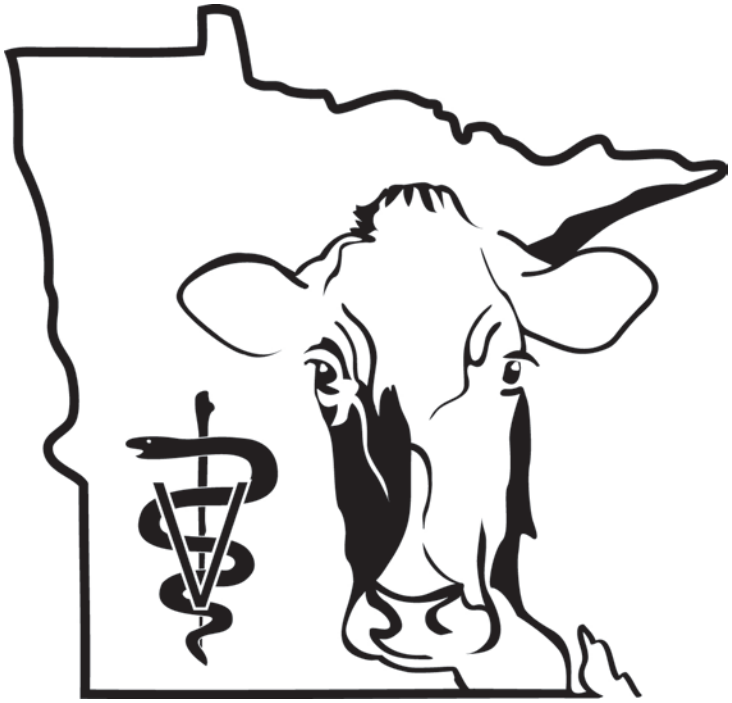
THIS ARTICLE IS SPONSORED BY THE
MINNESOTA DAIRY HEALTH CONFERENCE.

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION



ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Mastitis: Old Problems, New Approaches

Ynte H. Schukken*, Ruth N. Zadoks, Lisa White and Dörte D. Döpfer
Quality Milk Production Services
Cornell University
Ithaca, NY 14850

SUMMARY

In this presentation some recent development in the molecular and mathematical epidemiology of bovine mastitis are used as examples for the progress made in combining molecular and mathematical approaches to relevant research questions. Three examples are highlighted in some detail. First the arguments leading to the conclusion that in certain herds *S. uberis* infections may behave as contagious mastitis organisms are outlined. Second, the pathobiology of chronic coliform intramammary infections is discussed in some detail. The data appear to indicate that some reservoir of coliform bacteria in the mammary gland is necessary to be able to give rise to the observed data. Finally, the population dynamics and interaction of major and minor pathogenic bacterial species is examined. It is concluded that widespread infections of minor pathogens may lead to a reduction in transmission potential of major pathogens. Finally, the contribution of epidemiology and molecular microbiology to the better understanding of the pathobiology of intramammary infections is discussed.

INTRODUCTION

Epidemiologic research in bovine mastitis has been performed for many years , with very early studies relying mostly on clinical observations and linking certain pathogenic bacteria to the clinical signs that these bacteria typically produced in the cow (i.e. *Streptococcus agalactia* and *Arcanobacterium pyogenes*). Later, bacteria were grouped according to their typical behaviour in animal populations, identifying the 'contagious' and 'environmental' bacterial groups (Smith et al. 1985). More recent studies have used statistical techniques to associate certain risk factors with bacteria or groups of bacteria. For example, use of teat disinfection in low somatic cell count herds was associated with an increased risk of coliform mastitis (Lam et al. 1996, Schukken et al., 1991), and cows with very low somatic cell counts were more likely to get clinical mastitis or severe clinical mastitis compared with cows with somewhat higher cell counts (Peeler et al. 2001, Green et al. 1996, Suriayasathaporn et al. 2000). As is expected with such risk factor studies, not all studies result in associations in the same direction, making the cause and effect arguments somewhat suspicious. In addition, risk factor studies are often cross-sectional, so that the time order of events and causal relationships are impossible to prove.

In the last decade, two additional techniques have been added to the toolkit of the mastitis epidemiologists: mathematical modeling and molecular diagnostics. These new tools have added a lot of opportunities to contribute to a better understanding of the pathobiology of intramammary infections in the dairy cow. Molecular diagnostic tools allow the distinction between strains in the same bacterial species (see for example Wang et al. 1999 and Zadoks et al. 2000). This does, among other things, lead to more precise longitudinal follow up studies of infection occurrences in populations. Mathematical models have contributed especially in the

* Y.H. Schukken, Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, 14850 Email: yhs2@cornell.edu

area of contagious disease dynamics (Anderson and May 1985, De Jong 1995), both in terms of explaining observed infection (and/or disease) frequencies and predicting or simulating infection frequencies under a given set of preventive measures (De Jong 1995).

The objective of this presentation is to review a number of recent studies attempting to answer mastitis research questions using molecular and mathematical methods. Using these examples, we will also attempt to formulate some general experiences and suggestions that we have come to appreciate during the execution of these studies. The three areas of research are: 1) epidemiology of *Streptococcus uberis* infections 2) chronicity of *Escherichia coli* intramammary infections, and 3) impact of minor pathogens on the risk of new infections with major pathogens.

EPIDEMIOLOGY OF *S. UBERIS* INFECTIONS

The underlying question with regard to the epidemiology of *S. uberis* infections was whether they originate from other cows or whether they originate from the environment of the cow. The origin of infections must be known to choose adequate control measures for mastitis prevention in dairy herds. The question was approached with mathematical and molecular tools. The null-hypothesis that was tested in the mathematical approach to this study was that the number of new infections of *S. uberis* in a population does not depend on the number of existing shedders in that population. In statistical terminology, the infection dynamics are described by: $I_{\Delta t} = \theta P_{t-1}$, where I = incidence, P = prevalence, Δt = a given period of time, t-1 = beginning of time period, θ = regression parameter. The null hypothesis assumes $\theta = 0$. The alternative hypothesis indicates that $\theta > 0$ (possibly $\theta <> 0$). A similar model was previously used by Lam et al. (1996), to model the dynamics of *Staphylococcus aureus* infections in a dairy herd.

Data were from a dairy farm with 95 ± 5 lactating animals (mean ± s.d.) where an outbreak of *S. uberis* infections was observed (Zadoks, 2002). Data were collected during an 18-month observation period with 27 farm visits at 3-week intervals. These data are summarised in Figure 1. Initially, a low prevalence and very low incidence of *S. uberis* infections is evident. At approximately sampling period 10 the start of an exponential growth of new infections can be seen. This outbreak is halted around sampling 16. The prevalence remains high for a while, but then drops to a much lower level. Toward the end of the observation period, prevalence is still approximately three times as high as at the start of the study.

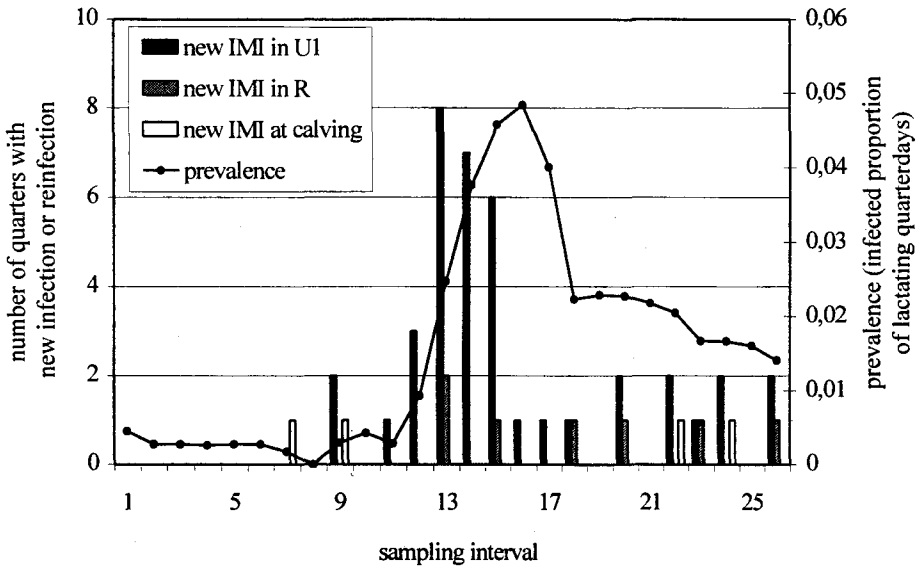


Figure 1 Outbreak of *Streptococcus uberis* infections in a dairy herd. U1 are previously uninfected quarters, R are quarters that already experienced a *S. uberis* infection (reinfection). All quarters of all cows were sampled approximately every 3 weeks.

To model these data in a biologically meaningful way it is necessary to assess whether spread of one specific strain has occurred. It has been described that multiple strains of *S. uberis* co-exist on a given dairy (Douglas et al. 2000, Wang et al. 1999). An outbreak consisting of cases from multiple molecularly distinguishable strains would indicate that spread did not occur from cow to cow, i.e. would favour the null hypothesis. Fingerprinting of the strains in the outbreak using RAPD fingerprinting techniques resulted in strong evidence for clonal spread (Zadoks et al. 2003). One large clonal outbreak with the strain named 'B' was observed (Figure 2). In additional, a number of single infections with a variety of different strains were observed on the farm. The data were modelled in a Poisson logistic regression model:

$$\varepsilon\,[\ln(IMI)] = \ln(\beta') + \ln(S/N) + \theta_1 * \ln(I) + \theta_2 * y + \theta_3 * U_m$$

where, ε = expected value, IMI = number of new intramammary infections with *Streptococcus uberis* in current time interval, β' = transmission parameter for model with ln(S/N) as offset, S = number of quarter-days susceptible in current time interval, N = total number of quarter-days in current time interval, I = number of quarter-days infected in preceding time interval, y = dummy variable for phase (y = 0 for early phase of the study up to and including outbreak, y = 1 for late phase of the study), U_m = dummy variable for compartment (U_m = 0 for R, U_m = 1 for U_1) and θ_i = regression coefficients. The estimates, standard errors and *P*-values for ln(β') and the three regression coefficients are shown in Table 1. Of specific interest is parameter θ_1 because this parameter tests the hypothesis that existing shedders contribute to the incidence of new infections.

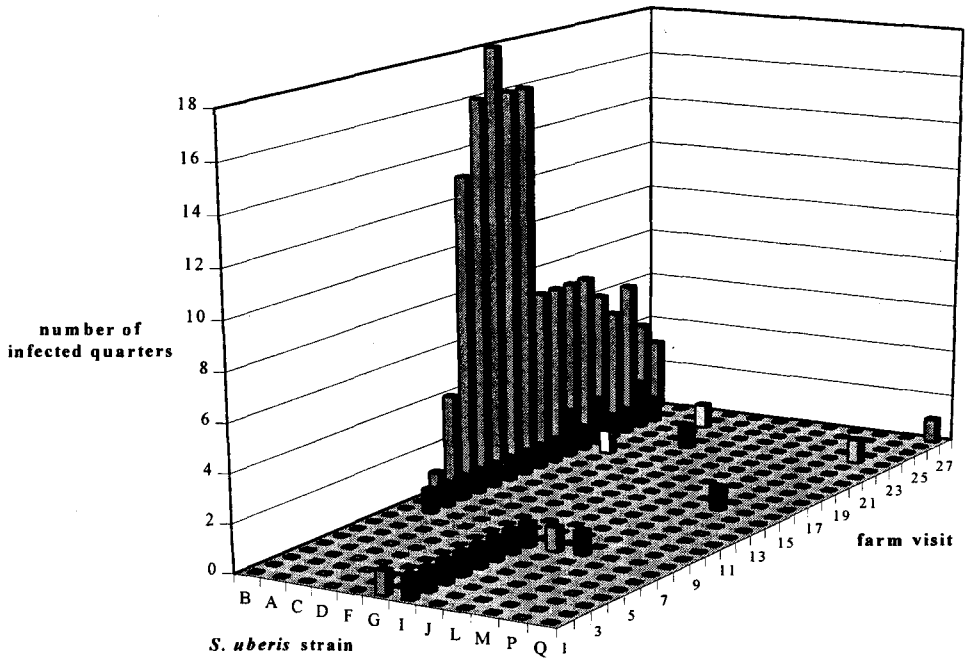


Figure 2. Number of quarters that was infected with a specified strain of *Streptococcus uberis*. Strains were specified by random amplified polymorphic DNA (RAPD) fingerprinting.

Table 1. Estimates, standard errors and *P*-values for ln(β') and regression coefficients. U_1 = never before infected with *Streptococcus uberis*; R = recovered from infection with *S. uberis*.

Model	Parameter	Coefficient	Estimate	Standard error	*P*-value
U1 vs. R	ln(β')		0.11	0.78	0.8793
	ln(I)	θ_1	0.68	0.15	< 0.0001
	study phase	θ_2	-1.55	0.35	< 0.0001
	compartment	θ_3	-2.06	0.42	< 0.0001

The regression results indicate that θ_1 is significantly different from 0, and hence the initial null hypothesis is rejected implying that the number of new infections is not independent of the number of existing infections. Therefore, the alternative hypothesis, indicating contagious transmission of *S. uberis*, appears to better fit the data. Additional arguments that favour cow-to-cow transmission and that indicate possible mechanisms of transmission are 1) finding identical strains of *S. uberis* in the milking liner up to 3 cow-milkings after milking of a *S. uberis* shedding cow, 2) finding only new infections with strain 'B' in lactation (in contrast to other strains that infect also during the dry period), and 3) the relative long duration of infection with strain 'B'. Survival analysis of observational data showed that infections with strain B lasted significantly longer than infections with other strains. Thus, infections with strain B

would have a larger window of opportunity for spread, most likely occurring during the milking process, than other strains. The molecular, mathematical and observational data analysis can be complemented by pathogenesis studies. *In vitro* studies have shown differences between *S. uberis* strains in their ability to adhere to and invade mammary epithelial cells (Almeida, 1999). If strain B had higher ability to adhere and invade than other strains, that ability could hypothetically result in a longer duration of infection and hence more time for contagious transmission, which would, in turn, lead to the phenomena that were observed using mathematical, statistical and molecular tools.

CHRONICITY OF *E. COLI* INTRAMAMMARY INFECTIONS

In recent publications, the occurrence of chronic *E. coli* intramammary infections was reported (Lipman et al. 1995, Döpfer et al. 1999, Döpfer et al. 2000). Using DNA fingerprinting, the presence of indistinguishable isolates from repeated cases of clinical mastitis in the same quarter of the same cow was shown. An example of such an infection is shown in Figure 3. The isolates in lanes 2-14 were repeated cases of clinical coliform mastitis from the same quarter from the same cow and the isolates in lanes 16 to 19 came from a different quarter in the same cow.

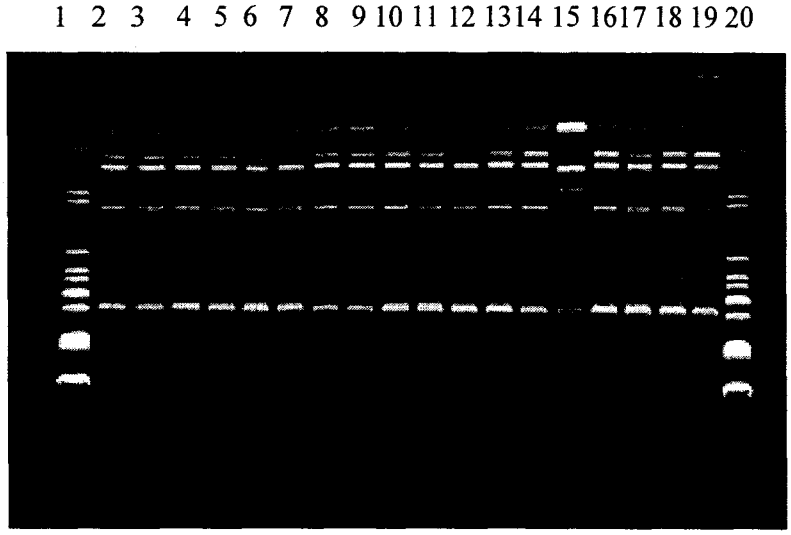


Figure 3. Genetically indistinguishable isolates (evaluated by DNA fingerprinting using PCR-REP and ERIC primers) from recurrent clinical *E. coli* mastitis cases. Lanes 1 and 20: molecular size marker. Lanes 2-14: Cow 1, quarter A. Lane 15: Cow 2, Lanes 16-19: Cow 1, quarter B.

Because of the high number of *E. coli* strains in the dairy environment, it is unlikely that recurrent isolation of one strain from the same quarter was the result of recurrent new infections. However, it is not impossible. To determine whether the infection was really persistent, longitudinal data on a single chronically infected cow were collected. Colony forming units and somatic cell concentrations from the infected quarter are shown in Figure 4. In colony forming units and somatic cell concentrations an apparent inverse cyclicity (with a Pearson correlation of -.36, $p<.05$) was observed.

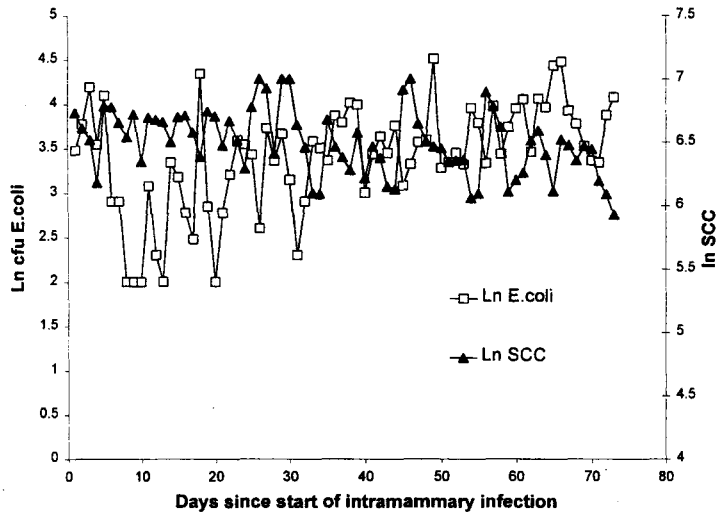


Figure 4. Time series of a chronic intramammary coliform infection. Daily observations on bacteria counts and somatic cell counts (mostly polymorphnuclear cells) in a chronically infected quarter. (D. Döpfer, 2000).

These data, although relatively sparse, provide initial evidence that milk leukocytes and bacteria show a dynamic behaviour. Similar behaviour has been suggested for *S. aureus* (Daley, 1991). Such dynamic behaviour may be modelled using 'predator-prey' type modelling techniques:

$$dR/dt = rR(1-R/K) - ENR^2/(R_0^2 + R^2) \qquad \text{(equation 1)}$$

$$dN/dt=N[-d+cER^2/(R_0^2 + R^2)] \qquad \text{(equation 2)}$$

where:

R = *E. coli* density in cfu per ml, t = time, r = growth rate of *E. coli* (logistic growth), K = carrying capacity of the system, E = SCC saturation level of "consuming" *E. coli,* N = SCC density in cells per ml, R_0 = half-saturation density of *E. coli* (where $R > R_0$), d = per capita rate at which SCC die out when no *E. coli* are present, c = "conversion efficiency" of *E. coli* to SCC - this may be considered as feeding efficiency.

To incorporate the concept of an intracellular reservoir for the bacteria, the model may be extended by including an additional state variable, Z, governed by the ODE's. Equations 3 and 4 are identical to equations 1 and 2, except for equation 3, where ϕz, the release of bacteria from the third state, z, at a rate ϕ per day, is added to and πR, the intracellular invasion of bacteria, R, at a rate π per day, is subtracted from dR/dt.

$$dR/dt = rR(1-R/K) - ENR^2/(R_0^2 + R^2) + \phi Z - \pi R \qquad \text{(equation 3)}$$

$$dN/dt=N[-d+cER^2/(R_0^2 + R^2)] \qquad \text{(equation 4)}$$

$$dZ/dt= - \phi Z + \pi R \qquad \text{(equation 5)}$$

Figure 5 shows the results of a simulation model using the above set of equations. Parameter estimates were obtained from literature or from the data. The model with the intracellular reservoir fitted the data considerably better when compared with a model without such a reservoir. Several potential reservoirs (i.e. dormant bacteria) may be envisioned. E.g. leukocytes that contribute to SCC ingest *E. coli*, and a failure to kill the coliform bacteria creates a reservoir. Studies have also shown that mammary epithelial cells may act as a reservoir (Almeida et al. 1999, Dopfer, 2001).

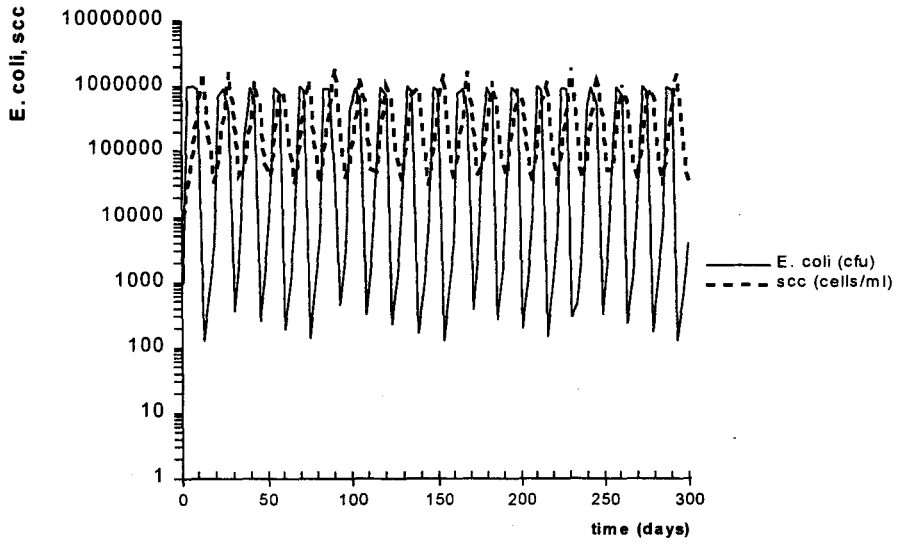


Figure 5. Simulated data for *Escherichia coli* concentration and SCC in a chronically infected quarter based on predator-prey model with an intracellular reservoir of bacteria.

Chronicity of *E. coli* infections would theoretically open up the potential of contagious spread of this pathogen that has hitherto always been considered an environmental pathogen. Recent field studies in the UK, where multiple chronic and partly subclinical infections were observed in dairy herds, also suggest that *E. coli* may be evolving to a more cow-adapted and possibly contagious organism (Bradley and Green, 2002). Further research in terms of collection of field data will be necessary to obtain better quantitative estimates for the model parameters. Stochastic models will also aid our understanding of infection chronicity versus fade out. Initial Monte Carlo simulations of the model presented above do support the conclusion of an *E.coli* reservoir in the mammary gland as a prerequisite for chronic infections. Molecular methods have provided the arguments that chronic coliform infections do exist. The mechanisms behind bacterial persistence are not clear at this point in time. As for the *S. uberis* example, further experimental studies and additional modelling work will be required to elucidate the biological mechanisms further.

IMPACT OF MINOR PATHOGENS ON THE RISK OF NEW INFECTIONS WITH MAJOR PATHOGENS

The bacterial pathogens responsible for infection of the mammary gland may be split into two main categories, major and minor. Infection with major pathogens generally results in clinical illness or strong inflammatory responses and reduced milk yields, whereas minor pathogen infection is usually subclinical with less severe SCC increase or yield loss. Previous investigations have considered the transmission of major and minor pathogens independently. Experimental evidence has in some cases shown cross protection between species of pathogens. A mathematical model for the transmission of both major and minor pathogens along with their interaction via the host was developed by White et al. (2001a,b 2002)to consider various methods for controlling the incidence of major pathogen infection (Figure 6). A stability analysis of the model equilibria provides explanations for observed phenomena. Previous modelling results focused on one species only. This multispecies model structure has provided a basis for quantifying the extent of cross protection between species and assessing possible control strategies against the disease.

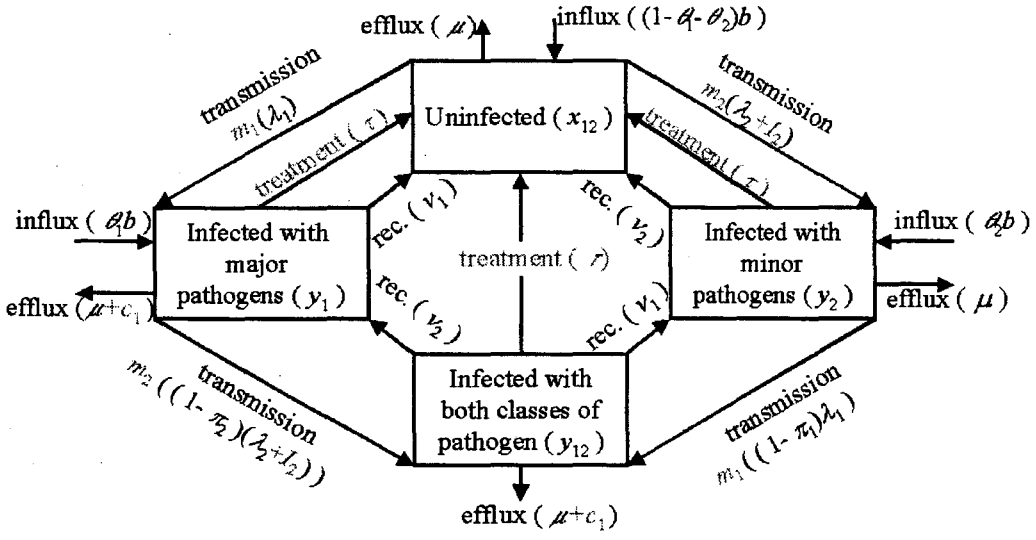


Figure 6. Multi species infection transmission model (White et al. 2001b). The variables and transmission parameters are identified in Table 2.

This analysis extends the work of Lam *et al.* (1996a) who modelled mastitis transmission in cattle using SIS (susceptible-infectious-susceptible) models that were fitted to prevalence and incidence data from herds of dairy cows. The results suggested some interaction in the transmission of the different pathogen species. The results indicated that , where both minor and major pathogens were being transmitted, the basic reproduction number of *S. aureus* (a major pathogen) decreased during the course of an outbreak of mastitis. This result could not be explained using decoupled (no interaction between species) models.

The objective of the extended work was to develop a simple multi-species model, where there is some cross-protection provided by infection by one class of pathogens (minor pathogens) against infection by another class (major pathogens) and examine the dynamic consequences of

the interaction (White et al. 2001a). The multi-species model that was used is presented in Figure 6. Other symbols in Figure 6 represent interventions: m_i represents a decrease in transmission by post milking teat disinfection, c_i additional culling, τ treatment of infections and I_i inoculation of quarters.

Table 2. Variables and parameters in the multi-species model.

	Symbol	Units	Definition
variables	x_{12}	normalised	Proportion of the lactating population not infected with either class of pathogens.
	y_1	normalised	Proportion of the lactating population infected with major pathogens.
	y_2	normalised	Proportion of the lactating population infected with minor pathogens.
	y_{12}	normalised	Proportion of the lactating population infected with both classes of pathogens.
	λ_1	day^{-1}	Force of infection for major pathogens.
	λ_2	day^{-1}	Force of infection for minor pathogens.
parameters	μ, b	day^{-1}	Average turnover of lactating cows in the herd.
	θ_1	normalised	Proportion of individuals entering the lactating herd already infected with major pathogens.
	θ_2	normalised	Proportion of individuals entering the lactating herd already infected with minor pathogens.
	θ_{12}	normalised	Proportion of individuals entering the lactating herd already infected with both classes of pathogens.
	ν_1	day^{-1}	Average recovery rate from major pathogen infection.
	ν_2	day^{-1}	Average recovery rate from minor pathogen infection.
	β_1	$capita^{-1}day^{-1}$	Transmission rate of major pathogens.
	β_2	$capita^{-1}day^{-1}$	Transmission rate of minor pathogens.
	π_1	normalised	Level of cross-protection against major pathogen infection provided by a minor pathogen infection.
	π_2	normalised	Level of cross-protection against minor pathogen infection provided by a major pathogen infection.

The model was fitted to the data observed by Lam et al. (1996a) using the computer package Facsimile. The raw data was in the form of spreadsheets for each of eighteen samplings. The number of colony forming units of each pathogen for each quarter of each cow was given. The system equations are given by:

$$\left.\begin{aligned}
\dot{x}_{12} &= (1-\theta_1-\theta_2)b-(\lambda_1+\lambda_2+\mu)x_{12}+\nu_1 y_1+\nu_2 y_2 \\
\dot{y}_1 &= \theta_1 b+\lambda_1 x_{12}+\nu_2 y_{12}-((1-\pi_2)\lambda_2+\nu_1+\mu)y_1 \\
\dot{y}_2 &= \theta_2 b+\lambda_2 x_{12}+\nu_1 y_{12}-((1-\pi_1)\lambda_1+\nu_2+\mu)y_2 \\
\dot{y}_{12} &= (1-\pi_1)\lambda_1 y_2+(1-\pi_2)\lambda_2 y_1-(\nu_1+\nu_2+\mu)y_{12}
\end{aligned}\right\} \qquad \text{(equation 6)}$$

where:

$$\left.\begin{aligned} 1 &= x_{12} + y_1 + y_2 + y_{12} \\ b &= \mu \\ \lambda_1 &= \beta_1(y_1 + y_{12}) \\ \lambda_2 &= \beta_2(y_2 + y_{12}) \end{aligned}\right\} \qquad \text{(equation 7)}$$

Steady state analysis has produced a "cross-protection curve" (figure 7) that has a similar form to those produced from other multistrain/species models (Feng & Velasco-Hernandez 1997; Gupta *et al.* 1994a; White *et al.* 1998). A similar analysis on the model equations extended to include various control procedures has given some theoretical insight into their possible effects.

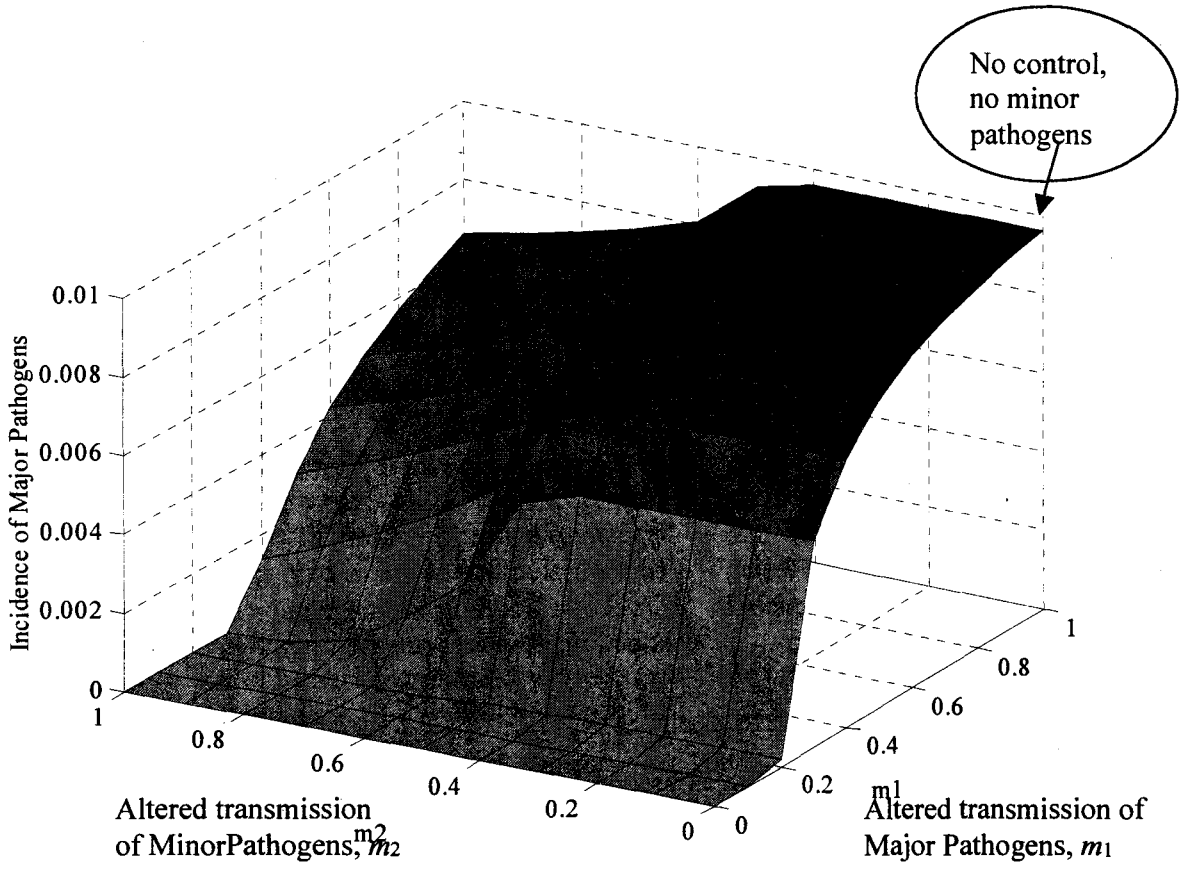


Figure 7. Predicted impact of transmission intervention on the incidence of mastitis with major pathogens. Transmission of 0 indicates fully successful intervention (no transmission), transmission of 1 indicates no intervention at all.

Figure 8 shows the boundary in parameter space between where the minor pathogen only equilibrium is stable and where it is unstable (and therefore the equilibrium where both major and minor pathogen classes are present is stable). The axes represent the basic reproduction numbers, R_{01} and R_{02}, for major and minor pathogens respectively. The narrow black line of the graph in Figure 8 shows the starting point of the comparison, a situation where no control procedures are in place. The cross (X) indicates a particular (uncontrolled) system with given basic reproduction numbers for major and minor pathogens. The cross is above the line, implying that major pathogens should be able to invade the system and persist at equilibrium. When treatment is included in the model, the boundary is shifted upwards, therefore decreasing the likelihood of invasion of major pathogens into the herd. In the example illustrated by Figure 8, the treatment cure rate was high enough to move the boundary above the cross and would therefore successfully eliminate major pathogens from the herd (given that pathogen spread is predominantly contagious, not environmental).

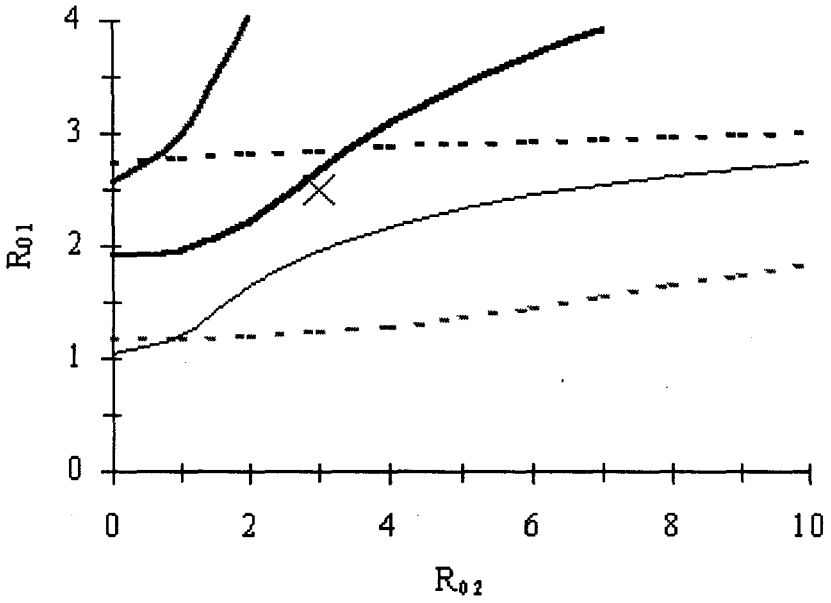


Figure 8. Graphs showing boundaries in (R_{02}, R_{01})-space between where the coexistence equilibrium is stable (above the line) and where the minor-pathogen-only equilibrium is stable (below the line). The axes represent the basic reproduction numbers, R_{01} and R_{02}, for major and minor pathogens respectively. The narrow black line shows the boundary for the parameter set θ_1=0.0 day^{-1}, θ_2=0.5 day^{-1}, $\nu_1=\nu_2$=0.01 day^{-1}, μ=0.0015, π_1=0.7 and π_2=0.0 where the model includes no control programs (i.e. $\tau=c1=I_2=0$ and $m_1=m_2=1$). Bold black line: treatment (at rate τ=0.01). Bold grey line: culling of major pathogen infected cows (at rate c_1=0.017). Grey dotted line: postmilking teat disinfection (with parameters m_1=0.9 and m_2=0.2). Black dotted line: inoculation of cows with minor pathogens (at rate I_2=0.1).

The culling of cows infected with major pathogens (at the same rate as the treatment cure rate) had a more pronounced effect moving the boundary much higher for increasing values of R_{02}. As shown in Figure 8, inoculation of cows with minor pathogen species would enhance the herd immunity against major pathogen infections (black dotted line). However, there must be a sufficiently high level of natural cross-protection against major pathogen infection provided by infection with the minor pathogens for them to out-compete the major pathogens. Although a theoretically feasible option, it is not logistically easy to infect animals with minor pathogens without at the same time increasing the risk of infection with major pathogens. Novel application systems would need to be developed to make this a feasible option.

DISCUSSION

The known udder pathogenic bacterial species show such a large variability within species that valid modelling of observed events is only possible with knowledge of the particular clones present in the data. This was shown to be important in the analysis of the observed *S. uberis* outbreak in a dairy. Several of the strains encountered in infected cows showed a very typical behaviour of single isolated infections without transmission between animals. However, one of the strains showed a very different epidemiology, with abundant evidence for clonal spread according to the laws of mass action. Modelling of these data was very helpful in providing quantitative evidence for contagious behaviour of this *S. uberis* strain. Using statistical testing, a formal argument can be made that this particular strain showed a rate of new infection that was dependent on the number of shedders. The argument still continues, because it is not impossible that a surge of growth of this particular *S. uberis* strain occurred in the environment of the cows. Several quantitative and non-quantitative arguments favour the contagiousness hypothesis but the undisputed proof of that may turn out to be impossible. It is interesting to note that the same arguments are used to "prove" contagiousness of *S. aureus*, and for reasons beyond the realm of reason, the contagiousness of *S. aureus* does not seem to be a matter of debate. Using molecular fingerprinting techniques, it was shown beyond doubt that chronic coliform infections occur in dairy cows. The observed data suggest a predator-prey type of cyclical system. Using fairly simple Lotka-Volterra type models, the observed data were reasonably well reproduced. Modelled somatic cell counts were relatively stable compared with the large fluctuation observed in the modelled bacterial count data. The addition of an intracellular reservoir somewhat dampened the fluctuation in predicted bacterial counts, to match the observed data better. The initial models suggest that an intracellular reservoir explains the observed data slightly better than a model without an intracellular reservoir of bacteria. Further biological data will need to be collected to better understand the location, if any, of reservoirs and the pathogenesis of this infection.

The multispecies model presented here is an extension of previous modelling work. It extended the specific modelling work, of Lam *et al.* (1996a, 1996b, 1997a, 1997b, 1997c), on the transmission of mastitis pathogens as well as providing some validation of a standard multispecies model structure (Lipsitch 1997). The multispecies model could be used to design an effective control strategy if its parameters were identified. The dynamical output of the model is consistent with the data from the biological system. Rather then looking at individual cows or quarters as is typically done in evaluating species competition, it is of much greater value to focus on ecological interactions between pathogen species (and strains) because they can have important influences on transmission dynamics. Infections with contagious organisms not only affect the infected individual, but they have an important impact on the population as well. When complex relationships between species exist, modelling is virtually the only option to look

at the ecology of the organisms in the population. Using this multi-species modelling approach, it has become clear that competition between species may be an important control option with regard to the transmission of clinically important pathogens (Ferguson *et al.* 1999; White *et al.* 1998). Such interactions can greatly enhance or reduce the effect of efficient control measures (Ferguson *et al.* 1999; McLean 1995; White *et al.* 1998). The premise of this applied population biology exercise is that a relatively small number of characteristics (e.g., major versus minor pathogens) suffice to account for major patterns in infection occurrence. Clearly these initial studies and models should be expanded further to reflect the complexity of the biology better. This study has not used any molecular diagnostics. More precise identification of minor pathogen species (corynebacteria are probably different in their protective effect compared to Coagulase Negative Staphylococci), and identification of major pathogen strains is warranted to better understand herd dynamics. There is evidence that strains within a species differ in transmission potential (R- value) under a given set of circumstances (i.e. for *S. aureus:* Middleton et al., 2001; *S. uberis*: Zadoks et al., 2002).

Some important developments for modern epidemiology are becoming evident when combining the results of these population studies on mastitis in cows:

The use of molecular methods is becoming a prerequisite for precise epidemiological studies on intramammary pathogens. Without a confirmation that clonal spread occurs through a population of animals, it is difficult to be convincing in novel arguments (paradigm shifts) about the epidemiology of strains (Zadoks et al. 2002, 2003).

Similarly, the use of generic species grouping to study epidemiological behaviour in populations should be used with care. As shown in these examples, some strains do not follow the conventional paradigms of bacterial species behaviour. Most *S. uberis* strains would have an environmental reservoir (Wang et al. 1999), but as we have shown, animal reservoirs may exist. Most coliform infections in the mammary gland are clinically severe and short-lived (Smith et al. 1985), but as shown, chronic infections truly occur.

Use of mathematical modelling to better understand the epidemiology and pathogenesis of intramammary infections has great potential. The examples presented all show an important additional understanding of the biology of infection in the population or in the host due to the additional tool of mathematical modelling.

Modeling of mastitis is challenging mainly due to the difference in dimensionality between the biological system and the available data. The dimension and complexity of deterministic (mechanistic) models describing mastitis in dairy cows can be increased *ad infinitum*. There are several overlapping and interacting levels of organization in the host (quarter, cow, herd, within cow, between cow) and parasite (species, strains) populations, not to mention all the external influences of the environment. Including random or structured variation in the proposed model structure would reduce its dimension as would biologically justifiable simplifying assumptions. The models that were used in the current examples are all either deterministic or stochastic compartmental models using differential equations. Koopman et al. (2001) recently described these as the first (simplistic) steps toward models that truly reflect field data. The next generation of models would include individual event history models and dynamic network models. For use in mastitis epidemiology, individual event history models are particularly appealing. The infection history and risk factor constitution (i.e. teat lesion, age, production level etc.) of an individual animal or preferably individual quarter are often known from data gathered in field studies, but cannot be incorporated in the current generation of infection

dynamics models. Multiple data sets including results from molecular work and model-induced experimental work as well the combined data from sets of observational studies would increase the dimension of the data. This is one of our challenges for the next years.

The combination of knowledge of the biology of infection, precise data from observational studies, molecular fingerprinting techniques and mathematical modelling provides an excellent basis for precise understanding of disease pathogenesis or pointing the way to additional hypotheses and research questions. At the same time, these tools can be utilized to understand the population impact of control strategies. An essential aspect of epidemiology is that the whole is more than the sum of it's parts. This is true for populations that are more than the sum of many individuals, and for the epidemiological toolkit, that is more than the sum of the individual techniques. It is through combined use of multiple approaches and through collaboration of experts in the different techniques that most progress in understanding of epidemiology and control of animal disease can be made.

REFERENCES

Almeida, R. A., W. Fang, and S. P. Oliver. (1999). Adherence and internalization of *Streptococcus uberis* to bovine mammary epithelial cells are mediated by host cell proteoglycans. FEMS Microbiol.Lett. 177:313-317

Anderson, R. M. and May, R. M. Infectious diseases of humans. (1992). Dynamics and control. Oxford: Oxford University Press

Bradley, A.J., and Green, M.J. Adaptation of Escherichia coli to the bovine mammary gland. (2001). J. Clin. Microbiol. 39, 1845-1849

Andreassen, V., Lin, J. and Levin, S. A. (1997) The dynamics of co-circulating influenza strains conferring partial cross-immunity. J. Math. Biol. 35, 825-842

Döpfer, D., Barkema, H.W., Lam, T.J.G.M., Schukken, Y.H., and Gaastra, W. (1999). Recurrent Clinical Mastitis Caused by Escherichia coli in dairy cows. J. Dairy Sci. 82,80-85

Döpfer, D., Almeida, R., Lam, T.J.G.M., Nederbragt, H., Oliver, S.P., and Gaastra,W. (2000). Adhesion and invasion of *E. coli* from recurrent clinical cases of bovine mastitis in vitro. Vet. Micr. 74, 331-343

Döpfer, D., (2000). Epidemiology and pathogenesis of repeated cases of *Escherichia coli* mastitis, PhD thesis, Utrecht University

Douglas, V.L., Fenwick, S.G., Pfeiffer, D.U., Williamson, N.B., and Holmes, C.W. (2000). Genomic typing of *Streptococcus uberis* isolates from cases of mastitis, in New Zealand dairy cows, using pulsed-field gel electrophoresis. Vet .Microbiol. 75:27-41

De Jong, M.C.M. (1995). Mathematical modelling in veterinary epidemiology: why model building is important.. Prev. Vet. Med. 25,183-193

Feng, Z. L. and VelascoHernandez, J. X. (1997) Competitive exclusion in a vector-host model for the Dengue fever. J. Math. Biol. 35, 523-544

Ferguson, N. M., Donnelly, C. A. and Anderson, R. M. (1999) Transmission dynamics and epidemiology of dengue: insights from age-stratified sero-prevalence surveys. Phil. Transact. Royal Soc. London Series B-Biol. Sci. 354, 757-768

Green M., Green, L. and Cripps. P. 1996. Low bulk milk SCC and toxic mastitis. Vet Rec. 138, 452-454

Koopman, J.S., Jacquez, G. and S.E. Chick. (2001). New data and tools for integrating discrete and continuous population modeling strategies. Annals NY Academy of Sciences: 954, 268-294

Lam, T. J., Lipman, L. J., Schukken, Y. H., Gaastra, W. and Brand, A. (1996b). Epidemiological characteristics of bovine clinical mastitis caused by Staphylococcus aureus and Escherichia coli studied by DNA fingerprinting. Am. J. Vet. Res. 57, 39-42

Lam, T. J., Schukken, Y. H., van Vliet, J. H., Grommers, F. J., Tielen, M. J. and Brand, A. (1997a) Effect of natural infection with minor pathogens on susceptibility to natural infection with major pathogens in the bovine mammary gland. Am. J. Vet. Res. 58, 17-22

Lam, T. G. M., Dejong, M. C. M., Schukken, Y. H. and Brand, A. (1996a). Mathematical modelling to estimate efficacy of post milking teat disinfection in split-udder trials of dairy cows. J. Dairy Sci. 79, 62-70

Lam, T. J. G. M., vanVliet, J. H., Schukken, Y. H., Grommers, F. J., vanVeldenRusscher, A., Barkema, H. W. and Brand, A. (1997c). The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds .1. Incidence of clinical mastitis. Vet. Quart. 19, 41-47

Lam, T. J. G. M., vanVliet, J. H., Schukken, Y. H., Grommers, F. J., vanVeldenRusscher, A., Barkema, H. W. and Brand, A. (1997d). The effect of discontinuation of postmilking teat disinfection in low somatic cell count herds .2. Dynamics of intramammary infections. Vet. Quart. 19, 47-53

Lipsitch, M. (1997). Vaccination against colonising bacteria with multiple serotypes. Proc. Nat. Acad. Sci. 94, 6571-6576

Macey, R., Oster, G. & Zahnley, T. (1999) Berkeley Madonna: University of California.

McLean, A. R. (1995). Vaccination, evolution and changes in the efficacy of vaccines: a theoretical framework. Proceedings of the Royal Society of London Series B 261, 389-393

Middleton, J. R., L. K. Fox, and T. H. Smith. (2001). Management strategies to decrease the prevalence of mastitis caused by one strain of Staphylococcus aureus in a dairy herd. J.Am.Vet.Med.Assoc. 218,1615-1622

Peeler E.J., Green M.J., Fitzpatrick J.L., Morgan K.L., and Green L.E. (2000) Risk factors associated with clinical mastitis in low somatic cell count dairy herds J Dairy Sci. 83 2464 - 2472

Schukken Y.H., Grommers F.J., van de Geer D., Erb H.N., and Brand A. (1991). Risk factors for clinical mastitis in herds with a low bulk milk somatic cell count. 2. Risk factors for Escherichia coli and Staphylococcus aureus. J. Dairy Sci. 74, 826-832

Sol, J., Sampimon, O. C., Snoep, J. J. and Schukken, Y. H. (1997). Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by Staphylococcus aureus. J. Dairy Sci. 80, 2803-2808

Smith K.L., Todhunter D.A., and Schoenberger P.S. (1985). Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci. 68, 1531-1553

Suriyasathaporn W., Schukken, Y.H. Nielen, M. and Brand, A.. (2000). Low somatic cell count: a risk factor for subsequent clinical mastitis in a dairy herd. J. Dairy Sci.. 83:1248

White, L. J., Cox, M. J. and Medley, G. F. (1998). Cross immunity and vaccination against multiple parasite strains. IMA J. Math. Appl. Med.Biol. 15, 211-233

White L.J., Schukken Y.H., Lam T.J., Medley G.F., and Chappell M.J (2001a). A multispecies model for the transmission and control of mastitis in dairy cows. Epidemiol. Infect.. 127, 567-576

White L.J., Evans N.D., Lam T.J., Schukken Y.H., Medley G.F., Godfrey K.R., and Chappell M.J. (2001b). The structural identifiability and parameter estimation of a multispecies model for the transmission of mastitis in dairy cows. Math. Biosci. 174, 77-90

White L.J., Evans N.D., Lam T.J., Schukken Y.H., Medley G.F., Godfrey K.R., and Chappell M.J. (2002). The structural identifiability and parameter estimation of a multispecies model for the transmission of mastitis in dairy cows with postmilking teat disinfection. Math. Biosci. 180,275-291

Wang S.M., Deighton M.A., Capstick J.A., and Gerraty N. (1999). Epidemiological typing of bovine streptococci by pulsed-field gel electrophoresis. Epidemiol Infect. 123, 317-324

Woolhouse, M. E. J., Haydon, D. T. and Bundy, D. A. P. (1997). The design of veterinary vaccination programmes. Vet. J. 153, 41-47

Zadoks R., van Leeuwen W., Barkema H., Sampimon O., Verbrugh H., Schukken Y.H., and van Belkum, A. (2000). Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human Staphylococcus aureus isolates. J. Clin. Microbiol. 38, 1931-1939

Zadoks R.N., Allore H.G., Barkema H.W., Sampimon O.C., Grohn Y.T., and Schukken, Y.H. (2001). Analysis of an outbreak of *Streptococcus uberis* mastitis. J Dairy Sci; 84, 590-599

Zadoks R.N., Allore H.G., Hagenaars T.J., Barkema H.W., and Schukken Y.H.. (2002). A mathematical model of Staphylococcus aureus control in dairy herds. Epidemiol Infect 129, 397-416

Zadoks, R. N.. Gillespie, B. E. Barkema, H. W. Sampimon, O. C. Oliver, S. P and Schukken, Y. H. (2003). Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* in press, Epidemiol. Infect.