments (Fig. 1). Habitats were selected based on reported potential for sustaining *E. coli* O157:H7 growth. Population dynamics are heterogenous between patches, reflecting diverse abilities at supporting *E. coli* O157:H7 replication:

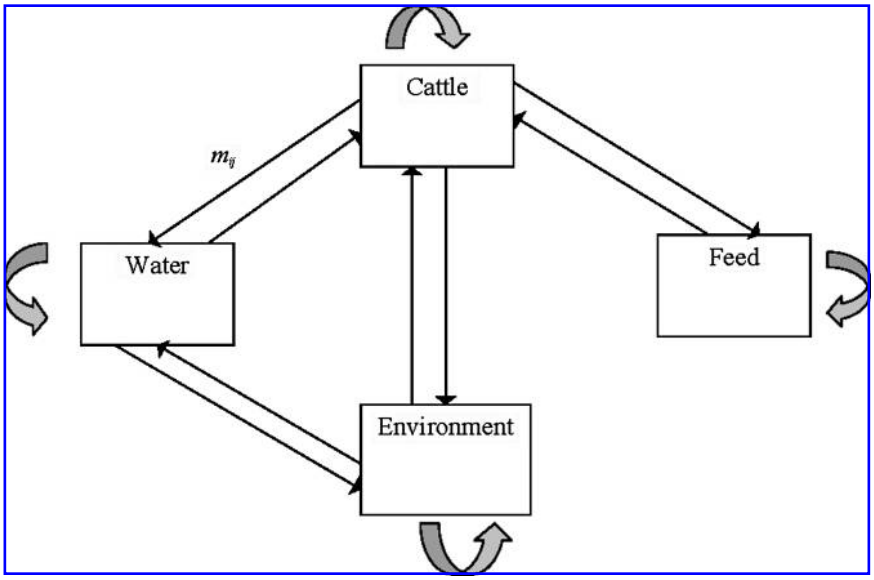

FIG. 1. Diagrammatic representation of model states and flows. Boxes represent patches where *E. coli* O157:H7 can reside. Arrows represent migration rates between patches with flow from patch *i* to patch *j* represented by m_{ij}. Large arrows indicate density-dependent exponential growth of bacteria within each patch.

$$\frac{dC}{dt} = r_c C\left[1 - \frac{C}{K_c}\right] - (\mu_c + p)C + m_{ec}E + m_{wc}W + m_{fc}F$$

$$\frac{dW}{dt} = r_w W\left[1 - \frac{W}{K_w}\right] - (\mu_w + m_{wc} + m_{we})W + m_{ew}E + m_{cw}pC$$

$$\frac{dF}{dt} = r_f F\left[1 - \frac{F}{K_f}\right] + m_{cf}pC - (\mu_f + m_{fc})F$$

$$\frac{dE}{dx} = r_e E\left[1 - \frac{E}{K_e}\right] + m_{we}W + m_{ce}pC - (\mu_e + m_{ew} + m_{ec})E$$

Bacteria are tracked and parameters calibrated to colony-forming units (CFU) as these are the most common quantified measures of bacterial presence reported in the *E. coli* O157:H7 literature. Parameters are defined and estimates presented in Table 1. Point estimates and distributions were derived from literature when possible. Distributions were selected to encompass both expected natural variation and uncertainty in point estimates. The parameter μ_j represents non–density-dependent death (population-level decay) in compartment j. Growth rates (r_j) in water troughs, feedbunks, animals, and surrounding environment are assumed to experience density-dependent adjustment through the logistic function dependent on the patch's carrying capacity (K_j). Growth rate in cattle (r_c) was set to 1 in the base model indicating neutral net

Table 1. Parameter Definitions and Values for Deterministic and Stochastic Analysis

Parameter	*Definition (units)*	*Baseline value*	*Sensitivity analysis*	*Reference*[a]
Water				
K_w	Carrying capacity (CFU/mL)	10^6	Log uniform (2, 7)	Amalaradjou *et al*., 2006; Besser *et al*., 2003; LeJeune *et al*., 2001a, 2001b
r_w	Replication (per day)	3.5	Normal (3.5, 0.75)	LeJeune *et al*., 2001b
μ_w	Death (per day)	0.06	Uniform (0, 0.13)	LeJeune *et al*., 2001a
V_w	Trough size (L)	50	Uniform (25, 3000)	Pers. comm.; Harner and Murphy, 1998
Environment				
K_e	Carrying capacity (CFU/g)	10^5	Log uniform (1, 6)	Berry and Miller, 2005; Besser *et al*., 1997; Jiang *et al*., 2002
r_e	Replication (per day)	1.5	Normal (1.5, 0.5)	Wang *et al*., 1996
μ_e	Death (per day)	0.3326	Uniform (0.1–0.9)	Jiang *et al*., 2002; Maule, 2000; McGee *et al*., 2001
V_e	Size of E compartment (kg)	500	Uniform (200, 1000)	Management
Feed				
K_f	Carrying capacity (CFU/g)	10^4	Log uniform (1, 5)	Besser *et al*., 1997; Lynn *et al*., 1998
r_f	Replication (per day)	0.75	Uniform (0.05–2.5)	Besser *et al*., 2003; Lynn *et al*., 1998
μ_f	Death (per day)	0.3	Uniform (0.05–1.5)	Estimated
V_f	Bunk capacity (kg)	2000	Uniform (500, 2500)	Pers. comm.
Cattle				
K_c	Carrying capacity (CFU/g feces)	10^6	Log uniform (1, 7)	Chase-Topping *et al*., 2007
r_c	Replication (per day)	1	Uniform (1, 3)	Estimated
μ_c	Death (per day)	3	Uniform (1, 12)	Estimated
P	Passage rate (per day)	1	Constant	Van Soest, 1994
S	Number steers (animals)	150	Uniform (50, 300)	Pers. comm.; Harner and Murphy, 1998
c_w	Water consumption [L/(animal/day)]	45	Normal (45, 7)	Harner and Murphy, 1998
c_f	Feed intake [kg/(animal/day)]	11	Normal (11, 2)	Pers. comm.
c_e	Fecal and soil ingestion [kg/(animal/day)]	0.75	Uniform (0.25, 1.25)	Estimated
Movement rates between patches[b]				
m_{wc}	Drinking (per day)	$\frac{S\times c_w}{V_w}$		NA
m_{fc}	Consumption(per day)	$\frac{S\times c_f}{V_f}$		NA
m_{ec}	Ingestion of environment (per day)	$\frac{S\times c_e}{V_e}$		NA
m_{we}	Spillage (per day)	0.1	Uniform (0.05, 5)	Estimated
m_{ew}	Water contamination(per day)	0.005	Uniform (0.001, 0.01)	Estimated
m_{cw}	Fecal shedding(to W; per day)	0.05	Uniform (0.01, 0.07)	Estimated
m_{cf}	Fecal shedding(to F; per day)	0.02	Uniform (0.005, 0.03)	Estimated
m_{ce}	Fecal shedding(to E; per day)	$m_{ce} = 1 - m_{cw} - m_{cf}$	NA	NA

[a]Pers. comm. indicates that estimates were arrived at from discussions with T. Besser, G. Loneragan, M. Baker, and J. VanDonkersgoed.
[b]m_{ij} is the movement rate from compartment i to compartment j where $i, j \in \{c, w, e, f\}$ and $i \neq j$.
CFU, colony-forming units.

growth. This parameter was however allowed to vary above 1 in the sensitivity analysis of the deterministic and stochastic models to assess the importance of pathogen replication in the host assuming long-term colonization and growth. The terms m_{ji} represent the rate of movement between habitat j to habitat i and p is the passage rate of ingested media through the cattle gastrointestinal tract. All elements outside the logistic functions in the model are linear with assumed exponential time to events.

Cattle ingest bacteria from and shed fecal matter into all other states. The model developed here to test the role replication plays in each compartment does not mechanistically model contact-based transmission between cattle. Patches were selected based on their abilities to sustain and/or propagate *E. coli* O157:H7, and cattle are considered a single patch experiencing homogenous growth. Spilled water is modeled as a vector for CFU to move into the environment, and bacteria are reciprocally able to enter water from the environment as troughs are assumed to be contaminated throughout the daily activities of the cattle. A single pen is considered.

All patches are subject to assumptions of homogenous mixing within the compartment. For example, water troughs and feedbunks are assumed to have uniformly distributed CFU throughout the water column and feed mixture, respectively, and all steers are assumed to eat and drink at equal rates evenly across a day. All patches also maintain a constant capacity reflecting an assumption that portions of the "habitat" consumed or removed are continuously refilled. Water sources, feedbunks, and surrounding environment (e.g., pen floor) are assumed to be the only sources from which cattle are exposed to *E. coli* O157:H7. The bulk of feces are assumed to enter the surrounding environment, thus fecal–oral contamination is largely modeled as vectored through this compartment.

The model was implemented and solved numerically in both Vensim Professional (Ventana Systems, Inc., Harvard, MA) and R 2.4.1 (freely available online at www.r-project.org) across a single summer season (120 days). Model output from both software systems agreed quantitatively with less than 1% variability at steady state. Bifurcations across parameters were explored and figures and data reported here were developed in R, Matlab (The Mathworks, Natick, MA) (2006), Microsoft Excel (Microsoft Corporation, Redmond, WA), and PowerPoint (Microsoft Corporation) (2003). Baseline parameter estimates as reported in Table 1 were used for all deterministic simulations and analyses unless otherwise noted. All analyses presented below were conducted with initial values of zero CFU in all patches except for feed ($F_0 = 10{,}000$), to mimic an introduction of *E. coli* O157:H7 via feed into an otherwise sterile feedlot.

Stochastic simulations

We generated stochastic simulation data from a range of parameter values in order to evaluate the robustness of results derived from the deterministic analysis. We used Monte Carlo techniques to conduct a global sensitivity analysis (as reviewed in Helton and Davis, 2003) by generating 10,000 iterations. Parameter distributions were sampled using Latin Hypercube methods with 1000 equiprobable stratifications in each distribution (McKay *et al.*, 1979). The simulation output was the steady state value for number of *E. coli* O157:H7 CFU shed per gram of cattle feces and was summarized with quartile box plots. For all analyses, model outputs reporting CFU per gram fecal material were calibrated to an average of 30 kg fecal production/(day/steer).

Spearman's rank correlation coefficient (ρ) was used to test a two-sided hypothesis of correlation between each parameter and output values. Significance was set at the $\alpha = 0.05$ level with Bonferroni correction for multiple hypothesis tests implemented (α corrected $= 0.00217$). Parameters influencing *E. coli* O157:H7 shedding rates in cattle were identified and are discussed below.

Model assessment

Model assessment and applicability were considered by comparing model outputs to population prevalences, a metric available in the *E. coli* O157:H7 animal agriculture literature. Real-world testing relates the number of CFU per fecal gram shed to animal level prevalence observations via imperfect culture tests. Similarly, we related the simulated number of CFU per gram of feces to pen-level prevalence estimates by the dose-dependent sensitivity of standard culture-based detection techniques (LeJeune *et al.*, 2006). This modification allowed us to examine model results across organismal scales. The number of CFU per gram of substrate in environment, water, and feed were validated against literature data.

Results

Deterministic analysis

The model demonstrated two steady states—one with *E. coli* present and one with *E. coli* absent (comparable to the disease-free state in host-level models). The *E. coli*–absent steady state is unstable at equilibrium; the system is readily invaded by *E. coli* organisms. The positive stable point is robust to changes in the initial value of state variables. The solution to the baseline model revealed a dynamic invasion process where *E. coli* moved throughout the system and grew approximately exponentially toward an equilibrium state, as demonstrated in Fig. 2. All patches outside the host maintained viable *E. coli* populations and thus contributed directly to CFU observed in cattle feces through CFU ingestion and subsequent expulsion.

Analysis of the deterministic form of the model revealed which environmental patches were influential for *E. coli* O157:H7 persistence. In the baseline model, the environmental patch dominated dynamics due to its relatively large size and slow turnover rate; environmental growth was necessary to maintain populations throughout the system, and served as the main reservoir for *E. coli* O157:H7 in the system. At equilibrium, the environmental patch acted as a reservoir for over 99.6% of the total *E. coli* population in the feedlot pen and accounted for over 98% of *E. coli* ingested by steers. Cattle shed 33 CFU/g feces at equilibrium.

In addition to the environmental patch, feed and animal growth rates and carrying capacities influenced systemwide shedding dynamics. High growth rates in the animal or feed patches can result in higher levels of shedding than the environmental compartment is able to produce alone (Fig. 3). However, the feed growth rates necessary to alter cattle

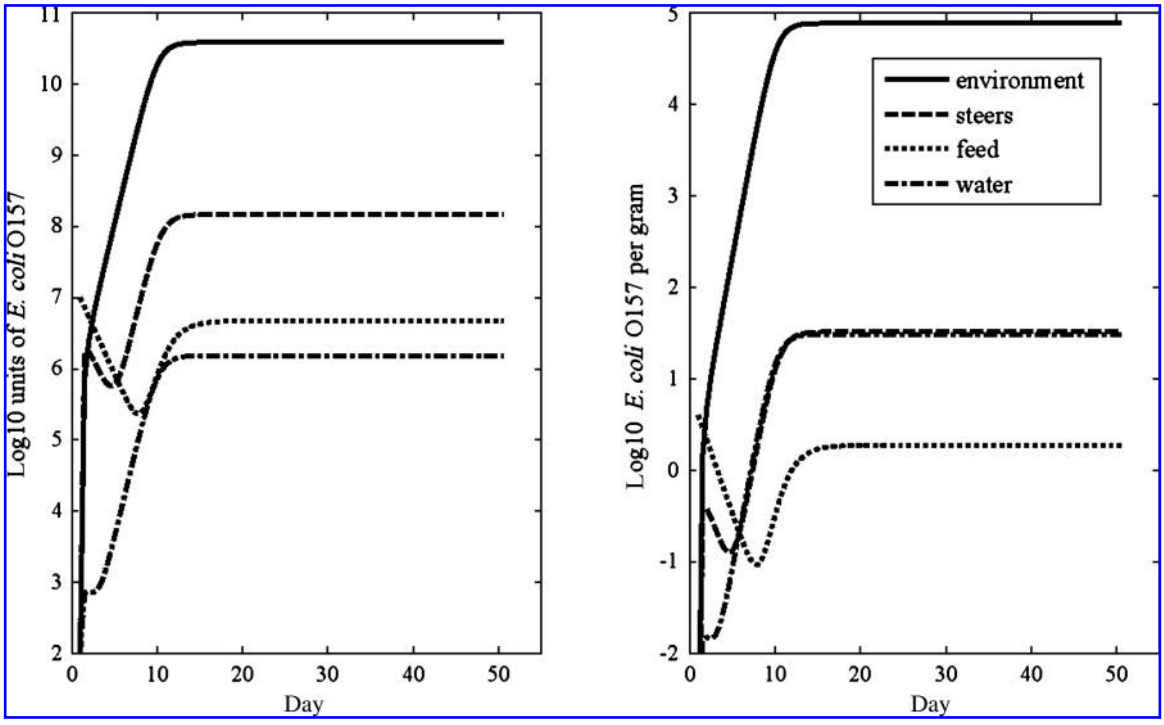

FIG. 2. The number of *E. coli* O157:H7 colony-forming units (CFU; log10) observed in each patch (left panel) and the number of log10 CFU per gram of substrate in each patch (right panel).

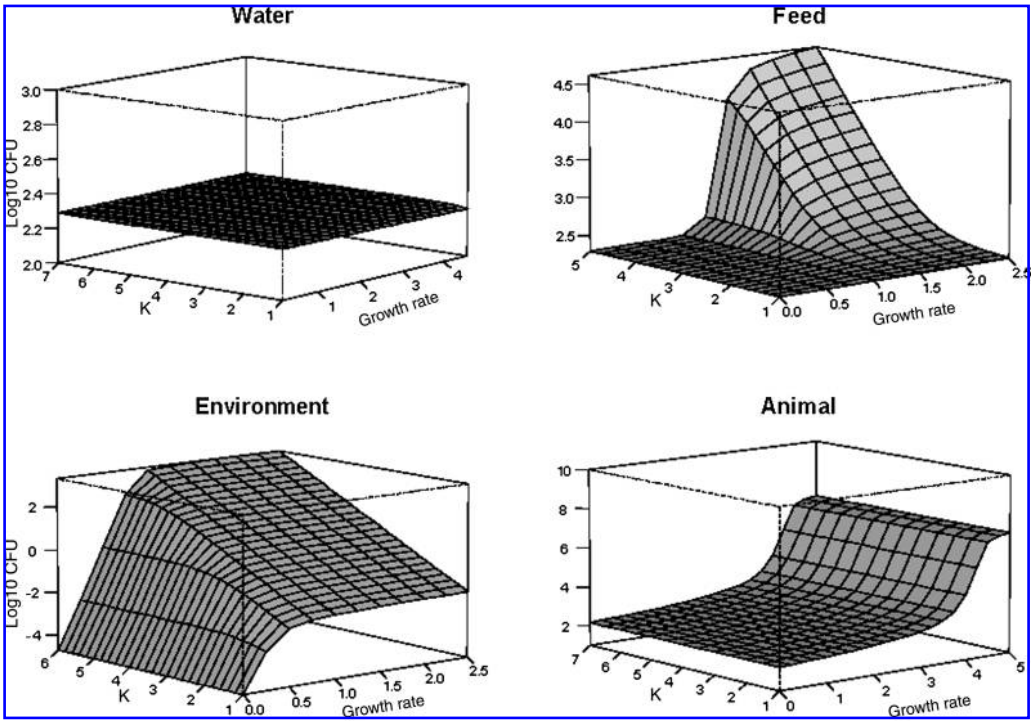

FIG. 3. Each panel is the bifurcation diagram of the respective patch comparing the growth rate and log carrying capacity per gram (K) with the effect on the number of colony-forming units (CFU) of *E. coli* O157:H7 per gram of fecal matter (note change in *x*-axis between panels). Changes in water parameters have little effect on the shedding dynamics, while changes in parameters of other patches do have substantial influence at values examined here.

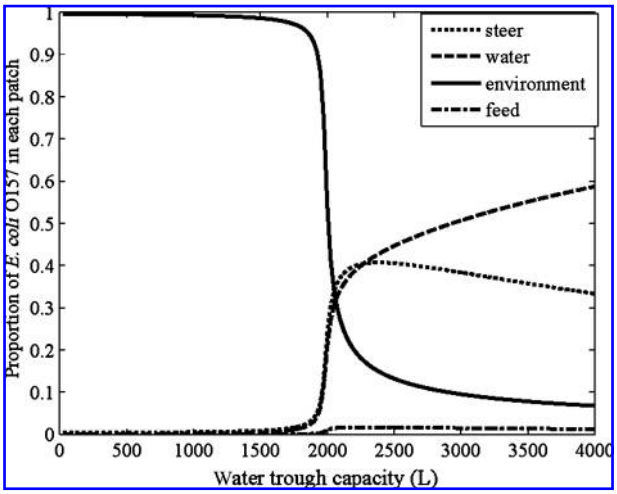

FIG. 4. Steady state solutions for proportion of *E. coli* O157:H7 population in each patch with different capacities of water trough in liters.

shedding patterns are likely infeasible and discussed below. Changes in growth parameters in water do not meaningfully change the CFU per fecal gram excreted by cattle.

When we considered values from the parameter distributions that result in a reduction in the turnover rate of water (changes in r_w, μ_w, V_w, c_w, S), a qualitative change in system dynamics (bifurcation) was reached whereby water came to dominate the invasion and equilibrium dynamics in the system as a whole. Crossing this bifurcation resulted in an almost fourfold increase in the number of *E. coli* O157:H7 in a gram of steer feces from approximately 10^1 to 10^5 CFU/g feces and system-wide growth during the invasion phase was slaved to growth seen in water (Fig. 4). The parameters most influential to determining whether the system was at the higher or lower level of shedding were those which most strongly influenced the exit rate of organisms in the water patch: numbers of steer (S; due to the high volume of water consumption imposed) and size of the water trough (V_w). The ratio of parameters S:V_w determined which regime the system operated under with the threshold occurring at approximately 10 L water/steer (Fig. 5).

Stochastic analysis

The average number of CFU shed per gram of feces for 10,000 simulated feedlots is reported in Fig. 6. Approximately 44.5% of the simulations demonstrated fewer than 10 CFU/g feces; given the sensitivity of the standard culture techniques, this would translate to an apparent prevalence of less than 10%. Fewer than 100 CFU/g feces was shed in 63.8% of the simulations, while the remaining 36.2% had shedding loads greater than 100 CFU/g, which would translate to an apparent prevalence as high as 80%. Growth and population dynamics in the animal and environmental patches were shown to be most influential from the sensitivity analysis (Fig. 7); high levels of growth in cattle and environment and environmental carrying capacity were found to be correlated with high levels of fecal shedding. Death rates in steers and the environment and the number of steers per pen were shown to be negatively correlated with fecal shedding per gram of feces.

Discussion

Interpretation and assessment

The results of this study indicate that *E. coli* O157:H7 growth dynamics in water, the external environment, and cattle hosts are influential to the population size of *E. coli* O157:H7 in a feedlot pen. We have further demonstrated that previously observed growth rates of *E. coli* O157:H7 populations outside the cattle host are adequate to maintain high levels of *E. coli* O157:H7 in the feedlot without net replication in cattle hosts. In the absence of these processes, we identify the carrying capacity of the environmental compartment, net growth/death rates in cattle, and number of steers in a pen as particularly vulnerable points for *E. coli* O157:H7 control at endemic levels of contamination. We predict that interventions targeting these parameters, such as higher cleaning rates and administration of growth inhibition agents in the environment and water troughs or probiotic treatments in cattle, would prove particularly efficacious at reducing the load of

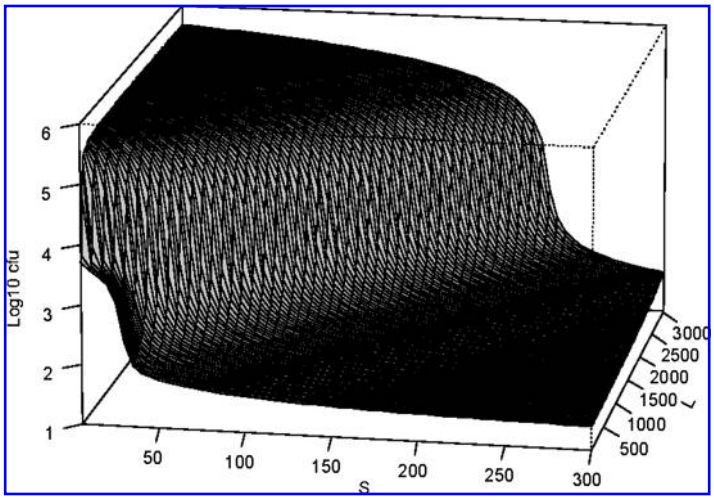

FIG. 5. Effect of varying the number of steers (S) in a pen and the capacity of the water trough (L) on the log10 colony-forming units (CFU) of *E. coli* O157:H7 observed per gram of fecal matter. Two clear planes emerge with a bifurcation resulting as the number of steers is decreased or capacity of water troughs increased.

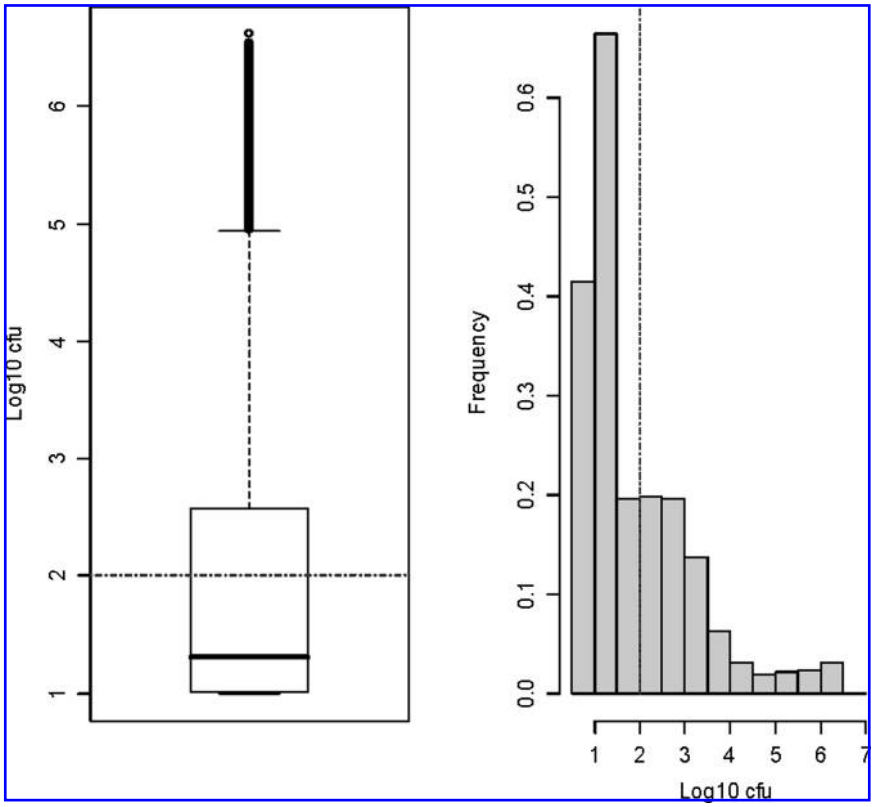

FIG. 6. A quantile box plot (left panel) and frequency histogram (right panel) of log colony-forming units (CFU) of *E. coli* O157:H7 per gram of fecal matter at equilibrium in stochastic simulations. The dotted line in both panels (horizontal on left and vertical on right) represents the minimum level of reliable detection in culture with immunomagnetic bead separation, the method most often used to test fecal samples for *E. coli* O157:H7.

E. coli on the farm, and subsequently the load introduced into the human food chain.

One of the most prominent results from this study is the ability of the model to maintain *E. coli* O157:H7 populations without net contributions from animal hosts. The predicted shedding levels compare favorably to those reported in the literature. For the deterministic baseline evaluation (assuming that all cows have the same shedding status and the number of bacteria shed per gram of feces), 33 CFU/g feces were observed that would lead to a prediction of approximately 20% prevalence observed in tested beef feedlot herds. This closely mirrors the recently observed prevalences in cross-sectional studies of feedlots. Elder and colleagues (2000) observed 28% prevalence in sampled feces of feedlot cattle, and Hussein (2007) found prevalence in beef feedlots ranged from estimates of 0.3–19.7% in a review of cross-sectional studies using fecal samples. Using most probable number analysis, Fegan and colleagues (2004) also demonstrated that the majority of cattle shedding *E. coli* O157:H7 (>90%) are shedding less than 500 CFU/g feces with 83% shedding less than 100 CFU/g. This has strong agreement with our deterministic and stochastic analyses that suggest most feedlots would support average cattle shedding at less than 100 CFU/g. Briefly, the *E. coli* O157:H7 predicted per gram of substrate in each compartment is also within the reported range of CFU in these media (Lynn *et al.*, 1998; LeJeune *et al.*, 2001a, 2001b; Jiang *et al.*, 2002; Besser *et al.*, 2003; Berry and Miller 2005; Amalaradjou *et al.*, 2006), as would be expected given the logistic constraints imposed within each patch of the model.

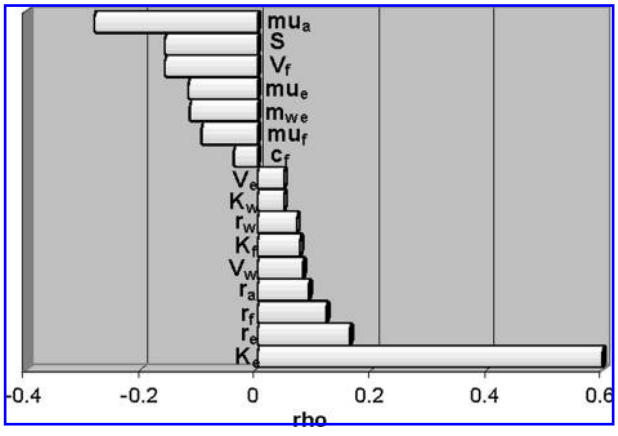

FIG. 7. Spearman's correlation (ρ) values indicating strength of the relationship between parameter and model output (colony-forming units [CFU] of *E. coli* O157:H7 per gram of feces at equilibrium) as determined by stochastic simulations. Only parameters with statistically significant correlations are shown.

Our work predicts that as the turnover rate of *E. coli* organisms in water (water available/steer ratio) is increased,

shedding levels will cross a threshold that results in higher individual prevalence levels observed. Thus, we would predict pens housing smaller numbers of animals, while maintaining trough capacities, would experience higher loads of *E. coli* O157:H7 in the system, per gram of feces with higher observed prevalences; as more steers drink from a single water trough, turnover rates of water are faster than *E. coli* is able to reproduce, and thus contributes very few organisms to the microbial population. When water ingestion by cattle remains constant but water turnover rate is slow enough to allow for *E. coli* growth in water, cattle may be ingesting 10^7 CFU/mL of water at a rate of 45–60 L/day. This bifurcation explains the negative correlation of herd size with fecal shedding per gram predicted from the stochastic analysis above and may account for differences seen between low and high *E. coli* O157:H7 burden farms.

The effect of herd size observed in this study also appears to be in agreement with reported observations; Khaitsa and colleagues (2003) observed 10 pens with only 10 steers apiece and tested for O157:H7 for 19 weeks. The observed prevalence across all pens was as high as 80% with 6 of 10 pens demonstrating 100% prevalence in at least one weekly sampling in the study period. These observations closely align with our model predictions that animals would be shedding at quantities that would demonstrate approximately 80% apparent prevalence when housed in numbers comparable to those in the study by Khaitsa *et al*. (2003).

The results of the model analysis suggest that positive exponential growth greater than approximately one ($r_a - \mu_a > 1$) in cattle could also lead to substantially increased amounts of fecal *E. coli* shedding. The model is not formulated to allow for individual host heterogeneity; however, when a group of steers experiences high net growth (>1), the average CFU per fecal gram reflect values seen in the high or "super" shedding animals observed in previous studies (Omisakin *et al*., 2003; Fegan *et al*., 2004; Low *et al*., 2005). The fate of *E. coli* O157:H7 organisms ingested by cattle is not clear. Studies demonstrating that large oral doses of *E. coli* can lead to variable shedding on the order of weeks in cattle have been used as evidence that within-host replication and host–host transmission are driving forces in *E. coli* O157:H7 maintenance on the farm. However, these studies have been conducted on experimental farms which are under the influence of the extra-host growth dynamics explored in this study and thus do not offer strong evidence for or against pathogen transmission or within host colonization and growth. Little information is available empirically regarding the net growth or death influencing *E. coli* populations moving through the bovine gastrointestinal (GI) tract. It is reasonable to assume orally ingested bacteria experience high rates of death in cattle given the diverse and relatively harsh environments of the cattle GI tract that O157:H7 must pass through before being able to colonize at the recto-anal junction. However the extent of this turnover and potential for subsequent colonization and growth in the recto-anal junction are unclear. The ability of relatively few animals to support a high level of *E. coli* O157:H7 growth following colonization may account for the diversity and relatively small number of animals demonstrating "super shedding" levels of *E. coli* in feces. An extension of this work will introduce a mechanism to scale heterogeneity to the host level to attempt to account for these observed rare high-end shedders.

The deterministic analysis (Fig. 3) indicated that growth rates in feed could significantly impact the population dynamics of *E. coli* O157:H7 across the pen at high values. However, the values needed to affect dynamics in other patches are likely biologically infeasible; the exponential growth rate in feed has been reported as high as 2.5 per day (Lynn *et al*., 1998), but later attempts to replicate reproduction observed in the earlier study were unsuccessful (Besser *et al*., 2003). Thus, we defined a more modest growth rate in line with these observations as the baseline and conclude interventions targeted to reduce growth in feed will likely have little impact to the system's load of *E. coli* O157:H7. Feedbunks also likely violate the assumption of homogenous mixing more so than did the other more viscous compartments considered which could result in an overestimate of growth in feeds in our model. Depending on trough design and sedimentation rates, water may also violate assumptions of homogenous mixing.

Limitations

The model performs well in simulations and compares favorably to observed data; however, limitations are introduced by modeling the pathogen population directly. Firstly, most real world pathogen observations occur at the host scale. In order to relate the model to observed data a pathogen testing regimen needed to be parameterized and modeled. Similarly, a large amount of data is necessary to parameterize the population dynamics of the pathogen. Fortunately, a large amount of laboratory work has been conducted on *E. coli* O157:H7 and parameter estimates were generally available for this study. Further, each individual stochastic model simulation reported here, even when drawn from a random distribution, is itself a deterministic realization of a single theoretical feedlot. As a result, parameter values that allowed for a meaningful invasion of *E. coli* O157:H7 into the system settled at a positive population equilibrium and did not allow for a systemwide fadeout of bacteria. We are not aware of any longitudinal data demonstrating long-term trends in feedlot environment, water, or feed *E. coli* O157:H7 populations; however, observed cattle shedding patterns are notoriously transient and highly variable. Stochastic implementation at the host level may be able to produce fadeout dynamics in future extensions of this work. Similarly, a biologically meaningful change at the *E. coli* O157:H7 population scale in salient parameters could lead to a lower equilibrium population value. This could be induced by any number of intrinsic or external factors to the microbial population (e.g., weather unfavorable to growth).

Recommendations and conclusions

We developed pathogen-based methods to evaluate where meaningful replication, and thus propagation of *E. coli* O157:H7, is occurring on the beef feedlot. By moving toward evaluating the *E. coli* system at the microbial scale, we were able to robustly address the question of *E. coli* propagation and replication from its mechanistic underpinnings. The results of this analysis highlight the influence of water and environment in maintaining *E. coli* O157:H7 populations on the farm. Additionally, we were able to identify levels of replication that would be necessary for the animal host to be influential in the maintenance of the microbial organism. Given the results of this study, we recommend expanding research

efforts to develop intervention strategies to target environmental growth and maintenance and limit the ability of cattle to harbor positive net *E. coli* O157:H7 replication. We identify the water available/cattle ratio as being particularly influential to high-level shedding patterns by affecting the turnover rate of water. Our model was able to demonstrate appropriate low- and high-level prevalences emergent from the mechanistic growth introduced in habitats outside the cattle host while reproducing realistic number of CFU per gram of feces, suggesting contact-based transmission and colonization of cattle is not solely responsible for *E. coli* O157:H7 propagation on feedlots, as is generally considered. Dynamics external to the host are likely impacting systemwide microbe propagation processes and thus affecting the load of *E. coli* O157:H7 presented to the human food chain.

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Disclosure Statement

No competing financial interests exist.

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