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Modelling the dynamics of intramammary *E. coli* infections in dairy cows: understanding mechanisms that distinguish transient from persistent infections

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Abstract – The majority of intramammary infections with *Escherichia coli* in dairy cows result in transient infections with duration of about 10 days or less, although more persistent infections (2 months or longer) have been identified. We apply a mathematical model to explore the role of an intracellular mammary epithelial cell reservoir in the dynamics of infection. We included biological knowledge of the bovine immune response and known characteristics of the bacterial population in both transient and persistent infections. The results indicate that varying the survival duration of the intracellular reservoir reproduces the data for both transient and persistent infections. Survival in an intracellular reservoir is the most likely mechanism that ensures persistence of *E. coli* infections in mammary glands. Knowledge of the pathogenesis of persistent infections is essential to develop preventive and treatment programmes for these important infections in dairy cows.

mathematical model / mastitis / *Escherichia coli* / persistent infection

1. INTRODUCTION

Mastitis is one of the most important production and welfare diseases affecting dairy cows. In recent decades, coliform infections have been identified in an ever increasing proportion of mastitis cases [22]. Among the coliform bacteria *Escherichia coli* is the dominant pathogenic species in dairy cow mastitis [5, 26]. The typical infection pattern for *E. coli* intramammary infection includes a clinically severe inflammatory response by the host with outcomes of either elimination of coliforms within 96 h of the initial infection [20, 23] or a deleterious outcome for the host including shock, sepsis and often death [8, 9]. In addition to severe disease several authors have reported persistent and clinically less severe *E. coli* infection [11, 12, 15].

Persistent *E. coli* infections are not uncommon in other organs or species. In particular, persistent infections of the human female urinary tract are often observed, and have inspired

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research into the pathogenesis of persistent *E. coli* infections [1, 6, 7, 14, 16, 21]. There is still debate about the precise mechanisms involved with persistent urinary tract infections, but it is clear that fimbriae that enable adhesion to bladder epithelial cells [6] and subsequent invasion of bladder epithelial cells, together with prolonged intracellular survival of the bacteria play an important role in persistence of infection [1]. A number of similarities between persistent urinary tract and persistent intramammary *E. coli* infections have been noted [11].

Recent studies on bovine infection patterns and immune response mechanisms have identified complex patterns of innate and adaptive immune mechanisms [2, 3] after intramammary challenge with *E. coli* and other mastitis – causing pathogens. Lipopolysacharides (LPS) in the bacterial cell wall are recognized by host cell receptors (such as Toll-like receptor-4) on macrophages that circulate in milk. The recognition of LPS by the host cell receptors leads to a cascade of events and eventually to an increased production of pro-inflammatory cytokines [2, 3]. These cytokines have, amongst other characteristics, a chemotactic effect on polymorphonuclear cells (PMN) in the bloodstream, which are, consequently, attracted into the mammary gland towards the site of infection where they attempt to phagocytise and kill the bacterial invaders [9, 23]. The outcome of the bacterial invasion into the mammary gland is eventually determined by the host response and the ability of the bacterial pathogen to circumvent or evade this host response [9, 20]. One of the mechanisms that bacterial pathogens employ to persist in the host is the creation of an intracellular niche [6], the importance of this in *E. coli* mastitis has not yet been established.

Mathematical modelling may be valuable in providing insight into the mechanisms of persistent infections, both at the population level [28] and the individual animal level [10, 17]. The model described by Detilleux et al. [10] models transient infections and includes the compartments: milk, blood leukocytes and bacteria in milk. This simple model was fitted to transient infection data, but it does not adequately describe the complex pathogenesis of bacterial infections that is essential to reproduce the dynamics of both transient and persistent intramammary infections.

In this study, we aim to improve understanding of the pathogenesis of transient and persistent intramammary *E. coli* infections. In particular, we are interested in understanding the processes that underlie the divergence in *E. coli* infection outcome (elimination or persistence): why do a majority of intramammary *E. coli* infections results in transient infections and how do other *E. coli* intramammary infections result in persistent infections?

2. MATERIALS AND METHODS

2.1. The model

We model the interaction between the bovine immune system and numbers of *E. coli* in milk and in intracellular reservoirs in a quarter (1 of the 4 compartments of the bovine mammary gland). We assume that the total number of colony forming units (cfu) of *E. coli* in milk (E) grows exponentially, but the bacteria are phagocytised and killed by macrophages and PMN [18]. Bacteria can move between milk and an intracellular mammary epithelial cell reservoir (I) [11]. From numerous challenge infections it is known that production of pro-inflammatory cytokines (C_+) is stimulated by contact between bacteria and immune cells, either macrophages (M) or PMN (P) [4]. The production of pro-inflammatory cytokines is inhibited by the presence of anti-inflammatory or inhibitory cytokines (C_-), which increase at a rate dependent on the concentration of pro-inflammatory cytokines, thus there is a negative feedback loop [2–4]. The macrophages present in the milk are assumed to remain at a constant concentration, whereas the concentration of PMN increases at a rate dependent on the concentration of pro-inflammatory cytokines present in the milk [23].

An additional process that determines the bacterial dynamics is the twice daily process of milking which rapidly depletes the volume of milk in the mammary gland, and so changes the population size of the bacterial cells. We assume a time dependent function, V, that defines the linearly changing volume of milk in the quarter (Fig. 1).

This simplified description of inflammatory dynamics leads to the following series of differential equations for the concentrations of cytokines (C_+ and C_-), and the numbers of PMN (P) and *E. coli*

cfu (E) in milk (which decreases due to milking, see Appendix for derivation) and intracellular *E. coli* (I):

$$\frac{dC_+}{dt} = \frac{g_{cp}}{(1 + i_{cp}C_-)} \frac{E}{V^2} (M + P) - d_c C_+$$

$$\frac{dC_-}{dt} = g_{ca} C_+ - d_c C_-$$

$$\frac{dE}{dt} = g_e E - kE \frac{(M + P)}{V} - aE + bI + \frac{E}{V} \frac{dV}{dt}$$

$$\frac{dP}{dt} = g_p C_+ V - d_p P + \frac{P}{V} \frac{dV}{dt}$$

$$\frac{dI}{dt} = aE - bI - d_i I$$

$$v = \left\{ \begin{array}{l} \frac{1 - p_{milk}}{0.5 - D_{milk}} \left[\frac{\mathrm{mod}(2t, 1)}{2} \right] + p_{milk} \\ \text{for } \mathrm{mod}(2t, 1) \leq 1 - 2D_{milk} \\ \frac{p_{milk} - 1}{D_{milk}} \left[\frac{\mathrm{mod}(2t, 1)}{2} - 0.5 \right] + p_{milk} \\ \text{for } \mathrm{mod}(2t, 1) > 1 - 2D_{milk} \end{array} \right\}$$

$$V = V_{max}\, v$$

$$\frac{dV}{dt} = \left\{ \begin{array}{ll} 0 & \text{for } \mathrm{mod}(2t, 1) \leq 1 - 2D_{milk} \\ \frac{p_{milk} - 1}{D_{milk}} & \text{for } \mathrm{mod}(2t, 1) \leq 1 - 2D_{milk} \end{array} \right\} \quad (1)$$

In all equations, t represents time in days. The initial values of these variables were set to 0, except for E, which had a low initial infection level of 1 cfu/mL (or 250 cfu in total immediately after milking) representing initial invasion. The baseline level for M was set at 5 000 and P was set at 25% of 50 000 (see below). The parameters are defined in Table I and discussed in more detail below.

To explore fadeout of the bacterial population, the model equations for the bacterial populations (E and I) were discretised using the Euler approximation with an interval, dt, of 1 s. The equations for the concentrations of immune cells and cytokines remained deterministic and continuous. Thus producing a stochastic version of the bacterial population section of the model as follows:

$$E_{t+dt} = \max \left\{ \Theta \left[E_t + \left(g_e E_t - kE_t(M + P) - aE_t + bI_t + \frac{dV}{dt} \frac{E_t}{V} \right) * dt \right], 0 \right\}, \quad (2)$$

$$I_{t+dt} = \max \{ \Theta [I_t + (aE_t - bI_t - d_i I_t) * dt], 0 \}$$

where $\Theta[x]$ is sampled from a Poisson distribution of mean x. The Poisson distribution is a discrete distribution and expresses the probability of a number of events (of the type that occur with a known average rate and independently of the time since the previous event) occurring in a fixed period of time. An event in this case is defined as a change in the population size due to growth, killing or milking. This distribution therefore appears to be the most appropriate for this system.

2.2. Biological data

2.2.1. *Differential cell counts in milk*

Basic milk somatic cell counts (SCC) at the time of initial infection were estimated using data from healthy non-infected quarters [13]. Differential milk leukocyte counts for healthy cows (n = 21) with low SCC milk (SCC < 200 000 cells/mL, [25]) in early lactation (22 ± 4 d after calving), mid lactation (185 ± 6 d after calving) and late lactation (245 ± 12 d after calving) were used [12]. Approximately 10% of all somatic cells in a healthy quarter are macrophages and 25% PMN. If we assume 50 000 cells/mL in a healthy quarter [24], then $\frac{M}{V}$ = 5 000 cells/mL and approximately 25% would be PMN.

Washout of cells and bacteria due to milking is estimated at about 90% (see also [10]). With a milk yield of 2.5 kg per quarter (i.e. V_{max} = 2 500 mL) and acceptable milk withhold of 0.25 kg at the end of milking, it is reasonable to assume that 90% of cells and bacteria are removed at each milking (i.e. p_{milk} = 0.1).

2.2.2. *Killing capacity of PMN*

No direct data on bacterial killing were available for bovine intramammary PMN or macrophages. To obtain a quantitative estimate of killing capacity we utilized data from a mouse model of *E. coli* infection published by Iwahi and Imada [16], in which the susceptibility of *E. coli* to killing by murine peritoneal exudate PMN was evaluated. A 0.05-mL volume of bacterial suspension (2×10^7 to

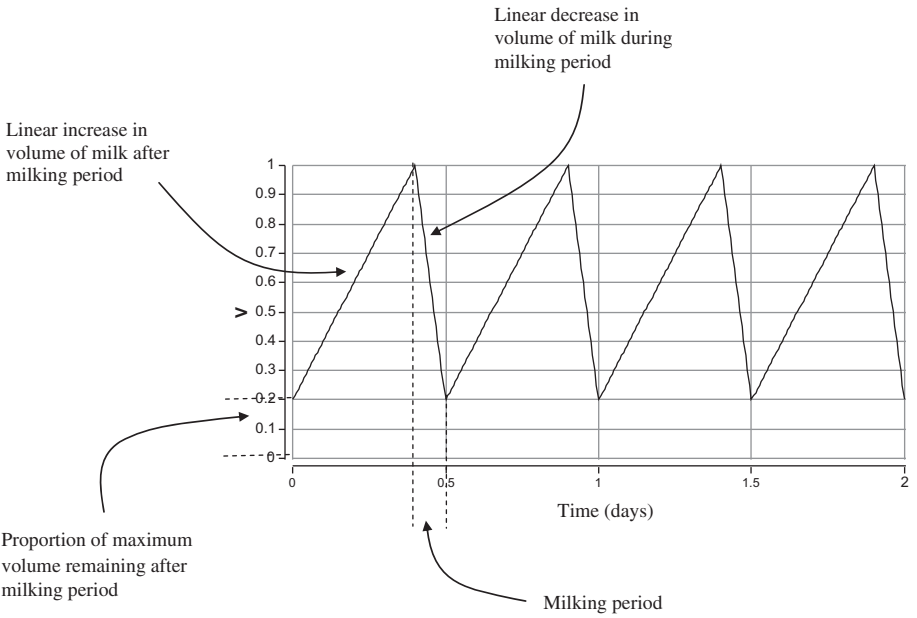

Figure 1. Annotated functional form (not to scale during milking periods) of the milk volume before, during and after milking.

8×10^7 cfu/mL) was mixed with 0.25 mL of PMN suspension (6×10^6 to 8×10^6 cells/mL). A killing rate of approximately 1 log per hour was observed, with a decreasing rate of killing over time. This estimate was very similar to an estimate used by Detilleux et al. [10]. This corresponds to an estimate of k between 4.8×10^{-6} and 3.6×10^{-6} cell/day (See Appendix for the derivation of these values).

2.2.3. *Growth of* E. coli *in vitro and in vivo*

Data on growth of *E. coli* both in vitro and in vivo were taken from experiments by Kornalijnslijper et al. [18, 19], in which 200 μL of bacterial suspension (aiming at an inoculum of 100 cfu) was added to 10 mL whole milk. Bacterial counts in whole milk after 6 h of incubation varied up to a 100-fold between individual cows (3.8–6.0 $\log_{10}$ cfu/mL) [18]. This corresponds to a value of g_e between 25.8 and 46.1 day^{-1} (See Appendix for the derivation of these values).

2.2.4. *Cytokine dynamics*

Data on cytokine dynamics during *E. coli* infection were taken from Bannerman et al. [2, 4]. In this study, cows were challenged with approximately 72 cfu of *E. coli*. Sterile milk samples were collected from all quarters at 0, 8, 16, 24, 32, 40 and 48 h after challenge and evaluated for cytokine profiles, cfu and SCC. An ELISA assay was used to measure the concentrations of IL-8 and TNF-α in milk obtained from quarters infused with *E. coli*, and saline controls. As expected, pro-inflammatory cytokines increased rapidly after intramammary challenge [2–4]. An increase was observed at 8 h after challenge and the concentration peaked at 16 h after challenge. Pro-inflammatory cytokine concentrations then dropped and reached baseline values between 32 and 48 h after challenge. Inhibitory cytokines (IL-10) showed a rapid increase at 16 h post-challenge (at the peak of pro-inflammatory cytokines) and peaked at 24 h post-challenge. Thereafter the concentration dropped and fell back to baseline at 72 h after challenge [2]. No data were available in peer-reviewed publications for cytokine profiles in persistent *E. coli* infections. However, a similar study using a *Klebsiella* challenge provides some insight in cytokine dynamics in more persistent gram-negative infections [3]. The model output was considered with reference to this general behavior and timescale. Cytokines have a half-life (T) of between 1 and 24 h [4]. Thus the rate of loss, d_c is given by $d_c = \frac{\text{Ln}(2)}{T}$. Then d_c is between 0.69

Table I. Model parameter definitions, initial values and model estimates.

Symbol	Definition	Initial value	Estimated value	Units
Parameters with the same value for all cows				
g_{cp}	Baseline growth rate of pro-inflammatory cytokines in absence of anti-inflammatory cytokines. Stimulated by contact between *E. coli* and macrophages	Unknown	0.0024	day^{-1}
i_{cp}	Inhibitory effect of anti-inflammatory cytokines on growth of pro-inflammatory cytokines	Unknown	0.035	none
g_{ca}	Rate of growth of anti-inflammatory cytokines (dependent on concentration of pro-inflammatory cytokines)	Unknown	9.8e-5	
g_e	Growth rate of *E. coli* in milk	25.8–46.1	34	day^{-1}
d_p	Rate of loss of PMN from milk	0.1–1.8	0.10	day^{-1}
d_c	Rate of loss of cytokines	0.7–17	10	day^{-1}
M	Baseline number of macrophages in milk	5000 (fixed)		Cells/mL
V_{max}	Maximum volume of milk in a quarter	2.5 (fixed)		Litre
D_{milk}	Duration of milking	$5/(60 \times 24)$ (fixed)		Day
p_{milk}	Proportion of milk remaining after milking	0.1 (fixed)		None
k	Killing rate by macrophages (M and P)	3.6e-6–4.8e-6	1.0e-5	Cell/day
σ_E	Mean standard deviation of the model fit from the data for log *E. coli* concentration	Unknown	0.54	log cells/mL
σ_S	Mean standard deviation of the model fit from the data for log somatic cell concentration	Unknown	0.75	log cells/mL
Parameters that have a different value for each type of infection (transient, persistent)				
a	Rate of movement of *E. coli* from milk to intra-cellular reservoirs	Unknown	0.51 (transient) 5.1 (persistent)	day^{-1}
b	Rate of movement of *E. coli* from intra-cellular reservoirs to milk	Unknown	0.55 (transient) 7.5e-3 (persistent)	day^{-1}
Parameters that have a different value for each individual cow				
d_i	Death rate of *E. coli* in intra-cellular reservoirs	$\sim$ 1 (transient) $\sim$ 0.1 (persistent)	2.1, 0.9, 1.4, 1.2, 1.4, 1.8, 1.9 (transient) 0.1 (persistent)	day^{-1}
g_p	Rate of influx of PMN into the milk in presence of pro-inflammatory cytokines	Unknown	0.02, 0.04, 0.02, 0.03, 0.03, 0.50, 2.0, 0.8, 6.0, 2.0, 0.3	day^{-1}

Table II. Data for seven transient infections. Cows 218, 4 003, 4 310, 7 036, 9 658, 2 058, and 2 109 are from Van Werven et al. [27]. Time is in days, lg(E) is $\log_{10}$ *E. coli* cfu/mL, lg(S) is $\log_{10}$ SCC/mL.

Time (days)	Cow number													
	218		4 003		4 310		7 036		9 658		2 058		2 109	
	Lg(E)	Lg(S)	Lg(E)	Lg(S)	Lg(E)	Lg(S)	Lg(E)	Lg(S)	Lg(E)	Lg(S)	Lg(E)	Lg(S)	Lg(E)	Lg(S)
0	0	5.0	0	4.4	0	4.0	0	4.0	0	4.4	0	4.0	0	5.4
1	9.0	7.0	5.9	7.0	7.7	6.9	8.7	5.6	8.6	6.7	5.3	7.0	3.7	6.3
2	5.2	7.0	6.5	7.0	6.2	7.0	6.4	7.0	4.9	7.0	2.4	7.0	0	7.0
3	4.1	7.0	6.0	7.0	5.0	7.0	5.4	7.0	4.9	7.0	2.4	7.0	0	6.9
4	3.6	7.0	5.7	5.5	5.1	6.9	5.5	6.8	4.5	6.5	2.2	6.8	0	6.4
5	2.9	7.0	5.0	6.8	4.5	7.0	4.5	7.0	4.2	6.8	0	7.0	0	6.3
6	3.2	7.0	4.9	6.4	3.3	7.0	3.9	7.0	3.7	6.8	0	7.1	0	7.0
7	0	7.0	4.0	6.2	3.0	7.0	2.3	7.0	3.1	7.0	0	6.6	0	6.3
8	0	7.0	3.4	7.0	2.4	7.0	2.3	7.0	3.4	7.0	0	6.4	0	5.8
9	0	7.0	3.1	7.0	2.3	7.0	1.7	7.0	2.9	7.0	0	6.2	0	6.0
10	0		0		0		0		0		0		0	

and 17, which are the upper and lower limits used for estimation of this parameter.

2.2.5. *Intracellular* E. coli

Data on intracellular survival of *E. coli* were taken from an in vitro study [11] in which *E. coli* strains from transient and persistent field infections were compared in adhesion and invasion assays. MAC-T cells were infected at a multiplicity of infection of 10 (10 bacteria per epithelial cell) for 1 h. The *E. coli* strains associated with persistent infections were 10 times more invasive than strains associated with transient infections. This translates to the constraint of the value for the parameter, a, for chronic infections being 10 times its value for acute infections.

Intracellular survival of *E. coli* strains in MAC-T cells was studied by gentamycin protection assays. MAC-T cells were infected with *E. coli* strains for 1 h, after which extracellular bacteria were killed by exposure to gentamycin (100 μg/mL), an extracellular antibiotic, for 2 h. MAC-T cells were incubated for a further 2, 24 and 48 h. The number of intracellular bacteria surviving was determined by disrupting the cell wall and plating and counting cfu. Strains of *E. coli* from transient infections showed a decrease of the number of viable bacteria to approximately 80% of the initial concentration after 48 h. In contrast, the number of cfu for persistent strains had almost doubled during the same time period (180% of baseline). These findings are in concordance with similar observations in mouse urinary tract epithelial cells [1, 21]. We use an initial value of $d_i = 0.1$ day^{-1} for chronic infections. This is equivalent to intracellular survival for an average of 10 days [21]. To be conservative with regard to the importance of the intracellular reservoir we initially assumed no growth in the intracellular reservoir.

2.2.6. E. coli *infection data*

E. coli intramammary challenge longitudinal infection data used in this modelling study were gathered from several published [11, 12, 19, 27] and unpublished studies[1]. We identified a total of 7 transient infections (cow numbers: 218, 4 003, 4 310, 7 036, 9 658, 2 058 and 2 109, all from Kornalijnslijper et al. [18] and VanWerven et al. [27]) and 4 persistent infections (cow numbers: 171 103 (from Dogan et al. [11]), 3a, 3b and 3c (from Döpfer et al. [12]). All data used for simulations are summarized in Tables II and III.

2.3. Parameter estimation

The model predictions for the logarithms of the *E. coli* and somatic cell concentrations generated using (1) were fitted to the data. The parameters were estimated within Berkeley-Madonna [28], by maximising likelihood assuming a normal distribution of error. The initial values for the parameter estimation

[1] Döpfer D., Ph.D. Thesis, Utrecht University, 2000.

Table III. Data for four persistent infections. Cow 171 103 is from Dogan et al. [11] and cows 3a, 3b and 3c are from Dopfer et al. [12]. Time is in days, lg(E) is 10 log *E. coli* cfu/mL, lg(S) is 10 log SCC/mL.

Cow number																							
Time	171 103					Time	3a					Time	3b					Time	3c				
(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)
0	4.3		41	3.9	7.1	0	3.5	6.7	41	3.6	6.4	0	2.0	6.7	41	3.6	6.9	0	3.0	6.8	41	2.0	5.7
1	5.5		42	2.0	7.1	1	3.8	6.6	42	3.4	6.2	1	2.8	6.6	42	2.6	6.8	1	2.0	6.6	42	2.0	6.5
2	3.5		43	1.9	6.7	2	4.2	6.5	43	3.8	6.1	2	2.3	6.8	43	3.2	6.6	2	0	6.4	43	0	6.1
3	3.0		44	2.8	7.2	3	3.5	6.2	44	3.1	6.9	3	2.5	6.8	44	3.4	6.8	3	0	6.1	44	2.3	5.8
4	3.1		45	3.2	7.0	4	4.1	6.8	45	3.3	7.0	4	2.5	6.6	45	3.3	6.7	4	0	5.6	45	2.0	5.4
5	5.8		46	3.3	7.3	5	2.9	6.8	46	3.6	6.6	5	3.5	6.9	46	2.4	6.8	5	2.0	5.5	46	2.3	5.5
6	4.3		47	3.7	7.1	6	2.9	6.7	47	3.6	6.5	6	3.3	7.0	47	2.5	6.8	6	2.5	5.5	47	3.1	7.0
7	4.7		48	4.8	7.3	7	2.0	6.5	48	4.5	6.5	7	3.5	6.8	48	2.6	6.6	7	0	6.3	48	2.3	6.7
8	3.9		49	4.3	5.2	8	2.0	6.7	49	3.3	6.5	8	3.2	6.8	49	2.7	6.4	8	2.0	6.2	49	2.3	6.6
9	3.9		50	4.4	5.5	9	2.0	6.3	50	3.3	6.3	9	2.4	6.7	50	6.3		9	2.0	6.4	50	2.3	6.5
10	4.4		51	4.3	7.4	10	3.1	6.7	51	3.4	6.4	10	2.3	6.7	51	3.4	6.4	10	0	6.1	51	3.3	6.2
11	4.5		52	4.7	7.4	11	2.3	6.7	52	3.3	6.4	11	2.2	6.7	52	3.3	6.4	11	2.0	5.8	52	0	6.4
12	4.2		53	5.2	7.4	12	2.0	6.7	53	4.0	6.1	12	2.2	6.5	53	4.0	6.1	12	2.3	6.2	53	2.0	5.9
13	4.7		54	5.4	7.0	13	3.3	6.5	54	3.8	6.1	13	2.1	6.5	54	3.8	6.1	13	0	5.9	54	0	5.7
14	4.0		55	5.4	7.0	14	3.2	6.7	55	3.3	6.9	14	2.1	6.3	55	3.3	6.9	14	2.3	5.7	55	2.6	6.9
15	3.9	5.2	56	5.5	7.0	15	2.8	6.7	56	4.0	6.8	15	2.0	6.0	56	4.0	6.8	15	0	6.2	56	0.0	6.6
16	4.1	7.3	57	3.2	7.2	16	2.5	6.6	57	3.4	6.6	16	2.0	6.1	57	3.4	6.6	16	2.0	5.3	57	2.3	6.3
17	3.0	7.0	58	2.3	6.6	17	4.3	6.4	58	3.7	6.1	17	2.5	6.7	58	3.7	6.1	17	0	5.4	58	2.0	6.4
18	3.0	5.9	59	2.6	6.7	18	2.8	6.7	59	4.0	6.2	18	4.2	6.9	59	4.0	6.2	18	2.3	5.8	59	0	6.0
19	3.6	7.4	60	2.9	6.9	19	2.0	6.7	60	4.1	6.3	19	4.4	6.8	60	4.1	6.3	19	0	6.4	60	0	5.9
20	3.7	7.4	61	1.8	7.2	20	2.8	6.5	61	3.5	6.5	20	4.7	6.8	61	3.5	6.5	20	2.0	6.1	61	0	5.5
21	4.1	7.5	62	3.1	7.4	21	3.2	6.7	62	4.1	6.6	21	3.9	7.0	62	4.1	6.6	21	2.0	5.9	62	2.7	5.5
22	2.4	7.2	63	3.5	7.2	22	3.6	6.5	63	4.0	6.4	22	3.6	7.0	63	4.0	6.4	22	2.3	6.5	63	2.0	6.7
23	1.9	6.9	64	2.1	7.0	23	3.5	6.3	64	4.4	6.1	23	4.1	6.7	64	4.4	6.1	23	2.0	6.3	64	2.0	6.3
24	2.3	7.1	65	2.0	6.4	24	3.4	6.8	65	4.5	6.5	24	4.4	7.0	65	4.5	6.5	24	2.5	6.4	65	2.0	6.0
25	1.3	7.1	66	3.5	7.3	25	2.6	7.0	66	3.9	6.5	25	3.5	6.7	66	3.9	6.5	25	0	6.3	66	2.5	6.5
26	2.4	6.6	67	2.6	7.2	26	3.7	6.9	67	3.8	6.4	26	3.7	6.9	67	3.8	6.4	26	0	6.0	67	0	5.7
27	0.0	7.3	68	2.3	6.6	27	3.4	6.4	68	3.5	6.5	27	4.2	7.0	68	3.5	6.5	27	2.3	5.8	68	6.8	

continued on next page

Table III. Continued.

Cow number																							
Time	171 103					Time	3a					Time	3b					Time	3c				
(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)	(days)	lg(E)	lg(S)	days	lg(E)	lg(S)
28	0.0	6.9	69	3.1	7.2	28	3.7	7.0	69	3.4	6.4	28	4.2	6.8	69	3.4	6.4	28	2.3	5.9	69	3.4	6.6
29	2.5	7.0	70	2.4	6.7	29	3.1	7.0	70	3.3	6.2	29	3.1	6.8	70	3.3	6.2	29	2.0	5.7	70	3.7	6.1
30	2.7	6.2	71	2.4	7.1	30	2.3	6.6	71	3.9	6.1	30	2.0	6.7	71	3.9	6.1	30	2.9	5.8	71	4.0	6.2
31	2.0	4.1	72	2.5	6.9	31	2.9	6.5	72	4.1	5.9	31	2.5	6.7	72	4.1	5.9	31	2.0	6.5	72	4.1	6.3
32	2.5	7.1	73	1.3	6.6	32	3.6	6.1				32	2.9	6.4				32	2.0	6.1	73	3.5	6.5
33	3.3	7.4	74	3.2	7.4	33	3.5	6.1				33	3.3	6.7				33	2.0	6.2	74	4.1	6.6
34	3.1	7.2	75	2.5	7.2	34	3.4	6.7				34	2.0	6.6				34	2.7	6.0	75	4.0	6.4
35	2.7	4.9				35	3.9	6.5				35	2.0	6.4				35	2.0	6.6	76	4.4	6.1
36	3.6	6.4				36	3.8	6.4				36	3.2	6.6				36	2.0	6.1	77	4.5	6.5
37	3.4	7.0				37	4.0	6.3				37	3.5	6.9				37	2.3	6.6	78	3.9	6.5
38	3.9	7.0				38	4.0	6.6				38	3.5	6.9				38	2.5	6.4	79	3.8	6.4
39	3.3	7.0				39	3.0	6.2				39	3.3	6.8				39	2.0	6.4	80	3.5	6.5
40	3.5	7.1				40	3.4	6.5				40	3.6	6.8				40	2.0	6.1	81	3.4	6.4

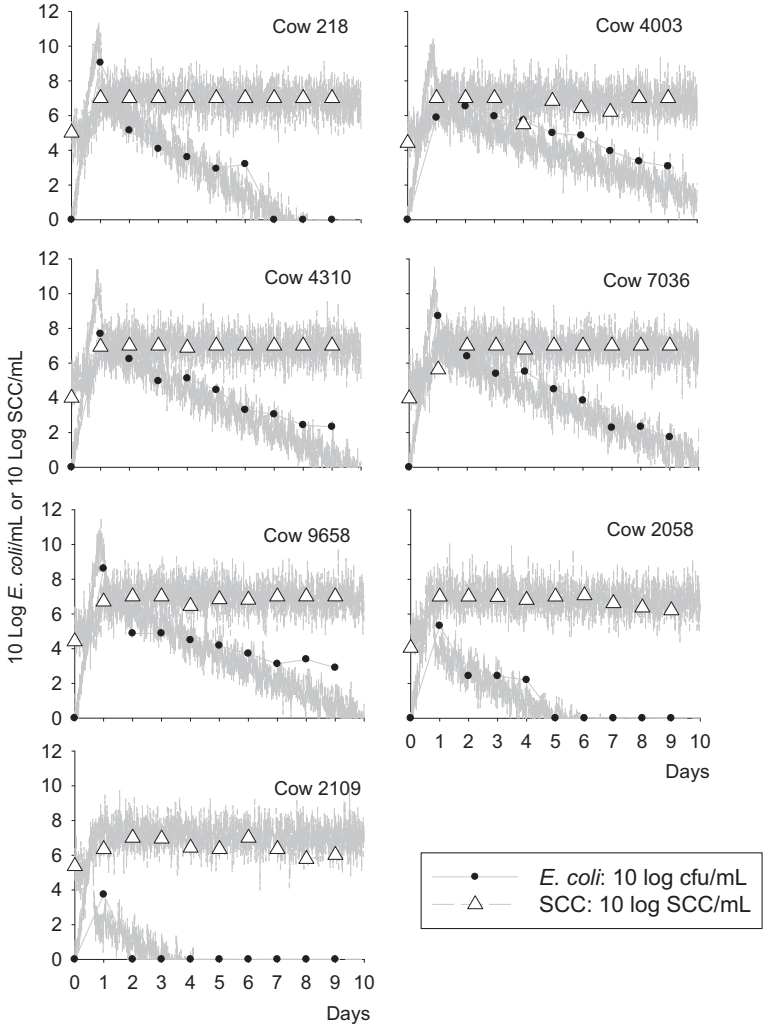

Figure 2. Observed (points) and model predicted (lines) bacteria count and SCC data for seven cows with transient infections. Cow numbers are shown on the top-right of each graph. The model prediction is a single realisation of the stochastic version of the model including variation in measurement based on the standard deviations of the model fit to the data (σ_E and σ_S).

process are given in Table I. We assume that if the dataset includes a zero value and the model predicts a value less than or equal to zero (that is less than or equal to 1 cell/mL), then this is the best fit for this point due to limits of detection. We also assume that the persistent infections had reached equilibrium when they were sampled.

3. RESULTS

The model reproduced the data with the estimated parameter values as given in Table I. The optimised negative log-likelihood was 650.35. The model also reproduced both transient and persistent infection behaviour (see Figs. 2 and 3).

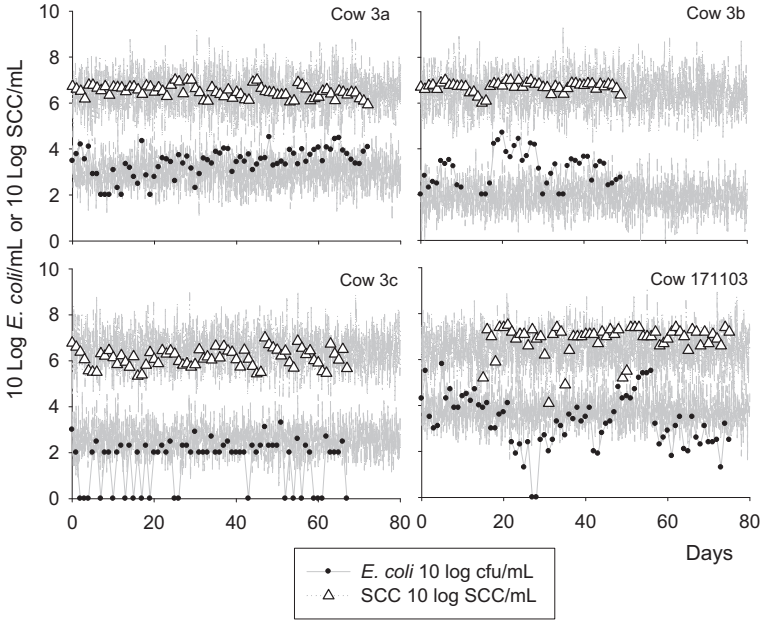

Figure 3. Observed (points) and model predicted (lines) bacteria count and SCC data for four cows with a persistent infection. The model prediction is a single realisation of the stochastic version of the model including variation in measurement based on the standard deviations of the model fit to the data (σ_E and σ_S).

For all cows, the model consistently predicted a 3 h difference between the timing of the peak of pro- and anti-inflammatory cytokine concentrations. The model also predicted that the pro-inflammatory cytokines peak within the range of 13 to 22 h after infection for all cows and that the anti-inflammatory cytokines show a rapid increase that started just before the peak in pro-inflammatory cytokines. Figure 4 shows an example of this predicted behaviour for cow number 218. The sampling frequency of the experiment by Bannerman et al. [4] was every 8 h. Therefore from the data we can conclude that the delay between peaks should be between 4 and 12 h. The model predicts an approximate 3 h difference which is somewhat lower than the range predicted by experimental data. Thus, the model is qualitatively but not quantitatively capturing the cytokine dynamics and a more complex model of the mechanism would be required for a more precise quantitative prediction of this phenomenon.

The key result from our model is the transition from transient to steady state dynamics for the two distinct types of infection. The output from the stochastic and deterministic versions of the model in Figure 5 is the model predictions for the total bacterial population in the milk and the intracellular reservoir combined. We use this output to demonstrate the potential for the animal to clear the infection due to very low numbers after the first 5 to 12 days. This would correspond with an observed clearance in 2 to 10 days since only the concentration in the milk was being observed in the experiment and so this value would drop below the detection limit (of about 1 cfu/mL) 2 or 3 days before true clearance by the animal. For transient infections, the number of bacteria drop below 10 cfu (not per mL but in the whole cow), whereas for the persistent infections the number of bacteria never drop below $10^{6.5}$ cfu in the whole cow: with approximately 2 500 mL of milk in the gland (quarter) this would equate to

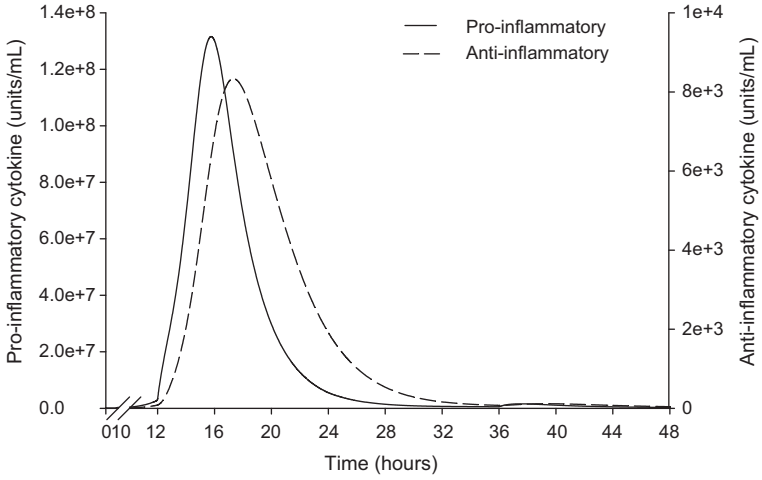

Figure 4. Dynamics of the concentrations of pro- and anti-inflammatory cytokines predicted by the model for cow number 218.

approximately 4×10^3 cfu/mL for a persistently infected quarter (see Fig. 3).

This value of the nadir in *E. coli* bacterial numbers was highly sensitive to the assumed duration of survival of bacteria in the intracellular reservoir. This duration of survival has a threshold value for simulations of the stochastic model of about 1 day where below the threshold fadeout was most likely and above the threshold persistence was more likely. For these sets of models we assumed this duration to be approximately 10 days (an alternative estimate of 2 days was also considered with similar results) for persistent infections and estimated (by fitting) the duration to be about 0.8 days for transient infections.

The discretized stochastic model was run 1 000 times and fadeout in the transiently infected cows and no fadeout in the persistently infected cows was predicted for 996 out of 1 000 runs. In four runs one or more of the modelled transient infections did not fadeout. In 1 000 runs the modelled persistent infections did not fadeout. This result confirms the conclusion from the deterministic model that the nadir in *E. coli* bacterial numbers for transient infections after the initial acute phase will generally result in fadeout of the bacterial population and thus clearance of infection.

4. DISCUSSION

The mathematical model presented reproduces the observed dynamic behaviour of both transient and persistent infections. The predicted time-dependent behaviour of the pro- and anti-inflammatory cytokine concentrations is also consistent with our current biological knowledge.

We show that for parameter values suitable for transient infections, the model predicts that the total number of bacteria in the mammary gland drops to values at which spontaneous clearance of the infection is highly likely due to stochastic effects. The predicted minimum level of bacteria present is sensitive to changes in the duration of survival in the intracellular reservoir. Long durations of survival in the intracellular reservoir correspond with high bacteria counts in the nadir of cfu following the acute phase, indicating a failure to spontaneously clear the infection and thereby leading to a persistent infection. Hence, the key parameters distinguishing transient from persistent infections were the parameters a, b and d_i (respectively the rates of movement of bacteria between the milk and the intra-cellular reservoir and the intracellular bacterial death rate). These three parameters were indeed different between

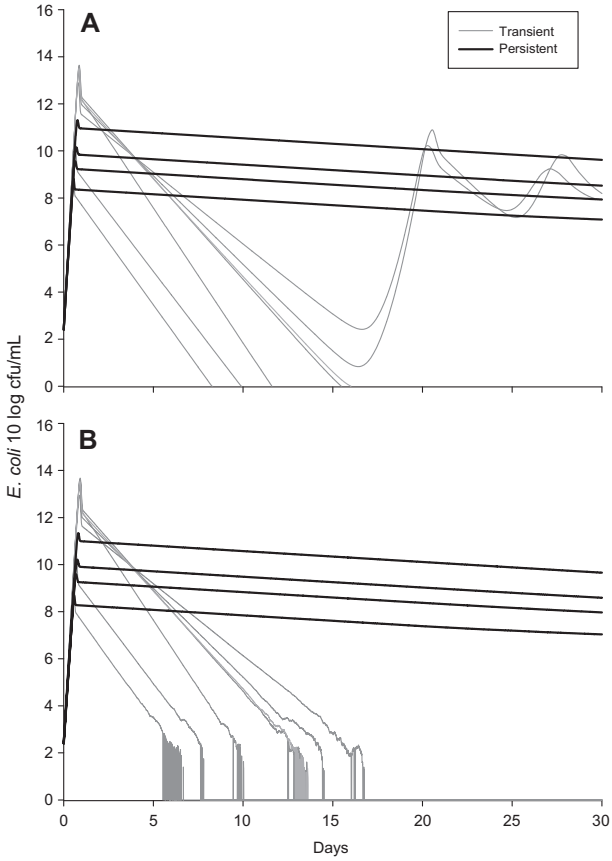

Figure 5. Predicted total numbers of *E. coli* cfu in the mammary gland and the intracellular reservoir combined for seven transient and four persistent infections from the deterministic (A) and stochastic (B) versions of the model.

transient and persistent infections when estimated from observed data (Tab. I).

Our parameter estimates were very similar to the estimates used by Detilleux et al. [10], although the current model is obviously more complex and needed additional input data. However, estimates of key parameters such as bacterial growth rate (1.40 per hour) and concentration reduction rate due to killing (240×10^{-6} μL/cell per hour) were very similar to previous simulation models [10] where the growth rate estimates ranged from 0.54 to 0.8 per hour and those for the killing rate ranged from 184×10^{-6} to 431×10^{-6} μL/cell per hour. Since both our model and previous models validated their estimates on actual observed data, this is not really surprising.

The purpose of this analysis was to propose the most likely explanation for why some *E. coli* infections persist in the mammary gland, whilst others do not. We propose that bacterial strains that result in persistent infections are more effective (by an order of magnitude) at surviving in an intra-cellular reservoir in mammary epithelial cells. This is consistent with a related *E. coli* survival mechanism where intracellular biofilm-like structures, called intracellular bacterial communities, were associated with persistent *E. coli* infections [7]. Other candidate explanations from the analysis are due to

differences at the host response rather than the bacterial strain level [8, 23]. Namely that (a) persistent infections occur when there is a lower or delayed influx of PMN in the presence of pro-inflammatory cytokines [20, 27], or (b) persistent infections result from a short half-life and decreased functionality of PMN such as impaired phagocytosis and/or killing capacity [9, 23]. However, these alternative explanations are unlikely to be a general mechanism responsible for creating the observed patterns. In the case of low or delayed PMN influx, the model predicts unrealistically high numbers of *E. coli* during the transient infection dynamics. In the case of a short life expectancy of PMN, our model would require an unrealistically short half-life to be able to fit data for cows with a persistent intramammary infection. Also, there is good in vitro experimental evidence to suggest that *E. coli* strains present in persistent infections have different attributes from those present in transient infections [11], thereby suggesting that the difference in pathogenesis between transient and persistent infections is due to the type of bacteria rather than the type of response mounted by individual cows.

Although macrophages, PMN and lymphocytes will probably undergo some homing towards the supramammary lymphnodes [24], we did not include this behaviour in the model. We also ignored the potential impact of memory dependent immunity, since it is generally assumed that antibody mediated immune response mostly reduces clinical severity and has relatively little impact on the incidence of infection or the distinction between transient and persistent infection [29]. In future generations of our infection model, more components of the immune response may be added and evaluated for their importance. The hypothesis of a longer survival time in an intracellular epithelial reservoir, resulting in persistent rather than transient infections, appeared to be supported by the data and our current knowledge of the biology of these infections.

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APPENDIX

Derivation of equation for *E. coli*

The variables are expressed as concentrations apart from bacterial load and PMN which is expressed in absolute numbers. We assume that milking does not alter the concentrations of any of these quantities and thus there is no loss term in the concentration formulae. However, milking will affect numbers of cells in the milk thus the following analysis was performed in order to predict total bacterial load. Let concentration of *E. coli* be X. Then the differential equation for concentration of *E. coli* is given by:

$$\frac{dX}{dt} = g_e X - kX(M+P) - aX + b\frac{1}{V}$$

Then the total number, E, is given by:

$$E = VX$$

Since the volume, V, also varies in time, the differential of E is derived using the product rule:

$$\frac{dE}{dt} = V\frac{dX}{dt} + X\frac{dV}{dt}$$

Thus combining the two previous equations gives the following formula for the rate of change of *E. coli* cfu in the milk:

$$\frac{dE}{dt} = V(g_e X - kX(M+P) - aX) + bI + X\frac{dV}{dt}$$

then

$$\frac{dE}{dt} = g_e E - kE(M+P) - aE + bI + \frac{E}{V}\frac{dV}{dt}$$

where $\frac{dV}{dt}$ is the rate of change of the volume of milk.

The differential equation for the numbers of PMN in the milk is derived similarly.

Killing rate, *k*:

Given 0.25 mL of p (6 to 8) $\times 10^6$ cells/mL PMN added to 0.05 mL of *E. coli*. solution. Then the number of PMN cells would be $0.25\ p \times 10^6$ and the volume would be 0.25 + 0.05 = 0.3 mL. Thus the concentration, P, of the resulting solution is $\frac{0.25 \times P.\,10^6}{0.3} = \frac{P \times 10^7}{12}$. Then, given a killing rate of k cell day^{-1}, the concentration of *E. coli* over time, y, is given by the following:

$$\frac{dy}{dt} = -kPy$$

$$y = y_0 \exp(-kPt)$$

$$\ln y = \ln y_0 - kPt$$

$$k = -\frac{\ln y - \ln y_0}{Pt}$$

Since a reduction of one log per hour is observed: t = 1/24, $P = \frac{p \times 10^7}{12}$, $\ln y_0 - \ln y = 1$ then $k = \frac{12 \times 24}{p \times 10^7}$, so for p = 6, $k = 4.8 \times 10^{-6}$ and for p = 8, $k = 3.6 \times 10^{-6}$.

Growth rate of bacteria in milk, g_e:

Let $x(t) = x_0 \exp(g_e t)$ where x is the concentration of *E. coli* over time, x_0 is the initial concentration and t is time, then

$$\ln x = \ln x_0 + g_e t$$

$$\frac{\log_{10} x}{\log_{10} e} = \frac{\log_{10} x_0}{\log_{10} e} + g_e t$$

$$g_e = \frac{\log_{10} x - \log_{10} x_0}{t \log_{10} e}$$

Then, the upper limit of g_e is $(6-1)/(0.25 \log_{10} e)$ or equal to 46.1, and the lower limit of g_e is $(3.8-1)/(0.25 \log_{10} e)$ or equal to 25.8.