# MATHEMATICAL MODELING OF INFLAMMATORY REACTION DURING BOVINE MASTITIS

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## INTRODUCTION

Infectious mastitis is defined as an inflammatory reaction of the mammary gland to injury mainly produced from bacteria and their toxins. It is the most prevalent (Hortet and Seegers, 1998) and the most costly (Fourichon *et al.,*1999) disease of dairy cows. Selection of cows naturally highly immunocompetent and most resistant to mastitis has been proposed as an alternative to therapeutic and prophylactic treatments (Detilleux *et al.*, 1994 ; Kehrli *et al.*, 1991 ; Kelm *et al,* 1997 ; Fitzpatrick *et al.* (1998). Neutrophils (PMN) are the principal line of defense against bacteria in the mammary gland (reviewed in Zecconi and Smith, 2000) but the relative efficiency of the different PMN functions in clearing infection is unknown. Laboratory assays for measuring PMN functions are available but data collection from which breeding values could be computed is too expensive and time-consuming to be applied in national breeding programs. Consequently, there is considerable potential for having a small number of assays measuring PMN ability to clear bacterial infection and identifying cows to be selected for mastitis resistance. The objective of this paper is to provide a mathematical framework to quantify bacteria and PMN dynamics during a typical mammary infection.

## MATERIALS AND METHODS

**The mathematical model.** The model was designed to keep track of the populations of PMN and bacteria at quarter level. It has 4 variables – bone marrow PMN precursor (P), blood PMN (B), milk PMN (M) and milk bacteria (C) – and consists of a system of 4 ordinary differential equations, whose structure reflects what we hypothesize to be the key features of the inflammatory response to infection during bovine mastitis. During the time period dt (= 1 hour), P cells are produced at a rate proportional to the abundance of blood B cells and migrate in the blood at constant per-capita rate $\omega$ (Equation 1). The second equation describes the rate of change in the B cells as the sum of rate of influx from the bone marrow and the rate at which they leave the blood in response to physiological stimuli ($\gamma B$) or to an infection ($\gamma'BC$). Equation 3 describes the rate of change in the population of M cells : of the cells that have entered into the milk compartment, some will be removed during milking at a rate $\mu M$ and some will die after ingesting bacteria at a rate $\epsilon C M$. In equation 4, the rate of change in the C population equals the sum of bacteria growth rate, which is assumed to be logistic with intrinsic reproductive rate $\beta$ and carrying capacity $C_{Max}$, the rate at which bacteria are flushed during milking ($\mu C$), and the rate at which they are ingested ($\alpha C M$) and released by M cells ($(1-\upsilon) \alpha C M$).  We expressed P, B, M, and C cells in units of cells/milk mm³.  In mathematical notation, we have :

BONE MARROW

$$\frac{dP}{dt} = \{\eta\ \phi\ /(B + \phi)\ -\ \omega\ \}P \qquad [1]$$

BLOOD

$$\frac{dB}{dt} = \omega\ P - \gamma\ B - \gamma'\ B\ C \qquad [2]$$

MILK

$$\frac{dM}{dt} = B\ \gamma - M\ \mu + \gamma'\ B\ C - \varepsilon\ C\ M \qquad [3]$$

$$\frac{dC}{dt} = \beta\ C\ (1 - C/C_{Max}) - C\ \mu - \alpha\ C\ M + (1 - \upsilon)\ \alpha\ C\ M \quad [4]$$

**Parameters of the healthy status.** Before infection, we assumed $C_0 = 0$. Values for $M_0$ were easily obtained from the literature because automated devices are available to measure PMN numbers or SCC in milk. Thus, PMN number varies from 20 to 50 cells/mm$^3$ (Shoshani *et al.*, 2000 ; Shuster and Kehrli, 1995) during a healthy lactation. he values for $B_0$ and $P_0$ were obtained from the steady state relationship between $B_0$, $P_0$ and $M_0$ during health: $P_0 = M_0\ \mu/\omega$, and $B_0 = M_0\ \mu/\gamma$. Laboratory studies showed blood PMN circulate during an average of 10 hr (4 - 12 hr) in the absence of bacteria (Duncan and Prasse, 1994) which gives $\gamma = 0.1$. Usually, 15% milk remains in the udder after milking (Lane *et al.*, 1970) and cows are milked twice daily (10- to 14-h intervals), so $\mu = 1/14$. There are almost five times more PMN in bone marrow storage pool than the circulating blood ($P_0 = 5*B_0$ and $\omega = \gamma/P_0$) and they remain in the bone marrow storage-pool for approximately 2 days (Duncan and Prasse, 1994), which gives $\omega = 1/48$. We let $\phi = B_0$ such that $\frac{1}{2}\ \eta\ P_0$ cells are produced every hour in the proliferating bone marrow compartment. Then, $\eta = 2*\omega = 0.04$ which corresponds to a length of stay in the proliferation-mitotic compartment of approximately 2 $\frac{1}{2}$ days, as stated in Duncan and Prasse (1994).

**Parameters of the inflammatory status.** At start, infection occurs with a certain amount of bacteria $C_1$. Values for the maximum bacterial growth rate ($\beta$) and the carrying capacity ($C_{Max}$) were obtained from pharmaco-dynamic studies of *S. aureus* growth in milk and liquid media free from PMNs (Ganiere *et al.*, 1991 ; Novak *et al.*, 2000 ; Fang *et al.*, 1993). Non-linear least-squares estimates were obtained, as $\beta = 0.28$ to 0.75, by solving the equation $C_t = C_1$ $C_{Max}/[C_1 + (C_{Max} - C_1)*exp(-\beta\ t)]$ (Proc NLIN, SAS/STAT software). During the inflammatory response to infection, milk PMN and bacteria interact. Encounters are at random and result in PMN ingesting ($\alpha$) and killing ($\upsilon$) bacteria before dying ($\varepsilon$) themselves. To obtain prior estimates for $\alpha$, $\upsilon$, and $\varepsilon$, a modified Holling functional response of Type 2 (Holling, 1959) was fitted to data obtained from *in vitro* studies in which PMN bactericidal activities against *S. aureus* and death after ingestion were determined (Aarestrup *et al.*, 1994 ; Barrio *et al.*, 2000 ; Herbelin *et al.*, 1997 ; Wolach *et al.,* 1998), which gave ordinary least-squares estimates of $\alpha = 0.075$ ($R^2 = 99.4$ %), $\upsilon = 0.97$ ($R^2 = 85.5\%$), and $\varepsilon = 0.019$ ($R^2 = 66.3\%$). Initial values for $\gamma'$ were obtained after simulating the model (STELLA, High Performance Systems, Inc.) and comparing the results with observations from experimental infection (Burton *et al.,* 1995 ; Daley *et al.,* 1995 ; Schukken *et al.,* 1999).

**RESULTS AND DISCUSSION**

The immunological aspects of the model are admittedly greatly oversimplified but, consequently, it can illustrate the main points without distracting details. As an example, we may observe that bacteria will establish an infection ($dC/dt > 0$) when $\beta > \mu + \alpha \upsilon M_0$. This means that initial concentration of bacteria will not influence the infectious development and that rate of bacterial growth must be high enough to overcome loss of bacteria through milking and phagocytosis. Such results are biologically plausible because doses used to challenge mammary glands vary across experimental studies even with identical bacterial strains. For example, doses for intra-mammary infusion through the streak canal with *S. aureus Newbould 305* varied from 40 CFU/ml (Erskine *et al., 1*990), 50 (Burton *et al., 1*995), 80 (Daley *et al.*, 1991), 100 (Riollet *et al.*, 2001), 300 or 600 CFU (Schukken *et al.*, 1999), 2000 (Leitner *et al.*, 2000), up to 8000 CFU/ml (Nickerson and Boddie, 1994). Moreover, similar quarter infection rates are achieved with 300 or 600 CFU *S. aureus Newbould 305*, with 79.3% of cows establishing infection (Schukken *et al., 1*999). Another result is that bacteria number will not increase if number of resident M cells is high, i.e. if $M_0 > (\beta-\mu)/\alpha \upsilon$. Indeed, low numbers of milk PMN before experimental infection have been associated with increased risk for clinical mastitis (Zecconi *et al.*, 2000 ; Schukken *et al.*, 1999). Milking frequency will prevent early infection if it is greater than bacteria multiplication rate. This result is also supported by field studies: Cows milked three times daily ($\mu = 1/8$) are generally less susceptible to mastitis than cows milked twice ($\mu = 1/12$) daily (Blowey and Edmonson, 1995). Whatever the value for $C_1$, the B cells will always decrease by a quantity $\gamma'B_0C_1$, which has been observed in numerous laboratory studies. The number M cells will increase only if $\gamma' > \varepsilon\gamma/\mu$, that is when the extra-diapedesis rate due to presence of bacteria ($\gamma'$) is greater than death rate of M cells ($\varepsilon$) weighted by the ratio of diapedesis ($\gamma$) over milking ($\mu$) rates under healthy conditions. This may be related with the important role played in clearing infection by PMN transmigration rate from blood to milk (Zecconi *et al.*, 2000).

**CONCLUSION**

These preliminary results clearly show the potentials of such methodology in identifying main immunological assays to be used in selection for mastitis resistance and provide a framework for answering the question of the adequacy of selection objective. Thus, according to our model, number of PMN at start of infection and speed with which PMN reach milk compartment are important in clearing infection.

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