THESIS

INVESTIGATING THE USE OF VACCINATION AS A TOOL FOR MANAGING PNEUMONIC PASTEURELLOSIS IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*)

Submitted by

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ABSTRACT

INVESTIGATING THE USE OF VACCINATION AS A TOOL FOR PREVENTING PNEUMONIC PASTEURELLOSIS IN ROCKY MOUNTAIN BIGHORN SHEEP (*OVIS CANADENSIS CANADENSIS*)

Currently one of the greatest threats to bighorn sheep (*Ovis canadensis*) populations across western North America is respiratory disease and the associated population-level impacts accompanying the illness. While lungworm (*Protostrongylus* spp.) and various bacteria and viruses likely contribute to the respiratory disease complex, probably the single most harmful group of pathogens is bacteria in the family *Pasteurellaceae.* It is well documented that these bacteria can and do cause disease, likely because of the leukotoxin (Lkt) produced by the bacteria which is toxic to ruminant leukocytes. Few ways exist to mitigate this problem, and to date, attempts by wildlife managers to intervene have been ineffective. In Colorado, while some herds of bighorn sheep are healthy, other herds have been crippled by the effects of respiratory disease and concomitant population-level impacts.

Previous research has focused on vaccines to protect bighorns from *Pasteurellaceae* and the associated Lkt produced by those species. Such vaccines have proven to be effective in domestic livestock and, while less research has been conducted

with bighorn sheep, some vaccines have been shown to provide a degree of protection. Because of this potential, I focused on evaluating 2 different *Pasteurellaceae* vaccines in bighorn sheep. One of those vaccines is an autogenous vaccine developed specifically for the Colorado Division of Wildlife (CDOW) for use in bighorn sheep while the other is a commercially available cattle vaccine (One Shot®). Both vaccines were evaluated experimentally in captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*), while the commercial vaccine was also evaluated in a free-ranging herd of bighorns where it was administered along with a suite of other health-related treatments.

Chapter 1 of my thesis is devoted to the evaluation of an autogenous vaccine manufactured by Newport Laboratories (Worthington, MN) and developed specifically to protect Rocky Mountain bighorn sheep in Colorado from several presumed pathogenic strains of *Pasteurellaceae*. In this chapter, I focused on serum antibody concentration responses to vaccination in ewes and lambs, the safety of vaccination, and passive transfer of maternal antibodies from ewes to their lambs.

This vaccine evaluation was carried out using Rocky Mountain bighorn sheep that were part of the CDOW's captive research herd in Fort Collins, Colorado, USA. All bighorn ewes were vaccinated approximately 1 month prior to expected parturition, and were boostered 6 months later, while lambs were vaccinated once at approximately 3 months of age. After vaccination, all animals were observed daily for signs of pneumonia or other adverse reactions to the vaccine. Serum samples were collected periodically from the ewes after vaccination and booster. Serum samples were also collected periodically from all lambs after birth and following vaccination. Colostrum samples were collected from 6 of the 9 study ewes within approximately 30 hours of

parturition. All serum samples were assayed for both Lkt neutralizing and *Mannheimia haemolytica* whole cell antibodies using an enzyme-linked immunosorbent assay (ELISA), while colostrum samples were assayed only for *Mannheimia haemolytica* whole cell antibodies using ELISA.

In the bighorn ewes, vaccination significantly increased Lkt neutralizing and *M. haemolytica* whole cell antibody concentrations for a period of less than 4 weeks. In addition, Lkt neutralizing antibodies were increased by an insignificant amount following booster 6 months after the initial vaccination.

No increase in passive transfer of maternal antibodies between ewes and lambs was detected as a result of vaccination. Antibody concentrations at < 30 hours of age were not different between lambs born to vaccinated or unvaccinated ewes. Likewise, antibody concentrations found in colostrum samples were not different between treatment groups.

I did not detect a statistically significant increase in antibodies after vaccinating the lambs. However, I did observe an increase in Lkt neutralizing antibodies from a mean OD value of 0.0011 (SE = 0.00067) to a mean OD value of 0.0044 (SE = 0.0014) after vaccination, and also an increase in *M. haemolytica* whole cell antibodies from a mean OD value of 0.0076 (SE = 0.0044) to a mean OD value of 0.029 (SE = 0.011) after vaccination.

In summary of Chapter 1, I did not detect differences between vaccinated animals and controls for many of the comparisons of interest. Nevertheless, vaccinating bighorn sheep with this new autogenous vaccine increases Lkt neutralizing antibodies as well as

M. haemolytica whole cell antibodies and therefore may confer some protective benefit to vaccinated animals.

Chapter 2 of my thesis was devoted to an opportunistic field-based evaluation of the commercial *Pasteurellaceae* vaccine One Shot. During the winter of 2008, wildlife managers discovered signs indicating an outbreak of respiratory disease in a herd of bighorn sheep, accompanied by apparently no recruitment of the previous summer's lambs. Three years of capture for herd treatment and/or vaccination followed this discovery in an effort to break the cycle of respiratory disease and to increase the survival of lambs beyond their first year. One Shot was administered the last 2 of those 3 years. In this chapter I focused on the evaluation of serum antibody responses to the vaccine as well as lamb recruitment in subsequent years.

Archived serum samples collected from animals at the time of capture were provided by the CDOW for this follow-up evaluation. These samples were all assayed for both Lkt neutralizing and *Mannheimia haemolytica* whole cell antibodies using ELISA. I compared serum antibody concentrations immediately prior to vaccination with the antibody concentrations 1 year post-vaccination. In addition, I compared the antibody concentrations immediately prior to booster with the antibody concentrations found several weeks after the booster.

Comparing serum antibody concentrations immediately prior to vaccination with the antibody concentrations 1 year post-vaccination, the hypotheses of no effect of vaccination were 2.5 and 2.7 times more likely than the hypotheses that Lkt neutralizing and whole cell *M. haemolytica* antibody levels were higher 1 year later, respectively, due to vaccination. In contrast, comparing pre-booster samples with paired samples collected

several weeks later, the hypothesis of a vaccination effect was 43.4 and 87.1 times more likely than the hypotheses of no vaccination effect for Lkt neutralizing and whole cell *M. haemolytica* antibody levels, respectively.

In addition to the above analysis, historic demographic data for this herd of bighorn sheep were provided by CDOW, and observational data were collected on ewe numbers and lamb recruitment in this herd during the winters following treatment and vaccination. Despite detecting a serum antibody response in adult bighorn sheep, lamb recruitment remained low even after 3 years of treatments and 2 years of vaccination.

The assay results from these first 2 elements of my project suggested that One Shot stimulated a greater increase in circulating antibodies than did the autogenous vaccine. This was interesting because a vaccine that stimulates a greater antibody response might provide more protection than a similar vaccine that stimulates less of an antibody response, assuming that they both properly target the same pathogen. Given this observation, I performed an experiment to directly compare One Shot to the autogenous vaccine; this study is detailed in Chapter 3 of my thesis. Specifically, I focused on the serum levels of Lkt neutralizing and *M. haemolytica* whole cell antibodies in response to vaccination.

Once again, I used CDOW's captive bighorn sheep for this experiment. Thirty sheep were divided into 3 groups of 10 animals each. One group received the vaccine One Shot, another group received the autogenous *Pasteurellaceae* vaccine, and the last group received a sterile saline placebo injection. Serum samples were collected from each animal at the time of vaccination, 2, 4, and 6 weeks after vaccination, and, as with previous experiments, serum samples were assayed for Lkt neutralizing antibodies as

well as *Mannheimia haemolytica* whole cell antibodies using ELISA. I discovered that vaccinating animals with One Shot increased Lkt neutralizing antibodies from a mean OD value of 0.049 to an OD value of 0.13 (95% credible interval [CI]: 0.11, 0.15) while vaccination with the autogenous vaccine increased antibody concentrations to an OD value of 0.067 (95% CI: 0.049, 0.085). In addition, an age effect was important: antibody levels were higher by an OD value of 0.0072 for each year of an animal's age (95% CI: 0.0032, 0.011) regardless of treatment group.

In this experiment I did not find either vaccine to increase *M. haemolytica* whole cell antibody concentrations. I did, however, detect an effect of age on antibody concentrations. Whole cell antibody concentrations increased by an OD value of 0.011 (95% CI: 0.0053, 0.016) for each year of an animal's age.

In summary, my work shows that both One Shot and the autogenous vaccine induced immunological responses in Rocky Mountain bighorn sheep in Colorado. Potential applications for these vaccines include vaccinating high-risk bighorn herds preventatively or vaccinating bighorn herds that are already experiencing chronic pasteurellosis. While this strategy is likely not feasible in all situations, free-ranging herds do exist where vaccines can be administered, as illustrated by the field evaluation of One Shot. Future research should be conducted to determine if the serological responses that I observed equate to protection from *Pasteurellaceae* infection and the associated damage caused by Lkt. Additionally, I suggest that future work should evaluate the efficacy of simultaneously administering both of these and potentially other *Pasteurellaceae* vaccines to provide broader protection against varied strains of *Pasteurellaceae*.

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TABLE OF CONTENTS

LIST OF TABLES

variance with a random intercept correlation structure. DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

LIST OF FIGURES

Figure 3.2: Estimated serum leukotoxin neutralizing antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with two different *Pasteurellaceae* vaccines based on the parameters of the top-ranked model. The dashed line represents antibody concentrations of animals vaccinated with an autogenous *Pasteurellaceae* vaccine, the dotted line represents antibody concentrations of animals vaccinated with the commercial *Pasteurellaceae* vaccine One Shot®, and the solid line represents antibody concentrations of control animals treated with a sterile saline placebo. Week 0 represents pre-vaccination antibody concentrations.

Figure 3.3: Estimated serum *M. haemolytica* whole cell antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with two different *Pasteurellaceae* vaccines based on the parameters of the top-ranked model. The dashed line represents antibody concentrations of animals vaccinated with an autogenous *Pasteurellaceae* vaccine, the dotted line represents antibody concentrations of animals vaccinated with the commercial *Pasteurellaceae* vaccine One Shot®, and the solid line represents antibody concentrations of control animals treated with a sterile saline placebo. Week 0 represents pre-vaccination antibody concentrations.

CHAPTER 1

EVALUATION OF AN AUTOGENOUS, MULTIVALENT, SELF–BOOSTERING, PASTEURELLACEAE VACCINE IN CAPTIVE BIGHORN SHEEP (OVIS CANADENSIS CANADENSIS)

INTRODUCTION

Pneumonic pasteurellosis epizootics and poor population performance currently present serious challenges to maintaining and successfully managing healthy, viable populations of bighorn sheep (*Ovis canadensis*; Singer et al., 2000; Miller, 2001; Cassirer and Sinclair, 2007; George et al., 2008; Wolfe et al., 2010). In many instances, epizootics result in high mortality for all age classes (Onderka and Wishart, 1984; Spraker et al., 1984; Festa-Bianchet, 1988; Cassirer et al., 2001; George et al., 2008). In these cases, disease appears to be acute and animals die despite being in apparently good body condition and without showing early signs of disease (Rush, 1927; Foreyt and Jessup, 1982; Onderka and Wishart, 1984; Festa-Bianchet, 1988; Miller et al., 1991). Additionally, these "all-age" mortality events usually precede years of depressed lamb recruitment (Spraker et al., 1984; Monello et al., 2001; George et al., 2008). In other instances, populations may experience many years of chronic or sporadic pneumonia, with individual animals exhibiting signs of illness such as coughing, weakness, loss of weight, shortness of breath, and lack of appetite, yet large-scale mortality events are not observed (Marsh, 1938; Cassirer and Sinclair, 2007). Recovery of these populations with chronic disease is also often hindered by many years of low lamb recruitment (Spraker et

al., 1984; Miller et al., 1997; McClintock and White 2007; George et al., 2008). In both situations, surviving ewes continue to bear lambs (Cassirer et al., 2001); however, lambs tend to succumb to acute pneumonia (Marsh, 1938; Spraker et al., 1984) between 6 and 11 weeks of age (Foreyt, 1990; Cassirer et al., 2001). The timing of lamb mortality is presumably due to the loss of passive immunity provided by maternal antibodies (Foreyt, 1990), although the ultimate cause of this depressed lamb recruitment is unknown. One likely hypothesis suggests that contagious adult bighorns that survive epizootics or have a chronic infection act as carriers and subsequently transmit the pathogen to their own or other offspring (Spraker et al., 1984; Foreyt, 1990; Miller et al., 1991).

Pasteurellaceae, a group of bacteria which include organisms from the genera *Mannheimia* and *Bibersteinia*, are commonly isolated from pneumonic bighorn sheep and have been implicated as the primary causative agents of many pneumonia epizootics in bighorns (Onderka and Wishart, 1984; Spraker et al., 1984; Foreyt, 1990; Rudolph et al., 2007; George et al., 2008). Other pathogens and stressors that may play a role in these epidemics include animal density, contact with domestic sheep (George et al., 2008), nutritional stress, habitat damage (Festa-Bianchet, 1988), concurrent lungworm (*Protostrongylus* spp.) infection (Spraker and Hibler, 1982), concurrent *Mycoplasma ovipneumoniae* infection (Besser et al., 2008; Dassanayake et al., 2010b), competition, and interactions with humans (Foreyt, 1990).

Vaccinating for pasteurellosis either preventatively, as in the case of chronic pneumonia and low lamb recruitment, or early in a pneumonia epizootic, have been suggested as ways to increase survival of infected bighorn sheep (Miller et al., 1997; Ward et al., 1999; George et al., 2008). In addition, passive antibody transfer from ewe

to lamb through colostrum of vaccinated ewes may increase lamb survival (Cassirer et al., 2001). At birth, lambs have essentially no circulating antibodies of their own and, instead, rely on maternal antibodies that are transferred from the mother to the lamb through colostrum during the first days of life. These antibodies provide protective immunity during development of the lamb's own immune system. This passive immunity wanes over time and is usually replaced by 6 months of age (Radostits et al., 2000). The idea of providing passive immunity to newborn animals by vaccinating the dam is not novel. In swine and cattle, vaccinating the dams to prevent *E. coli* infection in newborn offspring is generally regarded as effective (Moon and Bunn, 1993; Osek et al., 1995). A study evaluating the effectiveness of a combined rotavirus-*E. coli* inactivated vaccine in beef cattle concluded that vaccinating dams in the last trimester of pregnancy resulted in decreased calf morbidity (Cornaglia et al., 1992). In goats, passive immunity from an *E. coli* vaccine administered to pregnant dams provided kids with protection against experimental challenge (Vihan, 1993). It follows that vaccinating bighorn sheep for pasteurellosis may benefit not only the vaccinated dams, but also their offspring.

Vaccinating bighorn and domestic sheep for *Pasteurellaceae* has a history of mixed success. For example, a multivalent *Mannheimia haemolytica* vaccine produced elevated leukotoxin neutralizing antibody titers as well as agglutinating antibody titers in captive bighorn sheep (Miller et al., 1997), and later was demonstrated to provide protection and to reduce mortality in bighorns that were challenged with *Pasteurellaceae* (Kraabel et al., 1998). This vaccine also slightly enhanced survival of vaccinated animals relative to unvaccinated animals during a pneumonia epizootic in wild bighorn sheep that resulted from contact with an infected domestic sheep (George et al., 2008). This same

vaccine, however, failed to increase lamb survival when used in bighorn ewes that had survived a pneumonia outbreak (Cassirer et al., 2001) and did not protect bighorns commingled with domestic sheep (Foreyt 1998). The increase in antibodies stimulated by this vaccine also did not persist as long as did antibody increases resulting from natural *Pasteurellaceae* infections (Ward et al., 1999).

Two other *Pasteurellaceae* vaccines have been unsuccessful at protecting bighorn sheep from pasteurellosis. First, an experimental *M. haemolytica* vaccine failed to protect bighorn sheep placed in contact with apparently healthy domestic sheep; however, this lack of protection may have been due to a lack of cross-protection against infection from other strains of *Pasteurellaceae* harbored concomitantly by the domestic sheep (Foreyt, 1992). Second, vaccinating bighorns with a nonlethal cytotoxic strain of *M. haemolytica* failed to provide cross-protection from challenge with a known lethal strain of *M. haemolytica* (Foreyt and Silflow, 1996).

The history of mixed success applying *Pasteurellaceae* vaccines in bighorn sheep underscores the need for further research. For optimum protection, a vaccine should stimulate high levels of antibodies for a number of years (Ward et al. 1999). Resistance to pneumonic pasteurellosis and higher survival rates of experimentally challenged animals have been correlated with higher quantities of circulating serum leukotoxin (Lkt) neutralizing antibodies (Gentry et al., 1985; Kraabel et al., 1998). Antibody levels have been successfully increased by administering a second "booster" dose of a *Pasteurellaceae* vaccine 2 weeks after the initial dose (Miller et al., 1997); however, in a free-range situation it is not always possible or feasible to capture and revaccinate the same animals twice in such a short period of time. A vaccine that does not require a

classic second-dose booster to stimulate a protective immune response would have greater potential for field use in wild bighorn sheep (Miller et al., 1997). Here, the safety as well as serological responses stimulated by a new *Pasteurellaceae* vaccine that achieves self-boostering through the use of a partitioned design are evaluated in both ewes and their lambs.

MATERIALS AND METHODS

Study animals and animal handling

I used 9 captive Rocky Mountain bighorn ewes as well as their 9 newborn lambs for this study, which I conducted April – November 2009. These animals were part of the Colorado Division of Wildlife's (CDOW) captive bighorn sheep research herd. All animals were housed in 0.5–3.0-ha pastures at the Foothills Wildlife Research Facility (FWRF) in Fort Collins, Colorado, and were fed and cared for in accordance with facility animal husbandry protocols (T. Davis, unpublished data). Pregnancy was confirmed in the ewes via ultrasound 2 months prior to their vaccination.

Many of the bighorn sheep used in this study were hand raised and as a result were tractable. When possible, we physically restrained and handled animals on an enclosed scale without sedation. To prevent undue stress, we tranquilized less tractable animals with xylazine (0.6–1.5 mg/kg; Lloyd Laboratories, Shenandoah, Iowa), and reversed with tolazoline (2.0–3.0 mg/kg; Lloyd Laboratories, Shenandoah, Iowa). For rare situations warranting complete immobilization, we administered a cocktail of butorphanol (0.4–0.5 mg/kg; Wildlife Pharmaceuticals Inc., Fort Collins, Colorado),

azaperone (0.2–0.4 mg/kg; Wildlife Pharmaceuticals Inc., Fort Collins, Colorado), and medetomidine (0.1–0.2 mg/kg; Wildlife Pharmaceuticals Inc., Fort Collins, Colorado), and we administered atipamezole (2.0–2.5 mg/mg of medetomidine administered; Pfizer Animal Health, New York, NY) and tolazoline (2.0–3.0 mg/kg) as reversal agents. Animal care and use procedures were performed in accordance with CDOW and Colorado State University Institutional Animal Care and Use Committee guidelines (CDOW IACUC Approval #02-2009, CSU IACUC Approval # 09-088A-01).

Vaccination and sampling for serological responses

I evaluated a multivalent, autogenous, bacterin vaccine (Newport Laboratories, Worthington, MN) incorporating 4 isolates of *Pasteurellaceae* (2 *Mannheimia haemolytica* and 2 *Bibersteinia trehalosi*; Table 1.1; Wolfe et al., 2010; Miller and Wolfe 2011) collected by the CDOW from the Avalanche Creek (Pitkin County) and Fossil Ridge (Gunnison County) bighorn sheep herds of Colorado during the winter/spring of 2007–2008. To accomplish "self-boostering," this vaccine utilizes the partitioned SoliDose® implantable technology (SolidTech Animal Health, Inc., Newcastle, OK), consisting of 2 implantable pellet types. The pellets of the first type dissolve and release the initial dose of vaccine within an hour of implantation. The pellets of the second type booster the first dose by slowly dissolving and releasing the second dose of vaccine over several weeks.

I stratified the dams by age class and then randomly assigned 5 bighorn ewes to the treatment (vaccine) group and 4 ewes to the control (unvaccinated) group. After

restraint or immobilization, I used a SoliDoser® (SolidTech Animal Health, Inc., Newcastle, OK) applicator to implant the vaccine subcutaneously in the neck of each treatment ewe just in front of the left shoulder on 14 April 2009, approximately 1 month prior to expected parturition. Before injection, I shaved the injection site for ease of subsequent monitoring, and I examined the injection site for any immediate localized vaccine reaction each time the animal was handled for blood sample collection. Study animals were also observed daily throughout the study to assess general health and to document any adverse vaccine reactions. Specifically, animal care technicians monitored animals for signs of lethargy, stiffness or lameness, allergic reaction, and respiratory disease, including but not limited to coughing, nasal discharge, and respiratory distress. Blood samples (10–12 ml) were collected from each animal via jugular venipuncture on the day of vaccination (pre-vaccination), and at 2, 4, and 6 weeks, and 6 months post-vaccination.

To evaluate the serological effects of boostering the vaccine, I revaccinated the surviving ewes from the original vaccinate group (n = 4) with the autogenous vaccine on 13 October 2009, 6 months after they had received the initial vaccination. The second dose of vaccine was equivalent to the original dose and contained both the immediate-release and slow-release pellets. The original surviving control ewes (n = 3) again served as controls. Serum (10–12 ml) was again collected from all vaccinate and control animals at the time of boostering (pre-booster) and at 2, 4, and 6 weeks post-booster.

Prior to parturition, we separated vaccinate and control ewes into different pastures to prevent lambs from nursing and consuming colostrum from ewes of the other group. To assess passive transfer of maternal, vaccine-related antibodies, I collected

colostrum samples from 6 of the bighorn ewes (3 vaccinates, 3 controls) as well as serum samples (10–12 ml) from all of the lambs born to study ewes (n = 8), within approximately 30 hours of parturition. In addition, serum samples (10–12 ml) were collected every 2 weeks from each bighorn lamb up until the time of vaccination. All bighorn lambs were vaccinated with the vaccine on 18 August 2009 at approximately 3 months of age. The vaccine was administered subcutaneously in the left hindquarter of each animal. Serum samples were collected at the time of vaccination (pre-vaccination), 2, 4, 6, 8 weeks, and at 6 months post-vaccination.

Serological analyses were conducted at Oklahoma State University (OSU; Stillwater, OK). Samples were assayed for Lkt neutralizing and *M. haemolytica* whole cell antibodies using enzyme-linked immunosorbent assays (ELISA; Confer et al., 2003) using OSU's standard *M. haemolytica* whole cell strain. Results are reported as optical density (OD) values.

Colostrum samples were assayed for *M. haemolytica* whole cell antibodies using ELISA at Washington State University (Pullman, WA). These antibody concentrations are reported as the reciprocal of the $\log_2$ of the highest dilution yielding a positive reading (Appendix I).

Analysis

I used Bayesian hierarchical models (Gelman et al. 2004) with Markov Chain Monte Carlo (MCMC) methods in the WinBUGs statistical software package, version 1.4.3 (Lunn et al., 2000) to analyze the serum antibody data collected on the bighorn

ewes. I estimated the unnormalized joint posterior density for each model parameter based on the data likelihood and prior densities for the parameters.

To determine the structure of the data likelihood and to identify required prior densities, I used a 2-tiered approach to analysis in order to maintain a reasonably sized candidate model set. I used the first tier of analysis to determine what covariance structure, if any, was necessary to account for correlation that may have been induced by repeatedly sampling individual animals through time. I examined 4 structures to determine which best accounted for the potential correlation contained in the dataset by using each covariance structure in combination with the global model (Model 9; Tables 1.2–1.3; Figure 1.1). The 4 covariance structures that I examined included: an independent error model to determine if evidence for a correlation among samples existed; a random intercept model that modeled only within-individual correlation by treating each individual's intercept (z_i) as a random variable drawn from a common distribution (Jensen 2001); a random time effect model to determine if there was evidence for a correlation between samples collected closer in time; and a random intercept and a random time effect model that allowed for both within-individual and temporal correlation. I modeled the temporal correlation through the specification of a conditionally autoregressive (CAR) prior on the random time effect, τj (i.e., $t_j \sim N\left[t_{j-1}, \sigma^2_{time}\right]$ for $j > 1$, and $t_1 \sim N[0, \sigma^2_{time}]$; Bannerjee et al. 2004; Appendix II). Due to the limited data available for this study, I did not examine structures that were more complex. I used deviance information criterion (DIC) to conduct model selection and to determine which of these covariance structures was best supported by the information in the data (Spiegelhalter et al. 2002). I assumed that the model with the

lowest DIC value was the model best supported by the data, and I calculated differences in DIC values (ΔDIC) among models in the candidate set, model likelihoods, and model probabilities to determine the strength of evidence for each model (Burnham and Anderson 2002, Farnsworth et al. 2006).

I used this selected covariance structure in the second tier of the analysis, and incorporated it into a set of covariate models that represented the plausible antibody responses to vaccine treatment over time. This allowed me to examine 3 covariates of interest: effects of initial vaccine treatment, effects of an application of a second dose of the vaccine, and each application's associated trend effects. Specifically, the initial vaccine effects revealed changes in antibody concentration due to vaccination; the second vaccination effects examined the changes in antibody concentration resulting from boostering the initial vaccination with the application of a second vaccine dose; the trend effects revealed changes in antibody concentration through time following each application of the vaccine. My covariate model set consisted of 18 models that included acute, constant, or changing antibody levels across time as a function of initial treatment with the vaccine and subsequent treatment with a second dose of vaccine (Figure 1.1).

The data likelihood based on this two-tiered analysis described above specified each observed antibody concentration (OD value) as a normal random variable with parameters (μ_{ij}, Σ):

$$y_{ij} \sim N(\mu_{ij}, \Sigma),$$

where y_{ij} was the OD value for the i^{th} sheep for $i = 1, \ldots, n$ on the j^{th} sampling occasion for $j = 1, \ldots, 14$, μ_{ij} represents the mean effect for the i^{th} sheep on the j^{th} sampling occasion, and Σ was the selected covariance matrix. Thus, given these 2 parameters I assumed each sheep's antibody response was conditionally independent. To model the underlying biological processes of interest I modeled μ_{ij} as a function of the covariates described previously and, if included in the selected covariance structure in the first-tier of analysis, random effects that account for the sampling process:

$$\mu_{ij} = z_i + x_{ij}^{'}\beta + \tau_j,$$

where $z_i \sim N(0, \sigma_{int}^2)$ is a random intercept for each individual to account for within individual correlation, $x_{ij}^{'}$ is a $k \times 1$ vector of covariates for the i^{th} sheep across the j^{th} sampling occasion, β is a $k \times 1$ vector of parameter estimates for these covariates, and $\tau_j \sim N(0, \sigma_{time}^2)$ is a random time effect to account for temporal correlation of observations.

Estimating the unnormalized joint posterior density for each parameter required specifying prior densities for each parameter based on prior knowledge of the system. Because I lacked prior knowledge on the effect of these covariates, I used a diffuse prior distribution of N(0,1000) on the treatment, booster, and trend effect parameters where N represents a normal distribution. I specified a diffuse U(0,100) prior for the residual, the random intercept, and the random time effect standard deviations where U represents a uniform distribution. Additionally, I specified CAR priors in the models containing the random time effect models as described previously.

I used 3 chains with overdispersed starting values that were much larger than would be expected given the specified prior distribution. This aided in assessing chain convergence as all 3 chains should converge to the same value (Gelman et al., 2004). I also used a burn-in of 100,000 iterations before drawing 100,000 samples from the posterior distribution for inference about each of the model parameters. Results are based on the mean of parameter estimates for the top ranked model.

I assessed the convergence and mixing of top models using the boa package of program R (Smith 2007; R Development Core Team, 2010). Specifically I looked at sampler lag-autocorrelation plots, density plots, and trace plots for each model parameter, as well as the multivariate potential scale reduction factors and estimates of the corrected scale reduction factors (Gelman and Rubin, 1992).

A simpler approach was used to examine passive transfer of maternal antibodies as well as the effect of vaccination on lamb antibody concentrations due to the small amount of data available for these comparisons. I evaluated passive antibody transfer between the dams and their lambs by examining antibody levels found in lamb serum samples as well as ewe colostrum samples, both collected within 30 hours of parturition. In both cases, I used an information-theoretic approach (Anderson 2008) to compare hypotheses of no effect of dam vaccination status on colostrum or lamb antibody levels to a hypothesis that dam vaccination status affected antibody levels. I used a similar approach to evaluate lamb antibody response to vaccination, comparing a hypothesis of no effect of vaccination to a hypothesis that vaccination affected serum antibody levels. For all 3 comparisons, I calculated evidence ratios as the ratio of the model probability of

the top hypothesis and the model probability of the lower-ranked hypothesis (Anderson, 2008).

RESULTS

Safety

Mild stiffness or soreness was noted in vaccinated animals the day following 3 of the 13 vaccination or booster treatments. By 2 weeks post-vaccination, all vaccinated bighorns experienced injection site swelling < 5 cm in diameter. Swelling was resolved by 6 weeks post-vaccination for all vaccinated animals.

Two ewes died during the course of this study, including 1 vaccinate and 1 control. The control ewe's death was the result of a misplaced dart during immobilization for sample collection. The vaccinate ewe died 11 days post-vaccination and bronchopneumonia was discovered on necropsy. No signs of pneumonia were observed in any of the other study animals.

Bighorn dam serological response to vaccination and booster

Lkt neutralizing antibodies

The first tier of analysis was performed to assess the fit of each of the 4 covariance structures in combination with the global model (Model 9: unequal treatment and booster effects, unequal time trends; Table 1.2). The top ranked model included the random intercept covariance structure and carried 100% of the model weight (Table 1.2). This structure was used for the entire second tier of analysis. The random intercept and

random time effect model did not converge likely due to small sample sizes, while the remaining two models converged but were not supported by the information in the dataset (Table 1.2).

For the second tier of analysis, the top 2 highest ranked models for Lkt neutralizing antibody response to vaccination and booster of bighorn ewes carried essentially the entire weight of the model set (cumulative $w > 0.99$; Table 1.4). Both of these models incorporated acute treatment effects, meaning that a vaccine effect was detected only at 2 weeks post-vaccination. The top model ($w_i = 0.75$; Model 17, Figure 1.1) also included an acute booster effect in addition to the acute treatment effect from the initial vaccination. This model was 3.0 times more likely than the second ranked model, and no other model appeared to be supported by the information in the data (Table 1.4). Based on the top model, vaccination increased Lkt neutralizing antibody concentrations from a mean ELISA OD value of 0.0074 (95% credible interval [CI]: 0.0034, 0.011) by an OD value of 0.028 0.021, 0.035), while booster increased antibody concentrations by a much smaller OD value of 0.0027 (95% CI: -0.0001, 0.0055; Figure 1.2). In this case, the 95% CI for booster effect slightly overlapped zero indicating a weak effect of the booster dose of vaccine on Lkt neutralizing antibody levels.

I found no evidence of non-convergence in any parameters of the top ranked model. Plots of sampler lag-autocorrelations, estimated posterior density, and sampler trace all support chain convergence (Appendix III). In addition, the corrected scale reduction factors for the Brooks–Gelman–Rubin diagnostics were all essentially 1.0 (range: 1.000001, 1.000080), and the multivariate potential scale reduction factor was

1.000063. This suggests that the samples from each chain were approximating the target distribution of the unnormalized joint posterior density of the parameters.

Whole cell *M. haemolytica* antibodies

The first tier of analysis of *M. haemolytica* whole cell antibodies was performed to assess the fit of each of the 4 correlation structures in combination with the global model (Model 9; Table 1.3). The top ranked model included the random intercept covariance structure and carried 100% of the model weight (Table 1.3). This structure was used for the entire second tier of analysis. Like the analysis of Lkt neutralizing antibodies, the random intercept and random time effect model did not converge, while the remaining two models converged but were not supported by the information in the dataset (Table 1.3).

The second tier of analysis revealed 2 models that carried nearly all of the cumulative model weight (cumulative $w > 0.98$; Table 1.5) in the model set. Both of these models included acute treatment effects (i.e., 2 weeks post-vaccination) from the initial vaccination. In addition, the second ranked model ($w_i = 0.26$) demonstrated an acute effect following the booster dose of vaccine. Based on the top model ($w_i = 0.72$; Model 15, Figure 1.1), which included only an acute effect of initial vaccination, antibody concentrations increased from a mean ELISA OD value of 0.058 (95% CI: 0.0075, 0.11) by an OD value of 0.14 (95% CI: 0.080, 0.20) for the second sampling occasion before returning to pre-vaccination levels for the remaining sampling occasions (Figure 1.3). This model was 2.7 times more likely than the second ranked model and no other model was supported by the information in the data.

Convergence testing for the top model did not indicate non-convergence in any of the model parameters. The corrected scale reduction factors for the Brooks–Gelman–Rubin diagnostic were all essentially 1.0 (range: 0.999998, 1.000024), and the multivariate potential scale reduction factor was 1.000016. Plots of sampler lag–autocorrelations, estimated posterior density, and sampler trace all support convergence (Appendix III).

Passive transfer of antibodies to bighorn lambs

While the mean Lkt neutralizing and *M. haemolytica* whole cell antibodies were both lower in lambs born to vaccinated ewes than lambs born to unvaccinated ewes, I did not detect a difference between these groups. For serum samples collected within 30 hours of birth, the Lkt neutralizing antibody concentration for lambs born to vaccinated dams (n = 3) resulted in a mean OD value of 0.0032 (SE = 0.0015) while the mean antibody level for lambs born to unvaccinated dams (n = 4) was 0.0045 (SE = 0.00052; Figure 1.4). The hypothesis of no vaccination effect was 18.8 times more likely than the hypothesis of a vaccination effect on Lkt neutralizing antibodies.

For lamb serum samples collected within 30 hours of birth, whole cell *M. haemolytica* antibody concentrations resulted in a mean OD value of 0.016 (SE = 0.0068) for lambs born to vaccinated dams (n = 3) versus 0.084 (SE = 0.037) for lambs born to unvaccinated dams (n = 4; Figure 1.5). The hypothesis of no vaccination effect was 8.5 times more likely than the alternative hypothesis of vaccination affecting *M. haemolytica* antibody levels.

Similarly, I did not detect a difference in *M. haemolytica* whole cell antibody concentrations in colostrum samples collected from either vaccinated or unvaccinated ewes. The mean *M. haemolytica* whole cell antibody titer was 11.50 (SE = 0.94; n = 3) in colostrum samples collected from vaccinated ewes while it was 11.16 (SE = 0.92; n = 3) in unvaccinated ewes (Figure 1.6). The hypothesis of no vaccination effect was 141.2 times more likely than the hypothesis of a vaccination effect on colostrum antibody level.

Bighorn lamb serological response to vaccination

All vaccinated lambs (n = 4) responded to vaccination with an increase in circulating antibodies (Figures 1.7 and 1.8). Lkt neutralizing antibodies peaked 4 to 8 weeks post-vaccination while the *M. haemolytica* whole cell antibodies all peaked 2 weeks after vaccination. Despite observing an increase in antibodies after vaccination, a significant difference due to vaccination was not detected. I compared pre-vaccination antibody levels from the day of vaccination (day 0) to the observed peak antibody levels after vaccination. Lkt neutralizing antibodies increased slightly from a mean OD value of 0.0011 (SE = 0.00067) to a mean of 0.0044 (SE = 0.0014). The hypothesis of no vaccination effect was 9.8 times as likely as the hypothesis of a vaccination effect. Similarly, while *M. haemolytica* whole cell antibodies increased from a mean OD value of 0.0076 (SE = 0.0044) to a mean antibody level of 0.029 (SE = 0.011), the hypothesis of no effect of vaccination was 34.7 times as likely as the hypothesis of a vaccination effect.

By 6 months post-vaccination, serum antibodies had returned to levels similar to pre-vaccination antibody levels in vaccinated bighorn lambs (Figures 1.7 and 1.8).

DISCUSSION

I evaluated the apparent safety as well as serological antibody responses to a self-boostering vaccine to determine its potential utility as a tool for managing pasteurellosis in free-ranging bighorns where administering a booster dose of vaccine might be impractical. While *Pasteurellaceae* vaccines have been used or evaluated in both captive and free-ranging bighorn sheep on a number of occasions (Foreyt, 1992; Foreyt and Silflow, 1996; Miller et al., 1997; Kraabel et al., 1998; Ward et al., 1999; Cassirer et al., 2001; George et al., 2008; Wolfe et al., 2010), overcoming the need for booster would greatly increase the potential of such vaccines for use in free-ranging animals (Miller et al., 1997).

This vaccine appears to be safe for use in bighorns as the only reactions consistently attributable to the vaccine were mild, short-lived, and far less severe than the disease that the vaccine is designed to prevent. Despite this, it was not possible to either implicate or rule out vaccination as a contributing factor in the death of one treatment ewe that died 11 days after vaccination. This was the least tractable study animal and for handling and sampling purposes it was necessary to move her to a new holding pen for the duration of the study where she could be handled safely. Although she was provided with a companion ewe, she showed signs of distress in her new environment up until the morning she was found dead. Because of the circumstances surrounding her death, I cannot conclude with certainty whether the vaccine, the stress of a novel environment, the combination of both, or neither, was responsible for the pneumonia.

The results of this study indicate that a single dose of vaccine was adequate to increase both Lkt neutralizing and *M. haemolytica* whole cell antibody concentrations

significantly without the addition of a booster dose of vaccine in bighorn ewes. Administering a second dose of vaccine 6 months later resulted in a slight though insignificant increase in Lkt neutralizing antibodies, but no increase in *M. haemolytica* whole cell antibodies. Though brief (less than 4 weeks), the positive effect of vaccination, on Lkt neutralizing antibodies in particular, is highly encouraging as Lkt is considered by many to be the key virulence factor resulting from lung infection with *Pasteurellaceae* (Shewen and Wilkie, 1983, 1985; Lo et al., 1987; Petras et al., 1995; Lainson et al., 1996; Kraabel and Miller, 1997; Tatum et al., 1998; Narayanan et al., 2002; Kelley et al., 2007; Dassanayake et al., 2007, 2009, 2010).

The apparent positive effect of vaccination on both Lkt neutralizing and *M. haemolytica* whole cell antibodies in all of the bighorn lambs is also encouraging even though the effect was not statistically meaningful. The pattern of response in lambs was similar to that observed in bighorn ewes (i.e., an initial peak and return to near pre-vaccination levels by 6 to 8 weeks post-vaccination). A larger sample size (n = 4 vaccinated lambs in this study) would have allowed a better estimate of the degree and sources of variability in antibody levels among lambs, and a better estimate of the effect that vaccination had on antibody levels, similar to that observed in the ewes.

The weaker response to the second treatment with the vaccine in the bighorn ewes despite an initial response to the vaccine 6 months earlier is somewhat perplexing. Based on the boostering results obtained by others with *Pasteurellaceae* vaccines, I expected to see an antibody response similar too, or greater in magnitude than, the initial response to vaccination as Confer et al. (1997) observed in cattle, and Miller et al. (1997) observed with bighorn sheep. One difference between those studies and this one, however, is the

length of time between vaccination and booster. Confer et al. (2003) boostered animals at 3 weeks post-vaccination and Miller et al. (1997) boostered at 2 weeks post-vaccination while here the booster was administered at 6 months post-vaccination.

Cassirer and Sinclair (2007) speculated about the possibility of seasonal variation in immunocompetence of bighorn sheep based on the observation that pneumonia outbreaks tend to occur most often during fall and winter (Spraker et al., 1984; Cassirer and Sinclair 2007; George et al., 2008; Wolfe et al., 2010), and that bighorn sheep may be less capable of mounting an immune response during these seasons due to factors such as limited energy availability or additional stressors. This idea could explain the weak antibody response observed after administering the second dose of vaccine during the fall relative to the response observed after administering the first dose of vaccine in the spring. Additional evidence for this idea is that antibody concentrations in the bighorn ewes were already lower prior to booster in the fall than they were prior to vaccination the previous spring, hinting at the possibility of underlying seasonal variation in immunocompetence before the booster was even administered. Repeating this experiment but swapping the seasons of initial vaccination and subsequent vaccination, or by applying the second dose at a shorter time interval, may reveal that seasonal variation is a more important predictor of immune response to this vaccine than whether it is being administered for the first or second time.

In conclusion, the autogenous *Pasteurellaceae* vaccine that I evaluated appeared to be safe for use in captive bighorn sheep. A single dose of this self-boostering vaccine was adequate to stimulate an increase in antibody levels in the study ewes, and bighorn lamb antibody levels also increased slightly following vaccination. Even though the

increases in antibody concentration were short-lived, the potential for protecting bighorns in the wild with this vaccine motivates additional investigation. Further research with this vaccine as well as a commercial *Pasteurellaceae* vaccine will supplement these findings (see Chapter 3).

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Table 1.1: Origin and bighorn sheep herd history of the *Pasteurellaceae* isolates used in the production of an autogenous *Pasteurellaceae* vaccine administered to captive bighorn sheep in 2009. See Wolfe et al. (2010) and Miller and Wolfe (2011) for further information on isolates.

Herd of origin	Location	Herd History	Species and biogroup	Serotype	Isolate #
Avalanche Creek	39^0 13" 53" N 107^0 13" 32" W	Poor lamb recruitment	*B. trehalosi* 4^{CDS}	T3	08-0381-011
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*B. trehalosi* 4^{CDS}	unknown	07-1920-003
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*M. haemolytica* 1^G	A2	07-1920-001
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*M. haemolytica* 1^G	A2	08-0200-195

Table 1.2: Model selection results from the first tier of analysis of leukotoxin neutralizing antibody concentration responses stimulated by administering an autogenous *Pasteurellaceae* vaccine to captive bighorn sheep in 2009, intended to determine the best fitting covariance structure in combination with the global covariate model (Model 9: ≠ treatment and booster effects, ≠ time trends). DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	Covariance structure	DIC	Δ DIC	Likelihood	Probability
9	Random Intercept	-541.01	0.00	1.00	1.00
9	Random Time	-465.87	75.14	0.00	0.00
9	Independent Error	-460.52	80.50	0.00	0.00
9	Random Time & Random Intercept	did not converge			

Table 1.3: Model selection results from the first tier of analysis of *Mannheimia haemolytica* whole cell antibody concentration responses stimulated by administering an autogenous *Pasteurellaceae* vaccine to captive bighorn sheep in 2009, intended to determine the best fitting covariance structure in combination with the global covariate model (Model 9: ≠ treatment and booster effects, ≠ time trends). DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	Covariance structure	DIC	Δ DIC	Likelihood	Probability
9	Random Intercept	-276.29	0.00	1.00	1.00
9	Independent Error	-172.08	104.20	0.00	0.00
9	Random Time	-152.13	124.16	0.00	0.00
9	Random Time & Random Intercept	did not converge			

Table 1.4: Covariate model selection results for serum Lkt neutralizing antibody data collected from captive Rocky Mountain bighorn ewes administered an autogenous *Pasteurellaceae* vaccine in 2009. DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model #	Model	DIC	Δ DIC	Likelihood	Probability
model 17	$V_{I\ acute} + V_{B\ acute}$	-578.13	0.00	1.00	0.75
model 15	$V_{I\ acute}$	-575.95	2.18	0.34	0.25
model 6	$V_{I=B} + T_{I=B}$	-545.37	32.75	0.00	0.00
model 14	$V_{I=B} + T_{I=B+} T_C$	-543.25	34.88	0.00	0.00
model 7	$V_I + V_B + T_{I=B}$	-543.23	34.90	0.00	0.00
model 8	$V_{I=B} + T_I + T_B$	-543.17	34.95	0.00	0.00
model 16	$V_{I\ acute=B\ acute}$	-542.85	35.28	0.00	0.00
model 9	$V_I + T_I + V_B + T_B$	-541.01	37.11	0.00	0.00
model 2	V_I	-540.09	38.04	0.00	0.00
model 5	$V_I + V_B$	-539.59	38.54	0.00	0.00
model 13	$V_I + T_{I=C}$	-537.99	40.14	0.00	0.00
model 12	$V_I + T_C$	-537.93	40.19	0.00	0.00
model 3	V_B	-534.11	44.02	0.00	0.00
model 1	null	-532.32	45.80	0.00	0.00
model 18	$V_{B\ acute}$	-532.31	45.82	0.00	0.00
model 4	$V_B + T_B$	-531.99	46.14	0.00	0.00
model 10	$T_{I=C}$	-530.56	47.57	0.00	0.00
model 11	T_C	-530.25	47.88	0.00	0.00

T_B: linear time trend on booster vaccination effect
T_C: linear time trend on control antibody level
$T_{I=B}$: linear time trend on initial vaccination effect equal to linear time trend on booster vaccination effect
$T_{I=C}$: linear time trend on initial vaccination effect equal to linear trend on control antibody levels
T_I: linear time trend on initial vaccination effect
V_B: effect of booster vaccination
$V_{I=B}$: effect of initial vaccination equal to effect of booster vaccination
V_I: effect of initial vaccination
$V_{B\ acute}$: acute effect of booster vaccination
$V_{I\ acute=B\ acute}$: acute effect of initial vaccination equal to acute effect of booster vaccination
$V_{I\ acute}$: acute effect of initial vaccination

Table 1.5: Covariate model selection results for serum *M. haemolytica* whole cell antibody data collected from captive Rocky Mountain bighorn ewes administered an autogenous *Pasteurellaceae* vaccine in 2009. DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model #	Model	DIC	Δ DIC	Likelihood	Probability
model 15	$V_{I\ acute}$	-302.01	0.00	1.00	0.72
model 17	$V_{I\ acute} + V_{B\ acute}$	-300.02	2.00	0.37	0.26
model 11	T_C	-292.66	9.35	0.01	0.01
model 14	$V_{I=B} + T_{I=B} + T_C$	-292.23	9.78	0.01	0.01
model 12	$V_I + T_C$	-291.01	11.01	0.00	0.00
model 13	$V_I + T_{I=C}$	-288.51	13.50	0.00	0.00
model 10	$T_{I=C}$	-287.59	14.42	0.00	0.00
model 16	$V_{I\ acute = B\ acute}$	-283.34	18.67	0.00	0.00
model 1	null	-281.73	20.29	0.00	0.00
model 6	$V_{I=B} + T_{I=B}$	-280.70	21.31	0.00	0.00
model 2	V_I	-280.09	21.92	0.00	0.00
model 18	$V_{B\ acute}$	-279.67	22.35	0.00	0.00
model 3	V_B	-279.52	22.49	0.00	0.00
model 8	$V_{I=B} + T_I + T_B$	-278.48	23.53	0.00	0.00
model 7	$V_I + V_B + T_{I=B}$	-278.47	23.54	0.00	0.00
model 5	$V_I + V_B$	-277.85	24.17	0.00	0.00
model 4	$V_B + T_B$	-277.61	24.40	0.00	0.00
model 9	$V_I + T_I + V_B + T_B$	-276.29	25.73	0.00	0.00

T_B: linear time trend on booster vaccination effect
T_C: linear time trend on control antibody level
$T_{I=B}$: linear time trend on initial vaccination effect equal to linear time trend on booster vaccination effect
$T_{I=C}$: linear time trend on initial vaccination effect equal to linear trend on control antibody levels
T_I: linear time trend on initial vaccination effect
V_B: effect of booster vaccination
$V_{I=B}$: effect of initial vaccination equal to effect of booster vaccination
V_I: effect of initial vaccination
$V_{B\ acute}$: acute effect of booster vaccination
$V_{I\ acute = B\ acute}$: acute effect of initial vaccination equal to acute effect of booster vaccination
$V_{I\ acute}$: acute effect of initial vaccination

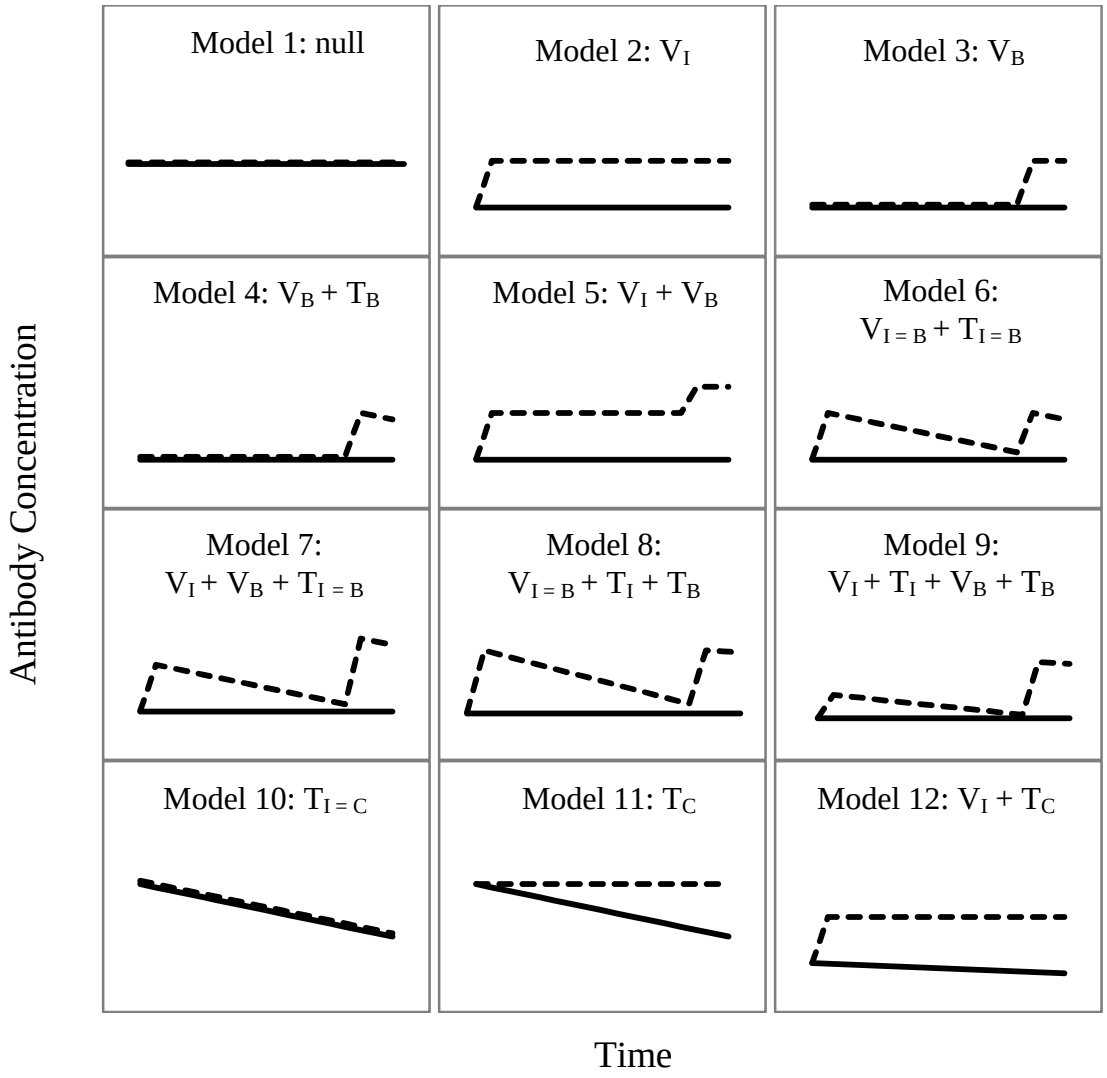

Figure 1.1: Graphical representation of the 18 models used in the analysis of captive bighorn ewe antibody concentration across time as a function of vaccination and booster with an autogenous *Pasteurellaceae* vaccine. Solid lines represent unvaccinated animal antibody concentrations; dashed lines represent vaccinated bighorn antibody concentrations. The model parameters are defined as follows: T_B: linear time trend on booster vaccination effect, T_C: linear time trend on control antibody level, $T_{I=B}$: linear time trend on initial vaccination effect equal to linear time trend on booster vaccination effect, $T_{I=C}$: linear time trend on initial vaccination effect equal to linear trend on control antibody levels, T_I: linear time trend on initial vaccination effect, V_B: effect of booster vaccination, $V_{I=B}$: effect of initial vaccination equal to effect of booster vaccination, V_I: effect of initial vaccination, $V_{B\ acute}$: acute effect of booster vaccination, $V_{I\ acute=B\ acute}$: acute effect of initial vaccination equal to acute effect of booster vaccination, $V_{I\ acute}$: acute effect of initial vaccination.

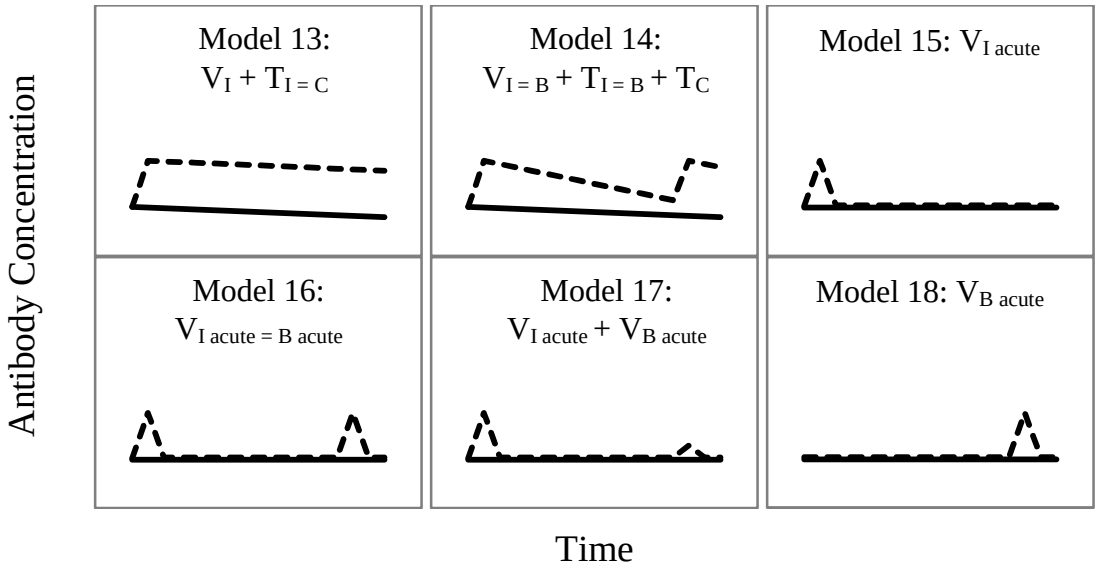

Figure 1.1: (continued)

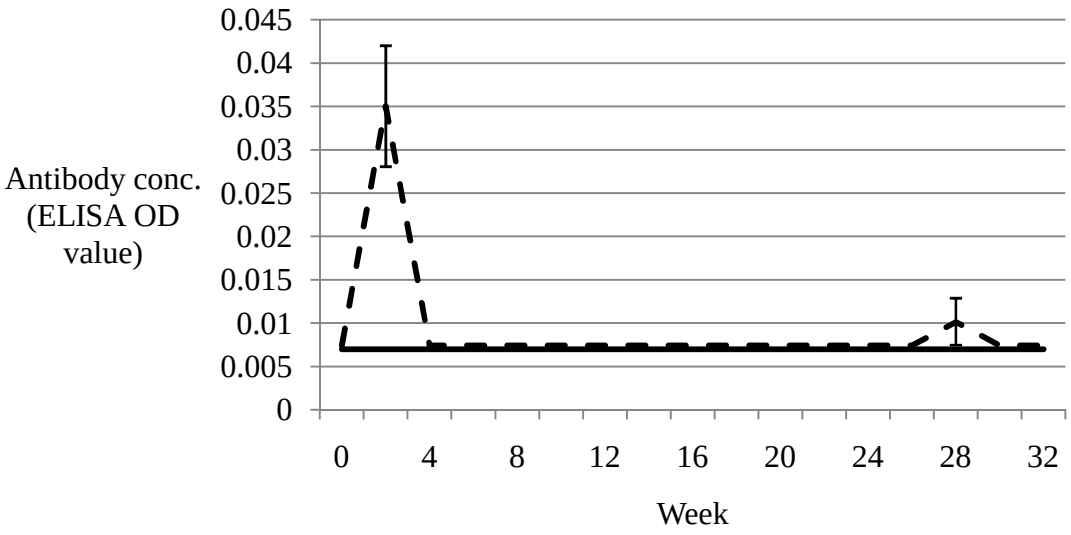

Figure 1.2: Estimated serum leukotoxin neutralizing antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with an autogenous *Pasteurellaceae* vaccine based on the parameters of the top-ranked model. The solid line represents antibody concentrations of control animals (n = 4) and the dashed line represents antibody concentrations of animals vaccinated with the autogenous vaccine (n = 5). Week 0 represents pre-vaccination antibody concentrations. Error bars represent 95% credible intervals.

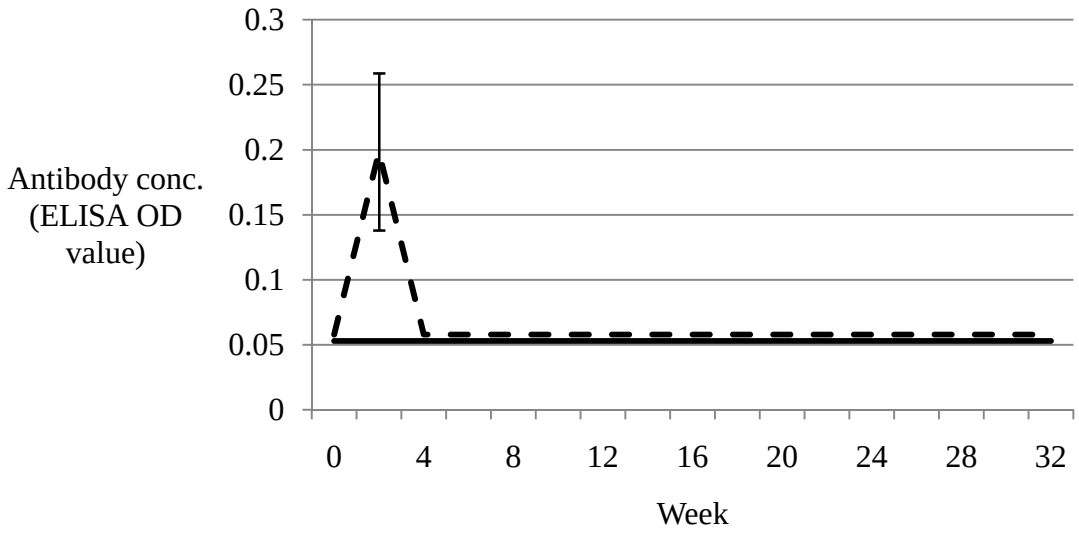

Figure 1.3: Estimated serum *M. haemolytica* whole cell antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with an autogenous *Pasteurellaceae* vaccine based on the parameters of the top-ranked model. The solid line represents antibody concentrations of control animals (n = 4) and the dashed line represents antibody concentrations of animals vaccinated with the autogenous vaccine (n = 5). Week 0 represents pre-vaccination antibody concentrations. Error bars represent 95% credible intervals.

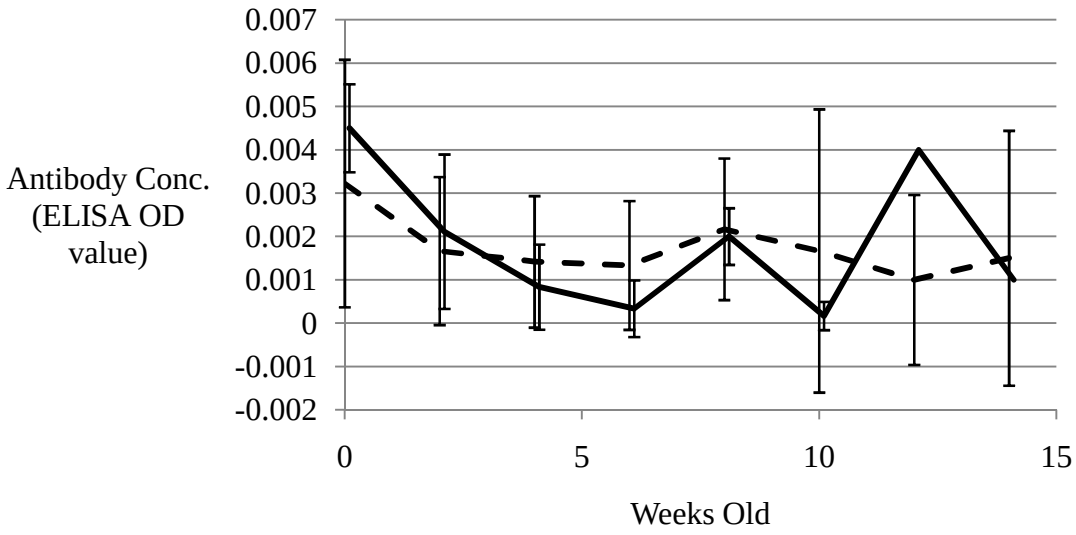

Figure 1.4: Leukotoxin neutralizing antibody concentrations from birth to 14 weeks old of captive bighorn sheep lambs born to either vaccinated or unvaccinated dams in 2009. Solid line represent antibody concentrations of lambs born to unvaccinated dams (n = 4). Dashed line represent antibody concentrations of lambs born to vaccinated dams (n = 4). Antibody concentrations measured by ELISA. Error bars represent 95% confidence intervals.

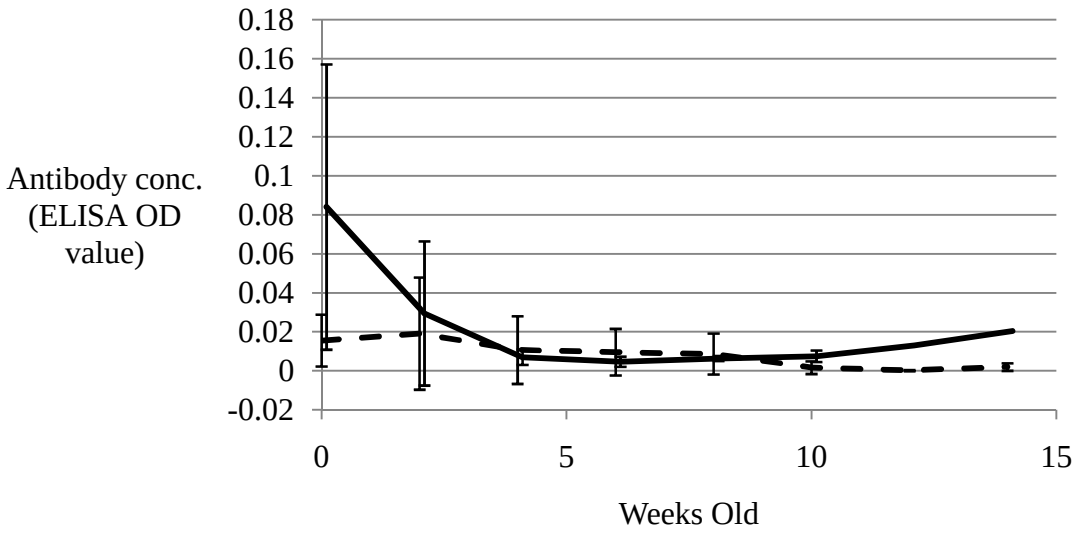

Figure 1.5: *M. haemolytica* whole cell antibody concentrations from birth to 14 weeks old of captive bighorn sheep lambs born to either vaccinated or unvaccinated dams in 2009. Solid line represent antibody concentrations of lambs born to unvaccinated dams (n = 4). Dashed line represent antibody concentrations of lambs born to vaccinated dams (n = 4). Antibody concentrations measured by ELISA. Error bars represent 95% confidence intervals.

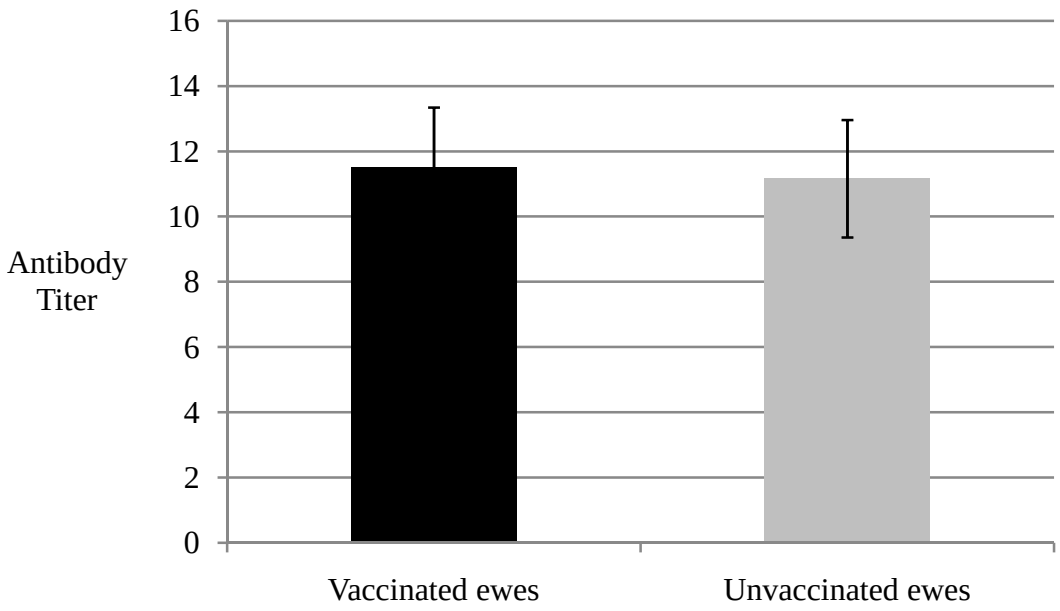

Figure 1.6: Mean *M. haemolytica* whole cell antibody titers for colostrum samples collected within 30 hours of parturition from vaccinated (n = 3) and unvaccinated (n = 3) bighorn sheep ewes administered an autogenous *Pasteurellaceae* vaccine in 2009. Error bars represent 95% confidence intervals. Antibody concentrations are reported as the reciprocal of the $\log_2$ of the highest dilution yielding a positive reading.

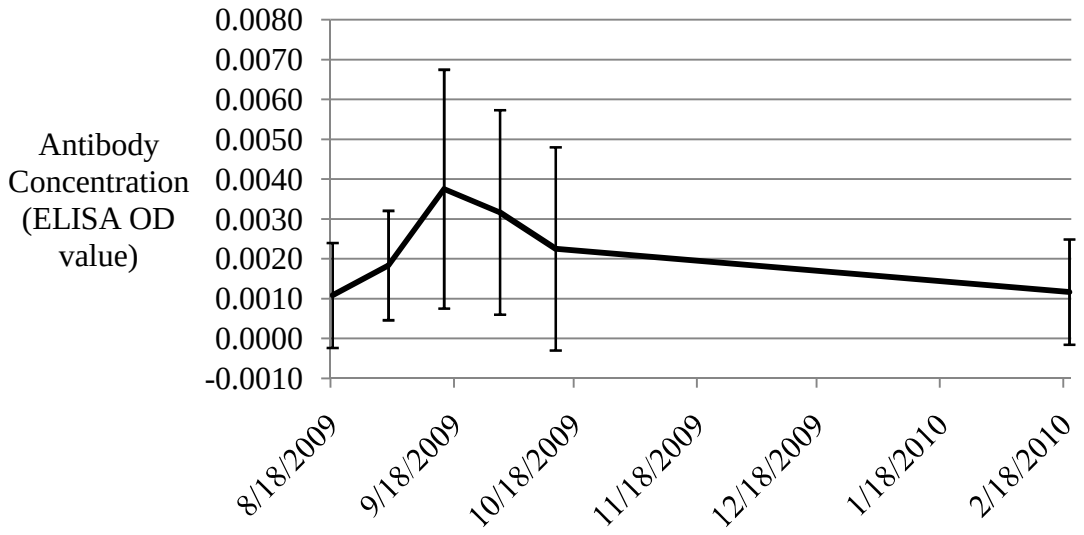

Figure 1.7 Mean serum Lkt neutralizing antibody concentrations of captive bighorn sheep lambs (n = 4) following vaccination with an autogenous vaccine on 8/18/2009. Antibody concentrations measured by ELISA. Error bars represent 95% confidence intervals.

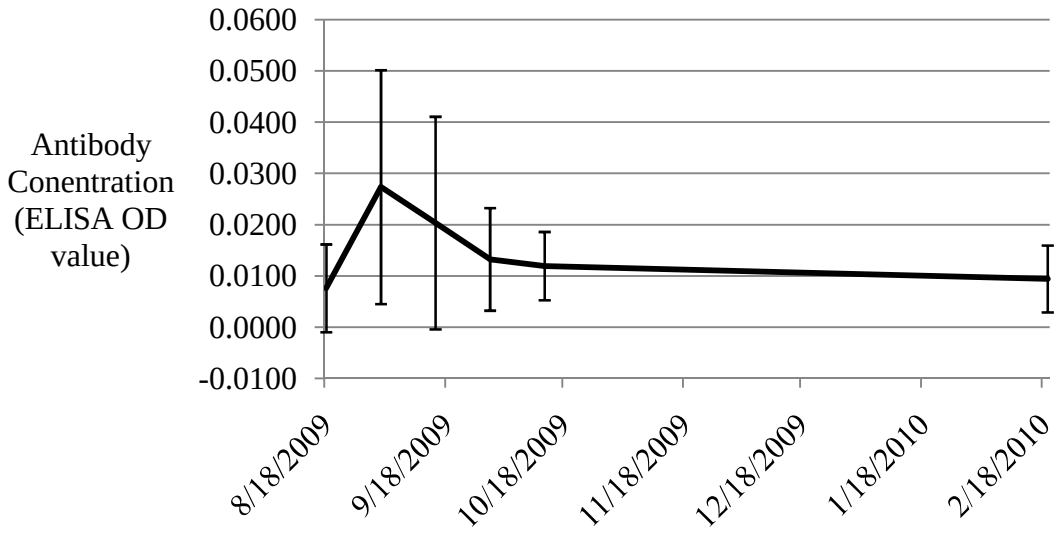

Figure 1.8: Mean serum *M. haemolytica* whole cell antibody concentrations of captive bighorn sheep lambs (n = 4) following vaccination with an autogenous vaccine on 8/18/2009. Antibody concentrations measured by ELISA. Error bars represent 95% confidence intervals.

CHAPTER 2

OPPORTUNISTIC EVALUATION OF A *PASTEURELLACEAE* VACCINE IN A FREE-RANGING ROCKY MOUNTAIN BIGHORN SHEEP HERD EXPERIENCEING CHRONIC PASTEURELLOSIS

INTRODUCTION

Respiratory disease represents one of the most significant threats to the successful long-term persistence of bighorn sheep (*Ovis canadensis*) populations throughout western North America (Singer et al., 2000; Miller et al., 2001; Cassirer and Sinclair, 2007; George et al., 2008; Wolfe et al., 2010). Pneumonia epidemics sometimes occur in bighorn herds, with a large number of otherwise apparently healthy animals succumbing to infection in a relatively short period of time (Onderka and Wishart, 1984; Spraker et al., 1984; Cassirer et al., 2001; George et al., 2008; Wolfe et al., 2010). Years of depressed lamb recruitment often follow the initial epidemic, further hindering population recovery (Spraker et al., 1984; Monello et al., 2001; George et al., 2008; Wolfe et al., 2010). In contrast to an acute outbreak, some bighorn herds experience extended periods of low lamb recruitment associated with more sporadic or chronic respiratory disease (Marsh, 1938; Cassirer and Sinclair, 2007). Bacteria species in the family *Pasteurellaceae*, including *Mannheimia haemolytica,* are often isolated from pneumonic bighorn sheep and have been implicated in the pathology associated with bighorn pneumonia (Spraker et al., 1984; Festa-Bianchet, 1988; George et al., 2008; Wolfe et al., 2010). Few tools are available for wildlife managers to use in protecting or

recovering bighorn sheep populations from these infections (George et al., 2009). Here we describe a suite of treatments, including a *Pasteurellaceae* vaccine managers used in an attempt to increase lamb recruitment in a bighorn sheep herd. In addition, we focus on an opportunistic analysis of serological responses resulting from the vaccine as well as subsequent lamb recruitment to the managed herd. The capture and handling procedures were exempted by the Colorado Division of Wildlife (CDOW) Animal Care and Use Committee because this work was part of ongoing herd health management (R. B. Davies, Chairman CDOW Animal Care and Use Committee, written communication, 11 16 2010).

Study Area

The Badger Creek bighorn sheep herd of the Brown's Canyon herd complex winters in the area of Gribbles Park (38° 38' 34" N 105° 47' 34" W), approximately 13 miles northeast of the town of Salida, Colorado, USA. This herd was started with a translocation of 19 sheep in 1990 (George et al., 2009) and reached a peak size of about 62 animals in 2005 (K. J. Woodruff, unpublished data). Following the transplant, annual lamb recruitment was generally maintained at ≥16 lambs prior to 2000; however, since at least 2004, lamb recruitment has been depressed, with 2 or fewer lambs surviving annually since 2006 (Table 2.1).

MATERIALS AND METHODS

From the late 1990's through 2007, the Colorado Division of Wildlife fed bighorns each winter at Gribbles Park for the purpose of treating the animals for lungworm (*Protostrongylus* spp.). Oral fenbendazole was administered to the sheep in the alfalfa hay and apple pomace feed (K. Woodruff, unpublished data). In March 2008, wildlife managers noted an absence of 2007 cohort lambs in the Badger Creek herd. Some adults exhibited signs of respiratory disease, including coughing and nasal discharge. Later that month, CDOW captured most of this herd under a drop net (22 females, 2 males), collected blood for serology and oropharyngeal swabs for bacterial culture (see Wolfe et al., 2010 for methods), and administered tulathromycin (200 mg subcutaneously; Draxxin®, Pfizer Animal Health, New York, New York, USA) and doramectin (10 mg subcutaneously; Dectomax®, Pfizer Animal Health) in an attempt to treat respiratory disease and improve lamb recruitment. Five additional animals that escaped capture (3 ewes and 2 rams) were later darted with tulathromycin. Samples from captured animals revealed evidence of β-hemolytic *Pasteurellaceae* strains (*M. haemolytica* biogroup 1^{A}, *Bibersteinia trehalosi* 4^{CDS}), *Mycoplasma* spp., and exposure to parainfluenza 3 virus. Based on bacterial culture results and similarities to patterns seen in other affected bighorn herds (Cassirer et al., 2001; George et al., 2008, 2009; Wolfe et al., 2010; L. L. Wolfe, unpublished data), we attributed the apparent herd health and recruitment problems to pasteurellosis. Despite treatment, recruitment to December of 2008 cohort lambs remained low (Table 2.1).

In February 2009, CDOW recaptured 18 of the approximately 34 total animals in the herd by drop net for treatment. In addition to repeating treatments with tulathromycin

and doramectin as described above, managers administered a *M. haemolytica* serotype 1 bacterin-toxoid (2 ml subcutaneously; One Shot®, Pfizer Animal Health) a multivalent, killed respiratory virus vaccine (2 ml intramuscularly; Triangle® 4 + type II BVD, Fort Dodge Animal Health, Fort Dodge, Iowa), and doses of 2 hyperimmune serum products (approximately 2 ml each, sprayed intranasally; RP-Bridge and M-Bridge, VDx, inc., Newburg, WI). Once again, blood and oropharyngeal swabs were collected. Samples revealed β-hemolytic *Pasteurellaceae* strains (*M. haemolytica* biogroup 1, *B. trehalosi* 4^{CDS} and 2^{CD}), *Mycoplasma* spp., and antibodies to parainfluenza 3 virus. Lamb recruitment to December remained poor in 2009 (Table 2.1).

During January and February 2010 wildlife managers captured 10 animals via darting and treatments of bacterin-toxoid (10 of 10 animals), multivalent respiratory virus vaccine (10 of 10 animals), tulathromycin (10 of 10 animals), and doramectin (6 of 10 animals) were administered as above. Between 2 weeks and 2 months later, 8 of those 10 individuals were captured again and the same 4 treatments were repeated to each animal. An additional 9 individuals were darted with doses of bacterin-toxoid that winter. Blood and oropharyngeal swabs were collected at each capture opportunity. β-hemolytic *Pasteurellaceae* strains (*M. haemolytica* biogroup 1^{B} and 1^{AE}, *B. trehalosi* 2^{BS}) were recovered from a portion of the samples. No lambs survived to the following winter (Table 2.1).

Overall, field conditions precluded our determining whether the treatments applied were ineffective or were simply insufficient or inappropriate to remedy the cause of poor lamb recruitment observed in the Badger Creek herd. To help assess whether vaccination with the bacterin-toxoid stimulated antibody responses, all serum samples

from 2009 and 2010 were assayed for both whole cell *M. haemolytica* antibodies and leukotoxin (Lkt) neutralizing antibodies using enzyme-linked immunosorbent assays (ELISA; Confer et al., 2003) at Oklahoma State University (Stillwater, Oklahoma, USA). We compared antigen-specific antibody concentrations between 2009 and 2010, as well as between treatments in 2010. For each analysis, we used a paired design and an information-theoretic approach to compare a set of 2 hypotheses (Burnham and Anderson 2002): a hypothesis of no effect of vaccination and a hypothesis incorporating an effect of the vaccination. We computed the residual sum of squares for each hypothesis and used these values to calculate Akaike's Information Criterion corrected for small sample sizes (AICc). We assumed that the hypothesis with the lowest AICc value was the best supported of the 2 hypotheses based on the strength of evidence provided by the information in the data. Lastly, we report evidence ratios, which we calculated as the model weight of the first ranked hypothesis with that of the second-ranked hypothesis (Burnham and Anderson 2002).

RESULTS

One year after vaccination with the bacterin-toxoid, neither the mean concentration of Lkt neutralizing nor whole cell *Mannheimia haemolytica* antibodies was different than prior to vaccination the year before (Figures 2.1 and 2.2). In 2009 the mean Lkt neutralizing antibody level before vaccination resulted in an ELISA optical density (OD) value of 0.029 (SE = 0.0049; C.I. = 0.019, 0.038) while it was 0.034 (SE = 0.0072; C.I. = 0.019, 0.048) in 2010 (n = 10). Our hypothesis of no effect (AICc = –83.73) was 2.5 times more likely than our hypothesis of an effect of vaccination (AICc =

–81.88). The whole cell *M. haemolytica* antibody level yielded an OD value of 0.045 (SE = 0.015; C.I. = 0.014, 0.075) in 2009 and 0.038 (SE = 0.013; C.I. = 0.014, 0.063) in 2010, and, in this case, the hypothesis of no effect carried an AICc of –76.57 and was 2.7 times more likely than our hypothesis incorporating an effect (AICc = –74.54; n = 10).

We did demonstrate a difference between the antibody levels before and after the vaccine booster in 2010 (n = 8). The Lkt antibody level resulted in a mean ELISA OD value of 0.028 (SE = 0.0041; C.I. = 0.020, 0.036) before booster and 0.10 (SE = 0.018; C.I. = 0.066, 0.14) after booster (Figure 2.1), and the hypothesis of a vaccination effect (AICc = –44.22) was 43.4 times more likely than the hypothesis of no effect (AICc = –36.68). Likewise, whole cell *M. haemolytica* antibody concentrations resulted in a mean OD value of 0.025 (SE = 0.0036; C.I. = 0.018, 0.032) before booster and 0.13 (SE = 0.020; C.I. = 0.087, 0.17) after booster (Figure 2.2), with the hypothesis of a vaccination effect (AICc = –41.01) being 87.1 times more likely than the hypothesis of no effect (AICc = –32.08).

DISCUSSION

I did not detect a difference in antibody levels 1 year post-vaccination. Based on data from paired samples collected in 2010, however, antibodies to *M. haemolytica* surface antigens and leukotoxin presumably increased initially after vaccination in 2009 as well. This response then likely waned through time preventing detection of an antibody response 1 year later, similar to bighorn responses to a multivalent *Pasteurellaceae* vaccine (Miller et al., 1997; Kraabel et al., 1998). Despite seeing evidence of an immune response to *M. haemolytica* antigens in ewes as a result of

vaccination, the vaccine and accompanying treatments apparently did not affect lamb recruitment, as recruitment remained low across all 3 years of intensive herd treatment (Table 2.1).

Because this was an observational study and not a designed experiment, I am limited in the conclusions I can draw. One potential explanation for my observations is that passive transfer of maternal antibodies from vaccinated dams to their lambs may have hindered the development of the lambs' own acquired immune response (Kiorpes et al., 1991; Vitour et al., 2011). It is this acquired immune response that would have been necessary to protect the lambs after the maternal antibodies had waned. The idea of maternal antibody interference, where maternally derived antibodies react with and eliminate vaccine antigens before the lamb's own immune system has an opportunity to recognize and respond to those antigens, is supported by one study conducted with domestic sheep. In that case, lambs born to vaccinated ewes had higher Lkt neutralizing antibodies shortly after birth, however 60 days later those same lambs had significantly lower concentrations of Lkt neutralizing antibodies than lambs born to unvaccinated ewes (Kiorpes et al., 1991).

My observation that vaccinating ewes did not increase lamb recruitment is similar to what Cassirer et al. (2001) discovered after attempting to increase lamb survival by vaccinating dams with 2 different *Pasteurellaceae* vaccines following a die off. In that case, survival of lambs born to vaccinated ewes was lower than lambs born to unvaccinated ewes and pneumonic pasteurellosis remained the principal cause of death.

Cassirer et al. (2001) concluded that vaccinating ewes prior to parturition was unlikely to increase recruitment of lambs following pneumonia epidemics, and my

observations do not provide any evidence to the contrary. As a result, wildlife managers should be aware that this may not be an effective strategy for increasing lamb recruitment.

Acknowledgements

We thank CDOW for providing the archived serum samples used in this analysis as well as the historic winter count data on the Gribbles Park bighorn sheep herd. We also thank Dr. Confer's lab at Oklahoma State University for performing the ELISA's.

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Table 2.1: Winter treatments and counts of Rocky Mountain bighorn sheep lambs and ewes in the Badger Creek herd, Gribbles Park, Colorado, present on winter range from 2004–2010. Effect of treatment on lamb recruitment, if any, is expected to occur in the year following treatment. See text for details of herd treatments.

Winter	Herd treatments [a]	Lambs	Ewes	Lambs:100 ewes
2004–2005	F,B	7	46	15.2 (SE = 5.3)
2005–2006	F,B	5	35	14.3 (SE = 5.9)
2006–2007	F,B	2	34	5.9 (SE = 4.0)
2007–2008	F,T,D	0	25	0.0
2008–2009	F,T,D,**M**,R,H	2	23	8.7 (SE = 5.9)
2009–2010	F,T,D,**M**,R	2	22	9.1 (SE = 6.1)
2010–2011	N	0	16	0.0

[a] F = fed hay and apple pulp; B = oral fenbendazole ; T = tulathromycin; D = doramectin; **M** = *Mannheimia haemolytica* serotype 1 vaccine; R = multivalent respiratory virus vaccine; H = intranasal hyperimmune sera; N = no treatments.

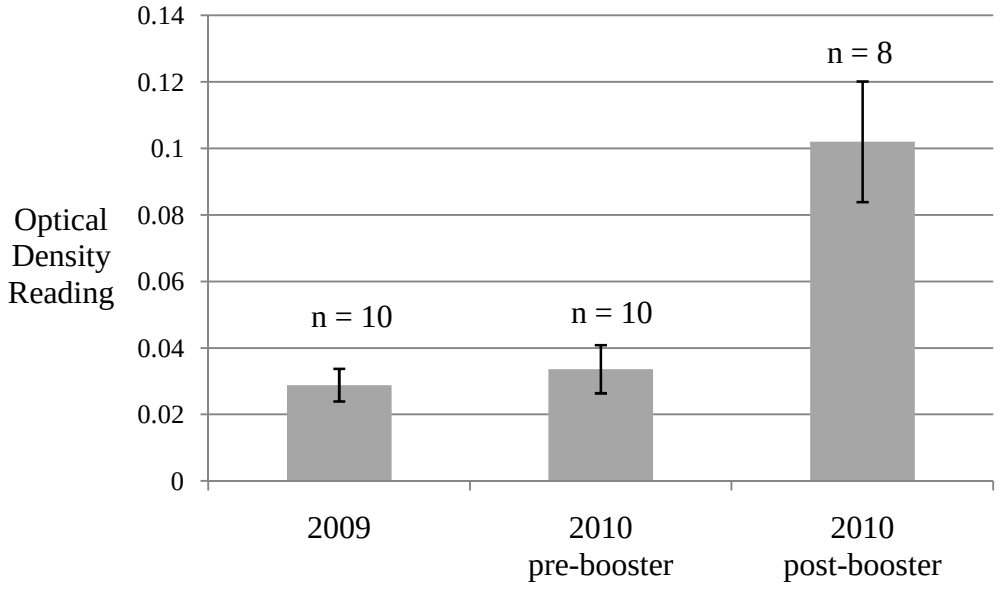

Figure 2.1: ELISA optical density values for LKT neutralizing serum antibody levels in Rocky Mountain bighorn sheep before vaccination with One Shot® in 2009 (n = 10), and before (n = 10) and after (n = 8) the vaccine booster in 2010. Error bars represent ± 1 Standard Error.

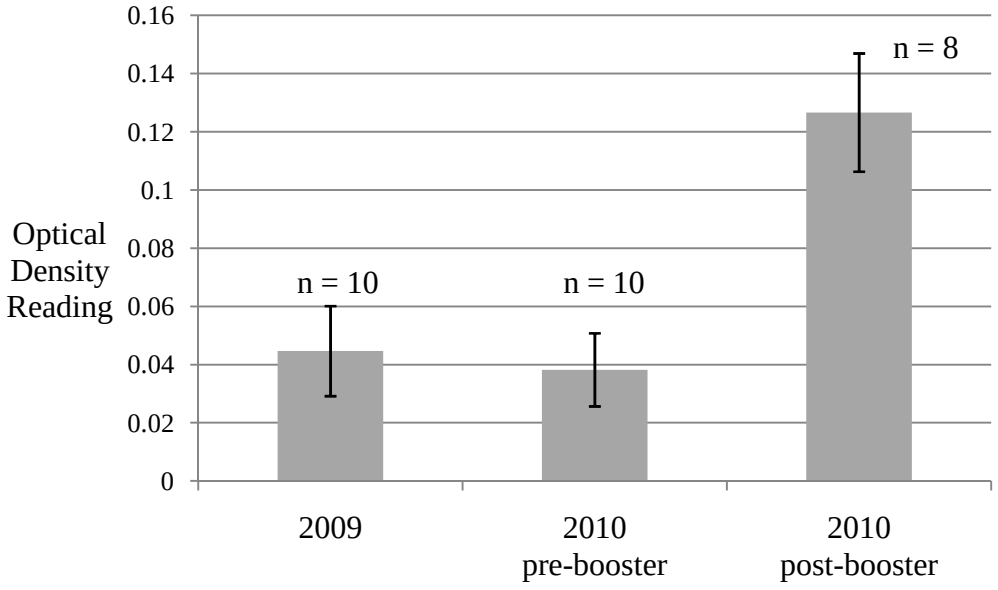

Figure 2.2: ELISA optical density values for whole cell *Mannheimia haemolytica* serum antibody levels in Rocky Mountain bighorn sheep before vaccination with One Shot® in 2009 (n = 10), and before (n = 10) and after (n = 8) the vaccine booster in 2010. Error bars represent ± 1 Standard Error.

CHAPTER 3

COMPARISON OF ANTIBODY RESPONSES STIMULATED BY TWO DIFFERENT *PASTEURELLACEAE* VACCINES ADMINISTERED TO CAPTIVE ROCKY MOUNTAIN BIGHORN SHEEP (OVIS CANADENSIS CANADENSIS)

INTRODUCTION

Pneumonic pasteurellosis epizootics and poor population performance currently present serious challenges to maintaining and successfully managing healthy, viable populations of bighorn sheep (*Ovis canadensis*; Singer et al., 2000; Miller, 2001; Cassirer and Sinclair, 2007; CAST 2008; George et al., 2008; Wolfe et al., 2010). The population impacts of pneumonic pasteurellosis on bighorn sheep are often quite severe. Epizootics can result in the mortality of greater than 70% of an entire herd in a matter of months, with mortality present within all age classes (Rush, 1927; Onderka and Wishart, 1984; Spraker et al., 1984; Festa-Bianchet, 1988; Cassirer et al., 1996; Cassirer et al., 2001; Enk et al., 2001; George et al., 2008; Wolfe et al., 2010). Reduced survival following a die-off (e.g., George et al., 2008), likely a result of either continued low levels of disease, or compromised health and body condition, can further reduce population size as animals continue to die at higher than normal rates. In the absence of an acute die-off, populations may experience years of chronic recurring or sporadic pneumonia (Marsh, 1938; Cassirer and Sinclair, 2007) as a result of the infection being maintained at low levels. Another impact to some herds is the loss of seasonal migration movements after a die-off, likely as a result of losing key dominant individuals. This can subsequently lead

to nutritional stress as a result of animals remaining on winter range year-round which impacts the amount of forage available to sheep during winter (Enk et al., 2001).

Whether chronic or acute, pasteurellosis invariably leads to years of depressed lamb recruitment (Woodard et al., 1974; Spraker et al., 1984; Monello et al., 2001; George et al., 2008), and is one of the primary factors limiting recovery of populations that have experienced a die-off (Enk et al., 2001). Although surviving ewes continue to bear lambs as usual each spring (Cassirer et al., 2001), those lambs tend to succumb to an acute pneumonia (Marsh, 1938; Spraker et al., 1984). The timing of mortality is often between 6 and 11 weeks of age (Foreyt, 1990; Cassirer et al., 2001), which coincides with, and may be the result of, waning passive immunity provided by maternal antibodies (Foreyt, 1990; Miller et al., 1997). The source of the agents causing infection in the lambs is likely adult bighorn sheep that have survived an infection themselves but are still carriers of the agent. These animals subsequently transmit the pathogen to their own or other offspring (Spraker et al., 1984; Foreyt, 1990; Miller et al., 1991), which in turn spreads the pathogen still further through nursery group interactions.

The signs of pneumonic pasteurellosis in bighorn sheep include depression, shallow or labored respiration, anorexia, weakness, and lack of mobility (Cassirer et al., 1996; Kraabel et al., 1998). In many cases, disease appears to be acute and animals die despite being in apparently good body condition and without showing preliminary signs of disease (Rush, 1927; Foreyt and Jessup, 1982; Onderka and Wishart, 1984; Miller et al., 1991). Bighorns that die of pneumonic pasteurellosis have been described as having histologic lesions of severe hemorrhagic, necrotizing, suppurative bronchopneumonia or acute fibrinous pneumonia (Onderka and Wishart, 1984; Onderka et al., 1988; George et

al., 2008; Wolfe et al., 2010); which is similar to necropsy findings in domestic livestock affected by pasteurellosis (Woolums et al., 2009).

Many biotypes of *Pasteurellaceae* are common inhabitants of the ruminant upper respiratory tract (Lainson et al., 1996; Woolums et al., 2009). The development of pneumonic pasteurellosis is often associated with various stressors that have been shown to shift this state of commensalism to one of virulent disease where respiratory clearance is impaired (Woolums et al., 2009), and *Pasteurellaceae* replicate rapidly, invade the lungs, and become pathogenic (Fowler 2003). Specific stressors linked to respiratory disease in bighorn sheep include animal density (Monello et al., 2001), contact with domestic sheep (George et al., 2008), nutritional deficiencies (Enk et al., 2001), habitat damage (Festa-Bianchet, 1988), environmental conditions, concurrent lungworm (*Protostrongylus* spp.) infection (Spraker and Hibler, 1982), concurrent *Mycoplasma ovipneumoniae* infection (Besser et al., 2008; Dassanayake et al., 2010b), and interactions with humans (Spraker et al., 1984; Foreyt, 1990). In captive bighorn sheep, Kraabel and Miller (1997) also showed that simulating stress by administering a long-acting adrenocorticotrophic hormone elevated plasma cortisol concentrations and increased neutrophil susceptibility to the leukotoxins produced by the key pathogenic bacteria linked to pneumonic pasteurellosis.

Alternatively, pneumonic pasteurellosis can be caused by the introduction of novel pathogenic strains of *Pasteurellaceae* to bighorn sheep, often through interactions with domestic livestock, primarily domestic sheep (Onderka er al., 1988; Foreyt and Jessup 1982; George et al., 2008; Lawrence et al., 2010; Wolfe et al., 2010). Contact between domestic sheep and bighorns often results in fatal pneumonia of the bighorns,

presumably through the transfer of *Pasteurellaceae* strains that are not harmful to the domestic sheep but are extremely pathogenic to bighorn sheep (Foreyt et al.,1994). While interactions with domestics are important, interactions among bighorn sheep within and among herds function to perpetuate infections (George et al., 2008).

Mannheimia haemolytica, a species of bacteria in the family *Pasteurellaceae*, is commonly isolated from pneumonic bighorn sheep and has been implicated as one of the primary pathogens of pneumonia epizootics (Onderka and Wishart, 1984; Festa-Bianchet, 1988; Foreyt, 1990; Wolfe et al., 2010). *M. haemolytica* may also be responsible for cases of pneumonia despite not being isolated from pneumonic animals as recent research indicates that *M. haemolytica* is readily overgrown and inhibited by *Bibersteinia trehalosi*, a common inhabitant of bighorn upper respiratory tracts. This situation is likely exacerbated by extended time between sample collection and laboratory analysis (Dassanayake et al., 2010), a common occurrence when sampling free-ranging bighorn sheep, and could conceivably lead to *B. trehalosi* being blamed for disease actually caused by *M. haemolytica.*

Regardless of the species of *Pasteurellaceae*, leukotoxin (Lkt) is considered by many to be the key virulence factor in lung infection (Shewen and Wilkie, 1983, 1985; Lo et al., 1987; Petras et al., 1995; Lainson et al., 1996; Kraabel and Miller, 1997; Tatum et al., 1998; Narayanan et al., 2002; Kelley et al., 2007; Rice et al., 2008; Dassanayake et al., 2010). This idea is further supported by a recent experiment using Lkt deletion in a highly pathogenic strain of *M. haemolytica* that resulted in a mutant strain of *M. haemolytica* that did not produce Lkt and did not kill bighorn sheep (Dassanayake et al., 2009).

Leukotoxin is a member of the repeats in toxin (RTX) family of exotoxins that is specifically lethal to ruminant leukocytes (Woolums et al., 2009). It is secreted during the logarithmic phase of bacterial growth (Shewen and Wilkie, 1985), and is dose-dependent (Narayanan et al., 2002; Davies and Baillie, 2003). At very low concentrations, Lkt triggers "respiratory burst" and degranulation of target cells. Moderate concentrations cause apoptosis of leukocytes, and high concentrations cause cell lysis (Narayanan et al., 2002; Woolums et al., 2009) as a result of pore formation (Narayanan et al., 2002). Lkt also inhibits proliferation of lymphocytes, which effectively prevents induction of a secondary immune response to the infection (Narayanan et al., 2002). All serotypes of *M. haemolytica* are capable of producing Lkt, and strains are only hemolytic when Lkt is produced (Shewen and Wilkie, 1983; Burrows et al., 1993; Narayanan et al., 2002). Extensive genetic variation in the structural Lkt gene (*lktA*) has been identified and likely results from mutations and horizontal gene transfer between bacterial strains (Davies and Baillie, 2003; Kelley et al., 2007). Despite the genetic variation, however, structure and function of Lkt is highly conserved among various serotypes of *M. haemolytica* (Davies and Baillie, 2003). This similarity in structure between serotypes is likely the reason that antibodies to Lkt of one serotype have been observed to cross-neutralize Lkt of other serotypes (Shewen and Wilkie 1983; Gentry et al., 1988).

Stimulating production of antibodies that are capable of neutralizing Lkt is probably the key presumed benefit of using vaccines to manage pasteurellosis. Vaccination has been shown to provide some level of protection against the organisms and Lkt that cause pneumonic pasteurellosis of captive and wild bighorn sheep in the past

(Kraabel et al., 1998; George et al., 2008), and vaccinating for pasteurellosis either preventatively, as in the case of chronic pneumonia and low lamb recruitment, or early in a pneumonia epizootic, has been suggested as a way to increase survival of infected bighorn sheep (Miller et al., 1997; Ward et al., 1999; George et al., 2008). In addition, passive antibody transfer from ewe to lamb through colostrum of vaccinated ewes may increase lamb survival (Cassirer et al., 2001).

My previous work with 2 different *Pasteurellaceae* vaccines, an autogenous vaccine in captive bighorn sheep, and One Shot® in free-ranging bighorn sheep (see Chapters 1 and 2) has demonstrated that both are capable of stimulating serum antibody responses. Although these data suggest that One Shot may have stimulated a greater antibody response to Lkt than did the autogenous vaccine, no direct comparison has been conducted. Even though the antibody responses to several different *Pasteurellaceae* vaccines have previously been compared in livestock (Srinand et al., 1996b; Confer et al., 1998) few if any comparisons have occurred with bighorn sheep. Here I test directly whether or not there is a difference in antibody response stimulated by these 2 vaccines when administered to a herd of captive bighorn sheep. Resistance to developing pneumonic pasteurellosis has been correlated with the quantity of circulating serum Lkt neutralizing antibodies (Gentry et al., 1985; Kraabel et al., 1998), and a vaccine that stimulates a greater antibody response might, in theory, provide more protection from pneumonic pasteurellosis than a similar vaccine that stimulates less of an antibody response.

MATERIALS AND METHODS

I used 30 captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) to compare the serum antibody responses elicited by 2 different *Pasteurellaceae* vaccines. All bighorns were part of the Colorado Division of Wildlife's (CDOW) captive bighorn sheep research herd, and were housed in 0.5–3.0 ha pastures at the Foothills Wildlife Research Facility (FWRF) in Fort Collins, Colorado. Animals were fed and cared for in accordance with FWRF animal husbandry protocols (T. Davis, unpublished data). Study procedures were approved by both Colorado State University (CSU) and CDOW Institutional Animal Care and Use Committees (CSU IACUC Protocol # 10-2062A, and CDOW IACUC # 11-2010).

I compared antibody responses stimulated by 2 different *Pasteurellaceae* vaccines for this experiment. The first vaccine is a multivalent, autogenous, bacterin vaccine (Newport Laboratories, Worthington, MN) incorporating 4 *Pasteurellaceae* isolates, (2 *Mannheimia haemolytica* and 2 *Bibersteinia trehalosi*; Table 3.1; Wolfe et al., 2010; Miller and Wolfe 2011) collected by CDOW from the Avalanche Creek and Fossil Ridge Rocky Mountain bighorn sheep herds in Colorado during the winter/spring of 2007–2008. The Avalanche Creek herd had been experiencing many years of poor lamb recruitment and was carrying a strain of *Pasteurellaceae* known to be pathogenic in bighorn sheep (Miller and Wolfe 2011). The Fossil Ridge herd experienced an all-age pneumonia-related die-off beginning in late 2007 that resulted in the mortality of approximately 2/3 of the herd (Wolfe et al., 2010). These strains were chosen for the vaccine because of these links to current and ongoing disease.

To accomplish "self-boostering," the autogenous vaccine incorporates the SoliDose® (SolidTech Animal Health, Inc., Newcastle, OK) implantable technology, consisting of an implantable bolus with multiple pellets. Half of the pellets release the initial dose of vaccine within an hour of implantation. The remaining pellets are designed to booster the first dose by slowly releasing the second dose of vaccine over the 2–3 weeks following implantation. I previously evaluated this vaccine under similar circumstances (see Chapter 1) and did not find the SoliDose technology to cause any apparent injury or harm to bighorn sheep.

The second vaccine is One Shot (Pfizer Animal Health, New York, NY), a commercially available bacterin-toxoid vaccine developed to protect cattle from *Mannheimia haemolytica* type A1 as well as the leukotoxin that is produced by the bacteria.

I stratified by age and sex prior to randomly assigning the study animals to 1 of 3 treatment groups of 10 animals each. The first treatment group received 2 simultaneous injections of the autogenous vaccine using a SoliDoser® (SolidTech Animal Health, Inc., Newcastle, OK) applicator, the second treatment group received 2 ml of One Shot, and the third group (control) received 2 ml of sterile saline.

Prior to vaccination and sample collection, most study animals (n = 24) were chemically immobilized via hand–injection with the tranquilizer xylazine (0.6–1.5 mg/kg; Lloyd Laboratories, Shenandoah, Iowa). The remaining 6 animals were considerably less tractable, and were anesthetized by remotely delivering a cocktail of butorphanol (0.4–0.5 mg/kg; Wildlife Pharmaceuticals Inc., Fort Collins, Colorado),

azaperone (0.2–0.4 mg/kg; Wildlife Pharmaceuticals Inc.), and medetomidine (0.1–0.2 mg/kg; Wildlife Pharmaceuticals Inc.) via 2ml darts (Pneu-dart inc, Williamsport, PA).

All bighorns were either vaccinated or injected with sterile saline (control group) the week of 9 August 2010 (week 0). The injection site was located on the left hind quarter of each animal, and was shaved prior to subcutaneously administering the injections. We also collected a 12 ml blood sample from each animal via jugular venipuncture.

After study procedures were performed, animals tranquilized with xylazine were reversed with tolazoline (2.0–3.0 mg/kg; Lloyd Laboratories, Shenandoah, Iowa) while the remaining 6 study animals were reversed with atipamezole (2.0–2.5 mg/mg of medetomidine; Pfizer Animal Health, New York, NY) and tolazoline (2.0–3.0 mg/kg).

I centrifuged blood samples for 10 minutes at 4000 RPM within several hours of sample collection. Subsequently the serum was stored in microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA, USA) at -20°F.

The immobilization and blood collection procedures were repeated for each bighorn at 2, 4, and 6 weeks post-vaccination as outlined above. In addition, injection sites were inspected for adverse reactions each time animals were sampled.

Serum samples were sent to Oklahoma State University (OSU; Stillwater, OK) for analysis. Samples were assayed for leukotoxin neutralizing antibodies as well as *M. haemolytica* serotype 1 whole cell antibodies using enzyme-linked immunosorbent assay (ELISA; Confer et al., 2003) and antibody concentrations were reported as optical density (OD) values. *Pasteurellaceae* isolate 09-185-003 collected from the Badger Creek

bighorn sheep herd (Chapter 2), was provided by the CDOW for OSU to use in the ELISA (Miller and Wolfe 2011).

I used Bayesian hierarchical models (Gelman et al. 2004) with Markov Chain Monte Carlo (MCMC) methods in the WinBUGs statistical software package, version 1.4.3 (Lunn et al., 2000) to analyze the serum antibody data collected on the bighorn sheep. I used prior densities and data likelihoods to estimate the unnormalized joint posterior density for each model parameter. Due to chain convergence issues, I standardized the response variables as well as all continuous covariates before analysis. Standardization was performed by subtracting the mean from each observation or covariate value and then dividing by the standard deviation. This resulted in the values having a variance of 1.0. Values were back-transformed to ELISA OD values prior to reporting results.

I used a 2-tiered approach to analysis in order to maintain a reasonably sized model set. The first tier of analysis was used to identify an appropriate correlation structure and was necessary to account for covariance in the data induced by repeatedly sampling individual animals. I examined 6 covariance structures to determine which best accounted for the potential correlation contained in the dataset by using each structure in combination with the global covariate model (Model 8_age_sex). The top ranked structure was subsequently used in the second tier of analysis. The 6 covariance structures that I examined included: an independent error model to determine if there was evidence for correlation among samples; a random intercept model that modeled only within-individual correlation by treating each individual's intercept (z_i) as a random variable drawn from a common distribution (Jensen 2001); a random intercept and a

random time effect model that included both within-individual and temporal correlation with the temporal correlation modeled through the specification of a conditionally autoregressive (CAR) prior on the random time effect, $\boldsymbol{\tau_j}$ (i.e., $t_j \sim N\left[t_{j-1}, \sigma^2_{time}\right]$ for $j > 1$, and $t_1 \sim N[0, \sigma^2_{time}]$; Bannerjee et al. 2004)) ; a first-order autoregressive structure for the covariance matrix (Σ), which creates a decreasing temporal correlation as a function of the time between observations (Diggle et al. 1994); and a heterogeneous variance model for Σ with and without a random intercept term. Setting up Σ in the heterogeneous variance model in this way creates a unique variance for observations collected in each sampling period with the exception that the variance for the last sampling occasion was constrained to be equal to the variance from the first sampling occasion to allow for parameter estimation. This last model induces further temporal correlation via a CAR prior specified for the inverse of the variances or the precision (i.e., $\frac{1}{\sigma^2_j} \sim \Gamma\left[\alpha_j, \mathrm{B}_j\right]$, $\alpha_j = \frac{1}{\sigma^2_{j-1}} \times \mathrm{B}_i$ and $\mathrm{B}_i = \frac{\mathrm{B}^2_1}{\alpha_1 \sigma^2_{j-1}}$ for $j > 1$, and $\frac{1}{\sigma^2_1} \sim \Gamma[0.1,0.1]$; Bannerjee et al. 2004). I provide a more detailed description of these 6 models in Appendix II.

In the second tier of analysis I used the best-fitting correlation structure identified in the first tier of analysis and applied that to a set of models that represented the plausible antibody responses to treatment over time that might occur as a result of vaccination. This allowed me to examine 2 main covariates of interest: vaccine effects and a trend effect. Specifically, the vaccine effects revealed changes in antibody concentration due to vaccination with each of the vaccines, while the trend effect revealed changes in antibody concentration through time following vaccination. My model set consisted of 12 primary models that included acute, constant, or changing

antibody levels across time as a function of treatment (Figure 3.1; Table 3.2). I added effects of age, sex, and both age and sex to each of these primary models for a total of 48 models. These 48 models included 1 strictly age-specific model, 1 strictly sex-specific model, and 1 strictly age + sex-specific model to determine whether age, sex, or age + sex explained antibody levels better than the specific vaccine treatment effects did.

The data likelihood in this analysis specified each standardized antibody concentration (OD value) as a normal random variable with parameters (μ_{ij}, Σ):

$$y_{ij} \sim N(\mu_{ij}, \Sigma),$$

where y_{ij} was the standardized value for the i^{th} sheep for $i = 1, \ldots, n$ on the j^{th} sampling occasion for $j = 1, \ldots, 4$, μ_{ij} represents the mean effect for the i^{th} sheep on the j^{th} sampling occasion, and Σ was the covariance matrix. Thus, given these 2 parameters I assumed each sheep's antibody response was conditionally independent. To model the underlying biological process of interest I modeled μ_{ij} as a function of covariates and, if included in the selected covariance structure in the first-tier of analysis, random effects that account for the sampling process:

$$\mu_{ij} = z_i + x_{ij}^{'}\beta + \tau_j,$$

where $z_i \sim N(0, \sigma^2_{int})$ is a random intercept for each individual to account for within individual correlation, x'_{ij} is a $k \times 1$ vector of covariates for the i^{th} sheep across the j^{th} sampling occasion, β is a $k \times 1$ vector of parameter estimates for these covariates, and $\tau_j \sim N(0, \sigma^2_{time})$ is a random time effect to account for temporal correlation of observations. Due to the standardization of the response variables, no intercept term was included in the β vector.

Estimating the unnormalized joint posterior density for each parameter required specifying prior densities for each parameter based on prior knowledge of the system. Because I lacked prior knowledge on the effect of these covariates, I used a diffuse prior distribution of N(0,1000) on the treatment, trend, age, and sex effect parameters. For the leukotoxin analysis, I specified a diffuse U(0,100) prior for the residual standard deviations and the random intercept standard deviation, and a U(0,10) for the random time effect standard deviation. Additionally, I specified CAR priors in the models containing the random time effect as well as the heterogeneous variance models as described previously. Lastly, for models containing first-order autogressive covariance matrices, the correlation parameter (ρ) was given a U(0,1) prior because only positive correlations are reasonable for this study. For the *M. haemolytica* whole cell analysis, all prior densities were the same as for the leukotoxin analysis with the following exceptions: I specified a U(0,10) prior for the residual standard deviations and the random intercept standard deviation, and a U(0,1) for the random time effect standard deviation. I specified less diffuse priors in this analysis because of a lack of variation in the observed data.

I used deviance information criterion (DIC) to conduct model selection and to determine which covariate and correlation model was best supported by the evidence in the data (Spiegelhalter et al. 2002). I assumed that the model with the lowest DIC value was the model best supported by the data, and I estimated differences in DIC values (ΔDIC) among models in the candidate set, model likelihoods, and model probabilities to determine the strength of evidence for each model (Burnham and Anderson 2002, Farnsworth et al. 2006).

I used 3 chains with overdispersed starting values, and a burn-in of 100,000 iterations before drawing 100,000 samples from the posterior distribution for inference about each of the model parameters. For the *M. haemolytica* whole cell analysis, I thinned the chains by 5 prior to making inference about the model parameters. Results are based on the mean of parameter estimates for the top ranked model.

I assessed the convergence and mixing of top models using the boa package of program R (Smith 2007; R Development Core Team, 2010). Specifically I looked at sampler lag-autocorrelation plots, density plots, and trace plots for each model parameter, as well as the multivariate potential scale reduction factors and estimates of the corrected scale reduction factors (Gelman and Rubin, 1992).

RESULTS

Leukotoxin neutralizing antibodies

The first step of the Lkt neutralizing antibody analysis involved assessing the fit of the 6 different correlation structures using the global covariate model to determine

which structure was the best fit. Results of model selection indicate that the "random intercept and random time effect" structure yielded the most appropriate covariance structure (Table 3.3). This structure carried 0.80 of the model weight and was 4.0 times more likely than the second ranked covariance model (Table 3.3). This structure was used for the remainder of the Lkt neutralizing antibody analysis.

For the second tier of analysis, the top 8 models carried essentially the entire cumulative weight of the model set (cumulative $w > 0.99$) while the remaining 40 models did not contribute appreciably to the cumulative model weight (cumulative $w < 0.01$; Table 3.4). These top 8 models were composed of all 4 variations of 2 different primary models, model 6 and model 8 (Model 6: unequal treatment effects, autogenous time trend = 0, One Shot time trend $\neq 0$; Model 8: unequal treatment effects, different time trends; see Figure 3.1).

My top model, model 6_age carried a model weight of 0.20. This model incorporates unequal vaccine treatment effects, a time trend on the effect of vaccination with One Shot over the 6 week sampling period, and a constant effect of vaccination with the autogenous vaccine over the same sampling period (Figure 3.1: Model 6). Animals vaccinated with either vaccine had higher Lkt neutralizing antibodies relative to controls in this experiment. Vaccination with One Shot increased antibody levels from a mean OD value of 0.049 to an OD value of 0.13 (95% credible interval [CI]: 0.11, 0.15) while vaccination with the autogenous vaccine increased antibody concentrations to an OD value of 0.067 (95% CI: 0.049, 0.085; Figure 3.2). After the initial response to vaccination with One Shot observed at 2 weeks post vaccination, Lkt neutralizing antibodies declined to an OD value of .10 (95% CI: 0.084, 0.12) by week 4 and 0.074

(95% CI: 0.053, 0.096) by week 6. At the same time, the antibody level of animals vaccinated with the autogenous vaccine remained constant over the 6 week sampling period after the initial response to vaccination. Antibody levels were higher by an OD value of 0.0072 for each year of an animal's age (95% CI: 0.0032, 0.011) regardless of treatment group.

Throughout the model set, the 4 variations of each primary model were ranked in order: age effects, age + sex effects, neither age or sex effects, and sex effects. This indicates that age was an important predictor of antibody level (cumulative *w* for models with age = 0.69; cumulative *w* for models without age =0.31). In addition, 95% credible intervals for the sex effects of the 2 highest ranking models that contained sex effects (Model 6_age_sex and Model 8_age_sex) both solidly included 0, indicating that any effect of sex on antibody level was weak at best.

Convergence diagnostics for the top ranked model did not provide any evidence of non-convergence for any of the model parameters. The corrected scale reduction factors for the Brooks, Gelman, and Rubin Convergence Diagnostics were all essentially 1.0 (range: 0.9999993, 1.0005986), while the multivariate potential scale factor was 1.000159. In addition, plots of sampler lag–autocorrelations, estimated posterior density, and sampler trace all supported convergence (Appendix IV).

Mannheimia haemolytica whole cell antibodies

The first stage of *M. haemolytica* whole cell antibody concentration analysis resulted in the "heterogeneous variance with a random intercept" structure being ranked

highest out of the 6 correlation structures (Table 3.5). This structure was subsequently used for the remainder of the *M. haemolytica* whole cell analysis.

Model selection results for the second stage of *M. haemolytica* whole cell antibody analysis revealed a large degree of model selection uncertainty, with no model carrying more than 10% of the model weight (maximum $w_i = 0.10$; Table 3.6). The top 4 models in the model set were the 4 variations of primary model 5 (Model 5: unequal treatment effects, no time trend; Table 3.6), and combined, those models yielded a cumulative weight of 34% (cumulative $w = 0.34$).

Making inference from the top model (Model 5_age), One Shot had a slightly negative effect on antibody levels, lowering whole cell antibody concentrations from a mean OD value of 0.126 to an OD value of 0.115 (95% CI: 0.105, 0.125), while the autogenous vaccine stimulated a negligible increase in antibody concentrations to an OD value of 0.127 (95% CI: 0.117, 0.137; Figure 3.3). The 95% CI's for the vaccine treatment effects overlapped indicating that there was no difference in the whole cell antibody response stimulated by these 2 vaccines. Likewise, the 95% CI for the effect of the autogenous vaccine on antibody levels was nearly centered on zero, indicating that the autogenous vaccine did not affect whole cell antibody levels. Antibody concentration did vary by age however, with concentrations being increased by an OD value of 0.011 (95% CI: 0.0053, 0.016) for each year of an animal's age. Nearly identical parameter estimates were obtained with the second ranked model (Model 5_age_sex), and the addition of the sex effect in that model revealed sex to be insignificant as the 95% CI included 0.

Convergence diagnostics performed on the top model did not indicate non-convergence in any of the model parameters. Plots of estimated posterior density, sampler lag–autocorrelations, and sampler trace all supported convergence (Appendix IV). The corrected scale reduction factors for the Brooks, Gelman, and Rubin Convergence Diagnostics were all essentially 1.0 (range: 0.9999985, 1.0001684) and the multivariate potential scale factor was 1.000152.

DISCUSSION

This study revealed that both One Shot and the autogenous vaccine were capable of stimulating Lkt neutralizing immune responses in captive bighorn sheep. This implies that either vaccine might provide some level of protection from the damaging effects of Lkt during infections with Lkt producing strains of *Pasteurellaceae* if administered at an appropriate time. Results of this study indicate that One Shot induced greater Lkt neutralizing antibody production than did the autogenous vaccine. Whether this equates to increased protection from the effects of Lkt was outside the scope of this study; however, past vaccine research with bighorn sheep indicates that Lkt neutralizing antibody concentrations are correlated with the degree of protection from experimental challenge with *Pasteurellaceae* (Kraabel et al., 1998), and the same has been found in domestic animals (Gentry et al., 1985; Srinand et al., 1996a).

I did not find any positive *M. haemolytica* whole cell antibody response to either vaccine. This failure contrasts my previous findings that the autogenous vaccine did stimulate an increase in *M. haemolytica* whole cell antibodies in captive bighorn sheep (Chapter 1), and that One Shot stimulated an increase in whole cell antibodies in free-

ranging bighorn sheep (Chapter 2). Several factors might partially account for these differences including small sample size (Chapter 1), lack of controls (Chapter 2), or strain differences in the isolates used for the antibody assay. Regardless, the lack of a whole cell antibody response that I observed may not necessarily be overly concerning. Past research has found *M. haemolytica* whole cell antibodies alone to be inadequate at providing protection from *Pasteurellaceae* (Kraabel et al., 1998) and Lkt neutralizing antibodies likely play a more important role in mitigating damage caused by pneumonic pasteurellosis.

I found age to be positively associated with serum concentrations of both Lkt neutralizing and *M. haemolytica* whole cell antibodies: older animals had consistently higher levels of both antibody types regardless of vaccination status. These associations have been observed previously in vaccinated bighorn sheep (Miller et al., 1997), and likewise, Delgado et al. (1996) found age to be positively correlated with the post-vaccination antibody levels of domestic sheep after receiving a *Brucella melitensis* vaccine. The extreme vulnerability of bighorn lambs to the effects of pneumonic pasteurellosis may be partially explained by their age-related lower antibody levels. Not only do lambs tend to have fewer protective circulating antibodies to provide a defense against *Pasteurellaceae* infections, but they also probably do not produce as many antibodies in response to infection with *Pasteurellaceae* as an older animal would. This may result in a diminished chance of successfully fighting off these infections for young-aged animals and could explain how the lingering effect of pasteurellosis in bighorn sheep might manifest itself in low lamb recruitment for many years after an outbreak.

In this study, even vaccination failed to successfully increase lamb antibody concentrations to the same level as adult animals. One potential explanation for this trend is that the higher antibody concentrations seen in older animals are the result of relatively longer term exposure to *Pasteurellaceae* over their lifetimes, and that their immune system responds to vaccination with a secondary immune response whereas lambs were exhibiting more of a primary immune response. Perhaps equivalent antibody concentrations could be obtained if lambs were administered 1 or more vaccine boosters over several weeks or months' time.

Another potential explanation for the poor antibody responses of bighorn lambs is that high antibody levels in adult bighorn ewes at the time of parturition may result in maternal antibody interference, where maternally derived antibodies react with and eliminate vaccine antigens before the lamb's own immune system has an opportunity to recognize and respond to those antigens. Vitour et al. (2011) document such a scenario with cattle where passively derived maternal antibodies from vaccinated dams blocked immune responses to an inactivated bluetongue vaccine in calves that were greater than 16 weeks old. This explanation also agrees with the findings of Cassirer et al. (2001) that bighorn ewes with lower antibody concentrations were more likely to recruit lambs than ewes with higher antibody concentrations, and the findings of Kiorpes et al. (1991) that domestic lambs born to unvaccinated ewes responded better to vaccination than lambs born to vaccinated ewes.

Based on the findings of this study, I suggest to wildlife managers that, when vaccinating ewes, timing relative to parturition and the potential for maternal antibody blocking should at least be considered. My earlier work with both vaccines (Chapters 1

and 2) demonstrated that antibody levels can wane to pre-vaccination levels in fewer than 4 weeks (autogenous vaccine) or 12 months (One Shot). Based on the trend parameter of the top Lkt neutralizing antibody model (Model 6_age) of this study, One Shot-induced antibody levels should return to pre-vaccination levels in less than 8 weeks following vaccination. If maternal antibody blocking is of concern, vaccinating as far in advance of parturition as possible may still prime the ewe's immune system in preparation for a later secondary immune response, while not exposing the lamb to higher than necessary antibody concentrations that may hinder its own immune response via interference. In the end, it may be necessary to weigh the potential trade-off between attempting to increase lamb survival during the early days of life while maternal antibodies provide the most protection, and attempting to increase lamb survival during the period of time after the maternal antibodies have waned and the lamb's own immune response is beginning to provide protection.

Despite the difference in immune responses stimulated by the 2 vaccines, there still may be reasons to consider using the autogenous vaccine, or using the autogenous vaccine in combination with One Shot. For one, the autogenous vaccine may provide a more specific immune response targeted toward the particular strains of bacteria that are currently causing pasteurellosis in bighorn sheep in Colorado. Secondly, the potential for using these 2 vaccines in combination may actually provide greater cross-protection from a wider array of *Pasteurellaceae* strains. Lastly, there was a declining trend on antibodies induced by One Shot over the 6 weeks of this study, and while the autogenous vaccine stimulated much less of a response, no trend was detected over the subsequent

sampling occasions, indicating that the antibody response to the autogenous vaccine may be of longer duration.

In conclusion, both One Shot and the autogenous vaccine stimulated immune responses that may confer some level of protection from the effects of pneumonic pasteurellosis in Rocky Mountain bighorn sheep. While this would certainly be useful in some circumstances, boosting antibody concentrations in bighorn ewes may be counterproductive in situations where lamb immune responses could be hindered, especially during the early weeks post-parturition, when lambs are most vulnerable. These findings should be supplemented with further work to unequivocally determine what, if any, effect of vaccinating bighorn ewes with these vaccines has on lamb immune responses.

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Table 3.1: Origin and bighorn sheep herd history of the *Pasteurellaceae* isolates used in the production of an autogenous *Pasteurellaceae* vaccine used in an experimental comparison with the commercial vaccine, One Shot® in captive bighorn sheep in 2010. See Wolfe et al. (2010) and Miller and Wolfe (2011) for further information on isolates.

Herd of origin	Location	Herd History	Species and biogroup	Serotype	Isolate #
Avalanche Creek	39^0 13" 53" N 107^0 13" 32" W	Poor lamb recruitment	*B. trehalosi* 4^{CDS}	T3	08-0381-011
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*B. trehalosi* 4^{CDS}	unknown	07-1920-003
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*M. haemolytica* 1^G	A2	07-1920-001
Fossil Ridge	38^0 33" 52" N 106^0 38' 03" W	Pneumonia Die-off, Dec 2007	*M. haemolytica* 1^G	A2	08-0200-195

Table 3.2: The 12 primary models used for the second tier of analysis of serum antibody levels in captive bighorn sheep vaccinated with the commercially available *Pasteurellaceae* vaccine One Shot®, an autogenous *Pasteurellaceae* vaccine, or a sterile saline placebo in 2010.

Model #	Description	Model
1	No treatment effects	$Y = 0$
2	Equal treatment effects, no time trend	$Y = V_{A=O}$
3	Equal treatment effects, equal time trends	$Y = V_{A=O} + T_{A=O}$
4	Equal treatment effects, unequal time trends	$Y = V_{A=O} + T_A + T_O$
5	Unequal treatment effects, no time trend	$Y = V_A + V_O$
6	Unequal treatment effects, autogenous time trend = 0, OS time trend ≠ 0	$Y = V_A + V_O + T_A$
7	Unequal treatment effects, equal time trends	$Y = V_A + V_O + T_{A=O}$
8	Unequal treatment effects, unequal time trends	$Y = V_A + V_O + T_A + T_O$
9	Equal acute treatment effects	$Y = A_{A=O}$
10	Unequal acute treatment effects	$Y = A_A + A_O$
11	Acute treatment effect autogenous vaccine only	$Y = A_A$
12	Acute treatment effect One Shot® only	$Y = A_O$

Y = change in antibody concentration
A_A = acute effect of vaccination with autogenous vaccine
A_O = acute effect of vaccination with One Shot®
$A_{A=O}$ = equal acute vaccination effects with either the autogenous vaccine and One Shot®
T_A = time trend on antibody concentration after vaccination with the autogenous vaccine
T_O = time trend on antibody concentration after vaccination with One Shot®
$T_{A=O}$ = equal time trends after vaccination with either the autogenous vaccine or One Shot®
V_A = effect of vaccination with autogenous vaccine
V_O = effect of vaccination with One Shot®
$V_{A=O}$ = equal vaccination effects with either the autogenous vaccine and One Shot®

Table 3.3: Model selection results from the first tier of an analysis comparing the leukotoxin neutralizing antibody concentration responses stimulated by an autogenous *Pasteurellaceae* vaccine and the commercial *Pasteurellaceae* vaccine One Shot®, intended to determine the best fitting covariance structure in combination with the global covariate model (Model 8_age_sex). DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	Covariance Structure	DIC	ΔDIC	Likelihood	Weight
Model 8_age_sex	random intercept and random time effect	210.22	0.00	1.00	0.80
Model 8_age_sex	heterogeneous variance with a random intercept	212.95	2.73	0.26	0.20
Model 8_age_sex	random intercept	225.55	15.33	0.00	0.00
Model 8_age_sex	first order autoregressive structure	254.12	43.90	0.00	0.00
Model 8_age_sex	heterogeneous variance	256.66	46.45	0.00	0.00
Model 8_age_sex	independent error	276.07	65.85	0.00	0.00

Table 3.4: Model selection results for the top 20 ranked covariate models of serum leukotoxin neutralizing antibody concentrations in captive bighorn sheep vaccinated with the commercially available *Pasteurellaceae* vaccine One Shot®, an autogenous *Pasteurellaceae* vaccine, or a sterile saline placebo in 2010, utilizing a random intercept and random time effect correlation structure. DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	DIC	ΔDIC	Likelihood	Weight
Model 6_age	209.35	0.00	1.00	0.20
Model 6_age_sex	209.58	0.23	0.89	0.18
Model 8_age	209.69	0.34	0.85	0.17
Model 8_age_sex	209.95	0.60	0.74	0.15
Model 6	210.98	1.63	0.44	0.09
Model 6_sex	211.15	1.80	0.41	0.08
Model 8	211.38	2.03	0.36	0.07
Model 8_sex	211.51	2.16	0.34	0.07
Model 7_age	222.20	12.85	0.00	0.00
Model 7_age_sex	222.46	13.11	0.00	0.00
Model 7	223.99	14.65	0.00	0.00
Model 7_sex	224.18	14.83	0.00	0.00
Model 10_age	227.03	17.69	0.00	0.00
Model 10_age_sex	227.27	17.93	0.00	0.00
Model 10	228.55	19.20	0.00	0.00
Model 10_sex	228.59	19.24	0.00	0.00
Model 12_age	228.76	19.41	0.00	0.00
Model 12_age_sex	229.08	19.73	0.00	0.00
Model 12	230.18	20.83	0.00	0.00
Model 12_sex	230.29	20.94	0.00	0.00

age: model includes age effect
sex: model includes sex effect
Model 6: unequal treatment effects, autogenous time trend = 0, One Shot® time trend ≠ 0
Model 7: unequal treatment effects, equal time trends
Model 8: unequal treatment effects, unequal time trends
Model 10: unequal acute treatment effects
Model 12: One Shot® acute treatment effect, no effect of autogenous vaccine

Table 3.5: Model selection results from the first tier of an analysis comparing *Mannheimia haemolytica* whole cell antibody concentration responses stimulated by an autogenous *Pasteurellaceae* vaccine and the commercial *Pasteurellaceae* vaccine One Shot®, intended to determine the best fitting covariance structure in combination with the global covariate model (Model 8_age_sex). DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	Covariance Structure	DIC	ΔDIC	Likelihood	Weight
Model 8_age_sex	heterogeneous variance with a random intercept	93.57	0.00	1.00	0.93
Model 8_age_sex	random intercept and random time effect	99.71	6.14	0.05	0.04
Model 8_age_sex	random intercept	100.48	6.91	0.03	0.03
Model 8_age_sex	first order autoregressive structure	168.64	75.08	0.00	0.00
Model 8_age_sex	heterogeneous variance	291.18	197.61	0.00	0.00
Model 8_age_sex	independent error	291.89	198.32	0.00	0.00

Table 3.6: Model selection results for the top 24 ranked covariate models of serum whole cell *M. haemolytica* antibody concentrations in captive bighorn sheep vaccinated with the commercially available *Pasteurellaceae* vaccine One Shot®, an autogenous *Pasteurellaceae* vaccine, or a sterile saline placebo in 2010, utilizing a heterogeneous variance with a random intercept correlation structure. DIC is Deviance Information Criterion, Δ DIC is the difference in DIC between the model of interest and the top ranked model, likelihood indicates the model likelihood, and the model weight is the probability that the model is the best model in the model set given the data.

Model	DIC	ΔDIC	Likelihood	Weight
Model 5_age	89.40	0.00	1.00	0.10
Model 5_age_sex	89.57	0.17	0.92	0.09
Model 5	89.87	0.47	0.79	0.08
Model 5_sex	89.97	0.56	0.76	0.07
Model 6_age	91.11	1.70	0.43	0.04
Model 6_age_sex	91.40	2.00	0.37	0.04
Model 6	91.63	2.23	0.33	0.03
Model 7_age	91.80	2.40	0.30	0.03
Model 2_age	91.81	2.41	0.30	0.03
Model 4_age_sex	91.81	2.41	0.30	0.03
Model 4_age	91.81	2.41	0.30	0.03
Model 6_sex	91.89	2.49	0.29	0.03
Model 7_age_sex	91.90	2.50	0.29	0.03
Model 2_age_sex	92.04	2.64	0.27	0.03
Model 4	92.13	2.73	0.26	0.03
Model 7	92.19	2.79	0.25	0.02
Model 2	92.20	2.79	0.25	0.02
Model 4_sex	92.30	2.89	0.24	0.02
Model 2_sex	92.48	3.07	0.22	0.02
Model 7_sex	92.51	3.11	0.21	0.02
Model 1_age	92.79	3.39	0.18	0.02
Model 1_age_sex	92.98	3.58	0.17	0.02
Model 1	93.15	3.75	0.15	0.02
Model 1_sex	93.36	3.96	0.14	0.01
Model 8_age	93.36	3.96	0.14	0.01
Model 8_age_sex	93.56	4.15	0.13	0.01
Model 8	93.86	4.46	0.11	0.01
Model 8_sex	94.03	4.63	0.10	0.01
Model 3_age	94.21	4.81	0.09	0.01

Table continued…

Table continued…

Model 3_age_sex	94.30	4.90	0.09	0.01
Model 3	94.57	5.17	0.08	0.01
Model 12_age	94.576	5.17	0.08	0.01
Model 3_sex	94.76	5.35	0.07	0.01
Model 12_age_sex	94.909	5.51	0.06	0.01
Model 12	94.942	5.54	0.06	0.01
Model 11_age	95.139	5.74	0.06	0.01
Model 9_age	95.156	5.75	0.06	0.01
Model 12_sex	95.265	5.86	0.05	0.01

age: model includes age effect
sex: model includes sex effect
Model 1: no effect
Model 2: equal treatment effects, no time trend
Model 3: equal treatment effects, equal time trends
Model 4: equal treatment effects, unequal time trends
Model 5: unequal treatment effects, no time trend
Model 6: unequal treatment effects, autogenous time trend = 0, One Shot® time trend ® 0
Model 7: unequal treatment effects, equal time trends;
Model 8: unequal treatment effects, unequal time trends
Model 9: equal acute treatment effects
Model 11: autogenous vaccine acute treatment effect, no effect from One Shot®
Model 12: One Shot® acute treatment effect, no effect of autogenous vaccine

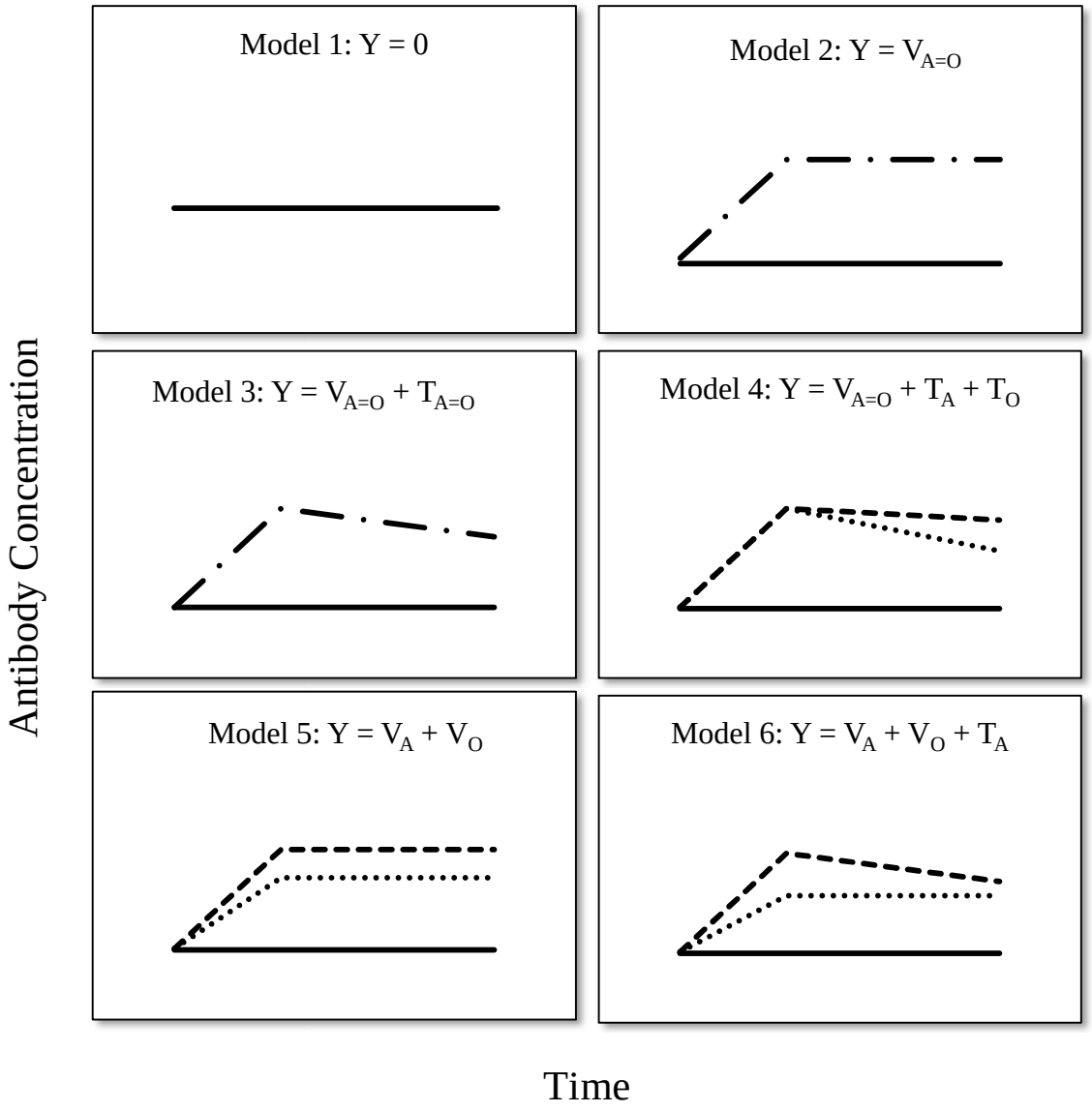

Figure 3.1: Graphical representation of the 12 primary models for the mean effect and time trend over the four weeks following vaccination that were used in the analysis of serum antibody concentrations in captive bighorn sheep vaccinated with the commercially available *Pasteurellaceae* vaccine One Shot®, an autogenous *Pasteurellaceae* vaccine, or a sterile saline placebo in 2010. The model parameters are defined as follows: Y = change in antibody concentration; A_A = acute effect of vaccination with autogenous vaccine; A_O = acute effect of vaccination with One Shot®; $A_{A=O}$ = equal acute vaccination effects with either the autogenous vaccine and One Shot®; T_A = time trend on antibody concentration after vaccination with the autogenous vaccine; T_O = time trend on antibody concentration after vaccination with One Shot®; $T_{A=O}$ = equal time trends after vaccination with the autogenous vaccine or One Shot®; V_A = effect of vaccination with autogenous vaccine; V_O = effect of vaccination with One Shot®; $V_{A=O}$ = equal vaccination effects with either the autogenous vaccine and One Shot®

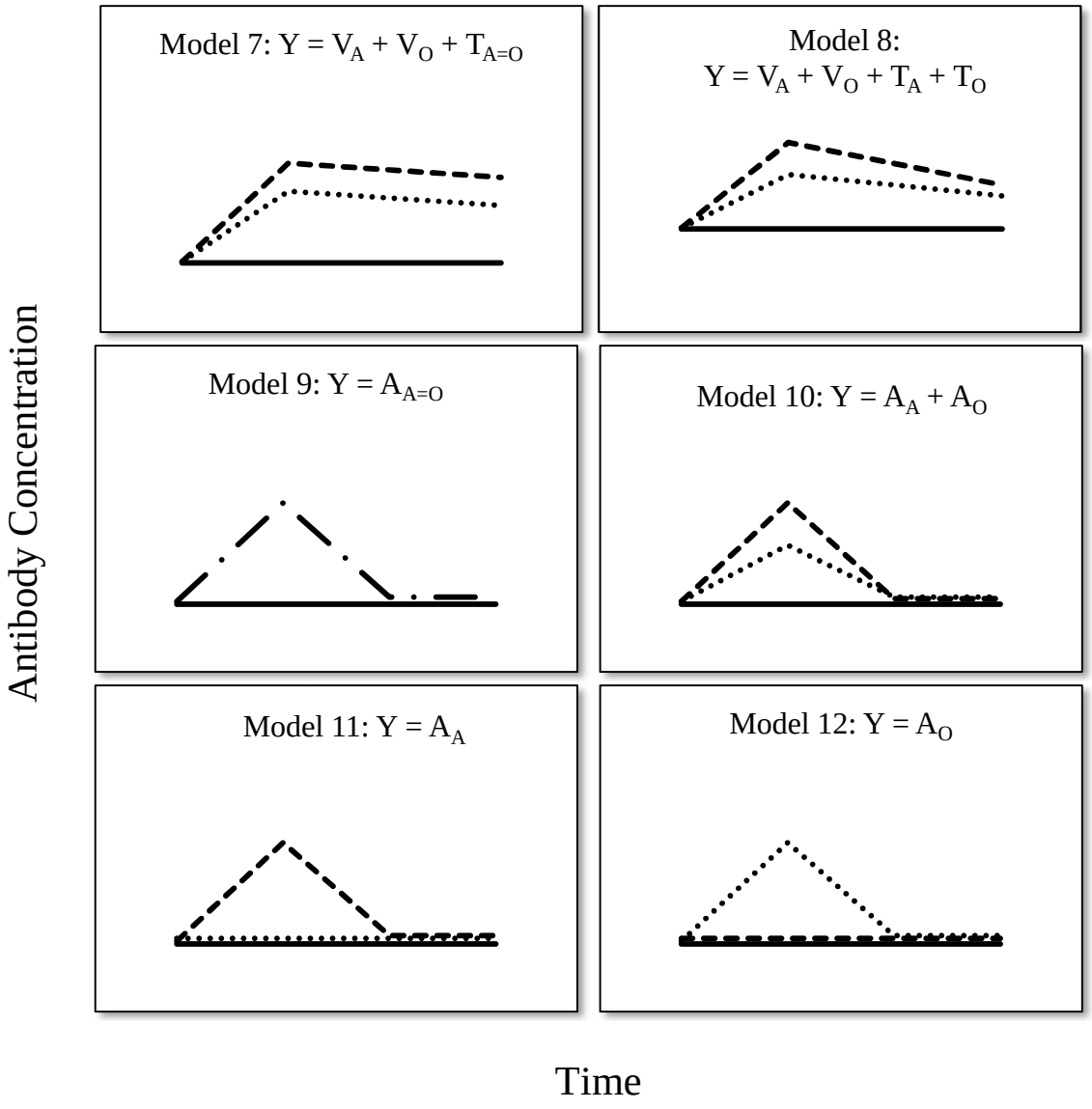

Figure 3.1: (continued)

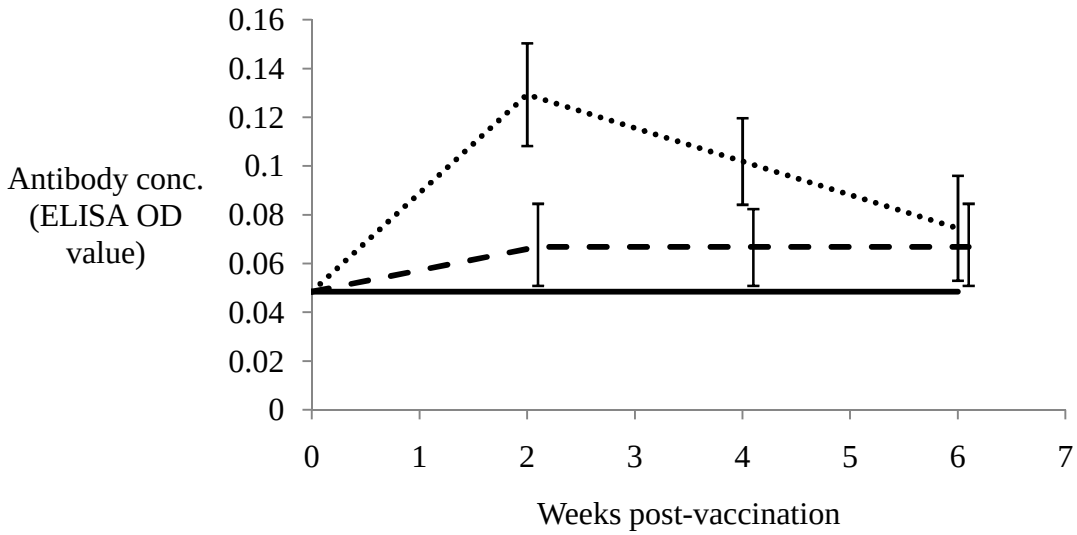

Figure 3.2: Estimated serum leukotoxin neutralizing antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with two different *Pasteurellaceae* vaccines based on the parameters of the top-ranked model. The dashed line represents antibody concentrations of animals vaccinated with an autogenous *Pasteurellaceae* vaccine, the dotted line represents antibody concentrations of animals vaccinated with the commercial *Pasteurellaceae* vaccine One Shot®, and the solid line represents antibody concentrations of control animals treated with a sterile saline placebo. Week 0 represents pre-vaccination antibody concentrations.

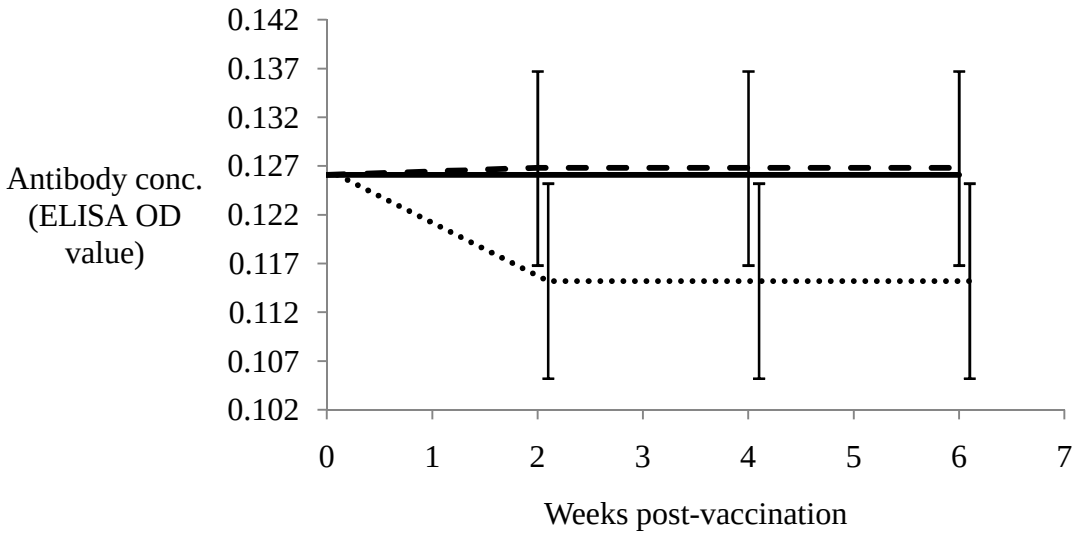

Figure 3.3: Estimated serum *M. haemolytica* whole cell antibody concentrations in captive Rocky Mountain bighorn sheep in the weeks following vaccination with two different *Pasteurellaceae* vaccines based on the parameters of the top-ranked model. The dashed line represents antibody concentrations of animals vaccinated with an autogenous *Pasteurellaceae* vaccine, the dotted line represents antibody concentrations of animals vaccinated with the commercial *Pasteurellaceae* vaccine One Shot®, and the solid line represents antibody concentrations of control animals treated with a sterile saline placebo. Week 0 represents pre-vaccination antibody concentrations.

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APPENDIX I: Lab Protocol

Indirect ELISA to measure antibodies to whole cell bacteria

1. Add 50 µl of poly-L-lysine (PLL) solution (1 mg/100 ml PBS, pH 7.2) to wells of 96 well polyvinylchloride microtiter plate and incubate for 2 hrs at RT.
2. Remove PLL and add bacterial suspension – 2.0×10^6 cells/50 µl (= 4.0×10^7 cells/ml)PBS to each well.
3. Centrifuge plates at 1000 x g for 10 min and remove supernatant.
4. Add 50 µl of 0.5% glutaraldehyde (v/v in cold PBS) to each well and incubate at RT for 30 min. Remove glutaraldehyde and wash plates twice with PBS.
5. Block unbound sites on plates with 100 mM glycine-1% BSA (pH 7.6) and incubate at RT for 1 hr.
6. Remove blocking solution and wash twice with PBS. Air dry plates at RT and store at 4° C until needed.
7. Dilute culture fluid, ascites fluid, or plasma appropriately. Add 50 ul of the preparation to each Ag coated well and incubate for 45 min at RT in a humified chamber.
8. Wash plates 3 times with PBS-T20. Add peroxidase labeled rabbit antibodies to sheep Ig. Incubate plates again for 45 min in humified chamber. Wash plates 3 times with PBS-T20.
9. Add substrate and incubate until sufficient color development. Read plates at 405 nm. Titer of Abs to WC Mh = reciprocal log of highest dilution yielding a positive reading.

APPENDIX II: Covariance Structures for the First Tier of Analyses

- First-order autoregressive structure (AR[1]): this corresponds to a model that attempts to model serial correlation in the residuals as a decreasing function of distance between observations. Matrix depicted below displays the variance matrix for the data of the samples for the first individual.

$$= \sigma^2 \begin{pmatrix} 1 & \rho & \rho^2 & \rho^3 \\ \rho & 1 & \rho & \rho^2 \\ \rho^2 & \rho & 1 & \rho \\ \rho^3 & \rho^2 & \rho & 1 \end{pmatrix}$$

- Heterogeneous Variance Model: this model assumes a different variance parameter for each sampling occasion and temporal autocorrelation is induced via the prior density on the precision parameter (inverse of the variance). The prior for each precision parameter was specified using a conditional autoregressive (CAR) structure with the exception of the precision for the first sampling occasion, which had was given a diffuse Gamma(0.1, 0.1) prior. Also the precision parameter for the fourth occasion was constrained to be equal to the precision parameter for the first sampling occasion to allow parameter estimation. Thus, the precision parameters with the CAR prior were specified to have the following distribution: $tau_i \sim Gamma(\alpha_i, \beta_i)$, where $\alpha_i = tau_{i-1} \times \beta_i$ and $\beta_i = \frac{tau_{i-1}}{10}$. This prior structure sets the mean of the i^{th} prior equal to the i-1 precision parameter and maintains the same variance as the Gamma(0.1,0.1) prior for the first precision parameter (i.e., a CAR structure on the prior).
- Random Intercept Model: this corresponds to the assumption that each individual has its own intercept/mean that is drawn from a common distribution, and is analogous to specifying a random individual effect. The model essentially means that observations made on an individual are correlated, but doesn't attempt to model any temporal correlation.

$$y_{ij} = \beta_0 + \beta_1 x_1 + \cdots z_i + e_{ij}$$

where $e_{ij} \sim N(0, \sigma^2)$ and $z_i = N(0, \sigma_{int}^2)$ for the i^{th} individual during the j^{th} sampling occasion.

- Random Time Effect Model: this includes a random effect parameter that has a CAR prior (i.e., $t_j \sim N\left(t_{j-1}, \sigma_{time}^2\right)$ and $t_1 \sim N(0, \sigma_{time}^2)$) to induce temporal correlation.

$$y_{ij} = \beta_0 + \beta_1 x_1 + \cdots + t_j + e_{ij}$$

where $e_{ij} \sim N(0, \sigma^2)$, and $t_j \sim N(0, \sigma_t^2)$ for the i^{th} individual during the j^{th} sampling occasion.

- Random Intercept and Random Time Effect Model: this model is a combination of the random intercept model and the random time effect model.
- Independent Errors Model: this corresponds to a normal regression model that assumes each observation is independent.

$$y_{ij} = \beta_0 + \beta_1 x_1 + \cdots + e_{ij}$$

where $e_{ij} \sim N(0, \sigma^2)$ for the i^{th} individual during the j^{th} sampling occasion.

APPENDIX III: Convergence Testing Plots for Chapter 1

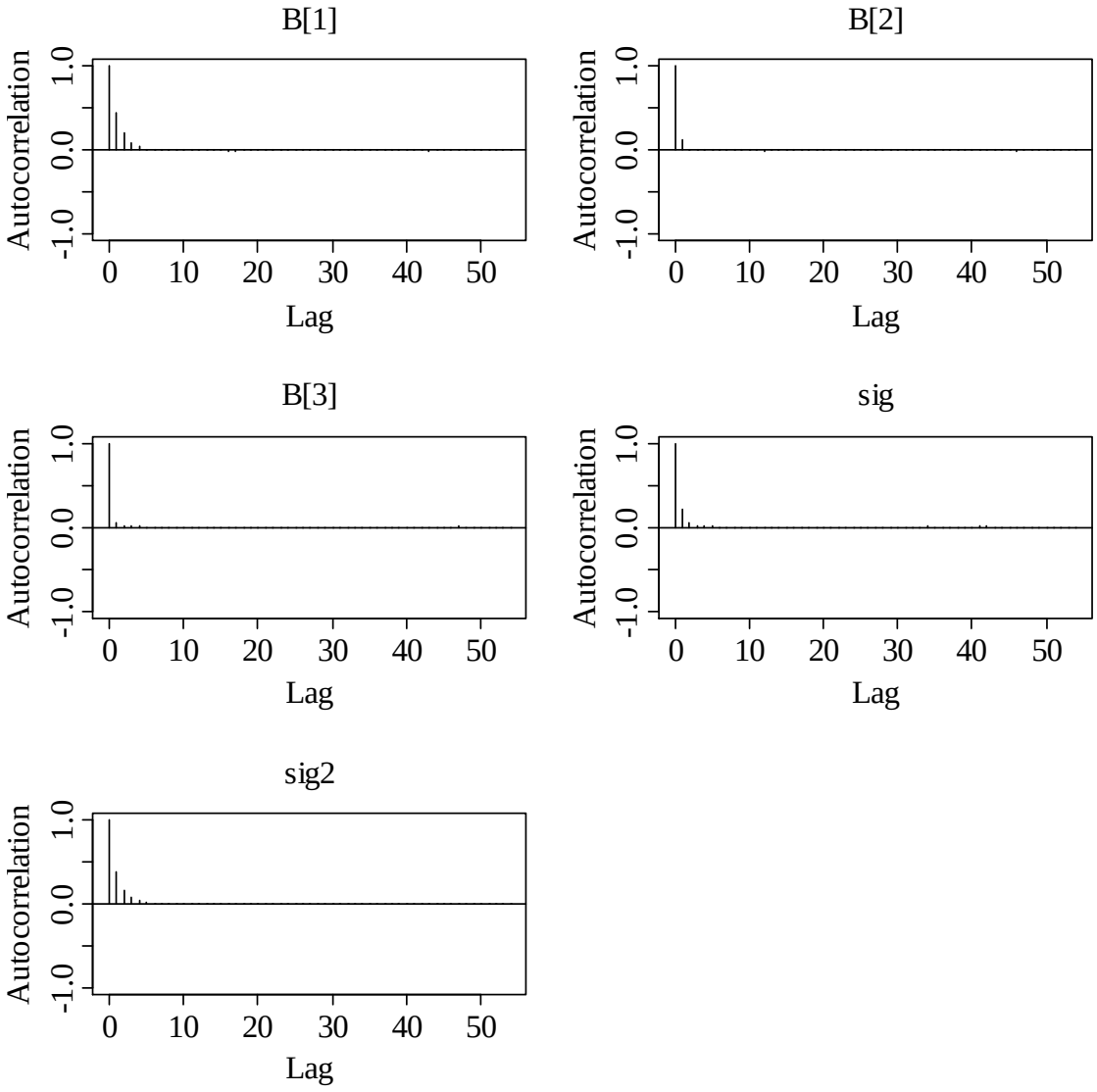

Figure III.1: Plots of sampler lag-autocorrelations for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the booster effect, B[3] is the mean of the random intercept, sig is the residual standard deviation, and sig2 is the standard deviation of the random intercept.

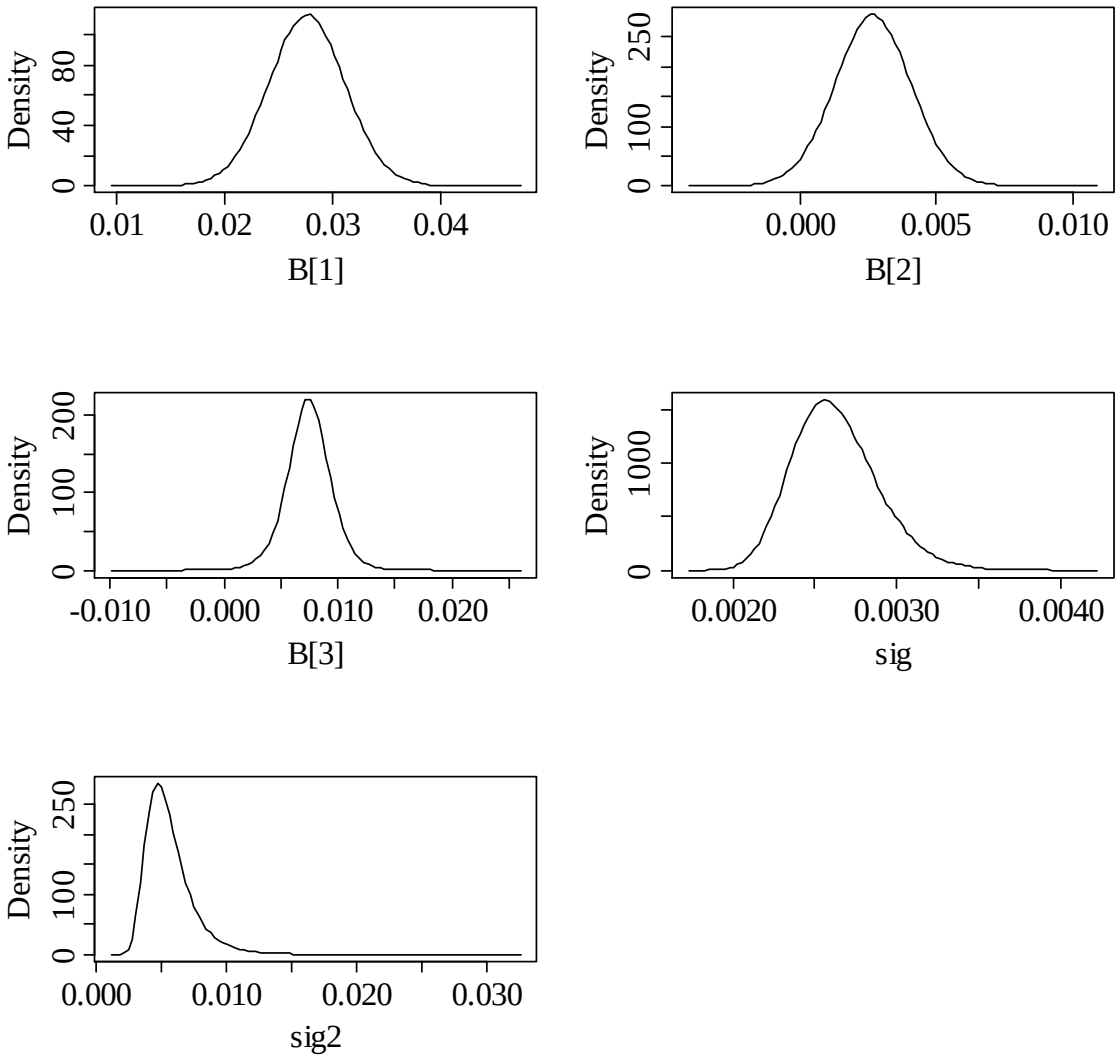

Figure III.2: Plots of estimated posterior density for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the booster effect, B[3] is the mean of the random intercept, sig is the residual standard deviation, and sig2 is the standard deviation of the random intercept.

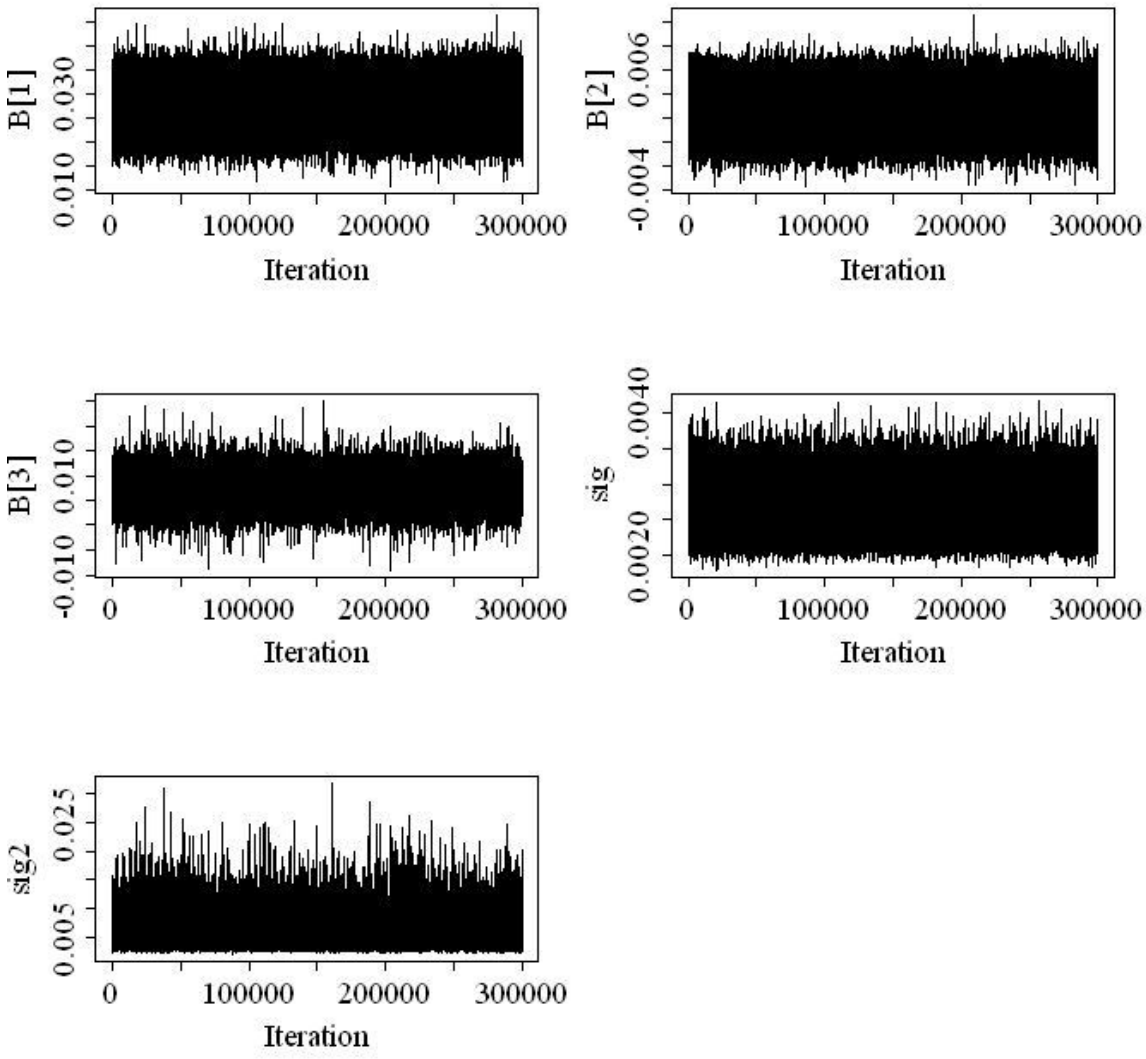

Figure III.3: Trace plots for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the booster effect, B[3] is the mean of the random intercept, sig is the residual standard deviation, and sig2 is the standard deviation of the random intercept.

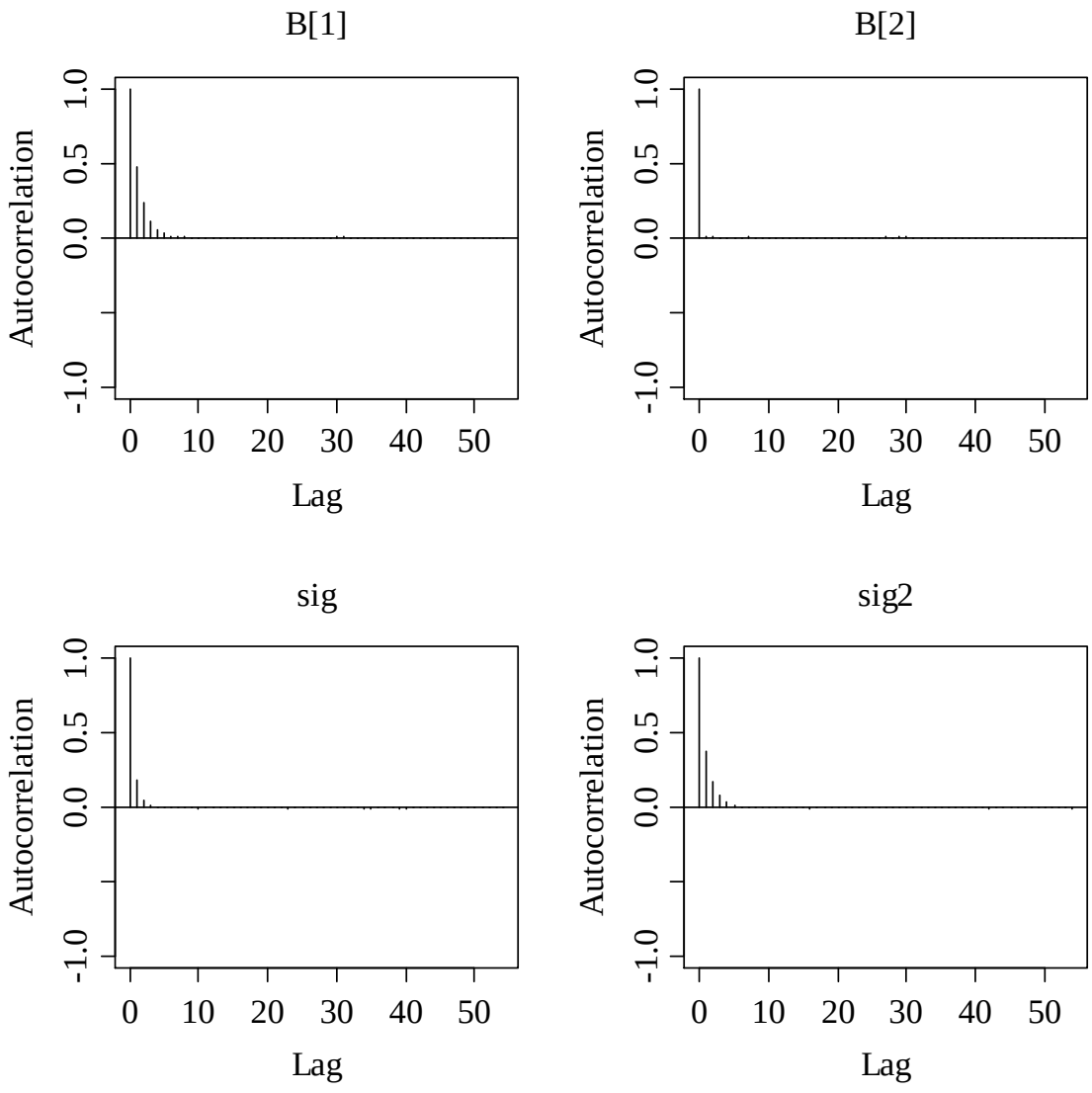

Figure III.4: Plots of sampler lag-autocorrelations for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the mean of the random intercept, sig is the residual standard deviation, and sig2 is the standard deviation of the random intercept.

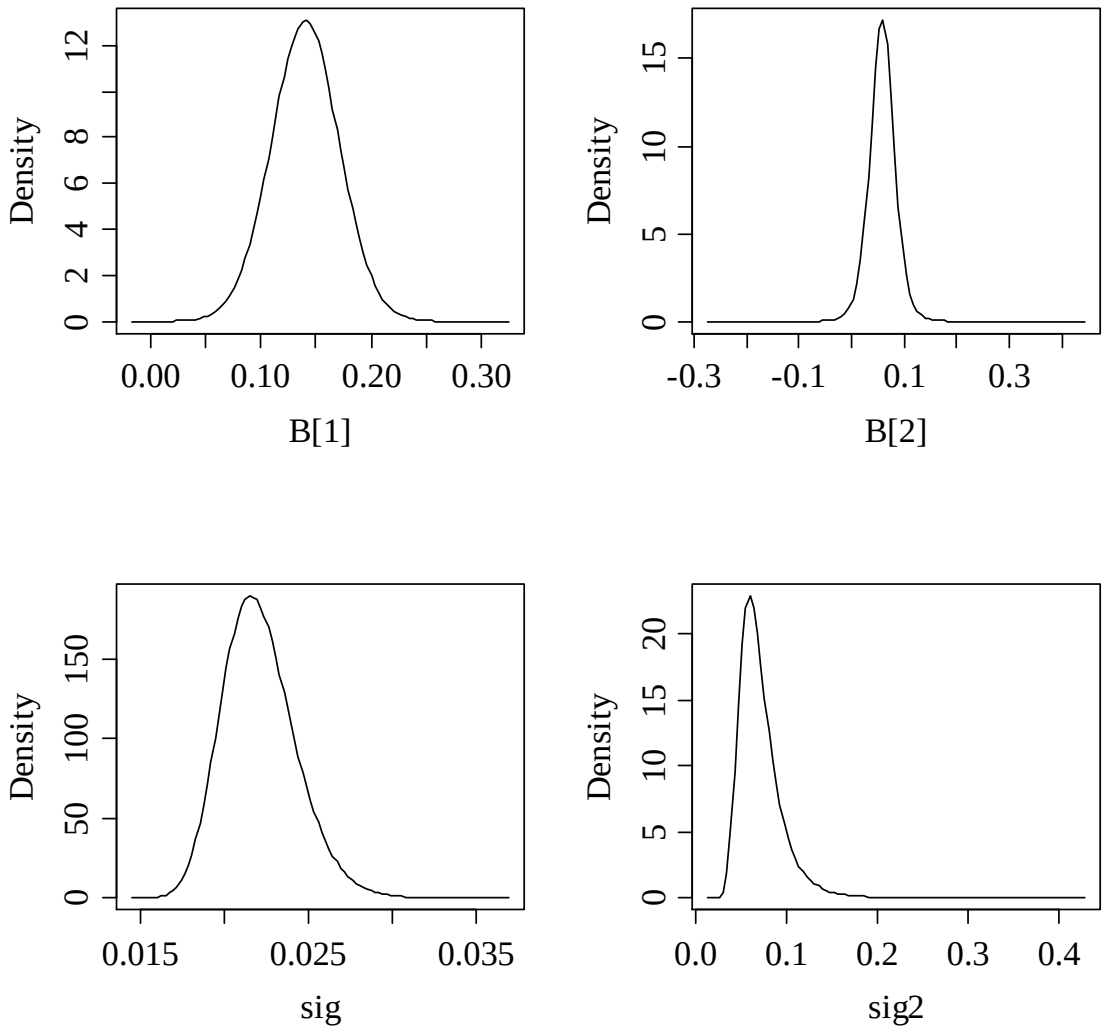

Figure III.5: Plots of estimated posterior density for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the mean of the random intercept, sig is the residual standard deviation, and sig2 is the standard deviation of the random intercept.

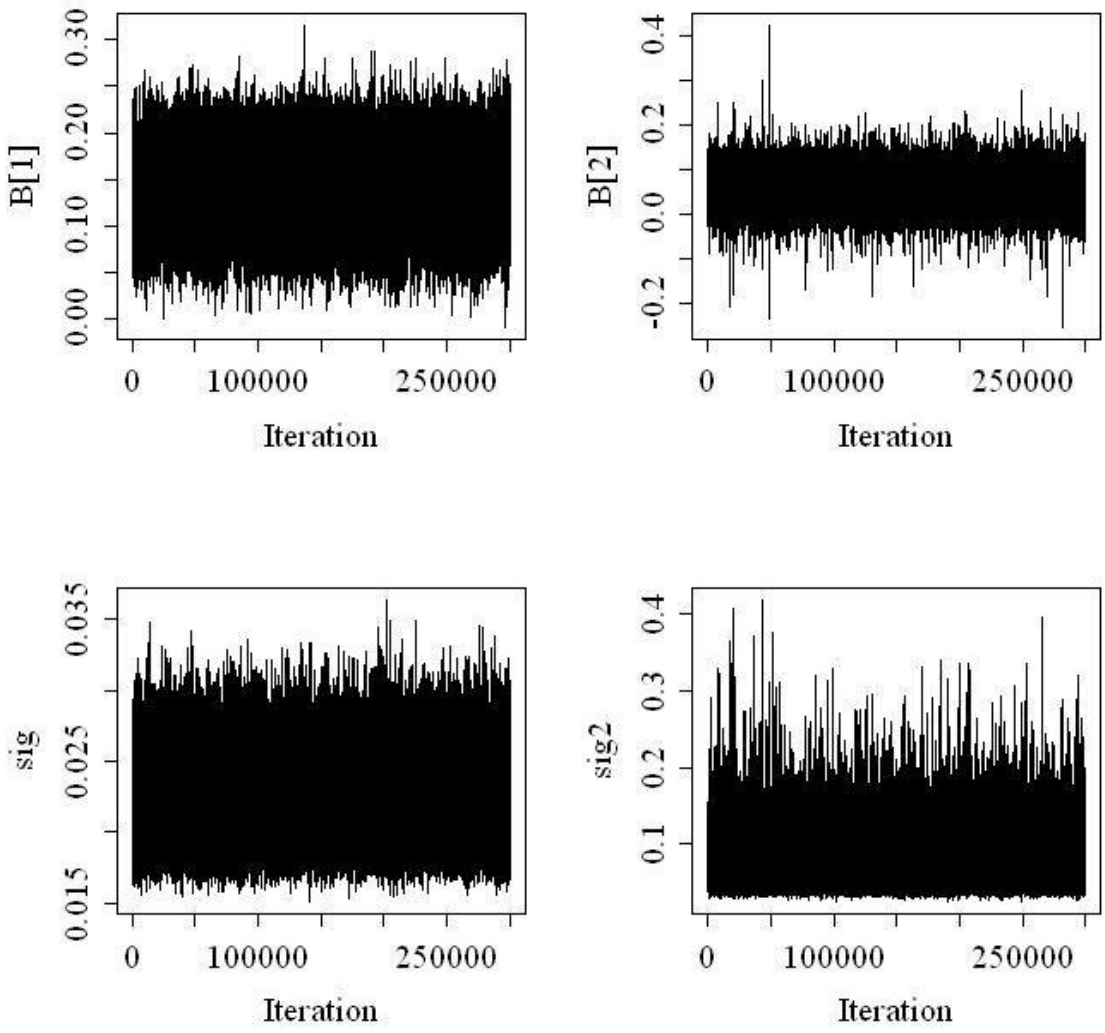

Figure III.6: Trace plots for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with an autogenous vaccine in 2009, where B[1] is the vaccine effect, B[2] is the mean of the random intercept, sig is the standard deviation, and sig2 is the residual standard deviation of the random intercept.

APPENDIX IV: Convergence Testing Plots for Chapter 3

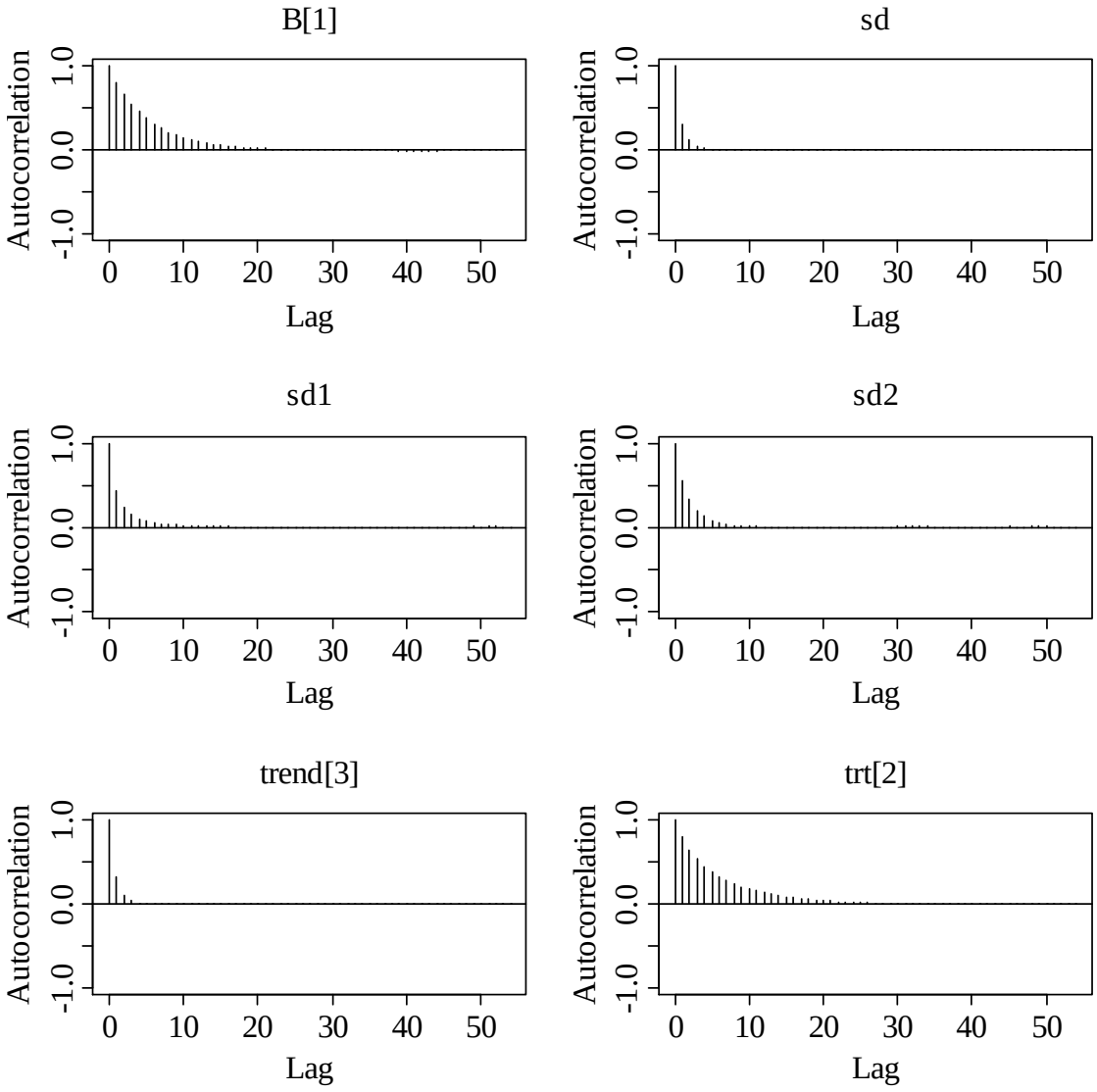

Figure IV.1: Plots of sampler lag-autocorrelations for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd is the standard deviations for the residuals, sd1 is the standard deviation for the random intercept, sd2 is the standard deviation of the random time effect, trend [3] is the time trend on the effect of vaccination with the commercial vaccine One Shot®, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with One Shot, and trtdiff is the difference in treatment effects between the two vaccines.

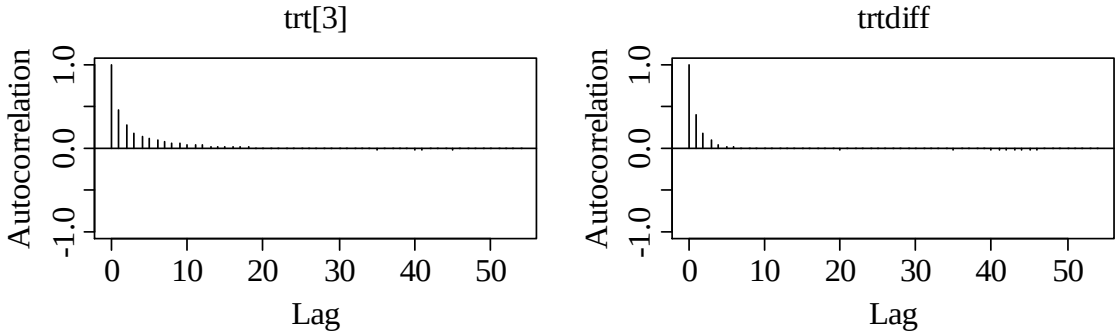

Figure IV.1: (continued)

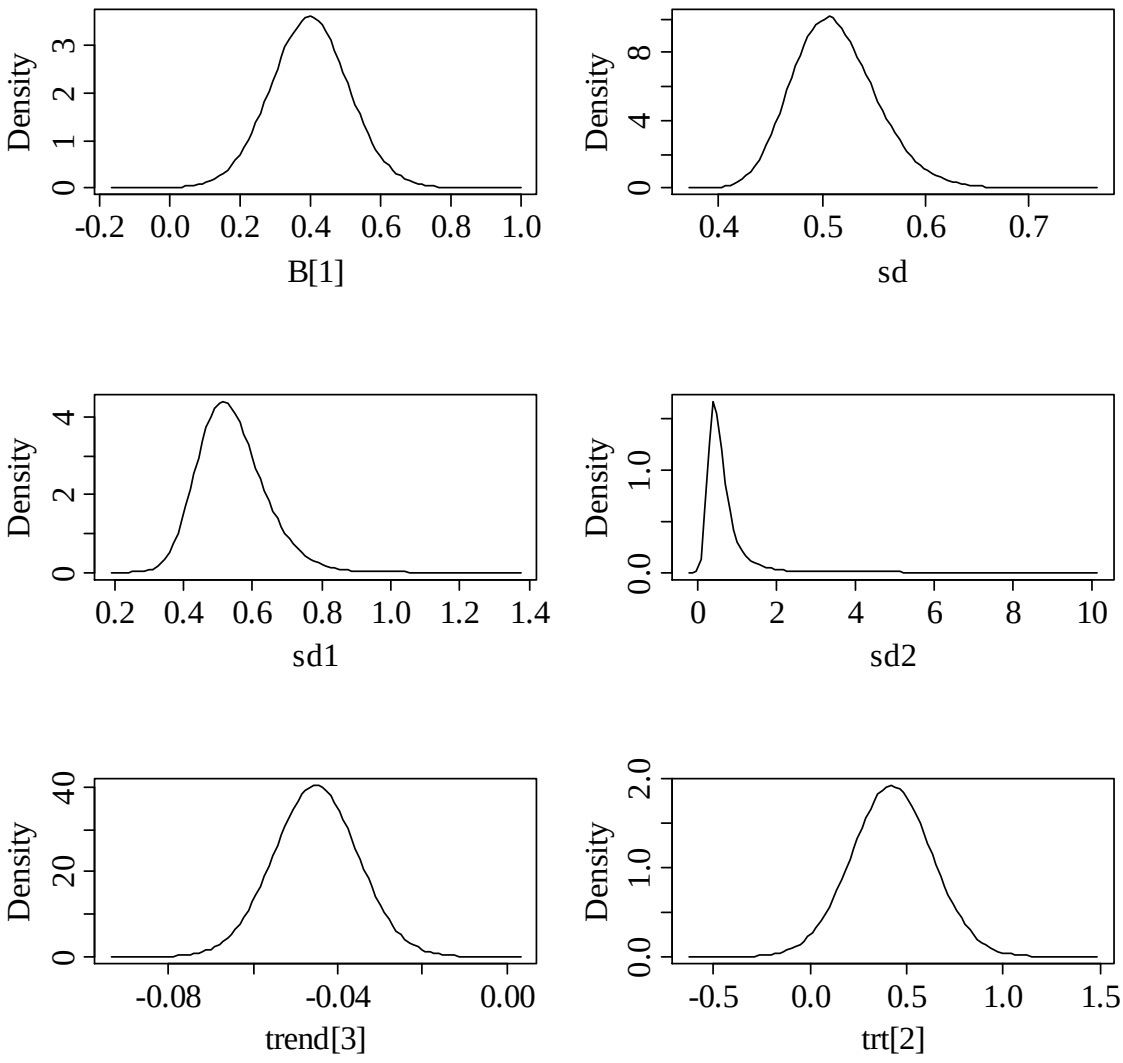

Figure IV.2: Plots of estimated posterior density for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd is the standard deviations for the residuals, sd1 is the standard deviation for the random intercept, sd2 is the standard deviation of the random time effect, trend [3] is the time trend on the effect of vaccination with the commercial vaccine One Shot®, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with One Shot, and trtdiff is the difference in treatment effects between the two vaccines.

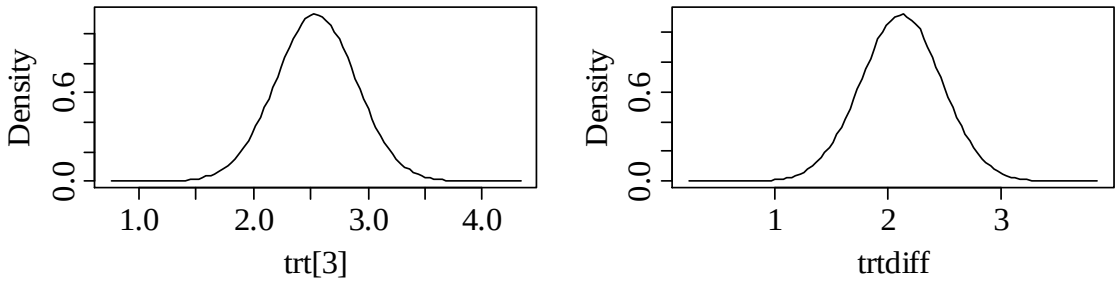

Figure IV.2: (continued)

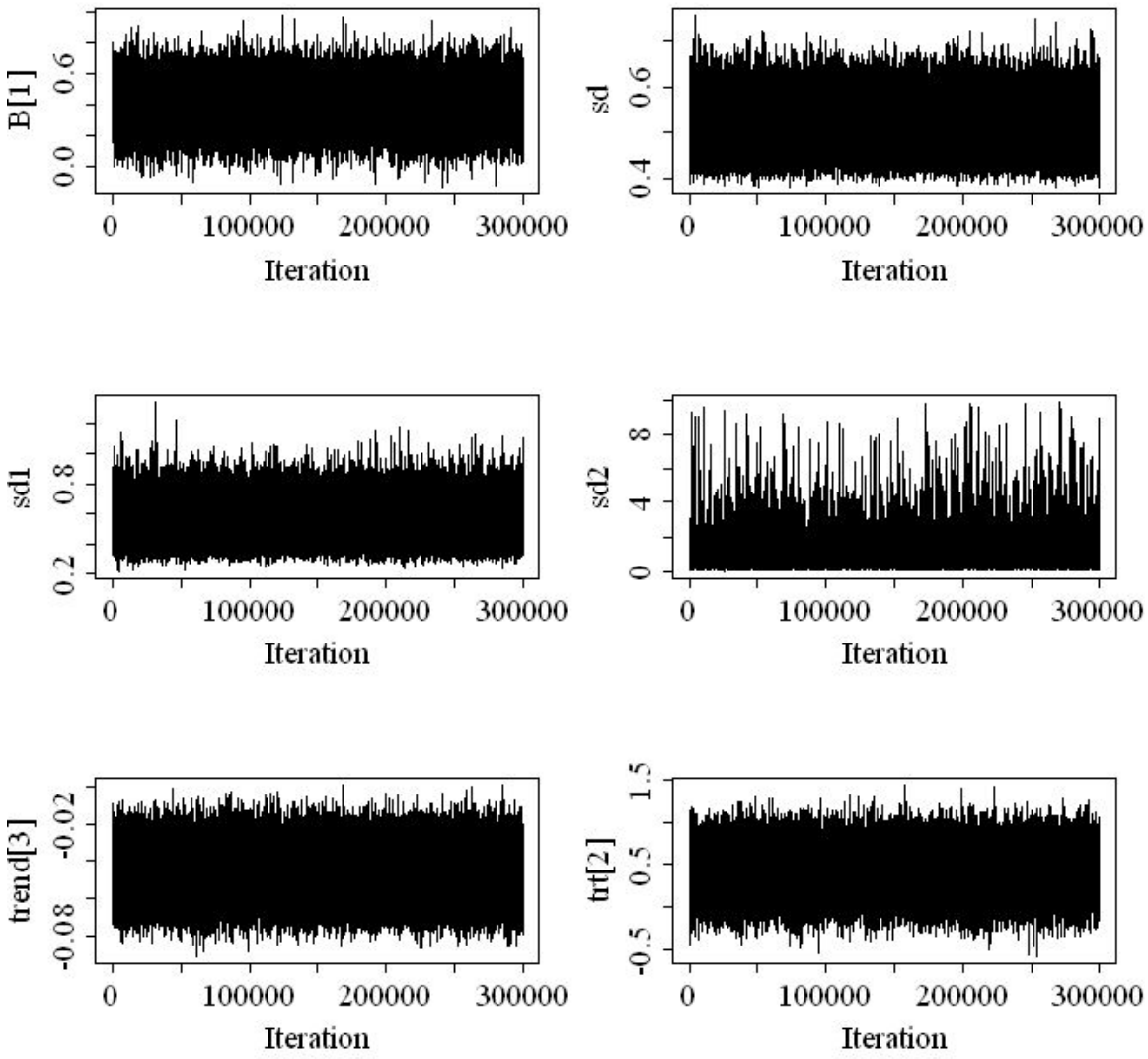

Figure IV.3: Trace plots for each model parameter of the top-ranked model used to estimate leukotoxin neutralizing antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd is the standard deviations for the residuals, sd1 is the standard deviation for the random intercept, sd2 is the standard deviation of the random time effect, trend [3] is the time trend on the effect of vaccination with the commercial vaccine One Shot®, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with One Shot, and trtdiff is the difference in treatment effects between the two vaccines.

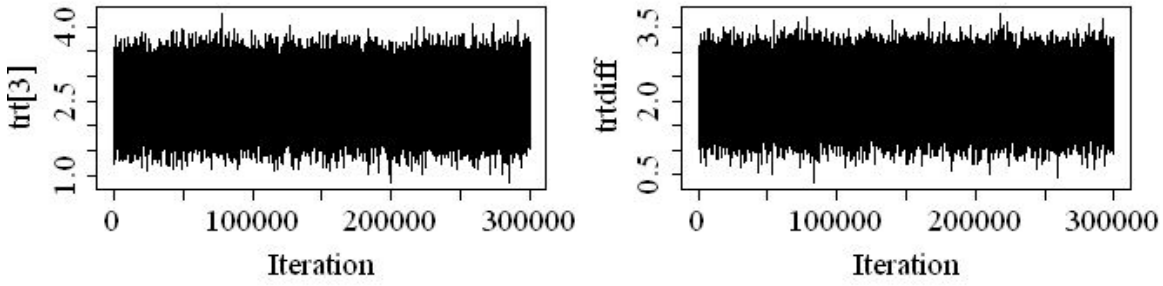

Figure IV.3: (continued)

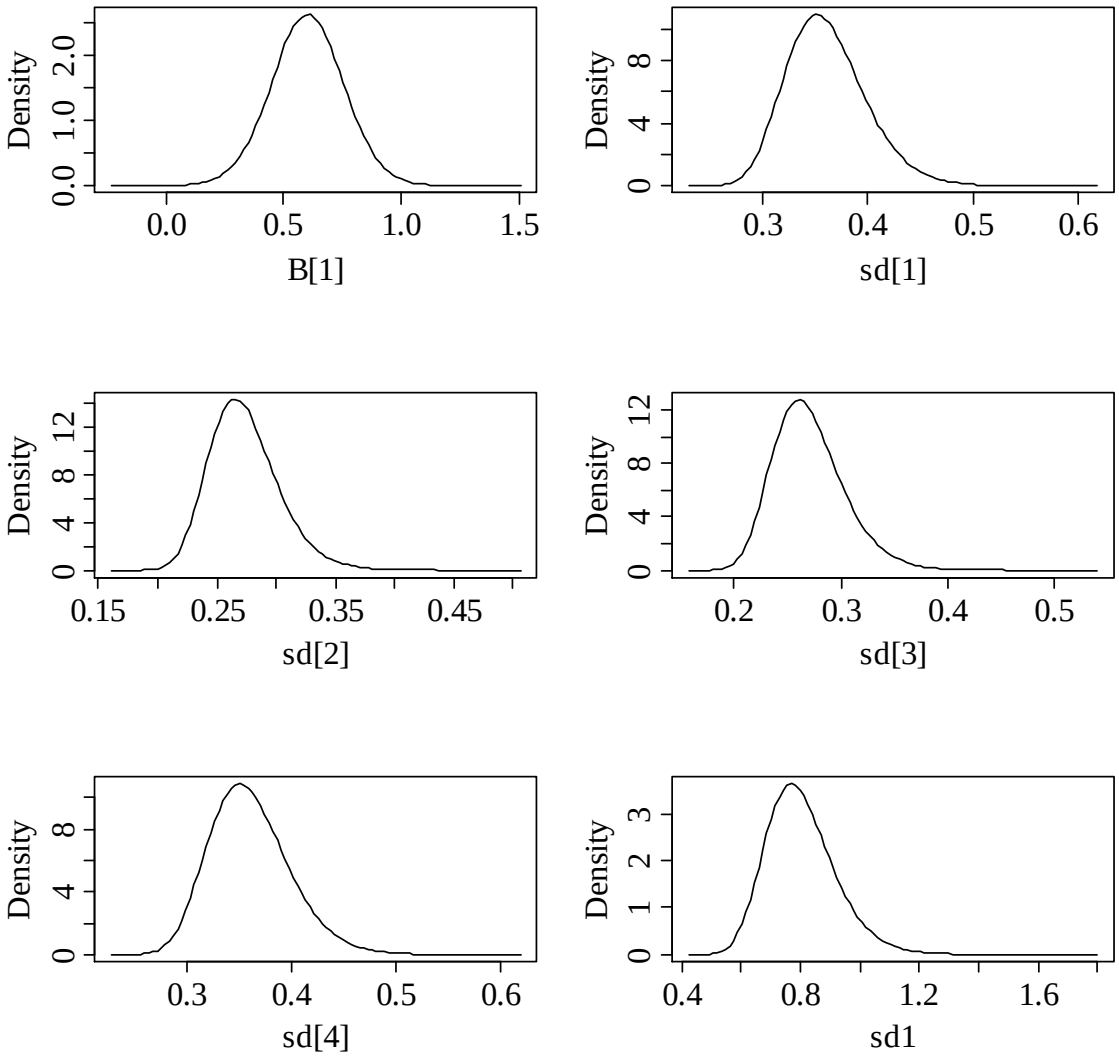

Figure IV.4 Plots of estimated posterior density for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd[*j*] is the standard deviations for the residuals during j^{th} sample occasion (j =1,…,4), sd1 is the standard deviation for the random intercept, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with the commercial vaccine One Shot®, and trtdiff is the difference in treatment effects between the two vaccines.

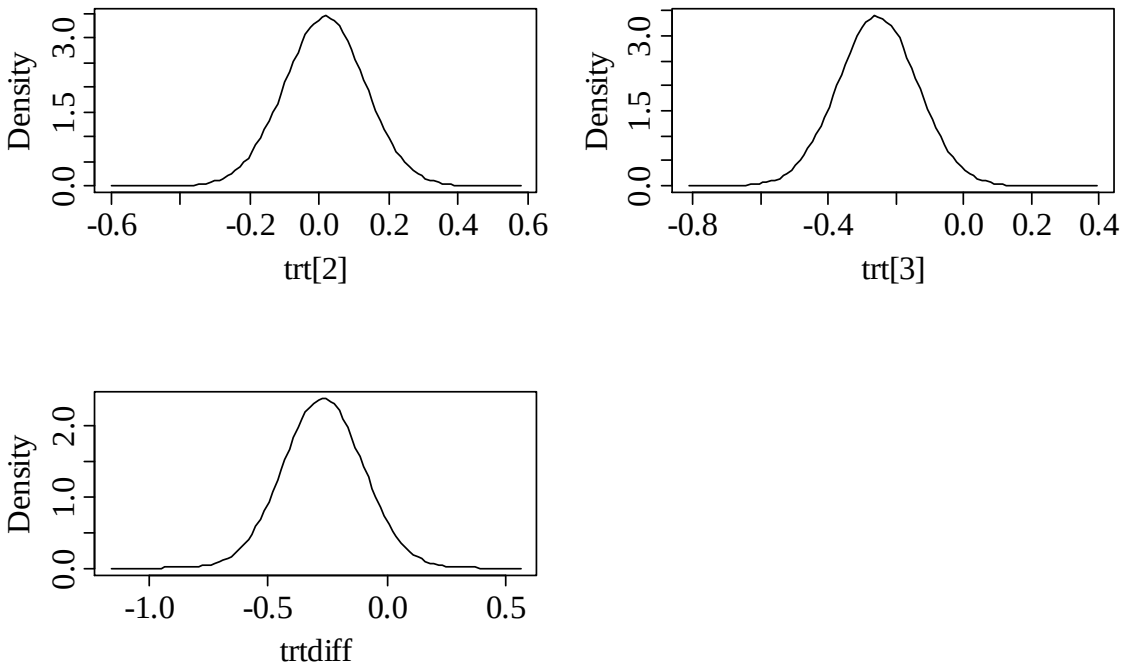

Figure IV.4: (continued)

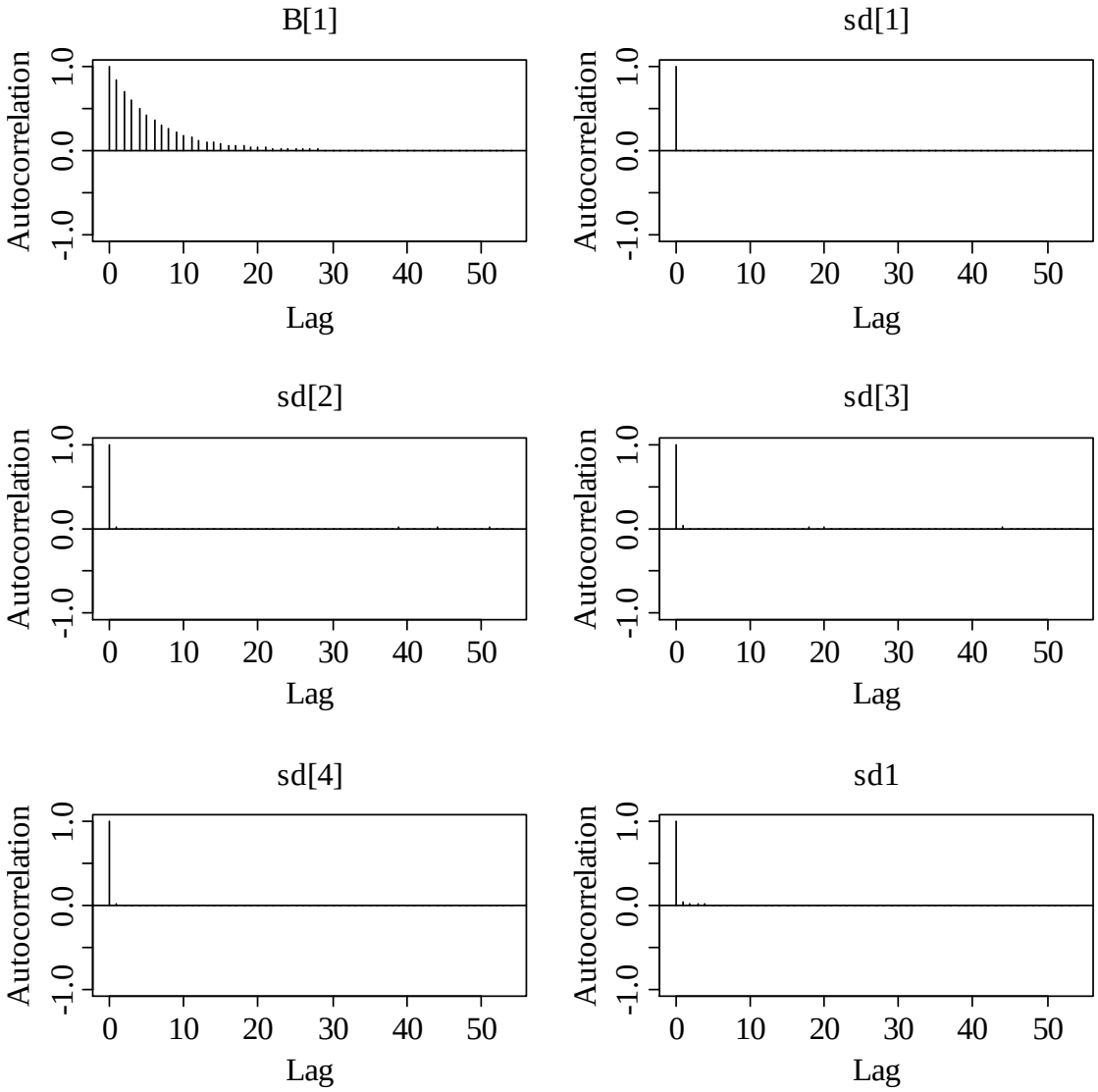

Figure IV.5: Plots of sampler-lag autocorrelation for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd[*j*] is the standard deviations for the residuals during j^{th} sample occasion (j =1,…,4), sd1 is the standard deviation for the random intercept, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with the commercial vaccine One Shot®, and trtdiff is the difference in treatment effects between the two vaccines.

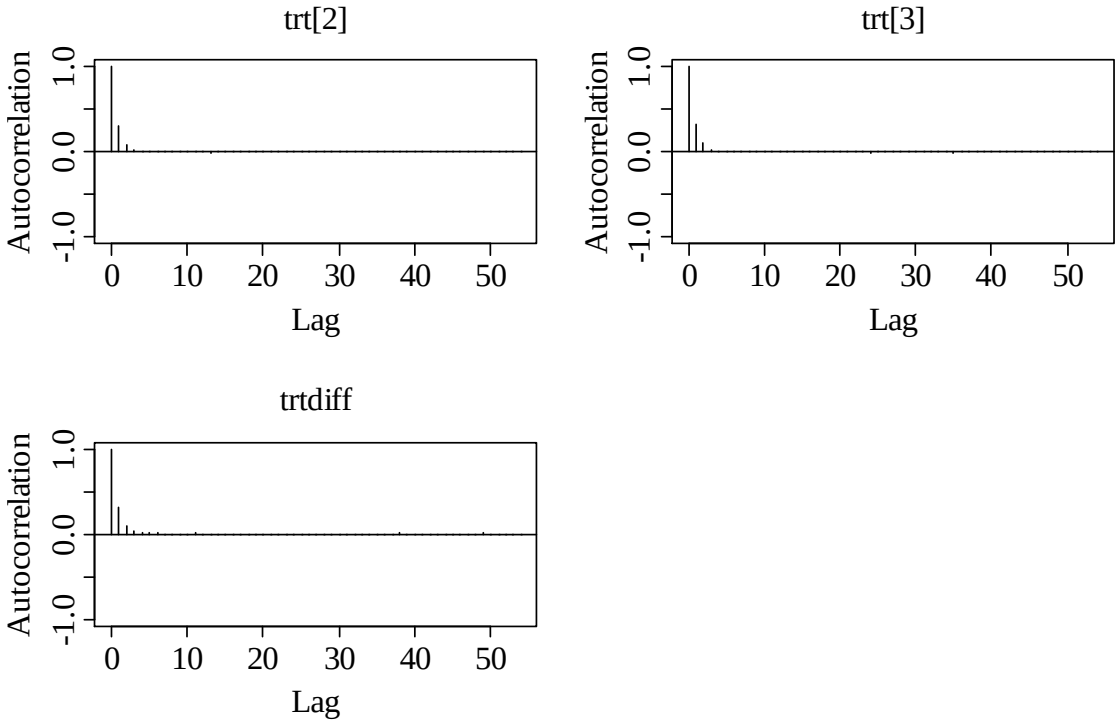

Figure IV.5: (continued)

Iteration

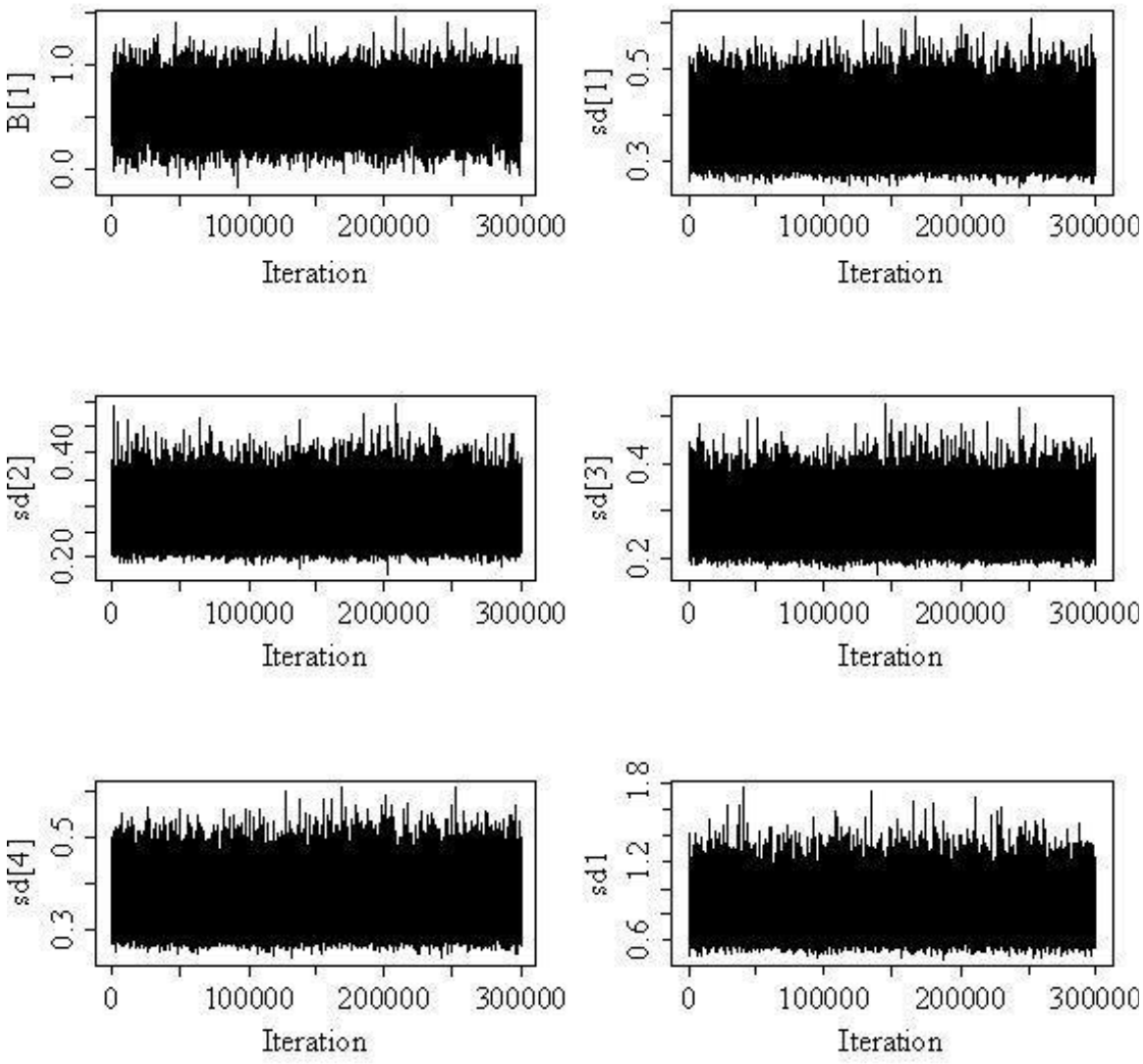

Figure IV.6: Trace plots for each model parameter of the top-ranked model used to estimate *Mannheimia haemolytica* whole cell antibody concentrations in captive bighorn sheep in response to vaccination with two *Pasteurellaceae* vaccines, where B1 is the age effect, sd[*j*] is the standard deviations for the residuals during j^{th} sample occasion (*j* =1,…,4), sd1 is the standard deviation for the random intercept, trt[2] is the effect of vaccination with an autogenous vaccine, trt[3] is the effect of vaccination with the commercial vaccine One Shot®, and trtdiff is the difference in treatment effects between the two vaccines.

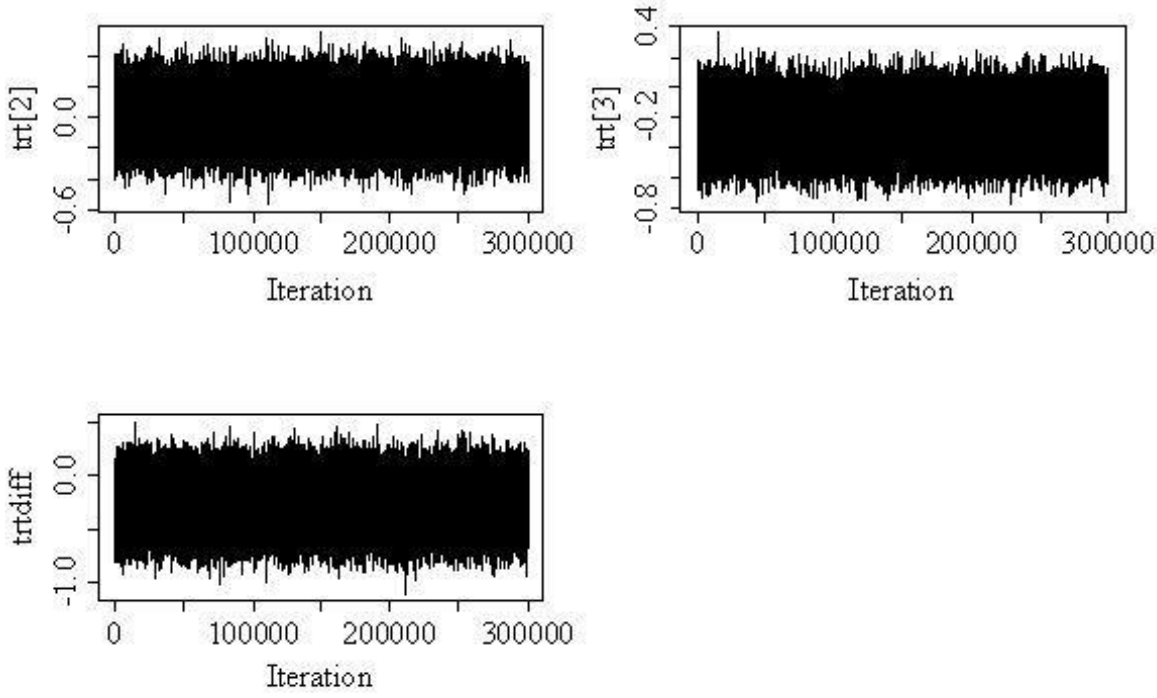

Figure IV.6: (continued)