Mathematical Model of the Acute Inflammatory Response to *Escherichia coli* in Intramammary Challenge

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ABSTRACT

We constructed a mathematical model of the early response to *Escherichia coli* infection of the mammary gland and explored the roles and interactions between inflammatory cells and bacteria. The model incorporates 3 equations that describe the interactions among bacteria, milk somatic cells, and blood leukocyte densities. These 3 equations were fitted to cell densities observed during acute inflammatory responses in unvaccinated and vaccinated heifers inoculated with 10^4 or 10^6 cfu of *E. coli*. The rates computed for the cellular transit from the storage sites to the blood and from the blood to the milk were lower in cows receiving 10^4 cfu but increased at approximately 6×10^{-6} and 30×10^{-6} $\mu\text{L/cfu per h}$ in nonvaccinated or vaccinated cows inoculated with 10^6 cfu, respectively. The cellular rates of bacterial killing were highest in unvaccinated cows ($\sim 400 \times 10^{-6}$ $\mu\text{L/cell per h}$) when compared with vaccinated cows (200 to 300×10^{-6} $\mu\text{L/cell per h}$). A critical density of milk somatic cells at which bacteria density is constant was computed from the model at 2×10^6 cells/mL, and a one-way sensitivity analysis revealed that the changes in milk cellular densities were mostly sensitive to variations in the rate of bacterial killing and in the rate of production of inflammatory cells.

INTRODUCTION

Escherichia coli is an important environmental pathogen causing clinical mastitis, with a rapid onset of clinical signs following inoculation (Erskine, 1993). The bacteria multiply in the mammary secretion without attachment to epithelial surfaces (Burvenich et al., 2003). Previous studies have shown that cows mount an inflammatory response to *E. coli*, but the efficiency of the response is variable in protecting the gland. Differences in severity have mainly been attributed to the rapidity of influx of polymorphonuclear neutro-

phils (PMN) from peripheral blood into milk and to the killing capacity of PMN (Burvenich et al., 2003). Indeed, PMN are the first line of immunological defense against *E. coli* invading the bovine mammary gland (Sordillo et al., 1997). They are produced in the bone marrow and are released into the blood circulation. After circulating for a few hours, the PMN migrate to the peripheral tissues, where they undergo apoptosis. During infection, PMN production is escalated by the action of inflammatory cytokines and growth factors that activate more immune cells and recruit them to the site of infection (Paape et al., 2003). After migrating out of the blood vessels into the udder compartment, PMN phagocytose bacteria and kill them by secreting bactericidal substances and producing oxidative metabolites. Next, PMN die by apoptosis and are phagocytosed by macrophages (Sladek and Rysanek, 2000), thereby minimizing the release of PMN granular contents that are damaging to tissues. Sometimes inflammation itself can damage healthy cells, which further stimulates inflammation and can lead to chronic inflammation, organ failure, and death.

Laboratory immunological assays are available to measure different PMN functions in the blood. There have been attempts to adapt those assays to milk PMN (Hoeben et al., 1997; Dosogne et al., 2001; Mehrzad et al., 2001; Vangroenweghe et al., 2001, 2002) but they are still expensive and time-consuming. Consequently, they cannot be applied in large-scale field studies, as required in programs of selection for mastitis resistance. Moreover, among the numerous PMN functions, some may be more important than others for clearing a mammary infection.

One solution may be to formalize the dynamics of the immune response process into a few relevant parameters on which breeders could apply selection pressure and on which bovine mammary gland immunologists could focus their studies. For example, in studies of the dynamics of *Staphylococcus aureus* and PMN, it has been shown that the ability of PMN to kill can be modeled with predator–prey models and that a critical PMN density is required for effectively killing bacteria (Li et al., 2002; Detilleux, 2004). Here, we designed a

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mechanistic model of the acute inflammatory response to the bacterial pathogen *E. coli*, for which we estimated parameters based on several studies of the cellular kinetics during an experimental infection. We also performed a one-way sensitivity analysis to determine which parameter might influence the inflammatory response the most.

MATERIALS AND METHODS

Stage 1: Model Development

In this section, we present a theoretical model derived to reflect the changes in cellular and bacterial densities during IMI. The system has density B (blood leukocyte count/unit of blood volume), density M (SCC/unit of milk volume), and density C [bacteria cfu/unit of milk volume] in a well-mixed volume. The dynamics of the system are described by the following ordinary differential equations:

$$dB/dt = \delta + \sigma CB - \theta B - \nu CB \quad [1]$$

$$dM/dt = \theta B + \nu BC - \alpha M - \rho MC \quad [2]$$

$$dC/dt = [\beta - \alpha - \varepsilon M]C \quad [3]$$

The first equation describes the rate of change in the density of blood leukocytes as the sum of the rate of influx of the cells from their production and storage sites and the rate at which they leave the blood. The first 2 terms describe the number of newly formed cells that migrate from the production and storage sites into the blood per unit of time and per unit of blood volume, either during health (δ) or during infection (σCB). The last 2 terms represent the number of cells that emigrate from blood vessels into the mammary gland in response to physiological stimuli (θB) and to infection (νBC), respectively. Equation 2 describes the rate of change in the population of milk somatic cells. Of the blood cells that have migrated into the milk compartment (θB), some will be removed during milking (rate = αM) and some will die after ingestion of bacteria (death rate = ρCM). In Equation 3, the rate of change in the population of bacteria (C) equals the sum of the growth rate (βC), the rate at which bacteria are flushed during milking (αC), and the rate at which bacteria are ingested and killed by PMN (εMC). All the variables and parameters are nonnegative.

The readers should be aware of the simplifications in the model. Let's first consider the healthy animal. The production or storage site (mainly the bone marrow) is imagined as a holding tank for cells that migrate into the peripheral blood at a time-independent

production rate (δ), and each cell in that pool has the same probability of entering the circulation (Rosinski et al., 2004). Once the newly produced cells gain access to the bloodstream, they can exit to the milk compartment at a time-dependent rate with no distinction between the circulating and marginal pools. The rate of exit, θB , is considered directly proportional to the density of blood leukocytes: Few leukocytes exit the blood (per unit of time) when their blood density is small, and many leukocytes exit the blood when their blood density is high. To model the interactions between bacteria (C) and inflammatory cells (M , B), we considered the rates as dependent on the product of the concentration of cells and the concentration of bacteria, according to the mass action principle (i.e., rates increase linearly during the time interval; Hamer, 1906). For example, in the equations describing the rates of change in the density of blood and milk cells, each rate increased proportionally with each newly produced bacterium: σB is the per-bacteria rate of blood PMN release by the bone marrow, νB is the per-bacteria rate of blood PMN migrating from the blood, and ρM is the per-bacteria rate of milk PMN death. In Equation 3 showing the rate of change in bacteria, εC is the rate of bacterial death for each newly migrated PMN. We assumed also that the bacteria had an unlimited food supply, so the growth rate (β) in the absence of PMN is exponential. In summary, the model represents the basic processes of the cellular response to IMI and could serve as a template on which to add other cells, cytokines, and their interaction as new knowledge becomes available.

The parameters of interest in this study are those characterizing the acute phase of the inflammation (σ , ν , ρ , β , ε), hereafter called the acute inflammation response (**AIR**) rates. Therefore, the other rates were fixed at values that would ensure equilibrium between cell populations before inoculation: During health, $\delta = \theta B_0$ and $\alpha = \theta B_0/M_0$, with B_0 and M_0 being the initial densities of blood and milk cells. The following equations were then fitted to the data described in stage 2:

$$dB/dt = \sigma CB - \nu CB \quad [4]$$

$$dM/dt = \nu BC - \rho MC \quad [5]$$

$$dC/dt = [\beta - \varepsilon M]C \quad [6]$$

Stage 2: Estimation of the Parameters

Data. Data used in the analyses were from studies published in 2004 by Vangroenweghe et al. (2004a, 2004b) in which primiparous cows were experimentally infected in the left quarters with 1×10^4 (groups

A, C) or 1×10^6 (groups B, D) cfu of *E. coli* P4:O32 per quarter. Ten cows in group C and 10 cows in group D were vaccinated with an *E. coli* J5 bacterin at -56, -28, and +7 d relative to calving, whereas nonvaccinated animals (groups A, B; $n = 20$) received pyrogen-free saline solution. All cows were considered moderate responders on the basis of a decrease in milk production after infection less than 50% of the preinfection level (Vandeputte-Van Messom et al., 1993). Blood and milk samples were collected from all cows at regular time intervals. The densities of *E. coli* (cfu/mL), of blood leukocytes (n cells/mL), and of milk somatic cells (n cells/mL) were determined either by direct counting using an electronic particle counter (Coulter Counter Z2; Coulter Electronics Ltd., Luton, UK) or by a fluoro-opto electronic method (Fossomatic 5000 cell counter; Foss Electric, Hillerød, Denmark), respectively. For the somatic cells, only counts from infected quarters were used in this study. Data were averaged over cows categorized into 4 groups according to their vaccination status (with or without vaccination) and inoculation dose (10^4 or 10^6). Observed averages are shown in Figure 1 for the first 24 h postinoculation (PIH).

Methods. Estimations were generated with the procedure MODEL on SAS (SAS 8.2, 2001; SAS Institute, Cary, NC). The system of equations (Equations 4, 5, and 6) was fitted to the observed data, separately for each group, with B , C , and M expressed in counts per microliter. The SAS codes are given in the appendix. The ordinary least-squares estimates of the AIR rates were obtained using the Gauss algorithm (FIT statement of the procedure MODEL; SAS Institute). The convergence criterion was set at 10^{-6} . A 95% confidence interval was computed for each parameter to determine whether the parameter was significantly different from 0. The goodness-of-fit of the models was evaluated by the R^2 values for the first 24 PIH.

By nullifying Equation 6, we computed the critical cell density (CCD), where $CCD = \beta/\varepsilon$. When $M_t = CCD$, bacteria grow and are killed at the same rate, so the bacterial density is in a steady state. When $M_t < CCD$, the bacterial density increases with time, whereas at $M_t > CCD$, the bacterial density decreases with time.

Differences in rates between groups were assessed with a weighted ANOVA, in which the weight was proportional to the inverse variance (Larson, 1992). Significance level was set at 5% for all statistical tests.

Stage 3: Sensitivity Analyses

Finally, we performed a one-way sensitivity analysis and studied the effects, on the dynamics of each cell type, of multiplying or dividing by 10 the values for the AIR rates (σ , ν , ρ , β , ε) obtained from the model

described above. The changes in cell density were simulated over a period of 100 h (SOLVE statement of the procedure MODEL; SAS Institute, Cary, NC), and the area under each curve (AUC) was computed by the trapezoidal method. The AUC were computed one rate at a time, with all other rates being kept at their base value (i.e., the value calculated from the data). The results were depicted in tornado diagrams that show, in rank order, the greatest to least effects of the extreme values of the AIR rates on each AUC. Each bar on the diagram is the percentage difference between a specific AUC and the base AUC. The largest bar, at the top of the chart, has the maximal impact on the AUC, whereas each successive lower bar shows a lesser impact.

RESULTS

Estimates of the parameters are presented in Table 1, along with the corresponding adjusted R^2 values and standard errors. The proposed system of equations fit the variation observed in the data very well, as demonstrated by the R^2 values close to 100%. The estimates are also plotted on Figure 2 for each group of cows to allow a comparison among the 4 groups. We observed that bacterial growth rates (β) were not statistically different across groups, even though it was the lowest in group D. In both groups inoculated with 10^4 cfu of *E. coli* (groups A, C), the apoptosis (ρ) rate was null and the bone marrow production (σ) was low or close to 0 (group A). When compared with the groups inoculated with 10^4 cfu, ρ and σ were higher in both groups inoculated with 10^6 cfu of *E. coli* (groups B, D), and were highest in the vaccinated group D. The blood-milk migration (ν) was approximately 2×10^{-6} $\mu\text{L}/\text{cfu}$ per h in groups A and C but increased to 6.3×10^{-6} $\mu\text{L}/\text{cfu}$ per h in group B and to 36×10^{-6} mL/cfu per h in group D (Table 1). Assuming $B_t = 10^4$ blood leukocytes/ μL of blood, we observed a release in the blood of 0.06 and 0.33 new leukocytes/h for each bacterial cfu in groups B and D, respectively. In all groups, the rates of entry into (σ) and egress from (ν) the blood compartment were equivalent. Values for the CCD were similar across groups and were close to 2×10^6 cells/mL. Finally, we noted that the killing rates (ε) were highest in both nonvaccinated groups and were lowest for the vaccinated group D. Assuming a bacterial density of 10^4 , such rates are equivalent to rates of 3.79, 4.31, 3.11, and 1.84 bacterial cfu killed per somatic cell and per hour in groups A, B, C, and D, respectively.

Results of the sensitivity analysis are shown in Figure 3. The tornado diagram indicates that, when compared with the other AUC, the AUC for bacterial cfu

Figure 1. Observed (diamonds) and predicted (lines) densities (per microliter) of blood leukocytes (BLC), of milk somatic cells (SCC) and of milk bacteria (cfu) for each group of cows. Groups A and C were inoculated with 1×10^4 cfu of *Escherichia coli*, and groups B and D were inoculated with 1×10^6 cfu of *E. coli*. Cows from groups C and D were vaccinated with an *E. coli* J5 bacterin. Data are for the first 24 h postinoculation (PIH).

Table 1. Estimates of the rates and critical cell densities (CCD) obtained with a system of equations describing the cellular dynamics during the acute inflammatory reaction to *Escherichia coli*¹

Statistic	Unit	Group A	Group B	Group C	Group D
R_C^2	%	89.40	89.18	85.22	74.73
R_B^2	%	94.62	93.89	87.93	87.37
R_M^2	%	98.67	85.30	97.20	88.37
σ	$\mu\text{L/cfu per h}$	0.91×10^{-6} (1.25×10^{-6})	6.07×10^{-6} (1.92×10^{-6})**	2×10^{-6} (0.57×10^{-6})**	33×10^{-6} (6.18×10^{-6})**
ν	$\mu\text{L/cfu per h}$	2.56×10^{-6} (1.02×10^{-6})*	6.35×10^{-6} (1.87×10^{-6})**	1.86×10^{-6} (0.53×10^{-6})**	36×10^{-6} (6.22×10^{-6})**
ρ	$\mu\text{L/cfu per h}$	0	26×10^{-6} (9.53×10^{-6})**	0	53×10^{-6} (12×10^{-6})**
β	per h	0.80 (0.07)**	0.70 (0.10)**	0.75 (0.10)**	0.54 (0.14)**
ε	$\mu\text{L/cell per h}$	379×10^{-6} (47×10^{-6})**	431×10^{-6} (95×10^{-6})**	311×10^{-6} (68×10^{-6})**	184×10^{-6} (53×10^{-6})**
CCD	SCC/ μL	2.11×10^3	1.62×10^3	2.41×10^3	2.93×10^3

¹Cows in groups A and C were inoculated with 1×10^4 cfu of *E. coli*; cows in groups B and D were inoculated with 1×10^6 cfu of *E. coli*; cows from groups C and D were vaccinated with an *E. coli* J5 bacterin. The goodness-of-fit values are given for each equation and describe the changes in bacterial cfu (R_C^2), blood leukocytes (R_B^2), and somatic cells (R_M^2) during the first 24 h postinoculation.

* $P < 0.05$; ** $P < 0.01$ for the null hypothesis that the value of the parameter is zero.

was the most sensitive to changes in the AIR rates. It was mostly sensitive to ε and to σ in cows inoculated with 10^4 and 10^6 cfu. For example, in cows from group B, the percentage differences were 1,000 and 100, respectively, when σ and ε were decreased by 10. The AUC for milk somatic cells was mostly sensitive to ε , whatever the inoculum dose. For example, in group A, the AUC for somatic cells was 100% lower when ε was multiplied by 10 and was 500% higher when ε was divided by 10, when compared with the base AUC. Finally, the AUC for blood leukocytes was more sensitive to flow rates in and out of the blood compartment in cows inoculated with 10^6 cfu than in those inoculated with 10^4 cfu.

DISCUSSION

Stage 1: Model Development

Mathematical modeling may facilitate our understanding of the complex interactions among the vari-

ous components of the AIR (Levin and Antia, 2001). Such models have been used, for example, to describe the inflammatory response in different shock states (Chow et al., 2005), to elucidate the cell-mediated immune response to *Mycobacterium tuberculosis* (Wigington and Kirschner, 2001), to predict the response to therapy (Clermont et al., 2004), and to support drug discovery (Vovodotz et al., 2004).

In this paper, we presented a simple model of the immune response to an infection with *E. coli*. To our knowledge, this is the first attempt to model the in vivo cellular kinetics observed during an IMI. Several assumptions were made to simplify the mathematical model. First, we modeled the dynamics of the blood leukocytes and milk SCC instead of the strict PMN densities, and this may have biased the estimates of the rates. However, during the acute inflammatory phase, somatic cells are composed mainly of PMN (Saad and Ostensson, 1990) and most of the blood leukocytes migrating to the inflamed udder are PMN.

Figure 2. Estimates of the rates obtained with a system of equations describing the cellular dynamics during the acute inflammatory reaction to *Escherichia coli*. Cows in groups A and C were inoculated with 1×10^4 cfu of *E. coli*; cows in groups B and D were inoculated with 1×10^6 cfu of *E. coli*; cows from groups C and D were vaccinated with an *E. coli* J5 bacterin.

Figure 3. Tornado diagrams for the changes in the area under the curves (AUC) describing the dynamics of blood leukocytes, milk somatic cells, and bacteria cfu during the first 100 h postinoculation for cows inoculated with 10^4 (A) or 10^6 (B) cfu/quarter. Changes are shown for extreme values of each rate.

Second, all rates were considered to be constant at all stages of the inflammatory response even though PMN may enter the blood and milk compartments at different period of cell maturation, with different killing and chemotactic abilities (Van Merris et al., 2002; Burvenich et al., 2003). The results of this study should also be restricted to the specific *E. coli* strain P4:O32 used to inoculate the cows because of the differences in susceptibility to phagocytosis among coliform strains (Hogan and Smith, 2003). However, it is generally accepted that the type of *E. coli* strain does not play a major role in the severity of mastitis (Burvenich et al., 2003). Finally, estimates of the rates are relevant only to cows “equivalent” to those used in the study, that is, primiparous cows with a moderate inflammatory response (Vangroenweghe et al., 2004a,b). Because the dynamics of the response are dependent on the initial values, results may be different in multiparous cows

or in cows with a more severe inflammatory response (Mehrzhad et al., 2005). Note also that the individual variability between cows within a group was not taken into account because deterministic models such as ours are developed to describe and explain what happens on the average at the population scale. Jacobsen et al. (2005) showed that 60% of the variation in blood leukocyte counts after an LPS challenge could be attributed to the differences between individual cows. Detilleux et al. (1994) demonstrated the heritable nature of these differences in immunological assays measured during the periparturient period.

Stage 2: Estimation of the Parameters

Rates. In spite of these assumptions, estimates of the rates compared nicely with the few published values. For example, our estimates of *E. coli* P4:O32

growth ($\beta = 0.5$ to $0.8/\text{h}$, with an average of $0.7/\text{h}$) are within the range of published values for the growth rate of *E. coli*. Indeed, Lohuis et al. (1990) observed a growth rate of $0.62/\text{h}$ for *E. coli* O111:B4 in whole milk from clinically healthy cows. Kornalijnslijper et al. (2003) reported in vitro growth rates varying from 0.4 to 0.74 cells/h for *E. coli* O157 cultured in a cell-reduced whole milk fraction. The growth rate of *E. coli* P4:O32 measured in UHT-treated milk was estimated at 0.61 and $0.31/\text{h}$ in normal (37°C , $\text{pO}_2 = 23.3$ mmHg) and abnormal (41°C , $\text{pO}_2 = 1.3$ mmHg) conditions (Goldberg et al., 1994). On the other hand, Fang et al. (1993) observed a rate of 0.16 and $0.09/\text{h}$ in whole milk from a healthy (California Mastitis Test-negative and $\text{SCC} < 200,000/\text{mL}$) and an infected (*Streptococcus dysgalactiae* and $\text{SCC} > 4.5 \times 10^6/\text{mL}$) cow, respectively.

Our estimates of the killing rates (ε) are also close to those observed by Li et al. (2004) in rabbit dermis inoculated with 10^8 *E. coli* cfu/mL. Indeed, they observed per capita killing rates of 2.2 to 4.0×10^{-9} mL/PMN per min ($= 132$ to 240×10^{-6} $\mu\text{L}/\text{PMN}$ per h), comparable to the values of 184 to 431×10^{-6} $\mu\text{L}/\text{cell}$ per h in this study. The values for ε may also be expressed per somatic cell and per hour and compared with published values of PMN killing indexes. It follows that, assuming $C_t = 10^4$ in our model, 4 *E. coli* cfu are killed on average per somatic cell and per hour in nonvaccinated cows (groups A, B). Likewise, in an in vitro experiment, Wise et al. (2003) observed 6 to 8 intracellular *E. coli* 727 per phagocytosing bovine PMN after incubating PMN and bacteria for 1.5 h. In humans, Braga et al. (1999) observed 2.33 ± 0.57 dead *E. coli* ATCC 25922 per PMN after 0.5 h of incubation.

Estimates for the production (σ), blood–milk migration (ν), and apoptosis (ρ) rates during *E. coli* infection were not found in the literature even though several papers stressed their important roles in the dynamics of the inflammatory reaction. For example, when investigating the production of leukocytes, Schalm and Lasmanis (1976) showed that the introduction of 0.05 mg of *E. coli* endotoxin into a normal quarter nearly depleted the marrow of mature PMN within 8 h. In mice, Rosinski et al. (2004) observed a 30-fold increase in the circulating population of PMN from 0.74 to 2.5×10^6 cells/h following induction of an acute systemic inflammatory response by burn injury. In humans, agents that stimulate release of PMN from the marrow (e.g., GCSF, GM-CSF, corticosteroids, or endotoxin) resulted in a doubling of the blood PMN count within 3 to 5 h (Dale, 2004), and in cattle, Cullor et al. (1990) observed a 3- to 4-fold increase in peripheral blood mature PMN counts in lactating cows administered 1 to $2 \mu\text{g}$ of human recombinant GCSF.

Rates of PMN migration from blood into milk compartments are different whether mastitis is experimentally induced with viable *E. coli* or endotoxin (Van Oostveldt et al., 2002). They also vary according to the strain and dose used to inoculate the quarter. Thus, milk SCC in quarters infused with $100 \mu\text{g}$ of *E. coli* O111:B4 endotoxin started to increase at 2 PIH, reaching an apex at 8 to 24 PIH (Diez-Fraile et al., 2003; Lehtolainen et al., 2003), whereas 68×10^6 and 6×10^6 leukocytes reached the gland 18 h after infusion of 20 mL of PBS with or without $5 \mu\text{g}$ of *E. coli* serotype O128:B12 endotoxin (Rysanek et al., 2005). In quarters inoculated with viable *E. coli*, milk SCC increased dramatically at 12 PIH in quarters inoculated with 21.3 (Scaletti et al., 2003) or 64.8 (Smith et al., 1999) cfu *E. coli* 727; it increased above $150,000$ cells/mL at 8 or 9 PIH in quarters inoculated with 30 to 50 cfu of *E. coli* O157 (Kornalijnslijper et al., 2003; van Werven et al., 1997); it started to increase at 6 PIH and reached maximum values at 12 h in quarters inoculated with approximately 10^4 *E. coli* P4:O32 (Monfardini et al., 1999), and in quarters inoculated with 72 cfu of the same *E. coli* P4:O32, the increase was evident within 16 h (Bannerman et al., 2004). Likewise, we observed that SCC started to increase at 6 to 9 PIH (Figure 1).

Differences Among Groups. In nonvaccinated cows inoculated with 10^4 *E. coli*, the per-cfu rate of leukocyte production (σ) was null, which may indicate that the number of leukocytes in circulating and marginal pools was large enough to mobilize the cells into milk without extra stimulation of the bone marrow and other storage sites. On the other hand, σ was highest in cows inoculated with 10^6 *E. coli*. Indeed, it is known that granulopoiesis and PMN emigration from bone marrow are dose-dependent (Paape et al., 2003). For example, Wenz et al. (2001) observed metamyelocytes in the blood of 22 to 45%, and $>100,000$ cfu/mL in the milk of 28 to 75%, of cows sampled at the time of identification of acute coliform mastitis. This corresponds roughly to a linear increase of 0.6% ($R^2 = 87\%$) in the percentage of cows with blood metamyelocytes for each percentage increase in the percentage of cows with $>100,000$ cfu/mL of milk.

The rate of leukocyte mobilization in response to infection (ν) was very low in cows from groups A and C compared with cows from groups B and D. This may be related to the observation by Rainard and Riollet (2003) and Vangroenweghe et al. (2004b) that increasing the inoculum of *E. coli* shortens the lag period before the inflammatory response manifests itself. Indeed, ν corresponds to exponentially distributed waiting times in the blood and is inversely related to the lag period (Hethcote, 2000): If the duration of the migration for each new bacteria is denoted D , then $\nu \sim$

$1/D$, since a blood cell migrates into the milk once in D units of time.

The per-cfu rate of apoptosis after ingestion of bacteria (ρ) was also nil in both nonvaccinated and vaccinated cows inoculated with 10^4 cfu, which indicates no enhancement in the PMN death rate at this infectious dose. In fact, the life span of PMN can be considerably extended by exposure to proinflammatory agents such as GMCSF, IL-1 β , IL-2, IL-4, IL-15, INF- γ , GCSF, LPS, sodium butyrate, and glucocorticoids (Edwards et al., 2004; Sladek et al., 2000). For instance, Boutet et al. (2004) observed enhanced survival of milk PMN from cows with SCC higher than 10^6 cells/mL; the rate of spontaneous apoptosis increased from 74.3 to 97.9% (in 24 h) in healthy cows and from 19.7 to 44.8% in cows with high SCC. Similarly, Van Oostveldt et al. (2002) observed, at 18 h after *E. coli* inoculation, that 20% of the blood PMN incubated in vitro for 3 h were apoptotic, compared with 5% before inoculation.

The rate of apoptosis (ρ) was different from zero in cows inoculated with 10^6 cfu. This may relate to the removal of PMN that have reached the end of their useful life span, thereby preventing the release of their potentially toxic contents. Indeed, Coxon et al. (1999) and Watson et al. (1996) have shown that human PMN phagocytosis of opsonized particles significantly accelerates apoptosis.

When comparing cows vaccinated or not vaccinated with the *E. coli* J5 bacterin, we observed higher rates of entry into (σ) and egress from (ν) the blood compartment and a lower rate of bacterial killing (ε) in vaccinated cows than in unvaccinated ones. This is related to the hypothesis of Dosogne et al. (2002) that the J5 vaccination might induce a T helper 1 (Th₁) response. Indeed, the Th₁ response is characterized by the production of cytokines that enhance PMN diapedesis, as reflected in the high ν values. The Th₁ cells also secrete IL-3 and GMCSF, which stimulate the bone marrow to produce more leukocytes, as indicated by the high values of σ . Subsequent to depletion of the storage pools, less mature granulocytes will appear in the milk compartment. Those cells are less capable of phagocytosis, show no respiratory burst activity, and do not produce reactive oxygen species (Van Merris et al., 2002), which justifies the observed low values for ε . This is markedly illustrated in group D, as characterized by the highest values for σ and ν , and the lowest rates for ε . The *E. coli* J5 vaccines also stimulate the production of antibodies directed against *E. coli*, which results in enhanced opsonization of the bacteria and more efficient phagocytosis. This should have been translated into higher values for ε , if we assumed a positive correlation between phagocytosis and killing, as observed by Barrio et al. (2000) in *Staph. aureus*

experimental infection. But this positive effect seemed to have been offset by the apparition of immature cells in the milk compartment.

A final comment is about the observation of a higher peak of cfu in vaccinated than in unvaccinated cows inoculated with 10^4 cfu. This phenomenon may be explained by the fact that the J5 vaccine reduces only the severity of clinical symptoms but not necessarily the rate of new infection (Hogan et al., 1992).

CCD. The model indicated that a density of about 2×10^6 somatic cells/mL is necessary for protection of the gland against *E. coli*. This is precisely the density of blood-derived PMN necessary to exert optimal bactericidal (*Staph. aureus*) activity in milk, as reported by Herbelin et al. (1997), but higher than 6×10^5 cells/mL, as reported by Postle et al. (1978). It is also close to the SCC value (1,151,000 cells/mL) reported in the meta-analysis of Djabri et al. (2002). Given our model, the bacterial density will increase with time if the density of somatic cells is lower than 2×10^6 cells/mL, in agreement with experimental infection studies reporting a negative correlation between SCC prior to experimental IMI and the severity of mastitis (Suriyasathaporn et al., 2000). For example, cows (6 to 10 d after calving) with prechallenge milk SCC significantly lower than midlactating cows revealed more rapid *E. coli* growth, higher peaks, and higher fevers after challenge with *E. coli* (Shuster et al., 1996).

Stage 3: Estimation

Our one-way sensitivity analysis identified ε and σ as the AIR rates to which cell densities were mostly sensitive (Figure 3). More specifically, we observed, for low values of ε , a persistent infectious inflammatory response characterized by a high inflammatory response (high AUC for somatic cells) and a high bacterial population (high AUC for bacterial cfu). This persistent and improper response will result in acute mastitis, as observed in 10% of the clinical coliform mastitis cases (Hogan and Smith, 2003). When σ was low, we observed, in cows inoculated with 10^6 cfu, some level of immunodeficiency in which bacteria grew to very high densities (with the highest AUC for bacteria cfu in group B) and little inflammatory response (no difference in AUC for somatic cells). This could happen when the immune system of the cows has been compromised and is unable to respond to the infection.

The AUC for blood leukocytes of cows inoculated with 10^4 cfu was, in contrast to cows inoculated with 10^6 cfu, much less sensitive to changes in blood flow rates (ν and σ). This is because a bacterial load of 10^4 cfu/quarter was not very effective in stimulating the flow of leukocytes into the blood compartment (Table

1). The AUC for somatic cells and bacteria cfu were also found to be much less sensitive to variations in ν , suggesting little influence of the infection-driven diapedesis rate on the outcome of *E. coli* inoculation. This is in agreement with the lack of differences found between BLAD carriers and noncarriers for IMI with coliforms (Wanner et al., 1998) although adherence of PMN to endothelium is reduced in BLAD carriers (Nagahata, 2004). The result may also be related to the univariate nature of the one-way sensitivity analysis, which does not reveal an interaction between rates. Here, it quantified the impact of ν , assuming that all other AIR rates were fixed at their base values.

CONCLUSIONS

Although simple, our model explained most of the variation observed in cell dynamics during the early response to *E. coli* infection. For the first time, AIR rates were quantified. Our findings were consistent with previously published results and could easily be explained by proven biological arguments. A first result of the model was the finding that more than 2×10^6 somatic cells/mL are necessary to successfully fight an *E. coli* infection. The sensitivity analysis identified cell-killing abilities and the flow rate from the production and storage sites into the blood compartment as the key parameters influencing the outcomes of an AIR. By doing so, it provided guidance on which PMN functions to consider for measurement in a selection program or in immunological studies. The model should be studied further to analyze the effects of other components of the AIR (macrophages, lymphocytes, cytokines, etc.), the effects of antibiotics, and individual variability, and it could be applied to other bacterial species.

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APPENDIX

SAS Code for Setting the System of Ordinary Differential Equations

```

proc model data=input_data out=output_data outactual outpredict;
dependent B B0 C C0 M M0;
parameters epsi s u bet;
bounds epsi s u bet>=0;
dert.B=(s*B*C) - min(B,u*B*C);
dert.M=(u*B*C) - min(M,r*M*C);
dert.C=bet*C - min(C,M*C*epsi);
fit C B M/time=hr maxiter=10000;

```

/* B0, C0, M0 are the initial densities */
 /* parameters to be estimated */
 /* restriction to be positive */
 /* average of blood cells */
 /* average of milk cells */
 /* average of bacteria */