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Nonlinear Hierarchical Models for Longitudinal Experimental Infection Studies

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NONLINEAR HIERARCHICAL MODELS FOR LONGITUDINAL EXPERIMENTAL INFECTION STUDIES

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Epidemiology and Biostatistics in the College of Public Health at the University of Kentucky

By
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ABSTRACT OF DISSERTATION

NONLINEAR HIERARCHICAL MODELS FOR LONGITUDINAL EXPERIMENTAL INFECTION STUDIES

Experimental infection (EI) studies, involving the intentional inoculation of animal or human subjects with an infectious agent under controlled conditions, have a long history in infectious disease research. Longitudinal infection response data often arise in EI studies designed to demonstrate vaccine efficacy, explore disease etiology, pathogenesis and transmission, or understand the host immune response to infection. Viral loads, antibody titers, symptom scores and body temperature are a few of the outcome variables commonly studied. Longitudinal EI data are inherently nonlinear, often with single-peaked response trajectories with a common pre- and post-infection baseline. Such data are frequently analyzed with statistical methods that are inefficient and arguably inappropriate, such as repeated measures analysis of variance (RM-ANOVA). Newer statistical approaches may offer substantial gains in accuracy and precision of parameter estimation and power. We propose an alternative approach to modeling single-peaked, longitudinal EI data that incorporates recent developments in nonlinear hierarchical models and Bayesian statistics. We begin by introducing a nonlinear mixed model (NLMM) for a symmetric infection response variable. We employ a standard NLMM assuming normally distributed errors and a Gaussian mean response function. The parameters of the model correspond directly to biologically meaningful properties of the infection response, including baseline, peak intensity, time to peak and spread. Through Monte Carlo simulation studies we demonstrate that the model outperforms RM-ANOVA on most measures of parameter estimation and power. Next we generalize the symmetric NLMM to allow modeling of variables with asymmetric time course. We implement the asymmetric model as a Bayesian nonlinear hierarchical model (NLHM) and discuss advantages of the Bayesian approach. Two illustrative applications are provided. Finally we consider modeling of viral load. For several reasons, a normal-errors model is not appropriate for viral load. We propose and illustrate a Bayesian NLHM with the individual responses at each time point modeled as a Poisson random variable with the means across time points related through a Tricube mean response function. We conclude with discussion of limitations and open questions, and a brief survey of broader applications of these models.
KEYWORDS: Bayesian nonlinear hierarchical model, infection response, viral challenge, simulation study, longitudinal data analysis

Michael Singleton

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NONLINEAR HIERARCHICAL MODELS FOR LONGITUDINAL EXPERIMENTAL INFECTION STUDIES

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To Zhenyu, Evan and Emma
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CHAPTER ONE

Literature Review

1.1. INTRODUCTION

In a 1951 article in Scientific American, the eminent Australian virologist Sir Frank MacFarlane Burnet sounded the death knell for infectious disease, writing:

One can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of infectious diseases as a significant factor in social life (Burnet 1951).

At the time there was just cause for such optimism. The preceding hundred years had brought astounding advances in the understanding of the etiology of infectious diseases and their prevention. Robert Koch and Louis Pasteur, in the latter half of the 19th century, had established that diseases such as anthrax, rabies, tuberculosis and cholera were caused by microorganisms and had successfully developed vaccines and medications to prevent infection (Koch 1884, Koch 1890a, Koch 1890b, Pasteur 1881, Suzor 1887). Smallpox vaccination programs had eliminated nearly all cases of that disease in the United States (Chapin 1913). Alexander Fleming had discovered penicillin in 1929 and subsequent work by Howard Florey, Ernst Chain and Norman Heatley made possible its mass production. At the same time Burnet wrote the above words, Jonas Salk was on the verge of commencing human testing of his killed-virus polio vaccine (Meldrum 1998).

However over the next forty years this confidence in the elimination of infectious disease would be steadily eroded. While many diseases were being successfully controlled, novel viruses were emerging. Examples include Ebola virus in the 1970’s (Simpson et al. 1978), the human immunodeficiency virus (HIV) in the early 1980’s
(Barre-Sinoussi et al. 1983), and more recently the severe acute respiratory syndrome or SARS (Fouchier et al. 2003) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Furthermore, it quickly became apparent that bacteria were far more resourceful than previously realized. As early as 1942 came the first reports of penicillin-resistant strains of Staphylococcus aureus (Rammelkamp and Maxon 1942). Within two decades as many as 80% of staphylococcal isolates were resistant to penicillin (Lowy 2003). In light of developments such as these, it has become clear that infectious disease research, epidemiology and prevention are as relevant and important as ever.

Experimental infection (EI) studies, involving the intentional inoculation of animal or human subjects with an infectious agent under controlled conditions, have played a central role in infectious disease research and epidemiology for more than two hundred years. Most of the early work of Koch and Pasteur cited above involved the experimental inoculation of small mammals such as rabbits and guinea pigs. Today there are hundreds of animal challenge models for infectious diseases (Zak and O’Reilly 1993). But experimental infection studies are not limited to animals. Certain diseases lack effective animal models, and even when they do exist, human models are often preferred when they can be safely and ethically employed. Human challenge models are used to study self-limiting respiratory infections such as rhinovirus, influenza and respiratory syncytial virus (Ramos et al. 2014, DeVincenzo et al. 2014, Wilkinson 2012), and models have been developed or proposed for Helicobacter pylori (Michetti 2004), cholera (Shirley and McArthur 2011), malaria (Sauerwein et al. 2011), gonorrhea (Hobbs et al. 2011), tuberculosis (Hokey 2014) and others.
In early EI studies the outcome of interest was usually the simple presence or absence of disease. Eventually investigators became interested in studying the time course of various aspects of the infection response. A major milestone in the development of a polio vaccine, for instance, was Dorothy Horstmann’s 1952 time course EI study which demonstrated the occurrence of viremia in monkeys following oral inoculation with poliomyelitis virus (Horstmann 1952). The knowledge that the virus reached the nervous system via the bloodstream led researchers to focus on development of an oral polio vaccine that would to elicit antibodies in the blood and gastrointestinal tract (Carleton 2011).

With advances in microbiology and technology, increasingly sophisticated questions are being investigated using time course EI studies. Yet the statistical approaches used to model and analyze time course data from EI studies have remained largely unchanged for at least thirty years, despite major advances in methods for analyzing longitudinal data that have occurred during that period. The most popular approaches traditionally have been 1) the analysis of variance for repeated measures (RM-ANOVA) and 2) multiple horizontal or vertical contrasts (Ludbrook 1994) using t-tests or their nonparametric equivalents (Paillot et al. 2013, Munhoz et al. 2012, Cray et al. 1995, Suter et al. 1985, Higgins et al. 1983). The aim of this dissertation is to propose an alternative approach to the analysis of longitudinal data from EI studies that draws upon recent developments in the areas of nonlinear hierarchical models and Bayesian statistics.

We begin by reviewing the various roles that EI studies have played in the study of infectious diseases. Next we narrow our focus by describing in detail four time course
EI studies on which we will draw in subsequent chapters for illustrative examples of our methods. We review some objections that have been raised to traditional methods of analyzing data from such experiments. Finally we outline the specific aims of this dissertation.

1.2. A BRIEF OVERVIEW OF THE USES OF EXPERIMENTAL INFECTION STUDIES

Long before the causal link between microorganisms and disease had been decisively proven, physicians and scientists were conducting EI studies. In 1796, in one of the earliest tests of vaccine efficacy, England physician Edward Jenner challenged an eight year-old boy with live smallpox two months after inoculating him with cowpox vaccine (Riedel 2005; Willis 1997). As part of his work on a rabies vaccine in the latter part of the nineteenth century in France, Pasteur performed EI studies on rabbits (Franco 2013). In the mid-twentieth century polio research and vaccine development relied heavily on the experimental infection of monkeys (Oshinsky 2006). Several candidate cholera vaccines have been tested in human challenge trials (Shirley and McArthur 2011, Herrington et al. 1990 Tacket et al. 1990). Recently, controlled human malaria infection has been used as a method of evaluating candidate malaria vaccines (Laurens et al. 2012, Roestenberg et al. 2013, Targett et al. 2013). Human challenge models for self-limiting respiratory infections such as influenza, human rhinovirus and respiratory syncitial virus are being used to accelerate proof of concept for vaccines against respiratory viruses (Ramos et al. 2014, DeVincenzo et al. 2014).

Many early EI studies were concerned with establishing the infectious etiology of a known disease. In a historic lecture in 1882, Koch demonstrated the infectious etiology
of tuberculosis and identified the causative agent, *Mycobacterium tuberculosis*. His discovery was accomplished by experimentally infecting guinea pigs with material from tuberculosis-infected human and animal sources (Koch *et al.* 1982). Similarly, his work on the etiology of anthrax involved the experimental infection of mice with *Bacillus anthracis* (Koch 1876). Koch would subsequently publish his postulates for causation in 1890. Since that time, EI studies have been used to demonstrate the etiological agents for many infectious disease. More recently an etiologic link between *Helicobacter pylori*, a gastrointestinal bacterium which was discovered in 1982 (Marshall and Warren 1984), and type B gastritis has been established largely through EI studies in both human volunteers and animal models (Blaser 1990).

Another historically important use of experimental infection has been to establish the route by which an infectious agent is transmitted to the host. Carlos Finlay in 1881 proposed that the *Culex* mosquito was the agent responsible for the transmission of yellow fever mosquito (Finlay 1881). To test his hypothesis he conducted a series of 102 experimental infections of human volunteers, the results of which were generally viewed as inconclusive (Sternberg 1891, Sternberg 1901). His experiments were subsequently repeated, and his theory confirmed, in further human volunteer experiments led by Walter Reed (Reed *et al.* 1900). Contemporary with Finlay and Reed, Ronald Ross 1897 conducted EI studies in birds to demonstrate transmission of malaria parasites by mosquito (Bynum 1999). Killingley et al (2011) recently reviewed the potential contributions of human challenge studies to research on mechanisms of influenza transmission.
EI studies have played a central role in experimental immunology, where they have helped to advance basic understanding of the immune response to infection. Representative examples include Wilkinson *et al.* 2012; Henriques *et al.* 2012; Gaunt *et al.* 2010; McMichael *et al.* 1981. Numerous journals, including Viral Immunology, Vaccine, Journal of Immunology and others, are dedicated to clinical, translational and basic research into the immune response to viral infections.

![Figure 1.1](image)

**Figure 1.1.** Google Scholar references containing the phrase “experimental infection” in the title by decade, 1890-2009

Scholarly papers containing the phrase “experimental infection” can be found from as early as 1899. Figure 1.1 shows the number of references by decade retrieved by Google Scholar (2013) containing the phrase “experimental infection” in the title. The number of published studies rose gradually through the mid-1940’s and then increased dramatically over the next four decades. This data indicates that hundreds of EI studies are currently being published annually in peer-reviewed journals around the world. The applications discussed in this section – vaccine efficacy, etiology, transmission and basic immunology – represent only some of the most common applications.
1.3. EXPERIMENTAL INFECTION CASE STUDIES

The focus of this dissertation is EI studies in which the response variable is measured repeatedly on one or more groups of subjects over time. We will refer to such experiments as repeated measures, time course, or longitudinal EI studies. In this section we give detailed descriptions of four recent longitudinal EI studies. These case studies will provide context for the discussion of our research aims as well as background for the illustrative examples in subsequent chapters. We consider these examples to be a representative cross-section of mainstream approaches to analyzing time course data from EI studies. Later chapters will explore alternative methods.

1.3.1. The role of host genetic factors in the pathogenesis of Equine Viral Arteritis

Equine arteritis virus (EAV) is the causative agent of a respiratory and reproductive disease of equids known as equine viral arteritis (EVA). The virus was first isolated in 1953 on a Standardbred breeding farm in Bucyrus, Ohio during an outbreak of respiratory illness and abortion (Doll et al. 1957). Numerous other outbreaks have occurred throughout the world since that time, including an epizootic among thoroughbreds in Kentucky in 1984 (Timoney et al. 1986). EAV is a major concern to horse breeders because it can cause spontaneous abortion in mares and temporary subfertility in stallions. Furthermore, 30% to 70% of exposed stallions will become long-term carriers of the virus, continuing to shed virus in semen after all clinical signs of infection have ceased (Timoney et al. 1986). Such carrier stallions constitute the natural reservoir for EAV, and venereal transmission from a carrier stallion, either directly or via artificial insemination, is the source of many outbreaks. The virus is also transmitted via the respiratory route (Holyoak et al. 2008).
It has been observed in natural outbreaks that the severity and clinical signs of EVA show considerable variation, both among individual horses in the same outbreak and between different outbreaks. Numerous factors may influence the course of infection including the age and physical condition of the horse, the viral strain and the received dose (Balasuriya et al. 2007, Holyoak et al. 2008). Recently it has been proposed that host genetic factors may also play a role in EAV pathogenesis. Balasuriya and colleagues have identified a haplotype that is associated with susceptibility of CD3+ T lymphocytes to infection by a recombinant virulent Bucyrus strain (VBS) of EAV. They classified horses as possessing the “resistant” or “susceptible” phenotype based on the ability of the VBS to infect in vitro their CD3+ T lymphocytes (Go et al. 2010). Recently, they conducted two related experiments to assess whether the response to EAV infection differs between horses with the resistant and susceptible phenotypes.

1.3.1.1. “Assessment of correlation between in vitro CD3+ T cell susceptibility to EAV infection and clinical outcome following experimental infection”

In the first experiment, by Go et al. (2011), four mares possessing the in vitro susceptible CD3+ T cell phenotype were identified along with four possessing the resistant phenotype. All eight mares were inoculated with the recombinant, virulent Bucyrus strain of EAV. Clinical signs, viral load, complete blood cell counts and serum neutralizing antibodies were monitored over a period of seven weeks, which included a seven-day baseline observation period. We focus here on comparison of the mean febrile response in the two groups as measured by core body temperature (CBT), which was observed on the morning of days -7, -4 and -2 prior to inoculation and on both morning and evening of days 0, 2, 4, 6, 8, 10, 12 and 14 post-inoculation (DPI). Figure 1.2 presents the observed febrile responses for all four mares in each group.
Figure 1.2. Observed core body temperature profiles by phenotype group for eight mares following experimental challenge with EAV (Go et al. 2011)

Figure 1.3. Mean core body temperature by phenotype group. Significant differences ($p < 0.05$) were reported on days 7 and 9 based on RM-ANOVA with post-hoc testing for group differences by Holm-Sidak method\(^1\).

\(^1\) Reprinted from Veterinary Microbiology 2012;157(1-2), Go et al, Assessment of correlation between in vitro CD3+ T cell susceptibility to EAV infection and clinical outcome following experimental infection, pages 220-225, Copyright 2012, with permission from Elsevier.
A global test of the hypothesis of no group difference in febrile response was conducted using two-way repeated measures analysis of variance (RM-ANOVA). The Holm-Sidak method (Holm 1979) was subsequently used to identify specific time points at which CBT differed significantly between phenotype groups. Significant differences (\(p < 0.05\)) were reported on days 7 and 9 (Figure 1.3). These findings were taken as evidence of an association between CD3+ T lymphocyte phenotype and febrile response.

1.3.1.2. “Semen quality of stallions challenged with the Kentucky 84 strain of equine arteritis virus”

The findings of a follow-up to the Go experiment with similar design, but using stallions instead of mares and a different strain of EAV, were published by Campos et al. in 2014. One of the primary aims of that experiment (the results of which have not been published to date) was to assess whether the CD3+ T cell phenotype may play a role in development of the long-term EAV carrier state in the stallion. Although not the primary focus of that particular paper, febrile response and other clinical signs were also recorded as was done in Go et al. (2011).

In Chapters 2 and 3 we re-analyze the EAV febrile response data from the two experiments by Go and Campos.

1.3.2. Effects of rhinovirus infection in chronic obstructive pulmonary disease

The first human rhinoviruses (HRV) were discovered in the 1950’s. Since that time 99 serotypes have been identified. HRV is the most common cause of upper respiratory infection worldwide. In recent years, rhinovirus infection has also been implicated as an important factor in exacerbations of asthma and chronic obstructive pulmonary disease (COPD) (Henderson 2013).
To overcome the difficulties involved in studying the role of respiratory infections in naturally occurring exacerbations, researchers have developed human challenge models. Studies employing HRV challenge models in asthma include those by Message et al. (2008); Contoli et al. (2006); Wark et al. (2005), Grunberg et al. (2001) and Bardin et al. (2000).

Similarly, Mallia and colleagues (2006, 2011, 2012, 2013) have developed an HRV challenge model for COPD. For their 2011 study, thirteen volunteers with COPD and thirteen controls with a similar smoking history, but normal lung function, were recruited. All participants tested negative for serum neutralizing antibodies to rhinovirus 16 (RV-16). All twenty-six subjects were inoculated with a 10 TCID$_{50}$ dose of RV-16 and followed for six weeks post-infection. Viral loads in nasal lavage and sputum, lung function, inflammatory markers, and upper and lower respiratory symptom scores were sampled at varying intervals and intensities. Twenty-three subjects displayed virological evidence of rhinovirus infection: eleven from the COPD group and twelve from the control group. Data from those 23 subjects were analyzed to assess whether experimental RV-16 infection induces exacerbation in persons with COPD.

Figure 1.4 presents the observed, individual profiles of viral load in nasal lavage (VLN) following inoculation over the course of six weeks. For most subjects, VLN increased to a peak very rapidly returned to baseline (zero) more gradually, as the host immune response neutralized and eliminated the virus. The figure suggests that the mean time to complete viral clearance may be greater in the control group – although this impression is based largely on just two control subjects with clearance times greater than
thirty days – and that the peak viral load may be slightly higher in the COPD group.

Figure 1.5 displays the group means at each measurement occasion.

**Figure 1.4.** Observed profiles of viral load in nasal lavage for eleven subjects with COPD and twelve health controls following experimental challenge with RV-16

**Figure 1.5.** Mean viral load in nasal lavage for COPD and control groups
In the original experiment, tests for differences in the population mean response at each time point were analyzed using unpaired $t$ tests or nonparametric Mann-Whitney tests. No adjustment for multiple comparisons was mentioned. Despite higher observed mean VLN in the COPD group on days 3 through 12, the authors reported a significant difference ($P < 0.05$) in population mean viral load on day 6 only. Thus there is some uncertainty about the interpretation of these findings. Group differences in age, gender and smoking status further complicate interpretation. We revisit the HRV viral load data in Chapter 4.

1.3.3. Effect of body condition on viral shedding in migratory birds

Mallard ducks (*Anas platyrhynchos*) are an important reservoir for influenza A virus (IAV), and their migration is a key factor in its global transmission (Webster *et al.* 1992). The physical strain of migration can cause declines in the birds’ body condition which may impair immune function (Latorre-Margalef *et al.* 2009a, Flint and Franson 2009, Latorre-Margalef *et al.* 2009b). Arsnoe and colleagues conducted an MC experiment to test the hypotheses that reduced body condition is associated with increased susceptibility to infection with IAV and with increased peak viral load and duration of infection (Ars noe *et al.* 2011). Thirty seronegative wild-caught mallards were randomly assigned to three treatment groups. By controlling food availability over a period of several weeks, differing levels of body condition were established in each group. Birds in the “normal” treatment group were maintained at $\pm 5\%$ of their baseline body mass; birds in the “lean” treatment group at $−10\%$ of baseline body mass; and birds in the “poor” treatment group at $−20\%$ of baseline body mass. A fourth treatment group consisted of ten captive-bred mallards given the normal treatment described above. Thus
four treatment groups were established: wild normal, wild lean, wild poor and captive-bred.

When target condition levels were reached, all birds were inoculated with 1.5 mL of $10^6$ PFU/mL low pathogenic AIV [strain A/Northern pintail/California/44221-761/2006 (H5N9)]. Body conditions were maintained over the course of the experiment. Viral shedding in pooled cloacal and oral swabs was measured by PCR and expressed in units of $log_{10}$(genome equivalent copy numbers), or $log_{10}(GEC)$, per 140 µl of swab fluid. Samples were collected on the first three days post-inoculation and every two days thereafter until 28 DPI. Mean shed virus by treatment group is presented in Figure 1.7. In general, groups with higher condition levels (i.e. greater food availability) shed more virus.

In original analysis, RM-ANOVA was used to compare virus excretion over the first five DPI. Significant variation among groups was detected ($F_{2,25}, p = 0.013$). Post-hoc group comparisons were carried out using Tukey’s Honestly Significant Difference procedure. The only reported difference was that birds in the wild poor treatment group shed less virus than birds in the wild normal treatment group (M=2.3, p=0.010).

Figure 1.7 presents the individual viral shedding profiles for the captive-bred control group and the wild lean treatment group. There is considerable inter-individual variability in shedding patterns, with one of the wild lean birds shedding high levels of virus and several of the control birds showing low levels. In Chapter 4 we analyze the viral shedding data in Figure 1.7.
Figure 1.6. Mean shed virus by treatment group

Figure 1.7. Observed viral shedding profiles for nine captive bred ducks with normal body condition and nine wild-caught ducks with lean body condition following experimental challenge with AIV (observations for days 11 through 28 not shown)
1.4. LONGITUDINAL EI DATA

1.4.1. Nature of longitudinal EI data

Unlike many types of longitudinal data, experimental infection data are inherently nonlinear, as exemplified by Figures 1.2 through 1.7. We restrict attention in this dissertation to experiments in which there is exactly one challenge point. In such experiments, for quantities such as body temperature or viral load there is typically a brief post-challenge incubation period followed by an interval of rapid increase (the “onset” phase of the response). As the host’s immune system responds to the infection, the increase slows and eventually peaks. This is followed by a return to the pre-challenge baseline level (the “recovery” phase). In the case of viral load typically the level returns to zero, whereas body temperature will return to the host’s normal baseline level, which will vary slightly from one individual to the next. This pattern has been referred to as a single-peaked response to distinguish it from growth curve data (Matthews et al. 1990). Peak responses may take other forms, but the present paper focuses on single-peaked responses with a common pre- and post-challenge baseline level.

Another way in which data arising from EI experiments differs from traditional longitudinal data is in the frequency with which responses are sampled. Clinical signs such as core body temperature or symptom scores are routinely observed on twenty or more occasions over a period of several weeks or months. Furthermore, advances in biomedical technology have made practical the intensive sampling of viral loads and various markers of the host immune response (Schochetmann et al. 1988).
1.4.2. Traditional approach to the analysis of longitudinal EI data

Prior to 1980, repeated measurements data from experimental infection studies were commonly presented without statistical analysis. By the late 1970’s investigators were beginning to employ basic statistical methods for comparing group mean responses (Makinde and Wilkie 1979; Higgins et al. 1983). By the mid-to-late 1990’s formal statistical analyses had become the norm for EI studies, RM-ANOVA apparently being the default method (Paillot et al. 2013; Munhoz et al. 2012; Cray and Moon 1995). However it is unclear whether, among practitioners, much critical thinking has gone into that decision or whether RM-ANOVA is simply used because it is familiar and straightforward to use.

Among statisticians the application of RM-ANOVA data to longitudinal data, in general, has been criticized on several grounds (Matthews et al. 1990, Gueorguieva and Krystal 2004, Fitzmaurice and Ravichandran 2008). There are concerns about the validity of certain underlying statistical assumptions. Furthermore, in the case of peaked data, it is readily apparent that any approach that involves averaging a group of single-peaked response profiles at each time point will tend to distort the very features of the infection response that are of greatest interest. Such naïve averaging will tend to produce estimated population response curves that understate peak intensity and overstate response duration, as has been described recently by DeVincenzo and colleagues (2010). This effect will increase as the variability in the time to peak response among subjects increases.

1.4.3. Alternative approaches to data analysis

Numerous alternatives to RM-ANOVA for analyzing longitudinal data exist, most notably including summary measures or response feature analysis (Matthews et al. 1990),
multivariate analysis of variance (MANOVA) and the linear mixed model. Each has strengths and weaknesses which depend on the particulars of the experimental design. Summary measures may be viable when the individual response profiles can be reduced to a single, meaningful measure, such as summarizing a linear trajectory by its slope. But a peaked response curve has numerous features of potential interest which cannot all be encompassed in a single measure.

MANOVA has the advantage that, unlike RM-ANOVA, it does not impose a restrictive assumption about the covariance structure of individual response vectors. Instead, an individual’s responses are treated as a vector arising from a multivariate normal distribution, the covariance structure of which can be explicitly specified by the analyst. However MANOVA requires larger numbers of individuals per group to equal the power of RM-ANOVA, and becomes increasingly unattractive as the number of observations per individual increases.

The linear mixed model (LMM) retains the ability to specify an appropriate individual covariance structure. Moreover, if the individual response trajectories are linear, the performance of the LMM improves as the number of measurement occasions increases, rather than degrading as with MANOVA. However with a nonlinear response, time in the LMM must be treated as a factor, which negates this advantage.

Nonparametric regression is an option that is capable of modeling the mean response curve quite flexibly (Wu and Zhang 2006). Campos et al. (2014) provides an example of this approach applied to data from an EI study. However the parameters in nonparametric models (and RM-ANOVA models also) bear no relationship to quantities of biological interest – such as the timing and intensity of the peak response, and the
response onset, recovery and duration – which limits inferential options. We propose another alternative, the nonlinear hierarchical model (NLHM), which among other features (a) permits direct modeling of the nonlinear mean response trajectories, (b) allows for meaningful parameterization of the response function, and (c) allows for flexible modeling of the covariance structure for individual response vectors.

1.5. NONLINEAR HIERARCHICAL MODELS FOR LONGITUDINAL EI DATA

1.5.1. Background

Nonlinear hierarchical models (NLHM) have a history of application in population pharmacokinetics and pharmacodynamics research (PopPK-PD) dating back to the early 1970’s (Sheiner et al. 1972). Pharmacokinetics is the study of drug absorption, distribution, metabolism and excretion (Roe 1997). A primary aim of PopPK modeling is to understand how patient characteristics affect these processes. Concentration is measured repeatedly over time in a sample of patients following ingestion of the drug. A NLHM is used to estimate the response of a typical patient as well as the degree of variation among individual responses and the influence of factors such as patient age, gender, and body weight on response parameters. Pharmacodynamics is concerned with the effects of a drug on the patient. The purpose of a PopPD model is to relate dose or drug concentration to pharmacodynamics effects of the drug (Bonate 2011). In 1980 Sheiner and Beal released NONMEM, a software package for NONlinear Mixed Effects Modeling which has become the standard for PopPK-PD modeling. An extensive literature on NLHM’s for PopPK-PD has developed over the past four decades.

Several developments since the early 1990’s have made the NLHM accessible to a wider audience and increased its range of application. Textbooks on the NLHM were
published by Davidian and Giltinan in 1995 and Vonesh and Chinchili in 1997. SAS Version 7, released in 1998, introduced the NLMIXED procedure. In 1999 Pinheiro and Bates released their package ‘nlme’ (Linear and Nonlinear Mixed Effects Models) in S-Plus and subsequently released a version for R, and in 2000 they published an accompanying textbook. Bayesian implementation of the NLHM has also been facilitated in recent years by the introduction of software packages for fitting Bayesian models using Markov Chain Monte Carlo sampling methods, such as BUGS (Bayesian Inference Using Gibbs Sampling) (MRC Biostatistics Unit 1989), WinBUGS (Lunn et al. 2000), JAGS (Just Another Gibbs Sampler) (Plummer 2003) and Stan (Stan Development Team 2014).

As a result of these developments, since Sheiner and colleagues’ seminal work on PopPK-PD the NLHM has been applied to a number of other areas in which nonlinear repeated measurements arise. Davidian and Giltinan (2003) provided a review of several of these including dairy science, forestry, toxicokinetics, prostate cancer, circadian rhythms, cardiology, fisheries science and plant and soil sciences. Of particular relevance to this dissertation is the application of the NLHM to the dynamics of human immunodeficiency virus (HIV) (Perelson et al. 1996, Wu et al. 1998, Fitzgerald et al. 2002, Huang 2013). The work on HIV dynamics has led to similar efforts to model Hepatitis B and C viral dynamics (O’Sullivan et al. 2008, Snoeck et al. 2010). In all three instances the focus is on the changes in viral load over time that can occur in chronically infected individuals. We are unaware of any previous efforts to apply the NLHM to the analysis of data from EI studies involving the intentional challenge of previously uninfected individuals.
In the following section we give a brief overview of one form of the NLHM which Vonesh and Chinchilli (1997) have referred to as the normal-errors NLHM. We adopt their descriptive term for this model and discuss it in the context of EI studies.

1.5.2. The normal-errors NLHM

Suppose that infection responses for \(n\) individuals are sampled on multiple occasions. Let \(m_i\) represent the number of sampling occasions for individual \(i\). Let \(j\) index the sampling times \(t_1, t_2, \ldots, t_{m_i}\) for individual \(i\). Denote the responses for individual \(i\) as \(y_{i1}, y_{i2}, \ldots, y_{im_i}\), and a vector of covariates for individual \(i\) at time \(j\) as \(x_{ij}\). The covariate vectors will include the sampling times, at a minimum, and may also include measurements on factors other than time – such age, gender or smoking status – thought to influence an individual’s response to infection. The individual mean infection response profiles are modeled as a nonlinear function \(f\) of the covariate vectors \(x_{ij}\) and a parameter vector \(\beta_i\) which is unique to each individual. The model for the individual infection responses can expressed in terms of the distribution of the response for individual \(i\) at time \(t\).

Level 1 (model of intra-individual variability)

\[
y_{ij} \sim N(\mu_{ij}, \sigma^2), \quad \mu_{ij} = f_i(x_{ij}, \beta_i)
\] (1.1)

In the normal-errors NLHM, intra-individual deviations from the mean response trajectory are assumed to follow a multivariate normal distribution. In the form specified in (1.1), it is further assumed that these errors are independent and identically distributed. It is possible to relax this assumption and allow for both correlation and heterogeneity in the intra-individual errors. The interested reader is referred to Davidian and Giltinan (1995) for details.
At Level 2 of an NLHM we specify a mechanism by which the individual response profiles are assumed to be related. This is accomplished by imposing a model on the response parameters $\mathbf{\beta}_i$. A common practice is to assume that they arise from a multivariate normal distribution with mean $\mathbf{\beta}$ and variance-covariance matrix $\mathbf{D}$. (We will refer to the components of $\mathbf{\beta}$ as the population infection response parameters and the components of $\mathbf{\beta}_i$ as the individual infection response parameters.) If all individuals in the study belong to a single group, then the model of inter-individual variability can be expressed as follows.

**Level 2 (model of inter-individual variability – one group)**

$$\mathbf{\beta}_i = \mathbf{\beta} + \mathbf{b}_i, \quad \mathbf{b}_i \sim N(0, \mathbf{D}) \tag{1.2}$$

Often in an MC experiment the primary objective is to compare the population response profiles for two groups – say treatment vs. placebo or presence vs. absence of a genetic trait thought to influence the response to infection – and interest in the individual response profiles is secondary. Suppose that the $n$ individuals are assigned to two groups indexed by $g \in \{1,2\}$, and that individual $i$ belongs to group $g$. For each group define a population parameter vector $\mathbf{\beta}^{(g)}$ which is assumed to give rise to the individual response vectors for members of group $g$. The Level 2 model can then be written as follows.

**Level 2 (model of inter-individual variability – two groups)**

$$\mathbf{\beta}_i = \mathbf{\beta}^{(g)} + \mathbf{b}_i, \quad \mathbf{b}_i \sim N(0, \mathbf{D}^{(g)}) \tag{1.3}$$

If we assume that the variance structure is same in both groups then we have

$$\mathbf{\beta}_i = \mathbf{\beta}^{(g)} + \mathbf{b}_i, \quad \mathbf{b}_i \sim N(0, \mathbf{D}) \tag{1.4}$$

Thus, the normal-errors NLMM provides estimates of the population response function (or functions) through $\mathbf{\beta}$ (or $\mathbf{\beta}^{(g)}$), estimates of the individual response functions
through the $\beta_i$, and estimates of both the intra-individual and inter-individual response variability through $\sigma^2$ and $D$ (or $D(g)$). In the two-sample infection response model, the components of the population response parameters $\beta^{(g)}$ can be contrasted to test for differences in the peak response, time to peak response, and response onset, recovery or duration between two groups.

The version of the normal-errors NLMM described above permits a great deal of flexibility in the specification of both the form of the individual infection responses and the nature of the relationship among them. The model can be extended to make it still more general. It is possible, for example, to allow for the dependence of the individual infection response parameters in $\beta_i$ on subject characteristics such as age or gender. One can also specify distributional assumptions on the $\beta_i$ other than multivariate normality, or indeed to avoid distributional assumptions at Level 2 altogether by specifying a nonparametric or semiparametric model for $\beta_i$. The interested reader is again referred to Davidian and Giltinan (1995) for details.

### 1.5.3. Application to EI studies

Kernel functions provide a convenient class of models for a single-peaked infection response. Figure 1.8 presents three common kernel functions: the Gaussian, Tricube and Epanechnikov. Each is bounded and symmetric, with a single peak. The Gaussian kernel approaches zero as the independent variable increases or decreases without bound, whereas the Tricube and Epanechnikov kernels are identically zero outside of the interval $[-1,1]$. Thus all three functions have the characteristic shape of a single-peaked infection response with a common pre- and post-infection baseline.
The parameters of these functions correspond to aspects of the infection response that are typically of focal interest in MC experiments. Consider for instance the Gaussian kernel, defined by the function

$$K(t) = \left(\sqrt{2\pi\sigma}\right)^{-1} e^{\frac{(t-\mu)^2}{2\sigma}}$$  \hspace{1cm} (1.5)

Here $\mu$ represents the time to peak response and $\sigma$ can be interpreted as a measure response duration. By replacing the normalizing constant $\left(\sqrt{2\pi\sigma}\right)^{-1}$ with a third parameter $I$ we obtain a measure of the peak response intensity. By incorporating a vertical shift parameter $B$ we add the capability of modeling responses with nonzero baseline values, such as body temperatures. Thus we can modify the basic Gaussian kernel to obtain a function that directly models the most salient features of the infection response.

$$K(t) = B + I e^{\frac{(t-\mu)^2}{2\sigma}}$$  \hspace{1cm} (1.6)

**Figure 1.8.** Gaussian, Tricube and Epanechnikov kernel functions

One advantage of a compact kernel function such as the tricube is that the endpoints of the interval on which it takes nonzero values provide direct estimates of the
timing of response onset and recovery. By allowing for rescaling of both the time and response variables, we obtain a basic, symmetric tricube kernel response function (1.7). We explore several variations and extensions of these infection response functions. 

\[ K(t) = B + I \left( 1 + \left| \frac{t-p}{s} \right|^3 \right)^3 \]  

(1.7)

In general, the choice of kernel is an aspect of model specification that the data analyst must decide upon. The best kernel for the application depends on the shape of the peaked response as well as the desirability of compactness. Overall, however, we found that the results were not particularly sensitive to choice of kernel for the applications we considered. For this reason we do not extensively explore the choice of kernel in this dissertation. Instead, we give examples of the use of each kernel at various places in the dissertation: Gaussian kernels are employed in chapters 2 and 3, and a tricube kernel is used for the viral load modeling of chapter 4. Clearly there are other possible choices of functions for modeling the infection response trajectories but in this dissertation we restrict our attention to kernel functions.

1.6. SCOPE AND OUTLINE OF DISSERTATION

Chapter 1 establishes the importance of EI studies for research, epidemiology and prevention of infectious diseases. Several case studies illustrate the nature of longitudinal EI data. Traditional approaches to the analysis of this type of data are discussed and an alternative approach is suggested based on nonlinear hierarchical statistical models.

Chapter 2 considers the case of a symmetric transient response variable, that is, a variable that can adequately be approximated by a symmetric mean response function. We introduce a version of the normal-errors NLHM that can be fit using standard procedures available in several commonly used statistical packages. We present a
simulation experiment comparing the performance of our Gaussian nonlinear mixed model with RM-ANOVA with respect to bias and precision of parameter estimation, power and Type I error control. Finally we provide an illustrative application to the equine arteritis virus data from § 1.3.1.

Many responses will not be adequately modeled by a symmetric function. In Chapter 3 introduces a version of the normal-errors NLHM model suitable for modeling an asymmetric infection response. We discuss several reasons for preferring to implement this model as a Bayesian NLHM rather than a nonlinear mixed model. We illustrate the asymmetric Bayesian NLHM using the equine arteritis virus data from § 1.3.1.

Frequently in MC experiments involving viral pathogens, the focus is the time course of the viral load in blood, feces or other fluids or excreta. For several reasons the normal-errors NLHM from Chapters 2 and 3 is not appropriate for modeling viral load. For the individuals in such experiments, viral load will be exactly zero at all observation times prior to inoculation. For those in whom the virus is completely cleared, viral load will return to exactly zero. During infection, however, viral load can be viewed as fluctuating randomly about some systematic trajectory. Thus the constant variance assumption is clearly violated. Further, because viral loads are nonnegative it makes no sense to model a subject’s viral load at each time point as a normal random variable. Finally, during infection it may happen the variability in viral load increases as the mean viral load increases. In Chapter 4 we propose an NLHM in which the viral load at each time point is modeled as a Poisson random variable with mean given by a compact kernel function. Because the variance of a Poisson random variable is equal to its mean, when the mean viral load is equal to zero (i.e. pre-onset or post-recovery) the variance will be
also. Similarly, when the mean viral load is positive (i.e. during infection) the variance will be positive, and the variance will increase with the mean. We illustrate the viral load NLHM using the rhinovirus data from § 1.3.2 and the influenza data from § 1.3.3.

1.7. ETHICAL CONSIDERATIONS IN EI STUDIES

Despite their obvious potential benefits for the study of infectious diseases, EI studies in both humans and animal models raise a host of ethical concerns. There have been numerous instances of ethically questionable practices involving the use of human subjects in infectious disease research (Zenilman 2013; Comfort 2009; Krugman 1986). In the United States, ethical guidelines for research involving human subjects have been established by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and summarized in its Belmont Report (1979). The National Institutes of Health have published a framework for evaluating the ethical aspects of infection-inducing challenge experiments (Miller and Grady 2001). In the United Kingdom, the Academy of Medical Sciences has published the report Microbial Challenge Studies in Human Volunteers which provides recommendations and guidance to ensure the safe and ethical conduct of MCE’s involving human volunteers (2005). The ethics of EI studies continue to be vigorously debated. Michetti has offered the following eloquent summary.

Challenge experiments have been an important method of studying the pathogenesis of many infectious diseases and of evaluating initial efficacy of vaccines before large scale field tests are conducted. In challenge experiments, infections are deliberately induced under carefully controlled and monitored conditions to healthy research volunteers. Induced infections are usually either
self-limiting or can be fully treated within a short period of time. Because physicians should be dedicated to alleviating disease and avoiding harm to patients, this type of experiment may cause uncomfortable symptoms and evoke serious moral concerns. It should be appreciated however that clinical research commonly involves risks to subjects that are not outweighed by medical benefits but are justified by the potential to acquire new knowledge. In that regard, infection inducing challenges are not necessarily more ethically problematic than phase I trials aimed at determining maximum tolerated doses of medications. Like any clinical research, challenge experiments should be conducted by competent investigators according to sound protocols that incorporate appropriate safeguards to ensure the safety of volunteers. Because these experiments may provide valuable information that might not be otherwise obtained, lead to novel therapies, or speed up vaccine development that will ultimately spare morbidity or death from infectious diseases and reduce exposure of large groups in field trials, challenge experiments may be justified. However, the scientific rationale should be carefully examined for any given pathogen and model. When such a rationale exists, then the question of risks and discomforts should be addressed (Michetti 2004).

For experiments involving animal subjects, the counterpart to the Institutional Review Board (IRB) is the Institutional Animal Care and Use Committee (IACUC). The IACUC has its origins in the Animal Welfare Act passed by the U.S. Congress in 1966. Today in the U.S. an IACUC is required of all institution that uses animals in federally funded research. Functions of the local IACUC include review of research protocols and
evaluation of the care and use of animals (Anderson 2007). A key resource for IACUC’s
is the Guide for the Care and Use of Laboratory Animals, which was published in 1963
by a group of veterinarians known as the Animal Care Panel (Barthold et al. 2011).
CHAPTER TWO

A nonlinear mixed model for a single-peaked, symmetric response

2.1. INTRODUCTION

Some quantities of interest in the response to an infection, such as body temperature and lymphocyte counts, fluctuate around a natural baseline value when an individual is not experiencing infection. The natural baseline level may vary slightly from one individual to the next. Furthermore, in the infected state, although the mean response level changes over time the variation about the mean tends to remain relatively consistent (e.g. Figure 2.2). The normal-errors model introduced in Chapter 1 will often be appropriate in such cases.

This chapter has three primary aims. First, we present a method for analyzing this type of longitudinal response data from experimental infection studies using a nonlinear mixed model (NLMM) that is straightforward to implement in popular statistical software packages. Second, we illustrate via Monte Carlo simulation the gains in accuracy, precision and power that this method provides over the traditional method of choice, RM-ANOVA. Third, we illustrate the application of the model to data from an actual EI study.

2.2. NORMAL-ERRORS MODEL FOR A SYMMETRIC RESPONSE

Suppose that infection responses for \( n \) individuals are sampled on multiple occasions. Let \( m_i \) represent the number of measurement occasions for individual \( i \). (Often in experimental infection studies \( m_i \) is constant across individuals, but we do not assume it here.) Let \( j \) index the sampling times \( t_1, t_2, \ldots, t_{m_i} \) for individual \( i \). Denote the
responses for individual \( i \) as \( y_{i1}, y_{i2}, \cdots, y_{im_i} \). We model the individual response profiles with the modified Gaussian function (1.6).

**Level 1 (model of intra-individual variability)**

\[
y_{ij} \sim N(\mu_{ij}, \sigma^2) \tag{2.1}
\]

\[
\mu_{ij} = B_i + I_t \exp \left[ -\left( \frac{t-p_i}{2s_i} \right)^2 \right] \tag{2.2}
\]

Here \( B_i \) represents the baseline response level for individual \( i \), \( p_i \) the time to peak response, and \( I_t \) the peak response intensity. \( s_i \) can be interpreted as a measure of response duration. A closely related, but more interpretable, measure of response duration is the full width at half maximum response (FWHM), given by \( FWHM_i = s_i \sqrt{2 \ln 2} \). This is the width of the response curve when the response is halfway between baseline and peak intensity.

To simplify the notation for Level 2 of the model, we collect the response parameters for individual \( i \), into a vector

\[
\beta_i = [B_i, I_t, p_i, \log(s_i)]^T. \tag{2.3}
\]

The scale parameter \( s_i \) is modeled on the log scale to restrict its range to the positive real numbers. Now suppose that each individual belongs to one of two groups indexed by \( g \in \{1,2\} \), and that individual \( i \) belongs to group \( g \). For each group \( g \) define a population parameter vector, which is assumed to give rise to the individual response vectors for members of group \( g \), as

\[
\beta^{(g)} = [B^{(g)}, I^{(g)}, p^{(g)}, \log(s^{(g)})]^T. \tag{2.4}
\]
Finally let $\sigma^2_B(\gamma), \sigma^2_I(\gamma), \sigma^2_p(\gamma), \sigma^2_{\log s}(\gamma)$ and denote the variances of the corresponding components of $\beta(\gamma)$. Then we specify the mechanism for the inter-individual response variability as follows.

Level 2 (model of inter-individual variability)

$$\beta_i = \beta(\gamma) + b_i$$ (2.5)

$$b_i \sim N(0, D(\gamma)), \text{ where } D(\gamma) = \text{diag}(\sigma^2_B(\gamma), \sigma^2_I(\gamma), \sigma^2_p(\gamma), \sigma^2_{\log s}(\gamma))$$ (2.6)

A diagonal structure for $D(\gamma)$ is specified because, due to the modest sample sizes typical of EI studies, there is usually an insufficient number of individuals to estimate a full 4 by 4 covariance matrix.

If we had reason to believe that the variance structure were the same in both groups then we could specify instead

$$b_i \sim N(0, D), \text{ where } D = \text{diag}(\sigma^2_B, \sigma^2_I, \sigma^2_p, \sigma^2_{\log s})$$ (2.7)

This is the approach taken in the illustrative example in Section 2.4.

2.2.1. Implementation as a nonlinear mixed model

The model (2.1)-(2.6) can be implemented as a nonlinear mixed model using widely available statistical computing systems including PROC NLMIXED (Nonlinear Mixed Models) in SAS, the ‘nlme’ (Linear and Nonlinear Mixed Effects Models) package in R, and numerous others.

2.3. SIMULATION

In order to assess the performance of the normal-errors NLMM relative to traditional approaches to modeling experimental infection data, we performed a Monte Carlo simulation experiment. In the one-sample setting we compared the accuracy and precision of parameter estimates for the normal-errors NLHM and the one-way RM-
ANOVA, and we computed the coverage rates of estimated confidence intervals for the parameter estimates from the former. (As we will see in §2.3.2, it is possible in RM-ANOVA to define ad hoc quantities analogous to the components of $\beta^{(g)}$ in the NLMM. However RM-ANOVA does not provide confidence intervals for those quantities so it is not possible to assess CI coverage for them.) In the two-sample setting we compared Type I error control and power for the normal-errors NLMM and the two-way RM-ANOVA.

2.3.1. Data-generating models

We explored cases where the functional form of the response trajectories is correctly specified, and also cases where it is not. For the former cases, we first simulated true parameter vectors $\beta_i$ for each individual subject as specified in (2.5)-(2.6), and then we simulated response profiles for those individuals according to (2.1)-(2.2).

To simulate incorrect specification of the functional form of the responses, we generated data from a normal-errors model with a parabolic response function (instead of Gaussian) as summarized in (2.8)-(2.11).

Level 1 (model of intra-individual variability)

$$y_{ij} \sim N(\mu_{ij}, \sigma^2)$$  (2.8)

$$\mu_{ij} = \begin{cases} a_i(t_j - h_i)^2 + (B_i - k_i) & \text{if } |t_j - h_i| \leq \sqrt{\frac{k_i}{a_i}} \\ 0 & \text{otherwise} \end{cases}$$  (2.9)

Level 2 (model of inter-individual variability)

$$\beta_i = \beta^{(g)} + b_i$$  (2.10)

$$b_i \sim N(0, D^{(g)})$$, where $D^{(g)} = \text{diag}(\sigma_{B^{(g)}}^2, \sigma_{k^{(g)}}^2, \sigma_{h^{(g)}}^2, \sigma_{a^{(g)}}^2)$  (2.11)
According to this data model, at the individual level, for time points in the interval 
\[ h_i \pm \sqrt{k_i/a_i} \] the mean response follows a parabolic trajectory. For time points outside of this interval, the mean response is equal to \( B_i \). The population parameters \( B, h \) and \( k \) correspond, respectively, to \( B, p \) and \( l \) in (2.3) with \( k = -l \). The parameter \( a \) governs the spread of the parabolic response. Although \( a \) itself has no direct relationship with \( s \) in (2.3), we can directly compare the estimation of response duration by comparing FWHM, which for the parabolic response function equals \( \sqrt{2k/a} \).

2.3.2. One-sample simulation: method

In the one-sample setting we compared the performance of the normal errors NLHM with \( g = 1 \) and the one-way RM-ANOVA model. The latter can be expressed as in (2.12)-(2.14), with \( \mu \) representing the fixed population mean, \( \pi_i \) a random effect for subject \( i \) that does not change with time, and \( \tau_j \) a fixed effect at time point \( j \) that is common to all subjects.

\[
y_{ij} = \mu + \pi_i + \tau_j + \varepsilon_{ij} \tag{2.12}
\]

\[
\pi_i \sim N(0, \sigma^2_{\pi}) \tag{2.13}
\]

\[
\varepsilon_{ij} \sim N(0, \sigma^2_{\varepsilon}) \tag{2.14}
\]

The focus of the one-sample simulation was to compare the accuracy and precision of estimation of population/fixed effects. We examined four scenarios which are described in detail below. For each scenario we randomly generated 1,000 data sets from the appropriate data model (i.e. Gaussian or parabolic). The normal-errors NLMM (2.1)-(2.6) and one-way RM-ANOVA models (2.12)-(2.14) were then fit to each simulated data set.
The means of the population baseline, intensity, time-to-peak and duration estimates over the 1,000 simulated data sets were calculated for both models, along with the bias, variance and mean squared error (MSE) for each of the means. For the normal-errors NLHM these quantities are directly estimated by the model. For RM-ANOVA, we must adopt *ad hoc* definitions for analogous quantities. The population baseline was taken to be the estimated response at the first time point, i.e. the estimated intercept. Peak response intensity was taken to be the difference between the largest estimated mean response and the baseline. Time to peak response was taken as the time point at which the group mean peak intensity was observed. FWHM was calculated by locating the time point on either side of the peak time where the estimated response was nearest in absolute value to one-half of the estimated peak intensity, and then taking the difference between those two times.

We also compared the mean standard errors ($\overline{SE}$) over the 1,000 data sets for the peak response intensity and FHWM estimates. These estimated standard errors are readily extracted from the fitted normal-errors NLMM. For RM-ANOVA, these quantities do not arise naturally from the model and can only be derived from the simulated data. Specifically, for RM-ANOVA, $\overline{SE}_B$ was taken to be the standard error of the estimated mean at the first time point, and $\overline{SE}_I$ was taken to be the standard error of the estimated mean at the time point where the peak response was observed.

Model fitting for the normal-errors NLMM was accomplished with the ‘nlme’ package in R (R core team 2012). Initial parameter estimates for ‘nlme’ were set to their true values from the data generating model. The RM-ANOVA model (2.12)-(2.14) was fitted via the lme() function in R.
The default data-generating parameters, unless otherwise specified, were set as follows.

Level 1 (*model of intra-individual variability*)
\[ \sigma = 5 \] (2.15)

Level 2 (*model of inter-individual variability*)
\[ \beta = [20, 30, 50, \log(3)]^T \text{ i.e. } B = 20, l = 30, p = 50, s = 3 \] (2.16)
\[ D = \text{diag}(9, 25, 4, 0.0625) \text{ i.e. } \sigma_B = 3, \sigma_l = 5, \sigma_p = 2, \sigma_s = 0.25 \] (2.17)

The default data collection schedule was every fifth day between days 0 and 40, then every second day between days 42 and 60, then every fifth time point again between days 65 and 100. This simulates the approach typically taken in experimental infection studies, where sampling is more intensive during the period when the response is expected to occur, and less frequent outside of that interval. We denote this sampling schedule by a vector \( t_0 \) to distinguish it from alternate schedules that were also considered.
\[ t_0 = [0, 5, \cdots, 35, 40, 42, \cdots, 58, 60, 65, \cdots, 100]^T \] (2.18)

### 2.3.3. One-sample simulation: results

*Effect of sample size on parameter estimation*

We simulated sample sizes of \( n = 5, 15 \) and 30 subjects while holding the true population response parameters constant as in (2.15)-(2.17) and the sampling schedule as in (2.18). Results are summarized in Table 2.1. Several points are noteworthy. 1) The parameter estimates from the NLMM are more precise at all three sample sizes, with relative efficiencies ranging from 1.5 to 25.9 in favor the NLMM. The difference in efficiency was greatest when estimating FWHM, a measure of response duration. The
efficiency advantage persisted at larger sample sizes. At the smallest sample size (n=5), neither model precisely estimated intensity (I), but NLMM was nevertheless 2.2 times more efficient than RM-ANOVA. 2) RM-ANOVA underestimates intensity between 13% and 18%, depending on sample size, and overestimates duration by 21%. Furthermore, neither bias diminishes as sample size increases, and the underestimation of intensity actually increases. This demonstrates that there is a systematic bias in the estimation of response intensity and duration by RM-ANOVA. 3) Both models estimate baseline and time to peak without bias at all three sample sizes, though again the NLMM estimates have uniformly greater precision. 4) For n=5, confidence interval coverage rates for population parameters in the NLMM were well below the nominal rate of 95% for, but for n=30 coverage was nominal or nearly so. The low coverage rates at small sample sizes are due largely to the fact that standard error estimates for the population parameters in the NLMM are conditional upon the variance component estimates. Thus, the standard error estimates do not reflect the uncertainty in the variance component estimates. 5) For RM-ANOVA, the average estimated standard error for the population baseline was more than double the average for NLMM at all three sample sizes, and the average estimated standard error for the population peak intensity was approximately 1.6 times larger.

Effect of sampling intensity on parameter estimation

Next we fixed the number of subjects at n=10 while varying the sampling schedule. We compared two schedules with similar numbers of total observations but different intensities: every third day over the entire study period and every second day within the true response interval but every fourth day outside the true response interval
We denote these two schedules by the vectors $t_{333}$ and $t_{424}$, which include 34 and 33 sampling occasions respectively.

$$t_{333} = [0, 3, 6, \cdots, 93, 96, 99]^T$$  \hspace{1cm} (2.19)

$$t_{424} = [0, 4, 8, \cdots, 36, 38, 40, \cdots, 92, 96, 100]^T$$  \hspace{1cm} (2.20)

The MSE’s in Table 2.2 show that estimation of peak intensity and duration were noticeably better for both models under $t_{424}$, and estimation of time to peak was improved for RM-ANOVA. Relative efficiencies still strongly favored the NLMM. We revisit the question of sampling intensity and schedule in Chapter 4.

**Effect of inter-individual response variability on parameter estimation**

Reports on experimental infection studies often mention considerable variability in the individual response trajectories, but the effect of this variability on the estimation of response parameters is rarely addressed. We analyzed the effect of inter-individual variation in the time to peak response. The number of individuals was held constant at N=15 and the sampling schedule at $t_0$ (2.18) while $\sigma_p$ was doubled from 2, as in (2.17), to 4. Results are summarized in Table 2.3. Increasing variability in the time to peak response negatively affects parameter estimation in both the NLMM and RM-ANOVA. However the former is far more robust to the increase, as can be seen by comparing the relative efficiencies of the parameter estimates for $\sigma_p = 2$ and $\sigma_p = 4$. In particular, the doubling of $\sigma_p$ results in a doubling of the underestimation of peak intensity for RM-ANOVA (from -4.8 to -11.0) and a tripling of the overestimation of FWHM (from 1.4 to 4.4).
Effect of incorrect response function specification on parameter estimation

Finally we examined performance of the NLMM (2.1)-(2.6) when the true response function is not Gaussian. To simulate the performance of the NLMM when correctly specified, the data were generated from the same model (2.1)-(2.6). To simulate performance when the response function is incorrectly specified, the data were generated from the parabolic data model in (2.8)-(2.11) with the following parameters.

Level 1 (model of intra-individual variability)

\[ \sigma = 5 \quad (2.21) \]

Level 2 (model of inter-individual variability)

\[ \beta = [20, -30, 50, -1.2]^T \text{ i.e. } B = 20, k = -30, h = 50, a = -1.2 \quad (2.22) \]

\[ D = \text{diag}(9, 25, 4, 0.04) \text{ i.e. } \sigma_B = 3, \sigma_k = 5, \sigma_h = 2, \sigma_a = 0.2 \quad (2.23) \]

The value of \( a \) in (2.23) for the parabolic response function was chosen so as to make the true FWHM the same as for the Gaussian response. Thus, the Gaussian and parabolic response functions are essentially identical with respect to baseline, timing of peak, peak intensity and FWHM. However the Gaussian is considerably wider in the tails and slightly narrower near the peak. The default sampling schedule \( t_0 \) (2.18) was used in both cases. Results are summarized in Table 2.4.

Comparing the estimated relative efficiencies we see that when the true underlying response function was parabolic rather than Gaussian, the NLMM was still much more efficient than RM-ANOVA.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>( n = 5 )</th>
<th>( n = 15 )</th>
<th>( n = 30 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NLMM</td>
<td>RM-ANOVA</td>
<td>Relative efficiency</td>
</tr>
<tr>
<td>B</td>
<td>20.0</td>
<td>20.0</td>
<td>-</td>
</tr>
<tr>
<td>Bias</td>
<td>-0.03 (0.05)</td>
<td>-0.02 (0.08)</td>
<td>-</td>
</tr>
<tr>
<td>Variance</td>
<td>2.2</td>
<td>6.7</td>
<td>0.7</td>
</tr>
<tr>
<td>MSE</td>
<td>2.2</td>
<td>6.7</td>
<td>3.0</td>
</tr>
<tr>
<td>CI coverage</td>
<td>86%</td>
<td>-</td>
<td>93%</td>
</tr>
<tr>
<td>I</td>
<td>30.1</td>
<td>26.1</td>
<td>-</td>
</tr>
<tr>
<td>Bias</td>
<td>0.14 (0.09)</td>
<td>-3.9 (0.13)</td>
<td>-</td>
</tr>
<tr>
<td>Variance</td>
<td>8.0</td>
<td>17.5</td>
<td>-</td>
</tr>
<tr>
<td>MSE</td>
<td>8.0</td>
<td>32.9</td>
<td>2.2</td>
</tr>
<tr>
<td>CI coverage</td>
<td>87%</td>
<td>-</td>
<td>92%</td>
</tr>
<tr>
<td>( p )</td>
<td>50.0</td>
<td>50.1</td>
<td>-</td>
</tr>
<tr>
<td>Bias</td>
<td>0.0 (0.01)</td>
<td>0.07 (0.04)</td>
<td>-</td>
</tr>
<tr>
<td>Variance</td>
<td>1.0</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>MSE</td>
<td>1.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>CI coverage</td>
<td>86%</td>
<td>-</td>
<td>93%</td>
</tr>
<tr>
<td>FWHM</td>
<td>7.0</td>
<td>8.5</td>
<td>-</td>
</tr>
<tr>
<td>Bias</td>
<td>-0.02 (0.02)</td>
<td>1.5 (0.09)</td>
<td>-</td>
</tr>
<tr>
<td>Variance</td>
<td>0.34</td>
<td>8.8</td>
<td>-</td>
</tr>
<tr>
<td>MSE</td>
<td>0.34</td>
<td>10.9</td>
<td>25.9</td>
</tr>
<tr>
<td>CI coverage</td>
<td>92%</td>
<td>-</td>
<td>92%</td>
</tr>
</tbody>
</table>

\( SE_B \) = \begin{array}{c|c|c|c|c|c} \hline SE_B & 1.3 (0.01) & 3.2 (0.01) & - & 0.8 (0.0) & 1.9 (0.0) & - & 0.6 (0.0) & 1.3 (0.0) & - \\
\hline SE_I & 2.5 (0.03) & 4.1 (0.02) & - & 1.5 (0.01) & 2.4 (0.01) & - & 1.1 (0.0) & 1.7 (0.0) & - \\
\hline \end{array}

B represents the population mean baseline level; I represents the population mean peak response intensity; \( p \) represents the population mean time to peak response; and FWHM represents the population mean full width at half maximum, which is a measure of the duration of the infection response.
Table 2.2. Effect of sampling intensity on parameter estimation in NLMM and one-way RM-ANOVA

| Parameter | Sampling schedule |  |  |
|-----------|------------------|------------------|------------------|------------------|------------------|
|           | $t_{333}$ | $t_{424}$ | Relative efficiency | $t_{333}$ | $t_{424}$ | Relative efficiency |
| $B$       | 20.0 | 20.0 | - | 20.0 | 20.1 | - |
| Bias      | -0.07 (0.03) | -0.03 (0.06) | - | 0.02 (0.03) | 0.09 (0.06) | - |
| Variance  | 0.98 | 3.5 | - | 0.94 | 3.4 | - |
| MSE       | 0.99 | 3.5 | 3.5 | 0.94 | 3.4 | 3.8 |
| $I$       | 30.5 | 24.7 | - | 30.3 | 25.4 | - |
| Bias      | 0.54 (0.07) | -5.3 (0.09) | - | 0.28 (0.06) | -4.6 (0.09) | - |
| Variance  | 4.8 | 8.8 | - | 4.0 | 9.0 | - |
| MSE       | 5.1 | 36.9 | 7.2 | 4.1 | 30.4 | 7.4 |
| $p$       | 50.0 | 50.2 | - | 50.0 | 50.0 | - |
| Bias      | -0.01 (0.02) | 0.21 (0.04) | - | 0.01 (0.02) | 0.03 (0.03) | - |
| Variance  | 0.44 | 1.8 | - | 0.48 | 1.1 | - |
| MSE       | 0.44 | 1.8 | 4.1 | 0.48 | 1.1 | 2.3 |
| $FWHM$    | 7.0 | 8.9 | - | 7.0 | 8.5 | - |
| Bias      | -0.07 (0.02) | 1.8 (0.06) | - | -0.04 (0.01) | 1.4 (0.04) | - |
| Variance  | 0.24 | 3.9 | - | 0.15 | 1.5 | - |
| MSE       | 0.25 | 7.2 | 28.9 | 0.16 | 3.5 | 21.9 |

$SE_B$ 0.9 (0.01) 2.1 (0.00) 0.93 (0.01) 2.2 (0.01)  
$SE_I$ 2.0 (0.01) 2.6 (0.01) 1.85 (0.01) 2.8 (0.01)  

B represents the population mean baseline level; I represents the population mean peak response intensity; $p$ represents the population mean time to peak response; and FWHM represents the population mean full width at half maximum, which is a measure of the duration of the infection response.
Table 2.3. Effect of variability in time to peak response on parameter estimation in one-sample NLMM and one-way RM-ANOVA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\sigma_p = 2$</th>
<th>$\sigma_p = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative efficiency</td>
<td>Relative efficiency</td>
</tr>
<tr>
<td>B</td>
<td>20.0 (0.02)</td>
<td>20.1 (0.02)</td>
</tr>
<tr>
<td>Bias</td>
<td>0.02 (0.00)</td>
<td>0.01 (0.00)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.7 (0.02)</td>
<td>0.74 (0.02)</td>
</tr>
<tr>
<td>MSE</td>
<td>0.7 (0.02)</td>
<td>0.8 (0.02)</td>
</tr>
<tr>
<td>I</td>
<td>30.3 (0.02)</td>
<td>28.9 (0.02)</td>
</tr>
<tr>
<td>Bias</td>
<td>0.32 (0.00)</td>
<td>-1.13 (0.00)</td>
</tr>
<tr>
<td>Variance</td>
<td>2.6 (0.02)</td>
<td>7.0 (0.02)</td>
</tr>
<tr>
<td>MSE</td>
<td>2.7 (0.02)</td>
<td>8.3 (0.02)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>50.0 (0.02)</td>
<td>49.9 (0.02)</td>
</tr>
<tr>
<td>Bias</td>
<td>0.02 (0.00)</td>
<td>-0.06 (0.00)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.3 (0.02)</td>
<td>1.3 (0.02)</td>
</tr>
<tr>
<td>MSE</td>
<td>0.3 (0.02)</td>
<td>1.3 (0.02)</td>
</tr>
<tr>
<td>FWHM</td>
<td>7.0 (0.02)</td>
<td>7.1 (0.02)</td>
</tr>
<tr>
<td>Bias</td>
<td>-0.05 (0.00)</td>
<td>4.4 (0.00)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.11 (0.01)</td>
<td>0.22 (0.01)</td>
</tr>
<tr>
<td>MSE</td>
<td>0.11 (0.01)</td>
<td>0.22 (0.01)</td>
</tr>
<tr>
<td>$SE_R$</td>
<td>0.8 (0.0)</td>
<td>0.8 (0.0)</td>
</tr>
<tr>
<td>$SE_I$</td>
<td>1.5 (0.0)</td>
<td>2.1 (0.0)</td>
</tr>
</tbody>
</table>

B represents the population mean baseline level; I represents the population mean peak response intensity; $p$ represents the population mean time to peak response; and FWHM represents the population mean full width at half maximum, which is a measure of the duration of the infection response.
Table 2.4. Comparison of NLMM and RM-ANOVA when NLMM response function is incorrectly specified

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gaussian response</th>
<th>Parabolic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NLMM</td>
<td>RM-ANOVA</td>
</tr>
<tr>
<td>B</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Bias</td>
<td>0.02 (0.03)</td>
<td>0.02 (0.05)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.7</td>
<td>2.3</td>
</tr>
<tr>
<td>MSE</td>
<td>0.7</td>
<td>2.3</td>
</tr>
<tr>
<td>I</td>
<td>30.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Bias</td>
<td>0.32 (0.05)</td>
<td>-4.8 (0.08)</td>
</tr>
<tr>
<td>Variance</td>
<td>2.6</td>
<td>6.0</td>
</tr>
<tr>
<td>MSE</td>
<td>2.7</td>
<td>28.8</td>
</tr>
<tr>
<td>( \mu )</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Bias</td>
<td>0.02 (-0.01)</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>MSE</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>FWHM</td>
<td>7.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Bias</td>
<td>-0.05 (0.01)</td>
<td>1.4 (0.03)</td>
</tr>
<tr>
<td>Variance</td>
<td>0.11</td>
<td>1.1</td>
</tr>
<tr>
<td>MSE</td>
<td>0.11</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\( S E_B \) = 0.8 (0.0) \( S E_I \) = 1.5 (0.01)

B represents the population mean baseline level; I represents the population mean peak response intensity; \( p \) represents the population mean time to peak response; and FWHM represents the population mean full width at half maximum, which is a measure of the duration of the infection response.
2.3.4. Two-sample simulation: method

We next compared the performance of the normal errors NLMM model with \( g \in \{1,2\} \), i.e. the two-sample NLMM model, with the two-way RM-ANOVA model. The latter can be expressed as follows, with \( \mu \) representing a fixed population mean, \( \gamma_g \) a fixed effect for group \( g \) that is common to all time points, \( \tau_j \) a fixed effect at time point \( j \) that is common to all subjects, \( (\gamma \tau)_{gj} \) a fixed effect unique to the members of group \( g \) that is specific to time \( j \), and \( \pi_{(i)g} \) a random effect for subject \( i \) within group \( g \) that does not change with time.

\[
Y_{igj} = \mu + \gamma_g + \tau_j + (\gamma \tau)_{gj} + \pi_{(i)g} + \epsilon_{igj}
\]  
(2.24)

\[
\pi_{(i)g} \sim N(0, \sigma_\pi^2)
\]  
(2.25)

\[
\epsilon_{igj} \sim N(0, \sigma_\epsilon^2)
\]  
(2.26)

\[
\sum_{g=1}^2 \gamma_g = 0, \ \sum_{j=1}^m \tau_j = 0, \ \sum_{g=1}^2 (\gamma \tau)_{gj} = 0, \ \sum_{j=1}^m (\gamma \tau)_{gj} = 0
\]  
(2.27)

In (2.27), \( m \) represents the final measurement occasion which, in the simulations, is the same for all individuals. The findings on parameter estimation from the one-sample simulation carry over to estimation in the two-sample setting. Our focus for the two-sample case, therefore, was a comparison of power and Type I error control for testing the null hypothesis of no difference between the true group infection responses. For RM-ANOVA this amounts to the hypothesis that the time-specific group effects are equal at all measurement occasions, i.e.

\[
H_0: (\gamma \tau)_{1j} = (\gamma \tau)_{2j} \text{ for all } j
\]  
(2.28)

We fit the two-sample RM-ANOVA model (2.24)-(2.27) and conducted the hypothesis test (2.28) using both the traditional approach, implemented in R by the
function aov() within the ‘stats’ package, and a mixed model approach using the R function lme() within the ‘nlme’ package, respectively.

For the NLMM (2.1)-(2.6) the hypothesis of no difference in the mean population infection responses can be expressed as

$$H_0: I^{(1)} = I^{(2)} \text{ and } p^{(1)} = p^{(2)} \text{ and } s^{(1)} = s^{(2)}.$$  \hspace{1cm} (2.29)

Following the recommendation of Pinheiro and Bates (2000) we tested this hypothesis via conditional F test.

Data were generated for each group independently using the same process described for the one-sample simulation.

2.3.5. Two-sample simulation: results

Type I error control for test of no difference in group responses

Response data for both groups were generated using the parameter values in (2.15)-(2.17) so that there was, in fact, no difference in the population infection responses. The experiment was repeated for group sizes ranging from N=5 to N=100 individuals. At each sample size 1,000 data sets were generated using both the Gaussian response function (2.2) and the parabolic response function (2.9). The hypotheses (2.28) and (2.29) were tested as describe above and the proportion of Type I errors was computed for each method along with 95% Clopper-Pearson confidence intervals. Thus we examined the Type I error rates for NLHM and RM-ANOVA at six different sample sizes when the response function in the NLHM was correctly specified and when it was incorrectly specified.

Results are summarized in Table 2.5. We consider first the case when the true response function was Gaussian. For larger sample sizes \(n = 15\), \(n = 30\) the Type I error
rate was near the nominal rate for both NLMM and RM-ANOVA [aov() implementation). However for smaller sample sizes \((n = 5, n = 10)\) the Type I error rate for RM-ANOVA was nearly twice the nominal rate and for NLMM it was two to three times higher. When the true response function was parabolic, NLMM was not much affected. However RM-ANOVA fared slightly worse at all sample sizes.

For the lme() implementation of RM-ANOVA, the rate remained two to three times above the nominal rate across all sample sizes from \(n=5\) to \(n=100\), irrespective of the true shape of the response function.

Table 2.5. Type I error rate by sample size for NLMM and RM-ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Gaussian data model</th>
<th>Parabola data model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NLMM</td>
<td>RM-ANOVA</td>
</tr>
<tr>
<td><strong>5</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(0.13, 0.18)</td>
<td>(0.07, 0.11)</td>
</tr>
<tr>
<td><strong>10</strong></td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.08, 0.12)</td>
<td>(0.06, 0.09)</td>
</tr>
<tr>
<td><strong>15</strong></td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.07, 0.11)</td>
<td>(0.05, 0.08)</td>
</tr>
<tr>
<td><strong>30</strong></td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.06, 0.09)</td>
<td>(0.06, 0.09)</td>
</tr>
<tr>
<td><strong>50</strong></td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>(0.04, 0.07)</td>
<td>(0.05, 0.08)</td>
</tr>
<tr>
<td><strong>100</strong></td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>(0.04, 0.07)</td>
<td>(0.05, 0.08)</td>
</tr>
</tbody>
</table>

Power to detect a true difference in group responses

To investigate the power of NLHM and RM-ANOVA to detect true differences in population infection responses between groups, we considered three scenarios:

\[
I^{(1)} \neq I^{(2)}, \quad p^{(1)} = p^{(2)}, \quad s^{(1)} = s^{(2)} \quad (2.30)
\]

\[
I^{(1)} = I^{(2)}, \quad p^{(1)} \neq p^{(2)}, \quad s^{(1)} = s^{(2)} \quad (2.31)
\]

\[
I^{(1)} = I^{(2)}, \quad p^{(1)} = p^{(2)}, \quad s^{(1)} \neq s^{(2)} \quad (2.32)
\]
Figure 2.1. Population response curves for two-sample simulation. (A) Peak intensity: $I^{(1)} = 30$ vs. $I^{(2)} = 36$. (B) Response duration: $s^{(1)} = 2.5$ vs. $s^{(2)} = 3$ [FWHM$^{(1)} = 5.9$ vs. FWHM$^{(2)} = 7.1$ days]. (C) Time to peak: $p^{(1)} = 49$ vs. $p^{(2)} = 51$ days.
As in the Type I error experiment we began with the true parameter values for both groups specified as in (2.15)-(2.17). For scenario (2.30), we increased $I^{(2)}$ from 30 to 36; for scenario (2.31) we decreased $p^{(1)}$ to 49 and increased $p^{(2)}$ to 51; and for scenario (2.32) we decreased $s^{(1)}$ from 3 to 2.5, which is equivalent to decreasing FWHM$^{(1)}$ from 7.1 days to 5.9 days. The three scenarios are visualized in Figure 2.1.

### Table 2.6. Effect of number of subjects on power to detect a true difference in response to infection in two-sample NLMM and two-way RM-ANOVA

<table>
<thead>
<tr>
<th>Parameter and model</th>
<th>$n=5$</th>
<th>$n=15$</th>
<th>$n=30$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak intensity ($I$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.40 (0.37, 0.43)</td>
<td>0.64 (0.61, 0.67)</td>
<td>0.92 (0.90, 0.93)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.14 (0.12, 0.17)</td>
<td>0.23 (0.20, 0.26)</td>
<td>0.42 (0.40, 0.45)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.21 (0.18, 0.23)</td>
<td>0.35 (0.32, 0.38)</td>
<td>0.57 (0.54, 0.60)</td>
</tr>
<tr>
<td><strong>Response duration (FWHM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.43 (0.40, 0.46)</td>
<td>0.71 (0.68, 0.73)</td>
<td>0.94 (0.92, 0.95)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.10 (0.08, 0.12)</td>
<td>0.11 (0.09, 0.13)</td>
<td>0.19 (0.16, 0.21)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.18 (0.15, 0.20)</td>
<td>0.20 (0.18, 0.23)</td>
<td>0.32 (0.30, 0.35)</td>
</tr>
<tr>
<td><strong>Time to peak intensity ($p$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.39 (0.36, 0.42)</td>
<td>0.67 (0.64, 0.70)</td>
<td>0.91 (0.89, 0.92)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.37 (0.34, 0.40)</td>
<td>0.74 (0.71, 0.77)</td>
<td>0.96 (0.94, 0.97)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.45 (0.41, 0.48)</td>
<td>0.82 (0.79, 0.84)</td>
<td>0.97 (0.96, 0.98)</td>
</tr>
</tbody>
</table>

For all scenarios we estimated the power of each method at three sample sizes (N=5, 15, 30). At each sample size 1,000 data sets were generated using both the Gaussian response function (2.2) and the parabolic response function (2.9). The hypotheses (2.28) and (2.29) were tested as described above and power was estimated as
the proportion of data sets for which the null hypothesis was correctly rejected, along with 95% Clopper-Pearson confidence intervals. The two data models correspond to correct and incorrect specification of the mean response function for the NLHM.

Results are summarized in Table 2.6. We find that the NLMM, when correctly specified, has between two and six times the power of RM-ANOVA to detect a true difference in response duration or intensity at sample sizes ranging from \( n=5 \) to \( n=30 \). Interestingly, RM-ANOVA outperformed the NLMM by a modest amount for detecting a difference in time to peak. Misspecifying the response function as Gaussian when it was truly parabolic did not adversely affect power (Table 2.7).

**Table 2.7.** Effect of number of subjects on power to detect a true difference in response to infection in two-sample NLMM and two-way RM-ANOVA – NLMM mean response function incorrectly specified

<table>
<thead>
<tr>
<th>Parameter and model</th>
<th>Sample size</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n=5 )</td>
<td>( n=15 )</td>
</tr>
<tr>
<td>Peak intensity (( I ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.44 (0.41, 0.47)</td>
<td>0.73 (0.70, 0.76)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.18 (0.15, 0.20)</td>
<td>0.36 (0.33, 0.39)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.25 (0.22, 0.28)</td>
<td>0.47 (0.44, 0.50)</td>
</tr>
<tr>
<td>Response duration (FWHM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.39 (0.35, 0.42)</td>
<td>0.67 (0.64, 0.70)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.10 (0.08, 0.12)</td>
<td>0.14 (0.12, 0.16)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.16 (0.14, 0.18)</td>
<td>0.24 (0.21, 0.26)</td>
</tr>
<tr>
<td>Time to peak intensity (( p ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLHM</td>
<td>0.39 (0.35, 0.42)</td>
<td>0.62 (0.59, 0.65)</td>
</tr>
<tr>
<td>RM-ANOVA – aov()</td>
<td>0.36 (0.33, 0.39)</td>
<td>0.77 (0.74, 0.79)</td>
</tr>
<tr>
<td>RM-ANOVA – lme()</td>
<td>0.45 (0.41, 0.48)</td>
<td>0.83 (0.80, 0.85)</td>
</tr>
</tbody>
</table>
2.4. ILLUSTRATIVE EXAMPLE

Referring to the EAV case studies from Chapter 1, we compare the population febrile responses to the Bucyrus and Kentucky-84 strains of EAV. In the experiments by Go and Campos, core body temperature (CBT) was observed on days -7, -4 and -2 prior to inoculation and on days 0, 2, 4, 6, 8, 10, 12 and 14 post-inoculation (DPI). Go et al challenged eight mares with the Bucyrus strain whereas Campos et al challenged eight stallions with the KY-84 strain. Figure 4 presents the observed, subject-specific febrile response profiles for the two groups.

![Figure 2.2. Observed febrile response profiles for eight mares challenged with the Bucyrus strain of EAV, and eight stallions challenged with the Kentucky-84 strain](image)

All sixteen profiles display a single-peaked response with a common pre- and post-infection baseline body temperature. We modeled the febrile responses using the NLHM in (2.1)-(2.7) with $g \in \{1,2\}$. The estimate of $\sigma_{\log(s)}$ was essentially zero, suggesting no need to include subject-specific scale parameters in the model. We refit the model with the scale parameters treated as purely fixed effects. Diagnostic checks did not
indicate any violations of distributional assumptions (see supplemental plots in Appendix A). The posterior estimates are summarized in Table 2.8.

**Table 2.8.** Estimates of population febrile response parameters following challenge of eight mares with Bucyrus strain of EAV and eight stallions with KY-84 strain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bucyrus strain (mares)</th>
<th>KY-84 strain (stallions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Population infection response parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( B^{(1)} )</td>
<td>99.4</td>
<td>(99.1, 99.7)</td>
</tr>
<tr>
<td>( i^{(1)} )</td>
<td>3.3</td>
<td>(2.8, 3.8)</td>
</tr>
<tr>
<td>( p^{(1)} )</td>
<td>6.3</td>
<td>(6.0, 6.6)</td>
</tr>
<tr>
<td>( \log(s)^{(1)} )</td>
<td>0.86</td>
<td>(0.71, 1.02)</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma_B )</td>
<td>0.33</td>
<td>(0.20, 0.56)</td>
</tr>
<tr>
<td>( \sigma_i )</td>
<td>0.47</td>
<td>(0.22, 0.99)</td>
</tr>
<tr>
<td>( \sigma_p )</td>
<td>0.23</td>
<td>(0.09, 0.57)</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>0.82</td>
<td>(0.75, 0.89)</td>
</tr>
</tbody>
</table>

The population parameters characterize the mean response to inoculation with the Bucyrus strain of EAV in mares (superscript \( g = 1 \)) and the KY-84 strain in stallions (superscript \( g = 2 \)). (More precisely, they represent the mean infection response parameter values for the populations from which the samples were drawn. If the samples were selected at random from a well-defined population then the posterior means can be interpreted as estimates of the true mean values within the source population.) The estimates of baseline CBT differ by only 0.03 °F (-0.50, 0.43), as might be expected. The duration of fever was similar also, with the scale parameter estimates differing by -0.05 (-0.24, 0.14) units on the log scale. This translates to a difference in FWHM of 0.3 (-0.8, 1.2) days. However they differed with respect to peak fever intensity and time to peak fever. On average, in the stallions challenged with the KY-84 strain, the febrile response
peaked 0.6 (-1.1, -0.2) days earlier with a maximum intensity 1.3 (0.6, 2.0) degrees higher. The 95% confidence intervals suggest that the true differences in significantly different than zero. Figure 2.3 illustrates the population febrile response curves for the two groups.

Figure 2.3. Population febrile response curves for mares challenged with the Bucyrus strain of EAV, and stallions challenged with the Kentucky-84 strain

Formally testing the hypothesis of no difference in population infection responses (2.29) with a conditional F test, we find evidence in favor of rejecting \([F=7.1, p=0.0001, \text{df}=(3,265)]\). Furthermore, testing hypothesis (2.28) with the two-way RM-ANOVA model (2.24)-(2.26) leads to the same conclusion \((F=4.3, p<0.0001)\).

The variance component estimates quantify the extent to which individual febrile responses may vary about their group’s population response. Thus for example, for a particular mare from the source population inoculated with the Bucyrus strain of EAV, it would be quite unusual to observe a fever that peaked at less than \(I^{(1)} - 1.96\sigma_I = 2.4\text{°F}\) above baseline CBT or at higher than \(I^{(1)} + 1.96\sigma_I = 4.2\text{°F}\) above baseline. The estimates of \(\sigma_B\) and \(\sigma_p\) have similar interpretations.
2.5. DISCUSSION

In this chapter we proposed a nonlinear mixed model (2.1)-(2.6) suitable for modeling a continuous, longitudinal response variable having normally distributed errors with constant variance across all measurement occasions. Example of such responses include quantities such as body temperature and lymphocyte counts. The model assumes that the systematic portion of the intra-individual response variation follows a Gaussian trajectory. Thus it is appropriate for situations in which the individual responses are approximately symmetric about a single peak. Implementation is straightforward using the NLMM framework (Lindstrom and Bates 1990, Davidian and Giltinan 1995) available in many standard software packages.

We proposed this model as an alternative to RM-ANOVA, which has traditionally been the most commonly used method of analyzing longitudinal response data from EI studies. The primary difference between the two approaches is that RM-ANOVA treats the responses at each time point as if they were unrelated to one another, whereas the NLMM models the relationship among the responses across time points through individual mean response functions (2.2). One consequence is that in RM-ANOVA each additional measurement occasion increases by one the number of degrees of freedom used to estimate the model. By contrast, the number of degrees of freedom in estimating the NLHM does not depend on the number of time points observed. We hypothesized that this difference would result in less efficient estimation of model parameters for RM-ANOVA, particularly when the number of measurement occasions is large. Another advantage of the NLMM is that its parameters represent aspects of the infection response of inherent biological interest: baseline, time to peak response, peak intensity and
response duration. In RM-ANOVA such quantities can only be approximated by ad-hoc methods.

2.5.1. Simulation: estimation

We tested our primary hypothesis via Monte Carlo simulation experiments. Simulation results indicated that the NLHM substantially outperformed RM-ANOVA across a broad range of scenarios, and that under certain conditions the improvement can be enormous. We found estimation in the NLHM to be more precise than RM-ANOVA for all model parameters, and less biased for peak intensity and duration parameters.

We also demonstrated the effect of increased inter-individual response variability on parameter estimation. It seems self-evident to us that, unless there is very little variation in the timing of the individual peaks, any approach that involves naïvely averaging a group of single-peaked response profiles in a pointwise manner will result in understated peak intensity and overstated response duration. When the individual peaks occur at different times the effect will be to dampen the estimate of the population peak intensity. Further, the occurrence of even a small number of responses of unusually long duration will give the appearance, under pointwise averaging, that the population response duration is longer than it truly is. This effect has been noted recently in an EI study by DeVincenzo and colleagues (2010). Specifically, in studying viral loads in subjects experimentally challenged with respiratory synctitial virus, they point out that when the individual viral load curves are naïvely averaged the breadth (duration) of the resulting population curves is misleadingly wide, and the magnitude (intensity) misleadingly low. (They attempted to correct for this effect by adjusting the individual viral load curves based on an arbitrary “incubation time”. Our method requires no such
ad-hoc adjustment.) Our simulation experiment confirmed that increasing variability in the time to peak response negatively affects parameter estimation in both the NLMM and RM-ANOVA, but that the former is far more robust to the increase.

Finally, we explored the effect of incorrect specification of the mean response function on parameter estimation. We found that when the true underlying response function was parabolic rather than Gaussian, the NLMM was still considerably more efficient than RM-ANOVA.

2.5.2. Simulation: type I error control and power

When the number of subjects per group was 5, the Type I error rate for RM-ANOVA was nearly twice the nominal rate and for NLMM it was three times the nominal rate. For \( n = 10 \) the Type I error rate for NLMM was still twice nominal. These results suggest that experimental infection studies with small numbers of individuals per group – which appear to be the norm in the literature – are subject to higher-than-nominal Type I error rates, with either NLMM or RM-ANOVA.

Our power analysis indicates that in certain scenarios the NLMM provides a large increase in power over RM-ANOVA with comparable Type I error control. In our simulation, in all scenarios the NLMM achieved 80\% power to detect differences in response duration and peak intensity with somewhere between 15 and 30 subjects per group. By contrast, with 30 subjects per group RM-ANOVA achieved only 42\% power for a true difference in peak intensity and 19\% power for a true difference in response duration.
2.5.3. Illustrative example

When applied to the EAV febrile response data, both the NLMM and RM-ANOVA detected a significant difference in the mean population febrile response of mares challenged with the recombinant Bucyrus strain compared to the response of stallions challenged with the KY-84 strain. Judging from Figure 2.3, the essential difference between the febrile responses appears to be in intensity of the peak. For the simulated data the relative change in intensity was \( \frac{I^{(2)}-I^{(1)}}{I^{(1)}} = \frac{36-30}{30} = 0.2 \), and for the febrile response data we can estimate \( \frac{4.6-3.3}{3.3} = 0.39 \). In other words, in the febrile response data we were searching for a relative change that was twice the size of the relative change in the simulated data. In this case the change was large enough to be detected by either method.
CHAPTER THREE

A Bayesian hierarchical model for a single-peaked, asymmetric infection response

3.1. INTRODUCTION

In Chapter 2 we presented the normal-errors NLHM as an alternative to repeated measures analysis of variance (RM-ANOVA) for analyzing time course data from experimental infection studies. We demonstrated, via Monte Carlo simulation experiments, scenarios in which the NLHM provides more accurate and precise estimation of population parameters, and greater power for detecting group differences, than RM-ANOVA. Furthermore, at moderate-to-large sample sizes (N=15 or greater) it provides near-nominal Type I error control.

Chapter 2 focused on the case of a symmetric response. The symmetric model was implemented as a nonlinear mixed model (NLMM) (Sheiner et al 1972, Lindstrom and Bates 1990, Davidian and Giltinan 1995, Vonesh and Chinchilli 1997). One attractive feature of this approach is the availability of several widely used software packages for fitting NLMM’s. However it is important to note that maximum likelihood estimation and inference for NLMM’s are based on asymptotic results which are not guaranteed to hold for the relatively small sample sizes typical of EI studies.

In the present chapter we consider asymmetric responses within the context of the normal-errors NLHM. A Bayesian approach to model implementation is introduced in part to avoid the large-sample assumptions of the NLMM approach, but it proves to have several additional benefits which we discuss.
3.2. NORMAL-ERRORS NLHM FOR AN ASYMMETRIC RESPONSE

3.2.1. Modeling an asymmetric infection response

In Chapter 2 we introduced a model for a symmetric infection response based on a Gaussian mean response function:

\[ f(t) = B + I \exp \left[ - \left( \frac{t-p}{2s} \right)^2 \right] \]  

(3.1)

One way to extend this model to accommodate asymmetric responses is to replace (3.1) with a piecewise function consisting of two half-Gaussian curves that are given different scale parameters but constrained to meet at their peaks, as in (3.2).

\[ f(t) = \begin{cases} 
B + I \exp \left[ - \left( \frac{t-p}{2l} \right)^2 \right], & t \leq p \\
B + I \exp \left[ - \left( \frac{t-p}{2r} \right)^2 \right], & t > p 
\end{cases} \]  

(3.2)

The interpretations of the parameters \( B, I \), and \( p \) are unchanged. The scale parameters \( l \) and \( r \) can be interpreted, respectively, as measures of the duration of the onset and recovery phases of infection. If more interpretable measures of these quantities are desired, \( l \) and \( r \) are directly proportional to the half-widths at half-maximum intensity (HWHM): \( HWHM_l = l \sqrt{2 \ln 2} \) and \( HWHM_r = r \sqrt{2 \ln 2} \). The full width at half-maximum intensity (FWHM) can be interpreted as an index of total response duration: \( FWHM = HWHM_l + HWHM_r = (l + r) \sqrt{2 \ln 2} \). Figure 3.1 demonstrates the flexibility of the piecewise half-Gaussian function to describe a wide range of response trajectories. Other applications of piecewise functions in nonlinear regression models have been described by Huisman (1993), Müller (1997) and Gössl (2001).
3.2.2. Statement of the model

We are now in a position to state the normal-errors NLHM for an asymmetric infection response, using a piecewise Gaussian kernel for the mean response function. Suppose that infection responses for $n$ individuals selected at random from a target population have been sampled on multiple occasions. For generality we allow the number of observations to vary among individuals and we denote by $m_i$ the number of sampling occasions for individual $i$. Let $j$ index the sampling times $t_1, t_2, \ldots, t_{m_i}$ for individual and denote the responses for individual $i$ as $y_{i1}, y_{i2}, \ldots, y_{im_i}$.
Level 1 (model of intra-individual variability)
\[ y_{ij} \sim N(\mu_{ij}, \sigma^2) \]  \hspace{1cm} (3.3)
\[ \mu_{ij} = \begin{cases} B_i + I_i \exp \left[ \frac{-(t_j-p_i)^2}{2l_i^2} \right], & t_j \leq p_i \\
B_i + I_i \exp \left[ \frac{-(t_j-p_i)^2}{2r_i^2} \right], & t_j > p_i \end{cases} \]  \hspace{1cm} (3.4)

Level 2 (model of inter-individual variability)
\[ \beta_i = \beta^{(g)} + b_i \]  \hspace{1cm} (3.5)
\[ b_i \sim N(0, D^{(g)}), \text{ where } D^{(g)} = \text{diag}(\sigma^2_{B^{(g)}}, \sigma^2_{I^{(g)}}, \sigma^2_{p^{(g)}}, \sigma^2_{\log l^{(g)}}, \sigma^2_{\log r^{(g)}}) \]  \hspace{1cm} (3.6)

The scale parameters \( l_i \) and \( r_i \) are modeled on the log scale to restrict their ranges to the positive real numbers. As in Chapter 2, we assume that individual \( i \) belongs to group \( g \), where \( g \in \{1,2\} \), and that \( \beta_i \) and \( \beta^{(g)} \) are defined as follows.
\[ \beta_i = [B_i, I_i, p_i, \log (l_i), \log (r_i)]^T. \]  \hspace{1cm} (3.7)
\[ \beta^{(g)} = [B^{(g)}, I^{(g)}, p^{(g)}, \log l^{(g)}, \log r^{(g)}]^T \]  \hspace{1cm} (3.8)

If there is reason to think that the inter-individual variability is not significantly different between the two groups then in 3.6 we can alternatively specify that \( D^{(g)} = \text{diag}(\sigma^2_{B^{(g)}}, \sigma^2_{I^{(g)}}, \sigma^2_{p^{(g)}}, \sigma^2_{\log l^{(g)}}, \sigma^2_{\log r^{(g)}}) \).

3.2.3. Bayesian implementation

In principle the NLHM (3.3)-(3.8) may be implemented within either a frequentist or Bayesian framework. We pursued a Bayesian approach in part to avoid the large-sample assumptions of the NLMM approach, but there are numerous additional benefits. Model specification is entirely under the control of the analyst. This can simplify diagnosis of the convergence problems that often arise in NLHM’s with small samples. It also provides finer control over the details of model specification than may be possible in
many off-the-shelf software packages. Furthermore, calculation of various secondary quantities of interest, such as credible intervals on mean response curves and timing of response onset and recovery, is straightforward.

A Bayesian perspective considers the model parameters $\beta, \sigma$ and $D$ not as fixed, unknown quantities, but as random variables with probability distributions. The investigator’s a priori knowledge about the likely values of these parameters is specified by prior distributions $p(\beta), p(\sigma)$ and $p(D)$. The joint posterior distribution of the model parameters is obtained via Bayes’ Theorem, which updates the prior based on the likelihood of the observed data obtained from the EI study (LeSaffre and Lawson 2012). Inference is then based on the resulting joint posterior distribution of $\beta, \sigma$ and $D$.

We specified uniform priors of the form $(0,c)$ for the residual standard deviation $\sigma$ and for all standard deviation parameters in $D$, thus reflecting vague prior knowledge about their likely values. Gelman (2006) has recommended uniform priors of this form for the variance component parameters in hierarchical models over the more traditional choices of inverse Gamma and Wishart priors. For the population infection response parameters in $\beta$ we also specified uniform $(0,c)$ priors for the scale parameters $\log l$ and $\log r$, the intensity parameter $I$, and the time to peak parameter $p$. Finally we specified a uniform $(a,b)$ prior for the baseline parameter $B$. The constants $a$, $b$ and $c$ reflect bounds on the plausible population mean values. Gaussian priors with large variances are often used for population parameters in hierarchical models (LeSaffre and Lawson 2012). This would be a reasonable alternative to the uniform prior for the $B$, but not for the other infection response parameters. Note that another advantage of the Bayesian approach is...
the ability to incorporate prior knowledge about the parameters, if available, into the model specification via informative prior distributions.

All models were fit using R version 2.15 interfaced with JAGS through the R2JAGS package (R core team 2012, Plummer 2003).

3.3. ILLUSTRATIVE EXAMPLES

3.3.1. Lymphocyte response to equine arteritis virus infection in horses

Our first example references the case studies on equine arteritis virus (EAV) from Chapter 1. In the experimental infection study by Go et al (2011), lymphocyte counts were determined from blood samples collected at 0, 2, 4, 6, 8, 10, 12, 14, 21, 28, 35 and 42 days following challenge with a virulent, recombinant Bucyrus strain of EAV. In the original study there was a total of eight mares belonging to two groups, one with the in vitro susceptible CD3+ T cell phenotype and one with the resistant phenotype, and the purpose was to compare the time course of lymphocyte counts (and other responses) between the two groups. For the present example we ignore the group structure and consider the eight mares as a single sample. Figure 3.2 presents the observed, subject-specific febrile response profiles for the two groups.

All eight profiles suggest a single-peaked response with a common pre- and post-infection baseline lymphocyte count, although the baseline level varies considerably among horses. Because the counts appear to be less variable during the response phase than during baseline when the individuals are not infected, we applied a square root transformation of the responses to stabilize the variance over the experimental period. We modeled the transformed lymphocyte responses using the asymmetric normal-errors NLHM in (3.3)-(3.6), with $g = 1$. Satisfactory convergence was achieved with 75,000
MCMC iterations following 25,000 burn-in. Convergence diagnostics and supplemental plots are included in Appendix A. The posterior estimates are summarized in Table 3.1.

**Figure 3.2** Observed lymphocyte response profiles for eight mares challenged with the virulent, recombinant Bucyrus strain of EAV

**Figure 3.3** Square root-transformed lymphocyte response profiles
The population parameters characterize the mean lymphocyte response of mares to inoculation with the Bucyrus strain of EAV. (More accurately, they represent the mean infection response parameter values for the populations from which the sample was drawn. If the samples were selected at random from a well-defined population then the posterior means can be interpreted as estimates of the true mean values within the source population.) On the transformed (square root) scale, the mean population baseline lymphocyte level was estimated to be 1.6 $K/\mu L$, with a posterior 95% credible interval (1.5, 1.7). Thus, the probability is 95% that the true (transformed) population baseline lymphocyte level is between 1.5 and 1.7 $K/\mu L$. The mean population time to peak response was 8.3 days (7.6, 9.0), and the maximum decline in lymphocyte level was -0.8 $K/\mu L$ (-1.0, -0.6). The mean population $FWHM$ was 5.5 days (4.4, 6.8), and the large difference in $HWHM_l$ [4.1 (3.1, 5.1)] and $HWHM_r$ [1.4 (1.2, 2.2)] indicates that the time from onset to maximum lymphocyte decrease was nearly triple the time for the return from peak to baseline lymphocyte level.

The variance component estimates quantify the extent to which individual lymphocyte response parameters may vary about their corresponding population mean values. Thus for example, for a particular mare from the source population inoculated with the Bucyrus strain of EAV, it would be unusual to observe a maximum drop in (square root-transformed) lymphocyte count of less than $I - 1.96\sigma_l = -0.4 K/\mu L$ below baseline count or more than $I + 1.96\sigma_l = -1.2 K/\mu L$ below baseline. The population mean response curve is presented in Figure 3.4.
Table 3.1. Posterior estimates of population lymphocyte response parameters following challenge of eight mares with the virulent, recombinant Bucyrus strain of EAV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>2.5% quantile</th>
<th>97.5% quantile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population infection response parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$B$</td>
<td>1.6</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>$I$</td>
<td>-0.8</td>
<td>-1.0</td>
<td>-0.6</td>
</tr>
<tr>
<td>$p$</td>
<td>8.3</td>
<td>7.6</td>
<td>9.0</td>
</tr>
<tr>
<td>$FWHM$</td>
<td>5.5</td>
<td>4.4</td>
<td>6.8</td>
</tr>
<tr>
<td>$HWHM_L$</td>
<td>4.1</td>
<td>3.1</td>
<td>5.1</td>
</tr>
<tr>
<td>$HWHM_R$</td>
<td>1.4</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma_B$</td>
<td>0.22</td>
<td>0.12</td>
<td>0.42</td>
</tr>
<tr>
<td>$\sigma_I$</td>
<td>0.21</td>
<td>0.05</td>
<td>0.47</td>
</tr>
<tr>
<td>$\sigma_p$</td>
<td>0.32</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>$\sigma_{log l}$</td>
<td>0.17</td>
<td>0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>$\sigma_{log r}$</td>
<td>0.92</td>
<td>0.09</td>
<td>1.94</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.68</td>
<td>0.03</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Figure 3.4. Population lymphocyte response curve, with pointwise 95% posterior credible intervals, for eight mares following experimental challenge with the virulent, recombinant Bucyrus strain of EAV. Circles indicate observed lymphocyte counts.
3.3.2. Febrile response to equine arteritis virus infection in horses

For our second example we revisit the comparison of population febrile responses to the Bucyrus and Kentucky-84 strains of EAV. This example was discussed in Chapter 2 where we modeled the febrile responses using the symmetric normal-errors NLHM (2.1)-(2.7). To review briefly, two separate experiments were conducted in which core body temperature (CBT) was observed on days -7, -4 and -2 prior to inoculation and on days 0, 2, 4, 6, 8, 10, 12 and 14 post-inoculation (DPI). In one of the experiments eight mares were challenged with the Bucyrus strain of EAV whereas in the second eight stallions were challenged with the KY-84 strain. Figure 3.5 presents the observed, subject-specific febrile response profiles for the two groups.

![Figure 3.5](image)

**Figure 3.5** Observed febrile response profiles for eight mares challenged with the Bucyrus strain of EAV, and eight stallions challenged with the Kentucky-84 strain

All sixteen profiles suggest a single-peaked response with a common pre- and post-infection baseline body temperature. We modeled the febrile responses using the asymmetric normal-errors NLHM in (3.3)-(3.6), with \( g \in \{1,2\} \). Satisfactory
convergence was achieved with 100,000 MCMC iterations per three chains, including 25,000 burn-in. Convergence diagnostics and supplemental plots are included in Appendix A. Posterior estimates are summarized in Table 3.2.

In many respects the febrile responses of the two groups were quite similar. The estimates of baseline CBT for example, $B^{(1)} = 99.4^\circ F$ and $B^{(2)} = 99.3^\circ F$, are nearly identical as might be expected. However they differed with respect to peak fever intensity and time to peak fever. On average, in the stallions challenged with the KY-84 strain, the febrile response peaked $p^{(2)} = 7.1$ days after inoculation at an intensity of $I^{(2)} = 4.7^\circ F$ above baseline CBT. The 95\% posterior credible intervals (CI) for $p^{(2)}$ and $I^{(2)}$ indicate that the true population means for time-to-peak and peak intensity could plausibly lie in the intervals (6.6, 7.4) and (4.1, 5.2), respectively. By comparison, in the mares challenged with the Bucyrus strain, the febrile response peaked $p^{(1)} = 7.8$ days (7.3, 8.4) after inoculation at an intensity of $I^{(1)} = 3.5^\circ F$ (2.7, 4.2) above baseline CBT.

Expressing the posterior means for the scale parameters $l^{(g)}$ and $r^{(g)}$ in terms of $HWHM$ and $FWHM$ increases their interpretability, so we report only those means in Table 3.2. We see that there was little difference between the groups in the mean duration of the febrile response, with $FWHM^{(1)} = 5.7$ days (4.8, 7.2) and $FWHM^{(2)} = 5.6$ days (4.8, 6.5). The response in both groups was strongly asymmetric as indicated by the posterior means for $HWHM_{l}^{(1)}$ and $HWHM_{l}^{(2)}$ being more than three times the posterior means for $HWHM_{r}^{(1)}$ and $HWHM_{r}^{(2)}$. In other words, recovery from fever was much more abrupt than onset.
Table 3.2. Posterior estimates of population febrile response parameters following challenge of eight mares with the Bucyrus strain of EAV and eight stallions with the KY-84 strain of EAV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bucyrus strain (mares)</th>
<th>Kentucky-84 strain (stallions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>2.5% quantile</td>
</tr>
<tr>
<td>Population infection response parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( B^{(1)} )</td>
<td>99.4</td>
<td>99.0</td>
</tr>
<tr>
<td>( I^{(1)} )</td>
<td>3.5</td>
<td>2.7</td>
</tr>
<tr>
<td>( p^{(1)} )</td>
<td>7.8</td>
<td>7.3</td>
</tr>
<tr>
<td>( FWHM^{(1)} )</td>
<td>5.7</td>
<td>4.8</td>
</tr>
<tr>
<td>( HWHM_{l}^{(1)} )</td>
<td>4.3</td>
<td>3.5</td>
</tr>
<tr>
<td>( HWHM_{r}^{(1)} )</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma_{B}^{(1)} )</td>
<td>0.46</td>
<td>0.17</td>
</tr>
<tr>
<td>( \sigma_{I}^{(1)} )</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>( \sigma_{p}^{(1)} )</td>
<td>0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>( \sigma_{\log l}^{(1)} )</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>( \sigma_{\log r}^{(1)} )</td>
<td>0.81</td>
<td>0.04</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>0.68</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Interpretation of the variance component estimates is the same as in the one-sample case. Thus for example, for a particular mare from the source population inoculated with the Bucyrus strain of EAV, it would be quite unusual to observe a fever that peaked at less than \( I^{(1)} - 1.96\sigma_{I}^{(1)} = 1.7\degree F \) above baseline CBT or at higher than \( I^{(1)} + 1.96\sigma_{I}^{(1)} = 5.3\degree F \) above baseline. The population response curves for the two groups are presented in Figure 3.6.
Figure 3.6. Population febrile response curves, with pointwise 95% posterior credible intervals, for mares challenged with the Bucyrus strain of EAV, and stallions challenged with the Kentucky-84 strain.

We can contrast the population response parameters for the two groups simply by monitoring the posterior distributions of their differences. These are presented in Table 3.3 for baseline, peak fever intensity, time to peak fever, and response duration as indexed by FWHM. We see that zero is excluded from the posterior 95% CI’s for the differences in both peak intensity and time to peak. We conclude that the febrile responses of the two groups differ with respect to these two characteristics.

Table 3.3. Posterior estimates of group differences in population parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>2.5% quantile</th>
<th>97.5% quantile</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B^{(2)} - B^{(1)}$</td>
<td>-0.11</td>
<td>0.28</td>
<td>-0.66</td>
<td>0.44</td>
</tr>
<tr>
<td>$I^{(2)} - I^{(1)}$</td>
<td>1.2</td>
<td>0.48</td>
<td>0.25</td>
<td>2.2</td>
</tr>
<tr>
<td>$p^{(2)} - p^{(1)}$</td>
<td>-0.72</td>
<td>0.34</td>
<td>-1.4</td>
<td>-0.1</td>
</tr>
<tr>
<td>$FWHM^{(2)} - FWHM^{(1)}$</td>
<td>-0.14</td>
<td>0.83</td>
<td>-1.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>
In comparing the asymmetric analysis in this section with the symmetric analysis in Chapter 2 we note that the asymmetric model placed the time to peak estimates for both groups about 1.5 days earlier than did the symmetric model.

3.4. ESTIMATION AND INference FOR ONSET AND RECOVERY TIMES IN THE NORMAL-ERRORS NLHM

The normal-errors NLHM developed in Chapters 2 and 3 was specified in such a way that its parameters would correspond to biologically meaningful properties of the infection response. In the asymmetric model (3.3)-(3.6), the baseline response level is represented by $B$, the peak response intensity by $I$, and the time to peak response by $p$.

The parameters $r$ and $l$ can be viewed as indices of, respectively, the duration of the onset and recovery phases of the response. Although adequate for testing hypotheses about differences in the duration of onset or recovery, or the total response duration, among treatment groups, these scale parameters lack satisfying descriptive interpretations. Quantities derivable from $r$ and $l$, such as HWHM and FWHM, can partially address the need for more intuitive measures of response duration. However for investigators interested in questions such as, “On average, how many days (or hours) after inoculation does the response onset (or recovery) occur?” or “Does treatment alter the timing of response onset (or recovery)?” none of these measures is adequate. In this section we propose a method of estimating the timing of response onset and recovery for the normal-errors NLHM.

3.4.1. Method

The model (3.3)-(3.6) is appropriate for response variables which vary in a consistent manner about some mean level prior to challenge, during infection, and
following recovery. This follows from the distributional assumption about the response variable specified in (3.3). Observe that for an individual in its baseline, uninfected state, the response distribution specified in (3.3) and (3.4) simplifies to

\[ y_{ij} | B_i \sim N(B_i, \sigma^2) \]  

(3.9)

Thus, in the baseline state 95% of an individual’s observed responses will fall within the interval \([B_i - 1.96\sigma, B_i + 1.96\sigma]\). Intuitively, a series of observations falling outside that interval in the same direction (either all above or all below) would be a strong indication that onset had occurred for that particular individual.

Now consider selecting an uninfected individual at random from the source population and sampling the response variable. Without knowing that particular individual’s true baseline level, a reasonable expectation for the observed value would be the population baseline \(B\). But the specific value we observe is influenced both by the inherent variability in the individual’s responses and the amount by which the individual’s baseline differs from the population baseline \(B\). In the model these quantities are represented by \(\sigma^2\) and \(\sigma_B^2\). Thus, marginally over the entire population, we have

\[ y_{ij} \sim N(B, \sigma_B^2 + \sigma^2) \]  

(3.10)

It follows that approximately 95% of all observations made under baseline (i.e. uninfected) conditions will fall within the interval

\[ [B - 1.96\sqrt{\sigma^2 + \sigma_B^2}, B + 1.96\sqrt{\sigma^2 + \sigma_B^2}] \]  

(3.11)

We can think of this interval as representing bounds on normal variability of the response variable among uninfected individuals in the sources population. When model (3.3)-(3.6) is fitted to data from an EI study we obtain an estimate of the population average response to infection. We define population average onset time as the earliest
time point at which the population average response \((O)\) departs from this interval of normal variability. Similarly, we define the population average recovery time \((R)\) as the earliest time at which the population average response returns to the interval of normal variability. Finally, we define population average response duration as the time between onset and recovery, i.e. \(D = R - O\).

### 3.4.2. Procedure

In the Bayesian setting implementation is trivial. After the final model is fit, posterior distributions for \(O\), \(R\) and \(D\) can be obtained as follows. For each MCMC iteration:

1. Reconstruct the population mean response curve from the parameter \(\beta^{(m)}\) obtained for that iteration. [Note that the \((m)\) superscript in this context refers to the sampled value obtained at MCMC iteration \(m\), and is not to be confused with the \((g)\) superscript notation used in (3.5) and (3.6) to denote group membership.]

2. Extract the parameter values \(B^{(m)}\), \(\sigma^{(m)}\) and \(\sigma_B^{(m)}\) obtained at that iteration.

3. Determine the earliest time at which the population mean response curve for iteration \(m\) escapes the interval (3.11). This is the estimated population average onset time for iteration \(m\), denoted \(O^{(m)}\);

4. Determine the earliest time at which the population mean response curve for iteration \(m\) returns to the interval (3.11). This is the estimated population average recovery time for iteration \(m\), denoted \(R^{(m)}\);

5. Define the estimated population average response duration for iteration \(m\) as \(D^{(m)} = R^{(m)} - O^{(m)}\).
Collect the MCMC estimates $O^{(m)}$, $R^{(m)}$ and $D^{(m)}$ into vectors $O$, $R$ and $D$. These vectors represent samples from the posterior distributions of $O$, $R$ and $D$ from which posterior means and credible intervals may be extracted.

3.4.3. Illustrative example: Lymphocyte count

Figure 3.7 presents population mean onset and recovery times of the lymphocyte response for mares challenged with the recombinant Bucyrus strain of EAV. The vertical blue line at 4.7 DPI on the x-axis indicates that the decline in lymphocyte count was first detectable, on average, at 4.7 days post-challenge. The lighter blue rectangular region surrounding this line indicates the posterior 95% credible interval (3.3, 6.9). Similarly, the vertical blue line at 9.4 DPI on the x-axis indicates that return to baseline lymphocyte count occurred, on average, 9.4 days post-challenge. The light blue region surrounding this line indicates the posterior 95% credible interval for recovery (8.5, 10.7). The total duration of the lymphocyte response – the time from initial onset to recovery to baseline level – was estimated to be 4.7 days (1.8, 6.8).

Figure 3.7. Estimated population mean onset and recovery times for lymphocyte response for mares challenged with the recombinant Bucyrus strain of EAV
3.4.4. Illustrative example: Febrile response

Figure 3.8 presents population mean onset and recovery times of the febrile response to EAV infection for mares challenged with the recombinant Bucyrus strain of EAV and stallions challenged with the KY-84 strain. The vertical blue line at 3.4 DPI on the x-axis indicates that in the Bucyrus group the onset of fever occurred at 3.4 days post-challenge. The lighter blue rectangular region surrounding this line indicates the posterior 95% credible interval (2.2, 4.9). Similarly, the vertical blue line at 9.1 DPI on the x-axis indicates that return to baseline body temperature in the Bucyrus group occurred, on average, 9.1 days post-challenge. The light blue region surrounding this line indicates the posterior 95% credible interval for recovery (8.5, 10.6).

![Figure 3.8](image_url)

**Figure 3.8.** Estimated population mean onset and recovery times for febrile response to EAV infection. Bucyrus strain (mares)

Onset and recovery estimates and accompanying credible intervals for the KY-84 group are indicated by the orange lines and rectangular regions. Estimated onset for that group was considerably earlier at 1.8 days (0.6, 3.1) and recovery was also slightly earlier.
at 8.7 days (8.2, 9.2). However as suggested by the overlapping credible intervals (illustrated by the brownish regions in Figure 3.8), neither of these differences was judged to be statistically significant. The estimated group difference in duration of the febrile response [Bucyrus 5.8 days (3.7, 7.7) and KY-84 6.8 (5.3, 8.2)] was likewise not significant. Note that the mean duration estimate for the KY-84 group is 21% higher than the estimate of mean FWHM from Table 3.2, but the estimate for the Bucyrus group is less than 2% greater.

3.5. DISCUSSION

Our primary aim in this chapter was to extend the normal-errors NLHM model to accommodate asymmetric responses. To accomplish this we proposed a mean response function consisting of two half-Gaussian curves constrained to meet at a common peak. There are several other examples in that statistical literature of piecewise models for peaked, asymmetric longitudinal responses. Huisman et al (1993) proposed a set of five response functions for modeling temporal variations in the abundance of vegetation species in response to environmental changes. The models are based on logistic equations of the form \((1 + e^{a+bx})^{-1}\). Two of the curves are analogous to our symmetric and asymmetric Gaussian-based response models. Müller and Rosner (1997) introduced a model for analyzing the hematologic (white blood cell) response of patients to chemotherapeutic medications. They used a piecewise linear and logistic response function consisting of a linear baseline component, a logistic “recovery” component, and a second linear “onset” component connecting the baseline and recovery portions of the curve. Gössl et al (2001) proposed a model for the hemodynamic response function (HRF) which arises in functional magnetic resonance imaging (fMRI). Neuronal
activation in the brain in response to an experimental stimulus results in local increases in blood flow and oxygen level. These changes follow a well-defined pattern known as the hemodynamic response. The HRF of Gössl and colleagues is a piecewise function consisting of two linear and three truncated Gaussian components for a total of five regions (baseline, increase, plateau, decrease and undershoot).

We implemented our infection response model as a Bayesian hierarchical nonlinear model. The primary reason for this decision was the inability of frequentist methods to achieve convergence when using the piecewise response function (3.4). Jang et al (2013) reported successfully fitting several piecewise mixed effects models of cardiac function using frequentist methods, specifically the nlme package in R. However their data set included 80 observations per individual. This suggests that our difficulties may have been due to the relatively sparsity of our data at the individual level. A second reason for preferring the Bayesian approach is that maximum likelihood estimation and inference for NHLM’s are based on asymptotic results that may not hold for the relatively small numbers of subjects typical of EI studies. The hematologic and hemodynamic response models mentioned above were both implemented as Bayesian hierarchical nonlinear models.

3.5.1. Illustrative examples

The illustrative examples highlight some concerns that can only be addressed by the investigator at the time of study design. Intensity of measurement occasions at the individual level appears to be a critical factor in the feasibility of applying piecewise NLHM’s to longitudinal EI data. Based on both the illustrative examples and the simulation results from Chapter 2, we can make some recommendations. In general, we
advise sampling as intensively as practically possible over the duration of the response. However there are certain periods during which sampling intensity is particularly relevant. First, we advise taking several baseline measurements both pre-challenge and post-recovery in order to better locate the individual baseline response levels. Second, we advise sampling intensively during periods when the response variable is expected to change rapidly, which for a single-peaked response means during the onset and recovery phases. In the febrile response example, core body temperature for all horses dropped rapidly after peaking. In many cases rapid onset will occur within the first two or three days post-inoculation, particularly with quantities like viral load as we will discuss in Chapter 4, so intensively sampling is advisable during this period.

The febrile response example illustrates yet another attractive feature of a Bayesian implementation. Recall that in Chapter 2 the symmetric NLHM was implemented as a frequentist nonlinear mixed effects model. It is well known that variance components tend to be underestimated by maximum likelihood (ML) methods for both linear and nonlinear mixed models, because they do not take into account the loss in degrees of freedom incurred in estimating the fixed effects. To enable a comparison of variance component estimates for the ML and Bayesian approaches, we reanalyzed the febrile response data from Section 3.3.2 using the symmetric NLHM from Chapter 2, but implemented as a Bayesian NLHM. We found that the posterior means for $\sigma_B, \sigma_f$ and $\sigma_p$ were between 12% and 26% higher than the ML estimates from Chapter 2. Restricted maximum likelihood (REML) estimation often produces more accurate variance component estimates than ML (Harville 1977). However in this particular example, the REML and ML estimates were nearly identical.
3.5.2. Estimation and inference for response onset and recovery

There are some examples in the literature of methods used to identify onset and recovery times in longitudinal responses. Fox (2008) used RM-ANOVA to estimate the time at which postural control (essentially, balance) returned to baseline level after exercise. They found that “the effects of fatigue persisted for up to 13 minutes before postural control returned to baseline.” As a result it was recommended that clinicians responsible for assessing athletes for concussions during play or practice should wait at least 13 minutes so that effects due to fatigue are not mistakenly interpreted as a sign of concussion.

Piecewise models such as those discussed above contain built-in change point parameters that can be used to estimate the timing of key transitions in the response trajectory. For example the hematologic response model by Müller and Rosner includes a parameter that marks the transition point from the baseline phase, modeled as a horizontal line, to the onset phase, which is modeled as a separate line connecting the baseline segment to the recovery segment.

Our approach aims to identify the population average time at which a clinically meaningful change in the response variable from the baseline, uninfected state is detectable. This is different from the time at which the underlying immune response to infection commences. We note also that in the frequentist setting, it would be possible to apply the same approach to estimating population mean onset, recovery and duration via the bootstrap (Efron 1979, Das 1999).
CHAPTER FOUR

A Bayesian nonlinear hierarchical model for viral load

4.1. INTRODUCTION

In Chapter 3, we presented a Bayesian normal-errors NLHM for asymmetric infection responses. Frequently in MC experiments involving viral pathogens, a primary focus is the time course of the viral load in blood, feces or other fluids or excreta. For the individuals in such experiments, viral load will be exactly zero at all observation times prior to inoculation and after clearance of the virus. During infection, however, viral load can be viewed as fluctuating randomly about some systematic trajectory (Figure 4.1).

Because viral loads are nonnegative, the assumption of identical, normally distributed errors is clearly untenable. We propose an NLHM in which the viral load at each time point is modeled as a Poisson random variable with mean given by a compact kernel function such as the Tricube or Epanechnikov. Because the variance of a Poisson random variable is equal to its mean, when the mean viral load is equal to zero (i.e. pre-onset or post-recovery) the variance will be also. Similarly, when the mean viral load is positive (i.e. during infection) the variance will be positive, and the variance will increase with the mean.

In this Chapter we propose a NLHM suitable for modeling viral load and other response variables that do not vary when the subject is not infected. We provide two illustrative examples in which the proposed model is applied to viral load data from actual experimental infection studies.
4.2. BAYESIAN NONLINEAR HIERARCHICAL MODEL FOR VIRAL LOAD

We model the individual response profiles with piecewise half-kernel functions, where $K$ in (16) is any nonnegative, compact kernel function. The normalizing constant is replaced with an intensity parameter $I_i$ which is specific to each individual and corresponds to mean peak intensity. Because viral load has a natural baseline value of zero, subject-specific baseline parameters, as in (8), are not required.

Level 1 (model of intra-individual variability)

\begin{align*}
y_{it} & \sim \text{Pois}(\mu_{it}) \\
\mu_{it} & = K^*(t,p_i,l_i,r_i) \\
K^*(t) & = \begin{cases} 
0 & \text{if } t \leq p_i - l_i \\
I_i \times K\left(\frac{t-p_i}{l_i}\right) & \text{if } p_i - l_i < t \leq p_i \\
I_i \times K\left(\frac{t-p_i}{r_i}\right) & \text{if } p_i < t < p_i + r_i \\
0 & \text{if } t \geq p_i + r_i
\end{cases}
\end{align*}

**Figure 4.1.** A typical nasal viral load response profile for a human subject experimentally infected with rhinovirus (Mallia 2012)
An attractive consequence of using a compact kernel $K$ in the specification of the mean infection response function $K^*$ is that we obtain estimates of response onset time for individual $i$ as $p_i - l_i$, recovery time as $p_i + r_i$, and response duration as $d_i = p_i + r_i - (p_i - l_i) = l_i + r_i$.

Level 2 (model of inter-individual variability)

Collect the response parameters for individual $i$, into a vector

$$\mathbf{\beta}_i = [l_i, p_i, l_i, r_i]^T.$$  (4.4)

Level 2 (model of inter-individual variability)

$$\mathbf{\beta}_i = \mathbf{\beta}^{(g)} + \mathbf{b}_i$$  (4.5)

$$\mathbf{b}_i \sim N \left(0, \mathbf{D}^{(g)}\right),$$

where $\mathbf{D}^{(g)} = \text{diag}\left(\sigma_{l_i}^2, \sigma_{p_i}^2, \sigma_{l_i}^2, \sigma_{r_i}^2\right)$  (4.6)

Level 3 (hyperprior distribution)

Prior distributions on $\mathbf{\beta}$, $\mathbf{D}$, and $\sigma$ were the same as for the normal-errors model in Chapter, with one exception. Because the response onset cannot occur prior time zero, we have $p - l \geq 0 \Rightarrow l < p$. Thus we can specify $l \sim Unif(0, p)$.

As previously, the following definitions are implicit in (4.1)-(4.6).

$$\mathbf{\beta}_i = [l_i, p_i, l_i, r_i]^T.$$  (4.7)

$$\mathbf{\beta}^{(g)} = [l^{(g)}, p^{(g)}, l^{(g)}, r^{(g)}]^T, \ g \in \{1,2\}$$  (4.8)

4.3. ILLUSTRATIVE EXAMPLES

4.3.1. Effect of body condition on viral shedding in migratory birds

This example refers to the case study from Chapter 1 on influenza A viral shedding in ducks. Recall that the original experiment included four treatment groups. For the sake of simplicity we focus here on a comparison of the viral shedding pattern of
the wild lean group to that of the wild normal group. Viral shedding profiles are presented in Figure 4.2. Peak shedding for birds in the wild normal group generally was higher than in wild lean birds, although there were exceptions. Mean duration of shedding also appears to be longer in the wild normal group.

Figure 4.2. Observed viral shedding profiles for nine wild-caught ducks with normal body condition and nine wild-caught ducks with lean body condition following experimental challenge with AIV (observations for days 11 through 28 not shown)

We modeled the viral shedding data using the Poisson NLHM in (4.1)-(4.6) with $g \in \{1,2\}$. Based on the observed profiles in Figure 4.2 we selected a piecewise Tricube kernel function as the model for the mean response functions. Thus the function $K$ in (4.3) is given by

$$K(u) = (1 - |u|^3)^3.$$  \hspace{1cm} (4.9)

Satisfactory convergence was achieved with 175,000 MCMC iterations following 25,000 burn-in, for all except the recovery parameters in the wild lean group, i.e. $r^{(2)}$ and
\( \sigma_r^{(2)} \). We discuss possible causes in §4.5. Convergence diagnostics and supplemental plots are included in Appendix A. Posterior estimates are reported in Table 4.1.

**Table 4.1.** Posterior estimates of population viral shedding parameters for wild normal and wild lean groups experimentally challenged with AIV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-caught normal</th>
<th>Wild-caught lean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 2.5% quantile</td>
<td>Mean 97.5% quantile</td>
</tr>
<tr>
<td>Population infection response parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( I^{(1)} )</td>
<td>470 355 584</td>
<td>( I^{(2)} ) 331 197 468</td>
</tr>
<tr>
<td>( p^{(1)} )</td>
<td>1.9 1.3 2.5</td>
<td>( p^{(2)} ) 2.5 1.4 3.7</td>
</tr>
<tr>
<td>( l^{(1)} )</td>
<td>1.3 0.4 2.0</td>
<td>( l^{(2)} ) 1.5 0.2 2.8</td>
</tr>
<tr>
<td>( r^{(1)} )</td>
<td>4.4 3.4 5.0</td>
<td>( r^{(2)} ) 3.4 1.3 4.9</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma_I^{(1)} )</td>
<td>166 98 291</td>
<td>( \sigma_I^{(2)} ) 189 110 341</td>
</tr>
<tr>
<td>( \sigma_p^{(1)} )</td>
<td>0.93 0.55 1.6</td>
<td>( \sigma_p^{(2)} ) 1.8 1.0 3.2</td>
</tr>
<tr>
<td>( \sigma_l^{(1)} )</td>
<td>1.3 0.8 1.9</td>
<td>( \sigma_l^{(2)} ) 2.7 1.6 4.5</td>
</tr>
<tr>
<td>( \sigma_r^{(1)} )</td>
<td>1.9 1.7 2.0</td>
<td>( \sigma_r^{(2)} ) 3.1 1.8 4.8</td>
</tr>
</tbody>
</table>

Figure 4.3 illustrates the population average time courses for influenza viral shedding in the wild normal and wild lean groups. The estimated time to peak was earlier in the wild normal group (1.9 days to 2.5 days) and the mean peak shedding intensity was 139 units \([log_{10}(GEC)/140 \mu l]\) greater.

Table 4.2 summarizes the posterior distributions for the group differences in the population infection response parameters. The posterior interval (-314, 38) for \( I^{(2)} - I^{(1)} \) includes zero, implying that peak viral shedding intensity was significantly greater in the captive normal group. The interval for \( p^{(2)} - p^{(1)} \) includes zero, implying no difference in mean time to peak shedding.
Table 4.2. Posterior estimates of differences in population viral shedding parameters for wild normal and wild lean groups challenged with AIV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>95% posterior credible interval</th>
<th>2.5% quantile</th>
<th>97.5% quantile</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I^{(2)} - I^{(1)} )</td>
<td>-139</td>
<td>-314</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>( p^{(2)} - p^{(1)} )</td>
<td>0.6</td>
<td>-0.7</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

(1) denotes normal body condition and (2) denotes lean body condition

Figure 4.3. Estimated population viral shedding curves, with pointwise 95% posterior credible intervals, for wild normal and wild lean groups challenged with AIV

4.3.2. Effects of rhinovirus infection in chronic obstructive pulmonary disease

Our second example refers to the case study from Chapter 1 on the effect of rhinovirus infection on persons with chronic obstructive pulmonary disease (COPD). To review briefly, Mallia and colleagues (2006, 2011, 2012, 2013) recently developed a human rhinovirus (HRV) challenge model for COPD. In their 2011 study, thirteen volunteers with COPD and thirteen controls with a similar smoking history, but normal lung function, were recruited. All participants tested negative for serum neutralizing
antibodies to rhinovirus 16 (RV-16). They were inoculated with a 10 TCID$_{50}$ dose of RV-16 and followed for six weeks post-infection. Viral loads in nasal lavage and sputum, lung function, inflammatory markers, and upper and lower respiratory symptom scores were sampled at varying intervals and intensities. Twenty-three subjects displayed virological evidence of rhinovirus infection: eleven from the COPD group and twelve from the control group. Data from those 23 subjects were analyzed to assess whether experimental RV-16 infection induces exacerbation in persons with COPD.

**Figure 4.4.** Observed profiles of viral load in nasal lavage for eleven subjects with COPD and twelve health controls following experimental challenge with RV-16

Our interest in this example is in the viral load in nasal lavage (VLN). Figure 4.4 presents the observed viral loads in nasal lavage (VLN) following inoculation over the course of six weeks, for all subjects in each group. In each group the viral load increases to a peak very rapidly for most subjects and decreases back to zero more gradually, as the host immune response neutralizes and eliminates the virus. The figure suggests that the mean time to complete viral clearance may be greater in the control group, although this
impression is based largely on just two control subjects with clearance times greater than thirty days.

As in the previous example we modeled the rhinovirus data using the Poisson model in (4.1)-(4.6) with $g \in \{1,2\}$. Again we selected a piecewise Tricube kernel function (4.9) as the model for the mean response functions, although other kernel functions could be used. Satisfactory convergence of the Markov chains was achieved with 200,000 MCMC iterations (50,000 burn-in) for all except for the onset parameters in both groups, i.e. $l^{(1)}$, $l^{(2)}$, $\sigma_{l}^{(1)}$ and $\sigma_{l}^{(2)}$. We discuss the cause of these exceptions in §4.5. Convergence diagnostics and supplemental plots are included in Appendix A.

Table 4.3. Posterior estimates of population viral load response parameters for controls and subjects with COPD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>2.5% quantile</td>
</tr>
<tr>
<td>Population infection response parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$I^{(1)}$</td>
<td>550</td>
<td>456</td>
</tr>
<tr>
<td>$p^{(1)}$</td>
<td>3.3</td>
<td>1.6</td>
</tr>
<tr>
<td>$l^{(1)}$</td>
<td>0.75</td>
<td>0.06</td>
</tr>
<tr>
<td>$r^{(1)}$</td>
<td>17.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Variance components</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma_{l}^{(1)}$</td>
<td>148</td>
<td>92</td>
</tr>
<tr>
<td>$\sigma_{p}^{(1)}$</td>
<td>2.7</td>
<td>1.7</td>
</tr>
<tr>
<td>$\sigma_{l}^{(1)}$</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>$\sigma_{r}^{(1)}$</td>
<td>4.95</td>
<td>4.82</td>
</tr>
</tbody>
</table>

Posterior estimates are reported in Table 4.3. Figure 4.5 illustrates the population-averaged time courses for RV-16 viral load in nasal lavage in the COPD and control groups. Peak viral load was reached earlier in COPD subjects, at 2.8 days (0.8, 5.0) compared to 3.3 days (1.6, 4.9) in controls. Peak intensity was estimated at 610 units
(442, 778) in the COPD group compared to 550 units (456, 644) in controls. Thus, the average response in the COPD group began earlier and peaked earlier at a higher level than the average response in the control group.

**Figure 4.5.** Estimated population time course for viral load in nasal lavage, following RV-16 challenge with pointwise 95% posterior credible intervals

Table 4.4 summarizes the posterior distributions for the group differences in peak intensity and time to peak. We see that both posterior 95% credible intervals include the null value of zero, suggesting that the differences described above are indistinguishable from random noise. Thus, although the original analysis detected a difference in viral loads between COPD subjects and controls at 6 DPI, our analysis found no significant difference in the population average response curves. This is noteworthy because it is unclear why one would expect COPD to influence the time course of viral load, and a false positive finding can be a distraction.
Table 4.4. Posterior estimates of differences in population viral load response parameters for controls and subjects with COPD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>95% posterior credible interval</th>
<th>2.5% quantile</th>
<th>97.5% quantile</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I^{(2)} - I^{(1)}$</td>
<td>60.0</td>
<td>-130.9</td>
<td>251.4</td>
<td></td>
</tr>
<tr>
<td>$p^{(2)} - p^{(1)}$</td>
<td>-0.5</td>
<td>-3.0</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
</table>

(1) denotes the control group and (2) denotes the COPD group

4.4. ONSET AND RECOVERY TIMES IN THE VIRAL LOAD NLHM

In Chapter 3, we introduced a method for estimating onset and recovery times for the normal-errors NHLM. The approach was based on identifying limits on the plausible values for the mean response when all the individuals in a sample are in the baseline, or nonresponsive, state. For the viral load NHLM (4.1)-(4.6) the solution to this problem is trivial: natural candidates for onset and recovery time can be obtained as direct functions of the model parameters. A glance at equation (4.3) shows that the transition from zero to positive viral load occurs at time $p_l - l_l$, and the return from positive to zero viral load occurs at time $p_l + r_l$. We define these times, respectively, as the population mean onset and recovery times, and we estimate them with posterior means and credible intervals.

Figure 4.6 presents population mean onset and recovery times for the captive normal group from the viral shedding example in §4.3.1. The vertical blue line at 0.59 DPI on the x-axis indicates that in the wild normal treatment group the onset of fever occurred at 0.59 days (or about 14 hours) post-challenge. The light blue shaded region surrounding this line indicates the posterior 95% credible interval (0.03 1.5). Similarly, the vertical blue line at 6.3 DPI on the x-axis indicates that viral shedding in the captive normal group ceased, on average, 6.3 days post-challenge. The posterior 95% credible interval for recovery is (5.1, 7.1).
Figure 4.6. Estimated onset and recovery times, with posterior 95% credible intervals, for wild normal ducks challenged with AIV

Figure 4.7 illustrates onset and recovery for the wild-caught, lean treatment group. Estimated onset for the wild lean group was later at 0.96 days (0.04, 2.64), or about 23 hours, and the point estimate for recovery was slightly earlier at 5.9 days (3.4, 7.9). However, the posterior credible intervals indicate that there is a great deal of uncertainty in these point estimates. Figures A.17 and A.18 are panel plots of the individual, observed viral shedding profiles. In both groups, there are very few observations within the time intervals where onset and recovery occur, but the problem is particularly evident in the wild lean group (Figure A.18). This likely explains the extreme lack of precision in the onset and recovery estimates for that group. As a result, although the point estimates of recovery time and response duration differ noticeably between the two groups, we cannot declare the differences to be significant. However, more intensive sampling during the onset and recovery phases may greatly increase precision. It is instructive to compare Figures A.18 and A.11 – the latter being a panel plot of the observed core body
temperature profiles for stallions challenged with the KY-84 strain of EAV from Chapter 3 – and the corresponding onset and recovery plots 4.7 and 3.7. The highly precise posterior credible interval for recovery in the KY-84 group in Figure 3.7 is due to the combination of (a) the similarity of the individual recovery trajectories in that group, and (b) the presence of adequate numbers of observations sampled during the recovery phase.

**Figure 4.7.** Estimated onset and recovery times, with posterior 95% credible intervals, for wild lean ducks challenged with AIV

### 4.5. DISCUSSION

For several reasons the normal-errors NLHM from Chapters 2 and 3 is not appropriate for modeling viral load in EI studies. Viral load does not vary in uninfected individuals prior to challenge nor after clearance, so that the constant variance assumption is clearly violated. Furthermore, viral load must be positive, so it makes no sense to model a subject’s viral load at each time point as a normal random variable. Finally, during infection it may happen the variability in viral load increases as the mean viral load increases. We have proposed a NLHM which better reflects viral load
dynamics in EI studies. Although we modeled the individual responses as Poisson random variables, there are other distributions that we might have chosen to address these shortcomings of the normal-errors NLHM.

Our model is not mechanistic, in the sense that the choice of response function is not informed by biological knowledge of viral kinetics. There are at least two other domains in which researchers have developed both mechanistic and empirical NLHM’s for studying the time course of viral load dynamics. A discussion of those cases will provide a helpful context within which to interpret our findings.

In AIDS research, early efforts to model HIV dynamics \textit{in vivo} date to the mid-1990’s. It was known that infected individuals treated with reverse transcriptase or protease inhibitors experienced an exponential decline of virus in plasma and a simultaneous increase in CD4 cell counts (Herz \textit{et al} 1996). Compartmental models, very much like the susceptible-infected-recovered (SIR) models used in the study of infectious disease transmission in epidemics, were developed to model the observed viral dynamics (Perelson \textit{et al} 1996, Ho \textit{et al} 1995, Wei \textit{et al} 1995). These models were based on differential equations that describe the interactions among cells susceptible to infection, infected cells and free virus (Huang \textit{et al}. 2006). They have been instrumental in understanding HIV pathogenesis and developing treatment strategies.

Wu \textit{et al} (1998) extended the work of Perelson \textit{et al}. by placing it in the framework of the normal-errors NLHM. Fitzgerald \textit{et al}. (2002) proposed a normal-errors NLHM of the HIV “rebound effect,” an increase in viral load which occurs in some patients following the initial decrease caused by initiation of antiretroviral therapy. Both of these were implemented in the nonlinear mixed model (NLMM) framework. Several
authors have reported on the application of Bayesian methods to the modeling of HIV dynamics including Frost et al. (2001), Han et al. (2002), Wolf et al. (2002), Wu (2005), Huang et al. (2006). A recent paper by Huang (2013) describes a model more similar to our own, namely, a piecewise Bayesian NLHM of viral load changes for HIV. The model includes parameters that identify transition points in the HIV trajectory, such as the previously mentioned rebound effect. Unlike the other HIV models we have described, it is empirical rather than mechanistic. Its purpose (like that of our infection response models) is to describe and estimate macro-level features of the response trajectories.

Efforts have also been made to apply NLHM to hepatitis viral kinetics. Neumann et al. (1998) proposed a mechanistic model of hepatitis C virus (HCV) dynamics in response to interferon-α therapy. O’Sullivan et al. (2008) developed a normal-errors NLHM based on a logistic response function to empirically quantify long-term within- and between-subjects HCV variation untreated, chronically infected individuals. Snoeck et al. (2010) introduced a mechanistic, normal-errors NLHM capable of flexibly modeling a range of HCV responses to treatment with peginterferon α-2α±ribivarin.

4.5.1. Illustrative examples

In both the HIV and the hepatitis C models discuss above, the focus is on the effect of therapeutic interventions on viral load in chronically infected individuals. The normal-errors assumption is reasonable in those cases because subjects have established a nonzero baseline level around which viral load fluctuates over time. In EI studies, the interest centers on the response to acute infection in individuals not infected at the beginning of the experiment. Thus normal-error models are not appropriate.
In both of the illustrative examples we noted a lack of convergence in the MCMC chains for certain of the onset and recovery parameters – both the population effects and their variance components. In particular, this was observed for the recovery parameters for the wild lean group in the influenza example, and the onset parameters in both the COPD and control groups in the RV-16 example. This problem was not encountered in the lymphocyte and febrile response examples in Chapter 3. These difficulties are symptomatic of two fundamental issues that can be addressed through improved study design. First, the response variable may have been sampled with insufficient intensity during the onset and recovery phases. Second, the individual responses within a particular group may be too dissimilar to permit them to be summarized by a single population response curve. The lesson is that, in addition to the number of individuals per group, the investigator should give careful thought to the sampling schedule and to the degree of heterogeneity permitted among the individuals in each group. For example, if there are significant differences in the way that male and females, or younger and older individuals, respond to a particular infection, then consideration should be given to restricting the samples accordingly, in order to reduce noise and increase power.

Wu et al (1998), Wu and Ding (2000) and Wu (2005) address the critical importance of the sampling schedule in viral load studies. Although their focus is studies of HIV dynamics, their comments are equally relevant to EI studies. For example Wu (1998) suggests “sampling as frequently as possible during the first 8 hours until one captures the drug effect time (the time that viral load starts to drop).” Obviously in EI studies viral load will increase following inoculation rather than dropping following treatment (as in HIV studies), but the message is the same: frequent sampling during the
incubation period is important for modeling the true onset trajectory accurately. Typically, however, in EI studies viral load is sampled at most daily during the first few days, and less frequently thereafter. A study by Harris and McGwaltney (1996) clearly demonstrates the rapid onset of rhinovirus infection. In eleven volunteers challenged with rhinovirus type 39, mean time of symptom onset occurred was less than 2 hours and the mean time to first detectable viral shedding was 11.3 hours. In this particular scenario it would seem advisable to sample viral load at least every three or four hours over the first day post-inoculation. Wu and colleagues suggest the use of Monte Carlo simulation as an aid to determining an adequate sampling schedule.
CHAPTER FIVE

Summary and future directions

5.1. Nonlinear hierarchical models for longitudinal EI studies

Longitudinal infection response data arise in many experimental infection studies including those designed to demonstrate vaccine efficacy, explore disease etiology, pathogenesis and transmission, or understand the host immune response to infection. Very often such data are analyzed with inefficient and arguably inappropriate statistical methods, frequently RM-ANOVA. This is not unique to EI studies; it has been noted by researchers in many fields (Jang 2013, Matthews 1990). Major advances in methods for analyzing longitudinal data that have occurred over the past fifty years, but investigators in some disciplines – including those that utilize EI studies – continue to rely almost exclusively on familiar, classical approaches. In some cases, such as EI where infection responses are inherently nonlinear, newer methods may offer substantial gains in accuracy and precision of parameter estimation and power. Our aim was to propose an alternative approach to the analysis of longitudinal data from EI studies that incorporates recent developments in the areas of nonlinear hierarchical models and Bayesian statistics.

In Chapter 2 we introduced a basic, single-peaked model for a symmetric infection response which was based on the normal-errors NLHM with a Gaussian mean response function. The parameters of that model correspond directly to biologically meaningful properties of the infection response, including baseline, peak intensity, time to peak and spread. In Monte Carlo simulation studies the NLHM outperformed RM-ANOVA across a broad range of scenarios, and under certain conditions the improvement was substantial. Estimation in the NLHM was more precise than RM-
ANOVA for all model parameters, and less biased for peak intensity and duration parameters. We demonstrated the effects of the sampling schedule, interindividual response variability and misspecification of the mean response function on estimation and inference. Finally we illustrated the application of the symmetric NLHM to real data as a frequentist nonlinear mixed model.

In Chapter 3 we extended the basic, symmetric response model from Chapter 1 by incorporating a piecewise, half-Gaussian response function. Piecewise longitudinal response models have been applied successfully in several fields including ecology (Huisman et al 1993), cancer research (Müller and Rosner 1997), HIV/AIDS research (Huang 2012) and functional magnetic resonance imaging (Gössl et al 2001). We chose to implement the model as a Bayesian hierarchical nonlinear model to overcome convergence problems and avoid reliance on asymptotic results associated with the frequentist framework. The latter concern is relevant given the generally small-to-moderate sample sizes associated with EI studies. Through illustrative examples we demonstrated the application to the description of lymphocyte decline following experimental challenge with EAV and the comparison of febrile responses to challenge with different strains of EAV. Finally we proposed a method for estimating infection response onset and recovery times from the fitted model.

In Chapter 4 we considered modeling of viral load in EI studies. Mechanistic viral load models have been developed for individuals chronically infected with HIV and hepatitis, and in these cases a normal-errors model is appropriate. In EI studies, however, it is not because viral load does not vary prior to challenge and during the incubation period, nor after elimination of the infection. We therefore proposed a NHLM with the
individual responses at each time point modeled as a Poisson random variable with the means across time points related through a Tricube mean response function. We demonstrated application of the model to compare the viral load trajectories in COPD patients and controls challenged with human rhinovirus, and the time course of influenza A virus shedding in birds with different, experimentally controlled body conditions. We discussed estimation of onset and recovery times within the viral load model.

5.2. Limitations and open questions

As we conjectured, the NLHM clearly offer potential gains in estimation and precision of parameter estimation and power over RM-ANOVA. The extent to which these gains will be realized in any particular example depends on a number of factors. Among them are the true effect size, the numbers of subjects per group, the number and configuration of measurement occasions for each individual, and the amount of interindividual response variability. Consultation with a biostatistician from the early stages of study design is highly advisable.

Numerous avenues exist for future work in this area. The asymmetric model accommodates many more response shapes than the symmetric model, but it is still limited to single-peaked responses. Some response variables may follow other trajectories. For example, a proportion of stallions infected with EAV will develop persistent infections. The viral load in such horses will rise strongly following challenge and decline after peaking, but will not return to the original zero baseline. Instead it will stabilize at a new, nonzero baseline level. Antibody responses will exhibit a similar pattern. In other cases there may be more than one peak, particularly in experiments involving more than one challenge point. These types of response variables and
experiments will require different forms for the mean response function. As the response function increases in complexity, issues of sample size and data collection schedules become increasingly critical due to the need to estimate a larger number of parameters.

The NLHM is a rich, highly flexible modeling framework with capabilities we have not explored in this dissertation. For example the effect of covariates such as subject age, gender and smoking history on the characteristics of the infection response can be modeled, and the response correlation structure can be explicitly modeled. Successful use of these features of the NLMH may require larger sample sizes and/or more intensive observations on individual subjects. Monte Carlo simulation studies can be utilized to explore feasibility and sample size requirements in the experimental design phase.

Another area we have not explored in great depth is model building and selection. The NLHM presents an impressive array of modeling options available to the data analyst. With this flexibility comes the need for tools to compare candidate models. Systematic and objective methods are needed to decide, for example, whether an assumption of equal variance across treatment groups is justified. Such questions were not a primary focus of this work.

5.3. Broader applicability

Peaked data arise in many contexts in which there is interest in experimentally studying an acute response to an external stimulus. Researchers in a number of fields have adopted the NLHM approach pioneered in pharmacokinetics and pharmacodynamics for analyzing this time of nonlinear, longitudinal data. Jang et al (2013) applied piecewise NLHM’s to the analysis of cardiac function (heart rate, coronary flow, and left ventricle developed pressure) in experiments on myocardial
ischemia/reperfusion injury (I/R). The goal of such models is to assess the effect of treatments designed to protect against I/R injury. Gössl et al (2001) developed a piecewise Bayesian NLHM of the hemodynamic response function (HRF) which arises in functional magnetic resonance imaging. The HRF reflects acute changes in blood flow and oxygenation levels in response to neuronal stimulation in the brain. Insulin and blood glucose levels follow an acute, single-peaked response pattern following food consumption, and insulin and blood glucose response curves are commonly used in nutrition and diabetes research (Fernandez-Raudales 2012). Salivary cortisol is a commonly used as a biomarker for stress, and salivary cortisol profiles are used to characterize the response to acute stressors (Sanchez 2012). Numerous other examples can be cited. There remain disciplines within which classical methods such as RM-ANOVA for modeling longitudinal data are still predominant. The models and approaches introduced in this dissertation may have applications in a number of domains outside of EI studies.
APPENDIX A.

Supplemental material for illustrative applications
§ 2.4. Febrile response to EAV in horses (symmetric model)

**Figure A.1.** Observed subject-specific core body temperature profiles for eight mares experimentally challenged with the Bucyrus strain of EAV

**Figure A.2.** Observed subject-specific core body temperature profiles for eight stallions experimentally challenged with the KY-84 strain of EAV
Figure A.3. Estimated subject-specific febrile response functions

Figure A.4. Composite residual plot
§ 3.4.1. Lymphocyte response to EAV infection in horses

```r
myModel <- function() {
  for (i in 1:n) {
    Response[i] ~ dnorm(f[i], pow(s.e,-2))
    f[i] <- step(p[Subject[i]]-DPI[i])*\{B[Subject[i]]+I[Subject[i]]*exp(-pow(DPI[i]-p[Subject[i]],2)/(2*pow(l[Subject[i]],2))) +
                           step(DPI[i]-p[Subject[i]])*(B[Subject[i]]+I[Subject[i]]*exp(-pow(DPI[i]-p[Subject[i]],2)/(2*pow(r[Subject[i]],2))))\}
    res[i] <- Response[i] - f[i]
  }

  # Hyperparameters for mean, standard deviation, baseline and intensity
  for (j in 1:8) {
    B[j] ~ dnorm(m.B,pow(s.B,-2))
    I[j] ~ dnorm(m.I,pow(s.I,-2))
    p[j] ~ dnorm(m.p,pow(s.p,-2))
    l[j] ~ dlnorm(m.log_l,pow(s.log_l,-2))
    r[j] ~ dlnorm(m.log_r,pow(s.log_r,-2))
  }

  # Hyperpriors
  m.B ~ dunif(0,5)
  m.I ~ dunif(-10,0)
  m.p ~ dunif(0,42)
  m.log_l ~ dunif(0,5)
  m.log_r ~ dunif(0,5)
  s.e ~ dunif(0,100)
  s.B ~ dunif(0,6)
  s.I ~ dunif(0,6)
  s.p ~ dunif(0,22)
  s.log_l ~ dunif(0,2)
  s.log_r ~ dunif(0,2)

  # FWHM
  HWHM.o <- 2.355*0.5*exp(m.log_l)
  HWHM.r <- 2.355*0.5*exp(m.log_r)
  FWHM <- HWHM.l + HWHM.r
}
```

**Figure A.5.** JAGS model for lymphocyte example
Table A.1. Posterior summary for lymphocyte example

<table>
<thead>
<tr>
<th>Parameter</th>
<th>mu.vect</th>
<th>sd.vect</th>
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<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>97.5%</th>
<th>Rhat n.eff</th>
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<tbody>
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<td>FWHM</td>
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<td>5.414</td>
<td>5.816</td>
<td>6.814</td>
<td>1.001 17000</td>
</tr>
<tr>
<td>HWHM.o</td>
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<td>0.528</td>
<td>3.064</td>
<td>3.711</td>
<td>4.036</td>
<td>4.374</td>
<td>5.116</td>
<td>1.001 26000</td>
</tr>
<tr>
<td>HWHM.r</td>
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<td>1.182</td>
<td>1.233</td>
<td>1.315</td>
<td>1.471</td>
<td>2.233</td>
<td>1.001 9100</td>
</tr>
<tr>
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<td>-0.890</td>
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<tr>
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<td>0.052</td>
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<td>0.148</td>
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<td>0.961</td>
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Figure A.6. Observed subject-specific lymphocyte profiles
Figure A.7. Estimated subject-specific lymphocyte response functions

Figure A.8. Composite residual plot
§ 3.4.2. Febrile response to EAV infection in horses (asymmetric model)

myModel <- function() {
  for (i in 1:n_A) {
    y_A[i] ~ dnorm(f_A[i], pow(s.e,-2))
    f_A[i] <- ifelse(p_A[Subject_A[i]]>=DPI_A[i],
                     B_A[Subject_A[i]]+I_A[Subject_A[i]]*exp(-pow(DPI_A[i]-
                     p_A[Subject_A[i]],2)/(2*pow(l_A[Subject_A[i]],2))),
                     B_A[Subject_A[i]]+I_A[Subject_A[i]]*exp(-pow(p_A[Subject_A[i]]-
                     DPI_A[i],2)/(2*pow(r_A[Subject_A[i]],2))))
  }
  for (i in 1:n_B) {
    y_B[i] ~ dnorm(f_B[i], pow(s.e,-2))
    f_B[i] <- ifelse(p_B[Subject_B[i]]>=DPI_B[i],
                     B_B[Subject_B[i]]+I_B[Subject_B[i]]*exp(-pow(DPI_B[i]-
                     p_B[Subject_B[i]],2)/(2*pow(l_B[Subject_B[i]],2))),
                     B_B[Subject_B[i]]+I_B[Subject_B[i]]*exp(-pow(p_B[Subject_B[i]]-
                     DPI_B[i],2)/(2*pow(r_B[Subject_B[i]],2))))
    res_B[i] <- y_B[i] - f_B[i]
  }

  # Hyperparameters for mean, standard deviation, baseline and intensity
  for (j in 1:8) {
    B_A[j] ~ dnorm(m.B_A, pow(s.B_A,-2))
    I_A[j] ~ dnorm(m.I_A, pow(s.I_A,-2))
    p_A[j] ~ dnorm(m.p_A, pow(s.p_A,-2))
    logl_A[j] ~ dnorm(m.logl_A,pow(s.logl_A,-2))
    logr_A[j] ~ dnorm(m.logr_A,pow(s.logr_A,-2))
    l_A[j] <- exp(logl_A[j])
    r_A[j] <- exp(logr_A[j])
  }

  for (j in 1:8) {
    B_B[j] ~ dnorm(m.B_B, pow(s.B_B,-2))
    I_B[j] ~ dnorm(m.I_B, pow(s.I_B,-2))
    p_B[j] ~ dnorm(m.p_B, pow(s.p_B,-2))
    logl_B[j] ~ dnorm(m.logl_B,pow(s.logl_B,-2))
    logr_B[j] ~ dnorm(m.logr_B,pow(s.logr_B,-2))
    l_B[j] <- exp(logl_B[j])
    r_B[j] <- exp(logr_B[j])
  }

  # Hyperpriors
  m.B_A ~ dunif(90,110); m.B_B ~ dunif(90,110)
  m.I_A ~ dunif(0,20); m.I_B ~ dunif(0,20)
  m.p_A ~ dunif(0,42); m.p_B ~ dunif(0,42)
  m.logl_A ~ dunif(0,5); m.logl_B ~ dunif(0,5)
  m.logr_A ~ dunif(0,5); m.logr_B ~ dunif(0,5)
  s.e ~ dunif(0,100)
  s.B_A ~ dunif(0,10); s.B_B ~ dunif(0,10)
  s.I_A ~ dunif(0,6); s.I_B ~ dunif(0,6)
  s.p_A ~ dunif(0,12); s.p_B ~ dunif(0,12)
  s.logl_A ~ dunif(0,4); s.logl_B ~ dunif(0,4)
  s.logr_A ~ dunif(0,4); s.logr_B ~ dunif(0,4)

  # Differences
  m.B_diff <- m.B_B - m.B_A
  m.I_diff <- m.I_B - m.I_A
  m.p_diff <- m.p_B - m.p_A
  HWHM.l_A <- 0.5*2.355*exp(m.logl_A); HWHM.l_B <- 0.5*2.355*exp(m.logl_B)
  HWHM.r_A <- 0.5*2.355*exp(m.logr_A); HWHM.r_B <- 0.5*2.355*exp(m.logr_B)
  FWHM_A <- HWHM.l_A + HWHM.r_A; FWHM_B <- HWHM.l_B + HWHM.r_B
  FWHM_diff <- FWHM_B - FWHM_A
  m.logl_diff <- m.logl_B - m.logl_A
  m.logr_diff <- m.logr_B - m.logr_A
}

Figure A.9. JAGS model for febrile response example
Table A.2. Posterior summary for febrile response example

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<th></th>
<th>mu.vect</th>
<th>sd.vect</th>
<th>2.5%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>97.5%</th>
<th>Rhat</th>
<th>n.eff</th>
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<td>0.694</td>
<td>4.813</td>
<td>5.322</td>
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<td>-1.620</td>
<td>-0.474</td>
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<td>0.353</td>
<td>1.155</td>
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<td>0.415</td>
<td>3.473</td>
<td>3.998</td>
<td>4.250</td>
<td>4.507</td>
<td>5.105</td>
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<td>1100</td>
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<td>HWHM_B</td>
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<td>1.092</td>
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<td>1.283</td>
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<td>1.092</td>
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<td>1.343</td>
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<td>0.369</td>
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Figure A.10. Observed subject-specific core body temperature profiles for eight mares experimentally challenged with the Bucyrus strain of EAV

Figure A.11. Observed subject-specific core body temperature profiles for eight stallions experimentally challenged with the KY-84 strain of EAV
Figure A.12. Estimated subject-specific febrile response functions for eight mares experimentally challenged with the Bucyrus strain of EAV

Figure A.13. Estimated subject-specific febrile response functions for eight stallions experimentally challenged with the KY-84 strain of EAV
Figure A.14. Composite residual plot for eight mares experimentally challenged with the Bucyrus strain of EAV

Figure A.15. Composite residual plot for eight stallions experimentally challenged with the KY-84 strain of EAV
§ 4.3.1. Effect of body condition on viral shedding in migratory waterfowl

myModel <- function() {
  for (i in 1:n_A) {
    y_A[i] ~ dnorm(f_A[i], pow(s.e,-2))
    Infected_A[i] <- (u_A[i] > -1) * (u_A[i] < 1)
    f_A[i] <- Infected_A[i] * I_A[Subject_A[i]] * (1-abs(u_A[i])^3)^3
  }

  for (i in 1:n_B) {
    y_B[i] ~ dnorm(f_B[i], pow(s.e,-2))
    u_B[i] <- ifelse(DPI_B[i] > p_B[Subject_B[i]], (DPI_B[i] - p_B[Subject_B[i]])/l_B[Subject_B[i]] - r_B[Subject_B[i]]< 1)* (u_B[i] < 1)
    Infected_B[i] <- (u_B[i] > -1) * (u_B[i] < 1)
    f_B[i] <- Infected_B[i] * I_B[Subject_B[i]] * (1-abs(u_B[i])^3)^3
    res_B[i] <- y_B[i] - f_B[i]
  }
}

# Hyperparameters for mean, standard deviation, baseline and intensity

for (j in 1:10) {
  I_A[j] ~ dnorm(m.I_A, pow(s.I_A,-2))
  p_A[j] ~ dnorm(m.p_A, pow(s.p_A,-2))
  l_A[j] ~ dnorm(m.l_A,pow(s.l_A,-2))
  r_A[j] ~ dnorm(m.r_A,pow(s.r_A,-2))
}

for (j in 1:9) {
  I_B[j] ~ dnorm(m.I_B, pow(s.I_B,-2))
  p_B[j] ~ dnorm(m.p_B, pow(s.p_B,-2))
  l_B[j] ~ dnorm(m.l_B,pow(s.l_B,-2))
  r_B[j] ~ dnorm(m.r_B,pow(s.r_B,-2))
}

# Hyperpriors
m.I_A ~ dunif(0,1000); m.I_B ~ dunif(0,1000)
m.p_A ~ dunif(0,10); m.p_B ~ dunif(0,10)
m.l_A ~ dunif(0,m.l_A); m.l_B ~ dunif(0,m.l_B)
m.r_A ~ dunif(0,5); m.r_B ~ dunif(0,5)
s.I_A ~ dunif(0,500); s.I_B ~ dunif(0,500)
s.p_A ~ dunif(0,5); s.p_B ~ dunif(0,5)
s.l_A ~ dunif(0,2); s.l_B ~ dunif(0,2)
s.r_A ~ dunif(0,2); s.r_B ~ dunif(0,2)

# Onset and recovery
onset_A <- m.p_A - m.l_A; onset_B <- m.p_B - m.l_B
recov_A <- m.p_A + m.r_A; recov_B <- m.p_B + m.r_B
dur_A <- recov_A - onset_A; dur_B <- recov_B - onset_B

# Differences
m.I_diff <- m.I_B - m.I_A
m.p_diff <- m.p_B - m.p_A
onset_diff <- onset_B - onset_A
recov_diff <- recov_B - recov_A
dur_diff <- dur_B - dur_A

Figure A.16. JAGS model for influenza A viral shedding example
Table A.3. Posterior summary for influenza A viral shedding example

<table>
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<tr>
<th>Parameter</th>
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<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>97.5%</th>
<th>Rhat</th>
<th>n.eff</th>
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<td>4.972</td>
<td>5.710</td>
<td>6.911</td>
<td>1.043</td>
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</table>
Figure A.17. Observed subject-specific viral shedding profiles for ten wild-caught, normal treatment mallards experimentally challenged with influenza A

Figure A.18. Observed subject-specific viral shedding profiles for nine wild-caught, lean treatment mallards experimentally challenged with influenza A
Figure A.19. Estimated subject-specific viral shedding responses for nine captive-bred, normal treatment mallards experimentally challenged with influenza A

Figure A.20. Estimated subject-specific viral shedding responses for nine wild-caught, lean treatment mallards experimentally challenged with influenza A
Figure A.21. Composite residual plot for nine captive-bred, normal treatment mallards experimentally challenged with influenza A (jittered to show overlapping values more clearly)

Figure A.22. Composite residual plot for nine wild-caught, lean treatment mallards experimentally challenged with influenza A (jittered to show overlapping values more clearly)
§ 4.3.2. Effects of rhinovirus infection in chronic obstructive pulmonary disease

```r
myModel <- function() {
  for (i in 1:n_A) {
    y_A[i] ~ dpois(f_A[i])
    Infected_A[i] <- (u_A[i] > -1) * (u_A[i] < 1)
    f_A[i] <- Infected_A[i] * I_A[Subject_A[i]] * (1-abs(u_A[i])^3)^3
  }
  for (i in 1:n_B) {
    y_B[i] ~ dpois(f_B[i])
    u_B[i] <- ifelse(DPI_B[i] > p_B[Subject_B[i]], (DPI_B[i] - p_B[Subject_B[i]])/r_B[Subject_B[i]], (DPI_B[i] - p_B[Subject_B[i]])/l_B[Subject_B[i]])
    Infected_B[i] <- (u_B[i] > -1) * (u_B[i] < 1)
    f_B[i] <- Infected_B[i] * I_B[Subject_B[i]] * (1-abs(u_B[i])^3)^3
    res_B[i] <- y_B[i] - f_B[i]
  }

  # Hyperparameters for mean, standard deviation, baseline and intensity
  for (j in 1:12) {
    I_A[j] ~ dnorm(m.I_A, s.I_A^(-2))
    p_A[j] ~ dnorm(m.p_A, s.p_A^(-2))
    l_A[j] ~ dnorm(m.l_A, s.l_A^(-2))
    r_A[j] ~ dnorm(m.r_A, s.r_A^(-2))
  }
  for (j in 1:11) {
    I_B[j] ~ dnorm(m.I_B, s.I_B^(-2))
    p_B[j] ~ dnorm(m.p_B, s.p_B^(-2))
    l_B[j] ~ dnorm(m.l_B, s.l_B^(-2))
    r_B[j] ~ dnorm(m.r_B, s.r_B^(-2))
  }

  # Hyperpriors
  m.I_A ~ dunif(0,1500); m.I_B ~ dunif(0,1500)
  m.p_A ~ dunif(0,42); m.p_B ~ dunif(0,42)
  m.l_A ~ dunif(0,50); m.l_B ~ dunif(0,50)
  m.r_A ~ dunif(0,50); m.r_B ~ dunif(0,50)
  s.I_A ~ dunif(0,500); s.I_B ~ dunif(0,500)
  s.p_A ~ dunif(0,12); s.p_B ~ dunif(0,12)
  s.l_A ~ dunif(0,m.l_A); s.l_B ~ dunif(0,m.l_B)
  s.r_A ~ dunif(0,5); s.r_B ~ dunif(0,5)

  # Onset and recovery
  onset_A <- m.p_A - m.l_A; onset_B <- m.p_B - m.l_B
  recov_A <- m.p_A + m.r_A; recov_B <- m.p_B + m.r_B
  dur_A <- recov_A - onset_A; dur_B <- recov_B - onset_B

  # Differences
  m.I_diff <- m.I_B - m.I_A
  m.p_diff <- m.p_B - m.p_A
  onset_diff <- onset_B - onset_A
  recov_diff <- recov_B - recov_A
  dur_diff <- dur_B - dur_A
}
```

Figure A.23. JAGS model for rhinovirus-COPD example
Table A.4. Posterior summary rhinovirus for COPD example

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<th></th>
<th>mu.vect</th>
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<th>2.5%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>97.5%</th>
<th>Rhat</th>
<th>n.eff</th>
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<td>0.140</td>
<td>4.480</td>
<td>4.784</td>
<td>4.889</td>
<td>4.933</td>
<td>4.996</td>
<td>1.001</td>
<td>450000</td>
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<tr>
<td>s.r_B</td>
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<td>4.889</td>
<td>4.933</td>
<td>4.996</td>
<td>1.001</td>
<td>450000</td>
</tr>
</tbody>
</table>
Figure A.24. Observed nasal viral load profiles for twelve healthy controls experimentally challenged with RV-16

Figure A.25. Observed nasal viral load profiles for eleven subjects with COPD experimentally challenged with RV-16
**Figure A.26.** Estimated subject-specific viral shedding responses for twelve healthy controls experimentally challenged with RV-16

**Figure A.27.** Estimated subject-specific viral shedding responses for eleven subjects with COPD experimentally challenged with RV-16
Figure A.28. Composite residual plot for twelve healthy controls experimentally challenged with RV-16 (jittered to show overlapping values more clearly)

Figure A.29. Composite residual plot for eleven subjects with COPD experimentally challenged with RV-16 (jittered to show overlapping values more clearly)
REFERENCES


Go YY, Zhang J, Timoney PJ, Cook RF, Horohov DW, Balasuriya UB. Complex interactions between the major and minor envelop proteins of equine arteritis virus


Koch R. The etiology of anthrax, based on the life history of Bacillus anthracis. Beitrage zur Biologie der Ppanzen 1876;2.2:277-310


Pasteur L. Summary report of the experiments conducted at Pouilly-le-Fort, near Melun, on the Anthrax vaccination (Dasgupta T, Trans.). Comptes Rendus de l’Academie des Science 1881;92:1378-1383.


Sternberg GM. Dr. Finlay's Mosquito Inoculations. American Journal of the Medical Sciences CII 1891;6: 629, 630


Suzor R (1887). Hydrophobia, an account of M. Pasteur’s system: containing a translation of all his communications on the subject, the technique of his method, and the latest statistical results.


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PEER-REVIEWED PUBLICATIONS:
1. Preston S, Riggs C, Singleton M, Troedsson M. Descriptive analysis of longitudinal
endoscopy for exercise induced pulmonary hemorrhage in Thoroughbred racehorses
training and racing at the Hong Kong Jockey Club. Equine Vet J 2014 Jul 23. doi:
10.1111/evj.12326. [Epub ahead of print]
2. Singleton M, Mannino D, Sanderson W. “Body mass index, asthma and exhaled
nitric oxide in U.S. Adults, 2007-2010.” J Asthma 2014 Sep;51(7): 756-61. doi:
10.3109/02770903.2014.912302
Crash, Emergency Department, and Inpatient Hospitalization Datasets in the
10.1080/15389588.2012.757310
of Driver Alcohol Involvement in United States Police and Hospital Records:
5. Slavova SS, Bunn TL, Singleton MD, Bush HM, Livingston SC. “Emergency
Department Visits and Hospitalizations with Concussion Diagnosis.” J KY Medical


NON-REFEREED PUBLICATIONS: