MEAT & LIVESTOCK AUSTRALIA

final report

Project code: B.FLT.0235

Prepared by: Paul Cusack
Australian Livestock Production Services

Date published: 15 March 2019

PUBLISHED BY
Meat and Livestock Australia Limited
PO Box 1961
NORTH SYDNEY NSW 2059

Effects of vaccination in backgrounded feedlot cattle

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

The primary aim of this study was to measure the effects of respiratory vaccines administered to cattle in local backgrounding facilities on feedlot health and growth rate. 7302 cattle across 6 sites in Australia were allocated to 8 respiratory vaccine groups, including negative controls, between November 2009 and February 2017 on entry to backgrounding facilities contiguous with each feedlot. The vaccines, against *Mannheimia haemolytica*, bovine herpesvirus 1 (BHV1), and bovine viral diarrhoea virus (BVDV) were given in various combinations at background entry and feedlot entry according to label recommendations. Blood samples were taken at these times to measure BHV1 and BVDV serum antibody concentrations. Cattle were held in the backgrounding facilities for a minimum of 28 days.

A high proportion (66%) of cattle were seropositive to BVDV at background facility arrival with more cattle seropositive in western Queensland than in sites south of this. 0.28% of the study population was persistently infected with BVDV. Only 13.5% of cattle were seropositive to BHV1 at arrival. With the exception of the *M. haemolytica* vaccine, Bovishield®, all other respiratory vaccine combinations decreased ($P < 0.001$) growth rate during backgrounding. Overall, feedlot growth rate was not affected by vaccine group (global Wald $P > 0.05$) with one point estimate improvement ($P = 0.003$) in response to Bovisheild® and Pestigard® (against BVDV) in combination. BRD risk was lowest ($P = 0.010$) in cattle vaccinated with Bovilis MH+IBR® (against *M. haemolytica* and BHV1; subhazard ratio or SHR: 0.47; 95% CI: 0.27-0.83), but other vaccine combinations had effects with the confidence limits overlapping nil effect.

Accounting for respiratory vaccine effects on growth rate during backgrounding and feedlot phases, financial analysis showed that routine use of the respiratory vaccines in this study, administered on entry to backgrounding and entry to the feedlot, with cattle held for at least 28 days in backgrounding facilities contiguous with their feedlots, is not warranted. Further research into the appropriate use of respiratory vaccines should be directed at their administration on the farm of origin.

Executive summary

Commingling (mixing) of cattle from multiple sources immediately before and at feedlot entry is a major determinant of the risk of bovine respiratory disease (BRD) when cattle are placed directly in a feedlot soon after arrival. In the Canadian Bruce County Project, incidences of clinical disease and death from BRD were greater with mixing of calves from different sources and assembly of calves from widely separated geographic locations (Martin et al., 1982). More recently, O'Connor et al. (2005) found a strong relationship between commingling and BRD (OR = 3, 95% CI = 2.5 to 3.6), and Sanderson et al. (2008) also found an increase in BRD incidence (Incidence Rate Ratio [IRR] = 2.0, $P < 0.001$) with cattle from multiple, rather than single, sources.

The primary aim of this study was to measure the effects of respiratory vaccines administered to cattle in local backgrounding facilities on feedlot health and growth rate.

The health and production effects of vaccination was evaluated separately from those associated with the other managerial and behavioural aspects of local backgrounding systems. The effects of this backgrounding system was evaluated by comparison with contemporary cattle of the same market specification that have been paced directly in the feedlot. This information was used to undertake a financial analysis of the various components of the backgrounding system.

These results do not support vaccination of feedlot beef cattle if they are to be locally backgrounded for at least 28 days before feedlot entry because all vaccine combinations decreased ADG during backgrounding with the exception of Bovishield®, and any increases in ADGs during the feedlot phase are probably insufficient to account for this and generate an overall marginal profit from vaccination. Overall, the use of the various vaccine combinations was not associated with an increase in ADG during the feedlot phase. However, point estimates were supportive of small increases in ADG, with the cattle vaccinated with the combination of Bovishield/Pestigard having a significantly higher ADG than controls. The positive effect of Bovishield/Pestigard was inconsistent with the effect of the other vaccine combinations and the reasons for this are unclear.

Tis result does not preclude a potential benefit from the use of respiratory vaccines in cattle that are placed directly in the feedlot. The likelihood of a benefit from on-farm administration of respiratory vaccines in the form of reduced BRD risk is supported by the NBRDI (Barnes et al., 2014) where modest reductions in BRD risk were observed with the use of Bovilis MH® and Pestigard®, and the finding from the same study that an increase in the number of viruses to which animals seroincrease was associated with an increase in the BRD risk. A high proportion of cattle naïve to several respiratory viruses is more likely in groups of cattle purchased directly from the farm of origin and placed directly in the feedlot. Cost-effective responses to respiratory vaccines are more likely in these cattle and this is where further research into the appropriate use of respiratory vaccines should be directed.

Table of contents

1 Background

1.1 Introduction

Commingling (mixing) of cattle from multiple sources immediately before and at feedlot entry is a major determinant of the risk of bovine respiratory disease (BRD) when cattle are placed directly in a feedlot soon after arrival. In the Canadian Bruce County Project, incidences of clinical disease and death from BRD were greater with mixing of calves from different sources and assembly of calves from widely separated geographic locations (Martin et al., 1982). More recently, O'Connor et al. (2005) found a strong relationship between commingling and BRD (OR = 3, 95% CI = 2.5 to 3.6), and Sanderson et al. (2008) also found an increase in BRD incidence (Incidence Rate Ratio [IRR] = 2.0, $P < 0.001$) with cattle from multiple, rather than single, sources.

Rather than placing cattle directly in a feedlot at or soon after arrival, 'backgrounding' is an alternative strategy. Backgrounding is defined as the holding of cattle in enclosures that provide at least 30 m^2 per animal and where they are fed, either pasture, or a mixed ration with no greater than 43% cereal grain (DM), or both). Contrary to the negative health effects of commingling where purchased cattle are placed directly in the feedlot, commingling might be used to reduce the risk of BRD where cattle can be mixed in backgrounding facilities for a sufficient period before placement in the feedlot. Barnes et al. (2014) showed that the timing of commingling determines its effect on the incidence of BRD. Mixing of saleyard-purchased cattle at least 28 days before feedlot entry was associated with a reduction in the incidence of BRD (OR dependent on subsequent mixing = 0.6 to 0.8). Conversely, mixing between 27 and 13 days before feedlot entry via a saleyard was associated with an increase in BRD incidence (OR = 1.9, 95% CI = 1.3 to 2.7, $P = 0.001$). With both these saleyard transits there was no evidence of a large direct effect, indicating that the effects were mediated primarily through mixing rather than direct saleyard effects. Cattle that were mixed through a saleyard 12 days or less before feedlot entry had a markedly increased risk of BRD (OR = 2.6, 95% CI = 1.6 to 4.1, $P < 0.001$). The direct effect of saleyard exposure within 12 days of feedlot entry was attenuated but important (OR = 1.6, 95% CI = 0.9 to 2.6, $P = 0.05$), indicating that there were negative effects specific to saleyard exposure in this period in addition to the effects of mixing. The protective effects of mixing at least 28 days before feedlot entry might be due to immunological responses to infections contracted during mixing that provide protection against the major respiratory viruses from feedlot entry. Seropositivity to the respiratory viruses, BHV1, PI3, BRSV and BVDV, at feedlot entry is protective against BRD during the feedlot period (Hay et al., 2016a). In addition, a longer period between saleyard exposure and mixing prior to feedlot entry provides additional time for the cattle to recover from the effects of these stressors. It is logical that recovery time would enhance the potential immunological benefits of prior exposure to respiratory viruses. Thus viral exposure long before feedlot entry has protective effects against BRD occurrence after feedlot entry whereas viral exposure soon before feedlot entry or early in the feedlot period has adverse effects. Associations were found (Barnes et al., 2014; Hay et al., 2016a) between risk of BRD during the feedlot period and an increase in antibody titres between feedlot entry and day 42 to the respiratory viruses, BHV1, PI3, BRSV and BVDV, either singly (OR = 1.4, 95% CI = 1.2 to 1.6, $P < 0.001$; OR = 1.4, 95% CI = 1.2 to 1.7, $P < 0.001$; OR = 1.4, 95% CI = 1.3 to 1.7, $P < 0.001$; OR = 1.3, 95% CI = 1.1 to 1.6, $P = 0.001$, respectively) or in combination. The risk of BRD increased with the number of these respiratory viruses to which serological increase occurred (Barnes et al., 2014; serological increase to one virus, OR = 1.3, 95% CI = 1.1 to 1.6, $P = 0.003$; serological

increase to two viruses, OR = 1.9, 95% CI = 1.5 to 2.3, *P* < 0.001; serological increase to three viruses, OR = 2.1, 95% CI = 1.6 to 2.6, *P* < 0.001; serological increase to four viruses, OR = 1.8, 95% CI = 1.1 to 2.7, *P* = 0.006). Thus, the risk of BRD was higher with increasing exposure to respiratory viruses of cattle with low respiratory virus antibody titres at feedlot entry. Backgrounding before feedlot entry involves commingling and close contact, which should increase respiratory virus transfer, but with more space and no ruminal acidosis challenge compared with direct feedlot entry. If backgrounding improves the immunological statuses of the animals at feedlot entry, this could reduce any potential benefits of vaccination in these systems. Accordingly, there is a need to assess effects of vaccination in cattle that have been backgrounded for a substantial period before feedlot entry. Modest effects of vaccination before feedlot entry in reducing the risk of BRD have been reported by Barnes et al. (2014) and Hay et al. (2016b) in response to vaccination against *M. haemolytica* (Bovilis MH, Coopers Animal Health, Macquarie Park, NSW; OR = 0.8, 95% CI = 0.6 to 1.0, *P* = 0.02) and BVDV (Pestigard, Zoetis Australia, Rhodes NSW; OR = 0.8, 95% CI = 0.5 to 1.1, *P* = 0.05). However, in that study, most cattle were not backgrounded, and benefits of vaccination might be greater in cattle that have not been backgrounded. In addition, vaccination may have been used in conjunction with other strategies such as associated commingling before feedlot entry. The current study was designed to assess effects of vaccination in backgrounded (rather than non-backgrounded) cattle, and to avoid any confounding of observed effects of vaccination due to associated commingling before feedlot entry, by assessing vaccination effects only in cattle commingled for at least 28 days before feedlot entry. It is possible that backgrounding could relocate the health challenges of the early feedlot period to the backgrounding facility. Accordingly, there is a need to also assess effect of vaccination on backgrounding growth rates.

With transport duration greater than 24 hours, increasing transport time was associated with higher BRD incidence in US cattle (Johnson, 1985). While no controlled studies in Australia have investigated the relationship between transport times and subsequent BRD outcomes, one study was conducted to assess metabolic changes in cattle subjected to transportation. Stanger et al. (2005) examined the immune status of *Bos indicus* steers after 72 hours of road transportation. The comparison of immunological functions before and after transport indicated a degree of dysfunction for 6 days post-transport. The authors concluded this could increase susceptibility to infectious agents for 6 days after transport, though this aspect was not tested. In keeping with this, a Polish study (Urban-Chmiel, 2006) found transport duration of 72 hours (1700 km) resulted in reduced (*P* < 0.05) leukocyte viability with samples exposed to leukotoxin from *Mannheimia haemolytica.* Most of the stress of transport of less than 24 hours duration was suggested by Cole et al. (1989) to be related to the loading and unloading process. However, Hay et al. (2014) found that cattle transported for 6 hours or more within 24 hours of feedlot entry were at slightly increased risk of BRD (OR = 1.2, 95% CI = 1.0 to 1.5, *P* = 0.02) compared with cattle transported for less than 6 hours within 24 hours of feedlot entry. Local backgrounding for at least 28 days, using feedlots with contiguous backgrounding facilities, removes these effects of transport immediately before feedlot entry.

There is a further reason why any benefits of vaccination might be less when cattle are backgrounded locally for at least 28 days. Lactic acidosis has been shown to increase the risk of BRD (Buczinski et al., 2015; Chako et al., 2015) and the likelihood of death in cattle diagnosed with BRD (Buczinski et al., 2015). These are most likely related to endotoxaemia (Plaizier, 2008; Ghozo, 2006; Andersen, 2003) and bacteraemia (Steele et al., 2011; Plaizier, 2008) arising from a loss of mucosal surface structural

integrity and therefore barrier function in the rumen (Steele et al., 2011; Penner et al., 2010) and large intestine (Gressley et al., 2011). The effects of ruminal acidosis are probably exacerbated in cattle that have been deprived of feed for greater than 24 hours before feedlot delivery because feed deprivation itself compromises gastrointestinal tract barrier function (Zhang et al., 2013; Gabel and Aschenbach, 2002). Thus, feed management that achieves high stable intakes during the first 2 to 3 weeks in the feedlot without inducing lactic acidosis appears to be important to immunocompetence and reducing the risk of BRD. It is possible that local backgrounding negates the negative effects on gastrointestinal tract barrier function of feed deprivation immediately before feedlot entry, and creates more uniform rumen characteristics associated with potential feed intake in starter cattle, thereby improving feed delivery management across the pen.

Many Australian feedlots vaccinate cattle against BHV1 at feedlot entry with a modified live intranasal vaccine (Rhinogard®, Zoetis Australia, Silverwater NSW). As there appears to be synergy between respiratory viruses in causing BRD (Barnes et al., 2014; Hay et al., 2016a), there is also a need to assess effects of vaccines against organisms other than BHV1 separately in cattle that receive Rhinogard® and within cattle that did not receive Rhinogard®.

The primary aim of this study was to assess the effects of vaccination within locally backgrounded beef feedlot cattle on feedlot average daily gain (ADG) and BRD risk. Secondary aims were: to assess interactions between vaccination and individual animal serological status at entry to backgrounding; to assess effects of vaccination on mortality; and to assess effects of vaccination on growth rates during the backgrounding phase. We also assessed effects of vaccines against organisms other than BHV1 on growth rates and BRD risk within cattle that received Rhinogard® at feedlot entry and within cattle that did not receive Rhinogard®. We also describe serological statuses to BVDV and BHV1 during the backgrounding phase by vaccination group and feedlot, and assess associations between each of BVDV and BHV serostatuses during the backgrounding phase and ADG.

2 Project objectives

2.1 Project objectives

1. Evaluated the health and production effects of vaccination separately from those associated with the other managerial and behavioural aspects of local backgrounding systems
2. Evaluated the effects of this backgrounding system by comparison with contemporary cattle of the same market specification that have been paced directly in the feedlot
3. Utilised the above information to undertake a financial analysis of the various components of the backgrounding system
4. Provided recommendations, based on the study outcomes, on how best to manage the backgrounding of feedlot cattle to optimise animal health outcomes and net returns from the practice

3 Methodology

3.1 Materials and Methods

3.1.1 Overview

A controlled trial was conducted in six Australian feedlots using cattle that had been comingled in the local backgrounding facilities contiguous to each feedlot for at least 28 days. Effects of eight vaccination groups were compared; these consisted of various combinations of vaccines against *Mannheimia haemolytica*, bovine herpesvirus 1 (BHV1), and bovine viral diarrhoea virus (BVDV). Serology was performed at entry to backgrounding and subsequent entry to the feedlots, and ADG and health outcomes compared.

A pilot study was conducted from November 2009 to July 2010 in one feedlot, cattle were enrolled from April to October 2014 in a further four feedlots, and from November 2016 to February 2017 in the sixth feedlot. In the pilot study, four of the eight vaccination groups were allocated and no serology was performed but otherwise the same protocol was used in all six feedlots.

3.1.2 Animal ethics compliance

This observational study conformed to the Australian Code for the care and use of animals for scientific purposes (8th edition, 2013; section 3). The cattle were purchased and managed under commercial feedlot operations and were not subjected to any differences in management due to the study, with a systematic allocation of vaccines routinely used on the feedlots and their backgrounding facilities, and data collection procedures, the same as routinely used for all cattle in these feedlots.

3.1.3 Feedlots and backgrounding facilities

The feedlots were in western Queensland, south-east Queensland (two feedlots), central-west New South Wales, the Murray-Mallee region of South Australia, and the Goldfields-Esperance region of West Australia. Backgrounding facilities were close enough to their feedlots so that cattle could be walked to the feedlot yards for feedlot entry. The backgrounding enclosures had lower stocking densities than the associated feedlots, ranging from 30 m^2 to 1250 m^2 per animal, and with mob sizes of 200 to 400 animals, feed trough spacings of 12 to 30 cm/animal where a mixed ration was provided, and minimum water trough spacings of 3 linear m/100 animals. Lower stocking densities were used on lower rainfall sites and the cattle were rotated through backgrounding enclosures to preserve ground herbage cover, with the exception of one site where a lack of rainfall resulted in ground cover depletion during the term of the study.

3.1.4 Cattle selection, allocation to treatment groups, and treatments

Cattle enrolled in the study were from various sources and held in receival or holding pens. Between zero and eight days later, cattle were ear tagged, weighed, and allocated to a vaccination group with the appropriate vaccine given. Vaccination group, weight and other animal-level characteristics (breed, sex and dentition) were recorded, blood samples were taken, and they were then placed in

the backgrounding facility. At each feedlot, cattle entering the backgrounding facility from the study start date were enrolled until target numbers of cattle were reached.

Eight vaccination groups were defined, consisting of various combinations of vaccines against *Mannheimia haemolytica* (Bovilis MH® and Bovilis MH+IBR®, Coopers Animal Health, Macquarie Park, NSW; Bovishield®, Zoetis Australia, Silverwater, NSW), bovine herpesvirus 1 (BHV1; Bovilis MH+IBR®, Coopers Animal Health, Macquarie Park, NSW), and bovine viral diarrhoea virus (BVDV; Pestigard®, Zoetis Australia, Silverwater, NSW), with vaccines administered on entry into backgrounding and feedlot entry as outlined in Table 1. For each batch of cattle entering backgrounding, vaccination group was allocated systematically, with group allocated sequentially in the order that cattle entered the backgrounding facility. The allocation of vaccination group started with group 1 for each new batch of cattle entering the trial. Five feedlots allocated all eight vaccination groups and the pilot study feedlot supplied 12% (816/7,011) of trial animals allocated to four of the vaccination groups: none, Pestigard® + Bovilis MH®, Pestigard®, and Bovilis MH®.

Each animal's vaccination group was recorded on the feedlot management software (StockaID®, Possum Gully®, Tru-test®, or Gallagher®, depending on the feedlot) and recorded on the visual ear-tag.

Table 1. Vaccinations given on entry into backgrounding and feedlot entry

Vaccination group	Vaccines given at entry into backgrounding	Vaccines given at feedlot entry*
Control	None	None
Bovishield	Bovishield®	None
Bovishield/Pestigard	Bovishield®+ Pestigard®	Pestigard®
Pestigard/Bovilis MH+IBR	Pestigard®+ Bovilis MH+IBR®	Pestigard®+ Bovilis MH+IBR®
Pestigard/Bovilis MH	Pestigard®+ Bovilis MH®	Pestigard®+Bovilis MH®
Pestigard	Pestigard®	Pestigard®
Bovilis MH+IBR	Bovilis MH+IBR®	Bovilis MH+IBR®
Bovilis MH	Bovilis MH®	Bovilis MH®

*In four of the six feedlots, Rhinogard® was also administered to all cattle other than those that received Bovilis MH + IBR® with or without Pestigard®.

Where animals lost their visual ear-tag during backgrounding, the animal's individual electronic identification was used with the feedlot management software to identify the correct vaccination boosters required. The vaccines were stored and administered according to label directions.

3.1.5 Blood collection

Blood was collected from each animal using caudal vein or jugular vein venipuncture with an 18 g 1" needle into a 5 ml vacuum tube (Terumo Corporation, Interleuvenlaan 40, Leuven, Belgium), with the individual animal identification recorded on the label, both on entry to the backgrounding facility, and at feedlot entry. These samples were packaged on ice and sent by courier to Swan's Veterinary Laboratory, Esperance, West Australia, for measurement of antibody concentrations against BVDV and BHV1, and for testing for BVDV antigen concentration.

3.1.6 Other treatments

The study feedlots administered their usual treatments at the time of entry to the backgrounding facility, which included as a minimum:

- clostridial vaccine (against *Clostridium perfringens, Cl. septicum, Cl. chauvoei, Cl. novyi*, and *Cl. tetani*)
- anthelmintic drench
- visual ear-tag

In addition, Rhinogard® vaccine, a modified live intranasal vaccine against BHV1, was administered at feedlot entry to those cattle that had not received Bovilis MH+IBR® at all feedlots except at one Queensland site and at the central west New South Wales site. At one feedlot, prophylactic Micotil® was administered at feedlot entry to animals assessed by feedlot staff as being at increased risk of BRD.

3.1.7 Management of cattle after enrolment in the study

Cattle Management During Backgrounding: The backgrounding phase was from entry to backgrounding until feedlot entry date, the latter being defined as the first day on feed (the first day that cattle were fed a feedlot diet in a feedlot pen; 'DOF1'). For each batch of cattle entering the backgrounding facility, study cattle from all 8 treatment groups were run together after entry. Where backgrounding enclosures were large, some cattle not included in the study but also from a variety of sources, were run with the study cattle. Typically, between 150 and 400 cattle (study cattle combined, in some cases, with non-study cattle) were in each backgrounding enclosure. Cattle in the backgrounding facilities were either fed pasture (minimum available herbage mass of 1500 kg DM/ha) or a high roughage total mixed ration with grain included at no greater than 44% (as fed) or 43% (dry matter). Where cattle were provided with a total mixed ration, they were fed to maximise intake whilst consuming their daily allocation with only crumbs of ration remaining at feed delivery and feed always available at the peak foraging periods of sunrise and sunset.

Feedlot Entry and Management in the Feedlot: After at least 28 days in backgrounding, cattle were selected, further treatments administered in some feedlots (Rhinogard® in 4 feedlots to those cattle that had not been given Bovilis MH+IBR®), weighed, blood sampled, moved to feedlot pens, and fed a feedlot ration. Cattle were placed directly into the feedlot on exit from the backgrounding facility. In some instances, it took more than one day to fill a feedlot pen. The time period when a feedlot pen was being filled was called the processing period. A study cohort was defined as the group of study animals located in the same initial feedlot home pen at the end of the processing period. Some cohorts contained only study cattle while other cohorts had a mixture of study cattle and non-study cattle. Not all cohorts had study cattle from all vaccination groups as cattle from the same batch entering the backgrounding facility were not always allocated to the same cohort. The commercial feedlot diets ordinarily fed to the given class of cattle in the feedlot were fed to the study cattle for the duration of their time in the feedlot.

3.1.8 Measurements

The following measurements were recorded for study animals during the feedlot phase –

- Treatments for bovine respiratory disease
- Treatments for all other diseases
- Mortalities
- Body weights (BW)

Days on feed (DOF) was defined as the number of days from the first day on feed until feedlot exit. Body weights were recorded at backgrounding entry ('entry to backgrounding weight'), second blood collection date (which was virtually always on or within 1 day of the first day on feed, so referred to as 'DOF1 weight'), and feedlot exit date or the final draft date (which was within a few days before feedlot exit; 'exit weight'). Average daily gains during the backgrounding phase were calculated as the average BW change per day from entry to backgrounding to DOF1. ADG during the feedlot phase was calculated for the majority of study animals as the average BW change per day on feed based on the BW change from DOF1 to exit. For 856 animals from two feedlots, rather than exit dates and exit weights, animal-level ADG during the feedlot phase was provided along with DOF and DOF1 weights. Exit dates and exit weights were back-calculated from these data. For animals that died during the study, and for other animals without BW data, ADG was not calculated.

Time at risk of BRD (TAR) was defined as the number of days from the first day on feed (DOF1) until the earliest of: death, first hospital treatment, or feedlot exit for slaughter. Deaths and first hospital treatments prior to exit were either classified as BRD (if they met the case definition below) or as competing risks.

Bovine Respiratory Disease Case Definition: Diagnosis of BRD at first pull was based on the pull reason or ailment provided by the feedlots in animal-level hospital records. Feedlot staff were asked to use the following case definition of BRD when identifying sick animals in the feedlot and allocating pull reasons or ailments: an animal with no clinical signs referable to systems other than the respiratory system, and two or more of the clinical signs of dyspnoea, nasal and/or oral discharge, lethargy and inappetence. Other diagnoses were based on the current feedlot diagnostic protocol, developed by the consulting feedlot veterinarian. For feedlots reporting both pull reasons and ailments, the animal was classified as having BRD only if both were consistent with BRD. Pull reasons or ailments classified as BRD included “BRD”, “BRD/Respiratory”, “Respiratory Problem”, “Tracheitis” and “Resp/Pneumonia”. The distribution of pull reason, ailment and death reason for animals classified as BRD cases is shown in Table 2.

Table 2. Cross-tabulation of pull reason and cause of death by ailment used to define treatments and deaths due to bovine respiratory disease

	Ailment						
	Not recorded	Respiratory	BRD/ Respiratory	Observe	Respiratory Problem	Tracheitis	Total
Pull reason							
BRD	2	0	0	0	0	0	2
BRD/ Respiratory	0	0	22	0	0	0	22
Buller	0	0	0	0	3	0	3

Heavy Breather	0	0	0	0	1	0	1
RESPIRATORY	0	15	0	0	0	0	15
Resp/ Pneumonia	6	0	0	0	0	0	6
Respiratory Problem	0	0	0	2	155	0	157
Tracheitis	0	0	0	0	0	13	13
Unknown	0	0	0	0	1	0	1
Not Recorded		1	20	0	0	0	21
Death Reason							
IBR	8						
Lung Abscess	2						
Pneumonia	9						
Total	27	16	42	2	160	13	260

Serology: Blood samples collected on entry into and exit from the backgrounding facility were tested for antibodies to two viruses commonly associated with BRD: bovine herpesvirus type 1 (BHV1), responsible for infectious bovine rhinotracheitis (IBR); and bovine viral diarrhoea virus (BVDV). Antibody concentrations in the second blood samples were assessed only in animals that were seronegative or weak positive at entry into the backgrounding facility. Serum concentrations for BVDV antigen were assessed in animals that were seronegative or weak positive to BVDV on backgrounding facility entry. Animals that were seronegative or weak positive at backgrounding facility entry and exit and antigen positive or weak positive at entry were again tested for BVDV antigen at backgrounding facility exit to identify those that were persistently infected with BVDV (PI).

The cut-points used to define serological status were:

- BHV1 antibody (blocking %): seronegative: <45; weak positive: 45-<55; seropositive ≥ 55
- BVDV antibody (S/P ratio): seronegative: <0.2; weak positive: 0.2-<0.3; seropositive ≥ 0.3
- BVDV antigen (sn): antigen negative ≤ 0.2; weak positive: >0.2 to <0.3; positive ≥ 0.3

Composite serological variables were derived separately for each of BVDV and BHV1 based on each animal's two serological results for the respective virus. Animals were classified as remaining seronegative if they were seronegative or weak positive at entry to backgrounding and were also seronegative or weak positive at second sampling. Animals that changed from negative or weak positive to positive were classified as seroincreased. Those seropositive at backgrounding facility entry were classified into a separate group, as were those that were seronegative at backgrounding facility entry and whose status at second sampling was unknown (as no sample was collected). Serostatuses were not allocated for animals if their sample(s) could not be verified due to animal identification number duplication. Serological status classification is shown in Table 3.

Table 3. Classification of composite serostatuses for each of bovine herpesvirus 1 and bovine viral diarrhoea virus

<table>
<tr><td>Result from first sample</td><td colspan="4">Result from second sample</td></tr>
<tr><td></td><td>Seronegative</td><td>Weak positive</td><td>Seropositive</td><td>Not tested</td></tr>
<tr><td>Seronegative</td><td colspan="2" rowspan="2">Remaining seronegative</td><td rowspan="2">Seroincrease</td><td rowspan="2">Composite serostatus not known</td></tr>
<tr><td>Weak positive</td></tr>
<tr><td>Seropositive</td><td colspan="3">Initially seropositive</td></tr>
</table>

Animals that were seronegative but antigen positive at both entry to backgrounding and second sampling were classified as persistently infected (PI) with BVDV. Animals with only a first sample collected that were seronegative but had very high antigen results (>0.65) were also classified as persistently infected. Cohorts in which identified BVDV-PI animals were placed were classified as exposed to a BVDV-PI animal.

3.1.1 Statistical methods

The two *a priori* primary outcomes of interest were ADG and risk of BRD during the feedlot phase. Secondary outcomes of interest were ADG during the backgrounding phase, and mortality (all-cause mortality and mortality attributed to BRD) during the feedlot phase. Due to ADG results, we also assessed ADG from entry to backgrounding to the end of the feedlot phase. The exposure variable was vaccination group (Group). Group was defined based on the group allocated to the animal at backgrounding entry and analysed as a categorical variable comprising eight categories as described in Table 1.

Effects of Group were assessed adjusted only for feedlot (fixed effect) and cohort (random effect). In supporting analyses of the two primary outcomes, effect estimates were adjusted for various potential confounders identified *a priori* based on published literature:

- breed
- BW (at feedlot entry)
- sex
- dentition (proxy for age)
- season of feedlot entry
- duration of backgrounding

Potential confounders measured in animals that had serological testing were:

- serological status to BVDV and BHV1 (animal level)
- the presence of at least one animal persistently infected with BVDV (BVDV-PI) in the cohort (cohort level)

Interactions specified as being of *a priori* interest were:

- Group*individual animal's serological status
- Group*Rhinogard® at feedlot entry

The potential confounders and interactions were the same for both primary outcomes. Breed was classified into four categories in the main analyses: i) British breeds (excluding Hereford), mainly comprised of Angus or Angus cross but also Murray Grey and Shorthorn; ii) Hereford or Hereford crosses; iii) European breeds and crosses; and iv) *Bos indicus* breeds or crosses. Breed was not recorded for one feedlot. All animals from this feedlot were omitted from models requiring breed.

Analyses were performed using the Stata statistical software package (versions 13 and 15). In primary analyses, the association between Group and ADG during the feedlot phase was assessed using mixed effects linear regression modelling fitted with Stata's -mixed- command. Feedlot was fitted as a fixed effect and cohort was fitted as a random effect. Two approaches to modelling were employed. Firstly, the derived ADG variable was used as a continuous outcome variable. In this analysis, only animals that had values for ADG at both feedlot entry and feedlot exit were included. Animals that died during the time on feed were excluded.

In further modelling, animal BW at known times was modelled as a function of time on feed, Group and the interaction between Group and time using three-level mixed effects linear regression modelling, with a random intercept for animal (level 2) and a random intercept and slope for cohort (highest level). Time was set to zero at each animal's weighing at or close to DOF1 and for subsequent weighings, at the number of days from that date to the subsequent weighing date. To ensure that ADG estimates for vaccination groups incorporated the effects of animals that died during the time on feed, their weights were set to zero on the date of their death. A scatter plot of weight by time revealed a non-linear relationship, especially for time on feed values above 160. Different approaches to modelling this non-linear function were explored. Firstly, the relationship during the first 160 days was analysed using a linear mixed model. This facilitated ease of interpretation of the interaction terms and assessment of the overall effects of vaccine group over this period. However, animals that remained on feed beyond 160 days were excluded, and if extended time on feed is related to vaccine group, estimates might be biased. Secondly, animals were stratified by target market and separate models were fitted for each. Target market was derived as a categorical cohort-level variable based on the mean time on feed for animals in the cohort, using cut-points of 80 and 155 days. Thirdly, the relationship was modelled using a fractional polynomial function of time. The Stata -fp- command was used within a simple linear regression of time on weight to determine the best fitting polynomial function. The best fitting time function was (time + time^2 + ln(time)*time^2). This function was then incorporated into the mixed effects model. The effects of Group and the Group*time interaction term were tested using global Wald tests. The derivatives with respect to time for each group, obtained post-estimation (using the Stata -margins- command with the -dy/dx()- option), provided estimates of the mean ADG for each group with the associated 95% confidence interval.

Model fit was assessed by checking the distributions of residuals and examining scatter plots of fitted versus predicted values for BW. As expected, the inclusion of zero BW values resulted in marked deviations from normality for the residuals. However, the number of dead animals was small compared to the total population and after excluding them from analyses, residuals were approximately normally distributed.

In secondary analyses, the effects of potential confounders listed above were assessed in models based on the derived ADG variable. Animal-level BW on the first DOF, breed, sex, and duration from entry to backgrounding to DOF1 were included in models. Further separate models were fitted for

cattle that did and did not receive Rhinogard® at feedlot entry. Effects of vaccines against organisms other than BHV1 relative to controls were also assessed with interaction between these vaccines and Rhinogard® fitted.

Effects of Group on ADG during the backgrounding phase, and from entry to backgrounding to feedlot exit, were assessed using the first approach described above. The derived ADG variable was fitted as a continuous outcome variable with Group and feedlot fitted as fixed effects and cohort fitted as a random effect.

The association between serological increase for BVDV and that for BHV1 during the backgrounding phase was assessed using control animals that were initially seronegative or weak positive for both viruses by fitting a logistic regression model with feedlot as a fixed effect, fitted with Stata's -xtlogit- command with cohort as a panel variable.

Associations between BVDV and BHV1 serostatuses during the backgrounding phase (i.e. remained seronegative, seroincreased or initially seropositive) and ADG during three periods (backgrounding phase, feedlot phase, and both phases combined) were assessed using mixed effects linear regression modelling fitted with Stata's -mixed- command in Stata. BVDV and BHV1 serostatuses were fitted in the same models, and interaction between the two was also assessed. Feedlot, vaccination group and the animal's BW at the commencement of the period were also fitted as fixed effects and cohort was fitted as a random effect. Likelihood ratio test *P*-values were used to assess the overall effects of BVDV and BHV1 serostatuses adjusted for each other without interaction terms fitted, and for the joint effect of all four terms for the interaction between BVDV and BHV1 serostatuses. Separate analyses were performed using only animals not vaccinated against BVDV or IBR, and animals not vaccinated against IBR. Animals receiving Rhinogard® at DOF1 were included only when analysing ADG during the backgrounding phase. For ADG during the feedlot phase, and for ADG during the backgrounding and feedlot phases combined, associations between a single composite variable that combined BVDV and BHV1 serostatuses and ADG were assessed using the same approach as described above. Likelihood ratio test *P*-values were used for these analyses.

The association between Group and BRD was analysed using competing risk regression fitted with Stata's -stcrreg- command, with the correlation between animals in the same cohort specified through the cluster variance option, and with feedlot fitted as a fixed effect. The “analysis time” variable was time until BRD or a competing event (defined above) occurred, calculated as date of first pull minus date of DOF1. Animals that did not experience BRD or a competing event were right-censored at date of leaving the study cohort prematurely (ie not feedlot exit) or feedlot exit. This method estimates subhazard ratios which provide comparisons of the cumulative incidence function between categories.

In secondary analyses, potential confounders listed above were tested in the model. To assess effects of vaccination by initial serological status, serological variables were incorporated into modelling. Vaccine components were described as three binary variables indicating vaccination against each of BHV1, BVDV and *Mannheimia haemolytica*. Terms for interaction between these and initial serostatus (ie at entry to backgrounding) were fitted. Predictive margins were obtained from this model for comparison of different combinations. In subset analyses, the effect of vaccine group was tested in animals that were seronegative to both BHV1 and BVDV on entry to backgrounding. Further separate models were fitted for cattle that did or did not receive Rhinogard® at feedlot entry. The *P*-value for

interaction between Group and Rhinogard® was calculated as a Wald *P*-value after excluding animals vaccinated with an IBR component.

The associations between each of all-cause mortality and BRD-related mortality (both during the feedlot phase) and Group were examined by fitting logistic regression models with feedlot as a fixed effect, fitted with Stata's -xtlogit- command with cohort as a panel variable. All cattle were included in this analysis regardless of time from DOF1 to exit from the cohort.

4 Results

4.1 Results

Descriptive Statistics: Of the 7302 animals allocated a vaccination group at entry to backgrounding, 7011 (96%) were retained in the study. Reasons for loss to follow-up or exclusion of the 291 animals were less than 28 days in backgrounding (n=275), hospital record on or before first DOF (n=13), death before feedlot entry (n=1) and lost to follow-up before feedlot entry (n=2). The distributions of animals by vaccination group within feedlot, animal characteristics and season are shown in Table 4. The majority of animals (70%) were from a single feedlot. Study cattle were in 47 cohorts (one from each of two feedlots, and two, five, 16, and 22 cohorts from four feedlots). The mean number of cattle per cohort was 149 (SD 130.2, range 1 to 511). Of the 31 cohorts from the five feedlots that had all eight vaccination groups, for 26 cohorts, numbers of cattle in each vaccination group were within three of that expected under perfectly balanced allocation. The remaining five cohorts had small excesses or deficits (three to five animals) for one (four cohorts) or two (one cohort) vaccination groups. Of the 16 cohorts in the feedlot that had four vaccination groups, six cohorts had excesses or deficits of three to 27 from that expected for one (one cohort) or more than one (five cohorts) vaccination groups. Most animals (71%) were started on feed in summer, with some feedlots only enrolling animals in a single season. Dentition was not recorded in one feedlot (comprising 816 animals). Most of the remaining animals had no permanent incisors and distributions by dentition did not differ markedly by vaccination group.

Table 4: Distribution of variables of interest by vaccination group (n (% within vaccination group))

Variable	Category/ measure	Control	Bovishield	Bovishield/ Pestigard	Pestigard/ BovilisMH+IBR	Pestigard/ BovilisMH	Pestigard	BovilisMH+IBR	BovilisMH	Total
Feedlot Location										
	W Qld	95 (9.9)	91 (11.7)	98 (12.4)	96 (12.3)	94 (9.3)	95 (10.0)	92 (12.0)	91 (9.2)	752 (10.7)(2)*
	CW NSW	60 (6.3)	54 (6.9)	56 (7.1)	53 (6.8)	54 (5.4)	54 (5.7)	55 (7.2)	51 (5.2)	437 (6.2)(5)
	Murray-Mallee	11 (1.2)	11 (1.4)	11 (1.4)	10 (1.3)	10 (1.0)	11 (1.2)	10 (1.3)	10 (1.0)	84 (1.2)(1)
	Esperance	6 (0.6)	6 (0.8)	6 (0.8)	6 (0.8)	5 (0.5)	4 (0.4)	5 (0.7)	3 (0.3)	41 (0.6)(1)
	SE Qld	171 (17.9)	0 (0.0)	0 (0.0)	0 (0.0)	236 (23.4)	179 (18.9)	0 (0.0)	230 (23.3)	816 (11.6)(16)
	SE Qld	612 (64.1)	618 (79.2)	618 (78.3)	613 (78.8)	609 (60.4)	604 (63.8)	606 (78.9)	601 (61.0)	4,881 (69.6)(22)
Breed	Angus/ Red Ang	459 (53.4)	398 (51.0)	403 (51.1)	399 (51.3)	468 (46.4)	451 (47.6)	388 (50.5)	453 (45.9)	3419 (48.8)
	British/Angus X	172 (20.0)	113 (14.5)	120 (15.2)	113 (14.5)	202 (20.0)	161 (17.0)	113 (14.7)	188 (19.1)	1182 (16.9)
	Hereford / X	110 (12.8)	93 (11.9)	78 (9.9)	85 (10.9)	126 (12.5)	102 (10.8)	90 (11.7)	101 (10.2)	785 (11.2)
	Murray Grey / X	32 (3.7)	13 (1.7)	21 (2.7)	20 (2.6)	24 (2.4)	38 (4.0)	18 (2.3)	38 (3.9)	204 (2.9)
	European	45 (5.2)	26 (3.3)	36 (4.6)	33 (4.2)	50 (5.0)	43 (4.5)	32 (4.2)	47 (4.8)	312 (4.5)
	Bos Indicus / X	26 (3.0)	19 (2.4)	13 (1.6)	11 (1.4)	30 (3.0)	37 (3.9)	13 (1.7)	51 (5.2)	200 (2.9)
	Shorthorn / X	15 (1.75)	26 (3.3)	20 (2.5)	19 (2.4)	14 (1.4)	18 (1.9)	21 (2.7)	16 (1.6)	149 (2.1)
	Not recorded	96	92	98)	98	94	97)	93)	92	760
Sex	Heifer	183 (19.2)	86 (11.0)	96 (12.2)	93 (12.0)	213 (21.1)	184 (19.4)	91 (11.8)	206 (20.9)	1152 (16.4)
	Steer	772 (80.8)	694 (89.0)	693 (87.8)	685 (88.0)	795 (78.9)	763 (80.6)	677 (88.2)	780 (79.1)	5859 (83.6)
Dentition – number of permanent incisors	0	673 (85.8)	663 (85.0)	682 (86.4)	667 (85.8)	665 (86.1)	664 (86.5)	662 (86.2)	660 (87.3)	5336 (86.2)
	2	108 (13.8)	109 (14.0)	98 (12.4)	101 (13.0)	100 (13.0)	96 (12.5)	99 (12.9)	91 (12.0)	802 (13)
	4-6	3 (0.4)	8 (1.0)	9 (1.1)	9 (1.2)	7 (0.9)	8 (1.0)	7 (0.9)	5 (0.7)	56 (0.9)
	Not recorded	171	0	0	1	236	179	0	230	817
Season of DOF1	Spring	74 (7.7)	63 (8.1)	66 (8.4)	65 (8.4)	73 (7.2)	74 (7.8)	63 (8.2)	71 (7.2)	549 (7.8)
	Summer	656 (68.7)	597 (76.5)	596 (75.5)	591 (76.0)	650 (64.5)	644 (68.0)	589 (76.7)	637 (64.6)	4960 (70.8)
	Autumn	156 (16.3)	92 (11.8)	95 (12.0)	91 (11.7)	225 (22.3)	176 (18.6)	87 (11.3)	227 (23.0)	1149 (16.4)
	Winter	69 (7.2)	28 (3.6)	32 (4.1)	31 (4.0)	60 (6.0)	53 (5.6)	29 (3.8)	51 (5.2)	353 (5)
Total (% of animals in each vaccination group)		955 (13.6)	780 (11.1)	789 (11.3)	778 (11.1)	1008 (14.4)	947 (13.5)	768 (11.0)	986 (14.1)	7,011

*Number of cohorts

The distribution of DOF for animals included in the study (excluding deaths) is shown in Figure 1. Summary statistics for continuous variables by vaccination group are shown in Table 5. Median days between entry to backgrounding and DOF1 was 35 (IQR: 33-48; minimum 29) days. Median days on feed was 149 (interquartile range 93-162). The range of DOF for two feedlots was low (median 28 (IQR: 25-28) days and median 42 (IQR: 20-69) days) compared to the remaining four feedlots with a median of 155 (IQR: 135-163) days. Mean BW at backgrounding facility entry and on DOF1 varied significantly across groups, but mean BW at exit did not. Of 7011 animals included in the study, ADG was determined from the BW's recorded at second blood sampling and feedlot exit (or proximate draft) for 5626 animals (80%), and was supplied by the feedlot for 856 animals (12%). A total of 144 animals died and average daily gain was missing for 385 animals (5%), including 333 animals from a single feedlot in which lots were combined and animal-level weights were not supplied.

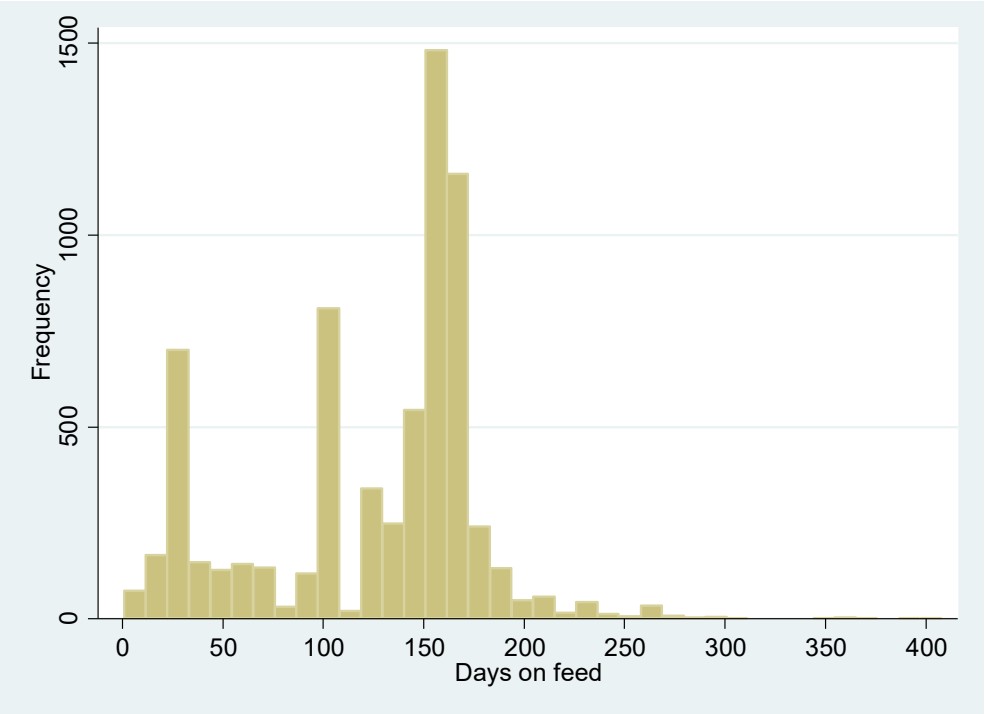


Figure 1. Frequency histogram of days on feed for animals included in the study (excluding deaths).

Table 5: Distribution of production outcomes and time at risk of BRD by vaccination group

Variable	Measure	Control	Bovishield	Bovishield + Pestigard	Pestigard + Bovilis + IBR	Pestigard + Bovilis	Pestigard	Bovilis + IBR	Bovilis	Total
Entry to backgrounding to DOF1 (days)	Median (IQR)	36 (34, 52)	34 (33, 38)	34 (33, 39)	34 (33, 39)	36 (34, 56)	36 (33, 52)	34 (33, 39)	36 (34, 56)	35 (33, 48)
Days on feed	Median (IQR)	135 (63, 161)	152 (105, 163)	152 (104, 163)	151 (105, 163)	126 (57, 161)	142 (70, 161)	152 (105, 163)	135 (54, 161)	149 (93, 162)
Time at risk of BRD	Median (IQR)	125 (41, 158)	149 (102, 161)	150 (102, 161)	149 (102, 161)	105 (35, 156)	125 (47, 158)	149 (102, 161)	107 (34, 156)	135 (54, 161)
Weight at entry to backgrounding	Mean (SD)	389 (76)	417 (53)	414 (52)	415 (54)	381 (80)	386 (76)	415 (53)	382 (78)	398 (70)
Weight at feedlot entry	Mean (SD)	456 (60)	472 (50)	466 (51)	466 (52)	445 (60)	449 (60)	466 (51)	449 (60)	458 (57)
Weight at exit	Mean (SD)	688 (109)	694 (105)	691 (108)	692 (109)	689 (109)	691 (107)	692 (105)	690 (108)	691 (107)
Average daily gain	Mean (SD)	1.7 (0.5)	1.7 (0.4)	1.8 (0.4)	1.7 (0.5)	1.7 (0.6)	1.8 (0.6)	1.7 (0.4)	1.8 (0.6)	1.7 (0.5)

Distributions of serological variables by vaccination group are shown in Table 6. The feedlot that only contributed animals to four vaccination groups, totalling 816 animals (12%) in the study did not have serological testing performed. Of the remaining 6195 animals, 5.8% (361) had missing serological results for BHV1 and 6% (373) had missing results for BVDV at backgrounding facility entry. Serological statuses at backgrounding facility entry were similar across vaccination groups. Overall, 13.5% (789/5834) of the animals tested were seropositive to BHV1 at backgrounding facility entry. Of the animals whose composite serostatus was known (ie their second blood sample result was available), 34.8% (1834) of all animals, and 36.4% of initially seronegative or weak seropositive animals, showed a serological increase. At backgrounding facility entry, 66% (3866/5822) of animals tested were seropositive to BVDV. Of the animals whose composite serostatus was known, 10.6% (588) of all animals, and 30.1% of initially seronegative or weak seropositive animals, showed a serological increase. A total of 0.28% (16/5668) of animals were classified as persistently infected with BVDV (BVDV-PI). These animals were placed on feed in the same cohorts as 26.5% of study animals. For cattle that did not receive Pestigard and which were seronegative or weak positive to BVDV at background entry: of the 349 that had a PI with them in their cohort, 79% (218) seroincreased during backgrounding; and of the 468 that had no PI with them in their cohort, 21% (59) seroincreased during backgrounding.

Table 6. Distributions of administration of Rhinogard® at DOF1, serological statuses, and BVDV in cohort by vaccination group (n (% within vaccination group))

Variable	Category/ measure	Vaccination group								Total	*P*-value[#]
		Control	Bovishield	Bovishield/ Pestigard	Pestigard/ Bovilis MH + IBR	Pestigard/+ Bovilis MH	Pestigard	Bovilis MH + IBR	Bovilis MH		
Rhinogard® DOF1	No	672 (70.4)	672 (86.2)	674 (85.4)	778 (100)	663 (65.8)	658 (69.5)	768 (100)	652 (66.1)	5318 (75.9)	
	Yes	283 (29.6)	108 (13.8)	115 (14.6)	0	345 (34.2)	289 (30.5)	0	334 (33.9)	1693 (24.2)	
BHV1 (entry to backgrounding)*	Negative	646 (87.2)	645 (87.4)	624 (84.8)	640 (87.6)	618 (84.9)	601 (83.4)	614 (84.5)	612 (86.0)	5000 (85.7)	
	Weak positive	4 (0.5)	5 (0.7)	5 (0.7)	9 (1.2)	3 (0.4)	4 (0.6)	6 (0.8)	9 (1.3)	45 (0.8)	
	Positive	91 (12.3)	88 (11.9)	107 (14.5)	82 (11.2)	107 (14.7)	116 (16.1)	107 (14.7)	91 (12.8)	789 (13.5)	
BHV1 (DOF1)*	Negative	372 (65.1)	389 (67.0)	386 (67.7)	149 (25.5)	356 (64.3)	360 (67.5)	115 (20.7)	376 (68.7)	2503 (55.7)	
	Weak positive	15 (2.6)	13 (2.2)	20 (3.5)	35 (6.0)	15 (2.7)	15 (2.8)	17 (3.1)	18 (3.3)	148 (3.3)	
	Positive	184 (32.2)	179 (30.8)	164 (19.5)	400 (68.5)	183 (33.0)	158 (29.6)	423 (76.2)	153 (28.0)	1844 (41)	
BHV1 composite*	Seronegative	387 (58.5)	402 (60.3)	406 (48.3)	184 (27.8)	371 (56.2)	375 (57.9)	132 (20.0)	394 (61.8)	2651 (50.3)	
	Seroincreased	183 (27.7)	177 (26.5)	163 (19.4)	397 (59.9)	182 (27.6)	157 (24.2)	422 (63.8)	153 (24.0)	1834 (34.8)	
	Seropositive	91 (13.7)	88 (13.1)	107 (12.7)	82 (12.4)	107 (16.2)	116 (17.9)	107 (16.2)	91 (14.3)	789 (15.0)	
	Seronegative, no second sample	80 (10.8)	71 (9.6)	60 (8.2)	68 (9.3)	68 (9.3)	73 (10.1)	66 (9.1)	74 (10.4)	560 (9.6)	
BHV1 seroincrease	Seroincreased of initially seronegative or weak positive	183 (32.1)	177 (30.6)	163 (28.7)	397 (68.3)	182 (32.9)	157 (29.5)	422 (76.2)	153 (28.0)	1834 (40.9)	<0.001
BVDV (entry to backgrounding)*	Negative	236 (32.0)	235 (32.0)	254 (34.6)	232 (31.7)	245 (33.7)	234 (32.5)	236 (32.5)	230 (32.3)	1902 (32.7)	
	Weak positive	8 (1.1)	6 (0.8)	8 (1.1)	8 (1.1)	9 (1.2)	5 (0.7)	4 (0.6)	6 (0.8)	54 (0.9)	
	Positive	494 (66.9)	494 (67.2)	473 (64.4)	491 (67.2)	472 (65.0)	480 (66.8)	487 (67.0)	475 (66.8)	3866 (66.4)	
BVDV (DOF1)*	Negative	136 (61.0)	128 (56.6)	143 (56.5)	124 (53.7)	141 (60.0)	119 (55.6)	140 (61.4)	119 (53.4)	1050 (57.3)	
	Weak positive	5 (2.2)	12 (5.3)	8 (3.2)	3 (1.3)	7 (3.0)	9 (4.2)	7 (3.1)	4 (1.8)	55 (3)	
	Positive	82 (36.8)	86 (38.1)	102 (40.3)	104 (45.0)	87 (37.0)	86 (40.2)	81 (35.5)	100 (44.8)	728 (39.7)	
BVDV composite*	Seronegative	138 (19.8)	138 (20.0)	149 (21.1)	126 (18.0)	145 (21.1)	128 (17.8)	143 (20.6)	121 (17.9)	1088 (19.7)	
	Seroincreased	64 (9.2)	66 (9.5)	85 (12.0)	85 (12.1)	74 (10.8)	67 (9.3)	66 (9.5)	81 (12.0)	588 (10.6)	
	Seropositive	494 (71.0)	493 (70.7)	472 (66.9)	490 (69.9)	469 (68.2)	480 (66.8)	484 (69.8)	474 (70.1)	3856 (69.7)	
	Seronegative, no second sample	42	37 (5.0)	28 (3.8)	29 (4.0)	35 (4.8)	44 (6.1)	31 (4.3)	34 (4.8)	280 (4.8)	
BVDV seroincrease	Seroincreased of initially seronegative or weak positive	64 (31.7)	66 (32.4)	85 (36.3)	85 (40.3)	74 (33.8)	67 (34.4)	66 (31.6)	81 (40.1)	588 (35.1)	0.086
BVDV-PI in cohort	No	444 (56.6)	446 (57.2)	444 (56.3)	432 (55.5)	437 (56.6)	431 (56.1)	431 (56.1)	426 (56.3)	3,491 (56.3)	
	Yes	340 (43.4)	334 (42.8)	345 (43.7)	346 (44.5)	335 (43.4)	337 (43.9)	337 (43.9)	330 (43.7)	2,704 (43.7)	
Total (% in each group)		955 (13.6)	780 (11.1)	789 (11.3)	778 (11.1)	1008 (14.4)	947 (13.5)	768 (11.0)	986 (14.1)	7,011	

*Totals differ due to missing values for serological variables; [#] P-values derived from global Wald test following fitting of mixed effects logistic models with a random effect for cohort and fixed effect for feedlot

Distributions of serological variables by feedlot are shown in Table 7. Seroprevalences to BHV1 at entry to backgrounding were low in all feedlots but there were marked differences in percentages of initially seronegative or weak seropositive animals that seroincreased by DOF1. In the three feedlots with substantial numbers of initially seronegative or weak seropositive animals, 20%, 30% and 70% seroincreased. Seroprevalences to BVDV at entry to backgrounding were high in the three feedlots with substantial numbers of animals (54% to 87%) and percentages of initially seronegative or weak seropositive animals that seroincreased by DOF1 in these feedlots ranged from 15 to 37%. Seroprevalence to BVDV at entry to backgrounding was higher in the more northern feedlots and highest in the western Queensland site.

Table 7. Distributions of serological variables by feedlot (n (% within feedlot))

		Feedlot					
Test	Result	W. Qld	CW NSW	SA	WA	SE Qld	Total
BHV1 (entry to backgrounding)*	Negative	677 (90.6)	366 (86.5)	50 (61.7)	40 (97.6)	3867 (85.1)	5000 (85.7)
	Weak positive	14 (1.9)	3 (0.7)	0 (0.0)	0 (0.0)	28 (0.6)	45 (0.8)
	Positive	56 (7.5)	54 (12.8)	31 (38.3)	1 (2.4)	647 (14.2)	789 (13.5)
BHV1 (DOF1)* #	Negative	363 (74.5)	41 (26.8)	32 (100.0)	23 (79.3)	1780 (67.0)	2239 (66.7)
	Weak positive	27 (5.5)	5 (3.3)	0 (0.0)	1 (3.4)	63 (2.4)	96 (2.9)
	Positive	97 (19.9)	107 (69.9)	0 (0.0)	5 (17.2)	812 (30.6)	1021 (30.4)
BHV1 composite*#	Seronegative	390 (73.4)	46 (24.0)	32 (58.2)	24 (80.0)	1843 (58.7)	2335 (59.1)
	Seroincreased	97 (18.3)	107 (55.7)	0 (0.0)	5 (16.7)	806 (25.7)	1015 (25.7)
	Seropositive at induction	44 (8.3)	39 (20.3)	23 (41.8)	1 (3.3)	493 (15.7)	600 (15.2)
	Seronegative, no second sample	28	126	6	0	266	426
	Seroincreased of initially seroneg or weak seropos	97 (19.9)	107 (69.9)	0 (0.0)	5 (17.2)	806 (30.4)	1015 (30.3)
BVDV (entry to backgrounding)*	Negative	91 (12.1)	193 (45.6)	30 (36.6)	35 (85.4)	1553 (34.3)	1902 (32.7)
	Weak positive	3 (0.4)	3 (0.7)	0 (0.0)	0 (0.0)	48 (1.1)	54 (0.9)
	Positive	655 (87.4)	227 (53.7)	52 (63.4)	6 (14.6)	2926 (64.6)	3866 (66.4)
BVDV (DOF1)*^	Negative	32 (62.7)	33 (57.9)	15 (83.3)	17 (85.0)	426 (56.5)	523 (58.1)
	Weak positive	1 (2.0)	1 (1.8)	2 (11.1)	0 (0.0)	24 (3.2)	28 (3.1)
	Positive	18 (35.3)	23 (40.4)	1 (5.6)	3 (15.0)	304 (40.3)	349 (38.8)
BVDV composite*^	Seronegative	33 (9.0)	34 (20.9)	17 (41.5)	17 (85.0)	439 (20.2)	| (19.6)
	Seroincreased	6 (1.6)	16 (9.8)	1 (2.4)	2 (10.0)	252 (11.6)	| (10.0)
	Seropositive at induction	326 (89.3)	113 (69.3)	23 (56.1)	1 (5.0)	1482 (68.2)	| (70.4)
	Seronegative, no second sample	3	51	0	0	90	144
	Seroincreased of initially seroneg or weak seropos	6 (15.4)	16 (32.0)	1 (5.6)	2 (10.5)	252 (36.5)	277 (33.9)
BVDV-PI in cohort	No	743 (99.9)	315 (100.0)	81 (100.0)	56 (100.0)	4551 (99.7)	5787 (99.7)
	Yes	1 (0.13)	0 (0.00)	0 (0.00)	0 (0.00)	15 (0.33)	16 (0.28)

*Totals differ due to missing values for serological variables; no serology was performed for one feedlot
#Results only for animals that did not receive BHV1 vaccines (ie Pestigard + Bovilis + IBR, or Bovilis + IBR) before DOF1

^Results only for animals that did not receive BVDV vaccine (ie Pestigard)

Only 189 study cattle were seronegative or weak positive to both BVDV and BHV1 at backgrounding entry. After excluding 7 animals not retested for BVDV at DOF1 and all animals from 2 feedlots where none seroincreased to BHV1 (n=9), seroincrease to BVDV was not associated with an increase in the proportion of animals with a seroincrease to BHV1 (Table 8; 33% versus 29%; odds ratio 2.4; 95% CI 0.7 to 8.1; *P* = 0.146).

Table 8. The association between seroincrease to BVDV during the backgrounding phase and seroincrease to BHV1 during that phase

BVDV status	BHV1 status		
	Number	Number seroincreased	% seroincreased
Initially and remained seronegative	112	33	29%
Seroincreased	61	20	33%

Average Daily Gain: Estimated effects of vaccination group on ADG during the backgrounding phase are shown in Table 9. Average daily gain was associated with vaccination group (*P* < 0.001). For each vaccination group relative to the control group, the estimated difference was negative (ie lower ADG), with significantly lower ADG's for each vaccination group compared to controls other than for Bovishield. Estimated reductions due to vaccination varied by group from 0.06 to 0.17 kg/animal/d.

Table 9. Effect estimates of vaccination group on average daily gain (kg/animal/d) during the backgrounding phase derived from mixed effects linear regression models (n=5982)

Vaccination group	Coefficient*	95% CI		*P*-value
Control	Reference category			
Bovishield	-0.062	-0.146	0.023	0.151
Bovishield/Pestigard	-0.155	-0.239	-0.070	<0.001
Pestigard/Bovilis MH+IBR	-0.173	-0.258	-0.089	<0.001
Pestigard/Bovilis MH	-0.149	-0.229	-0.070	<0.001
Pestigard	-0.128	-0.209	-0.048	0.002
Bovilis MH+IBR	-0.165	-0.250	-0.080	<0.001
Bovilis MH	-0.125	-0.204	-0.045	0.002
intercept	2.158	1.504	2.812	

*Coefficients for exposure variables estimate the difference in mean average daily gain between the respective category and the reference category; the coefficient for the intercept estimates the average daily gain in feedlot 1 for control animals; the global *P*-value for vaccination group was <0.001

The results of mixed effects linear regression modelling of vaccine group on ADG during the feedlot phase are shown in Table 10. All models were fitted with feedlot as a fixed effect and cohort as a random effect. Model 1 is the base model. Model 2 (Appendix 1) is additionally adjusted for weight on DOF1, Model 3 is adjusted for weight on DOF1, breed, sex, and days from entry to backgrounding to DOF1. Estimates in Model 4 (Appendix 1) are adjusted for dentition and BVDV and BHV1 serological status on feedlot entry in addition to all variables in Model 3 (Appendix 1). Results were generally

consistent between models. The global Wald *P*-values for Group did not reach statistical significance at the 0.05 level in any model. Point estimates were consistent with small increases in ADG for each vaccination combination relative to controls but mean ADG was significantly higher than controls only for the Bovishield/Pestigard group. Upper limits of 95% CIs for other vaccine groups indicate that if these other vaccine combinations do increase ADG, these increases are probably less than 0.06 to 0.08 kg/animal/d.

Table 10: Effect estimates of vaccination group on average daily gain (kg/animal/d) during the feedlot phase derived from mixed effects linear regression models (Global Wald *P* values for Group not statistically significant at the 0.05 level)

Model	Variable	Coefficient*	95% CI		*P*-value
Model 1 (n=6528) fixed effect for feedlot, random effect for cohort	Vaccination group				0.169
	Control	Reference category			
	Bovishield	0.018	-0.028	0.063	0.440
	Bovishield/Pestigard	0.068	0.023	0.114	0.003
	Pestigard/Bovilis MH+IBR	0.039	-0.007	0.084	0.094
	Pestigard/Bovilis	0.032	-0.010	0.074	0.139
	Pestigard	0.036	-0.007	0.078	0.101
	Bovilis MH+IBR	0.032	-0.014	0.077	0.171
	Bovilis MH	0.018	-0.024	0.061	0.398
	intercept	1.369	0.635	2.103	

*Coefficients for exposure variables estimate the difference in mean average daily gain between the respective category and the reference category; coefficients for intercepts estimate the average daily gain in feedlot 1 for animals at the reference values for all fitted categorical exposure variables and at values of 0 for continuous exposure variables

Based on further modelling of weight using three-level linear mixed effects models, the predicted marginal average daily gains by group are presented in Table 11 with the *P*-values derived from testing the interaction terms. None of the interaction terms tested were significant at the 0.05 level indicating that overall there were no significant differences in ADG between groups. Point estimates were consistent with, at most, small increases in ADG for each vaccination group relative to controls. Results from the model fitted with fractional polynomial terms were consistent with this (results not shown). Scatter plots of predicted versus observed values for each vaccine group within each cattle class are shown in Appendix 2.

Table 11: Estimated average daily gains (kg/animal/d) during the feedlot phase by vaccination group derived from three-level linear mixed effects models of BW for subsets indicated (DOF = days on feed)

	Animals <=160 DOF (n=6585) *P*=0.755*			Average DOF for cohort <80d (n=1530) *P*=0.394*			Average DOF for cohort 80 -155d (n=2643) *P*=0.248*			Average DOF for cohort >155d (n=2834) *P*=0.865*		
Vaccination group	Coefficient**	95% CI		Coefficient**	95% CI		Coefficient**	95% CI		Coefficient**	95% CI	
Control	1.36	1.15	1.57	1.31	0.94	1.67	1.29	0.91	1.67	1.66	1.57	1.75
Bovishield	1.36	1.15	1.57	1.36	0.96	1.76	1.35	0.97	1.74	1.66	1.57	1.75
Bovishield/ Pestigard	1.40	1.19	1.61	1.47	1.07	1.87	1.39	1.01	1.77	1.70	1.61	1.79
Pestigard/ Bovilis MH + IBR	1.40	1.18	1.61	1.30	0.90	1.70	1.45	1.07	1.83	1.62	1.53	1.71
Pestigard/ Bovilis MH	1.37	1.16	1.58	1.24	0.88	1.60	1.40	1.02	1.78	1.68	1.59	1.77
Pestigard	1.41	1.20	1.63	1.39	1.03	1.75	1.39	1.01	1.77	1.67	1.58	1.76
Bovilis MH + IBR	1.38	1.16	1.59	1.30	0.90	1.70	1.40	1.02	1.78	1.69	1.60	1.78
Bovilis MH	1.37	1.16	1.58	1.32	0.96	1.68	1.30	0.92	1.68	1.69	1.60	1.78

All models fitted with fixed effect for feedlot, random effects (intercept and slope) for cohort and random intercept for animal nested in cohort with main effects of group, time and group* time interaction; **P*-value from global Wald test for interaction term of group*time; **coefficients estimate the difference in mean average daily gain between the respective category and the reference category.

Estimated effects of vaccination group on ADG during the backgrounding and feedlot phases combined are shown in Table 12. Average daily gain did not vary significantly by vaccination group (*P* = 0.674). For each vaccination group relative to the control group, the estimated difference was close to zero, and 95% CI limits were all on or between -0.056 and +0.043.

Table 12. Effect estimates of vaccination group on average daily gain (kg/animal/d) during the backgrounding and feedlot phases combined derived from mixed effects linear regression models (n=5516)

Vaccination group	Coefficient*	95% CI		*P*-value
Control	Reference category			
Bovishield	0.004	-0.028	0.037	0.791
Bovishield/Pestigard	0.011	-0.022	0.043	0.520
Pestigard/Bovilis MH+IBR	-0.006	-0.039	0.026	0.699
Pestigard/Bovilis MH	-0.002	-0.035	0.031	0.912
Pestigard	0.003	-0.030	0.036	0.847
Bovilis MH+IBR	-0.002	-0.035	0.031	0.899
Bovilis MH	-0.023	-0.056	0.010	0.175
intercept	1.514	1.321	1.706	

*Coefficients for exposure variables estimate the difference in mean average daily gain between the respective category and the reference category; the coefficient for the intercept estimates the average daily gain in feedlot 1 for control animals; the global *P*-value for vaccination group was 0.674

For vaccines against organisms other than BHV1, there was no significant interaction between these vaccines and Rhinogard® for ADG in the backgrounding phase (*P* for interaction = 0.594), the feedlot phase (*P* = 0.216), or for both phases combined (*P* = 0.195). For these analyses, feedlot was fitted as a fixed effect and cohort as a random effect; no other covariates were fitted.

Effects of BVDV and BHV1 Serostatuses During Backgrounding on Average Daily Gain: Associations between BVDV and BHV1 serostatuses during the backgrounding phase and ADG are shown in Table 13. Amongst cattle not vaccinated against BVDV or BHV1, BVDV seroincrease during the backgrounding phase was associated with reduced ADG during that phase relative to animals that remained seronegative to BVDV (*P* = 0.002). This could be because seroincrease to BVDV reduces ADG, animals with low ADG are more likely to seroincrease to BVDV, or both. In contrast, there was evidence that animals that seroincreased to BHV1 had greater ADG during the backgrounding phase relative to animals that remained seronegative to BHV1 (*P* = 0.052). This could be because seroincrease to BHV1 increases ADG, animals with high ADG are more likely to seroincrease to BHV1, or both. Animals that were initially seropositive to BHV1 had greater ADG during the backgrounding phase relative to animals that remained seronegative to BHV1 (*P* < 0.001). The *P*-value for the interaction between BVDV and BHV1 serostatuses was 0.853.

For ADG during the feedlot phase, amongst cattle not vaccinated against BVDV or BHV1, BVDV seroincrease during the backgrounding phase was associated with increased ADG during the feedlot phase relative to animals that remained seronegative to BVDV (*P* = 0.022). The *P*-value for the interaction between BVDV and BHV1 serostatuses was 0.264.

Effects on ADG in the backgrounding and feedlot periods combined reflected the results for the separate periods. Amongst cattle not vaccinated against BVDV or IBR, there was evidence that animals that seroincreased to BHV1, and those that were initially seropositive to BHV1, had greater ADG over the entire period (*P* = 0.061 and 0.006, respectively). The *P*-value for the interaction between BVDV and BHV1 serostatuses was 0.091.

Table 13. Associations between BVDV and BHV1 serostatuses during the backgrounding phase and ADG during three periods (backgrounding phase, feedlot phase, and both phases combined in cattle not vaccinated against BVDV or IBR*

	ADG (kg/animal/d)		Coefficient**	95% CI		*P*-value***
	No. cattle	Mean (SD)				
ADG during backgrounding phase						
Effects of BVDV						
Pooled (no interaction; adjusted for BHV1 serostatus)						**0.001**
BVDV remained seronegative	339	1.73 (0.91)	Reference category			
BVDV seroincreased	193	1.24 (0.84)	-0.24	-0.39	-0.09	0.002
BVDV initially seropositive	1194	1.75 (0.85)	0.02	-0.09	0.12	0.744
Effects of BHV1						
Pooled (no interaction; adjusted for BVDV serostatus)						**<0.001**
BHV1 remained seronegative	1067	1.66 (0.90)	Reference category			
BHV1 seroincreased	427	1.69 (0.85)	0.10	0.00	0.20	0.052
BHV1 initially seropositive	232	1.84 (0.79)	0.25	0.14	0.37	<0.001

ADG during feedlot phase						
Effects of BVDV						
Pooled (no interaction; adjusted for BHV1 serostatus)						**0.047**
BVDV remained seronegative	301	1.69 (0.39)	Reference category			
BVDV seroincreased	189	1.82 (0.30)	0.08	0.01	0.14	0.022
BVDV initially seropositive	1042	1.74 (0.36)	0.01	-0.03	0.06	0.597
Effects of BHV1						
Pooled (no interaction; adjusted for BVDV serostatus)						**0.326**
BHV1 remained seronegative	887	1.74 (0.36)	Reference category			
BHV1 seroincreased	421	1.72 (0.37)	0.03	-0.02	0.07	0.240
BHV1 initially seropositive	224	1.74 (0.37)	0.03	-0.02	0.08	0.207

ADG during backgrounding and feedlot phases combined						
Effects of BVDV						
Pooled (no interaction; adjusted for BHV1 serostatus)						**0.521**
BVDV remained seronegative	289	1.62 (0.30)	Reference category			
BVDV seroincreased	187	1.66 (0.27)	0.03	-0.03	0.09	0.320
BVDV initially seropositive	1020	1.67 (0.30)	0.02	-0.02	0.06	0.300
Effects of BHV1						
Pooled (no interaction; adjusted for BVDV serostatus)						**0.013**
BHV1 remained seronegative	877	1.66 (0.29)	Reference category			
BHV1 seroincreased	401	1.65 (0.30)	0.04	0.00	0.08	0.061
BHV1 initially seropositive	218	1.69 (0.31)	0.06	0.02	0.10	0.006

*Cattle that received Rhinogard® at DOF1 were included for ADG during backgrounding, but not for ADG during the feedlot phase or ADG during the backgrounding and feedlot phases combined.

**Coefficients estimate the difference in mean average daily gain between the respective category and the reference category

***Bolded p-values are the overall p-value for the serostatus variable; unbolded p-values are for testing whether the mean ADG for that particular serostatus differs from the mean for animals that remained seronegative.

Deaths and Bovine Respiratory Disease: The distribution of deaths (all cause) across vaccination groups is shown in Table 14 with odds ratios derived from a logistic regression model adjusted for feedlot. Mortality incidences ranged between groups from 1.2% to 2.7%. After adjusting for feedlot, point estimates for all vaccination regimens were consistent with reduced risk of mortality compared to the control group but none of these differences were statistically significant.

Table 14. Distribution of deaths during the feedlot phase by vaccine group and effect estimates of vaccination group on all-cause mortality derived from logistic regression model adjusted for feedlot

		All deaths				BRD Deaths			
Group	Total	n (%)	OR*	95% CI	*P*-value**	n (%)	OR*	95% CI	*P*-value**
Control	955	25 (2.6)	Reference category			12 (15.8)	Reference category		
Bovishield	780	21 (2.7)	0.8	0.5-1.5	0.541	15 (19.7)	1.3	0.6-2.7	0.568
Bovishield/Pestigard	789	19 (2.4)	0.7	0.4-1.4	0.356	13 (17.1)	1.1	0.5-2.4	0.848
Pestigard/Bovilis MH+IBR	778	14 (1.8)	0.6	0.3-1.1	0.082	6 (7.9)	0.5	0.2-1.3	0.160
Pestigard/Bovilis	1,008	23 (2.3)	0.9	0.5-1.6	0.761	12 (15.8)	1	0.4-2.3	0.986
Pestigard	947	17 (1.8)	0.7	0.4-1.3	0.211	9 (11.8)	0.7	0.3-1.8	0.514
Bovilis MH+IBR	768	9 (1.2)	0.4	0.2-0.8	0.008	1 (1.3)	0.1	0.0-0.6	0.016
Bovilis MH	986	16 (1.6)	0.6	0.3-1.2	0.159	8 (10.5)	0.7	0.3-1.7	0.386
Total	7,011	144 (2.1)				76 (1.1)			

*Odds ratios derived from mixed effects logistic regression model with a random effect for cohort and adjusted for feedlot
**Global *P*-values for vaccination group derived from Wald test were 0.190 and 0.131 for all deaths and BRD deaths, respectively.

The classification of animals as BRD cases at first pull or as BRD pen deaths without a prior pull during the feedlot phase or as experiencing a competing risk is shown in Table 15. Amongst the 7011 animals included in the study, 3.7% (260) became BRD cases. These comprised 241 cases diagnosed at first pull, of which 49 later died, which together with 19 pen deaths attributed to BRD, resulted in a case fatality rate for BRD cases at first pull or BRD pen deaths without a prior pull of 26% (68/260). A further eight animals died of BRD after a non-BRD initial diagnosis, giving a total of 76 BRD deaths. Of a further 68 deaths not attributed to BRD, 36 animals died of an unknown cause without a prior hospital record. The total of 144 deaths gave an overall cumulative mortality risk of 2.1%. A total of 9.5% (665) animals had a pull or death record. Of these 665 pulled and dead animals, 39% (260) were classified as BRD cases. For BRD cases, the time from DOF1 until diagnosis ranged from 1 to 140 days with a median of 16 (IQR: 10-26) days.

Table 15. Detailed classification of animals used in competing risk analysis of time to first pull or pen death without prior pull where the reason for pulling or death was BRD by classification for analyses

Outcome	n	%	Classification
BRD at first pull	192	2.7	BRD case
BRD at first pull & subsequent death	49	0.7	BRD case
BRD pen death (not previously pulled)	19	0.3	BRD case
BRD death; first pull not for BRD	8	0.1	Competing risk

Pen death (not previously pulled) from unknown cause	36	0.5	Competing risk
Pen death (not previously pulled) from non-BRD cause	11	0.2	Competing risk
First pull not for BRD & subsequent death	21	0.3	Competing risk
First pull not for BRD	311	4.4	Competing risk
Inconsistent first pull reason & ailment	18	0.3	Competing risk
Not pulled, died in pen or early exit from cohort	6346	90.5	Right-censored
Total	7011	100.0	

Results of analysis of time to first pull or pen death without prior pull where the reason for pulling or death was BRD using competing risks regression analyses are shown in Table 16 and Appendix 3. All models were fitted with feedlot as a fixed effect and cohort was specified as the cluster variable. Models in Table 16 and Appendix 3 Table 1 were fitted with the full study population. Models in Appendix 3 Table 2 include only those animals with serological data. Vaccination group was significantly associated with the risk of BRD in all models. Estimates were generally consistent across models. Risk of BRD was lowest (*P* = 0.010) in the group vaccinated with Bovilis MH+IBR® (subhazard ratio or SHR: 0.47; 95% CI: 0.27-0.83). For other vaccine combinations, while subhazard ratio point estimates were consistent with reduced risk of BRD, the confidence limits on these estimates overlapped nil effect, so these results are compatible with both beneficial and adverse effects.

Table 16. Effect estimates of vaccine group on risk of BRD derived from competing risk regression models adjusted for feedlot using the full study population

Variable and Category	SHR*	95% CI		*P*-value
Group				<0.001
Control	Reference category			
Bovishield	0.78	0.51	1.21	0.265
Bovishield/Pestigard	0.75	0.52	1.07	0.116
Pestigard/BovilisMH+IBR	0.71	0.47	1.07	0.103
Pestigard/Bovilis MH	1.33	0.94	1.87	0.103
Pestigard	0.77	0.50	1.17	0.220
Bovilis MH+IBR	0.47	0.27	0.83	0.010
Bovilis MH	0.88	0.51	1.51	0.632

*Subhazard ratio

Results of subset analyses are shown in Appendix 3. Amongst animals that were seronegative at entry to backgrounding (Model F), there was weak evidence that group was associated with reduced risk for groups 3 (Bovishield/Pestigard) and 4 (Pestigard/Bovilis MH+IBR) compared to controls. Amongst animals given the modified live vaccine against BHV1, Rhinogard®, at feedlot entry, incidence of BRD was reduced for group 7 (Bovilis MH+IBR) but increased for group 5 (Pestigard/Bovilis MH) compared to controls (Model G). Amongst animals that did not receive Rhinogard® at feedlot entry, incidence of BRD was markedly reduced for group 3 (Bovishield/Pestigard; Model H).

Results from models fitted using the vaccine components and adjusted for serological statuses are shown in Appendix 3. Table 4. Rhinogard® vaccination was associated with moderately reduced

incidence of BRD (SHR: 0.66; 95% CI: 0.45-0.97; *P* = 0.033). Animals seropositive to BVDV were at markedly reduced risk compared to animals seronegative at entry to backgrounding (SHR: 0.56; 95% CI: 0.39-0.80; *P* = 0.001).

Cumulative incidences are shown graphically in Figures 2 (unadjusted), 3 (adjusted for covariates) and 4 (seronegative subset). The Pestigard/Bovilis MH group appeared to have the highest BRD risk in Figures 2 and 3, while in the seronegative subset, the cumulative incidence for this group overlapped with the control group (Figure 4). Lowest risk was observed for the Bovilis MH+IBR group.

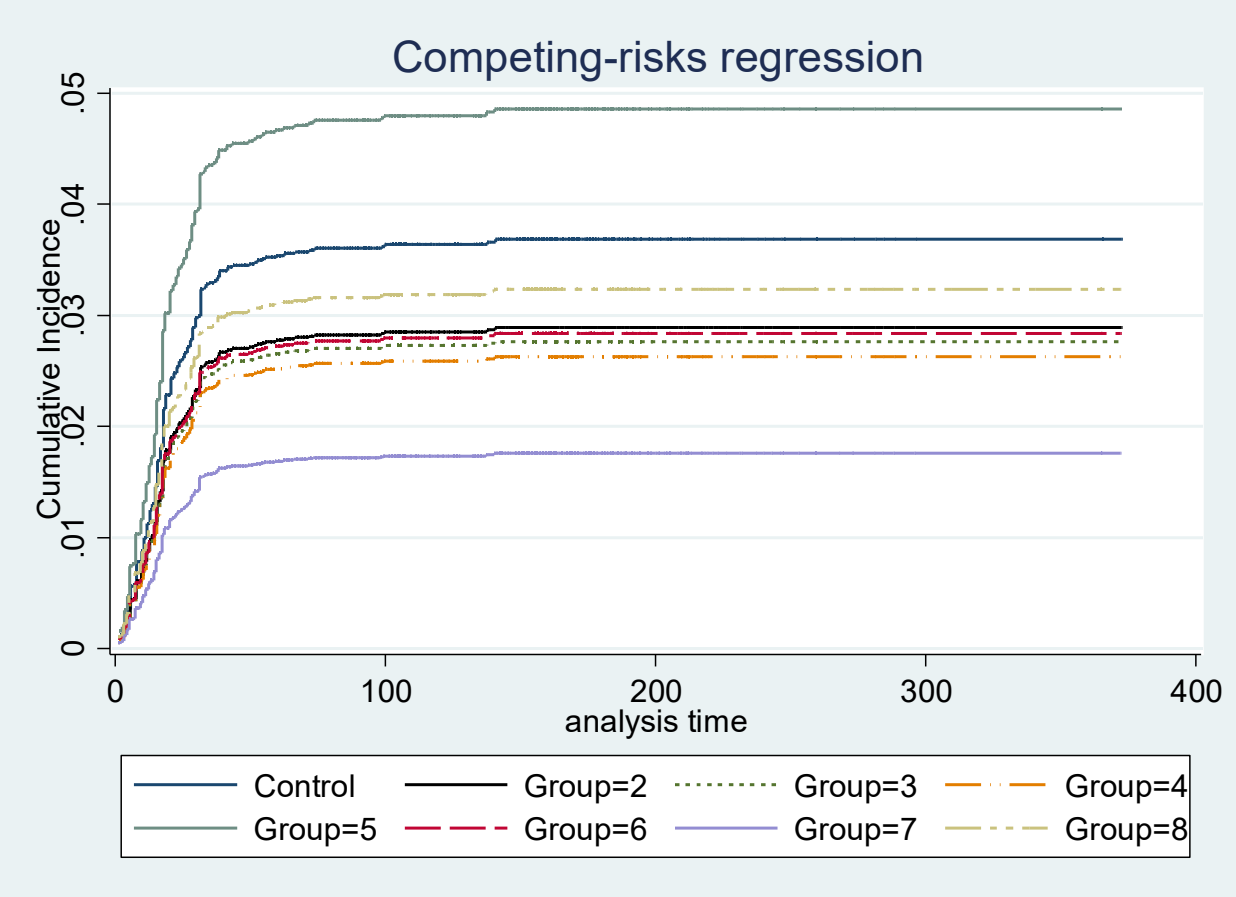


Figure 2: Cumulative incidences of BRD by vaccination group (adjusted for feedlot); group 1: Control; group 2: Bovishield; group 3: Bovishield/Pestigard; group 4: Pestigard/Bovilis MH+IBR; group 5: Pestigard/Bovilis MH; group 6: Pestigard; group 7: Bovilis MH+IBR; group 8: Bovilis MH).

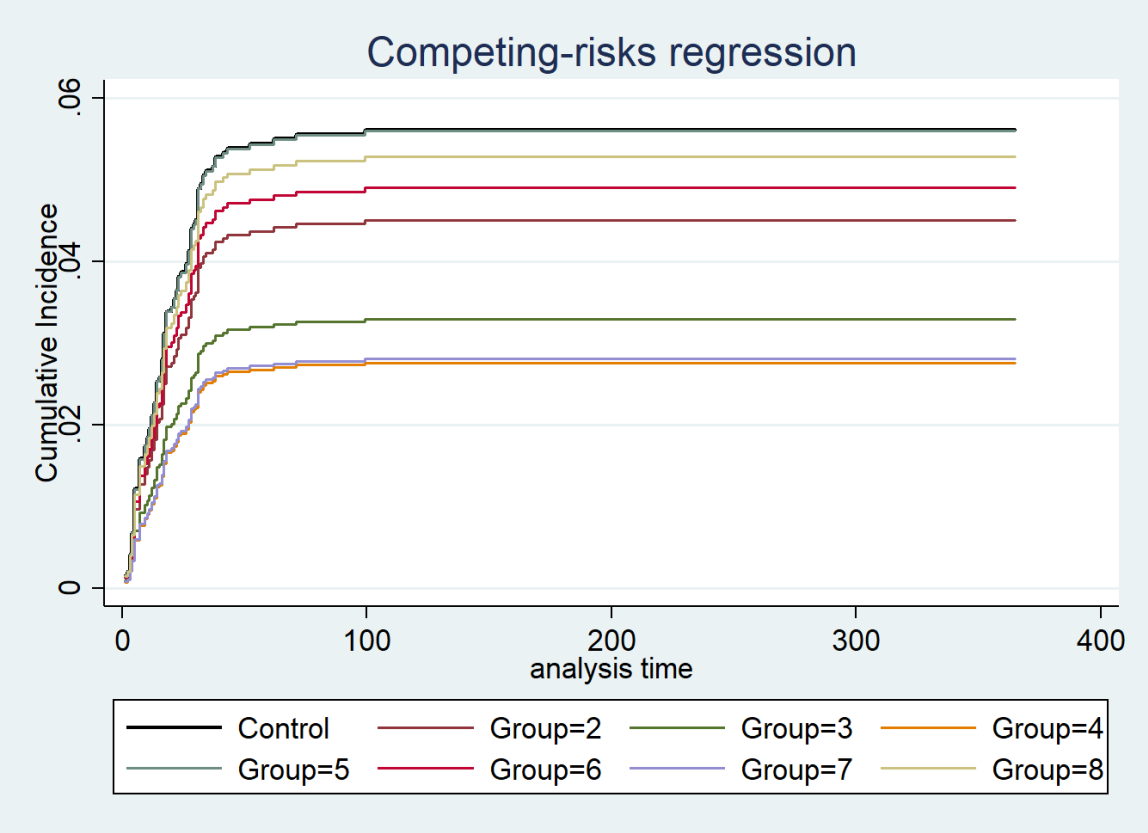


Figure 3: Cumulative incidences of BRD by vaccination group (adjusted for feedlot); subset analysis restricted to animals seronegative to both BVDV and BHV1 at entry to backgrounding (groups 1 and 5 overlap); group 1: Control; group 2: Bovishield; group 3: Bovishield/Pestigard; group 4: Pestigard/Bovilis MH+IBR; group 5: Pestigard/Bovilis MH; group 6: Pestigard; group 7: Bovilis MH+IBR; group 8: Bovilis MH).

The predicted relative subhazards derived from the model fitted with the vaccine component variables and their interaction terms are presented in Appendix 3. Table 3. The lowest incidence of BRD was observed in animals that were seropositive to BVDV and either seropositive to BHV1 or received Rhinogard® vaccine at feedlot entry. The highest incidence of BRD was observed in animals that were seronegative to both viruses at entry to backgrounding and did not receive a vaccine against IBR regardless of other vaccines administered.

For vaccines with no IBR component, estimated protective effects against BRD relative to controls were larger (i.e. sub-hazard ratios were further below 1.00) within cattle that received Rhinogard® at feedlot entry (sub-hazard ratios 0.00 to 0.57) than within cattle that did not receive Rhinogard® (sub-hazard ratios 0.88 to 1.52; Table 17). The joint *P*-value for the five terms for the interaction between Group and Rhinogard® was < 0.001).

Table 17. Effect estimates of vaccine group on risk of BRD derived from competing risk regression models for cattle that did and cattle that did not receive Rhinogard® at feedlot entry

Subset	n (%)	SHR*	95% CI	*P*-value
Rhinogard® at feedlot entry				

	Group					<0.001
1	Control	283 (19)	Reference category			
2	Bovishield	108 (7)	0.38	0.09	1.62	0.191
3	Bovishield/Pestigard	115 (8)	0.00	0.00	0.00	<0.001
5	Pestigard/Bovilis	345 (23)	0.57	0.16	1.95	0.367
6	Pestigard	289 (20)	0.12	0.02	0.94	0.044
8	Bovilis MH	334 (23)	0.35	0.12	0.97	0.048
	Total	1474 (100)				
No Rhinogard® at feedlot entry						
	Group					<0.001
1	Control	672 (12)	Reference category			
2	Bovishield	672 (12)	0.88	0.57	1.36	0.570
3	Bovishield/Pestigard	674 (12)	0.91	0.67	1.23	0.526
4	Pestigard/Bovilis MH + IBR	778 (14)	0.74	0.47	1.16	0.188
5	Pestigard/ Bovilis	663 (12)	1.52	1.06	2.18	0.022
6	Pestigard	658 (12)	0.93	0.63	1.37	0.720
7	Bovilis MH + IBR	768 (14)	0.50	0.28	0.88	0.017
8	Bovilis MH	652 (12)	1.01	0.58	1.75	0.973
	Total	5537 (100)				

4 Discussion

4.1 Discussion

These results do not support vaccination of beef cattle feedlot beef cattle if they are to be locally backgrounded for at least 28 days before feedlot entry because all vaccine combinations decreased ADG during backgrounding with the exception of Bovishield®, and any increases in ADGs during the feedlot phase are probably insufficient to account for this and generate an overall marginal profit from vaccination. Overall, the use of the various vaccine combinations was not associated with in an increase in ADG during the feedlot phase. However, point estimates were supportive of small increases in ADG, with the cattle vaccinated with the combination of Bovishield/Pestigard having a significantly higher ADG than controls. The positive effect of Bovishield/Pestigard was inconsistent with the effect of the other vaccine combinations and the reasons for this are unclear. The results do not preclude the possibility of greater feedlot weight gains in cattle vaccinated during the backgrounding phase in the order of 0.06 to 0.08 kg/animal/d. Taking the mid-range figure of 0.07 kg/animal/d, this would result in an additional 4.9 kg BW/animal at the conclusion of a 70 day feeding programme, or an additional 10.5 kg BW/animal at the conclusion of a 150 day feeding programme. Using 2018 values, the potential increase in feedlot weight gain could be worth $14.70/animal or $31.50/animal for cattle fed for 70 or 150 days respectively. Allowing for a feed conversion ratio of 5 kg feed dry matter/kg BW gain in cattle fed for 70 days or 6.5 kg feed dry matter/kg BW gain in cattle fed for 150 days, the additional feed cost would be approximately $8.65 or $22.98 for a mean diet dry matter of 85% at a cost of $300/tonne as fed. The cost of the vaccines must be added to this. In addition, the cost of reduced BW gain in vaccinated cattle during the backgrounding phase should be accounted for. All vaccine combinations decreased ADG during backgrounding with the exception of

Bovishield. The cost of the decreased BW and the cost of the vaccines is shown below in Table 18. The decreased feed cost is not included in the value of the decreased growth rate during the background phase because lower growth rate is strongly correlated with a higher feed conversion ratio, so the slower cattle grow, the more feed they require to reach the same BW end-point.

Table 18. Cost of decreased weight gain in vaccinated cattle during a 28 day background phase plus vaccine cost

Vaccine	Decrease in growth rate (kg/animal/d)	Value of decreased growth rate ($/animal)	Vaccine Cost ($)	Total Cost
Bovishield	-	-	4.26	4.26
Bovishield/Pestigard	0.16	13.44	17.22	30.66
Pestigard/Bovilis MH+IBR	0.17	14.28	27.71	41.99
Pestigard/Bovilis MH	0.15	12.60	20.13	32.73
Pestigard	0.13	10.92	12.96	23.88
Bovilis MH+IBR	0.17	14.28	14.75	29.03
Bovilis MH	0.13	10.92	7.17	18.09

Table 19. Optimistic scenario for potential return from increase in feedlot growth rate from background vaccination using the non-significant upper confidence intervals (ADG increase 0.07 kg/animal/d @ $3.00/kg = $14.70/animal for 70 DOF or = $31.50/animal for 150 DOF)

	70 DOF		150 DOF	
Vaccine	Value of increased growth rate ($)	Net profit (loss) after feed and vaccine costs ($)	Value of increased growth rate ($)	Net profit (loss) after feed and vaccine costs ($)
Bovishield	14.70	10.44	31.5	27.24
Bovishield/Pestigard	14.70	(15.96)	31.5	0.84
Pestigard/Bovilis MH+IBR	14.70	(27.29)	31.5	(8.49)
Pestigard/Bovilis MH	14.70	(18.03)	31.5	(1.23)
Pestigard	14.70	(9.18)	31.5	7.62
Bovilis MH+IBR	14.70	(14.33)	31.5	2.47
Bovilis MH	14.70	(3.39)	31.5	13.41

Table 20. Pessimistic scenario for potential return from overall non-significant increase in feedlot growth rate in response to background vaccination using the ADG increase for Bovishield/Pestigard of 0.07 kg/animal/d and zero for the non-significant vaccination combination point estimates (@ $3.00/kg = $14.70/animal for 70 DOF or = $31.50/animal for 150 DOF)

	70 DOF		150 DOF	
Vaccine	Value of increased growth rate ($)	Net profit (loss) after feed and vaccine costs ($)	Value of increased growth rate ($)	Net profit (loss) after feed and vaccine costs ($)
Bovishield	0	(4.26)	0	(4.26)
Bovishield/Pestigard	14.70	(15.96)	31.5	0.84

Pestigard/Bovilis MH+IBR	0	(41.99)	0	(41.99)
Pestigard/Bovilis MH	0	(32.73)	0	(32.73)
Pestigard	0	(23.88)	0	(23.88)
Bovilis MH+IBR	0	(29.03)	0	(29.03)
Bovilis MH	0	(18.09)	0	(18.09)

These analyses (Tables 19 and 20) indicate that investment in vaccination of cattle in local backgrounding systems is unlikely to generate a profit based on effects on growth rate in both the backgrounding and feedlot phases.

These calculations do not explicitly incorporate effects of vaccination on BRD incidence. However, as the major financial effect of BRD is reduced growth rate (Cusack, 2004), any effects on BRD are partly incorporated into these calculations. Further, the only treatment group that had a reduced risk of BRD was the group vaccinated with Bovilis MH+IBR®, where the subhazard ratio was 0.47 with a CI of 0.27 to 0.83. A halving in the incidence of BRD would markedly reduce production costs but this finding is not consistent with the lack of any marked effect of that vaccine combination on feedlot growth rate, nor with the lack of response for other vaccine combinations against the same organisms. Thus, the reason for this result remains unclear. This is especially so with this study design because, for our second method for analysing growth rates, deaths were allocated an exit weight of zero, which captured their effects on saleable BW and therefore growth rate. A reduction in BRD incidence would reduce the potential or the extent of the financial loss incurred from the use of vaccines in local backgrounding systems, but this effect is small compared with the effect on growth rate (Cusack, 2004).

Further, the only treatment group that had a reduced risk of BRD was the group vaccinated with Bovilis MH+IBR®, where the subhazard ratio was 0.47 with a CI of 0.27 to 0.83. A potential halving in the incidence of BRD would markedly reduce treatment costs but this is at odds with the lack of an effect on feedlot growth rate, considering that the greatest financial effect of BRD is expressed through reduced growth rate (Cusack, 2004). This is especially so with this study design because deaths were allocated an exit weight of zero, which captures their effects on saleable BW and therefore growth rate. A reduction in BRD incidence would reduce the potential or the extent of the loss incurred from the use of vaccines in local backgrounding systems, but this effect is small compared with the effect on growth rate (Cusack, 2004).

The evidence that use of vaccines in cattle placed in local backgrounding facilities does not markedly decrease the risk of BRD or increase growth rate during the feedlot phase is consistent with a recent US study (Bailey et al., 2016) using modified live viral respiratory vaccines, and the findings of the Australian National BRD Initiative (Hay et al., 2014; Barnes et al., 2014). In the Australian study, mixing for at least 28 days before placement in the feedlot was associated with a reduction in the risk of BRD. In the absence of vaccination, this effect could relate to generalised improvements in immune function through the re-establishment of rumen function and a commensurate correction of energy and protein balance, (Sahoo et al., 2009; Lochmiller and Deerenberg, 2000; Scrimshaw and SanGiovanni, 1997) and more specific effects with seroconversion or a serological increase to the commonly occurring respiratory viruses before placement in the feedlot (Hay et al., 2016a). As the concentrations of serum antibodies to alternative BRD respiratory viruses such as bovine respiratory syncytial virus

and bovine parainfluenza 3 were not measured, the extent and effects of seroconversion or seroincrease to these viruses in cattle that are backgrounded for at least 28 d, such as the study cattle, remain unknown. However, the NBRDI (Hay et al., 2016; Barnes et al., 2014) found 89% and 91% respectively, of predominantly non-backgrounded cattle were seropositive to these viruses at feedlot entry, leaving only 11% and 9% respectively that could seroconvert or have a serological increase to these viruses under the more intensive conditions during the first 42 days on feed. Of those initially seronegative, 65% and 54% seroconverted to bovine respiratory syncytial virus and bovine parainfluenza 3 respectively.

US studies (Chirase et al., 2001; Richeson et al., 2009; Arthington et al., 2013; Rodrigues et al., 2015) have shown variable effects on cattle production during the three weeks after vaccination, with a range of combined modified live respiratory virus vaccines, sometimes combined with killed bacterins against *Mannheimia haemolytica* or *Histophilus somni*, and sometimes in combination with a clostridial vaccine. Arthington et al. (2013; Exp 2) found no effect with the *M. haemolytica* killed vaccine, One Shot® (Pfizer Inc., New York, NY), which is the same vaccine as Bovishield®, on DMI for 15 days after vaccination, but ADG was lower ($P \leq 0.05$; 0.87 versus 1.14 kg/animal/d) in vaccinated heifers, and this was accompanied by increases in acute phase proteins. It appears, therefore, that the inflammatory response and stimulation of the immune system (Spurlock, 1997) might directly increase the nutrient requirements of vaccinated cattle. Further, it appears from these studies that modified live virus respiratory vaccines are not consistently associated with a decrease in ADG in the two to three weeks after vaccination (Bailey et al., 2016; Duff et al., 2000), but killed bacterins are (Arthington et al., 2013; Richeson et al., 2009; Chirase et al., 2001), and this effect might be enhanced by the endotoxins in killed vaccines against gram negative bacteria such as *M. haemolytica* (Tizard, 1996), specifically lipopolysaccharide (Garcia et al., 2017). The glucose demand in cattle immunostimulated with lipopolysaccharide has been measured by Kvidera et al. (2017, 2016) 12 hours after challenge at 1.00 to 0.66 g glucose/kg $BW^{0.75}$/h. It has also been proposed that the negative effect on ADG associated with the use of bacterins is due to the inflammatory effects of their adjuvants (Bailey et al., 2016) which are necessary to stimulate a vigorous immune response to the vaccine (Tizard, 1996). The negative effects on ADG during the backgrounding phase of most of the respiratory vaccines in this study are consistent with the conclusions we have drawn from these US studies. Further, the modified live vaccine against BHV1 (Rhinogard®) was shown in this study to improve the subhazard ratios for BRD risk when administered in combination with the range of vaccines investigated, and the vaccine itself was associated with a reduced BRD risk.

It is likely that the low incidence of BRD in this study (3.7% of the study cattle) reduced the likelihood of a response to vaccines in either BRD risk or ADG. This figure is low relative to commonly encountered BRD incidence with cattle placed directly in feedlots (Barnes et al., 2014). However, the BRD case fatality rate was elevated (20% of treated BRD cases (49/241); 26% with pen deaths due to BRD included (68/260)) suggesting that detection of cases on some sites might have resulted in a higher mortality rate (2.1% of study cattle; 1.2 to 2.7% by site) than would be anticipated for a low disease incidence (Barnes et al., 2014).

The serological screening in this study indicates that a high proportion of cattle entering Australian feedlots are seropositive to BVDV. The significance of this is illustrated by the finding of the lowest BRD incidence in cattle that were seropositive to BVDV and either seropositive to BHV1 or given a vaccine against BHV1. Conversely, an elevated incidence of BRD can be expected in cattle placed in a

feedlot that are seronegative and unvaccinated against both BVDV and BHV1. If the regional differences in serological status shown in this study are consistent over time, they should also be considered in BRD vaccination regimens. Whilst serological status to BVDV broadly conformed to a higher proportion of seropositive cattle moving from the south to the more extensive feeder cattle source properties of the north, serological status to BHV1 appears to be more variable. These observations suggest that the use of a BVDV vaccine in local backgrounding practices is unlikely to generate a health or production response, particularly in Queensland, but a vaccine against BHV1, to prevent infectious bovine rhinotracheitis (IBR) should routinely be included with locally backgrounded cattle. Further, the involvement of a single organism in IBR means that an effective vaccine can largely eliminate the condition, unlike the multifactorial condition of BRD which represents a microbial ecological niche which can be exploited by several viral and bacterial pathogens.

Serological increase to BVDV during backgrounding was associated with lower ADG during the backgrounding phase, but seroincrease to BHV1 was associated with greater ADG during the backgrounding phase. These findings are difficult to interpret during the backgrounding phase itself because the timing of seroincrease is unknown. Therefore, the effects of serostatuses during backgrounding on growth rates during the feedlot phase are more readily interpreted. Seroincrease to BVDV during the background phase was associated with greater ADG during the feedlot phase, but a seropositive status at background entry did not have a significant effect on feedlot growth rate. This difference might have been due to greater immunity to BVDV in response to the more recent challenge in those that seroincreased to BVDV during the background phase as seropositivity may not always indicate the same degree of immunocompetence. There were no significant effects of BHV1 serostatuses during backgrounding on feedlot growth rate. Based on previous findings (Hay et al., 2016a; Barnes et al., 2014) where risk of BRD was increased in association with seroincrease to an increasing number of respiratory viruses during the feedlot phase, a positive response to BHV1 seropositive feedlot entry might have been expected. However, this finding is in keeping with the lack of significant responses to a range of respiratory vaccines in locally backgrounded cattle, and emphasises the multifactorial nature of BRD and the as yet unquantified effects of backgrounding on the digestive and immune systems in terms of subsequent feedlot health and production. These effects might also partly explain the lack of significant interactions between serostatuses to BVDV and BHV1, in addition to the variable effects of BVDV on immunosuppression (Potgeiter, 1995; Brownlie, 1990).

The proportion of cattle identified by serology as being persistently infected with BVDV (PI), at 0.28% is comparable with the findings of overseas surveys (Bryant et al, 2011; Hessman et al., 2009; Loneragan et al., 2005; O'Connor et al, 2005; Taylor et al., 1995) and in one Australian survey (Bergman, 2007). However, the effects of this small proportion of PI cattle were spread over large numbers of in-contact cattle, with 26.5% of the study cattle having a PI in their cohort. The results of this study illustrate that the introduction of a PI to a backgrounding cohort promoted seroconversion, or serological increase, in purchase groups that had a low proportion of cattle seropositive to BVDV on backgrounding entry. Naivety at background entry to BVDV was associated in this study with increased risk of BRD during the feedlot phase, presumably due to an increased risk of initial seroconversion to BVDV either during backgrounding or during lot feeding. If the increasing awareness of the significance of BVDV to production and profitability in the grazing sector leads to an increase in the proportion of BVDV seronegative cattle arriving at feedlots or their backgrounding facilities over time, the potential benefit from a BVDV vaccine may need to be reassessed. Further, the role of PI

animals in contributing to BRD needs to be further explored within the context of the Australian lot feeding industry.

This study does not support the routine use of respiratory vaccines in locally backgrounded cattle. However, this result does not preclude a potential benefit from the use of respiratory vaccines in cattle that are placed directly in the feedlot. The likelihood of a benefit from on-farm administration of respiratory vaccines in the form of reduced BRD risk is supported by the NBRDI (Barnes et al., 2014) where modest reductions in BRD risk were observed with the use of Bovilis MH® and Pestigard®, and the finding from the same study that an increase in the number of viruses to which animals seroincrease was associated with an increase in the BRD risk. A high proportion of cattle naïve to several respiratory viruses is more likely in groups of cattle purchased directly from the farm of origin and placed directly in the feedlot. Cost-effective responses to respiratory vaccines are more likely in these cattle and this is where further research into the appropriate use of respiratory vaccines should be directed.

5 Bibliography

5.1 Bibliography

Andersen, P.H. 2003. Bovine endotoxicosis – some aspects of relevance to production diseases. A review. Acta Vet. Scand. Suppl. 98:141-155.

Arthington, J.D., R.F. Cooke, T.D. Maddock, D.B. Araujo, P. Moriel, N. DiLorenzo and G.C. Lamb. 2013. Effects of vaccination on the acute phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91(4):1831-1837.

Bailey, E.A., J.R. Jaeger, T.B. Schmidt, J.W. Waggoner, L.A. Pacheco, D.U. Thomson and K.C. Olson. 2016. Effects of number of viral respiratory disease vaccinations during preconditioning on health, performance, and carcass merit of ranch-direct beef calves during receiving and finishing. Prof. Anim. Sci. 32:271-278.

Barnes, T., K. Hay, J. Morton, M. Schibrowski, R. Ambrose, E. Fowler and T. Mahony. 2014. Epidemiology and management of bovine respiratory disease in feedlot cattle - final report. Meat and Livestock Australia Limited.

Bergman, E.L. 2007. Bovine viral diarrhoea virus. Proceedings, Beefworks, Australian Lot Feeders Association. Toowoomba, Queensland, September 19th and 20th, pages 149-153.

Brownlie, J. 1990. The pathogenesis of bovine virus diarrhoea virus infections. Rev. Sci. tech. Off. Int. Epiz. 9(1):43-59.

Bryant, T. C., J.R. Nichols, K.C. Rogers, T.D. Farmer, D.G. Miles, J. Campbell and J.T. Richeson. 2011. Effect of trivalent modified-live virus respiratory vaccine on performance, health, and carcass traits of lightweight feeder steers. Bovine Practitioner. 45(2):124-130.

Buczinski, S., R.D. Rademacher, H.M. Tripp, M. Edmonds, E.G. Johnson and S. Dufour. 2015. Assessment of L-lactatemia as a predictor of respiratory disease recognition and severity in feedlot steers. Prev. Vet. Med. 118(4):306-318.

Chako, C.Z., D.L. Step, J.R. Malayer, C.R. Krehbiel, U. Desilva and R.N. Streeter. 2015. Subacute ruminal acidosis and ruminal lactic acidosis: a review. Bovine Pract. 49(2):140-146.

Cole, N.A. 1989. Prearrival factors affecting feeder calf health and performance. Page 1 in Proc. Joint Meeting of the Academy of Veterinary Consultants/Plains Nutrition Council, Amarillo, Texas.

Chirase, N.K., L.W. Greene, G.D. Graham and J.M. Avampato. 2001. Influence of clostridial vaccines and injection sites on performance, feeding behaviour and lesion size scores of beef steers. J. Anim. Sci. 79:1409-1415.

Cusack, P.M.V. 2004. Effect of mass medication with antibiotics at feedlot entry on the health and growth rate of cattle destined for the Australian domestic market. Aust. Vet. J. 82:154-156.

Gabel, G. and J.R. Aschenbach. 2002. Influence of food deprivation on the transport of 3-O-methyl-α-D-glucose across the isolated ruminal epithelium of sheep. J. Anim. Sci. 80(10): 2740-2746.

Garcia, M., B.J. Bradford and T.G. Nagaraja. 2017. Invited Review: Ruminal microbes, microbial products, and systemic inflammation. Prof. Anim. Sci. 33:635-650.

Ghozo, G.N., D.O. Krause and J.C. Plaizier. 2006. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. J. Dairy Sci. 89:4404-4413.

Gressley, T.F., M.B. Hall and L.E. Armentano. 2011.RUMINANT NUTRITION SYMPOSIUM: Productivity, digestion, and health responses to hindgut acidosis in ruminants. J. Anim. Sci. 89:1120-1130.

Harland, R.J., G.K. Jim, P.T. Guichon, H.G.G. Townsend and E.D. Janzen. 1991. Efficacy of parenteral antibiotics for disease prophylaxis in feedlot calves. Can. Vet. J. 32:163-168.

Hay, K.E., T.S. Barnes, J.M. Morton, A.C.A. Clements, and T.J. Mahony. 2014. Risk factors for bovine respiratory disease in Australian feedlot cattle: Use of a causal diagram-informed approach to estimate effects of animal mixing and movements before feedlot entry. Prev. Vet. Med. 117:160–169

Hay, K.E., T.S. Barnes, J.M. Morton, J.L. Graveld, M.A. Commins, P.F. Horwood, R.C. Ambrosed, A.C.A. Clements and T.J. Mahony. 2016a. Associations between exposure to viruses and bovine respiratory disease in Australian feedlot cattle. Prev. Vet. Med. 127:121–133.

Hay, K.E., J.M. Morton, M.L. Schibrowski, A.C.A. Clements, T.J. Mahony, and T.S. Barnes. 2016b. Associations between prior management of cattle and risk of bovine respiratory disease in feedlot cattle. Prev. Vet. Med. 127:37–43

Hessman, B.E., R.W. Fulton, D.B. Sjeklocha, T.A. Murphy, J.F. Ridpath and M.E. Payton. 2009. Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. Am. J. Vet. Res. 70(1):73-85.

Johnson, E.G. 1985. Feedlot management practices and bovine respiratory disease. Vet. Clin. Nth Am. Food Anim. Pract. 1:413-419.

Kvidera, S.K., E.A. Horst, M. Abuajamieh, E.J. Mayorga, M.V. Sanz Fernandez and L.H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360-2374.

Kvidera, S.K., E.A. Horst, M. Abuajamieh, E.J. Mayorga, M.V. Sanz Fernandez and L.H. Baumgard. 2016. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci. 94:4591-4599.

Lochmiller, R. L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? – Oikos 88: 87–98.

Loneragan, G.H., D.U. Thomson, D.L. Montgomery, G.L. Mason and R.L. Larson. 2005. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. 226(4):595-601.

Martin, S.W., A.H. Meek, D.G. Davis. 1982. Factors associated with mortality and treatment costs in feedlot calves: The Bruce County Beef Project, years 1978, 1979, 1980. Can. J. Comp. Med. 46:341-349.

O'Connor, A.M., S.D. Sorden and M.D. Apley. 2005. Association between the existence of calves persistently infected with bovine viral diarrhea virus and commingling on pen morbidity in feedlot cattle. Am. J. Vet. Res. 66:2130-2134.

Penner, G.B., M. Oba, G. Gabel and J.R. Aschenbach. 2010. A single mild episode of subacute ruminal acidosis does not affect ruminal barrier function in the short term. J. Dairy Sci. 93:4838-4845.

Plaizier, J.C., D.O. Krause, G.N. Ghozo and B.W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Vet. Jnl 176:21-31.

Potgieter, L.N.D. Immunology of bovine viral diarrhea virus. 1995. Vet. Clin. Nth Am. 11(3):501-520.

Richeson, J.T., E.B. Kegley, M.S. Gadberry, P.A. Beck, J.G. Powell and C.A. Jones. 2009. Effects of on-arrival versus delayed clostridial or modified live respiratory vaccinations on health, performance, bovine viral diarrhoea virus type I titers, and stress and immune measures of newly received beef calves. J. Anim. Sci. 87(7):2409-2418.

Rodrigues, M.C., R.F. Cooke, R.S. Marques, B.I. Cappellozza, S.A. Arispe, D.H. Keisler and D.W. Bohnert. 2015. Effects of vaccination against respiratory pathogens on feed intake, metabolic, and inflammatory responses in beef heifers. J. Anim. Sci. 93(9):4443-4452.

Sahoo, A., A.K. Pattanaik and T.K. Goswami. 2009. Immunobiochemical status of sheep exposed to periods of experimental protein deficit and realimentation. J. Anim. Sci. 87(8):2664-2673.

Sanderson, M.W., D.A. Dargatz and B.A. Wagner. 2008. Risk factors for initial respiratory disease in United States' feedlots based on producer-collected morbidity counts.

Scrimshaw, N.S., and J.P. SanGiovanni. 1997. Synergism of nutrition, infection, and immunity: an overview. Am. J. Clin. Nut. 66 (2):464S-477S.

Stanger, K.J., N. Ketheesan, A.J. Parker, C.J. Coleman, S.M. Lazzaroni and L.A. Fitzpatrick. 2005. The effect of transportation on the immune status of Bos indicus steers. J. Anim. Sci. 83(11):2632-2636.

Steele, M.A., J. Croom, M. Kahler, O. AlZahal, S.E. Hook, K. Plaizier and B.W. McBride. 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. Am. J. Physiol. 300:R1515-R1523.

Taylor, L.F., J. Van Donkersgoed, E.J. Dubovi, R.J. Harland, J.V. van den Hurk, C.S. Ribble and E.D. Janzen. 1995. The prevalence of bovine viral diarrhea virus in a population of feedlot calves in western Canada. Can. J. Vet. Res. 59(2):87-93.

Tizard, I. R. 1996. Veterinary Immunology - An Introduction. 5th ed. W.B. Saunders, Sydney.

Urban-Chmiel, R., A. Puchalski and A. Wernicki. Influence of transport stress on susceptibility of bovine leukocytes to the cytotoxic effect of *M. haemolytica* leukotoxin. Medycyna Weterynaryjna. Polskiego Towarzystwa Nauk Weterynaryjnych, Lublin, Poland: 2006. 62(9):1045-1047.

Zhang, S., J.R. Aschenbach, D.R. Barreda and G.B. Penner. 2013. Recovery of absorptive function of the reticulo-rumen and total tract barrier function in beef cattle after short-term feed restriction. J. Anim. Sci. 91: 1696-1706.

6 Appendix

6.1 Appendix 1

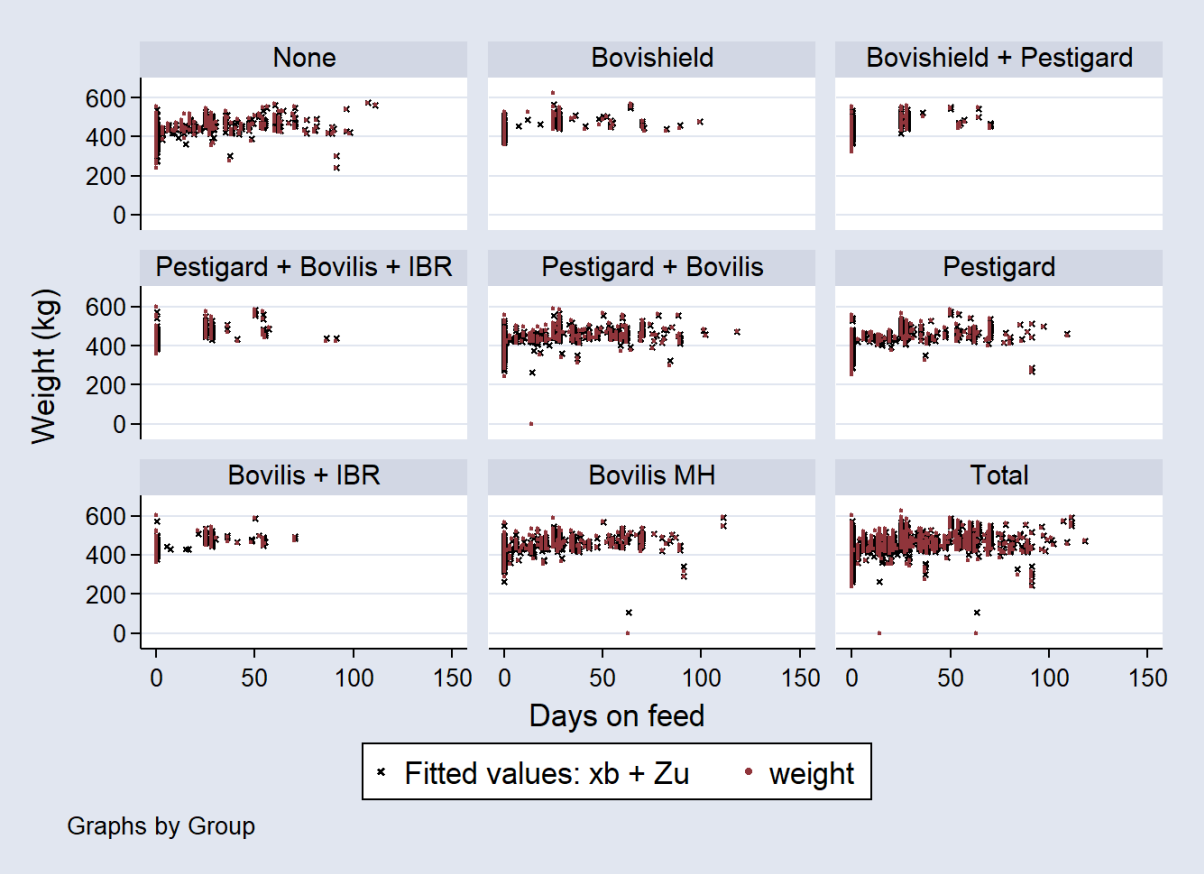
Effect estimates of vaccination group on average daily gain (kg/animal/d) during feedlot phase derived from mixed effects linear regression models

Model 2 (n=6528)					
Model 1 + weight on DOF1	Vaccination group				0.200
	Control	Reference category			
	Bovishield	0.019	-0.027	0.064	0.423
	Bovishield/Pestigard	0.067	0.022	0.112	0.004
	Pestigard/Bovilis MH+ IBR	0.037	-0.008	0.083	0.105
	Pestigard/Bovilis MH	0.031	-0.012	0.073	0.154
	Pestigard	0.034	-0.008	0.077	0.117
	Bovilis MH + IBR	0.031	-0.015	0.076	0.188
	Bovilis MH	0.018	-0.025	0.060	0.412
	Weight on DOF1	0.000	-0.001	0.000	0.023
	intercept	1.499	0.751	2.247	
Model 3 (n=6528)					
Model 2 + breed, sex, duration of backgrounding.	Group				0.185
	Control	Reference category			
	Bovishield	0.018	-0.027	0.063	0.432
	Bovishield/Pestigard	0.066	0.021	0.111	0.004
	Pestigard/Bovilis MH+ IBR	0.038	-0.007	0.083	0.101
	Pestigard/Bovilis MH	0.033	-0.009	0.075	0.124
	Pestigard	0.033	-0.009	0.076	0.122
	Bovilis MH+IBR	0.031	-0.014	0.076	0.183
	Bovilis MH	0.015	-0.027	0.057	0.487
	Breed				<0.001
	Angus/British	Reference category			
	Hereford	-0.125	-0.165	-0.084	<0.001
	European	0.036	-0.047	0.119	0.392
	Bos indicus	0.065	-0.013	0.144	0.103
	Weight on DOF1	0.000	-0.001	0.000	0.004
	Days from entry to backgrounding to DOF1	-0.002	-0.004	0.000	0.052
	Sex				
	Heifer	Reference category			
	Steer	0.170	0.109	0.231	<0.001
	intercept	1.601	0.848	2.354	
Model 4 (n=5462)					
Model 3 + dentition + BVDV & BHV1 serostatus at entry to backgrounding	Vaccination group				0.057
	Control	Reference category			
	Bovishield	0.026	-0.015	0.067	0.216

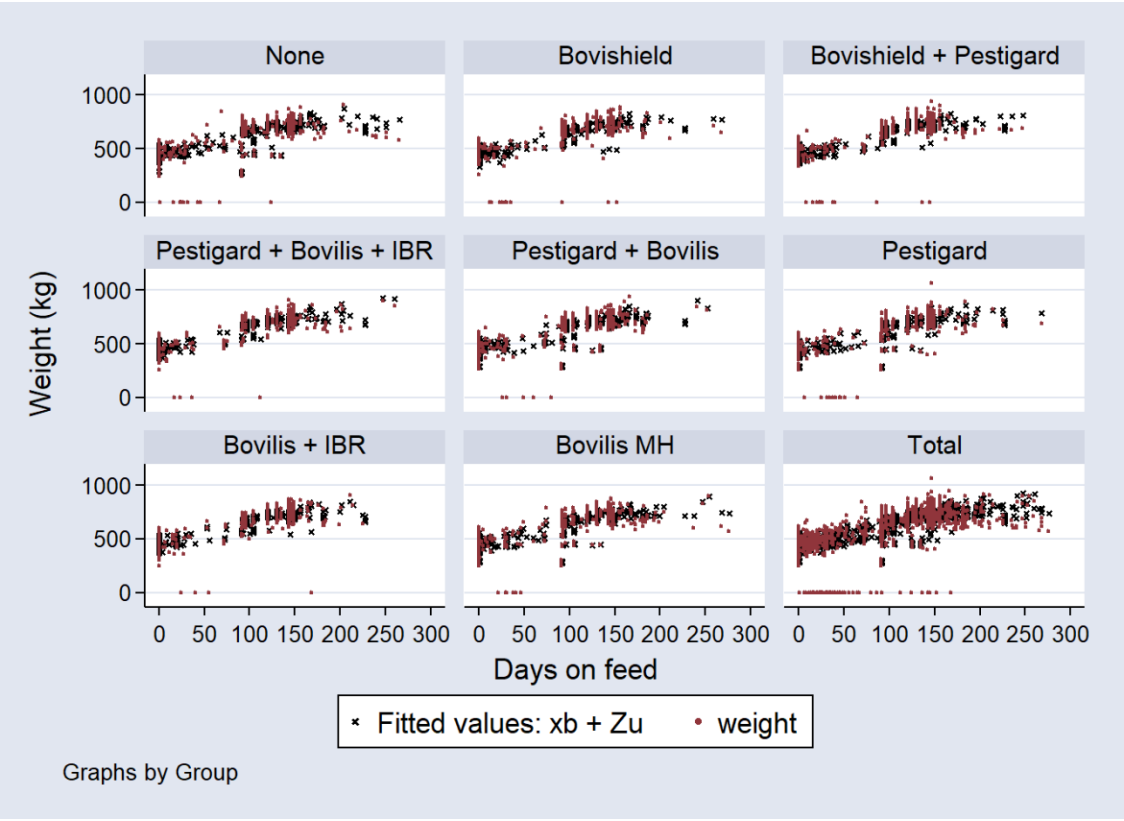
Bovishield/Pestigard	0.065	0.025	0.106	0.002
Pestigard/Bovilis MH+IBR	0.037	-0.004	0.078	0.076
Pestigard/ Bovilis MH	0.023	-0.018	0.064	0.281
Pestigard	0.050	0.009	0.091	0.017
Bovilis MH + IBR	0.033	-0.008	0.074	0.112
Bovilis MH	0.013	-0.028	0.054	0.549
Breed				<0.001
Angus/British	Reference category			
Hereford	-0.116	-0.155	-0.076	<0.001
European	0.088	-0.012	0.188	0.086
Bos indicus	0.078	-0.021	0.177	0.122
Weight on DOF1	-0.001	-0.001	0.000	<0.001
Days from entry to backgrounding to DOF1	0.019	0.010	0.027	<0.001
Dentition				<0.001
0	Reference category			
2	-0.102	-0.134	-0.069	<0.001
4+	-0.200	-0.312	-0.088	<0.001
Sex				
Heifer	Reference category			
Steer	-0.096	-0.196	0.004	0.059
BVDV serostatus at entry to backgrounding				0.196
seronegative	Reference category			
weak positive	0.086	-0.025	0.198	0.127
positive	0.015	-0.010	0.039	0.253
BHV1 serostatus at entry to backgrounding				0.031
seronegative	Reference category			
weak positive	-0.153	-0.274	-0.032	0.013
positive	0.012	-0.019	0.043	0.442
intercept	0.663	0.173	1.152	0.008

*Coefficients for exposure variables estimate the difference in mean average daily gain between the respective category and the reference category; coefficients for intercepts estimate the average daily gain in feedlot 1 for animals at the reference values for all fitted categorical exposure variables and at values of 0 for continuous exposure variables

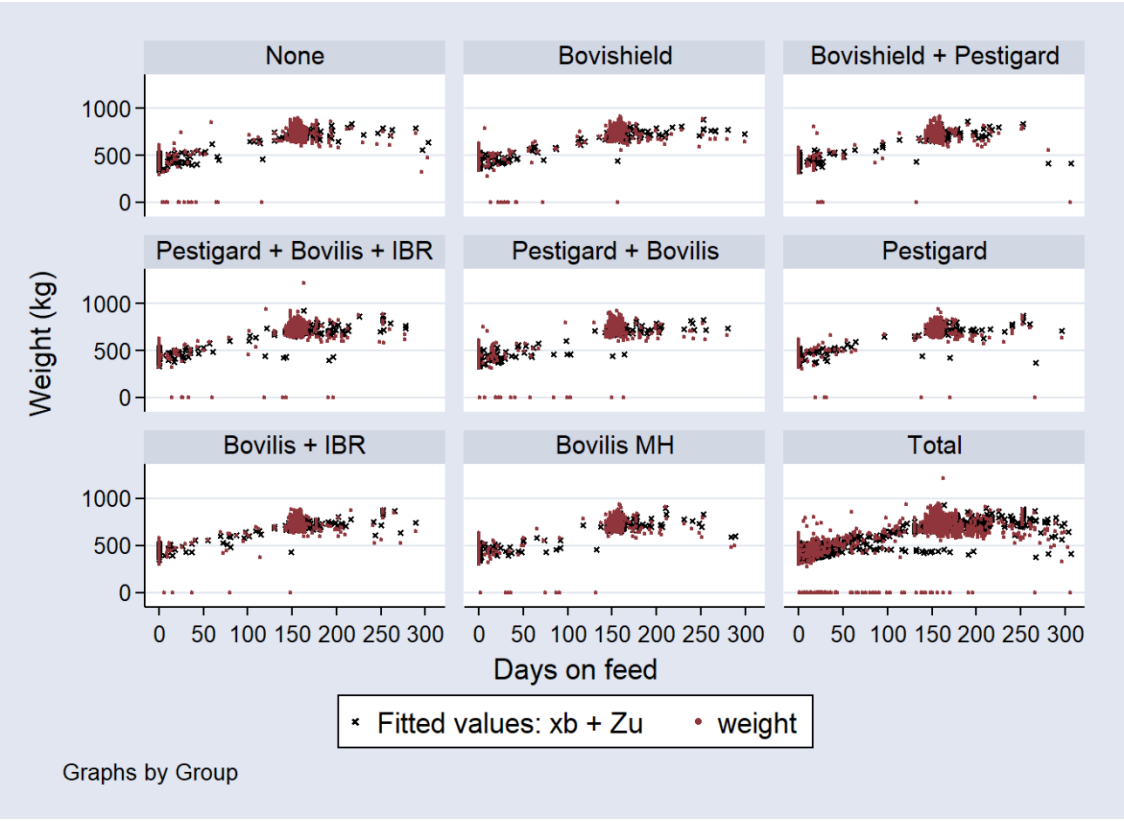
6.2 Appendix 2



Appendix 2. Figure 1: Scatter plots of predicted versus observed animal-level weights over time by vaccine group for animals in cohorts with average DOF<80 days



Appendix 2. Figure 2: Scatter plots of predicted versus observed animal-level weights over time by vaccine group for animals in cohorts with average DOF from 80-154 days



Appendix 2. Figure 3: Scatter plots of predicted versus observed animal-level weights over time by vaccine group for animals in cohorts with average DOF>=155 days

6.3 Appendix 3

Table 1. Effect estimates of vaccine group on risk of BRD during the feedlot phase derived from competing risk regression models adjusted for feedlot and variables indicated

Model	Variable and Category	SHR	95% CI		p-value
2 (Sex)					
	Group				<0.001
	Control	Reference category			
	Bovishield	0.77	0.50	1.20	0.254
	Bovishield/Pestigard	0.74	0.52	1.07	0.114
	Pestigard/Bovilis MH+IBR	0.71	0.47	1.07	0.102
	Pestigard/Bovilis	1.33	0.94	1.87	0.105
	Pestigard	0.76	0.50	1.17	0.219
	Bovilis MH+IBR	0.47	0.27	0.84	0.010
	Bovilis MH	0.87	0.50	1.50	0.621
	Sex				
	Heifer	Reference category			
	Steer	5.81	2.65	12.76	<0.001
3 (Weight on first DOF)	Group				<0.001
	Control	Reference category			
	Bovishield	0.76	0.48	1.21	0.250
	Bovishield/Pestigard	0.74	0.52	1.06	0.103
	Pestigard/Bovilis MH + IBR	0.69	0.46	1.04	0.075
	Pestigard/Bovilis	1.28	0.92	1.79	0.149
	Pestigard	0.76	0.49	1.17	0.205
	Bovilis MH+IBR	0.47	0.27	0.82	0.008
	Bovilis MH	0.86	0.50	1.48	0.591
	Weight on first DOF (kg)				<0.001
	<400	Reference category			
	400-<460	0.43	0.31	0.59	<0.001
	460-<500	0.35	0.21	0.59	<0.001
	≥ 500	0.29	0.15	0.57	<0.001
4 (breed)	Group				<0.001
	Control	Reference category			
	Bovishield	0.86	0.56	1.31	0.485
	Bovishield/Pestigard	0.89	0.67	1.20	0.456
	Pestigard/Bovilis MH+IBR	0.84	0.57	1.24	0.388
	Pestigard/Bovilis	1.44	1.02	2.02	0.037
	Pestigard	0.91	0.62	1.32	0.612
	Bovilis MH+IBR	0.43	0.24	0.77	0.005
	Bovilis MH	1.01	0.60	1.70	0.959
	Breed categroy (kg)				
	Angus/British/ Murray Grey/Shorthorn	Reference category			
	Hereford	1.21	0.84	1.76	0.308
	European or cross	0.23	0.03	2.13	0.198
	Bos indicus or cross	0.00	0.00	0.00	0.000
5 (duration of backgrounding)	Group				
	Control				
	Bovishield	0.78	0.50	1.21	0.264
	Bovishield/Pestigard	0.75	0.52	1.07	0.115

Pestigard/Bovilis MH+IBR	0.71	0.47	1.07	0.103
Pestigard/Bovilis	1.32	0.94	1.86	0.108
Pestigard	0.77	0.50	1.17	0.219
Bovilis MH+IBR	0.47	0.27	0.83	0.010
Bovilis MH	0.87	0.51	1.50	0.624
Backgrounding duration				
28-55 days	Reference category			
>55 days	0.51	0.03	8.04	0.636

*Subhazard ratio

Table 2. Effect estimates of vaccine group on risk of BRD derived from competing risk regression models using the subset population with serology data (n=5797). All models are adjusted for feedlot

	Model C:				Model D: adjusted for breed, sex, weight on DOF1, backgrounding duration and BVDV and BHV1 serostatus at entry to backgrounding				Model E: adjusted for breed, sex, weight on DOF1 and backgrounding duration			
	SHR*	95% CI		p-value	SHR	95% CI		p-value	SHR	95% CI		*P*-value
Group				<0.001				<0.001				<0.001
Control	Reference category				Reference category				Reference category			
Bovishield	0.74	0.47	1.18	0.206	0.77	0.48	1.24	0.190	0.72	0.44	1.16	0.174
Bovishield/ Pestigard	0.64	0.43	0.93	0.020	0.67	0.46	0.97	0.014	0.63	0.43	0.92	0.016
Pestigard/ Bovilis MH+IBR	0.70	0.45	1.08	0.109	0.68	0.45	1.04	0.325	0.68	0.45	1.04	0.074
Pestigard/ Bovilis MH	1.28	0.89	1.84	0.178	1.22	0.85	1.76	0.269	1.23	0.87	1.74	0.247
Pestigard	0.71	0.45	1.10	0.123	0.72	0.46	1.14	0.102	0.68	0.44	1.07	0.099
Bovilis MH+IBR	0.45	0.24	0.85	0.014	0.47	0.27	0.82	0.041	0.44	0.24	0.80	0.008
Bovilis MH	0.82	0.48	1.40	0.472	0.86	0.48	1.56	0.471	0.81	0.48	1.38	0.446

*Subhazard ratio

Table 3. Effect estimates of vaccine group on risk of BRD derived from competing risk regression models using the subsets indicated

Subset			n (%)	SHR*	95% CI		*P*-value
F: Seronegative to BVDV and BHV1 at entry to backgrounding							
		Group					0.073
	1	Control	227 (13)	Reference category			
	2	Bovishield	220 (13)	0.80	0.42	1.53	0.499
	3	Bovishield/Pestigard	240 (14)	0.58	0.34	0.99	0.048
	4	Pestigard/ Bovilis MH + IBR	212 (12)	0.48	0.24	1.00	0.049
	5	Pestigard/ Bovilis MH	221 (13)	1.00	0.61	1.63	0.994
	6	Pestigard	208 (12)	0.87	0.44	1.73	0.692
	7	Bovilis MH + IBR	206 (12)	0.49	0.21	1.14	0.098
	8	Bovilis MH	206 (12)	0.94	0.43	2.06	0.877
		Total	1740 (100)				
G: Rhinogard at feedlot entry							
		Group					<0.001
	1	Control	283 (19)	Reference category			
	2	Bovishield	108 (7)	0.38	0.09	1.62	0.191
	3	Bovishield/Pestigard	115 (8)	0.00	0.00	0.00	<0.001
	5	Pestigard/Bovilis	345 (23)	0.57	0.16	1.95	0.367
	6	Pestigard	289 (20)	0.12	0.02	0.94	0.044
	8	Bovilis MH	334 (23)	0.35	0.12	0.97	0.048
		Total	1474 (100)				
H: No Rhinogard at feedlot entry							
		Group					<0.001
	1	Control	672 (12)	Reference category			
	2	Bovishield	672 (12)	0.88	0.57	1.36	0.570
	3	Bovishield/Pestigard	674 (12)	0.91	0.67	1.23	0.526
	4	Pestigard/Bovilis MH + IBR	778 (14)	0.74	0.47	1.16	0.188
	5	Pestigard/ Bovilis	663 (12)	1.52	1.06	2.18	0.022
	6	Pestigard	658 (12)	0.93	0.63	1.37	0.720
	7	Bovilis MH + IBR	768 (14)	0.50	0.28	0.88	0.017
	8	Bovilis MH	652 (12)	1.01	0.58	1.75	0.973
		Total	5537 (100)				

*Subhazard ratio

Table 3. Effect estimates of vaccine components on risk of BRD derived from competing risk regression models using the population with serology results

Model	Group	SHR*	95% CI		*P*-value
1 n=5926	No vaccine & seronegative	Reference category			
	M H vaccine (Bovishield or Bovilis MH)	1.04	0.86	1.26	0.662
	Pestigard vaccine	1.08	0.88	1.34	0.469
	BHV1 vaccine	0.66	0.45	0.97	0.033
	Seronegative to BVDV at entry to backgrounding	Reference category			
	Seropositive to BVDV at entry to backgrounding	0.56	0.39	0.80	0.001
	Seronegative to BHV1 at entry to backgrounding	Reference category			
	Seropositive to BHV1 at entry to backgrounding	0.79	0.54	1.15	0.215

*Subhazard ratio

Table 4. Predicted relative subhazards derived from model fitted with the variables indicated with interactions terms for Pestigard*BVDV serostatus at entry to backgrounding and IBR vaccine*BHV1 serostatus at entry to backgrounding

Man haem vaccine	Pestigard vaccine	BVDV seropositive at entry to backgrounding	IBR vaccine	BHV1 seropositive at entry to backgrounding	Predicted subhazard	95% CI	
No	No	No	No	No	1.38	-0.38	3.13
No	No	No	No	Yes	1.00	-0.25	2.24
No	No	No	Yes	No	0.86	-0.19	1.91
No	No	No	Yes	Yes	0.92	-0.32	2.16
No	No	Yes	No	No	0.65	-0.17	1.46
No	No	Yes	No	Yes	0.47	-0.11	1.05
No	No	Yes	Yes	No	0.40	-0.10	0.91
No	No	Yes	Yes	Yes	0.43	-0.18	1.05
No	Yes	No	No	No	1.26	-0.34	2.86
No	Yes	No	No	Yes	0.91	-0.21	2.03
No	Yes	No	Yes	No	0.79	-0.21	1.78
No	Yes	No	Yes	Yes	0.84	-0.35	2.03
No	Yes	Yes	No	No	0.83	-0.27	1.94
No	Yes	Yes	No	Yes	0.60	-0.16	1.37
No	Yes	Yes	Yes	No	0.52	-0.16	1.20
No	Yes	Yes	Yes	Yes	0.55	-0.25	1.36
Yes	No	No	No	No	1.44	-0.37	3.25
Yes	No	No	No	Yes	1.04	-0.22	2.31
Yes	No	No	Yes	No	0.90	-0.17	1.97
Yes	No	No	Yes	Yes	0.96	-0.29	2.21
Yes	No	Yes	No	No	0.68	-0.16	1.51
Yes	No	Yes	No	Yes	0.49	-0.09	1.07
Yes	No	Yes	Yes	No	0.42	-0.08	0.93
Yes	No	Yes	Yes	Yes	0.45	-0.17	1.07
Yes	Yes	No	No	No	1.32	-0.31	2.95
Yes	Yes	No	No	Yes	0.96	-0.17	2.08
Yes	Yes	No	Yes	No	0.82	-0.17	1.82
Yes	Yes	No	Yes	Yes	0.88	-0.32	2.08
Yes	Yes	Yes	No	No	0.87	-0.28	2.02
Yes	Yes	Yes	No	Yes	0.63	-0.15	1.41
Yes	Yes	Yes	Yes	No	0.54	-0.15	1.24
Yes	Yes	Yes	Yes	Yes	0.58	-0.24	1.41