

**ANALYSIS OF TRAIT-BASED VARIATION IN BOVINE EXPOSURE TO  
VIRAL RESPIRATORY TRACT INFECTIONS AT THE WILDLIFE-  
LIVESTOCK INTERFACE IN THE MNISI COMMUNAL FARMING  
AREA OF SOUTH AFRICA**

**By**

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## **Declaration**

I, Kramer Manyetu declare that this dissertation which I am submitting in partial fulfillment of the Master of Science (Animal/Human/Ecosystem Health) at the University of Pretoria, South Africa is my own work. This work has not been submitted for a degree at any university.

.....

**Kramer Manyetu**

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## List of abbreviations

BAV-3: Bovine adenovirus-3

BoHV-1: Bovine herpesvirus-1

BRDC: Bovine Respiratory Disease Complex

BRSV: Bovine respiratory syncytial virus

BVDV: Bovine viral diarrhoea virus

ELISA: Enzyme-linked immunosorbent assay

FMD: Foot and mouth disease

GLTCA: Great Limpopo Transfrontier Conservation Area

HHWRS: Hans Hoheisen Wildlife Research Station

KNP: Kruger National Park

MCP: Mnisi Community Project

PI-3: Parainfluenza virus -3

PI: Persistently Infected

SADC: Southern African Development Community

UP: University of Pretoria

## SUMMARY

Animal diseases have always been one of the main constraints on animal production, especially in Africa where there are a variety of tropical and subtropical diseases. Knowledge of these diseases and the development of approaches to combat them is highly relevant to the socio-economic development of Africa and its fight against poverty. Serological tests were performed to determine seroprevalence and important risk factors for occurrence of respiratory pathogens in cattle on 423 biobanked sera collected from cattle at 11 dip tanks in the Mnisi communal farming area which is on the edge of the Kruger National Park. These pathogens are known to cause significant production losses in livestock by predisposing animals to secondary infections including pneumonia. A pentavalent, indirect ELISA test was performed to estimate seroprevalence of bovine herpesvirus-1, bovine respiratory syncytial virus, bovine viral diarrhoea virus, parainfluenza virus-3 and bovine adenovirus-3 infections in cattle at the wildlife-livestock interface in the Mnisi communal farming area. Previous exposure to the five pathogens was determined. Additionally, the data was analyzed using the statistical software R to determine important risk factors that predicted exposure to the pathogens in cattle, namely population factors (distance from interface and month of collection) and individual characteristics (age, sex, body condition and breed). Age and body condition of the animals were found to have an effect on seropositivity while breed, sex, spatial distribution of the animals and month of sample collection did not have an effect. Recommendations to reduce pathogen exposure and improve production are made to the livestock owners in the Mnisi community.

## CHAPTER 1

### 1.1 Introduction:

Livestock farming is important to the rural economy of most Southern African Development Community (SADC) member states (Devereux, 2012) and to many poor people from the developing countries contributing significantly to poverty reduction. Livestock farming contributes to personal nutritional security as well as income generation (Randolph et al., 2007). Additionally, the keeping of cattle, as opposed to the keeping of other types of livestock, is an important form of wealth accumulation and draught power, contributing to the maintenance of soil fertility and fulfilling a wide range of socio-cultural roles in sub-Saharan Africa (Jahnke and Jahnke, 1982). It is therefore critical to ensure survival and increased productivity of livestock in communities whose livelihoods largely depend on the health of their animals.

Animal production outputs in communal areas with livestock-wildlife interfaces areas are often low because of poor husbandry practices, pasture quality and transmission of infectious diseases (Devereux, 2012) mainly because the livestock is susceptible to the same pathogens that affect wildlife and vice versa (Palmer et al., 2012). As such, in communities which share boundaries with conservation areas, conflicts between livestock owners and conservation managers can occur when there is suspicion of disease spread (Bengis et al., 2002).

Respiratory diseases result in both direct and indirect losses to a farmer. Direct losses are derived from increased mortality and treatment costs for livestock. Indirect losses to a farmer are a result of disease impact on working animals, poor weight gains, the increased calving intervals (Wittum et al., 1996), forced culling

as well as reduced milk production (Elvander, 1996). Indirect losses to a region can be incurred through loss of trade of animals that are perceived to be sick by potential buyers (Otte et al., 2004).

The objective of this study was to describe the patterns of exposure in cattle to five viral respiratory tract pathogens namely bovine herpes virus-1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), parainfluenza virus -3 (PI- 3) and bovine adenovirus-3 (BAV-3) for the purpose of understanding their distribution and transmission at a wildlife-livestock interface. For this purpose we utilized the Mnisi Community in Mpumalanga Province of South Africa on the border of Kruger National Park (KNP) as a model wildlife-livestock system.

The overall aim of this study in conjunction with several related, on-going studies was to find out which pathogens may be shared between the African buffalo and cattle so as to minimize the possibility of pathogen transmission across the interface. Data from this study on patterns of exposure in cattle combined with data from concurrent studies will be used to identify which respiratory pathogens present the greatest likelihood of transmission from buffalo to cattle and vice versa. In cases where transmission is a possibility, recommendations would be developed to minimize pathogen transmission between the two species.

The study will determine seroprevalence and the important risk factors for the occurrence of these five respiratory pathogens in the cattle from the Mnisi community. A report to the community will be produced explaining the findings and recommendations which will subsequently be presented to the entire community with the hope that the recommendations will result in reduced disease occurrence and/or pathogen transmission and thus increase animal productivity.

This study will not do analyses of Bovine Respiratory Disease Complex (BRDC) although we are aware that co-infections are common. Without this study, the distribution and risk factors for occurrence of these pathogens in this area will remain unknown and as such no recommendations to minimize their occurrence would be proposed and be implemented.

Additionally, because the farming systems, climate and proximity to nature reserves are relatively similar in our study location as elsewhere in southern Africa, for example, the community adjacent to the Gonarezhou National Park in Zimbabwe and the community adjacent to Limpopo National Park in Mozambique, we suspect that information collected here will benefit animal management beyond the Mnisi area. When presenting the findings to the livestock owners in the Mnisi community it will also be a platform to engage the community and teach them about these neglected diseases which have likely been affecting their animals.

## **1.2 Problems and hypotheses:**

While several respiratory pathogens are known to affect cattle as well as both captive and wild African buffaloes elsewhere in southern Africa (Coetzer et al., 2006), knowledge of the identity and epidemiology of these pathogens in the Mnisi community will enable management suggestions for other livestock-wildlife interfaces throughout southern Africa. The study will examine the distribution of these pathogens and the most important risk factors for their occurrence in cattle in the Mnisi community area.

The study sought to determine whether distance from Kruger Park (herd location) predisposes cattle to the viruses, with cattle closer to the park being more likely to

make contact with wild buffaloes in which the viruses are now known to occur. The study will also assess whether our pathogens of interest were more prevalent in animals with certain traits (age, sex, body condition, and breed) or during certain month. Perhaps most importantly, the study aims to test the following hypotheses:

- (i) Cattle from the dip tanks nearest to Kruger Park should have high seroprevalences of the five viruses compared to cattle from dip tanks that are further away from the park. This is because cattle closer to the interface are more likely to be exposed to the pathogens which are now known to occur in the African buffalo.
- (ii) Cattle above one year of age should have high seroprevalences of the five viruses compared to cattle below one year of age because animals above one year would have been exposed for a longer period of time compared to animals below one year of age.
- (iii) Male cattle should have higher seroprevalences of the five viruses compared to female animals because the male animals are subjected to stressful conditions like castrations and some being used for draught power.
- (iv) Animals in poor body condition (score of 2 out of 5) should have higher seroprevalences of the five viruses compared to animals in good body condition (score of 3.5 and 4 out of 5) because the poor body condition maybe an indicator of an underlying infection by pathogens.
- (v) Sanga cattle should have lower seroprevalences for the five viruses compared to the Brahman cattle because it is an indigenous breed which has some resistance towards some pathogens.



- (vi) Cattle sampled in winter (May, June and July) should have higher seroprevalences for the viruses compared to cattle sampled during the other months (April, August and September) because some animals especially calves are housed during winter increasing chances of exposure to respiratory pathogens during that period.

## CHAPTER 2

### 2. Literature Review

#### 2.1 Pathogens

##### 2.1.1 Bovine herpesvirus (BoHV-1)

BoHV-1 belongs to the family *Herpesviridae* and subfamily *Alphaherpesvirinae*, and causes many diseases in cattle. The common ones are bovine rhinotracheitis, vaginitis, balanoposthitis, abortion, conjunctivitis, and enteritis (Fenner et al., 1993). The pathogen is transmitted directly via nose to nose contact from infected to susceptible animals. The pathogen is also spread during mating, artificial insemination and aerosol transmission as well as vertically across the placenta (Muylkens et al., 2007).

##### Clinical signs and pathogenesis

BoHV-1 infections are known to occur in cattle populations worldwide causing either clinical or subclinical infections, depending on the virulence of the strain. The virus enters the animal through the mucous membrane of the respiratory tract and genital tracts. The incubation period for the respiratory and genital forms varies from two to six days. Once an animal is infected, it is difficult to clear BoHV-1 because the virus has many ways of avoiding the host animal's innate and adaptive immune system, resulting in latent infections (Muylkens et al., 2007). A good example is when the virus suppresses interferon regulatory factor 3, effectively stopping transcription of interferon type 1 which is important in the innate immune response against viral infections (Nandi et al., 2009). Interferons

are a component of innate immunity involved in inhibiting viral replication in a host cell, as well as stimulating immune cells. BoHV-1 is also able to evade adaptive immune cells by inducing apoptosis in CD4+, which assist in activating T cells when antigens are present (Smits et al., 2000). This reduces the number of immune cells that recognize the virus, resulting in the virus evading detection and elimination.

BoHV-1 is initially shed in the nasal mucosa immediately after infection. Infection by the virus alone may not produce any clinical disease but predisposes the animals to secondary bacterial pneumonia, which may be fatal. The epithelial cells of the respiratory tract undergo viral induced apoptosis during the viral replication process. Damage to the epithelial cells provides an entry site for secondary bacterial infections and this happens mainly in cases of shipping fever, commonly known as Bovine Respiratory Disease Complex (BRDC), (Lovato et al., 2003).

The respiratory form of BoHV-1 infection (infectious bovine rhinotracheitis) is common in areas with high cattle density such as feedlots and overstocked communities. Clinical signs depend on whether infection progresses to secondary bacterial pneumonia. The common clinical signs are fever, conjunctivitis with corneal opacity, loss of appetite and a clear to mucopurulent nasal discharge causing coughing, sneezing and difficult breathing. The infection is characterized by ulcers in the mouth and nose producing an inflamed muzzle (red nose) and mortality can be as high as ten percent. Where there is no progression to secondary pneumonia, recovery generally occurs within a week (Fenner et al., 1993).

In breeding cattle, the infection commonly presents as abortions and genital infections. Abortions usually occur in mid gestation and may occur together with respiratory disease (Carter et al, 2002). Organ necrosis and early embryonic deaths

occur when the virus crosses the placenta during the viraemia phase. Genital infections (infectious pustular balanoposthitis in bulls and infectious pustular vaginitis in cows) are another clinical manifestation observed within one to three days after mating or close contact with an infected animal. The early signs are frequent urination, elevation of the tail head, a mild vaginal discharge, swollen vulva and small papules, and then later on erosions and ulcerations on the mucosal surface. In cases where no secondary bacterial infections occur, recovery occurs in ten to fourteen days. However, where bacterial comes in, swelling of the uterus with purulent vaginal discharge occurs for several weeks. This also results in transient infertility. The same lesions are seen on the penis and prepuce in bulls (Miller and Maaten, 1987).

BoHV-1 infection may sometimes be severe in young calves producing generalized disease. The usually observed clinical signs are fever, ocular and nasal discharges, respiratory distress, diarrhoea, incoordination and eventually convulsions and death may occur shortly after generalized viral infection. Cattle with latent BoHV-1 infections in herds, in which the virus is endemic, generally do not show clinical signs upon viral reactivation as a result of stress, immunosuppression or other infections, but are a source of infection for other susceptible animals (Muylkens et al., 2007).

### **2.1.2 Bovine respiratory syncytial virus (BRSV)**

BRSV is an enveloped virus pneumovirus from the *Paramyxoviridae* family. The virus derives its name from the characteristic cytopathic effect in which it uses fusion proteins to facilitate the formation of syncytial cells. Outbreaks are usually

associated with housing animals in facilities with inadequate ventilation and stressful periods like mixing of calves and transportation to feedlots. During outbreaks, morbidity is known to be high, and the mortality rate can be up to twenty percent (Silva, 2012). Subclinical re-infections are important in spreading disease (Radostits et al., 2000).

### **Clinical signs and pathogenesis**

BRSV is associated with respiratory disease in all ages but disease is severe in young stock (2 to 5 years) in both beef and dairy cattle (Brodersen, 2010).

Transmission occurs when an infected animal sheds the virus in secretions such as nasal discharges allowing susceptible animals to inhale the virus. The virus is also be spread by fomites or people that have had contact with infective secretions, though the virus is very labile and does not live long in the environment. The infection occurs worldwide and is a big threat to cattle health. BRSV is very common and studies have found that almost 70% of calves going into feedlots have been previously exposed to the infection (Bryson, 1999).

BRSV replicates in nasal epithelium before spreading throughout the upper respiratory tract and the whole bronchial tree. The virus then replicates in type 2 pneumocytes and alveolar macrophages, which are two cell types associated with lung epithelium (Kirchhoff, 2014). The virus interferes with phagocytosis of the alveolar macrophages and later causes damage to epithelial cells. This results in death or damage of the cilia cells. This compromises the immune system of the lungs and makes the animal more prone to secondary infections (Blood and Studdert, 1999).

Gross lesions associated with BRSV infections are found mostly in the lungs presenting as a diffuse interstitial pneumonia with sub-pleural and interstitial emphysema as well as interstitial edema. Secondary bronchopneumonia caused by bacterial invasion is also usually present (Baker et al., 1997). On microscopy, syncytial cells in bronchiolar epithelium and lung parenchyma, intracytoplasmic inclusion bodies, proliferation and/or degeneration of bronchiolar epithelium, alveolar epithelialization, edema, and hyaline membrane formation are usually present. Again secondary bacterial pneumonia is a frequent occurrence (Andrews et al, 2004).

While BRSV can cause mild disease on its own, it is also a component of the BRDC along with parainfluenza viruses and herpesviruses as well as the bacteria *Pasteurella multocida*, *Mannheimia haemolytica* and *Mycoplasma bovis*. Clinical signs for BRDC in which BRSV is involved include fever, decreased appetite, increased respiratory rate, cough, nasal discharges and lacrimation. In adult cattle, a drop in milk yield and dyspnea may occur in severe cases during the later stages (Divers and Peek, 2008).

### **2.1.3 Bovine viral diarrhoea virus (BVDV)**

BVDV is a very common disease of cattle with economic importance. The disease is endemic in several countries worldwide (Fray et al., 2000). The virus belongs to the genus *Pestivirus* and the family *Flaviviridae* (Lindenbach and Rice, 2001). It is an important disease of cattle across the whole world because of its high prevalence, persistence and clinical consequences (Moennig et al., 2005). Prevalence has been found to be positively associated with high stocking density in

cattle. The virus can be transmitted both horizontally and vertically. Both persistently and transiently infected animals shed infectious virus (Kirkland, 1991). However PI animals are the most important source of the virus as they continuously shed a high viral load which is one thousand more than the amount shed by acutely infected animals (Brownlie et al., 1987). Transmission of BVDV occurs through direct contact, bodily secretions and contaminated fomites because the virus can survive in the environment for more than two weeks (Bryson et al., 1983).

### **Clinical signs and pathogenesis**

Replication occurs in epithelial cells following viral invasion of oral and nasal mucosa. Viral replication also occurs in the palatine tonsils, lymphoid tissues and epithelium of the oropharynx. Phagocytes are responsible for taking up cells infected by the virus and transporting them to peripheral lymphoid tissues. The virus can also spread through the blood producing a viraemia two to four days after exposure (Fray et al., 1998). It is during this systemic spread that the virus enters most body tissues especially lymphoid tissues (Lanyon et al., 2014).

BVDV infection produces many clinical signs because of its suppression of the immune system (Schaut et al., 2015) and the direct effect on the respiratory system and fertility (Lanyon et al., 2014). The wide manifestations of clinical signs in older cattle include infertility, drop in milk yield, fever, diarrhoea and fetal infection. In younger cattle, BVDV may cause diarrhoea, calf pneumonia and cerebellar hypoplasia which presents as lack of voluntary coordination of muscle movements (ataxia), tremors, stumbling, failure to suckle and in severe cases death. BVDV may occasionally present as a severe acute form characterized by high morbidity and mortality. However, the clinical signs are usually mild and

infection insidious, recognized only as its immunosuppressive effects perpetuating other circulating infectious diseases, particularly scours and pneumonia (Maclachlan and Dubovi, 2010).

### **Persistently infected (PI) animals (Intrauterine infections)**

Animals can become PI with BVDV if they are initially infected transplacentally, before they develop a competent immune system such that the virus becomes accepted as self. A PI animal recognizes viral particles inside the cells as part of itself and sheds very huge quantities during the course of their life. This is critical in maintaining a high prevalence and is the major reason behind BVD success as a disease (Brownlie et al., 1984). Although PI animals are usually less than one percent of all animals in BVDV endemic areas, they are the ones that ensure viral persistence in the host population. The virus remains present in large numbers and is continuously shed throughout the animal's life in PI animals. These PI animals are more susceptible to other diseases, and less than 20% survive to two years (Voges, 2006). They are usually frail with poor growth rates, though at times they appear normal (Duffell and Harkness, 1985). If a PI dam reproduces, it will at all times give birth to persistently infected animals (Moennig and Liess, 1995).

The effect of BVDV infection on the fetus depends upon the stage of pregnancy at which the dam is initially infected. Infection before conception and during the first 18 days of gestation results in delayed conception which in turn increases the calving to conception interval. Infection of dam from day 29 to 41 of gestation (when the embryo is already attached to the placenta) can result in embryonic infection leading to early embryonic death. If the dam is infected between 30 and 120 days immune-tolerance may occur resulting in the birth of calves persistently infected with the virus (Grooms, 2004). BVDV infection between 80 and 150 days



of gestation may be teratogenic, with the type of birth defect dependent upon the stage of fetal development at infection. Abortion may occur at any time during gestation. Infection after day 120 of gestation can result in the birth of a normal fetus which is BVD antigen-negative and BVDV antibody-positive. This is because the fetus would have received maternal antibodies at this stage of gestation and is able to recognize and fight off the invading virus, producing anti-BVDV antibodies (Fray et al., 2000).

### **Transient, acute and chronic infections**

In cases of infection of a susceptible or non vaccinated cow or calf, one of many situations can occur; transient infection where shedding of the virus occurs for a few days without showing any clinical signs. The animal then develops immunity and is able to clear the infection. This animal is safe to keep in the herd. Acute infection may result in respiratory signs but the animal may clear the infection in 10 to 30 days. BVDV can also be maintained as a chronic infection within some immune-privileged sites following transient infection. These sites include ovarian follicles, testicular tissues, central nervous system and white blood cells. Cattle with chronic infections produce strong immune response, exhibited by extremely high antibody titers (Moennig and Liess, 1995).

### **Mucosal Disease**

Mucosal disease develops only in PI cattle (infected in the uterus) that survive and is usually fatal (Lanyon et al., 2014). The disease occurs when a PI animal is super-infected with a cytopathic biotype of the virus arising from mutation of the non-cytopathic strain of BVDV already circulating in that animal (Brownlie et al., 1987). The cytopathic BVDV invades to the gastro-intestinal epithelium and

causes necrosis of keratinocytes resulting in erosions and ulcers. There is leakage of fluid from the epithelial surface of the gastro-intestinal tract then causes diarrhoea and dehydration. Additionally, bacterial infection of the damaged epithelium results in secondary septicaemia and death within days or weeks (Peterhans et al., 2010).

#### **2.1.4 Parainfluenza-3 virus (PI-3)**

PI-3 is an RNA virus in the family *Paramyxoviridae*. PI-3 infections are common and affect all ages of cattle, but mostly calves or cattle housed during winter. Occurrence is usually associated with stressful conditions like housing of animals of different age groups, poor nutrition, transportation, winter housing and poor hygiene (Andrews et al., 2004). The virus on its own does not usually generate disease in cattle, but together with other pathogens such as those in the BRDC, it can cause enzootic pneumonia in calves (Blood and Studdert, 1999).

#### **Clinical findings and pathogenesis**

The most important role of PI-3 is to serve as an initiator facilitating development of secondary bacterial pneumonia (Babiuk, 1998). PI-3 causes epithelial necrosis and cessation of ciliary clearance, predisposing animals to secondary bacterial infections and ultimately pneumonia. The necrosis is a result of viral replication in airway epithelial cells causing initially bronchitis, and later bronchiolitis before extension into alveoli, causing bronchointerstitial pneumonia. Early stages usually show intracytoplasmic inclusions and the produced exudate is predominantly neutrophilic (Bridger and Russel, 2007).

PI-3 infection alone may cause rhinitis. However, clinical signs worsen with secondary infections and the onset of bacterial pneumonia in calf pneumonia complex producing fever, coughing, general malaise, nasal and lacrimal discharges, increased respiratory rate and breathing sounds. Deaths from uncomplicated PI-3 pneumonia are rare. The common lesions include cranio-ventral lung consolidation, bronchiolitis, and alveolitis with marked congestion and haemorrhage. Inclusion bodies may be identified and most fatal cases have a concurrent bacterial bronchopneumonia (Radostits et al., 2000).

### **2.1.5 Adenovirus 3 (BAV-3)**

BAV-3 belongs to the family *Adenoviridae* and causes disease in cattle. BAV-3 occurs across the whole world but is particularly common in Africa and Central America (Baber and Candy, 1981). Transmission occurs via aerosols as the virus is shed in respiratory secretions (Benko et al., 2000). Ten serotypes of bovine adenovirus have been identified across the world. However particular serotypes have been found to predominate from time to time in particular geographic regions. (Zee, 1999)

#### **Clinical signs & pathogenesis**

The primary targets for adenovirus infection are the respiratory and enteric tracts. The infection causes cell lysis and virus shedding, while some cells accumulate virus particles in their nucleus, establishing persistent infections (Benko et al, 1989). After infection, cattle can shed BAV-3 for approximately ten days in nasal secretions, tracheal fluids, intestinal contents and / or faeces. Some cattle become persistently infected, excreting the virus for much longer (Aldasy et al., 1965).

When the kidneys are involved, the virus is shed for more than ten weeks in urine. With persistent infection, lysis of fragile infected cells in the nasal cavity can result in infection of susceptible animals that come in contact with the virus in the discharge (Boros et al., 1985).

Gross lesions associated with BAV-3 include atelectasis and consolidation of the lungs and erosions, ulcerations and haemorrhage in the intestinal tract. There is enlargement of bronchiolar, mediastinal, and mesenteric lymph nodes.

Bronchiolitis with necrosis and sloughing occur early and hyperplasia occurring later on in the course of the infection. On microscopy, amphophilic, intranuclear inclusions are seen in swollen respiratory epithelium and sloughed cells in the lumen of the gut. In the gastrointestinal tract, the basic lesions are fibrinonecrotic plaques overlying foci of haemorrhage and necrosis (Lehmkuhl, 1979).

Successful infection produces disease of the gastrointestinal or respiratory tract and may cause ocular as well as generalized signs. BAV-3 is known to contribute to BRDC of calves. However, infection with BAV-3 does not always result in clinical disease as the virus can be isolated in healthy cattle (Pardon et al, 2011). Clinical signs in BRDC are common in calves at the time when levels of maternal antibodies begin to wane, from the age of five to six months. In cattle, the virus has been shown to be able to infect any breed, sex or age. The common gastrointestinal signs are diarrhoea, a reduced feed intake and distended abdomen while the respiratory signs include coughing, nasal discharges, laboured breathing and rapid breathing (Caldow et al., 1993). These clinical signs worsen when secondary infection comes in resulting in bronchopneumonia. The following classical signs of a generalized disease, like fever, rapid decrease in body condition, general weakness, lymphadenopathy and lethargy may be seen (Lehmkuhl, 1979).

Additionally, conjunctivitis, keratoconjunctivitis, weak calf syndrome and sudden death have been associated with adenovirus infections (Aldasy et al., 1965).

## **2. 2 Bovine respiratory disease complex (BRDC).**

BRDC is a combination of bacterial and viral infections that causes pneumonia mainly in calves and can be fatal. The condition is usually a result of three co-dependent factors namely stress, an underlying viral infection, and a new bacterial infection (Lillie, 1974). The viruses most frequently associated with BRDC include BoHV-1, BRSV, BVDV, PI-3 and BAV-3. Secondary bacterial pneumonia is typically attributed to *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma* (Frank, 1989).

The pathogenesis initially involves predisposing factors which compromises respiratory defense mechanisms. This coincides with infections by one or more respiratory viruses. Viral infection and the host's poor response to the infection further compromises the animal's defense and allows invasion of deeper pulmonary tissues by bacteria normally carried in the upper respiratory tract, mainly from the family *Pasteurellaceae* (Dyer, 1982). Outbreaks usually occur after stressful periods like shipment of calves to feedlots in large scale livestock farms and thus why it is commonly referred to as "shipping fever." However, a few clinical cases in calves from communal livestock and dairy animals are also recognized (Conlon et al., 1987). BRDC causes huge economic loss in the beef industry and a number of health problems in the dairy industry (Whiteley et al., 1992).

The common clinical signs after infection include lethargy or depression, reduced feed intake, fever, increased respiratory rate, and dyspnea, with or without nasal discharge (Frank, 1989). Recovery may occur if broad-spectrum antibiotics are used as treatment. However, feed conversion, weight gain, and the resulting economic return may be affected significantly (Potgieter et al., 1984). Vaccination against the various implicated bacteria and viruses can help to prevent BRDC occurrence (Taylor et al, 2010).

### **2.3 Costs of BRD to cattle owners**

The five selected pathogens can predispose cattle to secondary bacterial pneumonia which can cause death in livestock. Together, these viruses are major components of the BRDC as well as calf pneumonia syndrome resulting in huge calf mortalities. Bovine adenovirus infections have been reported to cause sudden deaths (Baber, 1981). Dairy farmers incur additional losses when they cull animals persistently infected with BVDV to reduce spread of the virus. However for resource-poor farmers in Africa, these animals remain poor doers and a source of infection for the healthy animals.

While the selected viruses are usually non-life-threatening on their own, they are economically important diseases as infection can cause reduced production as a result of decreased conception rate, abortions, weak and abnormally small calves, poor weight gains, low weaning weights, premature culling of animals, drop in milk yields and calf deaths shortly after birth. Presence of clinical diseases such as mucosal disease and pneumonia can also result in market exclusion as buyers avoid animals perceived to be sick (Graham, 2013; Ames,1997). Large scale

farmers incur a cost when they institute supportive treatment for any of the five pathogens with antibiotics and anti-inflammatory drugs to reduce pain and fever as well as rehydration in cases of BRSV and BVDV. Other losses are a result of the impact of the diseases on the animals such as reduced draught power (Wittum et al. 1996). It is possible to prevent occurrence of these pathogens at a cost through vaccination although the vaccines are not readily available worldwide to the rural farmers. However, there is need for annual booster vaccinations to maintain adequate immunity and that is an additional cost to the farmer.

## **2.4 Opportunity for spillover**

The wildlife-livestock interface has many challenges including disease transmission. The interface allows both direct and indirect contact of wildlife and livestock making it difficult to minimise interaction between the two without considerable spatial separation and huge infrastructure investment (Bengis et al., 2002; Hudson, 2002).

The five viral pathogens studied in this research project are shed in secretions such as nasal discharges, respiratory droplets, genital secretions, semen, fetal fluids and tissues resulting in contamination of pastures, objects, fences and water sources at the livestock-wildlife interface (Belknap, 1993). This allows the transmission of the infectious organisms to susceptible animals that will come to graze on the same pastures or drink from the same water sources. In situations where natural barriers like fences exist, fomites and scavengers may play an important role in disease transmission by contributing to contamination of pastures and water sources. There is also an opportunity for nose to nose viral transmission via aerosols at the interface from either the infected buffalo to cattle or vice versa. This is possible

during the dry seasons and during periods of drought when cattle walk long distances in search of pastures and come near the game fences. The dry season is also associated with nutritional stress which is one of the key factors for occurrence of these pathogens (Fratkin, et al, 1994.). Many disease outbreaks at wildlife/livestock interfaces have been observed during droughts when the direct and indirect contact rates between wildlife and livestock are high (Kock et al., 1999). Destruction of the fence barrier either deliberately by poachers and farmers or by big game animals presents an opportunity for contact between livestock and wildlife, increasing chances of pathogen transmission between the two species. Another basic concept in transmission dynamics is the immune status of the population at a particular time when the disease agent is present. It determines whether contact between infected animals and susceptible animals will allow multiplication of the pathogens leading to successful transmission. The immunity status of the animals is affected by many factors including vaccination, nutrition, age, neo-natal colostrum intake and physical stress (Thrusfield, 1997; Lemaire, 2000). One of the most important driving factors for disease occurrence at wildlife-livestock interfaces are human activities and effects on the environment like overstocking, deforestation and veld fires that change the spatial distribution of hosts and increasing contact rates between wildlife and livestock populations (Bengis et al., 2002; Kruse et al., 2004).

## **2.5 Known prevalence of the pathogens in southern Africa**

The five respiratory pathogens have been shown to occur in many countries in southern Africa. Serological studies on five traditionally managed herds of cattle in the Kafue flats in Zambia indicated high seroprevalences for BVDV (76.2%), PI 3



(94.4%), BoHV-1 (42.1%) and BAV-3 (87.4%) (Ghirotti et al., 1991). In another study in Zambia in traditional cattle in Southern Province of Zambia, prevalences were observed to be lower with BVDV (21%), PI 3 (25%), BAV-3 (25%) and BoHV-1 (23.28%), (Mweene et al., 2004). In 1990, Van Vuuren reported a 43% prevalence of BRSV in feedlot cattle in South Africa.

BVDV occurrence in southern Africa was demonstrated around 1970 through several serological tests that indicated wide spread infections in domestic cattle, wild ruminants including the African Buffalo and other species (Depner et al., 1991; Muvavarirwa et al., 1995). The presence of BVDV in southern Africa was confirmed by Muvavarirwa et al., 1995, who detected an average seroprevalence of 79, 2% in free range cattle in Zimbabwe.

In a study in Namibia, seroprevalence of BVDV antibodies was found to be 58 % in cattle (Depner et al., 1991). Other studies carried out on cattle in South Africa showed BVDV seropositivity of 51% to 77% for the different regions of the country (Theodoridis, Boshoff & Botha, 1973). In other southern African countries, a wider seropositivity range from 6% to 70% has been recorded over a number of years. Some notable upward and downward trends were observed depending on the years when sampling was done (Anderson & Rowe 1998; Depner et al., 1991; Hamblin & Hedger, 1979).

In wildlife, a number of serological surveys also produced BVDV seroprevalence ranging from 6% to 70% in southern African wild ruminants (Scott et al., 2013). Serological studies in wildlife showed that the prevalence of PI-3 in African buffalo ranged from 25% in the shrub land to 86% in the southern part of the KNP in the woodlands (Barnard, 1997). In a serological study in Zimbabwe, 3 out of 51

free-living African buffalo (*Syncerus caffer*) were positive for PI-3 virus (Hamblin et al., 1980).

## CHAPTER 3

### 3. Materials and Methods

#### 3.1 Study location

The Mnisi Community is on the periphery of the Great Limpopo TransFrontier Conservation Area (GLTCA) of South Africa (Figure 1). The majority of the livestock owners are very poor and many rely on agricultural activities such as crop production and livestock rearing (Van Rooyen, 2011). The Mnisi Community Project (MCP) is a research platform managed by the University of Pretoria (UP) for projects that focus on developing One Health approaches for conflicts that occur at human-livestock-wildlife-ecosystem boundaries. The community is in a foot and mouth disease protection zone with vaccination and the only available veterinary service is through the state veterinary service (Mpumalanga Veterinary Services) and the UP-run Hluvukani Animal Health Clinic which provides veterinary services at subsidized rates as part of student training.

In this study area, the majority of the livestock owners are resource poor farmers whose free-ranging animals typically experience seasonal nutritional stress, especially during the end of the dry season when it is necessary to range cattle further in order to obtain the few available nutrient resources remaining. As in many other rural locations, the sub-tropical climate and high cattle densities in communal farming systems in southern Africa also permit ticks to thrive resulting in an additional challenge of tick infestations and, as such, the potential for tick-borne diseases. The stress of seasonal resource shortages and tick infestation can have a direct, negative effect which lowers the immune system of cattle in this area which may then increase susceptibility or morbidity to pathogens (Dowell, 2001).

### 3.2 Target animals

Blood samples which were collected from 422 cattle from 11 different community dip tanks within the Mnisi Community (Table 1) were used. Cattle owners are required to bring all of their cattle for weekly inspection at registered facilities or dip tanks. During this inspection, tick control is also carried out either by plunge dipping or pour-on treatment where there is no dip tank at the inspection point. Dipping was introduced to prevent cattle losses from the buffalo-borne corridor disease (caused by *Theileria parva*) and other tick-borne diseases namely babesiosis, anaplasmosis and heartwater that are prevalent in the area (Choopa, 2015). Dipping also incentive farmers to participate in the weekly inspections as part of the foot and mouth disease control policy (Rikhotso et al., 2005). The categories of animals sampled were calves, heifers, cows, bulls and oxen. There were three distinct beef breeds namely pure Brahman, Brahman cross and pure Sanga.

### 3.3 Sample collection

422 blood samples were collected between March and September 2013 by Dr. Anne Meyer and Professor Darryn Knobel, both of the University of Pretoria (UP), during monthly tick-control at communal dip tanks in the Mnisi Community Project (MCP). Body condition scoring of the cattle was always done by Dr Anne Meyer on a scale of 1-5 (Herd and Sprott, 2012). Blood was collected either from the jugular vein or from the tail of cattle into heparinized vacutainer tubes. Samples were spun down less than five hours after collection, after which serum

was collected and aliquoted. Serum samples were then biobanked in  $-80^{\circ}\text{C}$  freezers at Hans Hoheisen Wildlife Research Centre (HHWRS) in the MCP prior to use.

Between 40 and 60 biobanked cattle samples per dip tank were selected to maximize variation in independent variables that may act as important infection and transmission risk factors in individual cattle (age, sex, breed and body condition) and at the population level (distance from nearest game fence and time of collection). The number of samples for this study was based on data from nearby buffaloes that have an estimated infection prevalence of 20-30% for each of the pathogens. Between 15 and 60 individual animals per township or dip tank were found necessary to give the required analytical power to make meaningful comparisons between groups with  $\pm 10\%$  precision and 90% confidence.

### **3.4 ELISA**

Bio-X Respiratory ELISA Pentavalent kits (IBRPA), manufactured by Bio-X Diagnostics (Rocheffort, Belgium), were used to evaluate the humoral immune response of cattle to the selected five pathogens commonly implicated in bovine respiratory tract infections: BoHV-1, BVDV, BRSV, PI-3 and BAV-3.

### **3.5 Principle of the test**

The ELISA makes use of 96-well micro-titration plates coated with monoclonal antibodies specific to one of the five pathogens listed above. The monoclonal antibodies are used to trap the capture antigens as well as to purify these antigens from lysates of the cells in which the viruses were grown. A genuine negative

control was provided to differentiate the virus-specific antibodies from those directed against the antigenic determinants of the kidney cells used for their replication. Using such a control reduces the number of false positives. Seropositivity on a 0-5 scale was assessed according to the manufacturer's instructions.

### **3.6 Method**

The serological assays (indirect ELISA) described above were performed on the 422 serum samples to test for exposure (seropositivity) to the five pathogens targeted in this study. The test sera were diluted 1:100 in an appropriate buffer and incubated on the plate for one hour at 21°C +/- 3°C. The plates were washed with phosphate-buffered saline before the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, was added to the wells. The plate was again incubated at 21°C +/- 3°C for 1 hour. After the second incubation, the plate was again washed and the chromogen (tetramethylbenzidine) was added. If pathogen-specific immunoglobulins were present in the test sera, the conjugate remains bound to the corresponding microwell and the enzyme catalysed the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signals recorded for the negative control microwells were subtracted from the corresponding positive microwells. The reactivity (intensity of the blue colour) of a serum sample was quantified on a scale ranging from 0 to 5(++++) as per the manufacturer's instructions.

### 3.7 Data formatting

The raw data from the plate reader was exported as excel spreadsheets (one per plate) which was then compiled into a single excel master data sheet. The following information was included in the master data sheet for each sample:

- (i) Sample Identity: numbering from 1 to 423.
- (ii) Seropositivity (presence or absence of antibodies to these pathogens):
  - (a) As a continuous variable: raw optical density data proportional to the amount of antibodies in each sample.
  - (b) As an ordinal variable: Correct, continuous seropositivity scores described above were transformed into a 0 to 5 scale as per manufacturer's instructions with 0 indicating negative sample with no antibodies and a 5 indicating highest amount of antibodies.
  - (c) As a binomial variable: The ordinal scale data above was compressed to indicate whether a sample was positive (+ve) (score of 1-5) or negative (-ve) (score of 0) for antibodies, as per manufacturer's instruction pamphlet.
- (iii) Age was initially recorded at sample collection as a continuous variable in months and years but was transformed to a binomial variable to indicate whether an animal was below and above one year of age so that we compare seropositivity in animals with maternal antibodies versus animals where the maternal protection is no longer present.

- (iv) Sex was recorded as a binomial (male or female) and as a categorical variable (bull, oxen, heifer or cow).
- (v) Body condition was recorded on an ordinal scale from 1 to 5 (where 1=poor, 2=fair, 3= average, 4=good and 5=best), (figure 2).
- (vi) Location was recorded as dip tank name (categorical), distance from nearest game reserve in km (continuous variable) and by surroundedness with a game park (ordinal variable, 0-4 scale). Surroundedness was a score for each dip tank determined by the number of times that dip tank was the closest to a game reserve fence for each of the four cardinal directions .
- (vii) Breed was recorded as a binomial variable by combining Brahman and Brahman cross into a single category and compared to Sanga; and as a categorical variable with each category: pure Brahman, Brahman cross and pure Sanga.
- (viii) Time of sampling was recorded as month from January to December (ordinal variable).

### **3.8 Statistical Analyses**

Seropositivity was used as an estimate of seroprevalence for each pathogen. Overall seroprevalence was calculated by expressing the number of positive samples for each pathogen as a percentage of the total number of animals tested (422). Seroprevalence at each dip tank was calculated by expressing the number of samples that were positive (scaled data score of greater than 1) for each pathogen at a particular dip tank as a percentage of samples tested at that dip tank. Excel



Pivot tables were used to construct tables to compare seropositivity by age, breed, body condition, location, sex and month of collection.

Data was analyzed using the statistical package R (version 3.1.3) to establish the relationship between single pathogens (the dependent variable) and the independent variables of interest (age, breed, location, body condition, sex and time of sampling). Mixed models were used to determine which individual (age, sex, breed and body condition) and population (location and month of collection) variables were important predictors of seroprevalence. When the independent variables were age, sex, breed, body condition and month of collection, stockcard number and diptank name were random variables. When location was the independent variable, only stockcard number was used as a random variable.

- (a) A general linear mixed model (glmm) was used when the pathogen data was binomial (either positive or negative). We used the glmer function in the R-package lme4 for these analyses with the family indicated as binomial.
- (b) A cumulative link mixed model (clmm) was used where the pathogen data (Seropositivity) was ordinal (0-5). The clmm function was used in R and the dependent variable needed to be a factor but the family was not specified.
- (c) Linear mixed-effects models were used when the pathogen was a continuous variable. For this model we used the R-package Lme4, with the lmer function. because the dependent variable did not have to be a factor and the family was not specified.

The package Multcomp was used to make pair-wise multiple comparisons for independent variables: breed, location, body condition and time of sampling. Bonferroni correction was built into the multiple comparisons to counteract the

problem of multiple comparisons by adjusting the P values in order to avoid a lot of spurious positives.

### **3.9 Data used for statistical analyses**

The binomial version of the seropositivity data (glmm) are the only analyses presented here because it showed the same patterns as the analyses that used the raw (lmer) or ordinal version (clmm) of the seropositivity data and has the benefit of being easiest to interpret in that animals have either been exposed or not. Raw and ordinal data are more difficult to interpret because it is impossible to know if high levels of antibodies are due to more recent exposures or because of a very strong reaction a long time prior. Similarly, it is impossible to know whether a low level of antibodies are because the exposure occurred far enough in the past that antibody levels are diminished or whether it is because the animal had a weak humoral immune response initially. On comparing seropositivity by sex, only the binomial version (male/female) comparison was presented as the categorical version (cow/heifer/bull/oxen) produced similar results. For location, only the categorical version (dip tank name) was reported because the other versions, continuous (distance from nearest game fence) and ordinal (surroundness with the game park) had similar results.

## Chapter 4

### 4. Results

#### 4.1 Overall seroprevalence (all dip tanks)

BAV-3 had the highest average seroprevalence of 83% (351/422), followed by BRSV with 82% (348/422). PI-3 had the third highest seroprevalence at 45% (188/422) and BoHV-1 had the fourth highest seroprevalence of 43% (183/422). BVDV had the lowest average seroprevalence of 31% (129/422) (Figure 3).

#### 4.2 Risk factors

##### 4.2.1 Age

Age was found to be an important predictor of infection exposure ( $p < 0.001$ ) for each of the five pathogens (Figures 4 through to Figure 13). Individuals above one year of age were more likely to be exposed (BoHV-1: 78%, BVDV: 70%, BRSV: 57%, PI-3: 73% and BAV-3: 57%) when compared to animals less than one year of age (BoHV-1: 29 %, BVDV: 42 %, BRSV: 16%, PI-3: 32% and BAV-3: 18 %) for all the five respiratory pathogens.

##### 4.2.2 Breed

There was no observed difference in seropositivity amongst the three breeds (pure Brahman typical, Brahman cross and pure Sanga) (Figure 14 through to Figure 23).

### **4.2.3 Body Condition**

Body condition was observed to have an effect on exposure to all the five pathogens ( $p < 0.001$ ) (Figure 24 through to Figure 33). Animals with body condition score of 3, 5 (good) and 4 (best/excellent) out of 5 were more likely to be seropositive for the five viruses. In fact, all animals with body condition score of 4 ( $n = 2$ ) were 100% seropositive for all five pathogens. Similarly, animals with a body condition score of 3.5 ( $n = 34$ ) were 100% seropositive for BRSV and BAV-3 and had relatively high seroprevalence for the remainder of the pathogens, BoHV-1 (82.4%), BVDV (82.4%) and PI 3 (61.7%).

### **4.2.4 Sex**

There were no significant differences in seropositivity in males compared to females for any of the five viruses (Figure 34 through to Figure 43).

### **4.2.5 Location**

Dip tank of origin did not predict seroprevalences of BoHV-1, BRSV, PI 3 and BAV-3 (Figures 44, 45, 48 through to figure 53). However, for BVDV, cattle from the Welverdiend B, Seville A and Dixie dip tanks had significantly lower seroprevalences of 0.05%, 10.5% and 12.5% respectively compared to the average seroprevalence of 37% for cattle from rest of the dip tanks (Figures 46 and 47).

### **4.2.6: Month of sampling**

The month in which the blood samples were collected did not have an effect on seroprevalence for the five viruses (Figure 54 through to Figure 63).

## CHAPTER 5

### 5.0 Discussion

#### 5.1 Seropositivity

The five viral pathogens considered in this research project are known from previous studies to occur worldwide. The seroprevalences from this study (BAV-3: 83%, BRSV: 82%, PI-3: 45%, BoHV-1:43% and BVDV: 31%) confirmed the presence of these viruses at the wildlife-livestock interface of the Mnisi communal farming area in South Africa. The prevalences are, however, different from findings from other studies, though it is common for seroprevalences of these pathogens to vary between regions or countries. The differences in prevalence are likely explained by factors such as cattle density, herd sizes and management practices (Talafha, 2009). Each of these pathogens can easily spread in large herds, especially if the animals are kept on relatively small pieces of land (high density) where transmission potential is high (Kadir and Burak, 2008). The number of animals susceptible to infections in large herds is obviously higher than in small herds, which could contribute to maintaining infections circulating within a herd over extended periods (Gulliksen, 2009). Additionally, some farmers house their animals in closed buildings with inadequate ventilation at night and this increases the chances of spread if one of the animals is sick or is persistently infected (Snowder et al., 2006).

Our reported seroprevalence of 43% for BoHV-1 is very similar to the seroprevalence of 42.1% recorded in Kafue in Northern Zambia in 1991. Other studies by Hassard and Durham (1990) and Duman et al., (2009), reported

relatively similar BoHV-1 seroprevalences of 37.8% and 35.3% in Canada and Turkey, respectively.

In a study in Northern Zambia, exposure to BAV-3 was found to be very common with a seroprevalence of 87.4% (Ghirotti et al., 1991) which is comparable to our finding of 83%. Kale et al., (2013) found an 82.1% seroprevalence for BAV-3 in Turkey which is close to the 83% obtained in this study. However, Valarcher and Hagglund, 2006 found a lower BAV-3 seroprevalence of 66.5% in France.

The high BRSV seropositive levels in this study (82%) concurred with a study for detection of antibodies against BRSV in beef cattle in Yucantan, Mexico which revealed that 90.8% of cattle were seropositive (Solis-Calderon et al., 2007). Other serological studies in Chahar Mahal Bakhtiary province in Iran showed that the BRSV infection rate was 80.9% and in Ethiopia, 92.5% seroprevalence was recorded in 2000 (Woldemeskel, 2000).

Our recorded seroprevalence of 45% for PI-3 is consistent with the seroprevalence of 51.37% recorded in Adamawa, Bauchi, Taraba and Borno states in North Eastern Nigeria in 2005 (Tiwari et al, 2016). It is however, high when compared to the 25% recorded in traditional cattle in southern provinces in Zambia and low compared to the findings by Duman et al., (2009) (92.8% ) and Gurses, (2008) both in Turkey (91.1%).

Seroprevalence for BVDV in this study (31%) are much lower than those figures recorded elsewhere in southern Africa with prevalences of 58%, 76.2% and 79% respectively for Namibia, Zambia and Zimbabwe (Anderson & Rowe, 1998; Ghirotti et al., 1991)

The prevalences recorded in this study are mainly due to the fact that there is no biosecurity in the Mnisi community and cattle are allowed to mix at dip tanks. These viruses are also known to circulate among healthy animals (Fulton et al., 2009). The presence of antibodies reactive to these viruses implies that exposure is a common occurrence. These viruses therefore represent a risk factor for development of viral-induced secondary bacterial infections in the cattle in the Mnisi community. During this study, no information on symptoms, morbidity or mortality from these exposures was collected. The information would be useful in future studies that can be aimed at assessing impact of the exposure to the viruses. Co-infections with infectious pathogens are very common in both wildlife and domestic livestock and are known to affect the host animal's immune response (Graham, 2008) with the common occurrence being BRDC. While it is likely that there were co-infections that may have affected results in this study, such analyses were outside the scope of this report and thus no analyses were carried out.

## **5.2 Variables**

Age and body condition were the two traits found to be important predictors of seropositivity for all the pathogens. The breed, sex, month of collection and residence of the animal had no effect on seropositivity for the pathogens except for BVDV where there were significant differences in seropositivity at different locations (dip tank).

### **5.2.1 Age**

Our hypothesis was that cattle above one year of age would have higher seroprevalences of the five viruses compared to cattle below one year of age and

this was found to be true. This is likely a result of a longer duration of exposure to these viruses. Older animals will have had longer duration of exposure and living together with persistently infected animals in some herds (Ohlson et al., 2010). Chances of older animals making contact with infected material and infected cattle several times are higher than in younger animals. It was observed from previous studies that serological prevalence of BoHV-1 increased with age, (Magana-Urbina et al., 2005).

### **5.2.2 Location**

The hypothesis was that cattle from the dip tanks near the game park have higher seroprevalences of the five viruses compared with cattle from the dip tanks further away from the park. This was found not true for the five viruses. However, for BVDV, cattle from the Welverdiend B, Seville A and Dixie dip tanks had significantly lower seroprevalences of 0.05%, 10.5% and 12.5% respectively compared to the average seroprevalence of 37% for cattle from rest of the dip tanks (Figure 25). These were however not the dip tanks nearest to the game park. There were no significant differences in seropositivity between dip tanks in the Mnisi community for BoHV-1, BRSV, PI-3 and BAV-3. The hypothesis is likely incorrect because animals from different dip tanks in the Mnisi community share grazing and water points and thus share the same pathogens. During the dry periods, all animals in this community travel long distances in search of grazing (Van Rooyen, 2011) and it is therefore possible that they are equally exposed. For this study, the furthest dip tank from the fence was 7.3 km (Clare A) and it is possible that animals from that dip tank can reach the game fences and cattle where they can pick up infections. It is also possible for escaped buffaloes or other



infected animals like pigs and impala to travel this distance to the furthest dip tank and equally exposing the cattle as the ones near the game fence.

### **5.2.3 Body condition**

Our hypothesis was that cattle with poor body condition scores (2/5) should have high seroprevalences compared to animals with good body condition scores (>3.5/5) but this hypothesis was found not true. Animals with body condition scores of 3.5 and 4 were more likely to be seropositive for the five viruses. We hypothesized that animals in poor body condition would have underlying problems resulting in increased susceptibility. However, it may be that animals in poor condition have high levels of immunosuppression and weak immune responses such that there are reduced rates of seroconversion or high levels of mortality after infection (Statham et al., 2015). Likewise, animals considered to have good body condition score likely have good health with sound immune systems and so they are more likely to produce more antibodies (Aiello & Moses, 2012) after exposure to these viruses as compared to animals in poor body condition. Additionally, healthy animals are expected to walk longer distances during grazing thereby making more contact with other cattle and exposing themselves more than the relatively unhealthy animals with poor body condition.

### **5.2.4 Sex**

Our hypothesis was that the male cattle would have higher seroprevalences of the five viruses compared to female cattle. This was found not true. There were no significant differences in seropositivity in different sex categories for the animals (male/female or bull/cow/steer/heifer). While there could be differences in immune status of the different sexes, the level of exposure might have been the same hence

no differences in seropositivity. Ghirotti et al., (1991) found that sex was not a significant factor for seroprevalence of BVDV, PI 3, BoHV-1 and BAV-3. Sex was found to have an effect on the occurrence of clinical respiratory tract disease, (Muggli-Cockett, et al., 1992) which is different from seroprevalence discussed here.

### **5.2.5 Breed**

Our hypothesis was that Sanga cattle would have lower seroprevalences of the five viruses compared to the Brahman cattle. This was also found not to be true as there were no significant differences between the different breeds of the animals included in this study (pure Brahman, Brahman cross or pure Sanga) even though indigenous breeds are known to have better resistance to the common pathogens like tick-borne diseases and parasites (Kariuki, 1991). As with sex, rather than differences in exposure there could be differences in clinical disease occurrence amongst the different breeds because it is possible for an animal to be exposed and produce antibodies (becoming seropositive) without necessarily developing clinical disease (Fulton et al., 2011). Our finding is consistent with another study that found no significant variation amongst twelve beef cattle breeds for seroprevalence of the pathogens responsible for bovine respiratory disease in the United States of America (Snowder et al., 2006).

### **5.2.6 Month of sample collection**

Our hypothesis was that cattle sampled in winter (May, June and July) should have high seroprevalences of the five viruses compared to cattle sampled during the other months (April, August and September). The hypothesis was not true as there was no difference in seropositivity in animals sampled during different months of

the year. High seroprevalences have been recorded in winter compared to summer (Baker et al., 1997; Van der Poel, 1993). However, Klem et al., (2013) found out that higher seroprevalences associated with winter are usually a result of new infections in animals that are housed during winter where susceptible animals come into close contact with infected animals. This is different from the situation in the Mnisi community where livestock is reared extensively and animals are housed in the same kraal throughout the year. It is likely that some of the antibodies detected by the serological test were from exposure that occurred previously and not at the time of sampling because antibodies do not become immediately detectable in serum on exposure. Also, it may be that animals are constantly being exposed to these pathogens from persistently infected animals. Such was the case with BoHV-1 (Muylkens, 2007).

## CHAPTER 6

### 6.0 Conclusions

Livestock diseases are one of the major constraints on animal production, especially in Africa where there are a variety of tropical and subtropical diseases. Knowledge of these diseases and ways to combat them is critical to the socio-economic development of Africa and its fight against poverty (Penzhorn and Verwoerd, 2005). Unfortunately, there are relatively few cost effective therapies for the treatment of most common viral respiratory diseases of cattle (Aiello and Moses, 2012). The results of this study provide evidence for the widespread circulation of the five bovine respiratory tract viruses namely BoHV-1, BVDV, BVDV BRSV, PI-3, and BAV-3 among the cattle in the Mnisi community. There are many places in southern Africa which share boundaries with conservation areas and the livestock farmers in those areas have challenges similar to those in the Mnisi community. These challenges include inadequate resources to afford veterinary services for their animals as well as nutritional stress during the dry season. The Mnisi community is therefore a representative of other southern African townships and communal farming areas where a livestock-wildlife interface may be present. Livestock is important to resource poor populations and there is a threat posed to their livelihoods by animal diseases that have an impact on livestock productivity and human food security. Any programme aimed at reducing such diseases that have adverse consequences for the poorest sections of the human population will have a major positive impact on poverty reduction (Devereux, 2012).

From concurrent studies, these pathogens are known to occur in African buffalo populations (Coon et al., under review). All stakeholders have to ensure that fence

barriers are maintained. Veterinary extension workers should conduct farmer training workshops where livestock owners must be advised to consider reducing their herd sizes to reduce grazing pressure and avoid driving animals for longer distances during the dry season when grazing is scarce. It has also been found out that for BRDC, the larger the herd, the more likely it is that both infectious and susceptible animals will be present, maintaining the spread of infection on the farm (Mourits et al., 2000).

It is now known that poor nutrition impairs animal immunity (Woolums, 2013). Livestock owners in this area should also stock crop residues for use as feed supplements for their animals during the dry season. A large proportion of the costs associated with pneumonia are hidden, such as reduced live-weight gain and feed conversion efficiency (Potter, 2010). Strategies to reduce pneumonia should target improving cattle immunity and reducing stress, as well as treating sick animals supportively to minimize the effect of the pathogens on the animals (Griffin, 1997). Farmers should quarantine sick cattle in order to minimize spread of the pathogens (Bowland and Shewen, 2000).

Additionally, livestock owners should ensure adequate colostrums intake to ensure that the calves are protected during the first 6 months of life. Calves where available, are supposed to be housed separately in buildings with adequate ventilation (Potter, 2010). There is a need to consider vaccination of all animals in future to minimize the effects of these pathogens on the animals although this is expensive. The farming systems in the Mnisi community are relatively similar to many places elsewhere in southern Africa and the data collected during this research project may be applicable and of benefit beyond the Mnisi area. It is also necessary to carry out further studies to find out more on symptoms, morbidity and

mortality in order to quantify the effects of these pathogens on animal health and productivity in the area.

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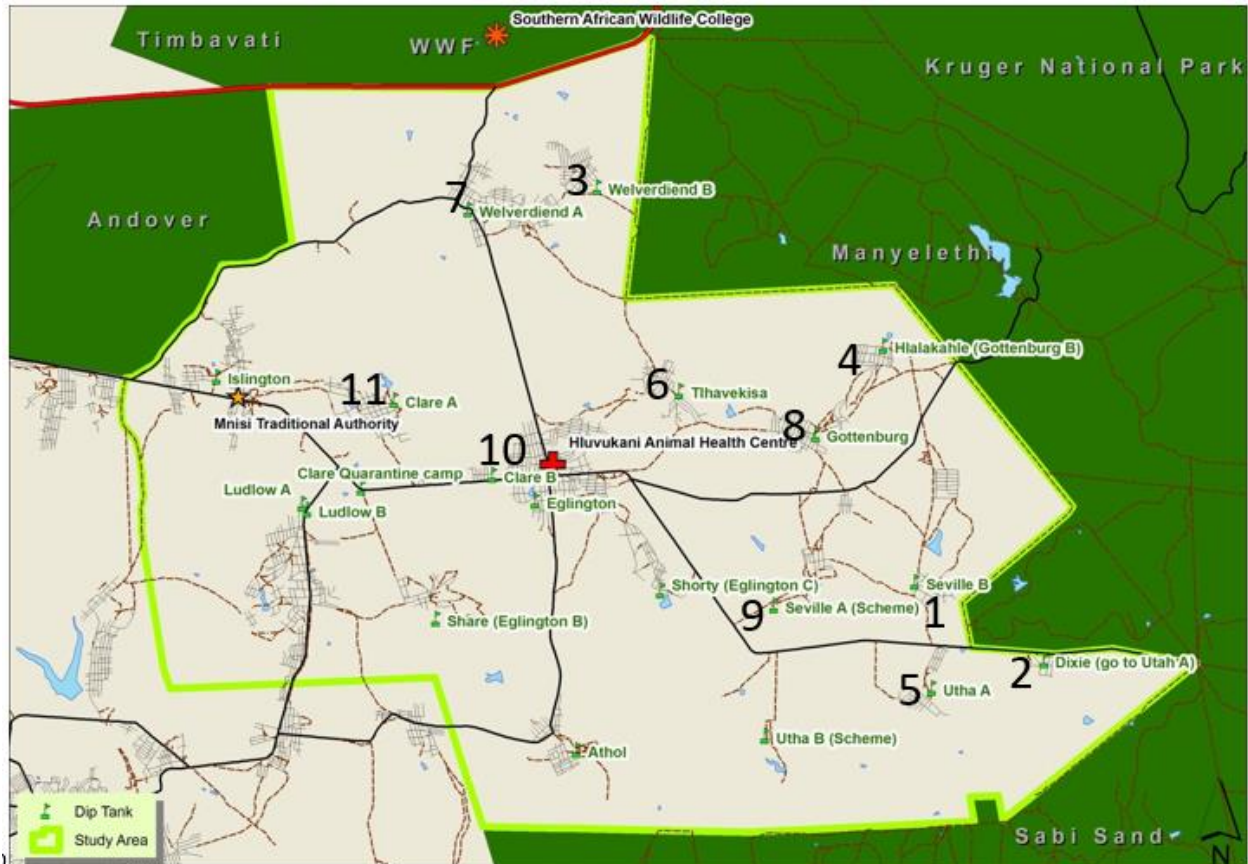
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## APPENDICES

**Figure 1: Map of the study area, the Mnisi Community relative to the wildlife preserves including Kruger National Park.**

(Numbers denote dip tanks).

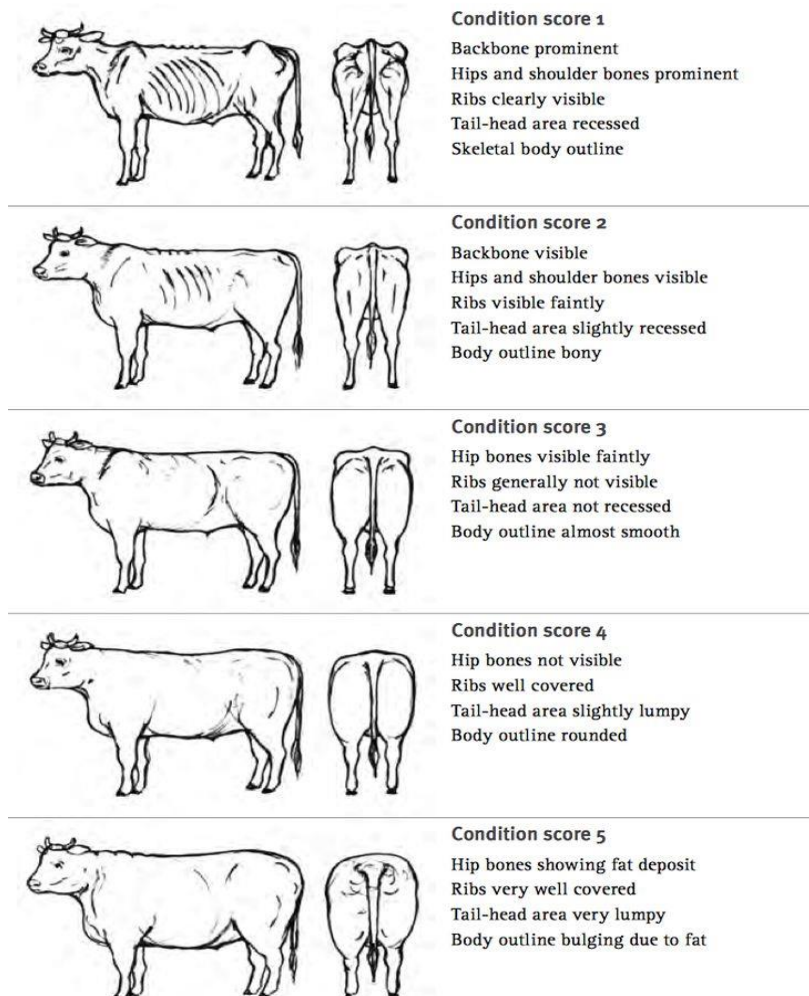


**Table 1: Key to Mnisi community map (Figure 1) along with approximate distances to the nearest wildlife game reserve fence in km.**

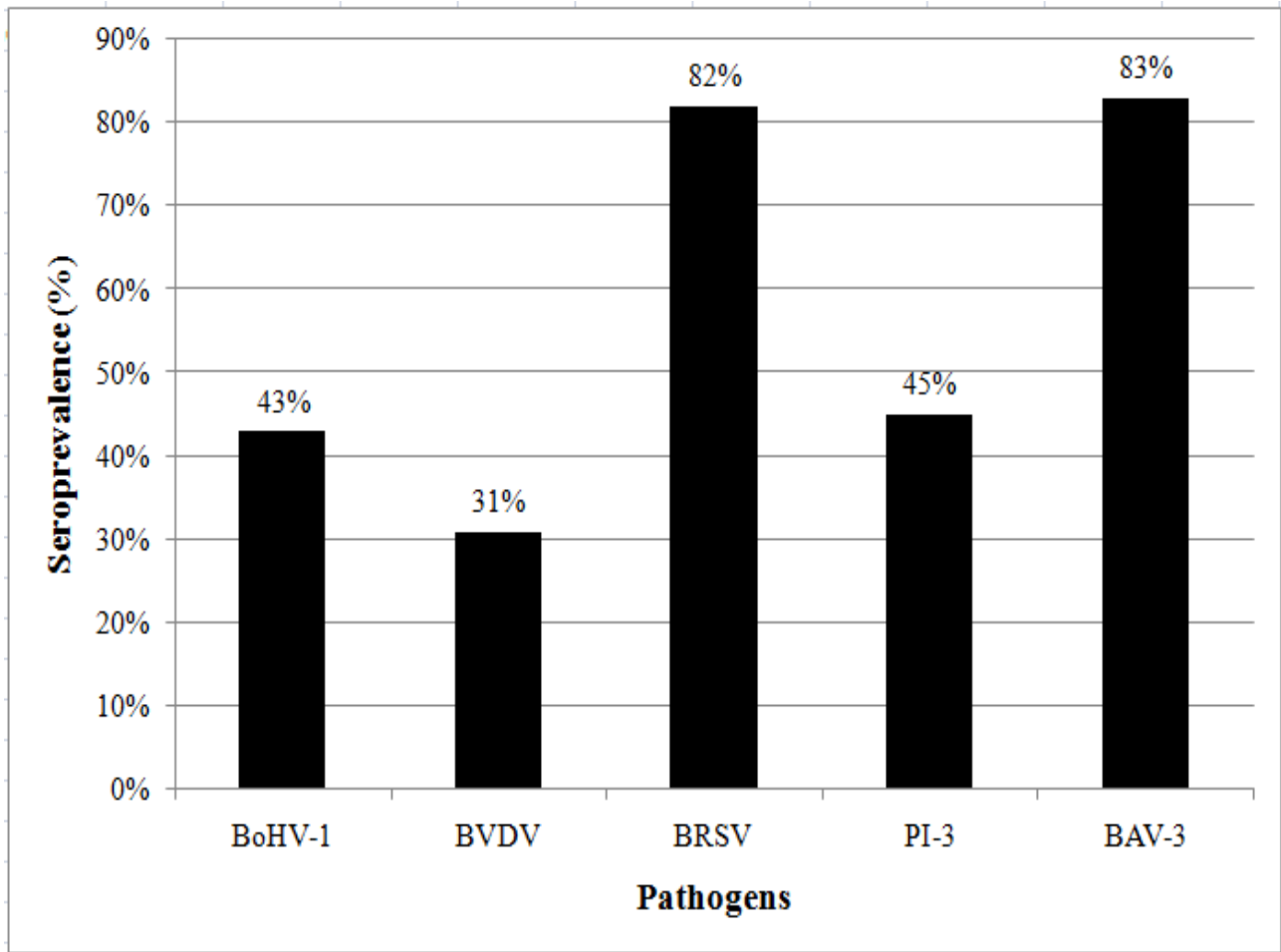
<b>Map ID</b>	<b>Dip tank</b>	<b>Distance to nearest game fence in km</b>
1	Seville B	0.5
2	Dixie	1
3	Wilverdiend B	1
4	Hlalakahle	1.2
5	Utha A	2.3
6	Tlhavekisa	3
7	Wilverdiend A	3.1
8	Gottenburg	4.2
9	Seville A	4.3
10	Clare B	6.4
11	Clare A	7.3

## Figure 2: Body condition score chart for beef cattle.

Cows were scored for body condition on a 1 to 5 scale based on physical characteristics by a veterinarian (<http://basicanimalhandling.com/body-condition-scoring>).



**Figure 3: Overall seroprevalence**



**Figure 4: BoHV-1 seropositivity by age glmm results**

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Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial ( logit )
Formula:
BHV_2 ~ factor(Age_lessthanone) + (1 | Diptank_name) + (1 | StockcardNumber)
Data: biobank

      AIC      BIC   logLik deviance df.resid
 482.3   498.4  -237.1   474.3     419

Scaled residuals:
   Min       1Q   Median       3Q      Max
-1.4293 -0.4851 -0.4851  0.6996  2.0615

Random effects:
Groups             Name                Variance Std.Dev.
StockcardNumber (Intercept) 0          0
Diptank_name     (Intercept) 0          0
Number of obs: 423, groups: StockcardNumber, 101; Diptank_name, 11

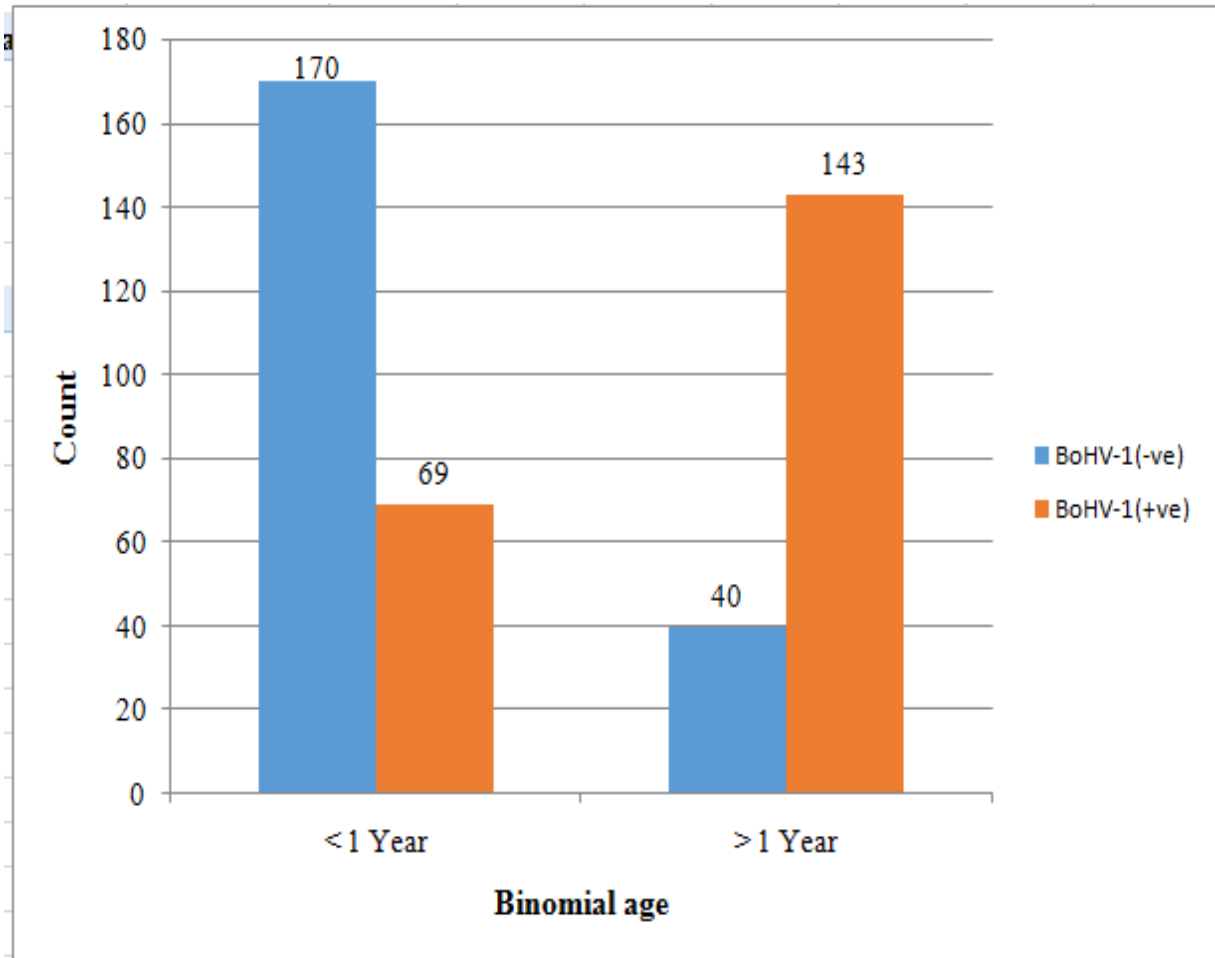
Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    -1.4469    0.1757  -8.234  <2e-16 ***
factor(Age_lessthanone)1  2.1613    0.2284   9.463  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
      (Intr)
fctr(Ag_1)1 -0.769

```



**Figure 5: BoHV-1 seropositivity by age graph**



**Figure 6: BVDV seropositivity by age glmm results**

```

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial ( logit )
Formula: BVDV_2 ~ factor(Age_lessthanone) + (1 | Diptank_name) + (1 |
StockcardNumber)
Data: biobank

      AIC      BIC   logLik deviance df.resid
 479.3    495.4   -235.6   471.3     419

Scaled residuals:
   Min       1Q   Median       3Q      Max
-1.1675 -0.5847 -0.4154  0.8919  3.6342

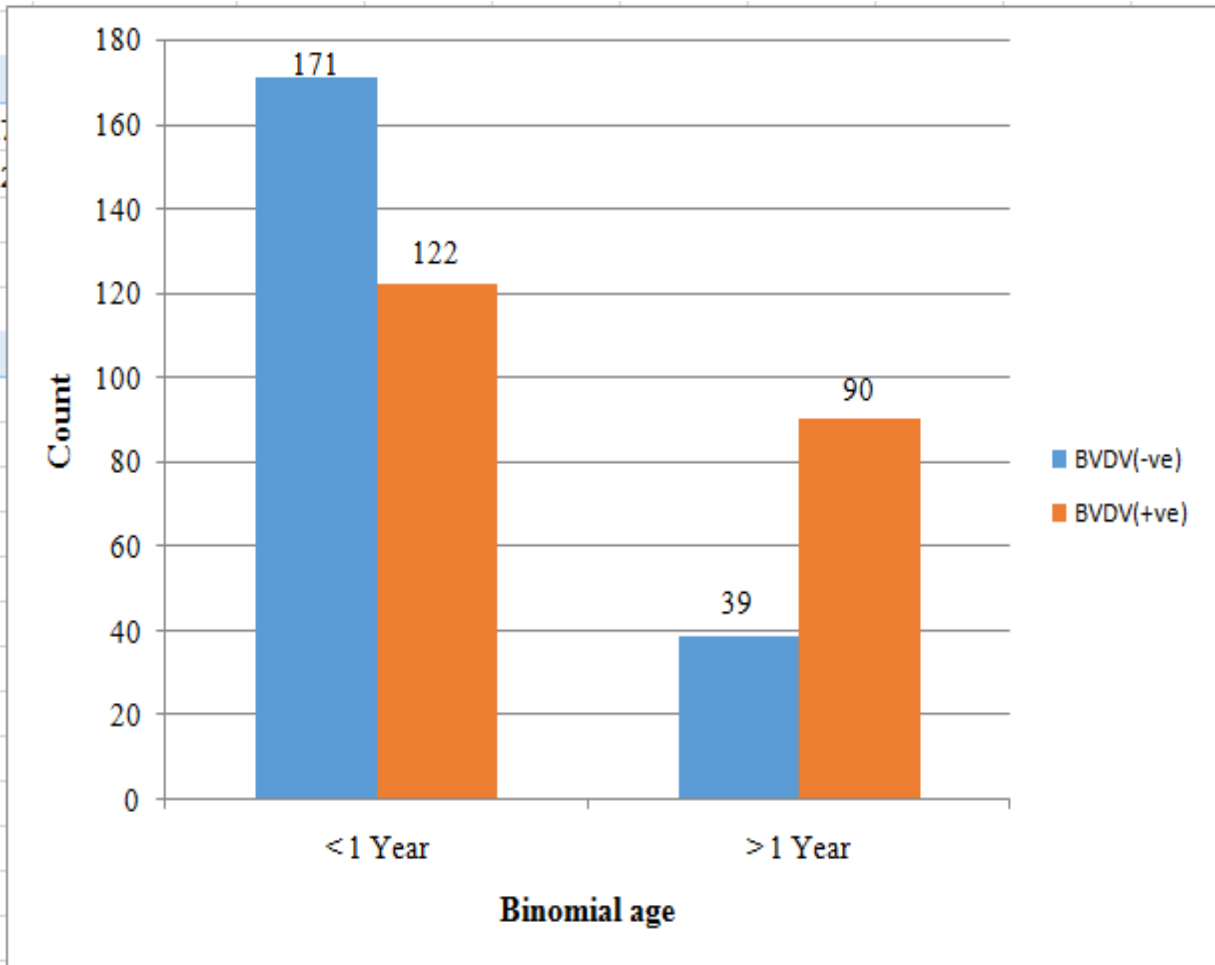
Random effects:
 Groups              Name              Variance Std.Dev.
 StockcardNumber (Intercept)  0.0000    0.0000
 Diptank_name     (Intercept)  0.6215    0.7884
Number of obs: 423, groups: StockcardNumber, 101; Diptank_name, 11

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    -1.6707     0.3100  -5.390 7.04e-08 ***
factor(Age_lessthanone)1  1.2550     0.2373   5.289 1.23e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fctr(Aq_1)1 -0.490

```

**Figure 7: BVDV seropositivity by age graph**



**Figure 8: BRSV seropositivity by age glmm results**

```

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial ( logit )
Formula: BRSV_2 ~ factor(Age_lessthanone) + (1 | Diptank_name) + (1 |
StockcardNumber)
Data: biobank

      AIC      BIC    logLik deviance df.resid
  355.2    371.4   -173.6   347.2     419

Scaled residuals:
   Min       1Q   Median       3Q      Max
-4.0927  0.2443  0.2443  0.6472  0.6472

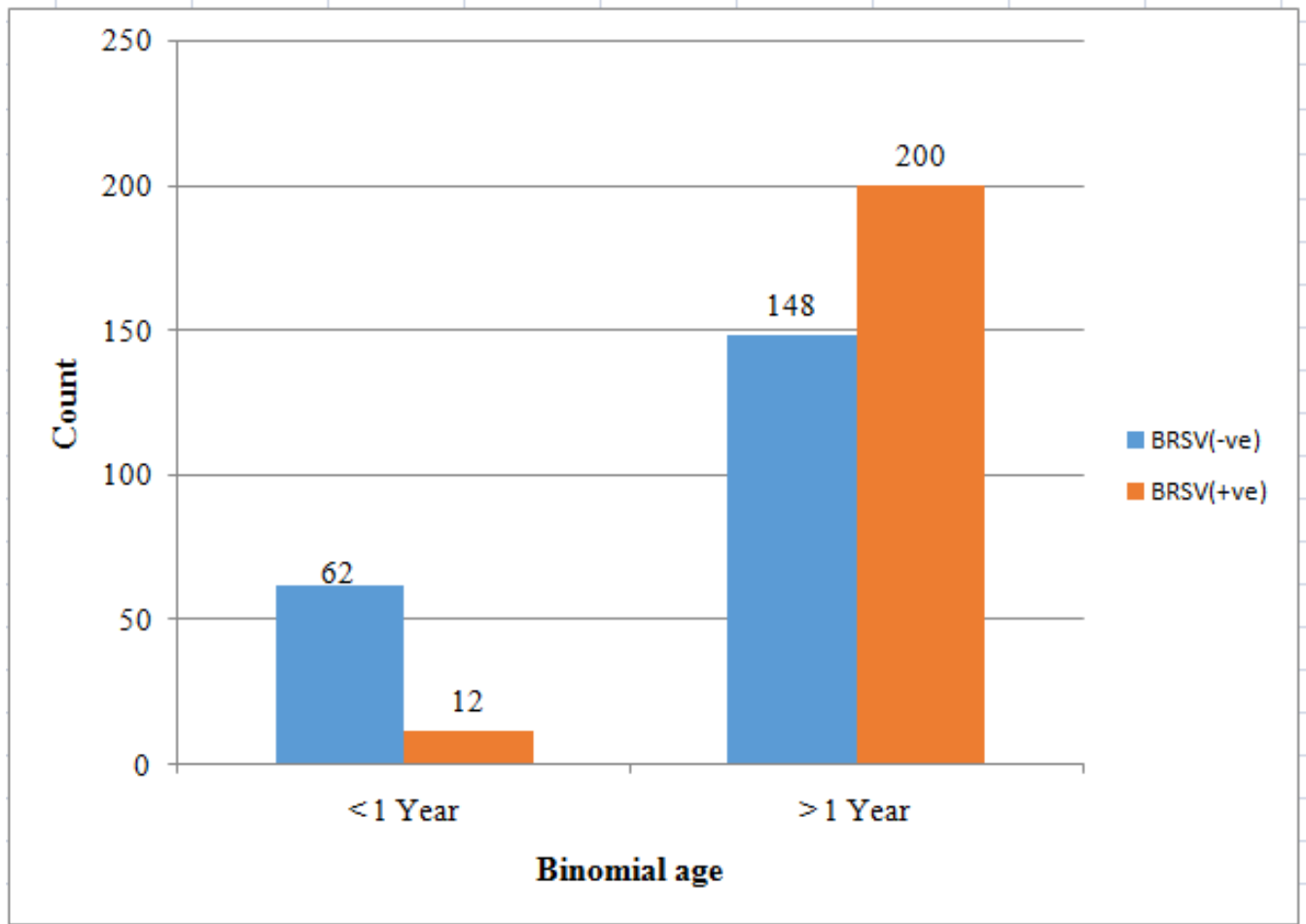
Random effects:
 Groups              Name            Variance Std.Dev.
StockcardNumber (Intercept) 1.966e-13 4.434e-07
Diptank_name     (Intercept) 0.000e+00 0.000e+00
Number of obs: 423, groups: StockcardNumber, 101; Diptank_name, 11

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)      0.8701     0.1513   5.751 8.85e-09 ***
factor(Age_lessthanone)1  1.9483     0.3335   5.843 5.13e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fctr(Ag_1)1 -0.454

```

**Figure 9: BRSV seropositivity by age graph**



**Figure 10: PI-3 seropositivity by age glmm results**

```

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial ( logit )
Formula:
PI3_2 ~ factor(Age_lessthanone) + (1 | Diptank_name) + (1 | StockcardNumber)
Data: biobank

      AIC      BIC    logLik deviance df.resid
 514.1    530.3   -253.0   506.1     419

Scaled residuals:
   Min       1Q   Median       3Q      Max
-1.4994 -0.5780 -0.5234  0.7312  2.0926

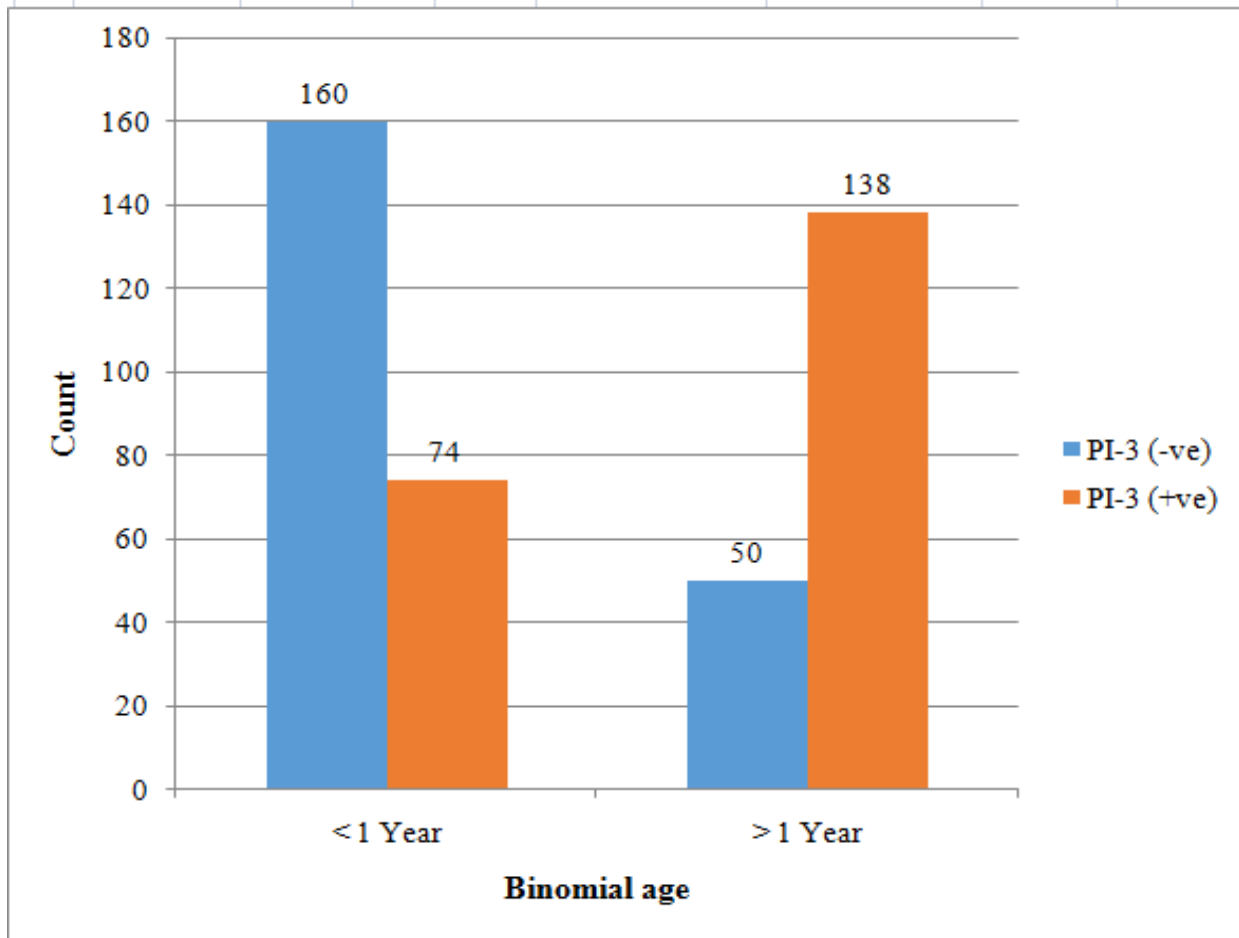
Random effects:
 Groups                Name            Variance Std.Dev.
 StockcardNumber (Intercept)  0.00000  0.000
 Diptank_name      (Intercept)  0.05474  0.234
Number of obs: 423, groups: StockcardNumber, 101; Diptank_name, 11

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)      -1.170      0.179  -6.535 6.37e-11 ***
factor(Age_lessthanone)1  1.786      0.219   8.155 3.50e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fcctr(Ag_1)1 -0.688

```

**Figure 11: PI-3 seropositivity by age graph**



**Figure 12: BAV-3 seropositivity by age glmm results**

```

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial ( logit )
Formula:
AD3_2 ~ factor(Age_lessthanone) + (1 | Diptank_name) + (1 | StockcardNumber)
Data: biobank

      AIC      BIC   logLik deviance df.resid
  352.5   368.7  -172.3   344.5     419

Scaled residuals:
   Min       1Q   Median       3Q      Max
-4.1036  0.2288  0.2505  0.5680  0.7699

Random effects:
 Groups             Name             Variance Std.Dev.
 StockcardNumber (Intercept)  0.2012   0.4485
 Diptank_name     (Intercept)  0.0000   0.0000
Number of obs: 423, groups: StockcardNumber, 101; Diptank_name, 11

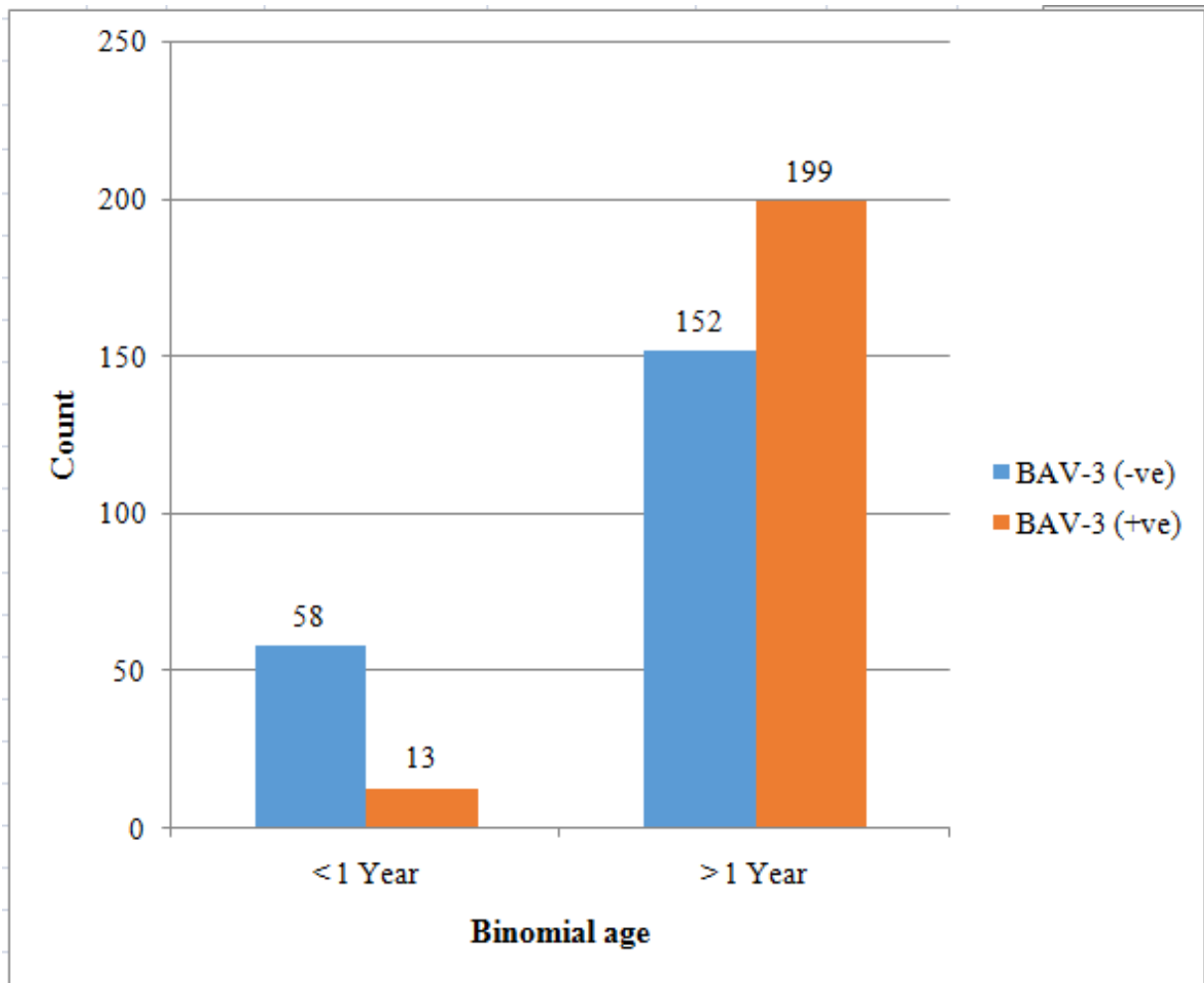
Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)         1.0084     0.1765   5.713 1.11e-08 ***
factor(Age_lessthanone)1  1.8251     0.3354   5.442 5.28e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fctr(Ag_1)1 -0.371

```



**Figure 13: BAV-3 seropositivity by age graph**



## Figure 14: BoHV-1 Seropositivity by breed glmm multicomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

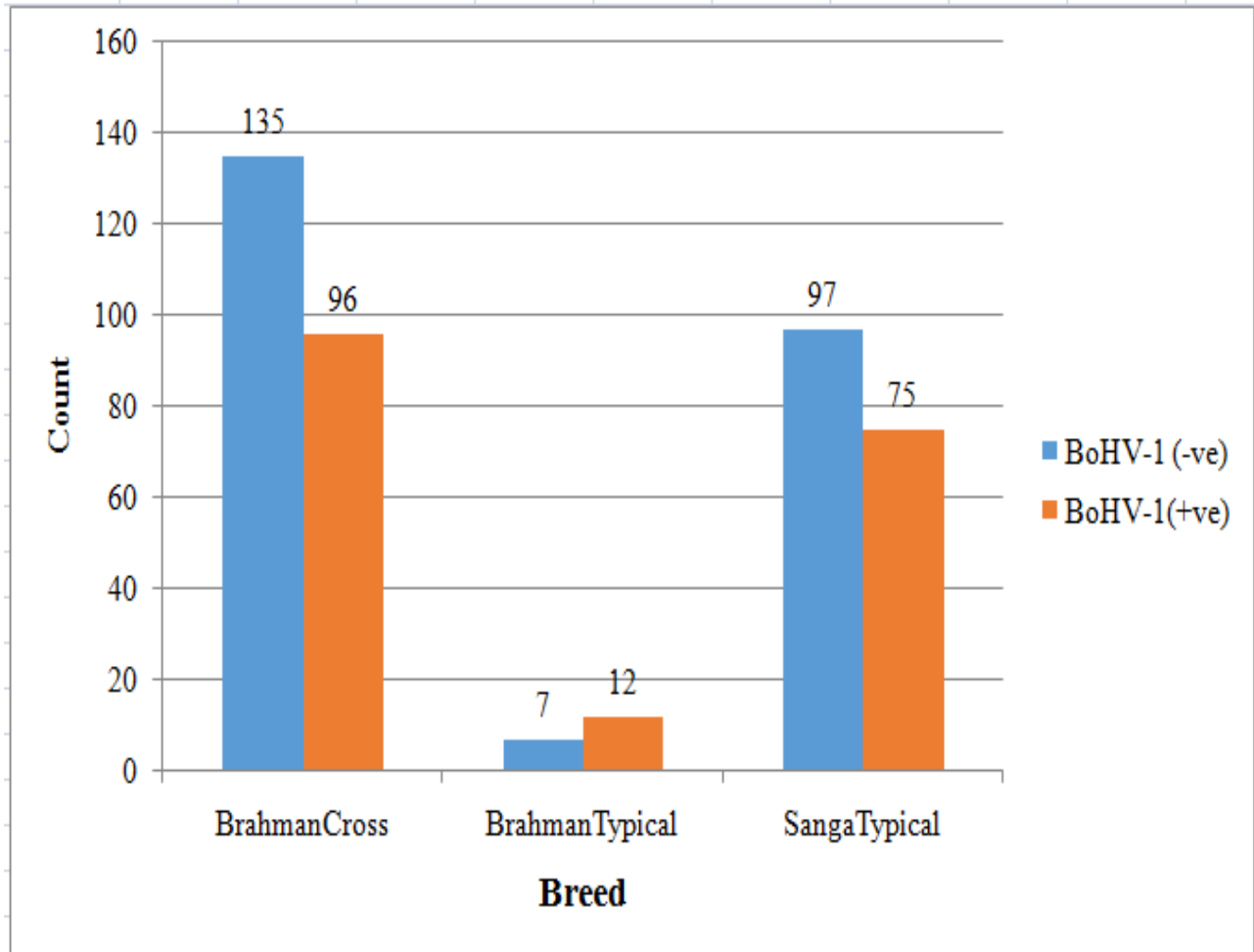
```
Fit: glmer(formula = BHV_2 ~ (AnimalType4) + (1 | StockcardNumber) +  
  (1 | Diptank_name), data = biobank1, family = binomial)
```

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
BrahmanTypical - BrahmanCross == 0	0.8799	0.4940	1.781	0.163
SangaTypical - BrahmanCross == 0	0.0837	0.2036	0.411	0.906
SangaTypical - BrahmanTypical == 0	-0.7962	0.4998	-1.593	0.233

(Adjusted p values reported -- single-step method)

**Figure 15: BoHV-1 seropositivity by breed graph**



## Figure 16: BVDV Seropositivity by breed glmm multcomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

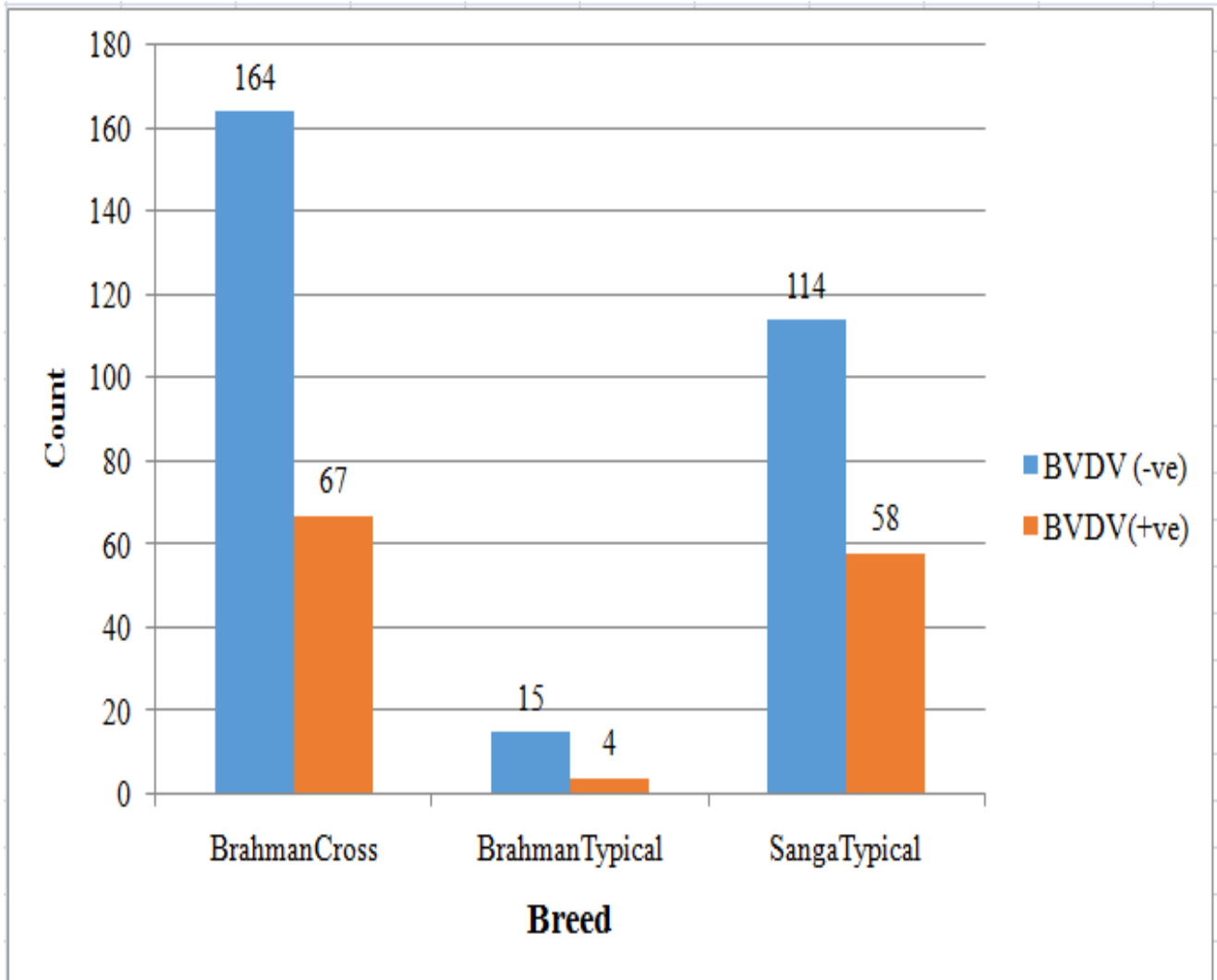
Fit: `glmer(formula = BVDV_2 ~ (AnimalType4) + (1 | StockcardNumber) + (1 | Diptank_name), data = biobank1, family = binomial)`

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
BrahmanTypical - BrahmanCross == 0	-0.4400	0.6007	-0.732	0.731
SangaTypical - BrahmanCross == 0	0.2502	0.2279	1.098	0.496
SangaTypical - BrahmanTypical == 0	0.6902	0.6070	1.137	0.471

(Adjusted p values reported -- single-step method)

**Figure 17: BVDV seropositivity by breed graph**



## Figure 18: BRSV Seropositivity by breed glmm multcomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

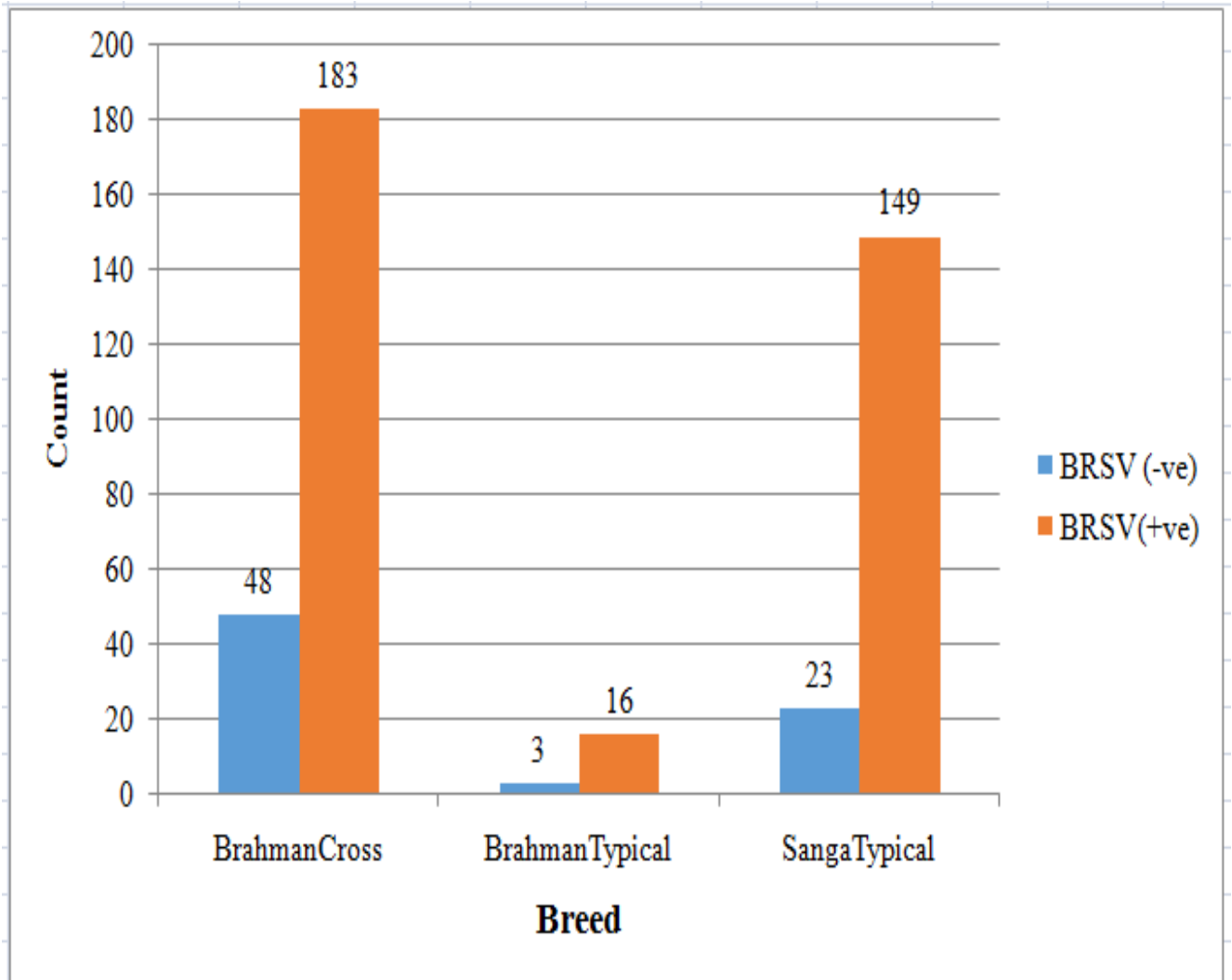
Fit: glmer(formula = BRSV\_2 ~ (AnimalType4) + (1 | StockcardNumber) +  
(1 | Diptank\_name), data = biobank1, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
BrahmanTypical - BrahmanCross == 0	0.3357	0.6497	0.517	0.856
SangaTypical - BrahmanCross == 0	0.5302	0.2766	1.917	0.123
SangaTypical - BrahmanTypical == 0	0.1945	0.6679	0.291	0.952

(Adjusted p values reported -- single-step method)

**Figure 19: BRSV seropositivity by breed graph**



## Figure 20: PI-3 Seropositivity by breed glmm multicomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

```
Fit: glmer(formula = PI3_2 ~ (AnimalType4) + (1 | StockcardNumber) +  
  (1 | Diptank_name), data = biobank1, family = binomial)
```

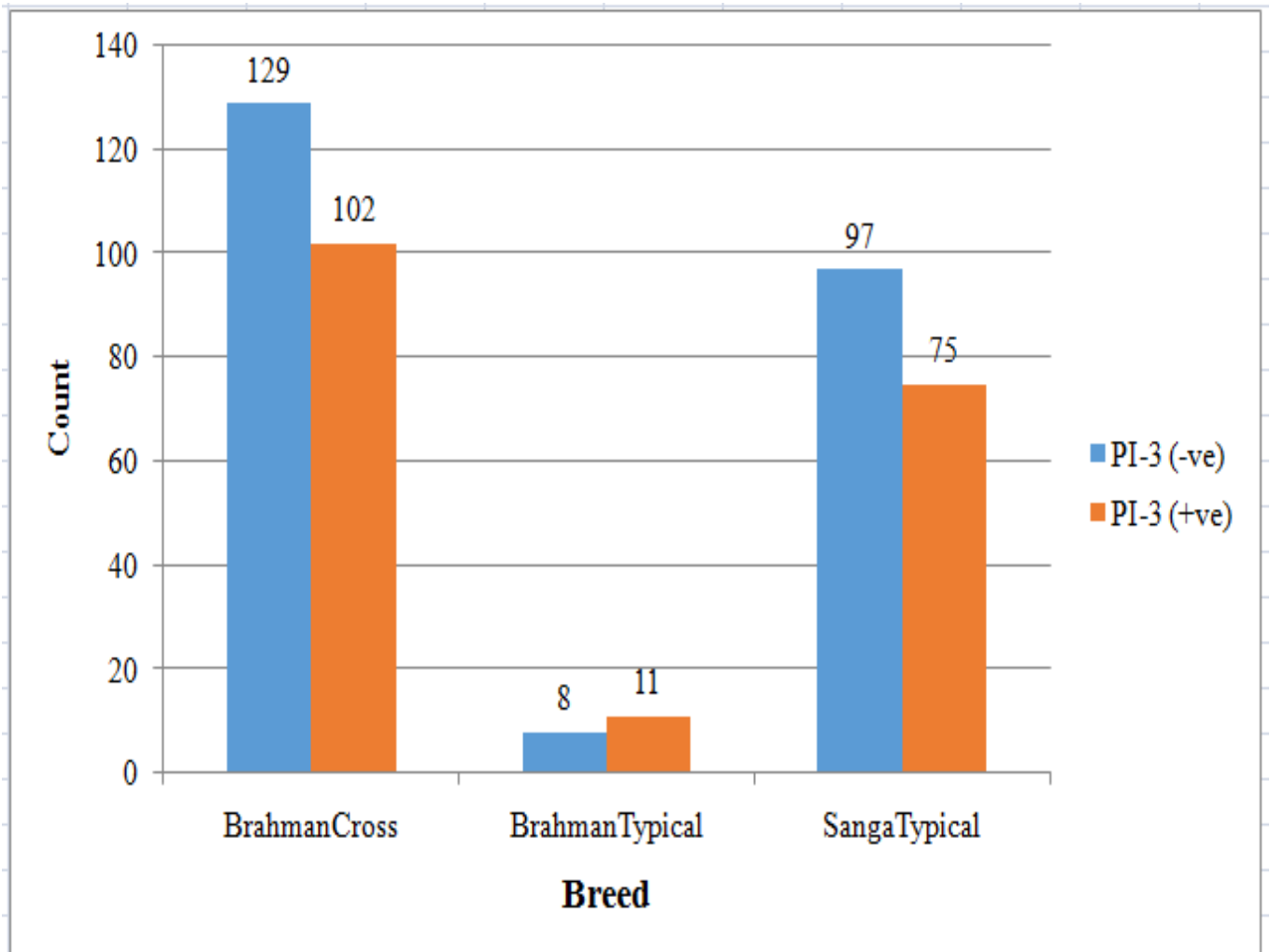
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
BrahmanTypical - BrahmanCross == 0	0.57985	0.48861	1.187	0.443
SangaTypical - BrahmanCross == 0	-0.02136	0.20479	-0.104	0.994
SangaTypical - BrahmanTypical == 0	-0.60122	0.49518	-1.214	0.427

(Adjusted p values reported -- single-step method)



**Figure 21: PI-3 seropositivity by breed graph**



## Figure 22 : BAV-3 Seropositivity by breed glmm multcomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

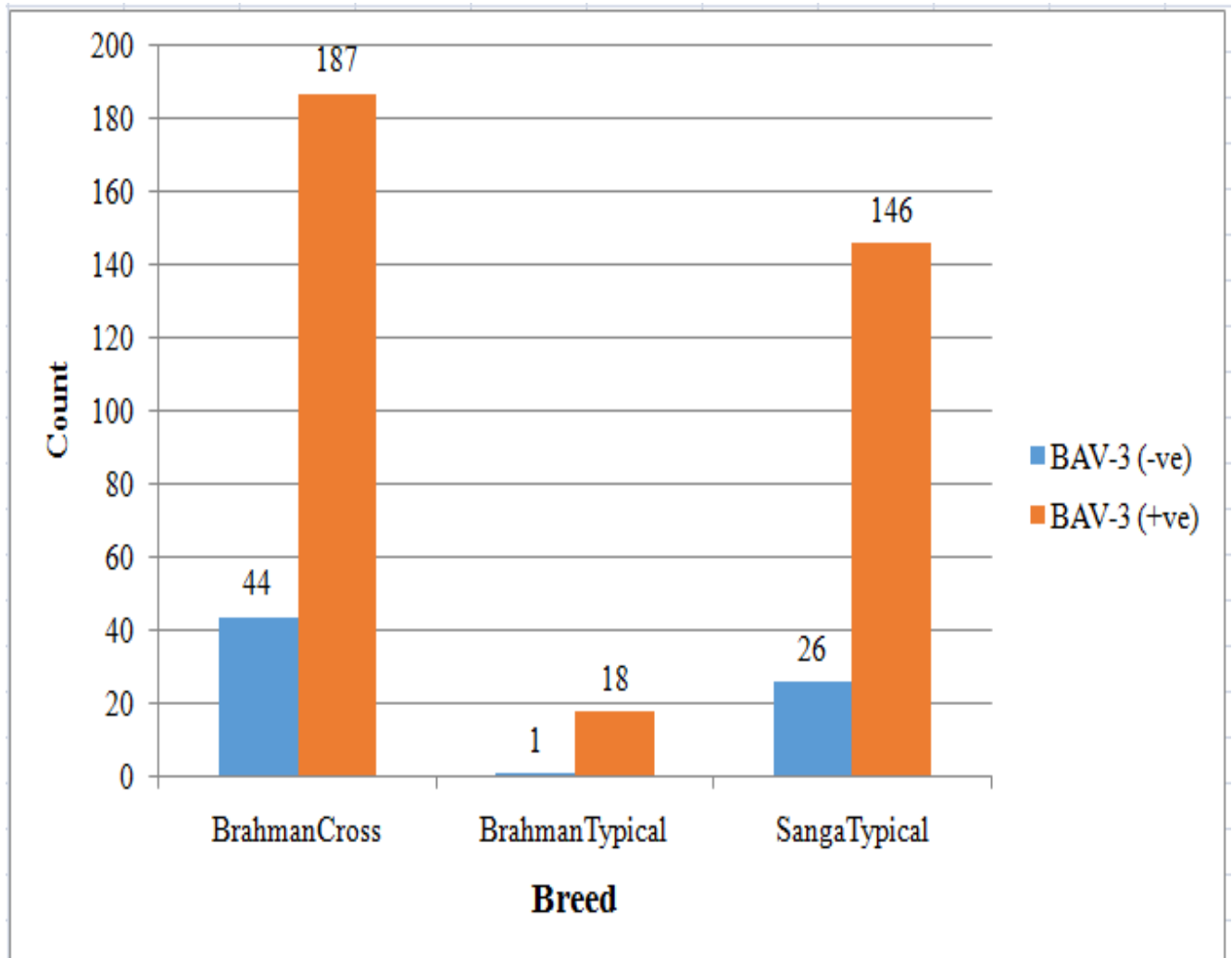
Fit: glmer(formula = AD3\_2 ~ (AnimalType4) + (1 | StockcardNumber) +  
(1 | Diptank\_name), data = biobank1, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
BrahmanTypical - BrahmanCross == 0	1.4386	1.0464	1.375	0.328
SangaTypical - BrahmanCross == 0	0.2835	0.2744	1.033	0.530
SangaTypical - BrahmanTypical == 0	-1.1551	1.0547	-1.095	0.491

(Adjusted p values reported -- single-step method)

**Figure 23: BAV-3 seropositivity by breed graph**



## Figure 24: BoHV-1 Seropositivity by body condition glmm multcomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

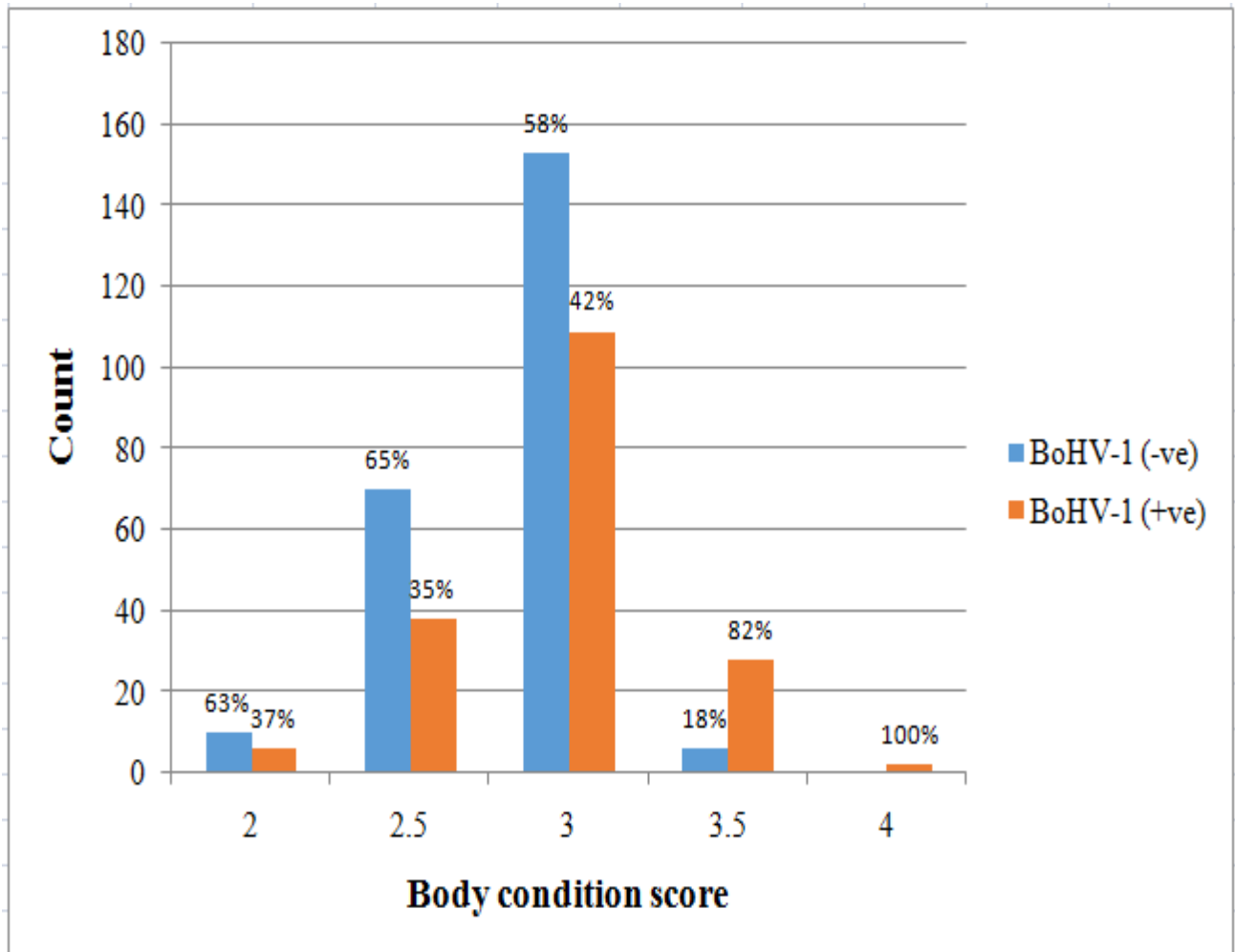
Fit: glmer(formula = BHV\_2 ~ (BodyCon) + (1 | StockcardNumber/Diptank\_name),  
data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
Excellent - Average == 0	3.023e+01	2.181e+06	0.000	1.000000
Fair - Average == 0	-2.653e-01	2.372e-01	-1.118	0.746928
Good - Average == 0	1.886e+00	4.670e-01	4.039	0.000335 ***
Poor - Average == 0	-1.652e-01	5.314e-01	-0.311	0.997189
Fair - Excellent == 0	-3.050e+01	2.181e+06	0.000	1.000000
Good - Excellent == 0	-2.834e+01	2.181e+06	0.000	1.000000
Poor - Excellent == 0	-3.039e+01	2.181e+06	0.000	1.000000
Good - Fair == 0	2.151e+00	4.929e-01	4.364	< 1e-04 ***
Poor - Fair == 0	1.001e-01	5.543e-01	0.181	0.999669
Poor - Good == 0	-2.051e+00	6.849e-01	-2.995	0.015572 *

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

**Figure 25: BoHV-1 seropositivity by body condition**



**Figure 26 : BVDV Seropositivity by body condition glmm multicomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = BVDV\_2 ~ (BodyCon) + (1 | StockcardNumber) +  
(1 | Diptank\_name), data = biobank1\_MANYETU, family = binomial)

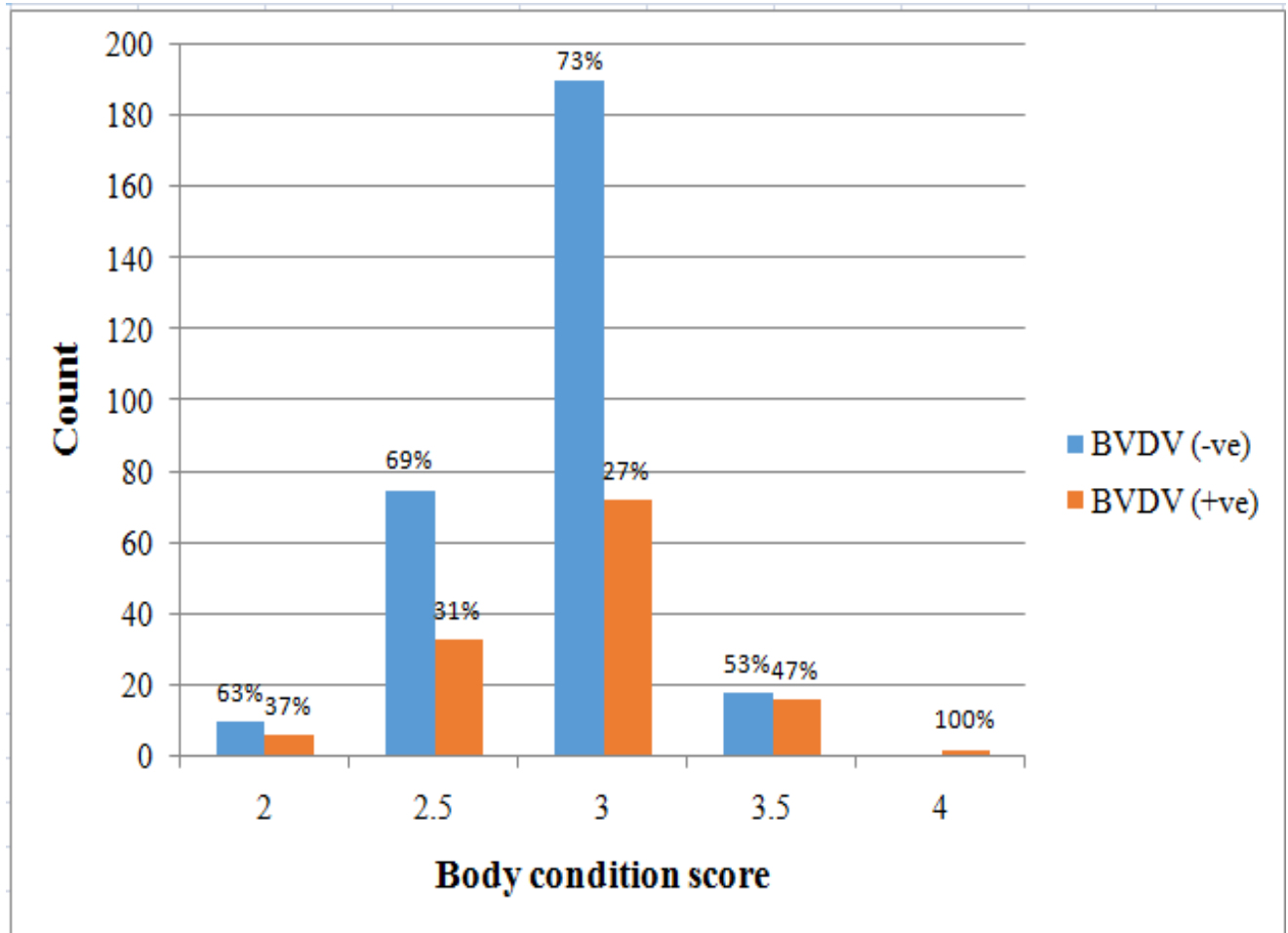
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )	
Excellent - Average == 0	1.473e+05	4.513e-03	3.263e+07	<2e-16	***
Fair - Average == 0	1.036e-01	4.512e-03	2.296e+01	<2e-16	***
Good - Average == 0	9.432e-01	4.513e-03	2.090e+02	<2e-16	***
Poor - Average == 0	7.191e-01	4.513e-03	1.593e+02	<2e-16	***
Fair - Excellent == 0	-1.473e+05	6.382e-03	-2.308e+07	<2e-16	***
Good - Excellent == 0	-1.473e+05	6.382e-03	-2.308e+07	<2e-16	***
Poor - Excellent == 0	-1.473e+05	6.382e-03	-2.308e+07	<2e-16	***
Good - Fair == 0	8.396e-01	6.381e-03	1.316e+02	<2e-16	***
Poor - Fair == 0	6.154e-01	6.382e-03	9.644e+01	<2e-16	***
Poor - Good == 0	-2.241e-01	6.382e-03	-3.512e+01	<2e-16	***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

**Figure 27: BVDV seropositivity by body condition graph**



**Figure 28: BRSV Seropositivity by body condition glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

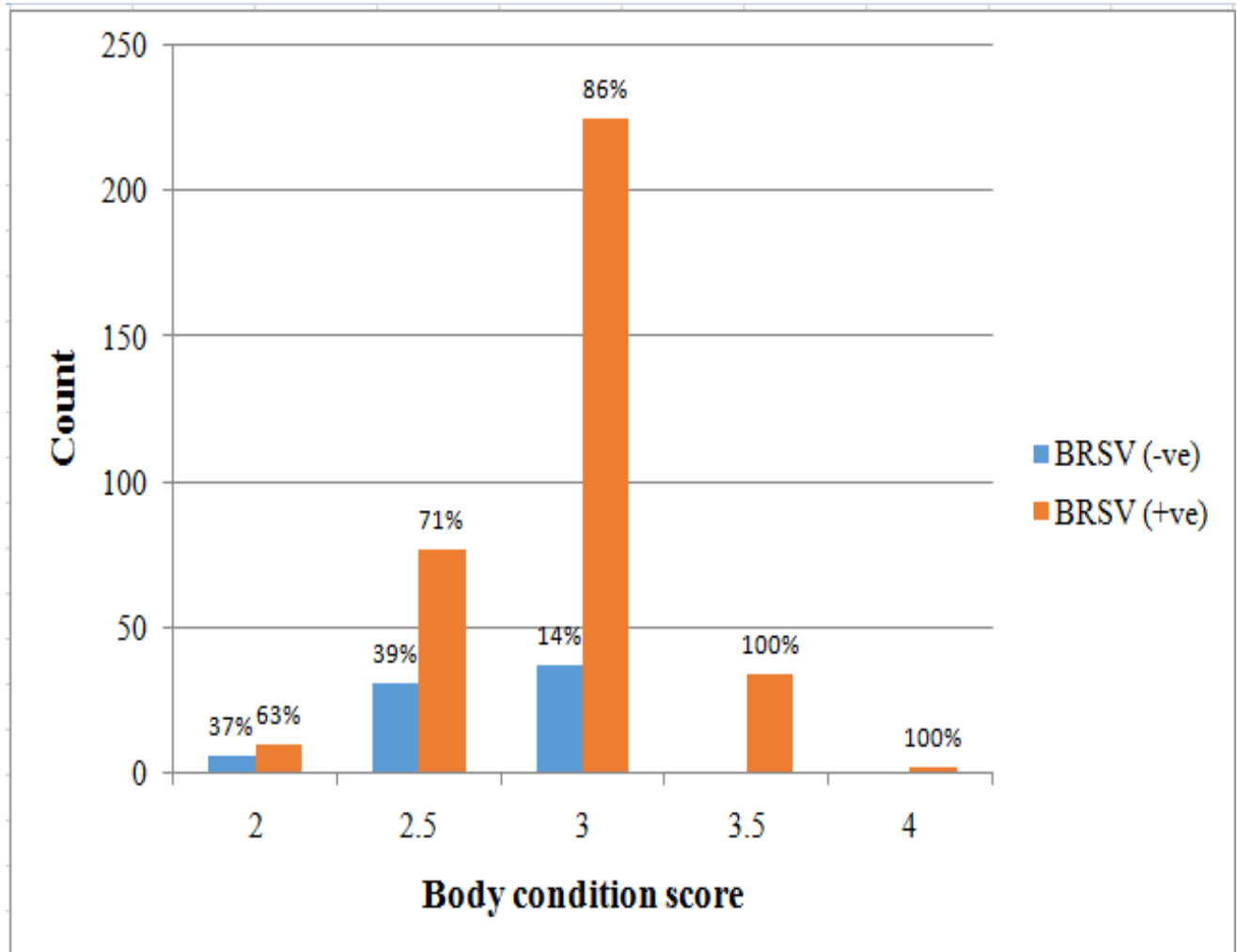
Fit: glmer (formula = BRSV\_2 ~ (BodyCon) + (1 | StockcardNumber) +  
(1 | Diptank\_name), data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )	
Excellent - Average == 0	16.9958	8560.2112	0.002	1.00000	
Fair - Average == 0	-0.9078	0.2785	-3.260	0.00569	**
Good - Average == 0	21.8626	23767.7541	0.001	1.00000	
Poor - Average == 0	-1.3091	0.5486	-2.386	0.07866	.
Fair - Excellent == 0	-17.9036	8560.2112	-0.002	1.00000	
Good - Excellent == 0	4.8668	25262.2911	0.000	1.00000	
Poor - Excellent == 0	-18.3049	8560.2112	-0.002	1.00000	
Good - Fair == 0	22.7704	23767.7541	0.001	1.00000	
Poor - Fair == 0	-0.4013	0.5608	-0.716	0.93319	
Poor - Good == 0	-23.1717	23767.7541	-0.001	1.00000	



**Figure 29: BRSV seropositivity by body condition graph**



### Figure 30: PI-3 Seropositivity by body condition glmm multcomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

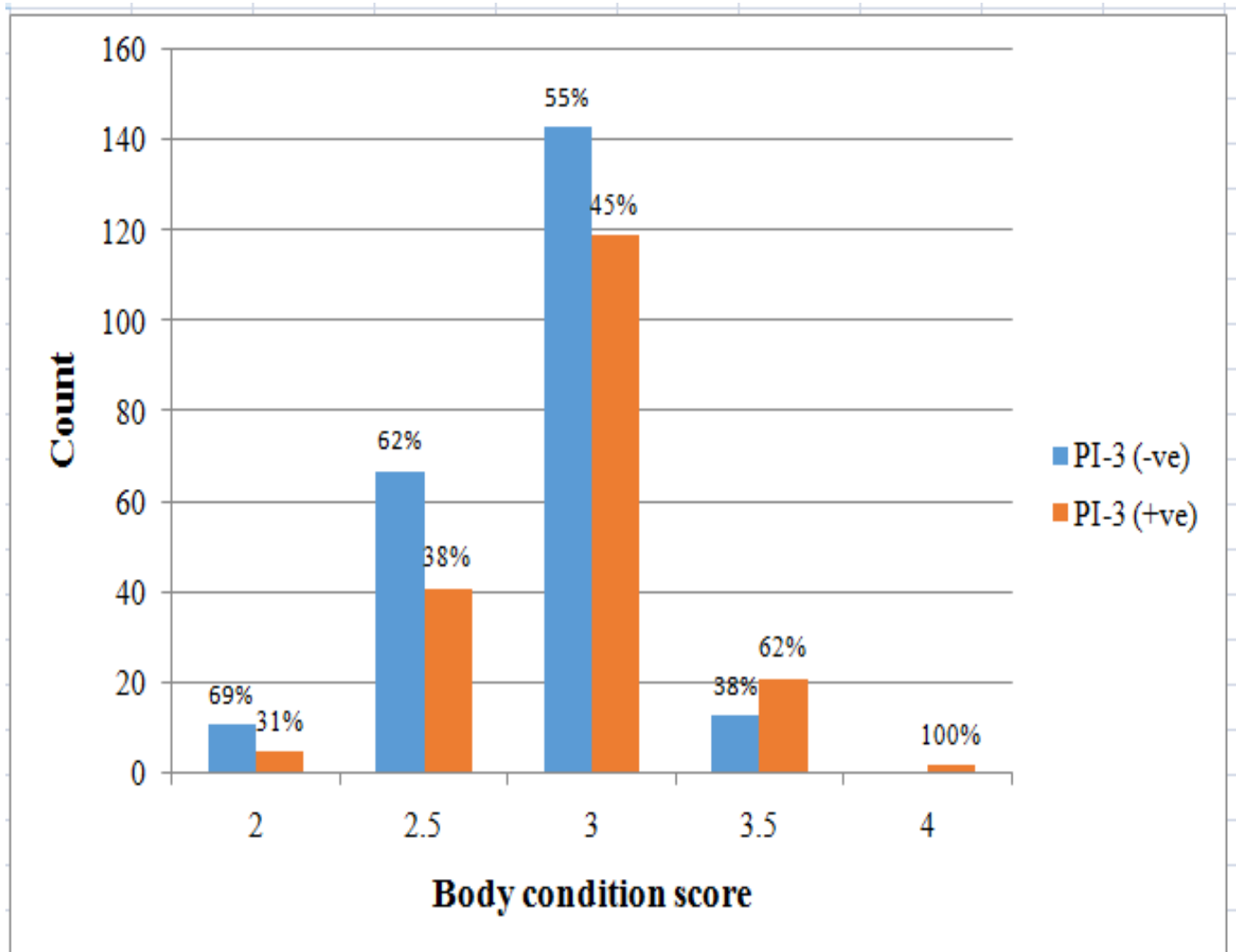
Fit: glmer(formula = PI3\_2 ~ (BodyCon) + (1 | StockcardNumber) + (1 | Diptank\_name), data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )	
Excellent - Average == 0	17.2786	3479.8049	0.005	1.000	
Fair - Average == 0	-0.3047	0.2356	-1.293	0.633	***
Good - Average == 0	0.6851	0.3762	1.821	0.295	
Poor - Average == 0	-0.5658	0.5573	-1.015	0.808	
Fair - Excellent == 0	-17.5833	3479.8049	-0.005	1.000	
Good - Excellent == 0	-16.5935	3479.8049	-0.005	1.000	
Poor - Excellent == 0	-17.8445	3479.8049	-0.005	1.000	
Good - Fair == 0	0.9898	0.4073	2.430	0.078	***.
Poor - Fair == 0	-0.2611	0.5790	-0.451	0.988	
Poor - Good == 0	-1.2509	0.6491	-1.927	0.242	

---  
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

**Figure 31: PI-3 seropositivity by body condition graph**



## Figure 32: BAV-3 Seropositivity by body condition glmm multicomp results

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = AD3\_2 ~ (BodyCon) + (1 | StockcardNumber) + (1 | Diptank\_name), data = biobank1\_MANYETU, family = binomial)

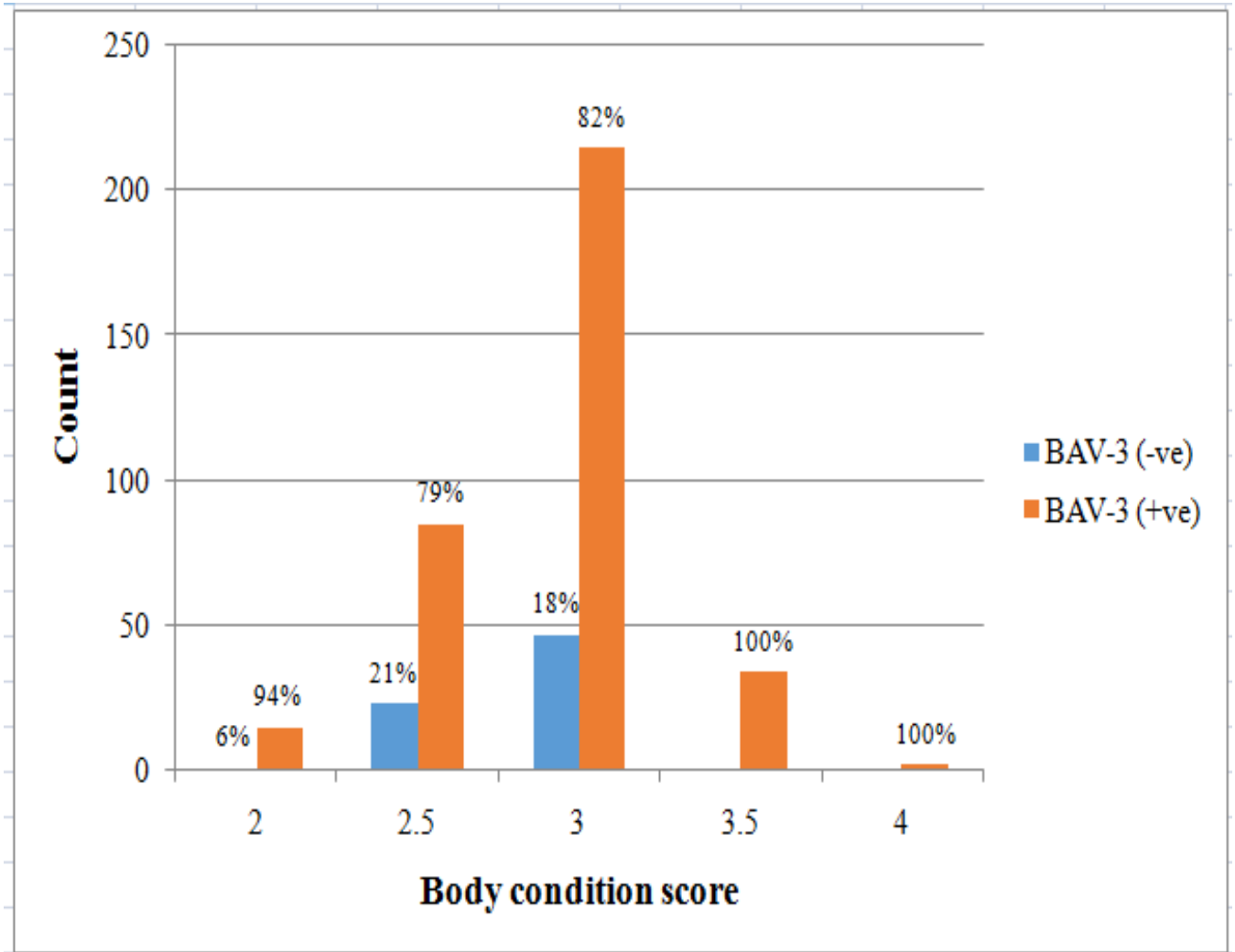
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
Excellent - Average == 0	18.6204	16490.7950	0.001	1.000
Fair - Average == 0	-0.2420	0.2913	-0.831	0.883***
Good - Average == 0	17.1218	1949.2844	0.009	1.000
Poor - Average == 0	1.1953	1.0728	1.114	0.723
Fair - Excellent == 0	-18.8625	16490.7950	-0.001	1.000
Good - Excellent == 0	-1.4986	16605.6023	0.000	1.000
Poor - Excellent == 0	-17.4251	16490.7950	-0.001	1.000
Good - Fair == 0	17.3638	1949.2844	0.009	1.000
Poor - Fair == 0	1.4373	1.0865	1.323	0.579
Poor - Good == 0	-15.9265	1949.2846	-0.008	1.000

(Adjusted p values reported -- single-step method)

summary(glht(AD339, linfct=mcp(BodyCon= 'Tukey'), p.adj="bonferroni"))

**Figure 33: BAV-3 seropositivity by body condition graph**



**Figure 34: BoHV-1 Seropositivity by sex glmm results**

```

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
Family: binomial ( logit )
Formula: BHV_2 ~ factor(sex2) + (1 | StockcardNumber) + (1 | Diptank_name)
Data: biobank1

      AIC      BIC   logLik deviance df.resid
 585.3    601.5   -288.7   577.3     418

Scaled residuals:
   Min       1Q   Median       3Q      Max
-0.8974 -0.8974 -0.8545  1.1143  1.1703

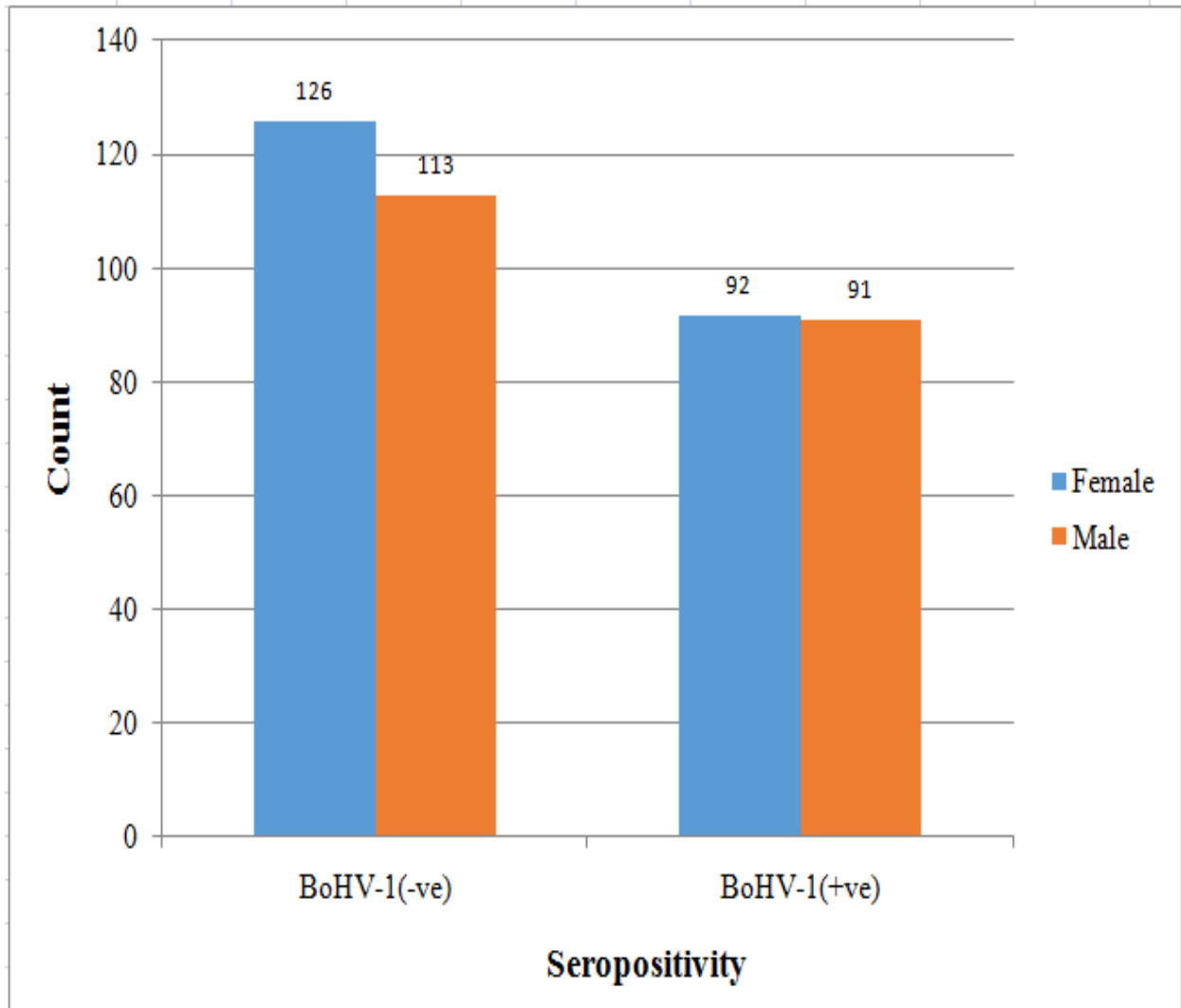
Random effects:
 Groups             Name             Variance Std.Dev.
StockcardNumber (Intercept) 0          0
Diptank_name    (Intercept) 0          0
Number of obs: 422, groups: StockcardNumber, 101; Diptank_name, 11

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)   -0.31449   0.13714  -2.293   0.0218 *
factor(sex2)Male  0.09797   0.19658   0.498   0.6182
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fctr(sex2)M1 -0.698

```

**Figure 35: BoHV-1 seropositivity by sex graph**



### Figure 36: BVDV Seropositivity by sex glmm results

```

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']
Family: binomial ( logit )
Formula: BVDV_2 ~ factor(sex2) + (1 | StockcardNumber) + (1 | Diptank_name)
Data: biobank1

      AIC      BIC  logLik deviance df.resid
  508.7   524.8  -250.3   500.7     418

Scaled residuals:
   Min       1Q   Median       3Q      Max
-0.9142 -0.7753 -0.4909  1.1594  3.0822

Random effects:
 Groups             Name                Variance Std.Dev.
StockcardNumber (Intercept) 7.725e-09 8.789e-05
Diptank_name    (Intercept) 5.331e-01 7.302e-01
Number of obs: 422, groups: StockcardNumber, 101; Diptank_name, 11

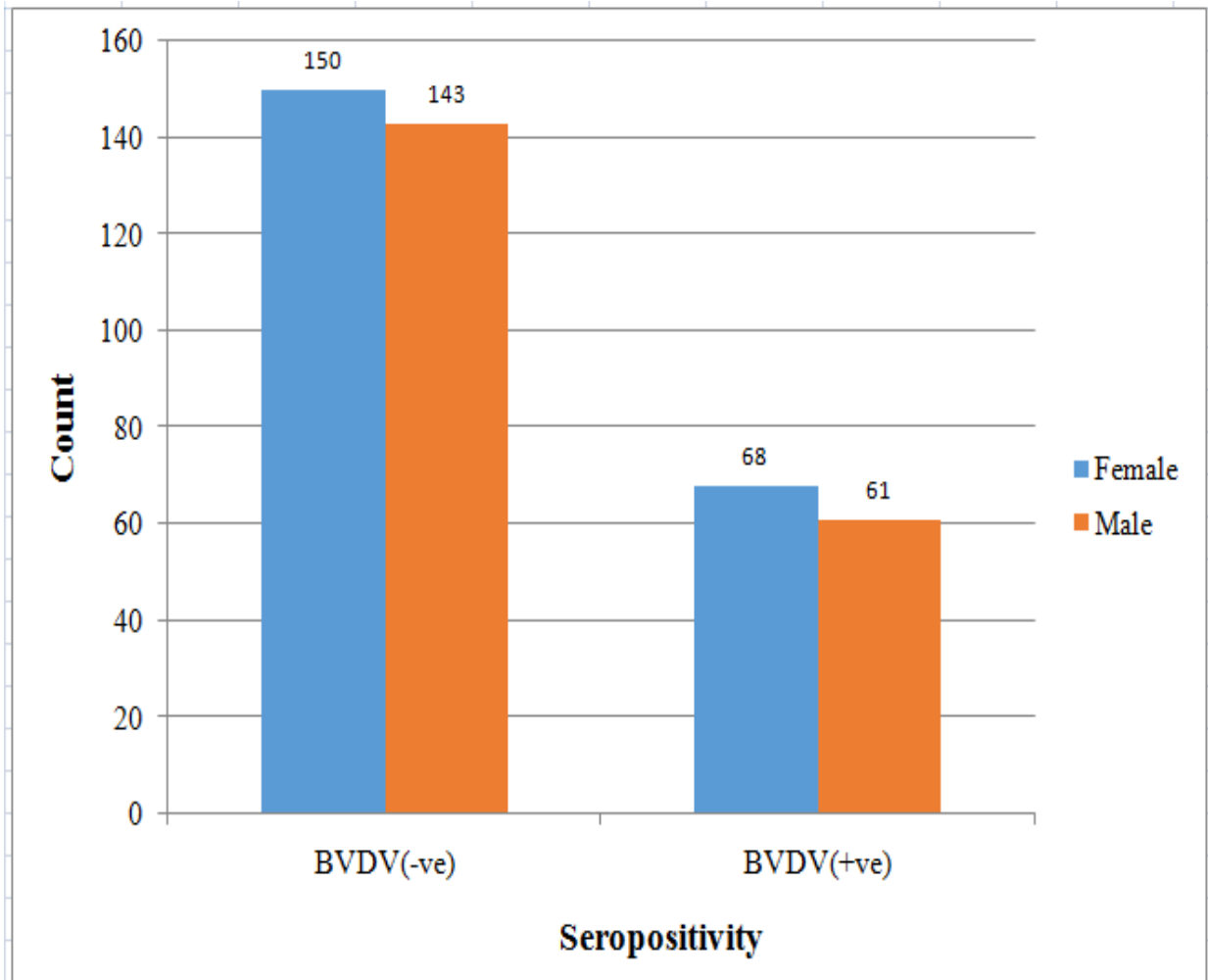
Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)   -0.8888    0.2725  -3.262  0.00111 **
factor(sex2)Male -0.1164    0.2210  -0.527  0.59837
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
              (Intr)
fctr(sex2)M1 -0.374

```



**Figure 37: BVDV seropositivity by sex graph**



### Figure 38: BRSV Seropositivity by sex glmm results

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']  
 Family: binomial ( logit )  
 Formula: BRSV\_2 ~ factor(sex2) + (1 | StockcardNumber) + (1 | Diptank\_name)  
 Data: biobank1

AIC	BIC	logLik	deviance	df.resid
399.5	415.7	-195.8	391.5	418

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.2485	0.4447	0.4447	0.4784	0.4784

Random effects:

Groups	Name	Variance	Std.Dev.
StockcardNumber	(Intercept)	0	0
Diptank_name	(Intercept)	0	0

Number of obs: 422, groups: StockcardNumber, 101; Diptank\_name, 11

Fixed effects:

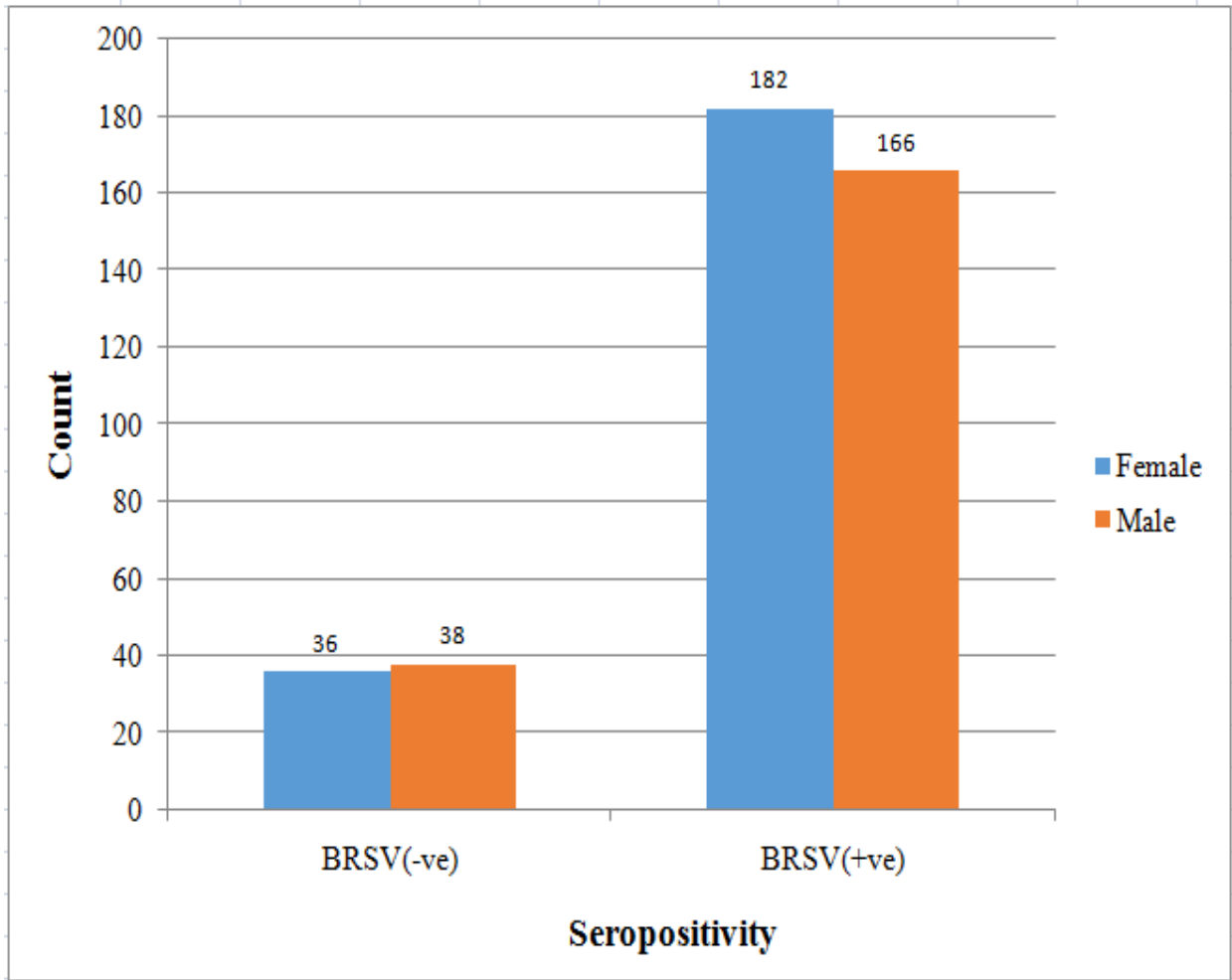
	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	1.6205	0.1824	8.884	<2e-16 ***
factor(sex2)Male	-0.1461	0.2561	-0.570	0.568

---  
 signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)
fctr(sex2)M1	-0.712

**Figure 39: BRSV seropositivity by sex graph**



### Figure 40: PI-3 Seropositivity by sex glmm results

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']

Family: binomial ( logit )

Formula: PI3\_2 ~ factor(sex2) + (1 | StockcardNumber) + (1 | Diptank\_name)

Data: biobank1

AIC	BIC	logLik	deviance	df.resid
582.6	598.8	-287.3	574.6	418

scaled residuals:

Min	1Q	Median	3Q	Max
-1.0910	-0.8565	-0.7636	1.0285	1.4465

Random effects:

Groups	Name	Variance	Std.Dev.
StockcardNumber	(Intercept)	0.00000	0.0000
Diptank_name	(Intercept)	0.04823	0.2196

Number of obs: 422, groups: StockcardNumber, 101; Diptank\_name, 11

Fixed effects:

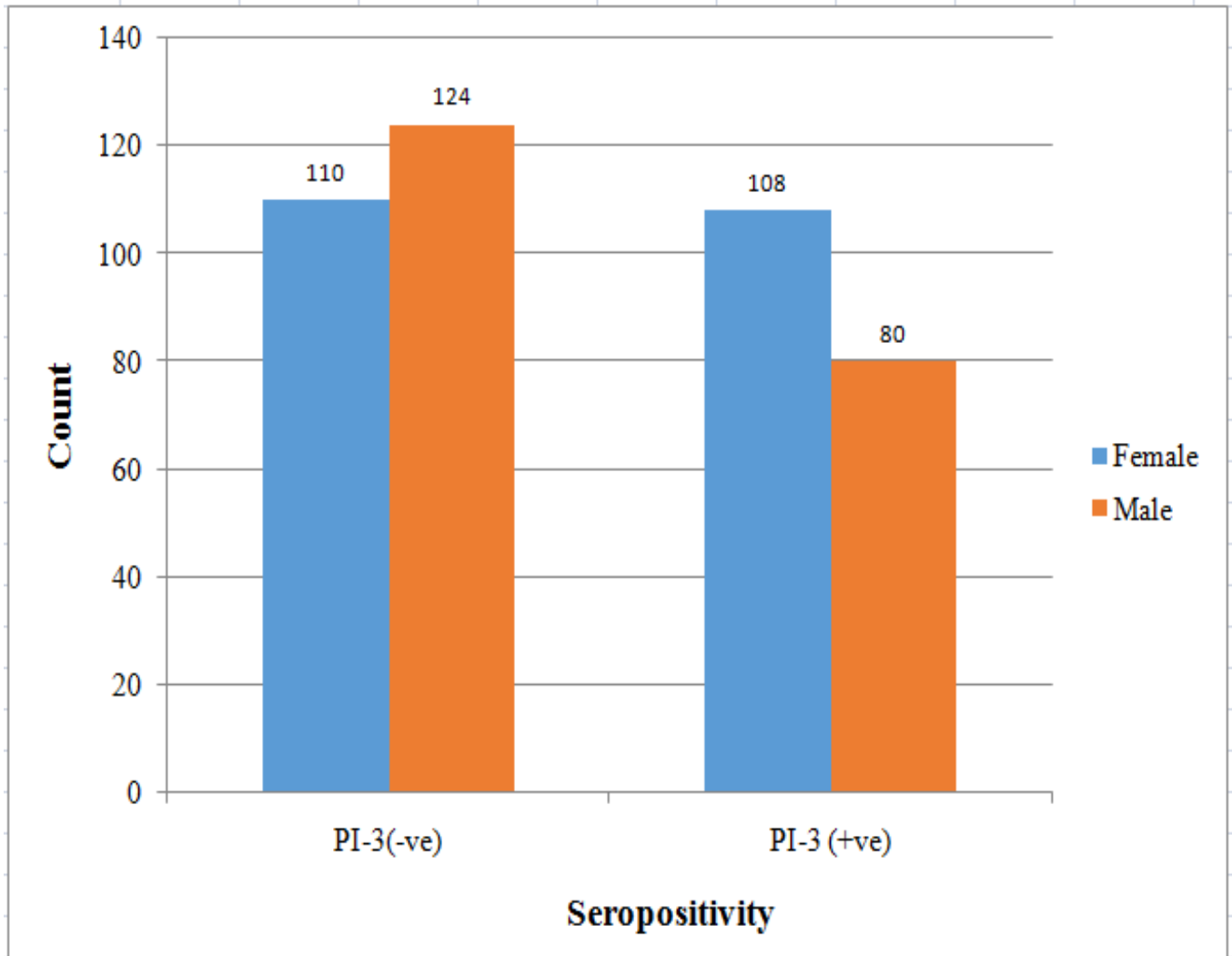
	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-0.01079	0.15221	-0.071	0.9435
factor(sex2)Male	-0.42850	0.19900	-2.153	0.0313 *

---  
signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)
fctr(sex2)M1	-0.614

**Figure 41: PI-3 Seropositivity by sex graph**



## Figure 42: BAV-3 Seropositivity by sex glmm results

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) ['glmerMod']

Family: binomial ( logit )

Formula: AD3\_2 ~ factor(sex2) + (1 | StockcardNumber) + (1 | Diptank\_name)

Data: biobank1

AIC	BIC	logLik	deviance	df.resid
390.1	406.3	-191.1	382.1	418

Scaled residuals:

Min	1Q	Median	3Q	Max
-2.3859	0.4173	0.4313	0.4452	0.5177

Random effects:

Groups	Name	Variance	Std.Dev.
StockcardNumber	(Intercept)	0.1046	0.3234
Diptank_name	(Intercept)	0.0000	0.0000

Number of obs: 422, groups: StockcardNumber, 101; Diptank\_name, 11

Fixed effects:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	1.65335	0.20001	8.266	<2e-16 ***
factor(sex2)Male	-0.03559	0.26411	-0.135	0.893

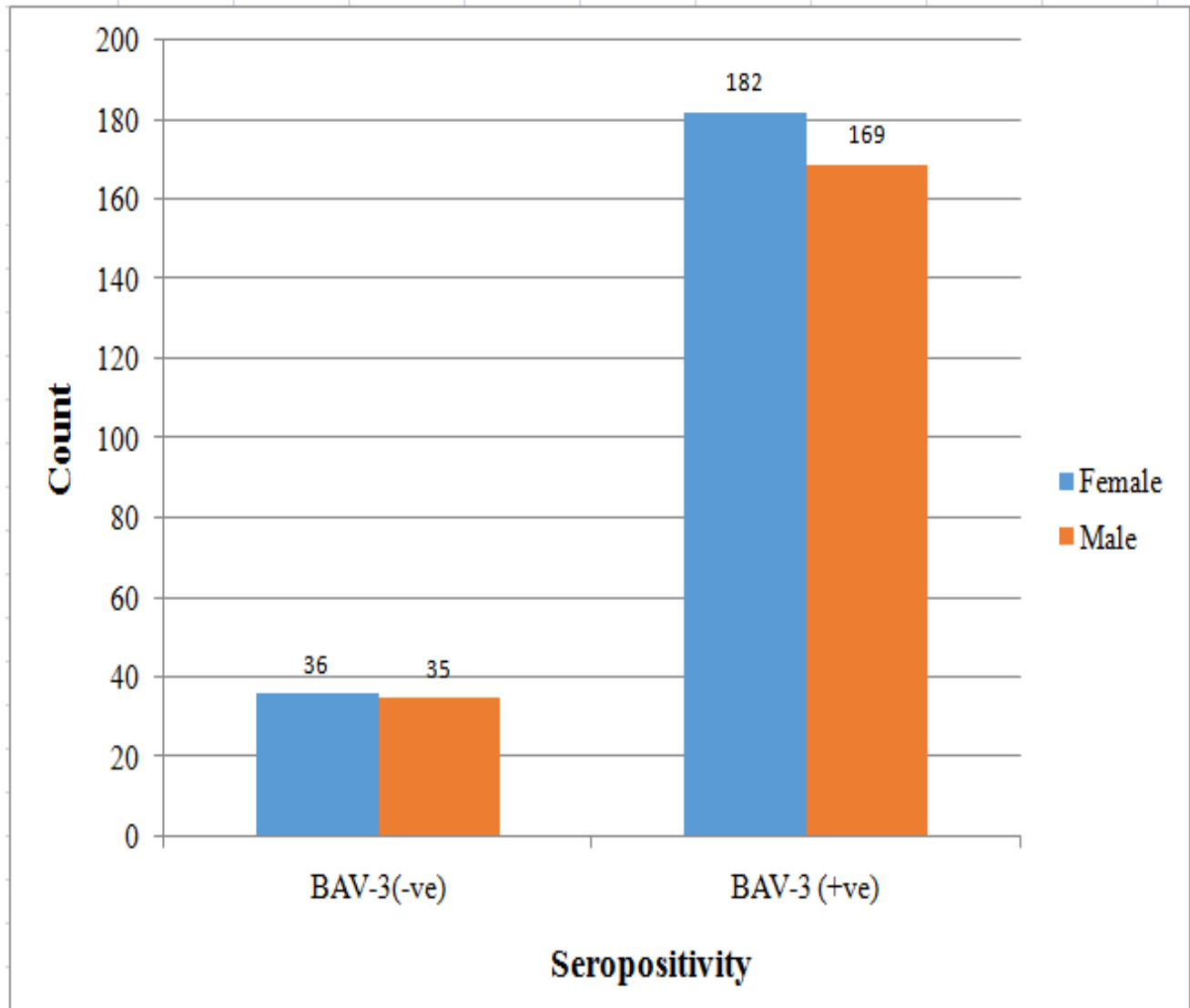
---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:

	(Intr)
fctr(sex2)M1	-0.621

**Figure 43: BAV-3 seropositivity by sex graph**



**Figure 44: BoHV-1 Seropositivity by location glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = BHV\_2 ~ (Diptank\_name) + (1 | StockcardNumber),  
data = biobank1, family = binomial)

Linear Hypotheses:

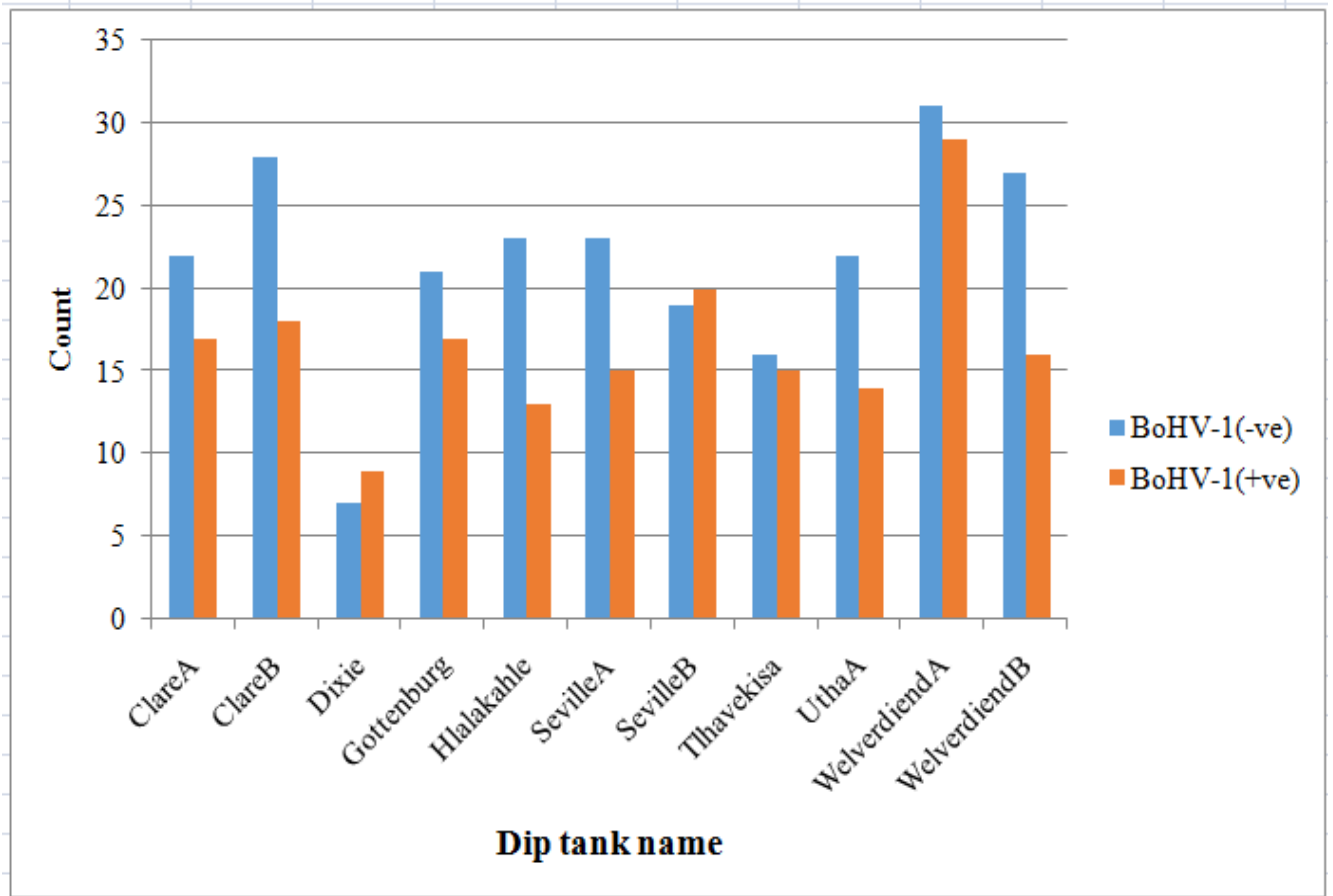
	Estimate	Std. Error	z value	Pr(> z )
ClareB - ClareA == 0	-0.184004	0.442207	-0.416	1.000
Dixie - ClareA == 0	0.509144	0.598535	0.851	0.999
Gottenburg - ClareA == 0	0.046520	0.459040	0.101	1.000
Hlalakahle - ClareA == 0	-0.312716	0.474004	-0.660	1.000
SevilleA - ClareA == 0	-0.169615	0.463056	-0.366	1.000
SevilleB - ClareA == 0	0.309122	0.454875	0.680	1.000
Tlhavekisa - ClareA == 0	0.193291	0.483162	0.400	1.000
UthaA - ClareA == 0	-0.194156	0.470273	-0.413	1.000
welverdiendA - ClareA == 0	0.191138	0.413543	0.462	1.000
welverdiendB - ClareA == 0	-0.265419	0.451456	-0.588	1.000
Dixie - ClareB == 0	0.693147	0.587569	1.180	0.984
Gottenburg - ClareB == 0	0.230524	0.444648	0.518	1.000
Hlalakahle - ClareB == 0	-0.128712	0.460078	-0.280	1.000
SevilleA - ClareB == 0	0.014389	0.448792	0.032	1.000
SevilleB - ClareB == 0	0.493126	0.440343	1.120	0.989
Tlhavekisa - ClareB == 0	0.377294	0.469508	0.804	0.999
UthaA - ClareB == 0	-0.010152	0.456237	-0.022	1.000
welverdiendA - ClareB == 0	0.375141	0.397506	0.944	0.997
welverdiendB - ClareB == 0	-0.081415	0.436814	-0.186	1.000
Gottenburg - Dixie == 0	-0.462624	0.600341	-0.771	1.000
Hlalakahle - Dixie == 0	-0.821859	0.611858	-1.343	0.960
SevilleA - Dixie == 0	-0.678758	0.603417	-1.125	0.989
SevilleB - Dixie == 0	-0.200021	0.597160	-0.335	1.000
Tlhavekisa - Dixie == 0	-0.315853	0.618979	-0.510	1.000
UthaA - Dixie == 0	-0.703300	0.608974	-1.155	0.987
welverdiendA - Dixie == 0	-0.318006	0.566311	-0.562	1.000
welverdiendB - Dixie == 0	-0.774563	0.594562	-1.303	0.968
Hlalakahle - Gottenburg == 0	-0.359236	0.476281	-0.754	1.000
SevilleA - Gottenburg == 0	-0.216135	0.465388	-0.464	1.000
SevilleB - Gottenburg == 0	0.262602	0.457246	0.574	1.000
Tlhavekisa - Gottenburg == 0	0.146771	0.485396	0.302	1.000
UthaA - Gottenburg == 0	-0.240676	0.472572	-0.509	1.000
welverdiendA - Gottenburg == 0	0.144618	0.416153	0.348	1.000
welverdiendB - Gottenburg == 0	-0.311939	0.453848	-0.687	1.000
SevilleA - Hlalakahle == 0	0.143101	0.480152	0.298	1.000
SevilleB - Hlalakahle == 0	0.621838	0.472265	1.317	0.965
Tlhavekisa - Hlalakahle == 0	0.506006	0.499569	1.013	0.995
UthaA - Hlalakahle == 0	0.118560	0.487118	0.243	1.000
welverdiendA - Hlalakahle == 0	0.503853	0.432600	1.165	0.986
welverdiendB - Hlalakahle == 0	0.047297	0.468976	0.101	1.000
SevilleB - SevilleA == 0	0.478737	0.461277	1.038	0.994
Tlhavekisa - SevilleA == 0	0.362905	0.489195	0.742	1.000
UthaA - SevilleA == 0	-0.024541	0.476473	-0.052	1.000



welverdiendA - SevilleA == 0	0.360753	0.420577	0.858	0.999
welverdiendB - SevilleA == 0	-0.095804	0.457909	-0.209	1.000
Tlhavekisa - SevilleB == 0	-0.115832	0.481456	-0.241	1.000
UthaA - SevilleB == 0	-0.503278	0.468523	-1.074	0.992
welverdiendA - SevilleB == 0	-0.117985	0.411550	-0.287	1.000
welverdiendB - SevilleB == 0	-0.574541	0.449632	-1.278	0.972
UthaA - Tlhavekisa == 0	-0.387447	0.496033	-0.781	0.999
welverdiendA - Tlhavekisa == 0	-0.002153	0.442615	-0.005	1.000
welverdiendB - Tlhavekisa == 0	-0.458710	0.478230	-0.959	0.997
welverdiendA - UthaA == 0	0.385294	0.428512	0.899	0.998
welverdiendB - UthaA == 0	-0.071263	0.465208	-0.153	1.000
welverdiendB - welverdiendA == 0	-0.456557	0.407771	-1.120	0.989

(Adjusted p values reported -- single-step method)

**Figure 45: BoHV-1 seropositivity by location graph**



**Figure 46: BVDV Seropositivity by location glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = BVDV\_2 ~ (Diptank\_name) + (1 | StockcardNumber),  
data = biobank1, family = binomial)

Linear Hypotheses:

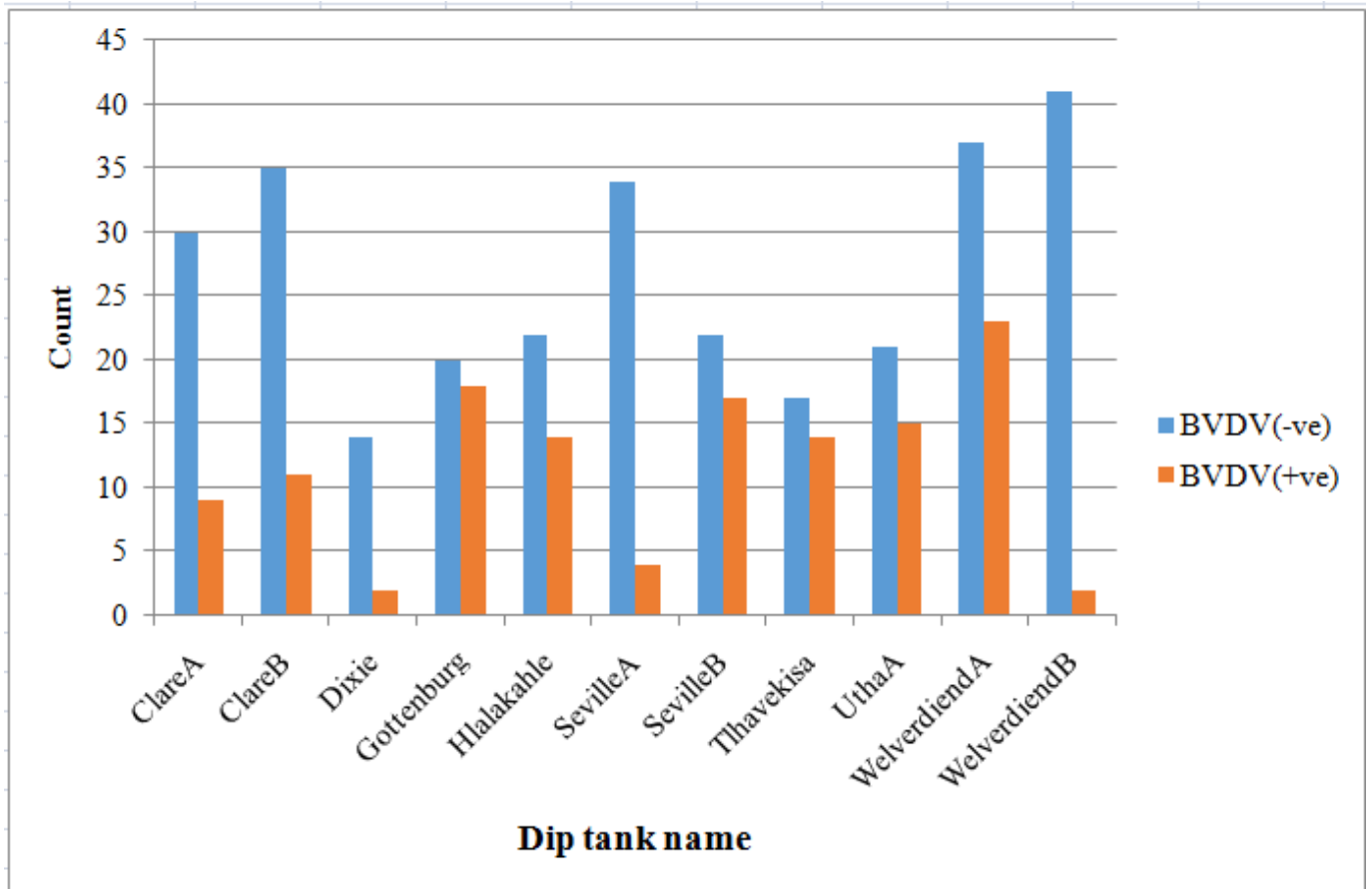
	Estimate	Std. Error	z value	Pr (> z )
ClareB - ClareA == 0	0.04652	0.51374	0.091	1.0000
Dixie - ClareA == 0	-0.74194	0.84609	-0.877	0.9984
Gottenburg - ClareA == 0	1.09861	0.50000	2.197	0.4845
Hlalakahle - ClareA == 0	0.75199	0.51120	1.471	0.9220
SevilleA - ClareA == 0	-0.93609	0.65104	-1.438	0.9325
SevilleB - ClareA == 0	0.94614	0.49872	1.897	0.7003
Tlhavekisa - ClareA == 0	1.00982	0.52411	1.927	0.6798
UthaA - ClareA == 0	0.86750	0.50865	1.705	0.8190
welverdiendA - ClareA == 0	0.72855	0.46362	1.571	0.8843
welverdiendB - ClareA == 0	-1.81645	0.81782	-2.221	0.4677
Dixie - ClareB == 0	-0.78846	0.83121	-0.949	0.9969
Hlalakahle - ClareB == 0	0.70547	0.48617	1.451	0.9284
SevilleA - ClareB == 0	-0.98261	0.63158	-1.556	0.8909
SevilleB - ClareB == 0	0.89962	0.47303	1.902	0.6973
Tlhavekisa - ClareB == 0	0.96330	0.49973	1.928	0.6788
UthaA - ClareB == 0	0.82098	0.48349	1.698	0.8232
welverdiendA - ClareB == 0	0.68203	0.43587	1.565	0.8869
welverdiendB - ClareB == 0	-1.86297	0.80241	-2.322	0.3963
Gottenburg - Dixie == 0	1.84055	0.82279	2.237	0.4547
Hlalakahle - Dixie == 0	1.49393	0.82965	1.801	0.7627
SevilleA - Dixie == 0	-0.19416	0.92241	-0.210	1.0000
SevilleB - Dixie == 0	1.68808	0.82202	2.054	0.5884
Tlhavekisa - Dixie == 0	1.75175	0.83767	2.091	0.5615
UthaA - Dixie == 0	1.60944	0.82808	1.944	0.6677
welverdiendA - Dixie == 0	1.47049	0.80121	1.835	0.7411
welverdiendB - Dixie == 0	-1.07451	1.04681	-1.026	0.9941
Hlalakahle - Gottenburg == 0	-0.34662	0.47163	-0.735	0.9997
SevilleA - Gottenburg == 0	-2.03471	0.62046	-3.279	0.0376 *
SevilleB - Gottenburg == 0	-0.15247	0.45808	-0.333	1.0000
Tlhavekisa - Gottenburg == 0	-0.08880	0.48560	-0.183	1.0000
UthaA - Gottenburg == 0	-0.23111	0.46887	-0.493	1.0000
welverdiendA - Gottenburg == 0	-0.37006	0.41960	-0.882	0.9983
welverdiendB - Gottenburg == 0	-2.91506	0.79369	-3.673	<0.01 **
SevilleA - Hlalakahle == 0	-1.68808	0.62952	-2.682	0.1927
SevilleB - Hlalakahle == 0	0.19416	0.47028	0.413	1.0000
Tlhavekisa - Hlalakahle == 0	0.25783	0.49713	0.519	1.0000
UthaA - Hlalakahle == 0	0.11551	0.48080	0.240	1.0000
welverdiendA - Hlalakahle == 0	-0.02344	0.43288	-0.054	1.0000
welverdiendB - Hlalakahle == 0	-2.56844	0.80079	-3.207	0.0466 *
SevilleB - SevilleA == 0	1.88224	0.61943	3.039	0.0759 .
Tlhavekisa - SevilleA == 0	1.94591	0.64005	3.040	0.0766 .
UthaA - SevilleA == 0	1.80359	0.62745	2.874	0.1202
welverdiendA - SevilleA == 0	1.66464	0.59154	2.814	0.1406

welverdiendB - SevilleA == 0	-0.88036	0.89655	-0.982	0.9959
Tlhavekisa - SevilleB == 0	0.06367	0.48428	0.131	1.0000
UthaA - SevilleB == 0	-0.07864	0.46751	-0.168	1.0000
welverdiendA - SevilleB == 0	-0.21759	0.41807	-0.520	1.0000
welverdiendB - SevilleB == 0	-2.76260	0.79288	-3.484	0.0189 *
UthaA - Tlhavekisa == 0	-0.14232	0.49451	-0.288	1.0000
welverdiendA - Tlhavekisa == 0	-0.28127	0.44806	-0.628	0.9999
welverdiendB - Tlhavekisa == 0	-2.82627	0.80910	-3.493	0.0182 *
welverdiendA - UthaA == 0	-0.13895	0.42987	-0.323	1.0000
welverdiendB - UthaA == 0	-2.68395	0.79917	-3.358	0.0291 *
welverdiendB - welverdiendA == 0	-2.54500	0.77129	-3.300	0.0354 *

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

Figure 47: BVDV seropositivity by location graph



**Figure 48: BRSV Seropositivity by location glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = BRSV\_2 ~ (Diptank\_name) + (1 | StockcardNumber),  
data = biobank1, family = binomial)

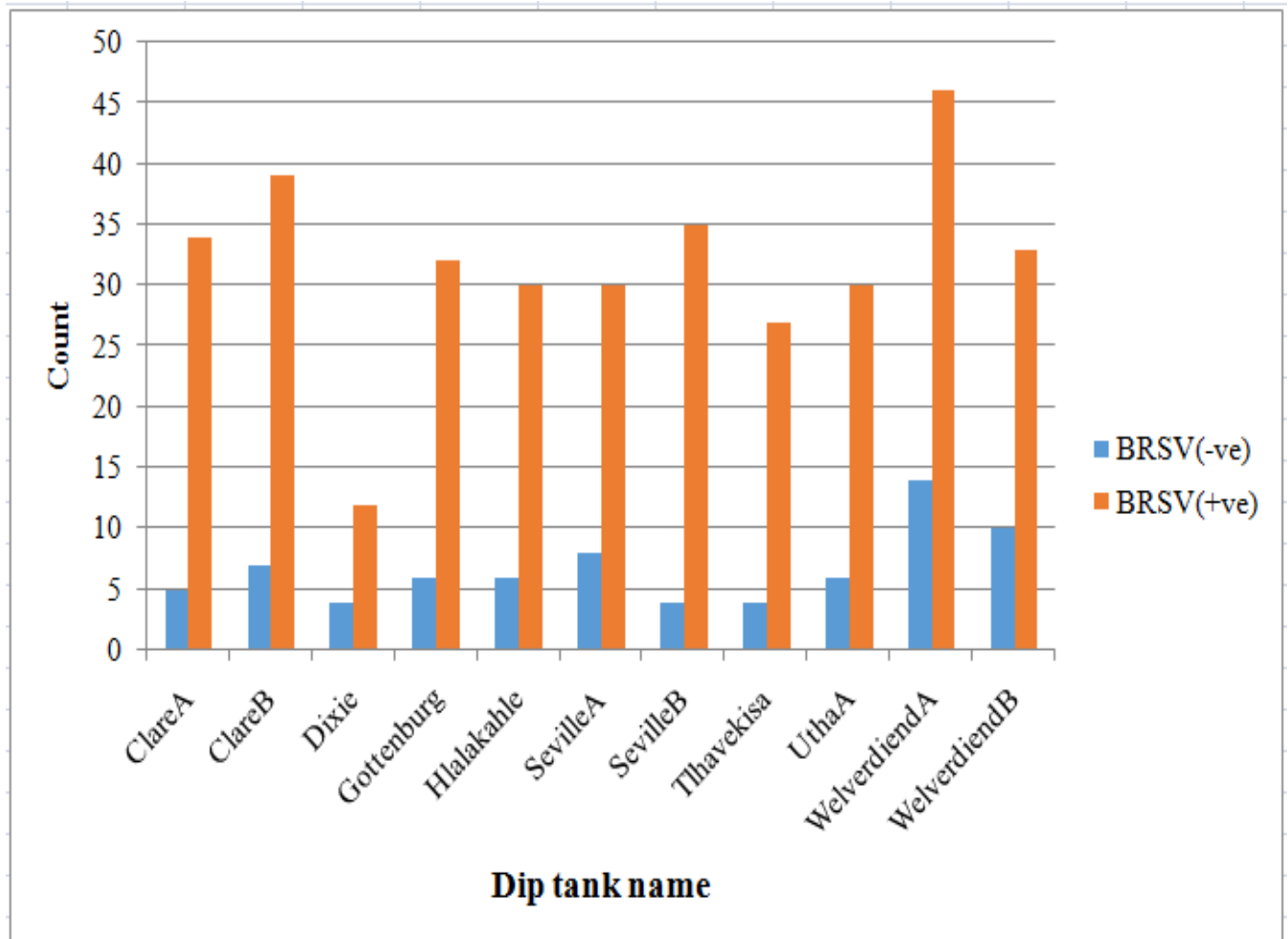
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
ClareB - ClareA == 0	-1.993e-01	6.308e-01	-0.316	1.000
Dixie - ClareA == 0	-8.183e-01	7.502e-01	-1.091	0.991
Gottenburg - ClareA == 0	-2.429e-01	6.537e-01	-0.372	1.000
Hlalakahle - ClareA == 0	-3.075e-01	6.553e-01	-0.469	1.000
SevilleA - ClareA == 0	-5.952e-01	6.227e-01	-0.956	0.997
SevilleB - ClareA == 0	2.521e-01	7.127e-01	0.354	1.000
Tlhavekisa - ClareA == 0	-7.380e-03	7.186e-01	-0.010	1.000
UthaA - ClareA == 0	-3.075e-01	6.553e-01	-0.469	1.000
welverdiendA - ClareA == 0	-7.273e-01	5.680e-01	-1.281	0.971
welverdiendB - ClareA == 0	-7.230e-01	5.998e-01	-1.205	0.981
Dixie - ClareB == 0	-6.190e-01	7.084e-01	-0.874	0.999
Gottenburg - ClareB == 0	-4.368e-02	6.053e-01	-0.072	1.000
Hlalakahle - ClareB == 0	-1.082e-01	6.070e-01	-0.178	1.000
SevilleA - ClareB == 0	-3.959e-01	5.717e-01	-0.692	1.000
SevilleB - ClareB == 0	4.514e-01	6.686e-01	0.675	1.000
Tlhavekisa - ClareB == 0	1.919e-01	6.749e-01	0.284	1.000
UthaA - ClareB == 0	-1.082e-01	6.070e-01	-0.178	1.000
welverdiendA - ClareB == 0	-5.281e-01	5.115e-01	-1.032	0.994
welverdiendB - ClareB == 0	-5.237e-01	5.466e-01	-0.958	0.997
Gottenburg - Dixie == 0	5.754e-01	7.289e-01	0.789	0.999
Hlalakahle - Dixie == 0	5.108e-01	7.303e-01	0.699	1.000
SevilleA - Dixie == 0	2.231e-01	7.012e-01	0.318	1.000
SevilleB - Dixie == 0	1.070e+00	7.822e-01	1.368	0.954
Tlhavekisa - Dixie == 0	8.109e-01	7.876e-01	1.030	0.994
UthaA - Dixie == 0	5.108e-01	7.303e-01	0.699	1.000
welverdiendA - Dixie == 0	9.097e-02	6.531e-01	0.139	1.000
welverdiendB - Dixie == 0	9.531e-02	6.809e-01	0.140	1.000
Hlalakahle - Gottenburg == 0	-6.454e-02	6.308e-01	-0.102	1.000
SevilleA - Gottenburg == 0	-3.522e-01	5.969e-01	-0.590	1.000
SevilleB - Gottenburg == 0	4.951e-01	6.903e-01	0.717	1.000
Tlhavekisa - Gottenburg == 0	2.356e-01	6.964e-01	0.338	1.000
UthaA - Gottenburg == 0	-6.454e-02	6.308e-01	-0.102	1.000
welverdiendA - Gottenburg == 0	-4.844e-01	5.395e-01	-0.898	0.998
welverdiendB - Gottenburg == 0	-4.801e-01	5.729e-01	-0.838	0.999
SevilleA - Hlalakahle == 0	-2.877e-01	5.986e-01	-0.481	1.000
SevilleB - Hlalakahle == 0	5.596e-01	6.918e-01	0.809	0.999
Tlhavekisa - Hlalakahle == 0	3.001e-01	6.979e-01	0.430	1.000
UthaA - Hlalakahle == 0	-3.859e-13	6.325e-01	0.000	1.000
welverdiendA - Hlalakahle == 0	-4.199e-01	5.414e-01	-0.775	0.999
welverdiendB - Hlalakahle == 0	-4.155e-01	5.747e-01	-0.723	1.000
SevilleB - SevilleA == 0	8.473e-01	6.610e-01	1.282	0.971
Tlhavekisa - SevilleA == 0	5.878e-01	6.674e-01	0.881	0.998
UthaA - SevilleA == 0	2.877e-01	5.986e-01	0.481	1.000
welverdiendA - SevilleA == 0	-1.322e-01	5.015e-01	-0.264	1.000
welverdiendB - SevilleA == 0	-1.278e-01	5.372e-01	-0.238	1.000
Tlhavekisa - SevilleB == 0	-2.595e-01	7.521e-01	-0.345	1.000
UthaA - SevilleB == 0	-5.596e-01	6.918e-01	-0.809	0.999
welverdiendA - SevilleB == 0	-9.795e-01	6.097e-01	-1.606	0.876
welverdiendB - SevilleB == 0	-9.751e-01	6.394e-01	-1.525	0.908
UthaA - Tlhavekisa == 0	-3.001e-01	6.979e-01	-0.430	1.000

welverdiendA - Tlhavekisa == 0	-7.200e-01	6.166e-01	-1.168	0.985
welverdiendB - Tlhavekisa == 0	-7.156e-01	6.460e-01	-1.108	0.990
welverdiendA - UthaA == 0	-4.199e-01	5.414e-01	-0.775	0.999
welverdiendB - UthaA == 0	-4.155e-01	5.747e-01	-0.723	1.000
welverdiendB - welverdiendA == 0	4.338e-03	4.727e-01	0.009	1.000

(Adjusted p values reported -- single-step method)

**Figure 49: BRSV seropositivity by location graph**





**Figure 50: PI-3 Seropositivity by location glmm multicom results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = PI3\_2 ~ (Diptank\_name) + (1 | StockcardNumber),  
data = biobank1, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
ClareB - ClareA == 0	-0.30010	0.43852	-0.684	0.9998
Dixie - ClareA == 0	0.30261	0.59716	0.507	1.0000
Gottenburg - ClareA == 0	-0.05407	0.45627	-0.118	1.0000
Hlalakahle - ClareA == 0	0.05129	0.46232	0.111	1.0000
SevilleA - ClareA == 0	-0.48770	0.46446	-1.050	0.9936
SevilleB - ClareA == 0	0.41420	0.45673	0.907	0.9981
Tlhavekisa - ClareA == 0	0.11583	0.48145	0.241	1.0000
UthaA - ClareA == 0	-0.40069	0.46852	-0.855	0.9988
welverdiendA - ClareA == 0	-0.08224	0.41181	-0.200	1.0000
welverdiendB - ClareA == 0	-1.14263	0.48263	-2.367	0.3826
Dixie - ClareB == 0	0.60271	0.58621	1.028	0.9946
Gottenburg - ClareB == 0	0.24604	0.44184	0.557	1.0000
Hlalakahle - ClareB == 0	0.35140	0.44808	0.784	0.9994
SevilleA - ClareB == 0	-0.18760	0.45029	-0.417	1.0000
SevilleB - ClareB == 0	0.71430	0.44232	1.615	0.8733
Tlhavekisa - ClareB == 0	0.41594	0.46780	0.889	0.9984
UthaA - ClareB == 0	-0.10059	0.45448	-0.221	1.0000
welverdiendA - ClareB == 0	0.21787	0.39577	0.550	1.0000
welverdiendB - ClareB == 0	-0.84252	0.46901	-1.796	0.7782
Gottenburg - Dixie == 0	-0.35667	0.59960	-0.595	1.0000
Hlalakahle - Dixie == 0	-0.25131	0.60422	-0.416	1.0000
SevilleA - Dixie == 0	-0.79031	0.60586	-1.304	0.9673
SevilleB - Dixie == 0	0.11159	0.59996	0.186	1.0000
Tlhavekisa - Dixie == 0	-0.18678	0.61898	-0.302	1.0000
UthaA - Dixie == 0	-0.70330	0.60898	-1.155	0.9865
welverdiendA - Dixie == 0	-0.38485	0.56651	-0.679	0.9998
welverdiendB - Dixie == 0	-1.44524	0.61990	-2.331	0.4066
Hlalakahle - Gottenburg == 0	0.10536	0.46547	0.226	1.0000
SevilleA - Gottenburg == 0	-0.43364	0.46760	-0.927	0.9977
SevilleB - Gottenburg == 0	0.46827	0.45993	1.018	0.9950
Tlhavekisa - Gottenburg == 0	0.16990	0.48448	0.351	1.0000
UthaA - Gottenburg == 0	-0.34662	0.47163	-0.735	0.9997
welverdiendA - Gottenburg == 0	-0.02817	0.41535	-0.068	1.0000
welverdiendB - Gottenburg == 0	-1.08856	0.48565	-2.241	0.4690
SevilleA - Hlalakahle == 0	-0.53900	0.47350	-1.138	0.9879
SevilleB - Hlalakahle == 0	0.36291	0.46593	0.779	0.9995
Tlhavekisa - Hlalakahle == 0	0.06454	0.49018	0.132	1.0000
UthaA - Hlalakahle == 0	-0.45199	0.47749	-0.947	0.9972
welverdiendA - Hlalakahle == 0	-0.13353	0.42199	-0.316	1.0000
welverdiendB - Hlalakahle == 0	-1.19392	0.49134	-2.430	0.3416
SevilleB - SevilleA == 0	0.90190	0.46805	1.927	0.6939
Tlhavekisa - SevilleA == 0	0.60354	0.49220	1.226	0.9790
UthaA - SevilleA == 0	0.08701	0.47956	0.181	1.0000

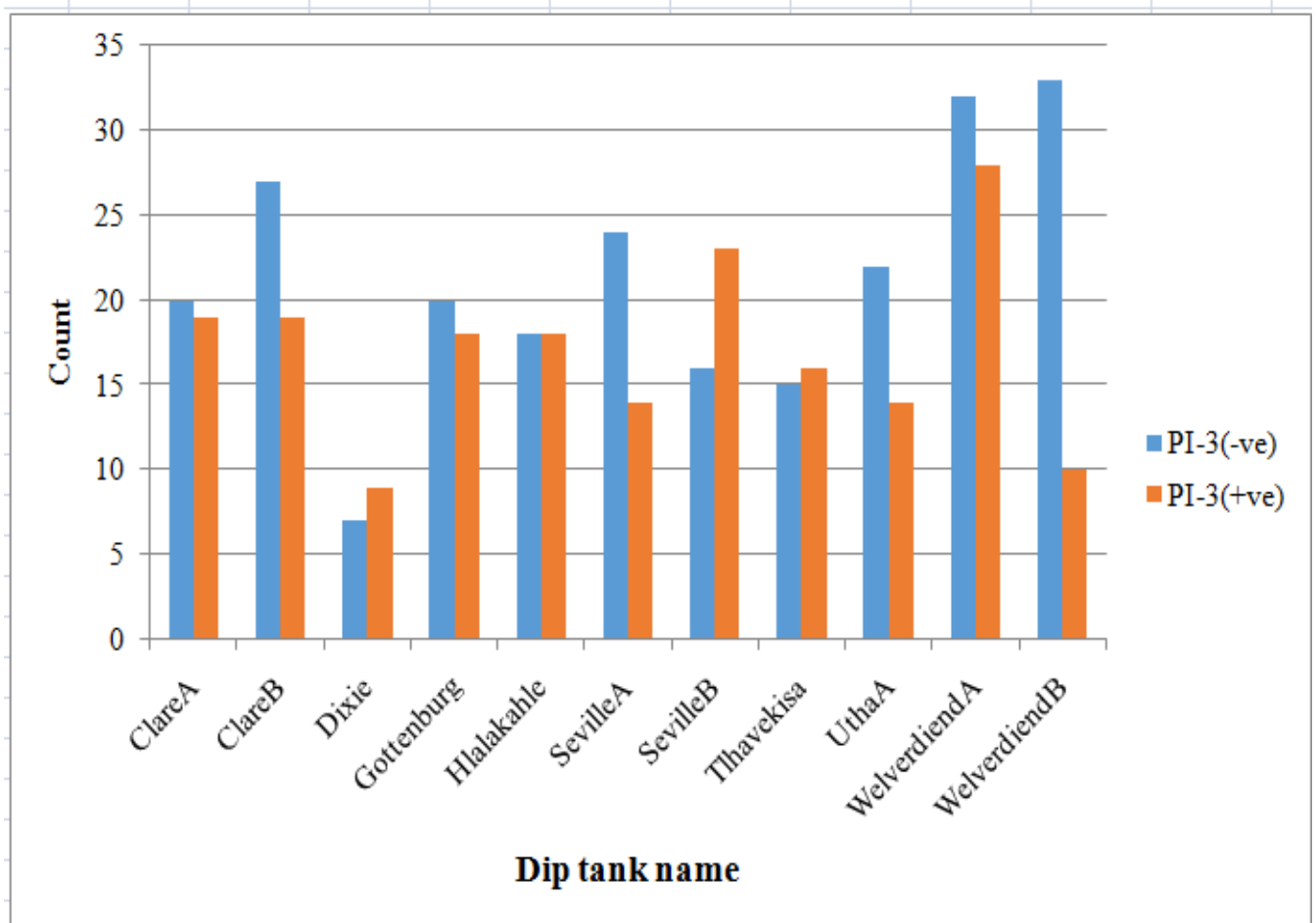
```

wilverdiendA - SevilleA == 0      0.40547      0.42433      0.956      0.9970
wilverdiendB - SevilleA == 0     -0.65493      0.49336     -1.327      0.9630
Tlhavekisa - SevilleB == 0       -0.29837      0.48492     -0.615      0.9999
UthaA - SevilleB == 0           -0.81489      0.47208     -1.726      0.8187
wilverdiendA - SevilleB == 0     -0.49644      0.41586     -1.194      0.9828
wilverdiendB - SevilleB == 0     -1.55683      0.48609     -3.203      0.0499 *
UthaA - Tlhavekisa == 0         -0.51652      0.49603     -1.041      0.9940
wilverdiendA - Tlhavekisa == 0   -0.19807      0.44287     -0.447      1.0000
wilverdiendB - Tlhavekisa == 0  -1.25846      0.50938     -2.471      0.3165
wilverdiendA - UthaA == 0        0.31845      0.42877      0.743      0.9997
wilverdiendB - UthaA == 0       -0.74194      0.49718     -1.492      0.9207
wilverdiendB - wilverdiendA == 0 -1.06039      0.44415     -2.387      0.3688

```

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

Figure 51: PI-3 seropositivity by location graph



**Figure 52: BAV-3 Seropositivity by location glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: glmer(formula = AD3\_2 ~ (Diptank\_name) + (1 | StockcardNumber),  
data = biobank1, family = binomial)

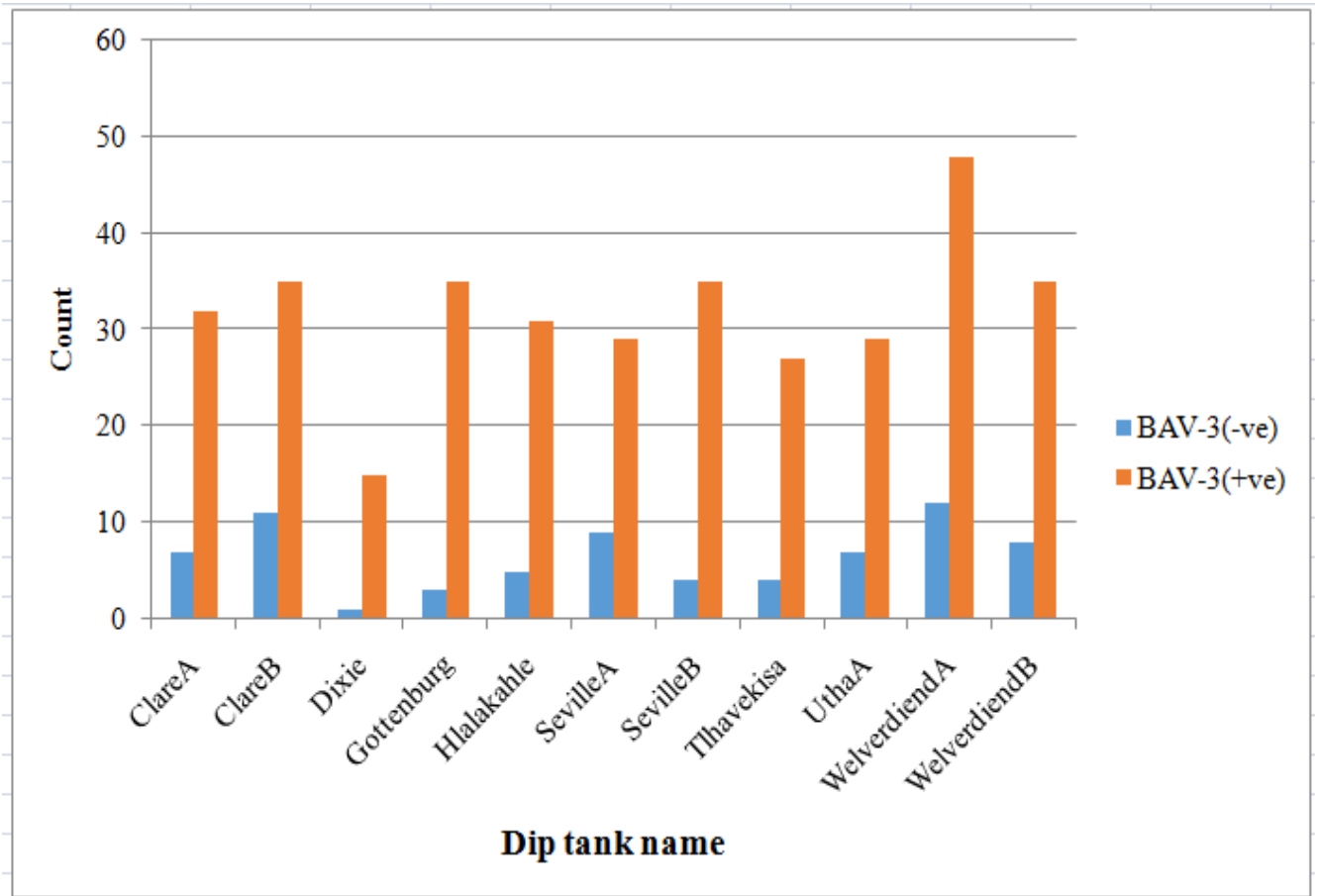
Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
ClareB - ClareA == 0	-0.38053	0.55605	-0.684	1.000
Dixie - ClareA == 0	1.20706	1.12803	1.070	0.992
Gottenburg - ClareA == 0	0.96870	0.74443	1.301	0.966
Hlalakahle - ClareA == 0	0.34471	0.65395	0.527	1.000
SevilleA - ClareA == 0	-0.33376	0.57876	-0.577	1.000
SevilleB - ClareA == 0	0.64647	0.68316	0.946	0.997
Tlhavekisa - ClareA == 0	0.41225	0.69469	0.593	1.000
UthaA - ClareA == 0	-0.09800	0.60697	-0.161	1.000
welverdiendA - ClareA == 0	-0.13417	0.53982	-0.249	1.000
welverdiendB - ClareA == 0	-0.04821	0.58547	-0.082	1.000
Dixie - ClareB == 0	1.58760	1.10412	1.438	0.933
Gottenburg - ClareB == 0	1.34924	0.70780	1.906	0.696
Hlalakahle - ClareB == 0	0.72525	0.61260	1.184	0.983
SevilleA - ClareB == 0	0.04678	0.53156	0.088	1.000
SevilleB - ClareB == 0	1.02700	0.64291	1.597	0.875
Tlhavekisa - ClareB == 0	0.79278	0.65528	1.210	0.980
UthaA - ClareB == 0	0.28253	0.55858	0.506	1.000
welverdiendA - ClareB == 0	0.24637	0.48463	0.508	1.000
welverdiendB - ClareB == 0	0.33233	0.53254	0.624	1.000
Gottenburg - Dixie == 0	-0.23836	1.20746	-0.197	1.000
Hlalakahle - Dixie == 0	-0.86235	1.15509	-0.747	1.000
SevilleA - Dixie == 0	-1.54082	1.11465	-1.382	0.948
SevilleB - Dixie == 0	-0.56060	1.17141	-0.479	1.000
Tlhavekisa - Dixie == 0	-0.79482	1.17810	-0.675	1.000
UthaA - Dixie == 0	-1.30507	1.13002	-1.155	0.985
welverdiendA - Dixie == 0	-1.34123	1.09586	-1.224	0.978
welverdiendB - Dixie == 0	-1.25527	1.11809	-1.123	0.988
Hlalakahle - Gottenburg == 0	-0.62399	0.77992	-0.800	0.999
SevilleA - Gottenburg == 0	-1.30246	0.72452	-1.798	0.767
SevilleB - Gottenburg == 0	-0.32223	0.81265	-0.397	1.000
Tlhavekisa - Gottenburg == 0	-0.55645	0.81609	-0.682	1.000
UthaA - Gottenburg == 0	-1.06670	0.74541	-1.431	0.935
welverdiendA - Gottenburg == 0	-1.10287	0.69341	-1.590	0.877
welverdiendB - Gottenburg == 0	-1.01691	0.72940	-1.394	0.945
SevilleA - Hlalakahle == 0	-0.67847	0.62701	-1.082	0.991
SevilleB - Hlalakahle == 0	0.30176	0.73055	0.413	1.000
Tlhavekisa - Hlalakahle == 0	0.06754	0.73406	0.092	1.000
UthaA - Hlalakahle == 0	-0.44271	0.65635	-0.675	1.000
welverdiendA - Hlalakahle == 0	-0.47888	0.59551	-0.804	0.999
welverdiendB - Hlalakahle == 0	-0.39292	0.63718	-0.617	1.000
SevilleB - SevilleA == 0	0.98023	0.66378	1.477	0.921
Tlhavekisa - SevilleA == 0	0.74601	0.67349	1.108	0.989

UthaA - SevilleA == 0	0.23576	0.58515	0.403	1.000
welverdiendA - SevilleA == 0	0.19959	0.51449	0.388	1.000
welverdiendB - SevilleA == 0	0.28555	0.56171	0.508	1.000
Tlhavekisa - SevilleB == 0	-0.23422	0.76711	-0.305	1.000
UthaA - SevilleB == 0	-0.74447	0.68816	-1.082	0.991
welverdiendA - SevilleB == 0	-0.78064	0.62935	-1.240	0.975
welverdiendB - SevilleB == 0	-0.69468	0.66873	-1.039	0.994
UthaA - Tlhavekisa == 0	-0.51025	0.69677	-0.732	1.000
welverdiendA - Tlhavekisa == 0	-0.54642	0.63915	-0.855	0.999
welverdiendB - Tlhavekisa == 0	-0.46046	0.67834	-0.679	1.000
welverdiendA - UthaA == 0	-0.03617	0.54120	-0.067	1.000
welverdiendB - UthaA == 0	0.04979	0.58764	0.085	1.000
welverdiendB - welverdiendA == 0	0.08596	0.51783	0.166	1.000

(Adjusted p values reported -- single-step method)

**Figure 53: BAV-3 seropositivity by location graph**



**Figure 54: BoHV-1 Seropositivity by time of collection glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

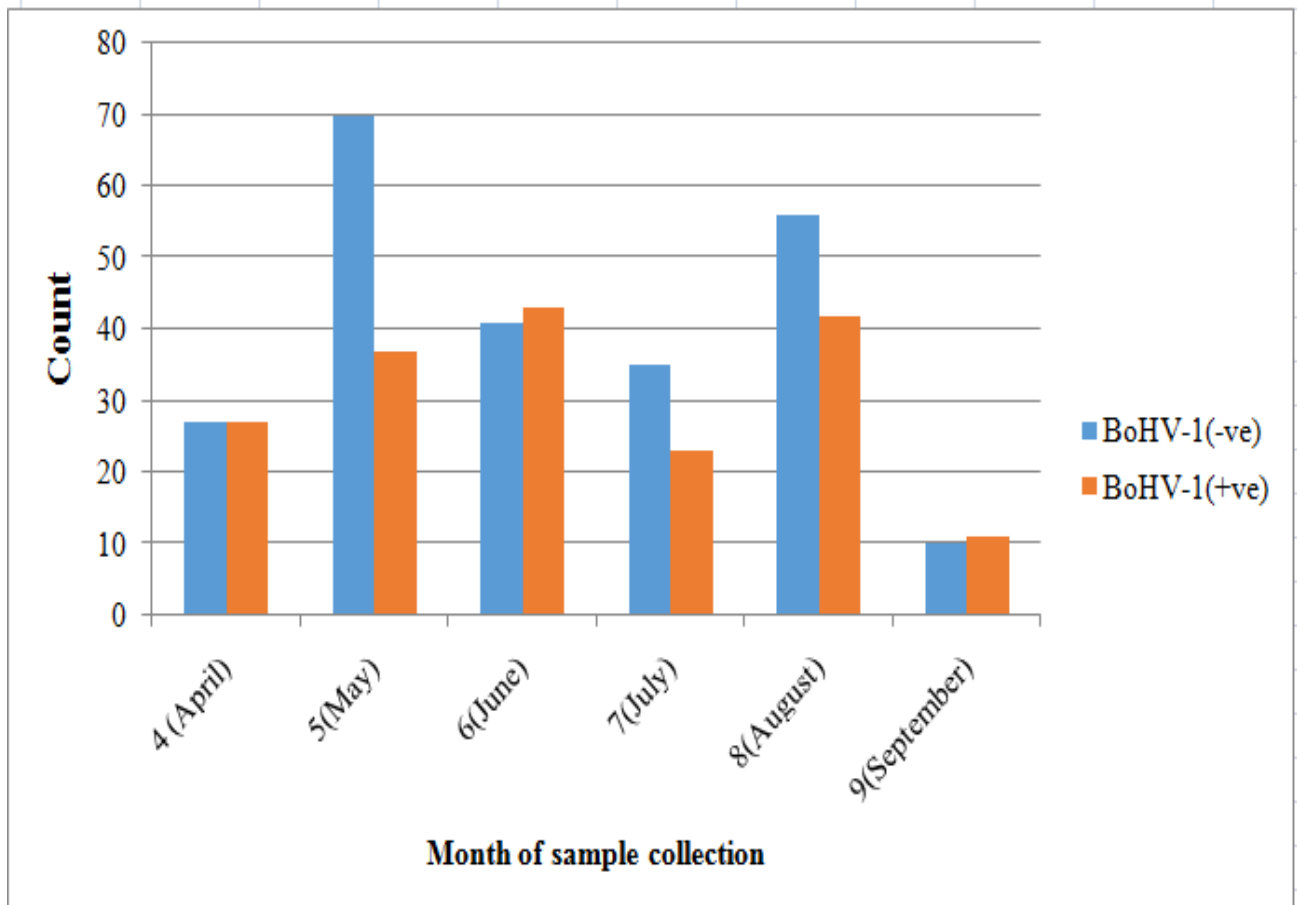
Fit: glmer(formula = BHV\_2 ~ (collectionmonth) + (1 | StockcardNumber/Diptank\_name), data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
August - April == 0	-0.28768	0.34020	-0.846	0.957
July - April == 0	-0.41985	0.38226	-1.098	0.877
June - April == 0	0.04763	0.34888	0.137	1.000
May - April == 0	-0.65176	0.33939	-1.920	0.379
September - April == 0	0.09531	0.51476	0.185	1.000
July - August == 0	-0.13217	0.33722	-0.392	0.999
June - August == 0	0.33531	0.29885	1.122	0.867
May - August == 0	-0.36408	0.28771	-1.265	0.797
September - August == 0	0.38299	0.48226	0.794	0.967
June - July == 0	0.46748	0.34597	1.351	0.748
May - July == 0	-0.23191	0.33639	-0.689	0.982
September - July == 0	0.51516	0.51279	1.005	0.913
May - June == 0	-0.69939	0.29792	-2.348	0.168
September - June == 0	0.04768	0.48842	0.098	1.000
September - May == 0	0.74707	0.48168	1.551	0.621

(Adjusted p values reported -- single-step method)

**Figure 55: BoHV-1 seropositivity by month of collection graph**





**Figure 56 : BVDV Seropositivity by time of collection glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

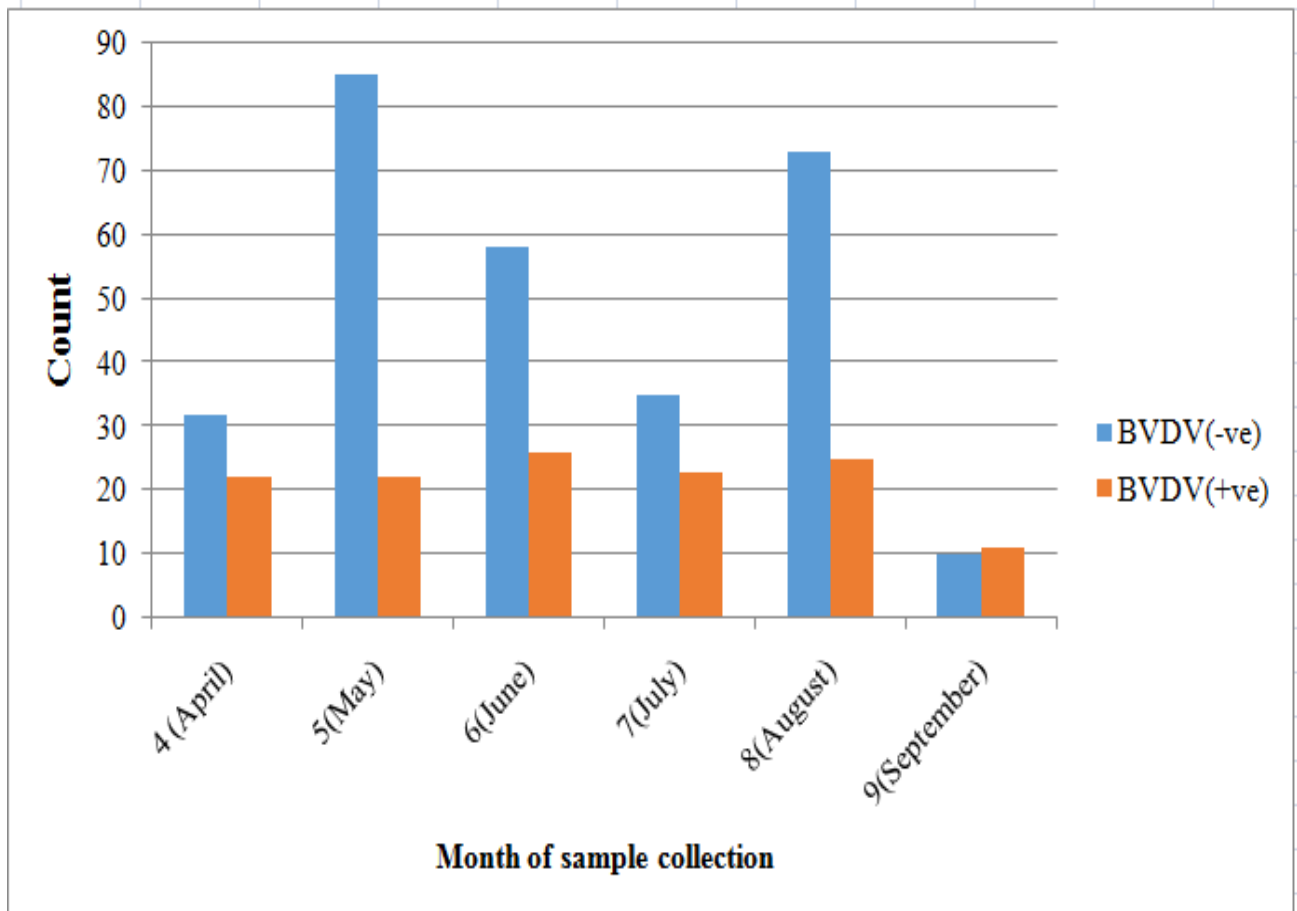
```
Fit: glmer(formula = BVDV_2 ~ (collectionmonth) + (1 |
StockcardNumber/Diptank_name),
  data = biobank1_MANYETU, family = binomial)
```

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
August - April == 0	-0.715043	0.002836	-252.166	< 0.001
July - April == 0	0.015875	0.002835	5.600	< 0.001
June - April == 0	-0.431173	0.002835	-152.071	< 0.001
May - April == 0	-1.083655	0.002836	-382.132	< 0.001 *
September - April == 0	0.597753	0.508485	1.176	0.80404
July - August == 0	0.730918	0.004010	182.290	< 0.001
June - August == 0	0.283869	0.004010	70.797	< 0.001 *
May - August == 0	-0.368613	0.004010	-91.929	< 0.001
September - August == 0	1.312796	0.508493	2.582	0.07594 .
June - July == 0	-0.447049	0.004009	-111.499	< 0.001 *
May - July == 0	-1.099531	0.004010	-274.211	< 0.001
September - July == 0	0.581878	0.508493	1.144	0.82106
May - June == 0	-0.652482	0.004010	-162.723	< 0.001 *
September - June == 0	1.028927	0.508493	2.023	0.26700
September - May == 0	1.681408	0.508493	3.307	0.00857

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Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1  
(Adjusted p values reported -- single-step method)

**Figure 57: BVDV seropositivity by time of sample collection graph**



**Figure 58: BRSV Seropositivity by time of collection glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

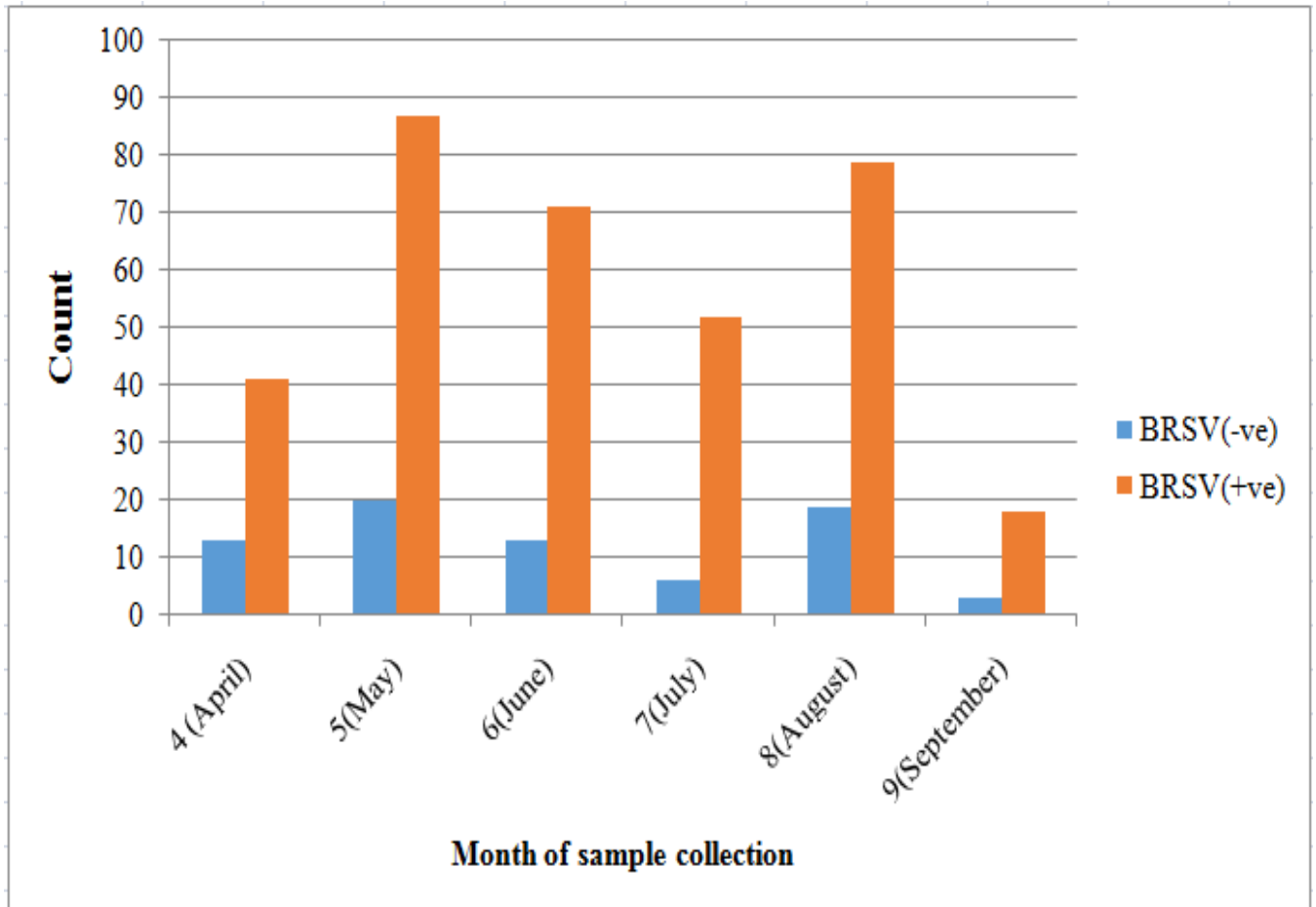
Fit: glmer(formula = BRSV\_2 ~ (collectionmonth) + (1 | StockcardNumber),  
data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
August - April == 0	0.27659	0.41009	0.674	0.984
July - April == 0	1.01138	0.54508	1.855	0.415
June - April == 0	0.54934	0.44087	1.246	0.804
May - April == 0	0.33341	0.41155	0.810	0.963
September - April == 0	0.64345	0.70289	0.915	0.939
July - August == 0	0.73479	0.50487	1.455	0.679
June - August == 0	0.27275	0.39545	0.690	0.982
May - August == 0	0.05682	0.35850	0.158	1.000
September - August == 0	0.36686	0.67441	0.544	0.994
June - July == 0	-0.46204	0.52911	-0.873	0.950
May - July == 0	-0.67797	0.49763	-1.362	0.738
September - July == 0	-0.36793	0.75928	-0.485	0.996
May - June == 0	-0.21593	0.39216	-0.551	0.994
September - June == 0	0.09411	0.69306	0.136	1.000
September - May == 0	0.31004	0.67148	0.462	0.997

(Adjusted p values reported -- single-step method)

**Figure 59: BRSV seropositivity by time of sample collection graph**



**Figure 60: PI-3 Seropositivity by time of collection glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

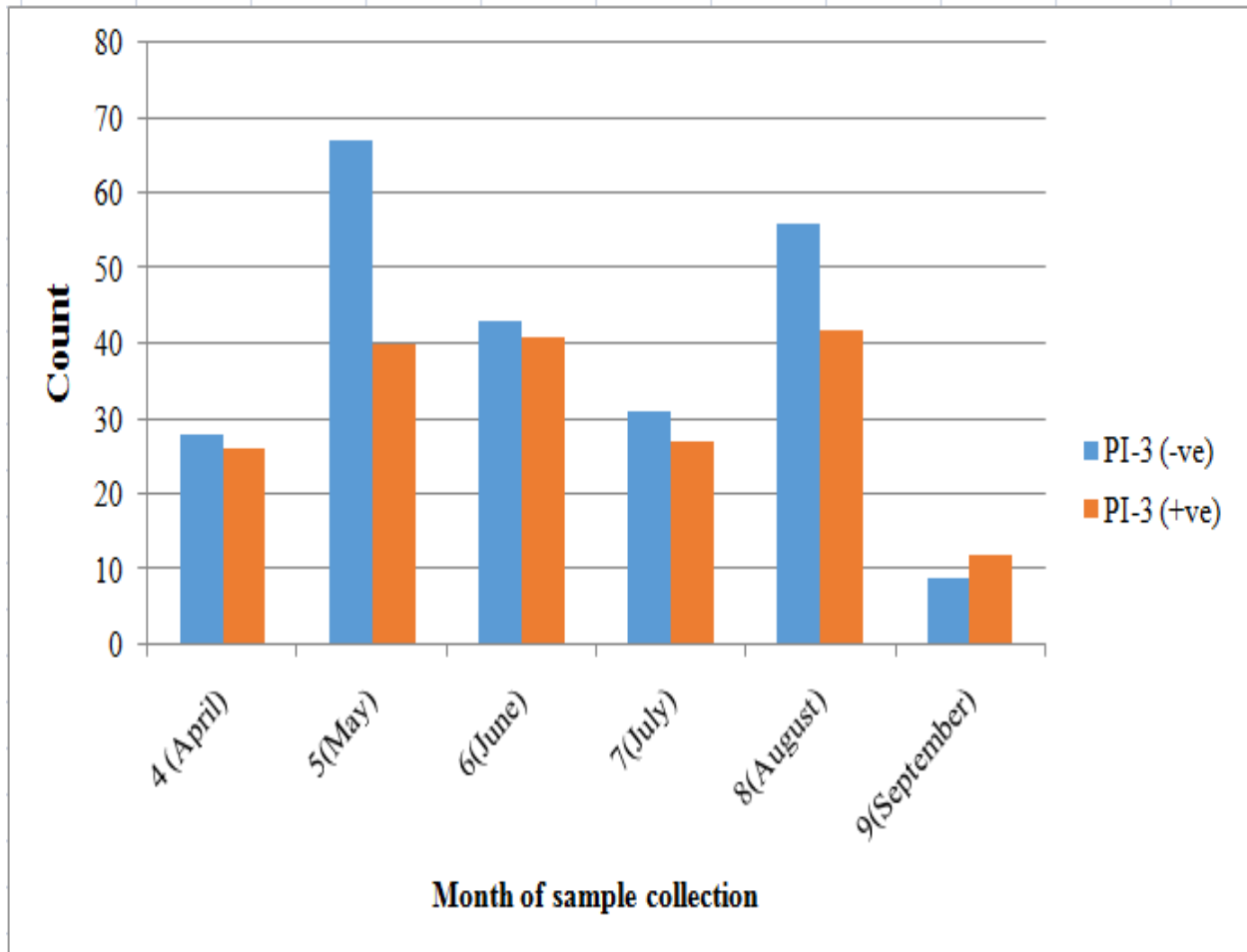
Fit: glmer(formula = PI3\_2 ~ (collectionmonth) + (1 | StockcardNumber),  
data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
August - April == 0	-0.21357	0.34035	-0.628	0.988
July - April == 0	-0.06404	0.37877	-0.169	1.000
June - April == 0	0.02648	0.34903	0.076	1.000
May - April == 0	-0.45652	0.33746	-1.353	0.747
September - April == 0	0.36179	0.51829	0.698	0.981
July - August == 0	0.14953	0.33311	0.449	0.998
June - August == 0	0.24005	0.29885	0.803	0.965
May - August == 0	-0.24295	0.28526	-0.852	0.956
September - August == 0	0.57536	0.48591	1.184	0.838
June - July == 0	0.09052	0.34197	0.265	1.000
May - July == 0	-0.39248	0.33015	-1.189	0.836
September - July == 0	0.42583	0.51356	0.829	0.960
May - June == 0	-0.48300	0.29555	-1.634	0.565
September - June == 0	0.33531	0.49203	0.681	0.983
September - May == 0	0.81831	0.48389	1.691	0.526

(Adjusted p values reported -- single-step method)

**Figure 61: PI-3 seropositivity by time of sample collection**



**Figure 62: BAV-3 Seropositivity by time of collection glmm multcomp results**

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

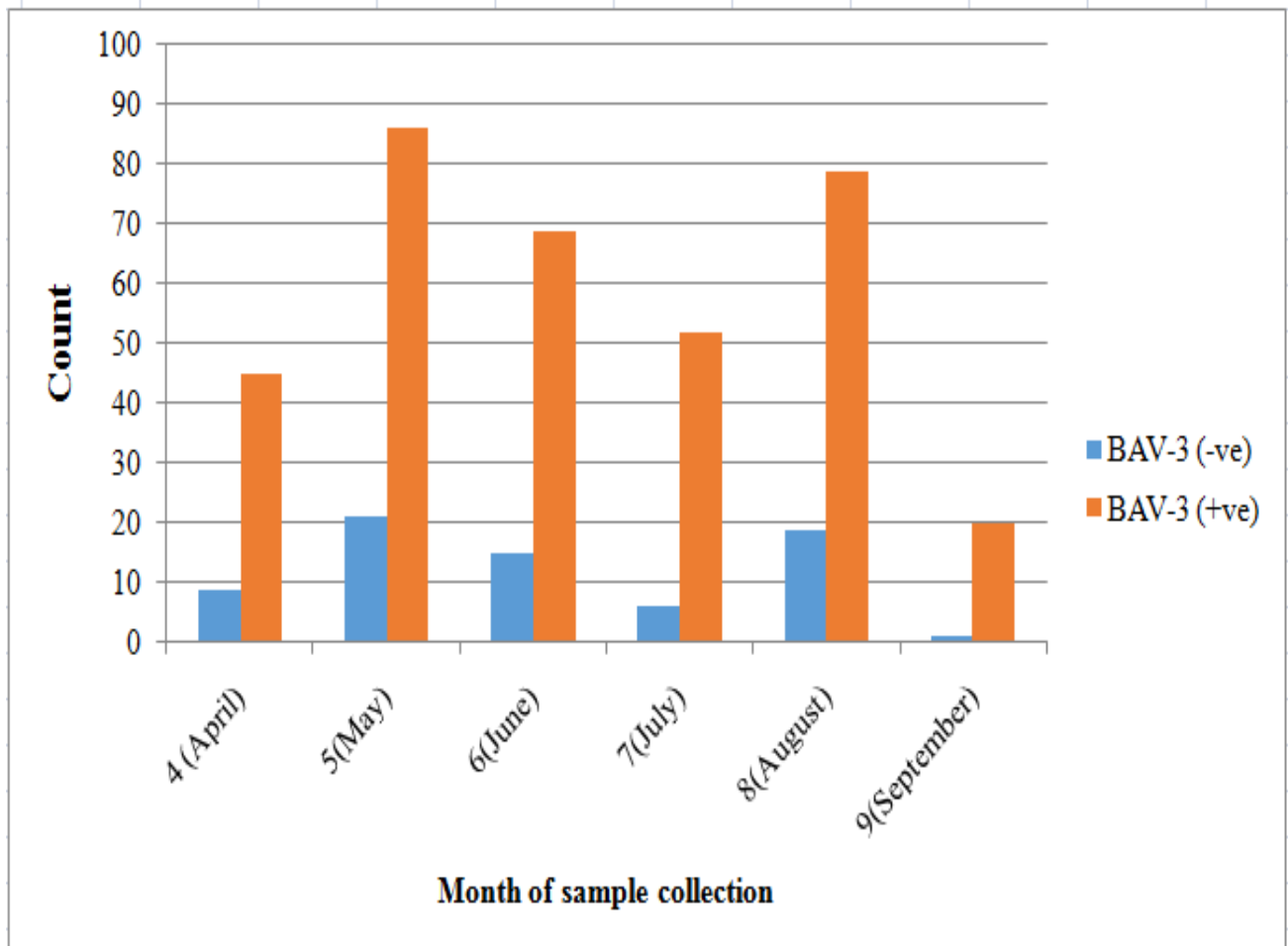
Fit: glmer(formula = AD3\_2 ~ (collectionmonth) + (1 | StockcardNumber),  
data = biobank1\_MANYETU, family = binomial)

Linear Hypotheses:

	Estimate	Std. Error	z value	Pr(> z )
August - April == 0	-0.185772	0.453450	-0.410	0.998
July - April == 0	0.576660	0.575148	1.003	0.908
June - April == 0	-0.088588	0.470741	-0.188	1.000
May - April == 0	-0.183187	0.446328	-0.410	0.998
September - April == 0	1.388978	1.097151	1.266	0.786
July - August == 0	0.762432	0.511421	1.491	0.646
June - August == 0	0.097184	0.389622	0.249	1.000
May - August == 0	0.002585	0.359855	0.007	1.000
September - August == 0	1.574750	1.064165	1.480	0.653
June - July == 0	-0.665248	0.526744	-1.263	0.787
May - July == 0	-0.759847	0.503587	-1.509	0.634
September - July == 0	0.812318	1.122128	0.724	0.976
May - June == 0	-0.094599	0.381153	-0.248	1.000
September - June == 0	1.477566	1.072027	1.378	0.719
September - May == 0	1.572165	1.061526	1.481	0.653

(Adjusted p values reported -- single-step method)

**Figure 63: BAV-3 seropositivity by time of sample collection graph**





## ANIMAL ETHICS COMMITTEE APPROVAL



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

### Animal Ethics Committee


PROJECT TITLE	A statistical analysis of spatial and trait based variation in exposure to selected upper respiratory viral infections in domestic cattle at the wildlife-livestock interface for the purposes of reducing infectious disease transmission within and between cattle and buffalo: a case study of Mnisi Community Project, South Africa
PROJECT NUMBER	V099-14 C
RESEARCHER/PRINCIPAL INVESTIGATOR	K Manyetu

STUDENT NUMBER (where applicable)	UP_15393781
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	n/a	
NUMBER OF ANIMALS	n/a	
Approval period to use animals for research/testing purposes	May 2016 – May 2017	
SUPERVISOR	Prof. M Jansen van Vuuren	

**KINDLY NOTE:**

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

<b>APPROVED</b>	Date	14 June 2016
CHAIRMAN: UP Animal Ethics Committee	Signature	

CA199E-1C