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Re-shaping models of *E. coli* population dynamics in livestock faeces: increased bacterial risk to humans?

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1 **Abstract**

2 Dung-pats excreted directly on pasture from grazing animals can contribute a significant
3 burden of faecal microbes to agricultural land. The aim of this study was to use a
4 combined field and modelling approach to determine the importance of *Escherichia coli*
5 growth in dung-pats when predicting faecal bacteria accumulation on grazed grassland.
6 To do this an empirical model was developed to predict the dynamics of an *E. coli*
7 reservoir within 1 ha plots each grazed by four beef steers for six months. Published
8 first-order die-off coefficients were used within the model to describe the expected
9 decline of *E. coli* in dung-pats. Modelled estimates using first-order kinetics led to an
10 underestimation of the observed *E. coli* land reservoir, when using site specific die-off
11 coefficients. A simultaneous experiment determined the die-off profiles of *E. coli* within
12 fresh faeces of beef cattle under field-relevant conditions and suggested that faecal
13 bacteria may experience growth and re-growth in the period post-defecation when
14 exposed to a complex interaction of environmental drivers such as variable temperature,
15 UV radiation and moisture levels. This growth phase in dung-pats is not accounted for in
16 models based on first-order die-off coefficients. When the model was amended to
17 incorporate the growth of *E. coli*, equivalent to that observed in the field study, the
18 prediction of the *E. coli* reservoir was improved with respect to the observed data and
19 produced a previously unquantified step-change improvement in model predictions of
20 the accumulation of these faecal bacteria on grasslands. Results from this study suggest
21 that the use of first-order kinetic equations for determining land-based reservoirs of
22 faecal bacteria should be approached with caution and greater emphasis placed on
23 accounting for actual survival patterns observed under field relevant conditions.

24

25 **Keywords:** cattle faeces, die-off, *E. coli*, grazing, growth, health, pathogen, soil

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1 **Introduction**

2 Livestock are an integral feature of the farmed landscape and a key component of the
3 human food chain and rural economy. However, management of livestock and their
4 manure must be undertaken with a view to ensure the sustainability of key ecosystem
5 services, such as the provision of clean and safe recreational and drinking water (Pretty,
6 2008). Catchments dominated by agriculture have consistently been shown to generate
7 high faecal indicator organism (FIO) pollutant concentrations in receiving waters (Sinclair
8 *et al.*, 2009; Kay *et al.*, 2008a). Thus, microbial contamination of watercourses
9 represents a critical component of diffuse water pollution (Kay *et al.*, 2008b; Defra, 2007)
10 and routine agricultural practices such as livestock grazing and manure spreading can
11 introduce a range of bacterial, protozoan and viral contaminants to land via faecal
12 material (Wilkes *et al.*, 2009; Oliver *et al.*, 2005). In particular, faeces excreted directly
13 on pasture from grazing animals can contribute a significant burden of faecal microbes
14 to agricultural land, often in excess of 10^{12} *E. coli* per hectare during each grazing
15 season (Oliver *et al.*, 2009). Importantly, dung-pats excreted by livestock undergo no
16 microbial treatment phase (in contrast with stored manures) and so the microbiological
17 content of faeces deposited directly to pasture is often high, though numbers vary with
18 livestock type, diet and season (Chadwick *et al.*, 2008, Donnison *et al.*, 2008, Weaver *et*
19 *al.*, 2005). Dung-pats from livestock are therefore critical reservoirs of FIOs, such as *E.*
20 *coli*, which are key regulatory determinands for assessing the microbiological quality of
21 bathing and shellfish harvesting waters as specified in EU directives (CEC, 2006a,
22 2006b).

23
24 To reduce microbial contamination of watercourses and ultimately meet compliance
25 requirements at designated bathing waters there is a need to target agricultural
26 management options where they are likely to have most effect on mitigating FIO impact
27 (e.g. Monaghan *et al.*, 2009; Kay *et al.*, 2007; Oliver *et al.*, 2007). Monaghan *et al.*
28 (2008) propose that the most effective mitigation strategies are those that target the

1 main *sources* of contaminants in farm systems. Being able to reliably predict FIO
2 accumulation on grazed pastures would therefore prove useful in identifying potential
3 microbial reservoirs, indicative of high risk critical source areas (CSAs) if combined with
4 appropriate drivers such as rainfall events (Moriarty *et al.*, 2008). The importance of on-
5 farm microbial reservoirs has been reinforced at the international level with highest
6 priority given to ‘investigating the fate of faecal microbes on farms’ in a recent and timely
7 workshop to establish research priorities for coordinating management of food safety
8 and water quality (Crohn and Bianchi, 2008). Predicting the balance between
9 accumulation and depletion of *E. coli* within land-based reservoirs is thus crucial for
10 understanding the dynamics of (or risk from) diffuse microbial pollution from agriculture.

11

12 Since the early 20th century (Bigelow, 1921) first-order kinetics have been used to
13 describe the population decline of bacteria in research fields as diverse as medicine,
14 food biotechnology and environmental microbiology (Peleg, 2003). When used to
15 describe populations of faecal bacteria and pathogens in livestock faeces, these kinetics
16 are commonly referred to as ‘die-off’, reflecting the generally held view that populations
17 decline after faeces has been deposited. Consequently any potential risk of transfers to
18 the wider environment and humans is thought to lessen with the passing of time after
19 faeces deposition and thus models and policies reflect this. However, studies
20 investigating naturally occurring bacterial survival in livestock faeces have tended to
21 report on laboratory scale microcosm experiments (e.g. Echeverry *et al.*, 2006;
22 Himathongkham *et al.*, 1999) which remove the complexity and heterogeneity of
23 interacting natural processes. Fortunately, there has been a recent emergence of
24 studies, particularly in North America and New Zealand, to investigate *field-relevant*
25 bacterial die-off in faeces deposited on pasture (Muirhead *et al.*, 2009; Soupier *et al.*,
26 2008; Sinton *et al.*, 2007; van Kessel *et al.*, 2007; Meays *et al.*, 2005; Muirhead *et al.*,
27 2005). These studies have suggested that bacterial growth may be occurring, thus

1 questioning the suitability of approximations of FIO die-off in line with traditional first-
2 order decline. If bacterial growth does occur then the potential underestimation resulting
3 from first-order approximations can be theorised for a single dung-pat as shown in Fig
4 1;b. The aim of this study was to test the suitability of first-order inactivation curves by: (i)
5 modelling *E. coli* dynamics on grassland grazed by cattle using a traditional first-order
6 die-off equation and comparing the output with field data of *E. coli* accumulation within
7 replicated 1-ha plots; (ii) determining an approximate growth value for *E. coli* in freshly
8 deposited faeces via field experimentation in order to amend the first-order modelled
9 predictions of *E. coli* dynamics on a grazed plot; and (iii) providing a first-approximation
10 of the magnitude of potential error associated with adoption of first-order decline for
11 predicting *E. coli* reservoirs on pasture whilst appreciating uncertainty within model
12 parameters.

13

14 **Materials and methods**

15 **Field monitored *E. coli* levels on pasture**

16 A field study was undertaken to compare modelled predictions of *E. coli* burden to land
17 with observed data. The field study reported here used the Rowden Research Platform
18 (UK National Grid Reference (NGR): SX 650 995) reported previously in others studies
19 (e.g. Oliver *et al.*, 2005). Four replicated 1-ha plots were sampled at two week intervals
20 throughout the May to November 2003 grazing season for *E. coli* and soil moisture
21 content. Cattle were introduced onto the plots on May 9th and removed on November
22 5th, equivalent to Julian day 129 and 305, respectively. Each 1-ha plot was subdivided
23 into a 6 x 6 grid and 12 soil cores (7 cm deep) were bulked from each sub-sector
24 sampled. None of the plots had been grazed for over a year prior to 2002 because of the
25 UK outbreak of Foot and Mouth Disease (FMD) in 2001 and pre-experiment soil
26 concentrations of *E. coli* were below detection levels in 2003. To calculate the total
27 reservoir of *E. coli* within each plot, mean cell concentrations per gram of dry weight soil

1 were multiplied by the estimated dry weight of soil in each 1 ha plot (to a depth of 7 cm,
2 as per soil core depth).

3

4 **Field monitored dung-pats**

5 An experiment was conducted to determine *E. coli* content in cattle faeces. This served
6 two purposes. Firstly it provided a site-specific *E. coli* content for fresh dung-pats from
7 beef cattle as model input. Secondly, given the potential for bacterial growth to impact on
8 the model results, a field study of *E. coli* die-off was needed to determine a first
9 approximation for the implementation of bacterial growth into the model; it was not
10 conducted to provide a detailed account of FIO population increase in dung-pats or
11 identify causal effects. Fresh dung-pats from beef cattle were collected during the
12 grazing season from the experimental site. Dung-pats (n = 6) were monitored to assess
13 changes in the number of *E. coli* within faeces over time. Six different animals each
14 contributed a single faecal deposit to serve as a replicate dung-pat. The deposits were
15 collected from cattle that had been allowed to graze for over two months, allowing the
16 gut microbial community of livestock to develop from that of a housed diet to one typical
17 of grazing animals. Each of the six dung-pats was collected from pasture within five
18 minutes of excretion from each animal and all six dung-pats were collected within two
19 hours of each other. The six fresh dung-pats were transferred, intact, to a grassland plot
20 adjacent to the grazed plots because this: (i) prevented cattle treading through the
21 excrement which may have resulted in destruction of a replicate deposit; and (ii) allowed
22 for a more convenient and rapid sampling protocol as each dung-pat was placed in
23 relatively close proximity to the next (~ 1 m spacing). Dung-pats were transferred using a
24 sterilised spade (70% industrial methylated spirit [IMS], rinsed with sterile water). The
25 dung-pats were not protected from rainfall, thus allowing a population change in
26 accordance with field conditions.

27

28 **Sample collection from dung-pats**

1 Each of the dung-pats was repeatedly sampled on days 0, 1, 4, 8, 14, 28, 48 and 70
2 post defecation. This repeated strategy was adopted because a destructive sampling
3 approach was deemed impractical due to the number of dung-pats required to be
4 obtained for t = 0 days and also because of the limit on the number of dung-pats one
5 animal makes per day. It was considered that the most dramatic changes in population
6 numbers may occur during the early stages of voidance from the warm and moist gut
7 environment because environmental conditions on the field surface become variable and
8 not optimal (see also Wang *et al.*, 1996). Thus, sampling was skewed so as to obtain
9 cell counts more regularly at the start of the experiment. Approximately 2 g of faeces
10 was collected (0.5 g for bacteriological analysis, 1.5 g for dry weight analysis). Samples
11 were randomly taken from the middle depth region of the dung-pat below the formed
12 crust. Faecal material was removed with a sterile spatula (70% IMS, rinsed with sterile
13 water) and placed into sterile MacCartney bottles (autoclaved at 121°C for 15 minutes).
14 Moisture content of faeces was determined by drying 1.5 g of faeces at 105°C for 24
15 hours in an oven and then weighing the residual.

16

17 **Microbiological analysis of samples**

18 *Soil analysis:* All bacterial analysis of samples was conducted within 4 hr of collection.
19 Briefly, fresh soil samples were crumbled and 10 g was added to 90 mL sterile water
20 prior to mixing for 40 min on a rotary agitator. The resulting soil suspensions were
21 serially diluted in sterile water then spread-plated onto MacConkey agar and incubated
22 at 37°C for 24 hr before enumeration of colony forming units (CFU). *Faecal analysis:* a
23 mass of 0.5 g of faeces was added to 4.5 mL of sterile water in a sterile 15-mL
24 centrifuge tube and shaken for 60 minutes on a rotary shaker, before being shaken
25 vigorously on a Whirlpool mixer for a few additional minutes. Serial dilutions were then
26 made in sterile water and 0.1 mL (or 0.2 mL in cases of low counts) spread-plated onto
27 MacConkey agar (Oxoid) as per soil analysis. Those colonies characteristic of *E. coli*
28 growing on MacConkey agar were enumerated and seven random isolates were used to

1 confirm their identity using both MicroPlate test panels (Biolog, Hayward, CA) and API
2 20E biochemical identification kits (bioMerieux Vitek, Hazelton, MO). Both these
3 procedures rely on the biochemical profiles exhibited by the test isolates for confirmation
4 of their identity through database comparison.

5

6 **Modelling *E. coli* dynamics on grassland plots**

7 An empirical model (Oliver *et al.*, 2009) was adapted to estimate the accumulation of an
8 *E. coli* reservoir on four replicated 1 ha paddocks grazed by four beef steers during a
9 typical six month grazing season in the UK. This empirical model was constructed using
10 biological parameters of die-off, faecal excretion and *E. coli* shedding rate and was
11 informed by previous field experimentation reported in the literature. The model accounts
12 dynamically for the accumulation and depletion of FIO burden to land at daily time-steps.
13 The quantity of *E. coli* on a defined plot (Equation 1) was calculated as the sum of two
14 terms (i) the daily fresh input of *E. coli* by all livestock; and (ii) the *E. coli* burden
15 deposited on previous days and now declining as a result of first-order die-off:

16

$$17 \quad E_{(x)} = Ein_{(x)} + E_{(x-1)}e^{-bx} \quad (1)$$

18

19 Where E_x is the magnitude of the *E. coli* store on day x , Ein is the *E. coli* input of fresh
20 deposits, e is a mathematical constant (base of natural log), b is the exponential die-off
21 constant. Specifically, daily *E. coli* loading was calculated by multiplying the number of
22 cattle ($n = 4$) by both the daily dry matter excreted per beef steer and a typical value for
23 *E. coli* per gram of dry cattle faeces (see Table 1). Many literature values for *E. coli*
24 content of livestock faeces exist and McDowell *et al* (2008) provide a succinct summary
25 table from herds across the world. However, in order to constrain uncertainties for this
26 specific study we analysed the *E. coli* content of dung-pats from beef steers grazing the
27 field site used to evaluate the model to provide an average value for this parameter.
28 Estimates of the error associated with dung-pat *E. coli* content were based on the

1 distribution of measured values from this study and on the range of existing literature
2 values for cattle faeces (see Table 2).

3

4 Seasonal die-off profiles for *E. coli* under field conditions typical of the UK are sparse
5 and only one study by Avery *et al.* (2004) provided appropriate data for use in the model
6 outlined here (0.061 day^{-1}). Other laboratory based studies do exist but these were
7 considered unsuitable to extrapolate to field conditions due to the degree of uncertainty
8 in translating controlled experimental data to the field. Die-off coefficients from lowland
9 areas of New Zealand (which can experience similar climatic conditions as the UK)
10 provided first-order die-off rates of similar range ($0.050\text{-}0.060 \text{ day}^{-1}$) for spring, summer,
11 autumn and winter seasonal experiments (Sinton *et al.*, 2007) to help constrain our
12 estimates of the range of error associated with the die-off parameter. Given the scarcity
13 of die-off data we allowed a $\pm 33\%$ error in this coefficient. The die-off data was used to
14 determine the daily *E. coli* decline within all deposited faecal material for each
15 successive day within a six month grazing period.

16

17 The model was run 500 times using randomly chosen parameter scenarios from the
18 error ranges (Table 2). Each scenario was given a different weighting, based upon its
19 deviation from the nominal parameter values (Table 1). Each scenario weighting was
20 calculated using triangular fuzzy membership functions for each parameter, summed to
21 give an overall weighting (e.g. see the approach of Page *et al.*, 2004). When sampling
22 the *E. coli* concentration distributions a day-to-day correlation of 0.7 was assumed as it
23 is unlikely that cattle excrete exactly the same number of cells each day owing to
24 biological variability and fluctuations reported in the literature (Robinson *et al.*, 2009;
25 Donnison *et al.*, 2008). This allowed a general 'drift' in shedding rate, but did not allow
26 large, unrealistic short-term fluctuations.

27

1 The model was modified to incorporate post-deposition growth using experimental
2 results from this study (see above). This was achieved by taking the average growth
3 observed in the present study for the six days after deposition. As fresh deposits were
4 input on each grazing day this equates to a six-day moving-window of growth through
5 the grazing season. The additional *E. coli* burden is discussed above and is included as
6 specified by Equation 2.

7

$$8 \quad E_{(x)} = Ein_{(x)} + E_{(x-1)}e^{-bx} + ER_{(x)} \quad (2)$$

9

10 Where ER is the magnitude of *E. coli* growth for any given day.

11

12 The multiple parameter scenarios and associated fuzzy weightings provided a
13 distribution of values for the *E. coli* reservoir at each time-step, expressed as percentiles
14 of these distributions in the sections below.

15

16 **Results**

17 **Measured *E. coli* in the faecal store (dung-pats)**

18 Overall, *E. coli* was present on the day of excretion at a mean concentration of 7.12 log₁₀
19 *E. coli* CFU g⁻¹ dry faeces and showed fluctuation in population numbers, rather than
20 first-order die-off kinetics, during the 70 day period of investigation. The average growth
21 recorded in the first 10 days post defecation was approximately 0.5 log₁₀ CFU g⁻¹ dry
22 faeces before cell numbers gradually declined to a level of 6.06 log₁₀ CFU g⁻¹ dry faeces
23 by day 48. However, between day 48 and day 70 cell numbers recovered to a mean of
24 6.29 log₁₀ CFU g⁻¹ dry faeces; a level greater than observed 28 days after deposition.

25 The individual die-off profiles for all six dung-pats are shown in Figure 2 along with
26 rainfall and air temperature conditions recorded throughout the die-off study. It was not
27 possible to use linear or non linear regression analysis on the replicate faecal deposits
28 because the percentage variation accounted for by the model fittings was inappropriate

1 for all replicates, demonstrating poor applicability of both linear and non-linear model fits
2 to the data points plotted. Deposit 1, 5 and 6 accommodated a final *E. coli* concentration
3 similar to that of the initial concentration on day 0. Five of the six replicates experienced
4 an increase in *E. coli* concentrations between day 48 and day 70. Changes in the dry
5 matter content of the dung-pats during the experimental period are shown in Table 3.

6

7 **Measured *E. coli* in the soil store**

8 Bacterial analysis of the topsoil layers (0 - 7cm), which were sampled between dung-
9 pats, detected *E. coli* numbers ranging between levels below detection ($< 5 \times 10^2$ CFU g⁻¹
10 dry soil) through to 10^6 CFU g⁻¹ dry soil. No water drained from the 1 ha plots during this
11 grazing period because brief spells of rainfall (Figure 3) were insufficient to initiate
12 drainage. On 10 of the 12 sampling dates the mean measured *E. coli* levels in the plots
13 exceeded the *upper* levels predicted by the model. Only on Julian Days 133 (near the
14 onset of grazing), 217 and 309 (end of grazing) were predicted *E. coli* values of the
15 same order of magnitude as those predicted by the model using 1st order die-off
16 coefficients. Time-series values (mean, 5th and 95th percentiles) of the measured *E. coli*
17 within the four replicate plots are shown in Figure 3 by the vertical bars. A paired *t*-test to
18 check for differences in mean *E. coli* stores within observed versus predicted datasets
19 on the 12 sampling dates showed that the values within the measured dataset were
20 significantly higher ($P < 0.05$) than those of the predicted dataset.

21

22 **Model output**

23 Predicted *E. coli* levels on pasture using first-order die-off coefficients and first-order die-
24 off coefficients combined with the moving-window representation of growth are shown in
25 Figures 3 and 4, respectively. The plots show 5th, 50th and 95th percentile of predicted *E.*
26 *coli* values. Within approximately 50 days from the onset of grazing the rate of *E. coli*
27 accumulation was seen to reach a near equilibrium (i.e. excreted *E. coli* and total die-off
28 were approximately in balance) and during each successive day through to Day 305 (the

1 end of grazing), the accumulating *E. coli* deposition rate exceeded or equalled that of the
2 combined die-off rate for all cells. Using first-order die-off coefficients the maximum
3 mean potential *E. coli* reservoir was predicted to be approximately $3 \times 10^{12} \log_{10} E. coli$
4 on day 305. Incorporation of growth in the order of $0.5 \log \text{CFU g}^{-1}$ dry faeces into the
5 model increased the maximum value of predicted *E. coli* burden to approximately $8 \times$
6 $10^{12} \log_{10} E. coli$ (Figure 4) and reduced the sum of absolute errors between observed
7 and predicted *E. coli* levels by 3×10^{13} CFU; a significant underestimation did, however,
8 remain ($P < 0.05$). On day 305 (the end of grazing) no fresh faecal material was added
9 to pasture and the *E. coli* reservoir declined following a first-order profile for both model
10 scenarios.

11

12 **Discussion**

13 An empirical model governed by biological parameters of die-off, shedding and excretion
14 rate is presented. Moriarty *et al.*, (2008) have suggested that for the management and
15 mitigation of bacterial pollution of watercourses, and for on-farm microbial risk
16 assessment, it would be useful to model the size of faecal microbe reservoirs on
17 pasture. To do this they propose that data is needed on the bacterial content of fresh
18 faeces and associated die-off data. The research reported here offers a critical first step
19 towards accounting for land-based reservoirs of *E. coli*.

20

21 A key finding of this study was reflected in the difficulty of extracting suitable die-off
22 coefficients for the model. The lack of data informing reliable *E. coli* die-off estimates for
23 faecal pats under farm conditions meant that we were restricted in our ability to
24 parameterise the model with UK field relevant coefficients. An alternative approach
25 would have been to use laboratory derived die-off coefficients but these are less
26 representative of environmental conditions (e.g. McGechan and Vinten, 2003; Oliver *et*
27 *al.*, 2006). However, New Zealand derived data from Christchurch (Sinton *et al.*, 2007),
28 associated with similar meteorological conditions to the UK, provided a die-off rate

1 constant of almost equal value to that used in this study (previously reported by Avery *et*
2 *al.*, 2004) and within the margin of uncertainty embedded into the model structure. This
3 provided reassurances with regard to the suitability of the die-off parameter used. This
4 lack of field relevant data to parameterise basic fundamental models of faecal bacteria
5 accumulation on land is alarming. Faecal bacteria such as *E. coli* represent basic
6 microbial determinands for policy drivers such as the rBWD in Europe (CEC, 2006a) and
7 TMDL assessments in the US (Chin *et al.*, 2009) and are key indicators of faecal
8 contamination of water. However, critical data on the most basic behavioural traits of
9 these bacteria – namely die-off under field relevant conditions – for UK climatic
10 conditions is limited to one study (Avery *et al.*, 2004). Unfortunately the aforementioned
11 study only covered die-off profiles for faeces deposited in November and so no account
12 of differential die-off profiles for differing months, or even seasons, was provided. As a
13 result, in our model we used a step-change approach in die-off coefficients between
14 seasons, whereas had sufficient data been available we could have developed a better
15 understanding of die-off fluctuations over an annual time-course (e.g. used a sine wave
16 approximation of seasonal die-off fluctuations). New Zealand-based research has now
17 provided key month-by-month assessments of FIO decline in faeces (e.g. Muirhead,
18 2009) but in the UK there appears to be unfounded complacency about the survival of
19 bacterial indicators, largely stemming from the fact the much work has been done in the
20 laboratory to study *E. coli*. Muirhead (2009) detailed the first example of FIO decline in
21 dung-pats deposited throughout each month of the year providing a much needed
22 resource for researchers investigating bacterial pollution from agriculture. Muirhead
23 (2009) therefore represents a key study which other geographic regions across the world
24 should replicate to provide equivalent and comparable datasets of faecal indicator
25 population dynamics under field conditions.

26

27 The discrepancy in assumed first-order die-off and actual field persistence of FIOs was
28 highlighted using our plot experiment and associated model. As noted by Beven (2007),

1 more information can often be learned from model rejection than acceptance, leading to
2 inference of key processes, in this case the potential for *E. coli* growth. However, even
3 when the model was amended with a growth phase the predictions still underestimated
4 observed values on pasture. The accumulating observed total of *E. coli* within the plot
5 soil should, in theory, have been lower than that of the maximum number of cells
6 predicted by the model to be within the faecal reservoir. This is because first-order die-
7 off would make it impossible to detect larger numbers of *E. coli* in the soil store (given
8 that concentrations were below detection on day 0) than in the maximum predicted
9 faecal store as first-order die-off implies a decline of cell numbers rather than an
10 increase. The fact that soil *E. coli* levels exceeded that of the maximum predicted input
11 levels on over 80% of sampling dates suggested that first-order die-off did not
12 satisfactorily describe changes in *E. coli* populations within cattle faeces and that higher
13 order approximations and complex growth patterns operate under field conditions. The
14 argument is reinforced further given that a proportion of the FIO population may have
15 entered a viable-but-non-culturable state yet detected numbers were still significantly
16 higher than those predicted. This revealed a major underestimation of diffuse source
17 bacterial risks from cattle to soil and water quality, with increased threats to public health
18 that may worsen if combined with expected climate change outcomes (Hulme *et al.*,
19 2002; Boxall *et al.*, 2009).

20
21 Overall the study identified post defecation *E. coli* growth equivalent to a magnitude 0.5
22 \log_{10} CFU g^{-1} dry faeces. It is possible that the discrepancy between the observed and
23 modelled *E. coli* reservoir was a function of erroneous die-off coefficients but the
24 complementary dung-pat die-off experiment would suggest that growth is a factor often
25 ignored in faecal microbe fate models, and this is reinforced by other studies (Soupir *et*
26 *al.*, 2008; Muirhead, 2009). Both Sinton *et al.* (2007) and Van Kessel *et al.* (2007)
27 recorded potential growth of up to 1.5 orders of magnitude, more than that observed in
28 our die-off study. This difference may be related to the timing of the experiments, the UV

1 radiation intensity and variations in ambient temperature among other environmental
2 factors experienced during the period of study but highlights that different levels of
3 growth may take place during different periods of the year on pasture and may explain
4 why our amended model still under predicted.

5

6 We appreciate that the die-off study reported here represents only a first step in what
7 should be a continued integrated field and modelling research programme and as such it
8 has provided data to help form a first approximation analysis of the impact of ignoring
9 growth in model predictions. The repeated use of a single value for *E. coli* growth
10 through time was somewhat limited because growth may in fact vary day-by-day for
11 each deposited dung-pat depending on the complex array or interacting environmental
12 variables. Soupier *et al.* (2008) observed differing levels of growth and times to reach
13 growth peak for different seasons so the 'moving window' of growth used in the model is
14 perhaps too regular but current knowledge prevented us from modifying this *E. coli*
15 growth approximation.

16

17 Furthermore, the complementary *E. coli* die-off experiment using replicated dung-pats
18 highlighted fluctuations of *E. coli* numbers within faeces over a 70 day period, including a
19 growth phase not only in the immediate period post defecation, but also a secondary re-
20 growth phase over 40 days after deposition. This was not accounted for in the original or
21 the amended model. During brief periods of precipitation, cell numbers did not decline as
22 might be expected due to wash-out from the faecal deposit. Instead, the resurgence of
23 cells in the secondary re-growth phase appeared to coincide with rainfall and may be a
24 function of the conditions brought about by rehydration of the faecal material and
25 requires further investigation (c.f. Sinton *et al.*, 2007).

26

27 It has been argued that first-order approximations do not account for *adaptation* of
28 bacterial communities. In fact, *E. coli* are a notable example of bacterial cells capable of

1 adjusting their metabolism in response to stress in order to increase their survivability
2 (Corradini and Peleg, 2009), and may explain the observed growth ignored in traditional
3 log-linear models. A key objective is now to investigate field relevant die-off of *E. coli* for
4 varying UV radiation and rainfall typologies to start to account for variable die-off rates
5 and potential for growth as a function of fluctuating field conditions. As noted by Soupir
6 *et al.* (2008), higher-order approximations and the inclusion of weather variables are
7 likely to improve predictions of bacterial decline when compared to first-order
8 approximations: this will however require an increase in high quality data to constrain
9 model structures and parameter values. The current lack of representation of growth
10 dynamics in models of bacterial die-off, and the general assumption of first-order decline
11 equates to a bias or structural error that leads to potential underestimation of diffuse-
12 source microbial risks to soil and water quality at the field and catchment scale.

13

14 **Conclusion**

15 The availability of fundamental field relevant data for *E. coli* population dynamics in
16 faeces deposited onto pasture by grazing livestock is currently poor and the reliance on
17 1st-order 'die-off' approximations will in some instances significantly underestimate the
18 size of *E. coli* populations on grassland. Information derived from laboratory experiments
19 is not satisfactory to underpin the development of models of bacterial fate and dynamics
20 at farm and catchment scales. Given that *E. coli* are monitored across the world,
21 because of regulatory drivers and legislation criteria for water quality and human health,
22 this emphasises the need to understand better the behaviour of faecal bacteria in the
23 environment. This critical finding has wide-ranging implications that may be applicable to
24 other faecally-derived bacteria and disease causing microorganisms such as *E. coli*
25 O157:H7 (c.f. Pryor Williams *et al.*, 2008). It is therefore relevant to microbial
26 ecologists, policy makers, agronomists and those working in soil and water science who
27 can use this data to frame future evaluations of bacterial risks to public health, the
28 human food chain and key ecosystem services such as the provision of clean and safe

1 recreational and drinking water. The potential of *E. coli* population increase under
2 environmental conditions highlights that we should focus on understanding bacterial
3 population dynamics and their ability to proliferate and persist in the environment rather
4 than deriving traditional distinct coefficients that focus only on 'die-off', which are both
5 misleading in terminology and erroneous in nature. This is especially pertinent given
6 predicted changes in surface soil temperature and moisture under grasslands which may
7 favour survival further and thus exacerbate human health risks in the future.

8

9 **Acknowledgements**

10 Funding was provided by Lancaster University, The University of Sheffield and the
11 Biotechnology and Biological Sciences Research Council (BBSRC). The authors are
12 grateful to the staff of North Wyke Research for support especially with access to the
13 Rowden field site and also acknowledge the valuable and constructive comments made
14 by the editor and anonymous referees.

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Figure Captions

Figure 1: Theorised dynamics of faecal bacterial re-growth and errors relative to first-order die-off approximation for a single dung-pat

Figure 2: Die-off patterns of *E. coli* within six dung-pats (FD1-6) under field conditions in Devon, UK. Average rainfall and temperature data are shown on the secondary y axis. Day 0 = July 31st 2003.

Figure 3: Modelled *E. coli* reservoir (mean, 5th and 95th percentile shown by solid and dashed lines respectively) on a 1 ha plot grazed by 4 beef steers over a 6 month grazing season. May 9th (day 129) and November 5th (day 305) represent the start and end of grazing, respectively. Bar-plots show actual soil *E. coli* levels measured in 1 ha plots (Horizontal dashes represent median and upper and lower values).

1 **Figure 4:** Modelled *E. coli* reservoir (mean, 5th and 95th percentile shown by solid and
2 dashed lines respectively) on a 1 ha plot grazed by 4 beef steers over a 6 month grazing
3 season with re-growth accounted for. May 9th (Day 129) and November 5th (Day 305)
4 represent the start and end of grazing, respectively. Bar-plots show actual soil *E. coli*
5 levels measured in 1 ha plots (Horizontal dashes represent median and upper and lower
6 values). Faded data plot shows previous modelled output excluding re-growth.
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1 **Table 1:** Model parameters and their associated values

Parameter	Value	Reference
<i>E. coli</i> concentration in fresh bovine faeces	1.3 x 10 ⁷ CFU g ⁻¹ dry weight	This study
Faecal dry matter excreted per day by 1 beef cow (assuming 10% dry matter content)	3200 g	Chambers <i>et al.</i> , (2001)
<i>E. coli</i> die-off coefficient in bovine faeces on pasture	0.0606 day ⁻¹	Avery <i>et al.</i> , (2004)

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Table 2: Magnitude of uncertainty associated with model parameters

Parameter	Potential parameter error
Die-off coefficient	+/- 33 %
Day-to-day correlation in excretion	0.7
Daily <i>E. coli</i> excretion	+/- 1 log ₁₀ CFU g ⁻¹ dry weight

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Table 3: Change in % dry matter content for the six faecal deposits

Day since deposited	% dry matter within each faecal deposit					
	1	2	3	4	5	6
0	12.4	14.9	15.8	13.3	12.2	9.1
1	10.9	13.1	13.5	10.9	12.5	12.1
4	19.3	20.3	19.9	6.1	26.3	24.6
8	21.6	22.6	20.4	16.7	76.9	27.0
14	36.0	25.0	35.8	19.3	79.3	67.3
28	78.0	61.4	60.8	25.1	81.0	71.3
48	40.6	42.1	48.5	21.9	90.6	84.7
70	26.5	24.0	28.1	26.8	31.8	28.1

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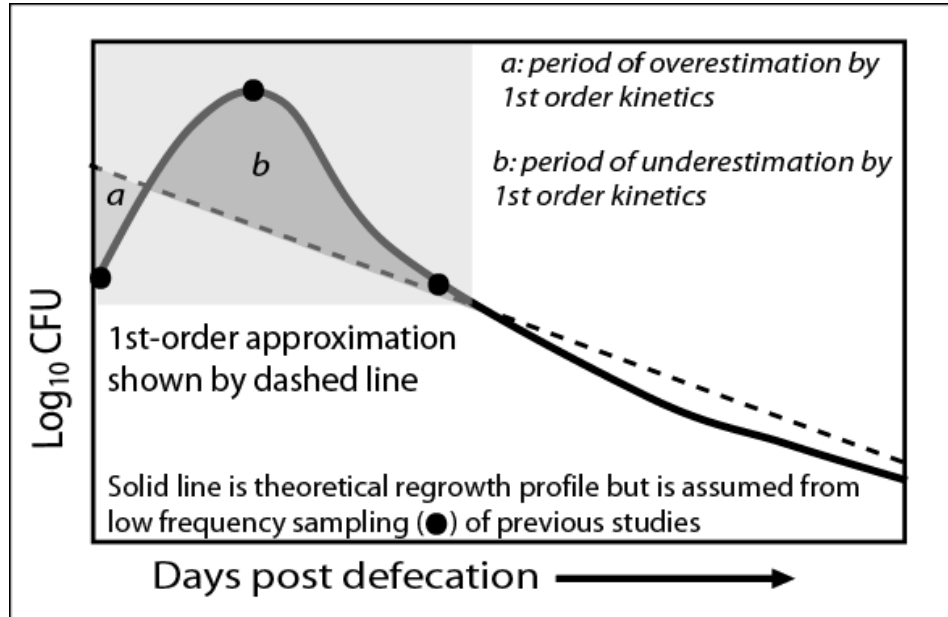
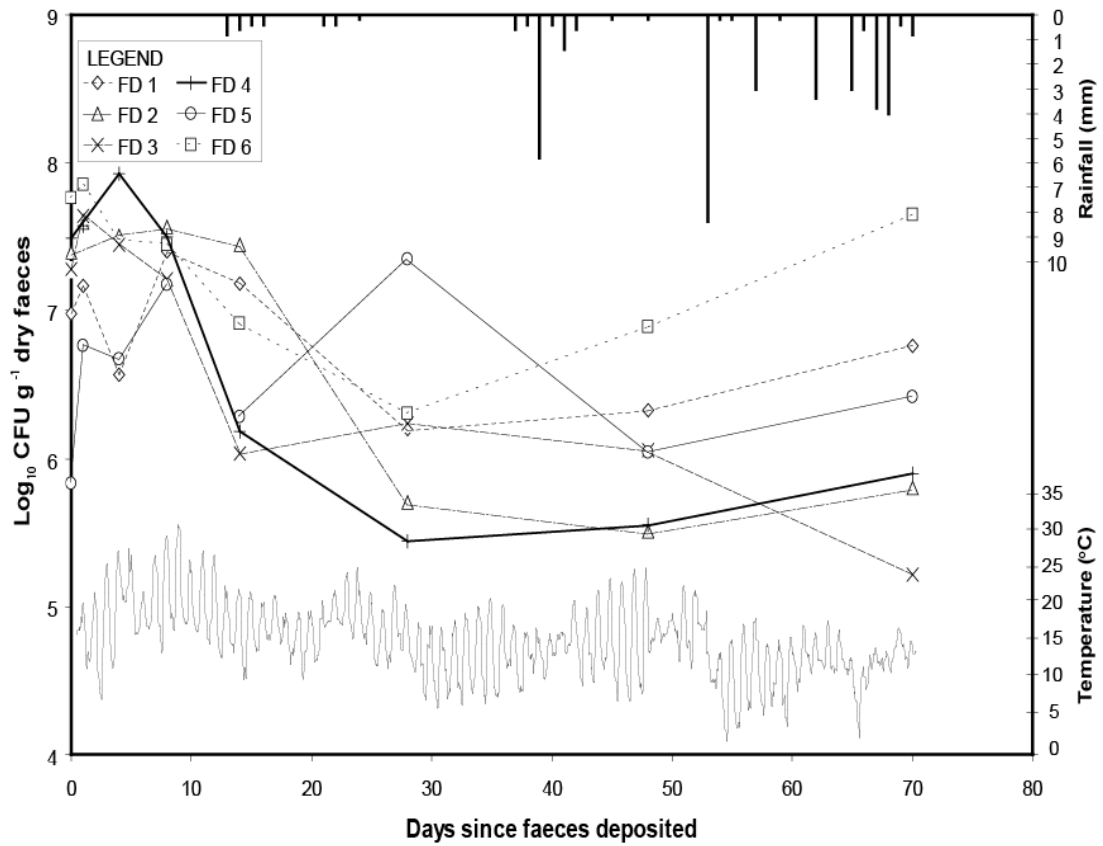
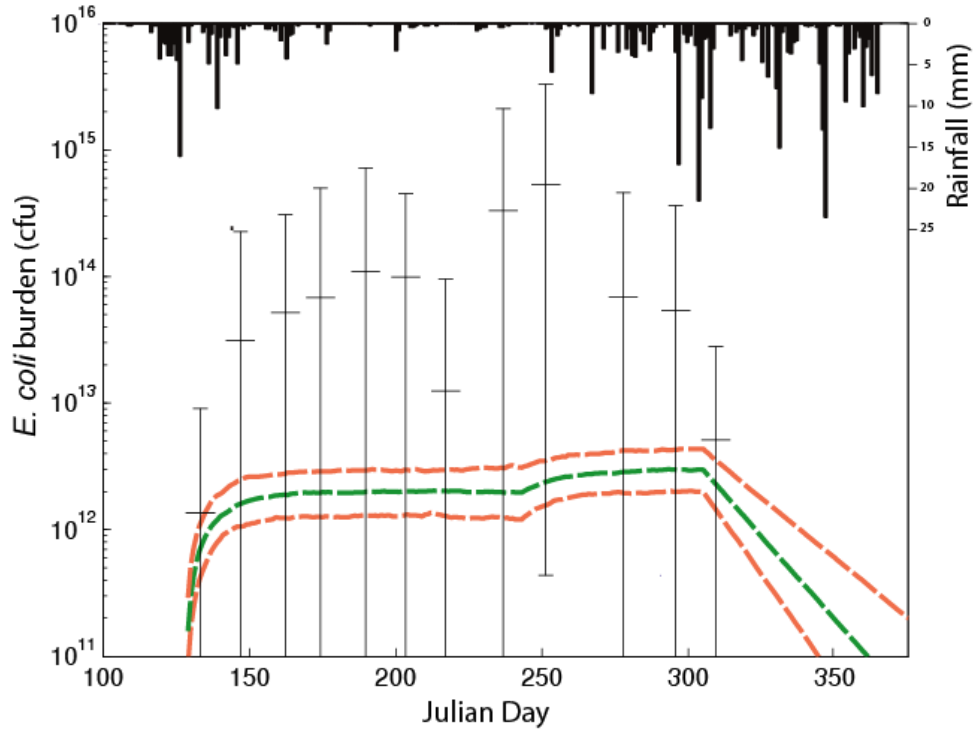


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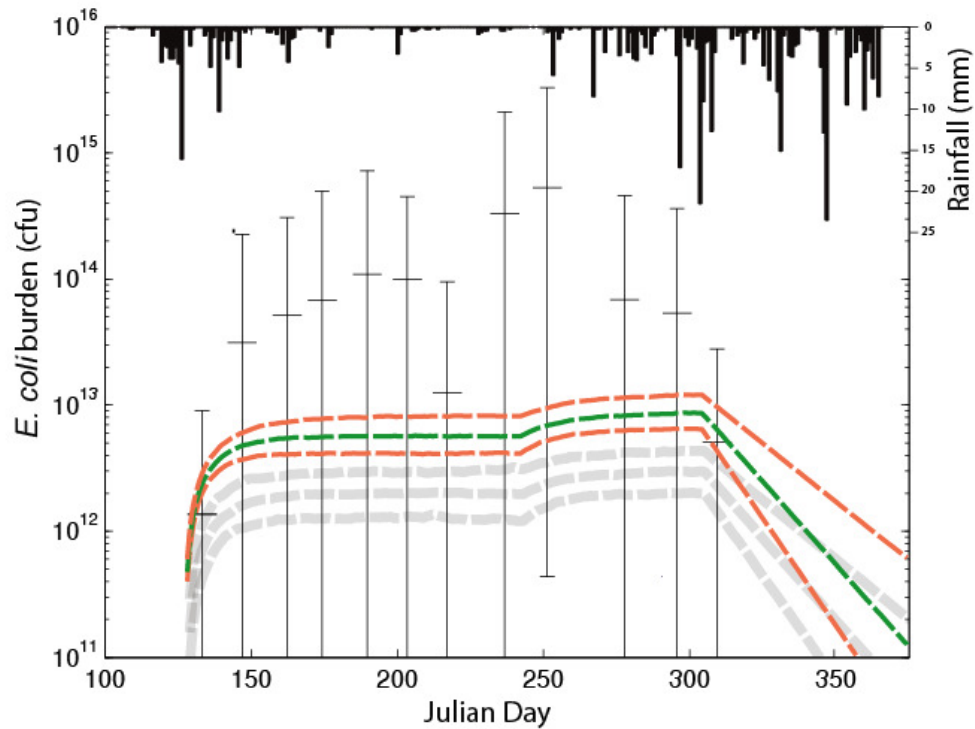
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Figure 3: Modelled *E. coli* reservoir (mean, 5th and 95th percentile shown by solid and dashed lines respectively) on a 1 ha plot grazed by 4 beef steers over a 6 month grazing season. May 9th (day 129) and November 5th (day 305) represent the start and end of grazing, respectively. Bar-plots show actual soil *E. coli* levels measured in 1 ha plots (Horizontal dashes represent median and upper and lower values).



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