Spatial sero-survey of respiratory tract viral infections in cattle at the wildlife-livestock interface in the Mnisi communal farming area of South Africa

by

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A thesis submitted in partial fulfilment of the requirements
for the degree of

Master of Science (Animal/Human/Ecosystem Health)

in the

Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
University of Pretoria

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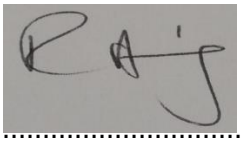
Co-Supervisor: **Dr Courtney Coon**

2018

Declaration

I declare that this dissertation hereby submitted to the University of Pretoria for the degree of Master of Science (Animal/Human/Ecosystem Health) has not been previously submitted by me for the degree at any other University, that it is my own work in design and in execution, and that all material contained therein has been duly acknowledged.



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Rauna Athingo

Date: **31 August 2018**

Acknowledgements

I would first like to thank my supervisor, Professor Emeritus Moritz van Vuuren who has been supportive throughout the duration of this project. He consistently kept me grounded and instilled discipline to keep to the deadline of submission of all the required documents for this project.

I also would like to sincerely thank my co-supervisor, Dr Courtney Coon, who has been incredibly supportive throughout the duration of this project. Her inputs, prompt feedback and willingness to help were just fantastic. She consistently allowed this paper to be my own work, but steered me in the right direction whenever she thought I needed it for which I will forever be grateful. I could not have asked for a better co-supervisor and consider myself very lucky to have had her supervision throughout the project.

I would also like to thank the State Veterinarian for the Mpumalanga Province, Dr Bjorn Reininghaus, as well as Ms Jeanette Wentzel and Dr Greg Simpson, managers at the Hans Hoheisen Wildlife Research Centre (HHWRS), for making available the stored serum samples and the laboratory for sample analysis. Without their support this project would not have been possible.

I am very grateful to the University of Pretoria, Department of Veterinary Tropical Diseases (DVTC) and Institute of Tropical Medicine (ITM) firstly for according me an opportunity to further my career and secondly for the funding I received during my MSc program.

To the staff of the Department of Veterinary Tropical Diseases, I would like to express my appreciation for the diverse knowledge acquired during the two years. A special thanks to Ms Rina Serfontein and Ms Leonie Johnson from student administration for outstanding logistical support and assistance with all the queries during this project.

I would like to thank my sister Mrs Anna Haifete for setting a good example and instilling a good culture of acquiring knowledge through research.

Last but not least, I would like to thank my husband, Mr Petrus Athingo for believing in me and his encouragement to further my career. I thank him for being supportive throughout my studies.

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List of abbreviations

BAV-3	Bovine mastadenovirus-3
BoHV-1	Bovine alphaherpesvirus-1
BRDC	Bovine Respiratory Disease Complex
BRSV	Bovine respiratory syncytial virus
bTB	Bovine tuberculosis
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
OD	Optical density
PAHC	Primary animal health care
PI-3	Parainfluenza-3 virus
UP	University of Pretoria

Summary

Spatial sero-survey of respiratory tract viral infections in cattle at the wildlife-livestock interface in the Mnisi communal farming area of South Africa

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Degree: **MSc (Animal/Human/Ecosystem Health)**

Animal diseases impact on livestock production and threaten food security through loss of animal protein. Additionally, disease impacts may cause major production losses by adding to the cost of livestock production through the necessity to apply costly disease control measures. Taken together, farm animal diseases have been shown to increase poverty levels particularly in poor communities in Africa that have a high dependence on livestock farming for sustenance (Perry et al., 2009). Research to learn more about animal diseases is necessary for the development of appropriate policies and strategies to prevent, control and possibly eradicate costly animal diseases in order to increase socio-economic development and improve livelihood, especially in Africa (Perry et al., 2009).

The classification used in this document is the same as that currently used in the OIE Terrestrial Code (for bovine herpesvirus- 1 (BoHV-1) and bovine viral diarrhea virus (BVDV) and Merck manuals bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (PI-3) and bovine adenovirus (BAV-3) which is consistent with the documentation from the ELISA kit handout used to test samples for this study. New virus names have been accepted by the International Committee for Taxonomy of Viruses for the four viruses in this study as follows; bovine alphaherpes virus 1 (BHV-1), bovine orthopneumovirus (BRSV), bovine respirovirus (PI-3) and bovine mastadenovirus C (BAV-C).

The purpose of this study was to investigate five viruses that cause upper respiratory tract infections in cattle: bovine alphaherpesvirus-1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza-3 virus (PI-3) and bovine mastadenovirus-3 (BAV-3), in the rural Mnisi farming community in the Mpumalanga Province, South Africa which is located adjacent to the Kruger National Park (KNP) and private game reserves (Figure 1). The Mnisi Community Project (MCP) is a University of Pretoria initiative that is based on an One Health approach at the human/livestock/wildlife/ecosystem interface. Within the Mnisi community there are a number of dip tanks to which cattle are obligated to attend weekly for FMD inspection. In return, cattle are plunge-dipped free of charge in acaricides, as an aid to control tick-borne diseases such a theileriosis, anaplasmosis, heartwater and redwater. These viruses are known to cause pathology of the respiratory tract and lead to morbidity and even mortality in some cases. In addition, two of the viruses studied here, BoHV-1 and BVDV, can suppress the immune system of the host and also increase the risk of secondary bacterial infections (Valarcher & Hägglund, 2006).

This study used a cross sectional design to determine the spatially explicit herd-level antibody seroprevalence of five respiratory tract viruses. A total of 423 sera stored in the Hans Hoheisen Wildlife Centre biobank were collected at 11 dip tanks in the Mnisi communal farming area. A commercially available pentavalent, indirect enzyme linked immunosorbent assay (ELISA) was performed to estimate the seroprevalence of each.

The overall proportion of sera that contained antibodies against each pathogen were as follows: 43.3% for BoHV-1; 30.5% for BVDV; 82.5% for BRSV; 44.4% for PI-3 and 83.2% for BAV-3. The prevalence of antibodies against the five respiratory viruses did not appear to be influenced by location, distance from the adjacent wildlife conservation area, time of the year, or sex. However, age was a risk factor as antibodies appeared less frequently in animals less than 12 months of age compared to animals between 12 and 24 months, or older than 24 months.

Findings from this study should provide information for the cattle farmers and animal health sector that provide animal health and extension services about the risk of bovine respiratory disease in the Mnisi communal farming area. Appropriate measures to minimize exposure to viral respiratory tract infections are discussed.

1. Introduction

Livestock play an important role in rural livelihoods and the economies of developing countries (Herrero et al., 2013), especially among the poor farming communities where livestock contribute indirectly to food security. Such contributions arise from sales of animals and their products, providing cash for the purchase of staple foods, pay for education and other needs, provision of manure to fertilize the soil, draft power, and income for purchase of farm inputs to boost sustainable crop production in mixed crop livestock systems widely practiced in Africa (Smith et al., 2013). Hence, sustainable livestock production among resource poor farmers whose livelihood depends on sales of animals and their products requires good farming practices inclusive of animal health. Animal diseases, on the other hand, threaten livestock production and market access, impacting local livelihood and national economies (Nin et al., 2007).

When farmers have information about the prevalence of bovine respiratory diseases (BRD) they can participate in disease surveillance, enabling them to identify and manage respiratory infections accordingly. Such participation through training may improve awareness and knowledge about the distribution and appearance of respiratory infections, allowing them to predict the source of infection and monitor diseases by using well devised control measures.

Respiratory diseases are known to result from a variety of causes, although infectious agents predominate (Scott et al., 2011). A number of respiratory tract infections in cattle have been studied, but of interest to this study were viral infections caused by bovine alphaherpevirus-1 (BoHV-1), bovine viral diarrhea (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus-3 (PI-3) and bovine mastadenovirus-3 (BAV-3) because viral infections can cause primary disease on their own but some also are frequent co-infections, especially in association with animal stress factors, which in turn can trigger more serious secondary bacterial infections such as bovine pneumonic pasteurellosis. These secondary infections are most common in young cattle and can lead to malnourishment, morbidity and even mortality (Blowey, 2011).

Respiratory tract viruses may pose risks for spill-over from cattle to wildlife and *vice versa* in the Mnisi communal farming area a shared and unmanaged grazing area, as a result of its proximity to wildlife conservancies. Among other challenges faced by livestock farmers on communal land in Mnisi includes limited grazing, which may lead to under-nutrition of the livestock, especially during dry periods rendering livestock prone to many infections (Van Rooyen, 2011). In addition to limited grazing, communal farming systems are also subject to sharing other resources, especially water and grazing, which in turn can cause other adverse issues within the herd and community. According to the livestock farmers in the Mnisi communal farming area, the most important limiting factors to livestock production (reasons for cattle losses) are diseases (26%), lack of feed and water (25%), stock theft (17%) and abortion (6%) (Van Rooyen, 2011). Lack of knowledge by farmers on how to treat and prevent diseases among their livestock is one of the biggest constraints in livestock production in the Mnisi area.

The study was conducted in the Mnisi communal farming community, Mpumalanga Province which is located adjacent to the Kruger National Park (KNP) and private game reserves in South Africa (Figure 1). The Mnisi Community Project (MCP) is a University of Pretoria initiative that is based on an One Health approach at the human/livestock/wildlife/ecosystem interface. The main agricultural activity in the Mnisi community is livestock farming, of which cattle are by far the most important species (Van Rooyen, 2011). Within the Mnisi community there are a number of dip tanks to which cattle are obligated to attend weekly for FMD inspection. In return, cattle are plunge-dipped free of charge in acaricides, as an aid to control tick-borne diseases such a theileriosis, anaplasmosis, heartwater and redwater.

The purpose of this study was to address disease as one of the farmers' main issues. Specifically, our goal was to determine the seroprevalence of five upper respiratory tract viruses by performing serological tests using an indirect pentavalent ELISA kit. Important risk factors for occurrence of respiratory pathogens in cattle on 423 biobanked sera collected from cattle at 11 dip tanks were also examined so that it would be enable suggestions for measures that would minimize the introduction or dispersal of these viral infections.

Samples from the late wet (April) and dry (May to September) seasons were used in this study, as this should be the time of peak viral infection for two main reasons:

- If we assume that maternal immunity wanes around six months of age (Hamblin & Hedger 1982; Chase, Hurley & Reber 2008), these seasons should be the period during which calves born in July to September become susceptible.

- Cattle most likely experience nutritional deficits during this period (Lazarus, 2014) and may experience suppressed immunity due to lack of available forage.

1.1 Problem and hypotheses

In addition to bovine tuberculosis (bTB) and foot and mouth disease (FMD), which are well studied and known to affect cattle throughout southern Africa, several other contact-transmitted, upper respiratory disease are known to occur in cattle. Without adequate research, the distribution and risk factors for occurrence of the pathogens in this Mnisi area remain unknown and as such no recommendations to minimize their occurrence would be proposed and be implemented. Researched knowledge of the prevalence of these pathogens in the Mnisi community will enable management suggestions to be adapted and result in reduced disease occurrence and pathogen transmission and thus increase animal productivity. When presenting the findings to the livestock owners in the Mnisi community it will also be a chance to engage the community and teach them about these neglected diseases which have affected their animals.

This project examined five upper respiratory tract viral infections, as they are known to cause respiratory diseases in cattle (Brahmbhatt et al., 2012). A cross-sectional study was carried out to establish whether cattle from the Mnisi community area were exposed to the five pathogens, and determine whether location (dip tanks), sex, age and time of the year (months) influenced the seroprevalence. The hypotheses can be stated as:

- Seroprevalence would differ between dip tanks as a result of herd capacity, age and disease management measures which may exist between different dip tanks.

- Seroprevalence should be higher in cattle over one year of age when compared to younger animals.

- We hypothesize that female cattle would have higher seroprevalence than male cattle because the female animals are vulnerable especially during gestation and lactating period when more nutritional resources may be utilized to support pregnancies and for the production of milk to support the calves than immunological responses of the mothers.

- We hypothesize that cattle sampled in the dry winter period (June, July and August) would have a higher seroprevalence compared to cattle sampled in April and May months because some cattle, especially lactating female and calves that have higher caloric requirements, may face nutritional deficits due to limited grazing during the dry winter season.

2.1 Objectives

The aim of this study was:

- To determine whether cattle in Mnisi community were exposed to five viral respiratory tract pathogens, namely bovine herpesvirus-1 (BoHV-1), bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza-3 virus (PI-3) and bovine adenovirus-3 (BAV-3), through a serosurvey to understand their distributions and investigate whether the following factors were correlated with seroprevalence.
    - Location in relation to distance from the Kruger National Park fence.
    - Sex.
    - Age.
    - Time of the year (month of sampling).

2. Literature review

2.1 Introduction to bovine respiratory tract viruses

Bovine respiratory infections are known to cause morbidity in cattle, but they also predispose them to more serious secondary bacterial lung infections, which account for high mortality rates, especially when animals have been subjected to poor nutrition and compromised biosecurity such as are usually faced in rural farming (Hodgins et al., 2002).

Bovine respiratory viral infections are economically important diseases, and more research is necessary to investigate possible predisposing factors under different farming conditions (Taylor et al., 2013). Taylor et al. (2013) pointed out that age has been considered a risk factor for respiratory infections, especially BRSV, which affects mostly calves. Poor farming practices, including poor management practices, compromised biosecurity as well as unfavourable environmental conditions, characterized by extreme weather and poor grazing, makes animals more vulnerable to respiratory infections and its subsequent diseases (Taylor et al., 2010).

Respiratory tract infections have been reported to occur in both the commercial and small-scale farming sectors in South Africa (Njiro et al., 2011). BoHV-1 is an important disease of cattle in most regions of the world, because of its endemic infection with varying seropositivity rates (Daniel et al., 2016) sometimes approaching 40% of animals within herds (Hodgins et al., 2002). BRSV has been established as a common pathogen in respiratory disease and has been demonstrated to interact with bacterial pathogens in establishing pneumonia in cattle and occurs within the cattle population around the world (Hodgins et al., 2002). Infections with BVDV are widespread throughout the world. Although the prevalence of infection varies among surveys, the infection tends to be endemic in many populations, reaching a maximum level of 60-85% of the cattle being antibody positive (Houe, 1999). BAV-3 infections of cattle were documented before 1960 and the virus is encountered worldwide in cattle (Hodgins et al., 2002).

2.2 Viral pathogens

2.2.1 Bovine alphaherpesvirus-1 (BoHV-1)

Bovine alphaherpesvirus-1 (BoHV-1) belongs to the family *Herpesviridae,* subfamily *Alphaherpesvirinae*. BoHV-1 infections are widespread in cattle populations and have been associated with several diseases in cattle, such as infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, balanoposthitis, conjunctivitis, abortion, encephalomyelitis and mastitis (Hodgins et al., 2002). BoHV-1 transmission can occur by direct contact, through aerosols, through mating and artificial insemination with semen from subclinically infected bulls (Murphy et al., 1999). Cattle become infected with the virus through the mucous membrane of the respiratory and genital tracts.

BoHV-1 infections can be either clinical or subclinical, depending on the virulence of the strain. Risk factors among other include sale markets and co-mingling. It is not clear whether or not such practices increase susceptibility and exposure to respiratory tract viruses, or are linked to poor management (Taylor et al., 2010). The incubation period for the respiratory and genital forms varies from two to six days. In the respiratory form, clinical signs range from mild to severe, depending on the presence of secondary bacterial infections (Stott et al., 1978). Possible clinical signs include: fever, anorexia, coughing, excessive salivation, serous nasal discharge that becomes mucopurulent, conjunctivitis with lacrimal discharge, inflamed nares and dyspnoea if the larynx becomes occluded with purulent material (Hodgins et al., 2002). Alternatively, conjunctivitis with corneal opacity may occur as the only manifestation of BoHV-1 infection. In the absence of bacterial pneumonia, recovery generally occurs four to five days after the onset of signs (Hodgins et al., 2002).

Abortions may occur concurrently with respiratory disease and occur most often during the second half of pregnancy, while foetal death is possible. With a secondary bacterial infection, there may be inflammation of the uterus and transient infertility with purulent vaginal discharge, and/or preputial and penile lesions. The infection can cause severe generalized disease in calves characterized by pyrexia, ocular and nasal discharges, respiratory distress,

diarrhoea, incoordination, and eventually convulsions and death may occur in a short period after generalized viral infection (Alkan et al., 2000).

2.2.2 Bovine viral diarrhea virus (BVDV)

Bovine viral diarrhea viru (BVDV) is an economically important viral pathogen of cattle worldwide, causing a wide range of clinical syndromes. The virus belongs to the genus *Pestivirus* in the family *Flaviviridae* (Scott et al., 2011). BVDV prevalences are well documented globally and have been receiving high priority as the pathogen affects reproductive organs (Lillie, 1974). Studies on BVDV conducted in southern Africa, described the prevalence varying as between 49% and 89% and are associated with diarrhoea, mucosal disease, abortion, teratogenic defects, stillbirths and respiratory disease (Njiro, 2011).

Its economic importance is due to both direct costs, such as treatment and mortalities, as well as indirect costs, such as retarded growth (Scott et al., 2011). BVDV plays an important role in the bovine respiratory disease complex (BRDC) by suppressing the host immunity and predisposing cattle to secondary bacterial pneumonia (Hodgins et al., 2002). BVDV also has direct implications on the reproductive system. Specifically, BVDV can infect a developing foetus and is capable of causing abortions in pregnant animals, teratogenic defects, and stillbirths (Njiro, 2011). It is one of the major causes of abortions in cattle.

2.2.3 Bovine respiratory syncytial virus (BRSV)

Bovine respiratory syncytial virus (BRSV) is an RNA virus in the *Pneumoviridae* family. The virus has a cytopathic effect, causing the formation of syncytial cells (Scott et al., 2011). Stress caused by movement, crowding and temperature changes plays a role in BRSV outbreaks (Van der Poel, 1994). The virus is often transmitted by contact with nasal secretions, but may also be transmitted by aerosols (Taylor et al., 2010). BRSV is distributed worldwide, and is common in young beef and dairy cattle (Njiro, 2011). In addition to cattle, sheep and goats can also be infected by respiratory syncytial viruses. Passively derived immunity does not appear to prevent BRSV infections, but limits the clinical signs of the disease (Hodgins et al., 2002). Initial exposure to the virus is associated with severe respiratory disease; subsequent exposures result in mild to subclinical

disease. BRSV is an important virus in the bovine respiratory disease complex because of its frequency of occurrence, predilection for the lower respiratory tract, and ability to predispose the respiratory tract to secondary bacterial infection (Hodgins et al., 2002). During outbreaks, morbidity tends to be high, and the case fatality rate can range between 0-20%.

Clinical signs of infection include fever, depression, decreased feed intake, increased respiratory rate, cough, and nasal and lachrymal discharge. Dyspnoea, possibly with open-mouthed breathing, may become pronounced in the later stages of the disease due to a congested nasal cavity. Gross lesions include a diffuse interstitial pneumonia with subpleural and interstitial emphysema along with interstitial oedema. Bronchopneumonia of bacterial origin is usually present (Hodgins et al., 2002).

BRSV has been reported to cause annual outbreaks of respiratory disease in cattle all over the world with most severe cases observed in calves less than six months of age (Van der Poel, 1994). While BRSV can cause mild disease on its own, it is also a component of the BRDC together with parainfluenza viruses and herpesviruses as well as the bacteria *Pasteurella multocida*, *Mannheimia haemolytica* and *Mycoplasma bovis* (Sacco et al., 2014). Apart from infections and death in cattle, BRSV can cause long-term losses in production performance (Hodgins et al., 2002).

2.2.4 Bovine parainfluenza virus 3 (PI-3)

Bovine parainfluenza virus 3 (PI-3) is an RNA virus classified in the *Paramyxovirus* family. PI-3 is an economically important respiratory infection. It was first described in the USA, but has since been reported globally. Infections caused by PI-3 are common and affect all ages of cattle, but calves housed during the autumn and winter are infected most frequently (Scott et al., 2011). PI-3 has been associated with mild and subclinical infections, but plays a role as an initiator that can lead to development of secondary bacterial pneumonia.

Clinical signs of infection include fever, nasal discharge, cough, lacrimation, conjunctivitis and loss of appetite (Elankumaran, 2013). PI-3 infection together with other viruses and bacterial infections with *Mannhemia* (*Pasteurella* species) could result in respiratory disease (Scott et al., 2011). Factors such as environmental temperature, transportation, hygiene, stocking status, co-mingling, and host immune status can predispose animals to secondary bacterial infection and severe clinical diseases post-PI-3 infection (Elankumaran, 2013). A study by Stott et al. (1978) suggested that, although PI-3 infection occurs during outbreaks of respiratory disease complex, it may not play an important role in actually causing the disease.

2.2.5 Bovine mastadenovirus 3 (BAV-3)

Bovine mastadenovirus type 3 (BAV-3) belongs to the *Mastadenovirus* genus of the family *Adenoviridae* and is involved in respiratory and enteric infections of calves (Mohanty, 1971). The first isolation of BAV-3 was reported by Darbyshire and co-workers in Britain (Zhu et al., 2011). Although adenovirus infections of cattle were documented before 1960, their role in clinical diseases in cattle remains disputable (Scott et al., 2011). Ten serotypes of bovine adenovirus have been identified. Although adenoviruses have been isolated from the respiratory tracts of pneumonic calves, isolation from clinically healthy cattle is more frequent (Scott et al., 2011). Some serological studies have supported a role for bovine adenoviruses in bovine respiratory disease, while evidence was lacking in other studies (Hodgins et al., 2002). BAV-3 is a non-enveloped icosahedral particle of 75-80 nm in diameter and has a double-stranded linear genomic DNA (Zhu et al., 2011). Serologic surveys indicated widespread distribution of BAV throughout the world.

Bovine adenoviruses (BAVs) cause a variety of clinical signs including conjunctivitis, pneumonia, diarrhoea, and polyarthritis (Calcedo et al., 2009). Other clinical signs reported include pyrexia, respiratory distress, and nasal and conjunctival discharges. BAV-3, a member of subgroup 1, is considered one of the important respiratory tract pathogens of cattle, particularly newborn calves (Scott et al., 2011).

2.3 Socio-economic impact for livestock owners

Respiratory disease is a major cause of financial loss, especially in growing cattle industries around the globe. The financial losses are as a result of vaccination costs, antibiotic treatment and veterinary clinical services, poor growth rates and mortality (Scott et al., 2011). For example, respiratory disease is the fourth largest cause of antibiotic use for treatment of animal diseases globally (Valarcher et al., 2006). In areas considered resource-poor where farmers have limited access to affordable veterinary services, the economic losses are valued in terms of the direct costs of treatment, labour and mortalities due to respiratory infection. Although the viruses included in this study are associated mostly with low mortality rates, their socio-economic impacts are a result of poor production, abortion, poor growth rates, low conception rates, still births and low milk production.

2.4 Role of dip tanks

Dip tanks are facilities constructed in most parts of South Africa with the aim of providing comprehensive disease control and opportunities for surveillance. Historically, it also provided for weekly inspection of cattle for specific clinical signs associated with FMD in those regions of the country where the disease was present. In return, the South African veterinary services provide the opportunity for farmers to plunge dip their cattle in anti-tick solution in an effort to control tick-borne diseases (Simela, 2012). Although dip tanks are necessary for the control of certain diseases, the gathering and mixing of cattle from different herds may also present an opportunity for pathogen transmission via aerosol, due to close and frequent contact of animals.

3. Materials and methods

3.1 Study location

During this study, serum samples collected from cattle at all eleven dip tanks distributed throughout the Mnisi community (Figure 1) were analysed. The sera were tested at the Hans Hoheisen Wildlife Research Station (HHWRS) in the KNP close to Mnisi. This research and training facility is managed by the University of Pretoria, in collaboration with the Peace Parks Foundation and Mpumalanga Tourism and Parks Agency. HHWRS facilities include, among others, laboratories and accommodation, a library, wildlife housing and handling facilities.

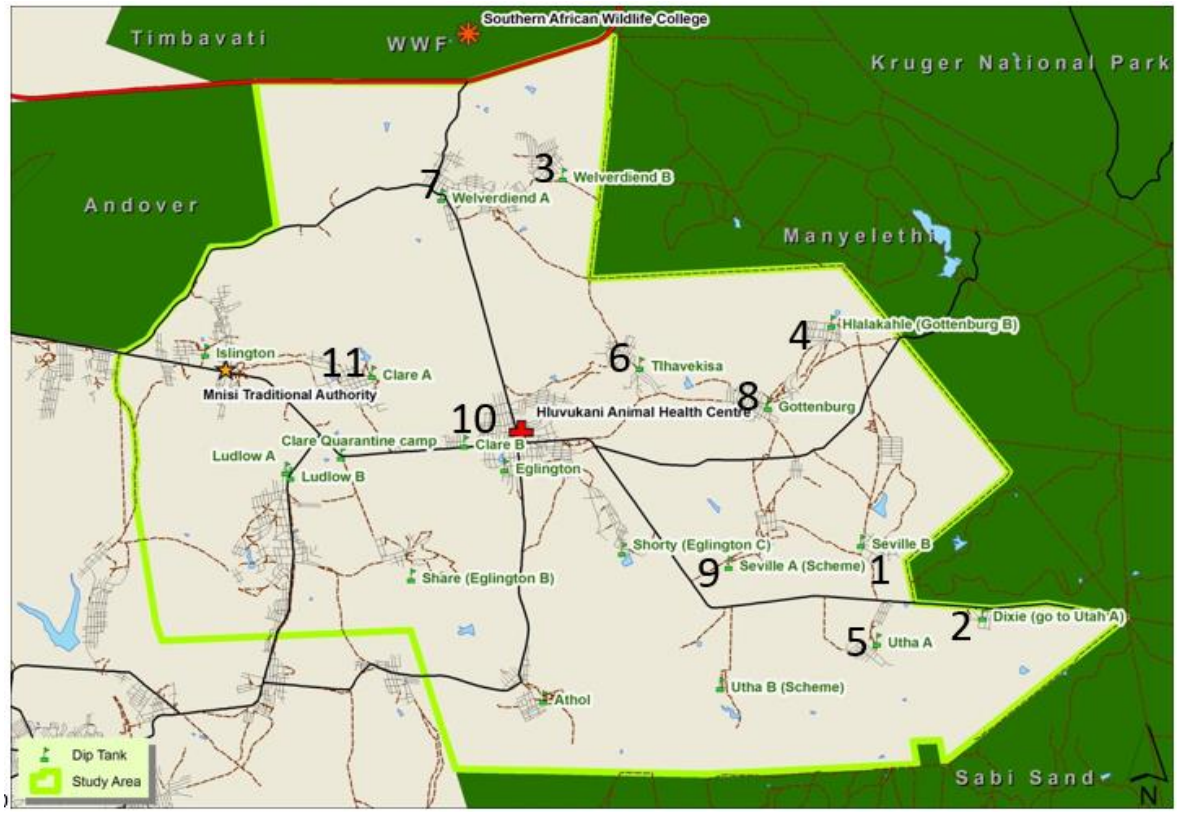


Figure 1 Map of the study area, the Mnisi community (outlined by a light green border) in relation to the Kruger National Park and adjacent conservation areas. The numbers (1-11) depicted represents the dip tanks (sampling locations).

3.2 Sample collection and storage in a biobank

The sample and basic biometric data collection were done by a team of researchers under the supervision Dr Anne Meyer and Prof Darryn Knobel, both from the University of Pretoria (UP) in 2013. About 20-30 animals were sampled per day/per dip tank. Variation in numbers of samples between dip tanks was based on number of animals presented. Upon collection, either from the jugular vein or from the tail vein, blood samples were centrifuged to obtain serum, which was aliquoted and then stored in a biobank freezer at -80°C at HHWRS prior to use. The samples size for this study was based on data from a previous study on nearby buffaloes that have an estimated infection prevalence of 20-30% for each of the pathogens. A subset of 15-60 biobanked sera per location or diptank was selected to give the required analysis for meaningful comparisons between groups with ±10% precision and 95% confidence.

3.3 Target animals

From the sera available in the HHWRS biobank, we randomly selected 423 samples collected from 11 different dip tanks (Figure 1) within the Mnisi Community. All samples had been collected between April and September 2013 (Table 1). The sample set included 219 females and 204 male, with ages ranging from 7 days to 159 months (less than a month to thirteen years). Information on cattle ages was obtained from owner's stock cards, which are updated at every inspection session by veterinary officials. Cattle breeds used in the study were mainly Sanga and Brahman crosses. The categories of cattle sampled were calves, heifers, cows, bulls and oxen. For analytical purposes, samples were stratified into three age categories as follows: from 1-12 months of age for calves and weaners, older than 12-24 months of age for pre-reproductive animals and older than 24 months for reproductive animals, because these age classes are known to be associated with different susceptibility rates (Hodgins et al., 2002).

Table 1 Total number of serum samples analysed for each of the 11 dip tanks

Figure 1 Map ID	Diptank name	Total # of samples	Sampling time
1	Seville B	39	July-September 2013
2	Dixie	16	Aug 2013
3	Welverdiend B	44	May 2013
4	Hlalakahle	36	May 2013
5	Utha A	36	July and August 2013
6	Tlhavekisa	31	June and July 2013
7	Welverdiend A	60	April and May 2013
8	Gottenburg	38	July 2013
9	Seville A	38	Aug and September 2013
10	Clare B	46	May and June 2013
11	Clare A	39	June 2013
Total		**423**	

3.4 Laboratory testing

Serological samples were screened using the Bio-X Respiratory ELISA Pentavalent kit (IBRPA) developed by BIO-X Diagnostics (Belgium). These kits were designed to evaluate the humoral immune response of cattle to BoHV-1, BVDV, BRSV, PI-3 and BAV-3.

The procedure is based upon a solid phase indirect ELISA (ELISA) and was performed and analysed according to the manufacturer's instructions, as described below. A total of 30 plates were used to analyse the 423 samples. Each plate consisted of 96 wells coated with antigens specific to the five pathogens analysed. A negative control was provided in the kit 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. Test sera and control samples were diluted 1:100 before being placed into the wells of microtitre plates. Plates were then covered and incubated on the bench at room temperature for one hour to allow for the binding of any respiratory pathogen antibodies in the sample being analysed.

After incubation, each microtitre plate was washed three times using 200 µl washing solution. Then 100 µl anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase-labelled anti-bovine immunoglobulin monoclonal antibody) was added to each well and incubated as above. After a further three washing steps, 100 µl of substrate containing chromogen tetramethylbenzidine (TMB) was added to each well. The reaction was stopped with a 50 µl of stop solution (phosphoric acid) added to each micro-well after 10 min.

The optical density in the microwells of each plate was read using a plate reader with a 450 nm filter. The generated optical density (OD_{raw}) readings were saved in a Microsoft Excel spreadsheet (Appendix 1). Each plate was validated using the mean value of the positive control ODs were as follows (as suggested by the manufacturer): BoHV-1 > 0.700, BVDV > 1.000, BRSV > 0.800, PI-3 > 0.800 and BAV-3 > 0.600. The negative control was used in every fifth plate to validate the plate and ensure no cross contamination had taken place. The sample OD_{raw} values were converted to degree of positivity (percentage) using the following formula: Val = (OD_{raw} sample/OD_{raw} positive control) × 100. The samples with the following valences were considered positive, as per the manufacturer's guideline: BoHV-1 > 30%, BVDV, BRSV and PI-3 > 20% and BAV-3 > 10% (Appendix 3). The quality of the test was also scored from 0-5 based on the degree of positivity. A '0' score was considered negative while the 1-5 score considered positive (Appendix 3).

3.5 Preparation of raw data for analysis

The generated raw data from the plate reader was exported as Excel spreadsheets for each plate. Microsoft Excel was used to transform and organize a master data sheet and to generate pivot tables and bar charts used in the Results section. The master data sheet captured the following information for each sample (animal) used in this study:

- Sample identity as a unique number for each sample recorded on the tube.

- Seropositivity as presence or absence of antibodies to the pathogens on a binary scale. The initial raw optical density data (continuous variable) relative to the amount of antibodies in each sample was transformed into ordinal variable on a 0-5 scale as prescribed by the manufacturer. On this scale a '0' indicated a negative sample with no antibodies and a '5' indicated the highest amount of antibodies measured by the test. To determine the prevalence of each pathogen, the seropositivity was transformed into a binary variable from ordinal variable using an Excel formula to indicate whether a sample was positive (positive; score of 1-5) or negative (negative; score of 0) for antibodies, as per manufacturer's instruction pamphlet.

- Age was initially recorded at sample collection as a continuous variable in days and months but was transformed to categorical ordinal variable to indicate whether an animal was below twelve months (< 12), between 12 and 24 months (12-24) or above 24 months (> 24) of age so that we compared seropositivity in animals with maternal antibodies, animals in the waning phase of maternal antibodies and animals where the maternal protection was no longer present, respectively (Scott et al., 2011).

- Sex was recorded at sample collection and used as a categorical binary variable (male or female).

- Location was recorded as dip tank name (categorical).

- Time of sampling was recorded as a continuous variable (date/month/year) then converted to an ordinal variable as month (April to September). For analysis purposes, the data was also converted into a categorical variable of winter (April to July) and dry summer (August to September).

3.6 Statistical Analyses

Descriptive statistics were used to explain both the total samples and the seroprevalence of the five pathogens. Overall seroprevalence (expressed as percentage) was calculated as the number of positive samples for each pathogen out of the total number of samples tested (n = 423). Seroprevalence of a pathogen at each dip tank was calculated by dividing the number of samples that were positive for each pathogen at a particular dip tank by the total number samples tested at that dip tank. Seroprevalence by sex was calculated by expressing the number of positive samples for each of the pathogens divided by the total number of samples tested for each sex (female: n = 219; male: n = 204). Seroprevalence for each month of the study (April to September) was calculated by expressing the number of samples that were positive for each pathogen in a particular month divided by the total number of samples tested in that particular month. Excel Pivot tables were used to construct tables and generate bar charts for each variable of interest. Excel was further used to filter and format data (as per analytical test used) for independent variables (grouping) in columns corresponding to rows of ELISA scores and each row of data is a different sampling point.

Numerical data was analysed using the statistical program EpiTool (Ausvet), an epidemiological calculator. A non-parametric (Wilcoxon rank sum) test was used to establish whether independent variables (sex) had an effect on our binary dependent variable (seropositivity). A non-parametric (Kruskal-Wallis rank sum) test was used to summarise and compare ELISA scores (continuous ELISA output) between groups (age, location and time of sampling). Data were formatted in a specific way as prescribed by EpiTools and were submitted for automated calculation.

4. Results

4.1 Overall seroprevalence

The highest number of positive sera were found for BAV-3 83.2% (353/423) and BRSV 82.5% (349/423), followed by PI-3 44.4% (188/423) and BoHV-1 43.3% (183/423). The lowest seroprevalence was observed for BVDV 30.5% (129/423) (Figure 2).

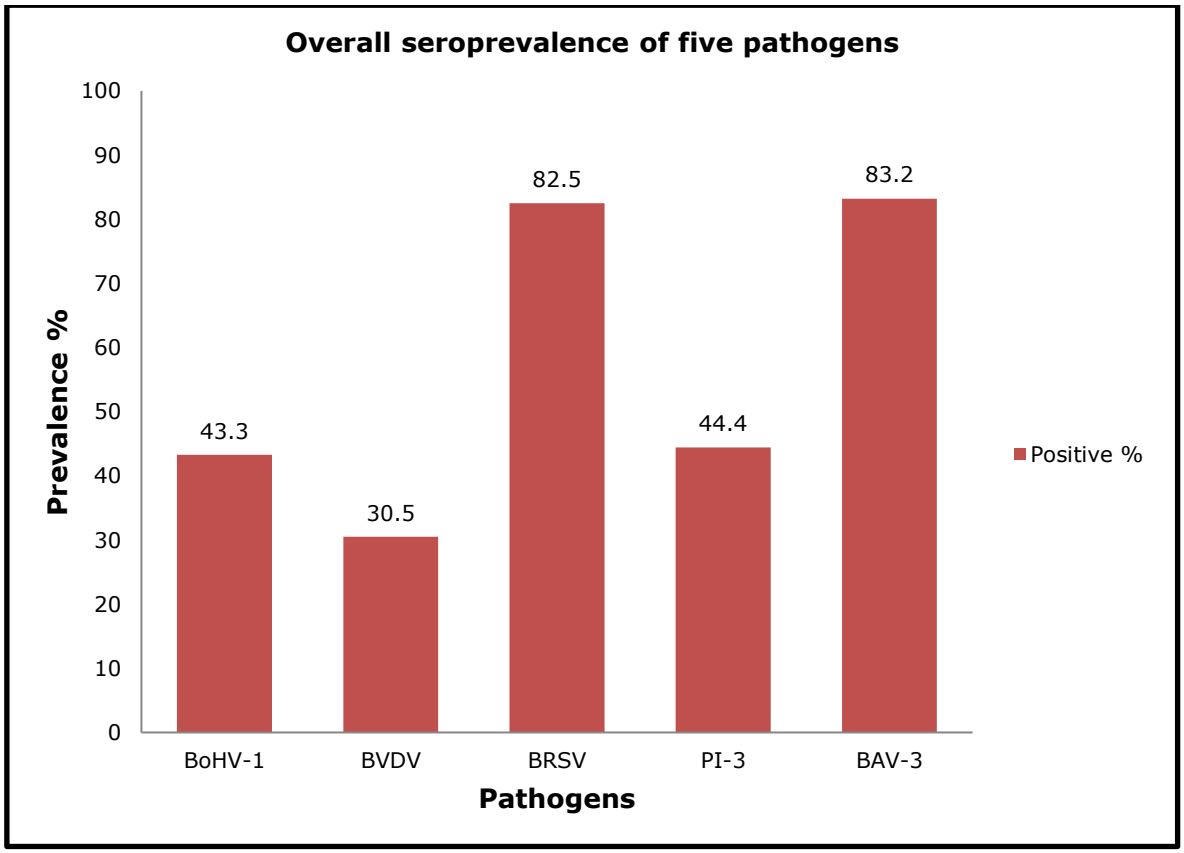


Figure 2 Overall seroprevalence of the five pathogens in 423 cattle surveyed

In the tested population, 95.7% of animals tested positive for at least one of the five pathogens. It was found that 15.1% of the of the cattle had antibodies for a single pathogen, 24.6% had antibodies for two of the pathogens, 21.3% had antibodies for three of the pathogens and 18% had antibodies for four pathogens. Nearly 17% of the cattle had been exposed to all five pathogens under investigation (Figure 3).

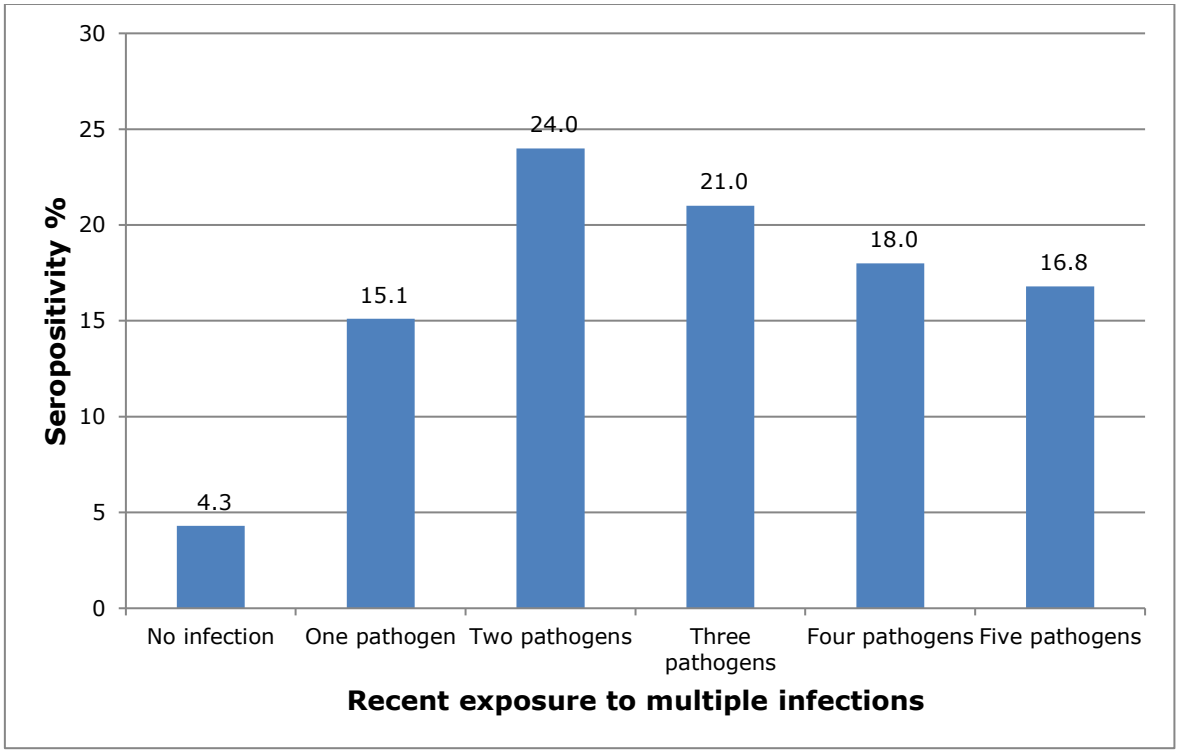


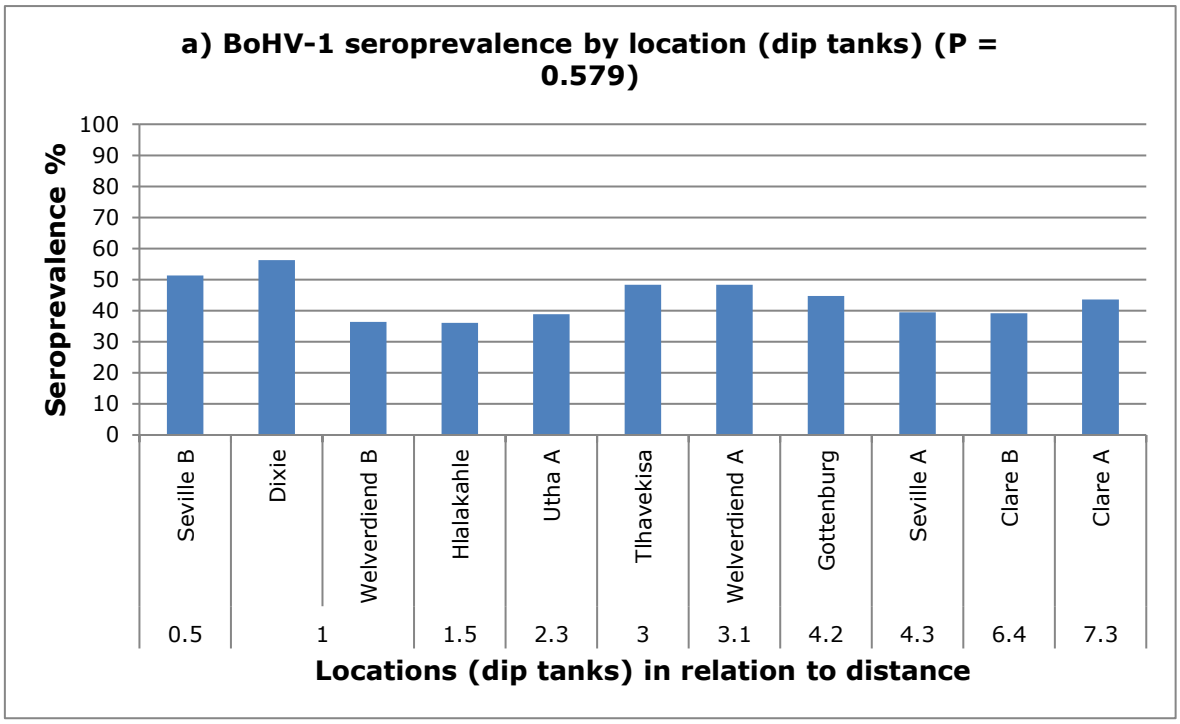
Figure 3 Seroprevalence of multiple infections with the five pathogens

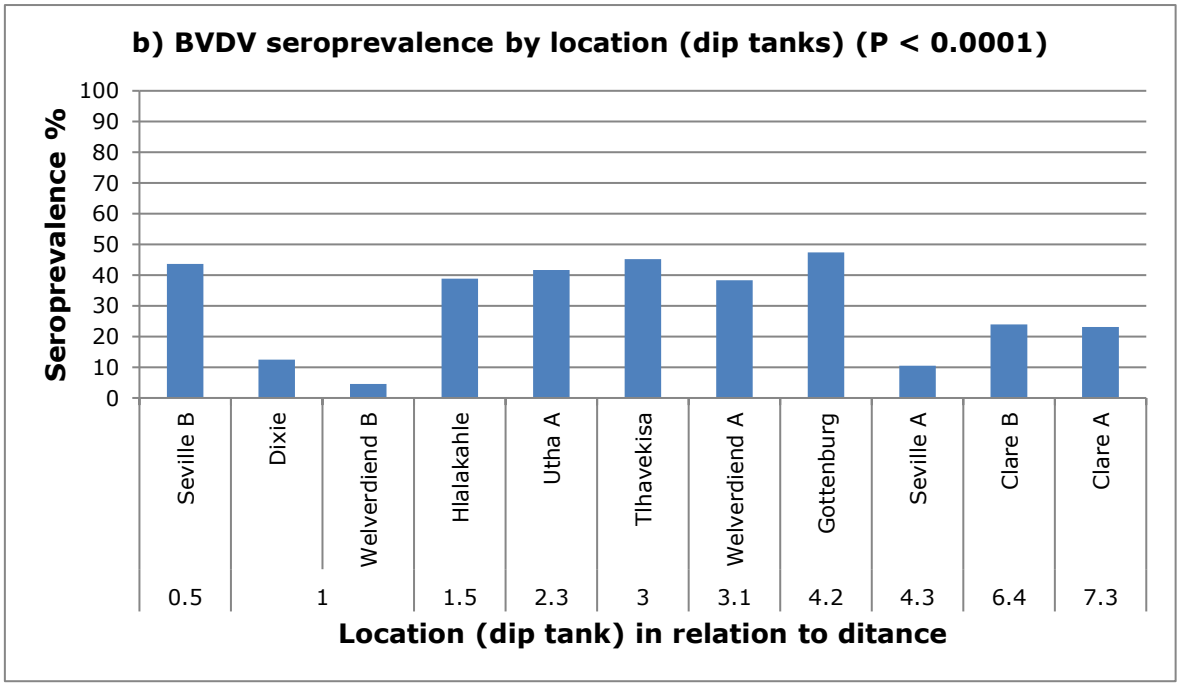
4.2 Pathogen prevalence in relation to specific risk factors

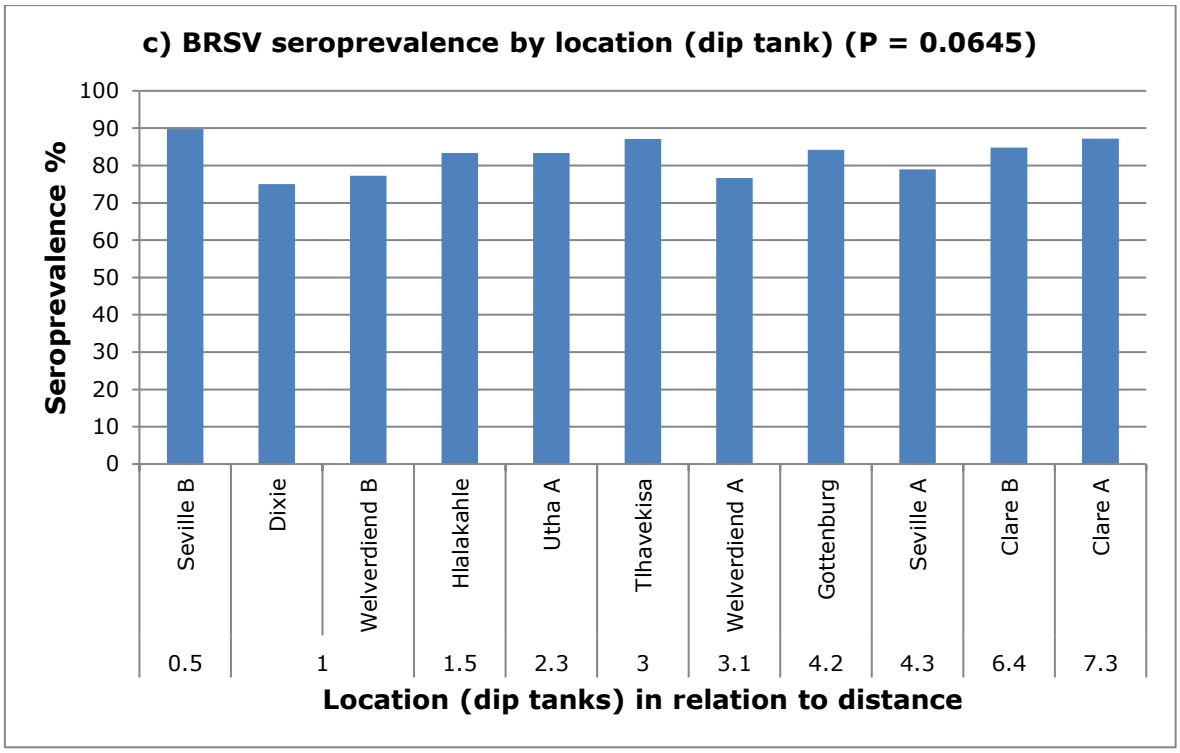
4.2.1 Seroprevalence by location (dip tanks)

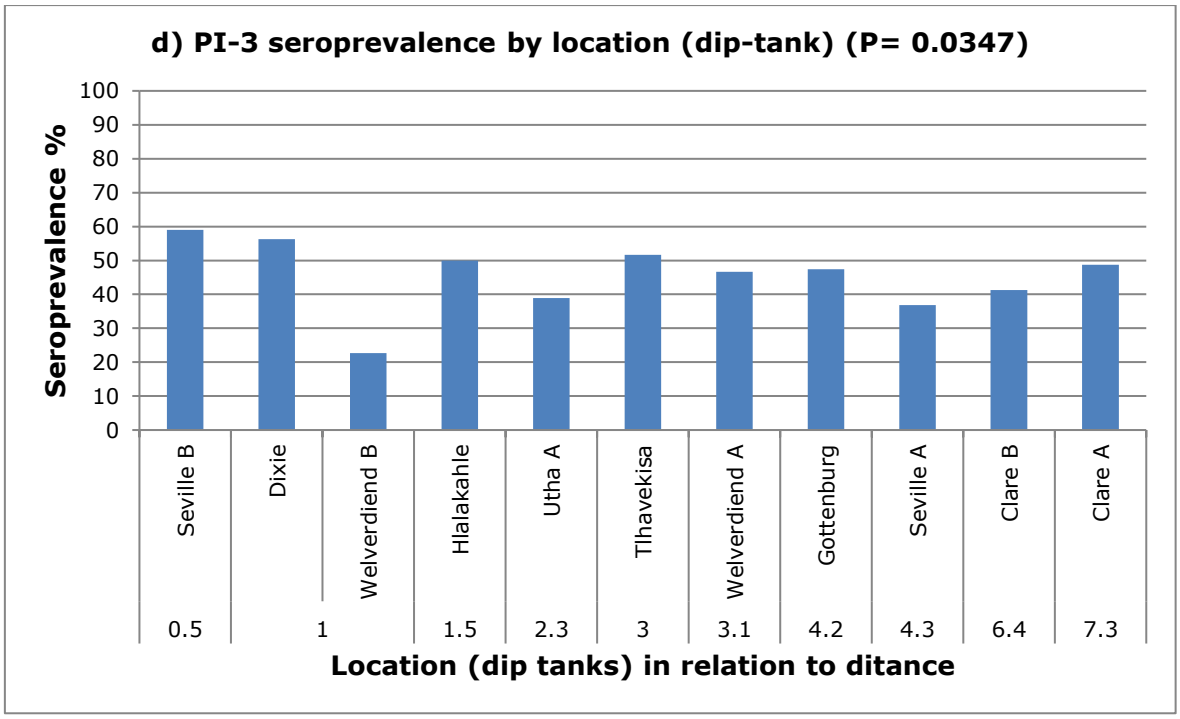
The seroprevalence for each of the five pathogens was analysed to determine whether location (dip tank) had an effect on the prevalence of the pathogens analysed. Seroprevalences for BoHV-1 and BRSV was observed to be independent of location [Figures 4 (a and c)] while PI-3 and BAV-3 was observed not to be independent of location [Figure 4 (d and e)]. However, there was significant difference in seroprevalence for BVDV ($p < 0.0001$) with lower

seroprevalence observed in cattle from Welverdiend B (2/44: 4.5%), Seville A (4/38: 10.5%) and Dixie (2/16: 12.5%) [Figure 4 (e)].






c) BRSV seroprevalence by location (dip tank) (P = 0.0645)
Seroprevalence %
100
90
80
70
60
50
40
30
20
10
0
Seville B
Dixie
Welverdiend B
Hlalakahle
Utha A
Tlhavekisa
Welverdiend A
Gottenburg
Seville A
Clare B
Clare A
0.5
1
1.5
2.3
3
3.1
4.2
4.3
6.4
7.3
Location (dip tanks) in relation to distance


d) PI-3 seroprevalence by location (dip-tank) (P= 0.0347)
Seroprevalence %
100
90
80
70
60
50
40
30
20
10
0
Seville B
Dixie
Welverdiend B
Hlalakahle
Utha A
Tlhavekisa
Welverdiend A
Gottenburg
Seville A
Clare B
Clare A
0.5
1
1.5
2.3
3
3.1
4.2
4.3
6.4
7.3
Location (dip tanks) in relation to ditance

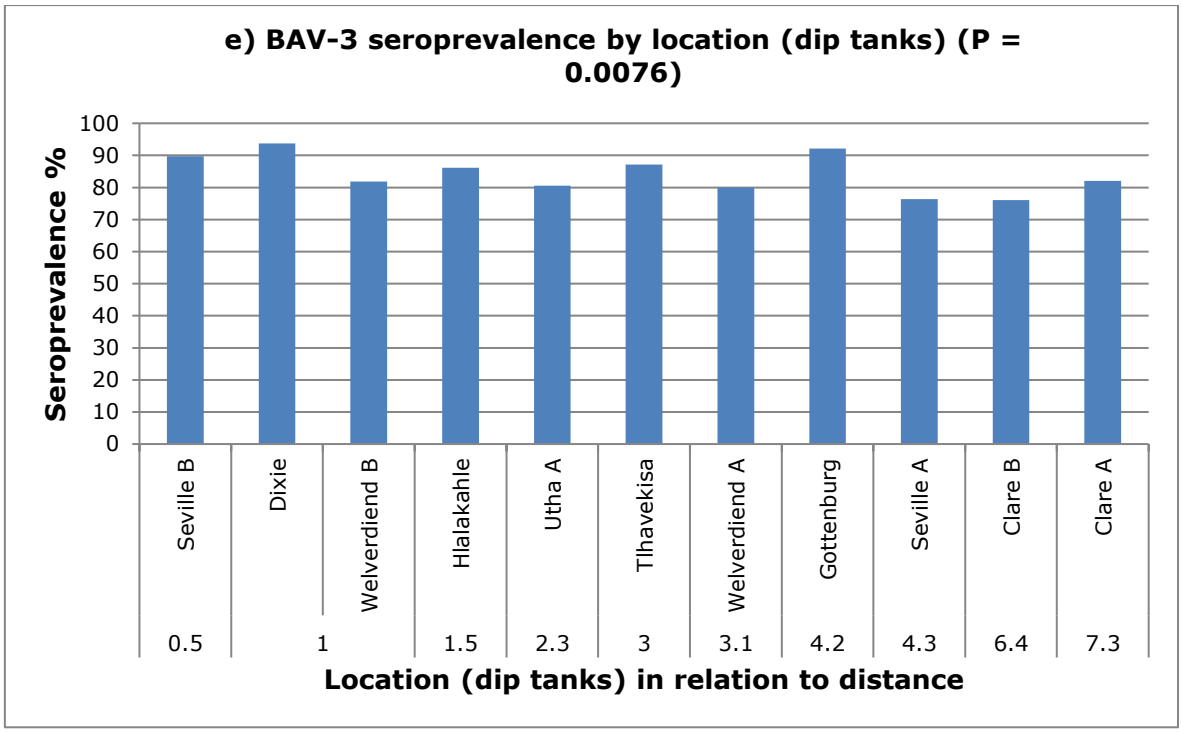


Figure 4 Seroprevalence of pathogens by locations (dip tanks)

a) BoHV-1 seroprevalence at each dip tank; b) BVDV seroprevalence at each dip tank; c) BRSV seroprevalence at each dip tank; d) PI-3 seroprevalence at each dip tank; e) BAV-3 seroprevalence at each dip tank

4.2.2 Seroprevalence by age group

Most samples (n = 210; 50%) were from cattle ≤ one year of age. Cattle between one and two years made up 15% of samples (n = 65), and cattle over two years made up the remaining 35% of samples (n = 148).

Age was found to have an effect on the dependent variable (seroprevalence) for all five pathogens ($p < 0.0001$). Individual cattle above 24 months of age were found to have a higher prevalence of antibodies against all five pathogens in comparison to cattle below 12 months and between 12-24 months of age (Figure 5). Likewise, cattle less than 12 months had lower levels of exposure than cattle between 12 and 24 months (Figure 5).

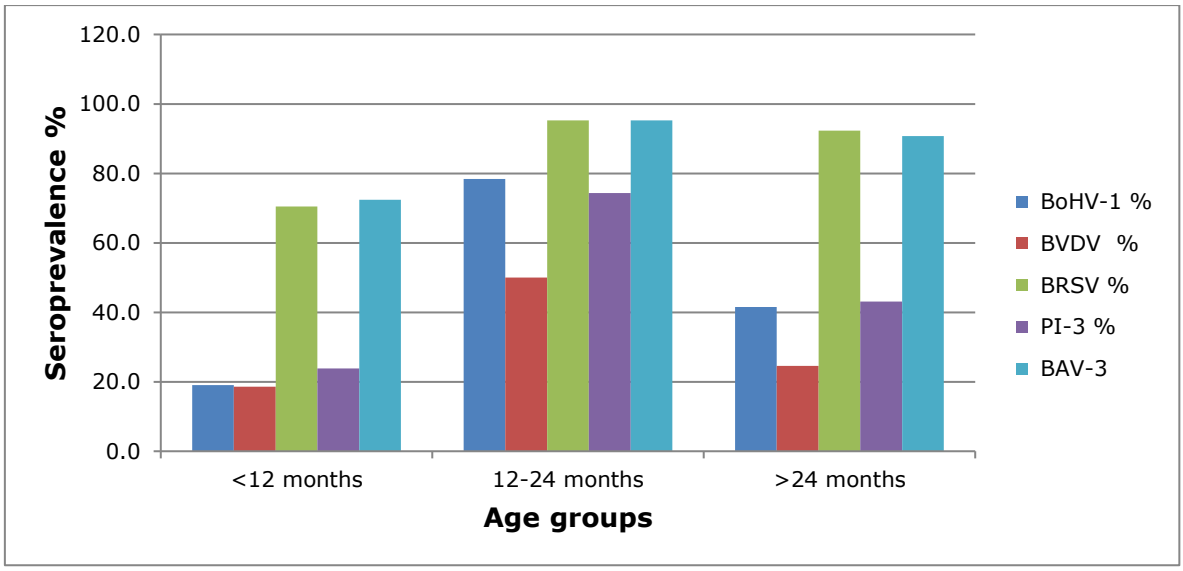
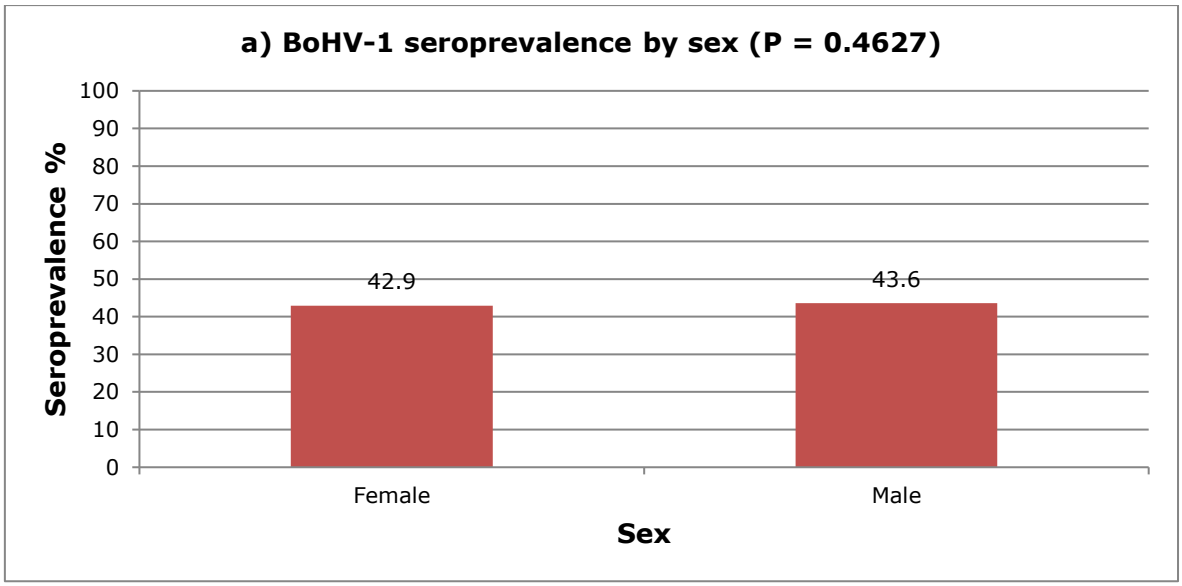


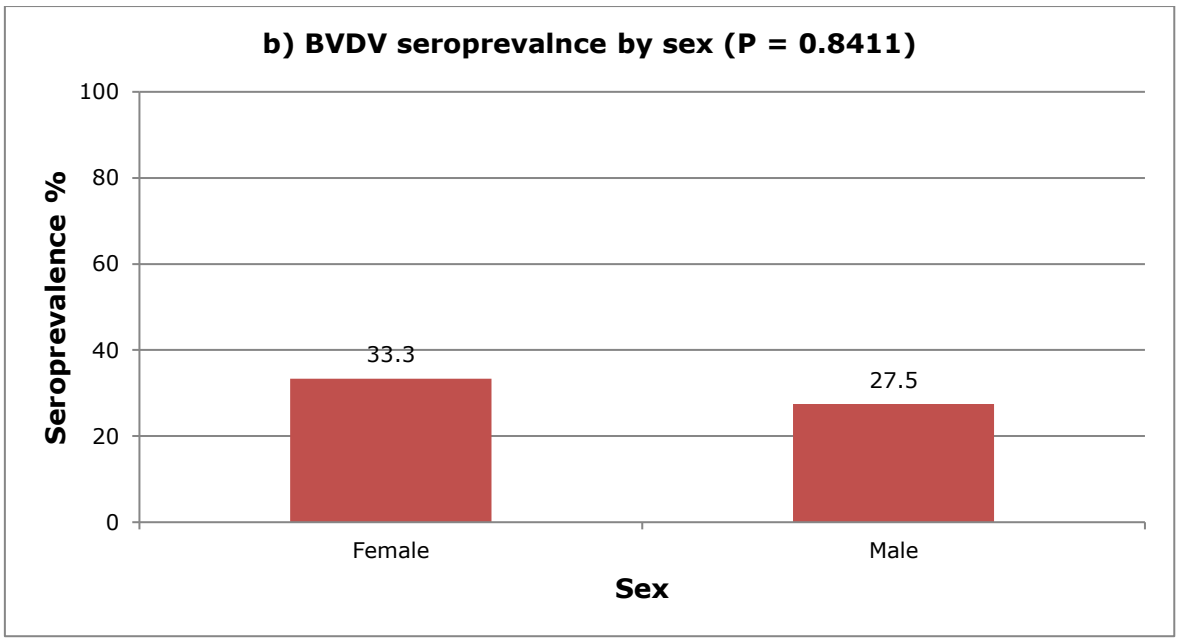
Figure 5 Seroprevalence of the five pathogens by age group

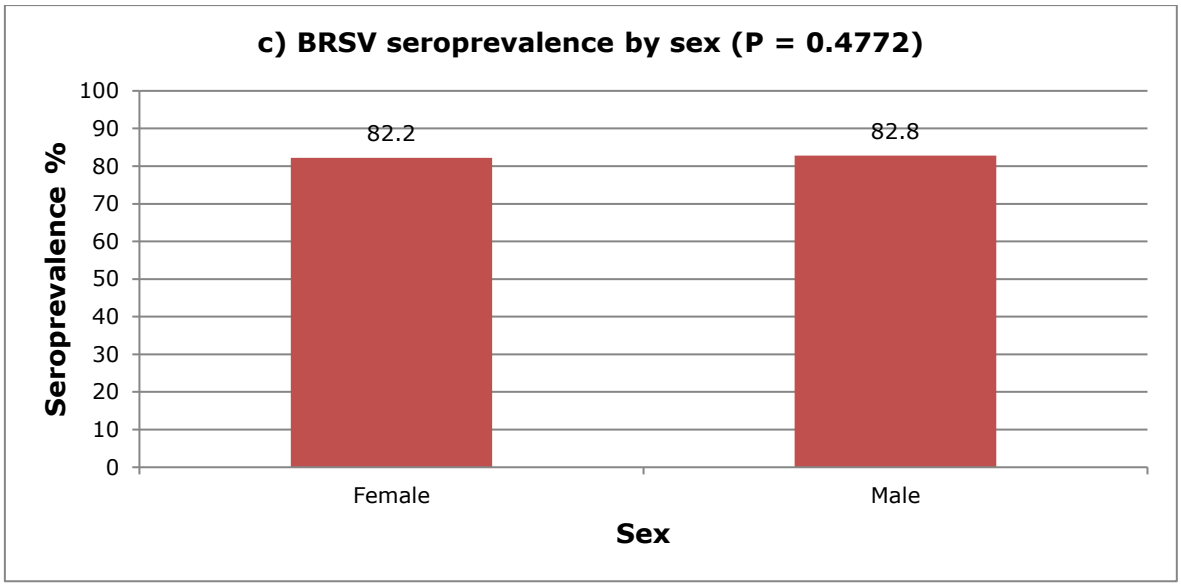
Of the 68 samples that tested positive for all five pathogens, 72.1% were over the age of two years (n = 49), 10.3% were between one and two years (n = 7), and 17.6% were under one year of age (n = 12). Of the 68 samples that tested positive to all five pathogens, 20% were recorded at Welverdiend A (n = 14), 16.2% were recorded at Tlhavekisa (n = 11), 13.2% were recorded at Seville B (n = 9), 11.8% were recorded at Clare B (n = 8) and 8.8% were recorded at Clare A and Gottenburg (n = 6). The lowest seroprevalence for all five pathogens were recorded at Welverdiend B at 2.9% (n = 2) followed by Seville A at 4.4% (n = 3) then Utha A and Hlalakahle at 5.9% (n = 4) and 7.4% (n = 5) respectively.

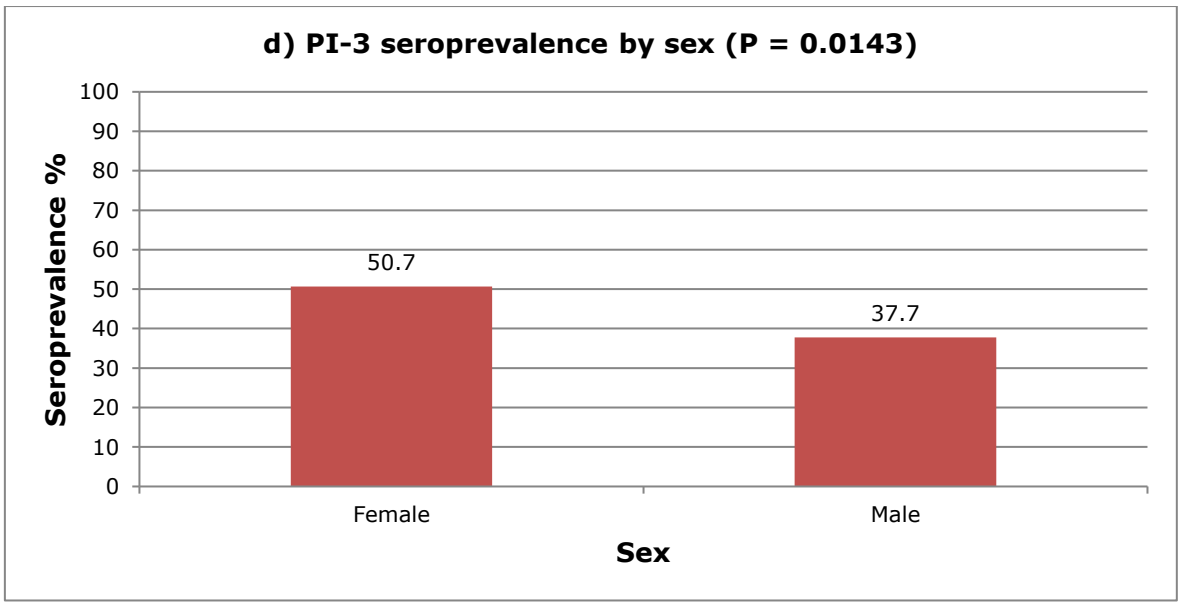
4.2.3 Seroprevalence by sex

Seroprevalences for BoHV-1, BVDV, BRSV and BAV-3 were found to be fairly similar between the two sexes [P = 0.4627, 0.8411, 0.4772 and 0.068 respectively; Figures 6 (a, b, c and e respectively)]. The seroprevalences for PI-3, on the other hand, was significantly different between the two sexes (P = 0.0143) with prevalences higher in females than males [Figure 6 (d)].


a) BoHV-1 seroprevalence by sex (P = 0.4627)
Seroprevalence %
100
90
80
70
60
50
40
30
20
10
0
42.9
43.6
Female
Male
Sex


b) BVDV seroprevalnce by sex (P = 0.8411)
Seroprevalence %
100
80
60
40
20
0
33.3
27.5
Female
Male
Sex


c) BRSV seroprevalence by sex (P = 0.4772)
Seroprevalence %
100
90
80
70
60
50
40
30
20
10
0
82.2
82.8
Female
Male
Sex


d) PI-3 seroprevalence by sex (P = 0.0143)
Seroprevalence %
100
90
80
70
60
50
40
30
20
10
0
50.7
37.7
Female
Male
Sex

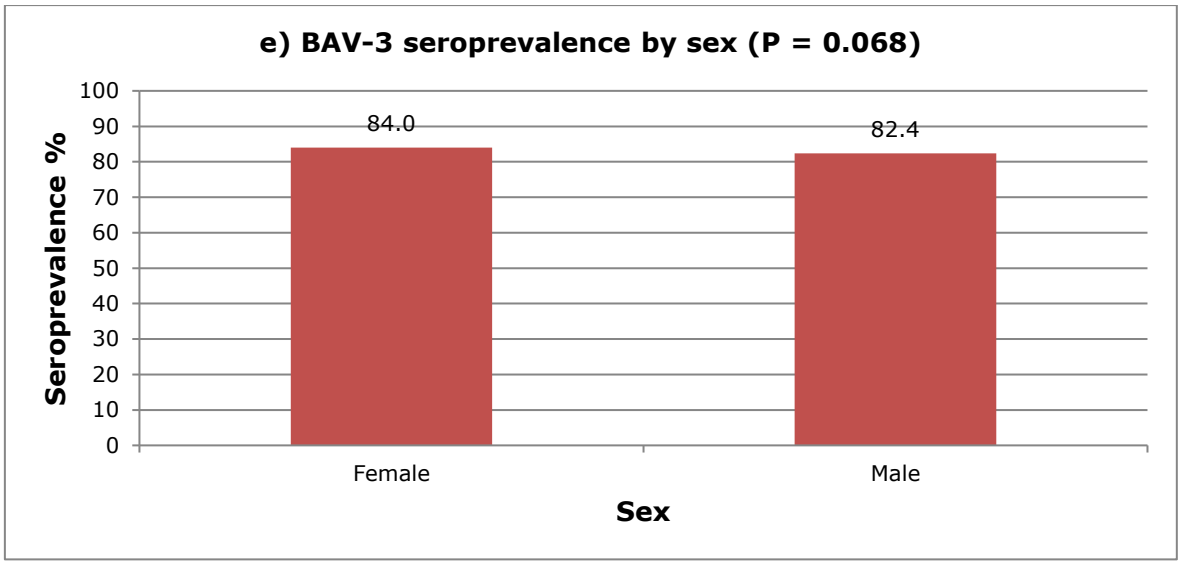
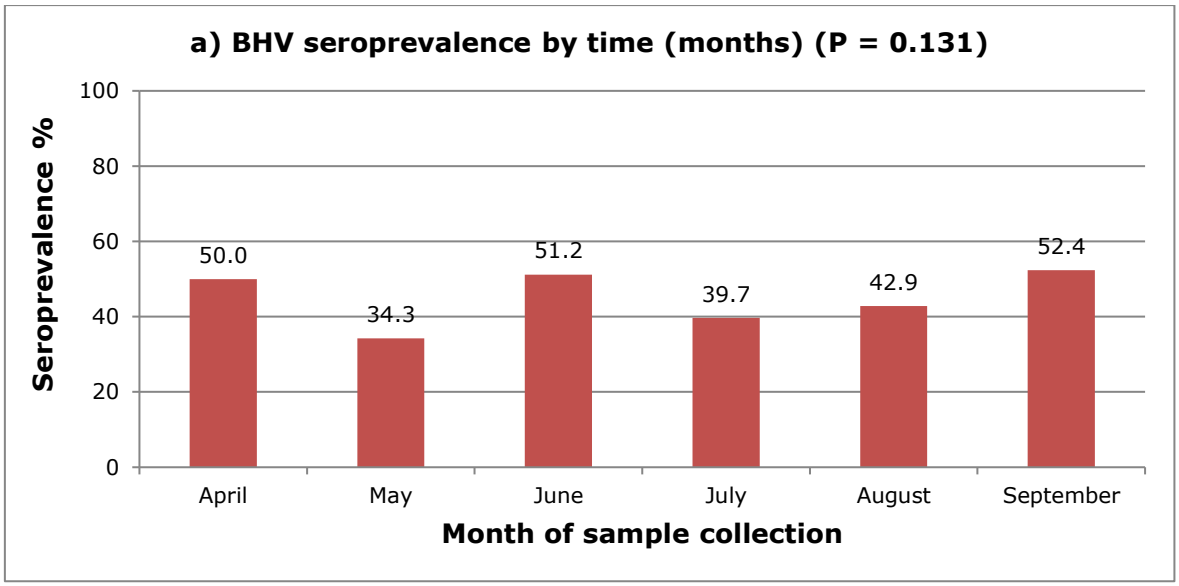


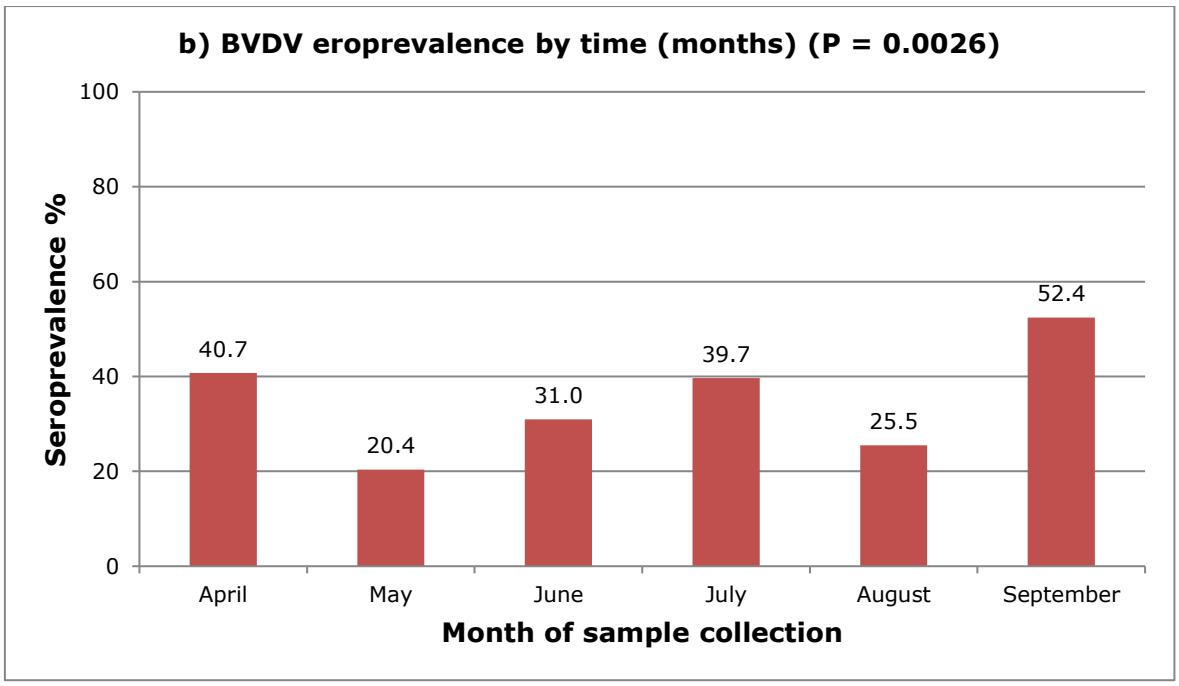
Figure 6 Seroprevalence of five pathogens by sex

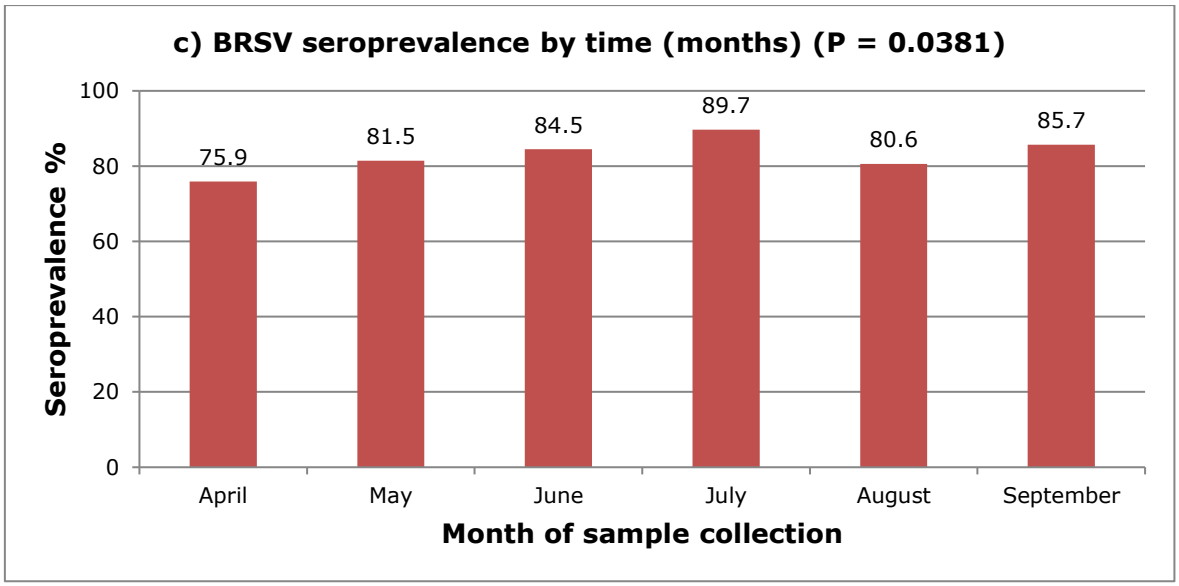
a) BoHV-1 seroprevalence by sex fairly similar between the two sexes; b) BVDV seroprevalence by sex fairly similar between the two sexes; c) BRSV seroprevalence by sex fairly similar between the two sexes; d) PI-3 seroprevalence by sex with significant difference between the two sexes; and e) BAV-3 seroprevalence by sex with significant difference between the two sexes.

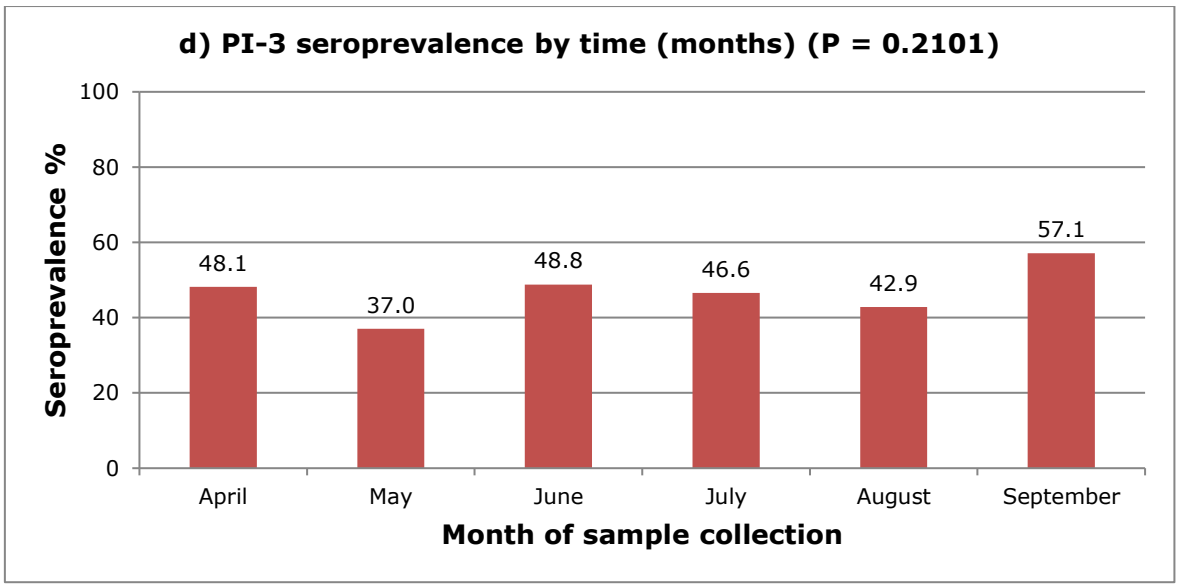
4.2.4 Seroprevalence by time of sampling

The seroprevalence was analysed to determine whether month of sampling had an effect on the prevalence of the pathogens analysed. Seroprevalence for BoHV-1, BRSV, and PI-3 was found to be independent of sampling time [Figure 7 (a, c and d) respectively]. However, a significantly lower seroprevalence for BVDV was observed in May [P = 0.0026; Figure 7 (b)] and a significantly higher seroprevalence of BAV-3 was observed in September [P = 0.0137; Figure 7 (e)].


a) BHV seroprevalence by time (months) (P = 0.131)
Seroprevalence %
100
80
60
40
20
0
50.0
34.3
51.2
39.7
42.9
52.4
April
May
June
July
August
September
Month of sample collection


b) BVDV eroprevalence by time (months) (P = 0.0026)
Seroprevalence %
100
80
60
40
20
0
40.7
20.4
31.0
39.7
25.5
52.4
April
May
June
July
August
September
Month of sample collection


c) BRSV seroprevalence by time (months) (P = 0.0381)
Seroprevalence %
100
80
60
40
20
0
75.9
81.5
84.5
89.7
80.6
85.7
April
May
June
July
August
September
Month of sample collection


d) PI-3 seroprevalence by time (months) (P = 0.2101)
Seroprevalence %
100
80
60
40
20
0
48.1
37.0
48.8
46.6
42.9
57.1
April
May
June
July
August
September
Month of sample collection

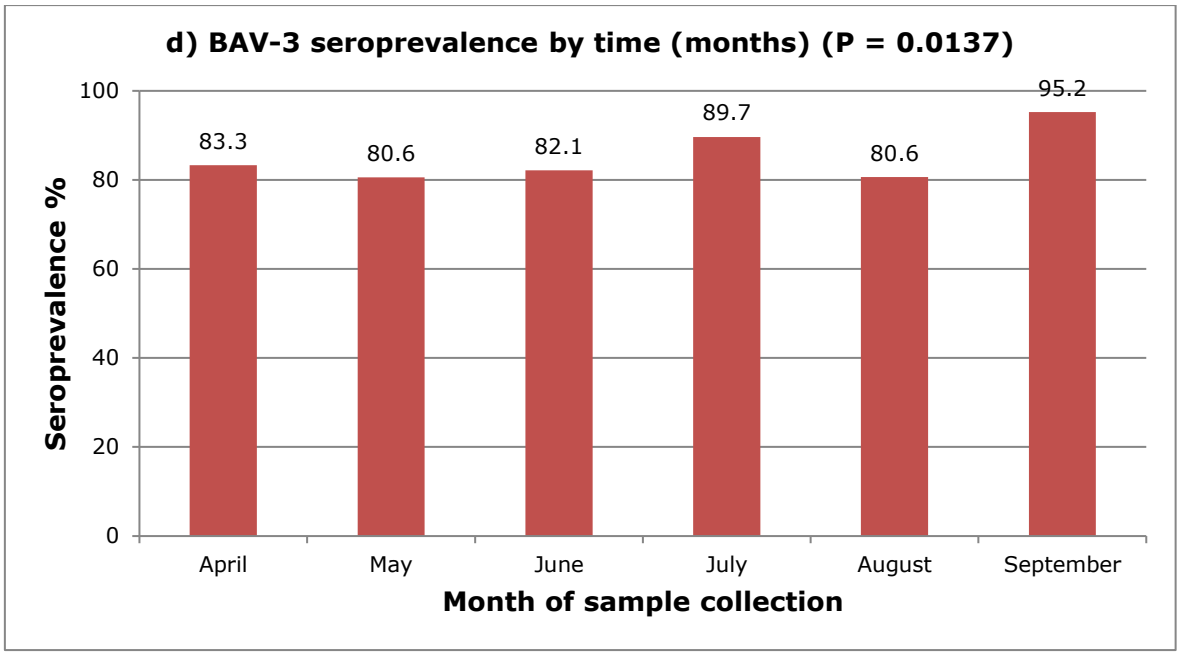


Figure 7 Seroprevalence of five pathogens by time of sampling (months)

a) BoHV-1 seroprevalence; b) BVDV seroprevalence; c) BRSV seroprevalence; d) PI-3 seroprevalence; and e) BAV-3 seroprevalence

5. Discussion

The five upper respiratory tract viral pathogens studied in this research project are known from previous studies to occur worldwide and are considered important health problems in cattle. The results described the serological prevalence of five respiratory viruses in the cattle population of the Mnisi farming community of South Africa. Humoral antibodies specific to BoHV-1, BVDV, BRSV, PI-3 and BAV-3 were investigated. Among the 423 serum samples tested and obtained from cattle at 11 dip tanks, 83.2% were positive for BAV-3, 82.5% for BRSV, 44.4% for PI-3, 43.3% for BoHV-1 and the lowest seroprevalence of 30.5% was found for BVDV. These findings confirmed the circulation of these viruses in cattle of the Mnisi communal farming area. The differences in seroprevalence could be attributed by many factors such as farming practices, climatic condition and herd sizes (Taylor et al., 2010). The anxiety of mixing of cattle and dipping every week well be sufficient to severely suppress body defence mechanisms, rendering cattle susceptible to infections. Mixing of larger number of cattle from multiple sources or rather different herds may be responsible for the increased risk of respiratory infections (Taylor et al., 2010). Equally, herd size can easily plays a role in the spread of respiratory infections, potentially resulting in an increased seroprevalence, especially if the cattle are kept in small capacity farms (piece of land) as opposed to large (Yeşilbağ et al., 2008).

The seroprevalence of 43.3% of BoHV-1 reported in this study is similar to the seroprevalence of 41.1% observed in Zambia under similar farming practices (Ghirotti et al., 1991). However, a study by Yeşilbağ et al. (2008) reported a very low seroprevalence of 17.1% in North-Western Turkey while Njiro et al. (2011) and high seroprevalence of 75.9% in Gauteng Province, South Africa. The different seroprevalences are likely explained by different farming practices in the respective countries. Additionally, BoHV-1 transmission can occur by direct contact, through aerosols and through mating with subclinically infected bulls (Murphy et al., 1999), which is likely to happen under communal farming practice, as different herds are likely to mix and share water and grazing resources.

In a study carried out by Ghirotti et al. (1991), cattle under traditional farming conditions in Zambia, a seroprevalence of 76.2% for BVDV was observed, which was much higher than the finding of 30.5% in Mnisi. In other studies by Ferreira et al. (2000) and Njiro et al. (2011) in dairy cattle and cattle from resource poor farmers in the South Africa respectively, seroprevalences of 36.8% and 49.4% were documented, which were similar to the 30.5% of BVDV seroprevalence observed in this study. However, a much lower prevalence of 19.8% was observed in calves in Western Kenya (Callaby, 2016).

In a study of feedlot cattle in South Africa, it was documented that 43.3% of cattle were exposed to BRSV (Van Vuuren, 1990), which was lower than the seroprevalence obtained in this study of 82.5%. Yeşilbağ et al. (2008) observed a 73.0% seroprevalence for BRSV in Turkey, which was closer to the 82.5% seroprevalence for BRSV in Mnisi.

The 44.4% seroprevalence observed in this study for PI-3 was higher than the seroprevalence of 20.1% observed in calves in Western Kenya (Callaby, 2016). However, our seroprevalence of 44.4% for PI-3 was much lower in comparison to the findings from a resource poor area in Zambia of 94.4% (Ghirotti et al., 1991).

In the same study (Ghirotti et al., 1991), a seroprevalence of 87.4% was reported for BAV-3. In another study by Yeşilbağ et al., (2008) a seroprevalence for BAV-3 of 89.5% was observed, which was also similar to our findings.

A seroprevalence value reflects the extent of seroconversion in a group of animal at the time of the study, as opposed to incidence which follows a group of animals over a period of time. The differences in seroprevalence can be attributed by many factors such as farming practices, climatic conditions, herd sizes and age groups at the time of study. The range of seroprevalences reported in various studies throughout sub-Saharan Africa and elsewhere may be explained further by the levels of biosecurity involved in the different farming systems. The seroprevalence results obtained in this study may be as a result of co-mingling of cattle from different herds during dipping, which compromises biosecurity and exposes cattle to viruses. These viruses often are transmitted

through contact with nasal secretions, but may also be transmitted by aerosols and through mating. Stress caused by movement, crowding and temperature changes may for example play a role in BRSV outbreaks (Van der Poel, 1994).

Cattle in the Mnisi area have to travel long distances in search of water and grazing. In the process, they may be exposed to varying weather conditions, unlike cattle under intensive farming systems. Though information on symptoms, morbidity or mortality from exposures to respiratory tract viruses was not collected during this study, such information would be useful in future studies that can be aimed at assessing the impact of exposure to the viruses.

Co-infections with infectious pathogens are common in cattle especially with BRDC and are known to affect the host animal's immune response (Okur-Gumusova et al., 2007). In a study by Okur-Gumusova et al., (2007), they reported the one, two, three, four and five multiple virus infection rates at 6.91%, 59.04%, 58.5%, 39.3% and 35.8%, respectively. Alkan et al. (1997), in their antibody surveillance study for nine viruses (IBR, PI-3, BRSV, BVDV, BAdV1, BAdV2, BAdV3, enterovirus 1 and enterovirus 2), reported 9.38%, 11.46% and 72.0% infection rates against single, double and 3-8 multiple viruses, respectively. Yavru et al. (2005) reported 14.7%, 36.22%, 29.92%, 14.56%, 3.93%, 1.57% and 0.39% seropositivity against one, two, three, four, five, six and seven multiple viruses, respectively. In the Mnisi study, the exposure rates to one, two, three, four and five pathogens were 5.1%, 24.0%, 21.0%, 18.0% and 16.8%, respectively. The numbers of cattle exposed to more than one pathogen were more than the numbers exposed to one or no pathogen. The obtained data indicates the widespread presence of multiple infections. The hot weather conditions and animal health management peculiar to the Mnisi farming community, such as tick control by dipping and frequent commingling of different herds could predispose cattle to mixed infections.

The higher prevalence of BoHV-1 antibodies among cattle in Mnisi, and its ability to suppress immunity (Taylor et al., 2010) may explain some of the mixed infections among the cattle. Additionally, the virus remains latent and it may be reactivated and spread to susceptible cattle (Obando et al., 1999).

Age was found to be an important predictor of seropositivity for all the viruses. Sex, month of collection and location 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). Additionally, lower seropositivity for BVDV was observed in cattle from Welverdiend B, Seville A and Dixie dip tanks at a level of 4.5%, 10.5% and 12.5% respectively. The lack of correlation with the other viruses may be because Mnisi is a communal area where farmers share available resources such as water, grazing and space and whereby cattle mingle every week during FMD surveillance and tick control activities at dip tanks. Additionally, the dip tanks with high seroprevalences for BVDV are located on the outskirts of Hluvukani Township where mixing with other cattle may not be as frequent as at other dip tanks.

Communal lands are characterized by vast open space with no distinctive boundaries. This allows cattle to travel long distances in search of food and water without barriers and in the process, they could be exposed to changing weather conditions unlike cattle under intensive farming systems. Additionally, it is common practice that cattle may not be herded during the dry period, thus mixing with other cattle is inevitable. In the Mnisi community, farmers practice extensive farming where cattle are allowed to roam freely especially during the dry period.

A high seroprevalence was recorded in older individual cattle above 24 months of age followed by middle aged cattle (12-24 months) while cattle younger than 12 months had lower seroprevalences to all five respiratory pathogens investigated. This may be explained by the fact that they have had a longer duration of exposure, and more time to acquire antibodies. Possibilities of older animals making contact with infected material and infected cattle several times are higher than in younger animals.

The hypothesis was that sex is predictor for seroprevalence with female cattle expected to have a higher seroprevalence that male counterparts as female animals are vulnerable especially during gestation and lactating period when more nutritional resources may be utilized to support pregnancies and for the production of milk to support the calves than immunological responses of the

mothers. This was not confirmed in this study. Seroprevalences for BoHV-1, BVDV, BRSV and BAV-3 were found to be similar between two sexes however, a significant difference in prevalence was observed for PI-3, with higher prevalences in females than males. Seroprevalences for the other pathogens was independent of sex.

The hypothesis was that cattle sampled in the dry winter period (June, July and August) would have a higher seroprevalence compared to cattle sampled in April and May months because some cattle, especially lactating female and calves may face nutritional deficits due to limited grazing during the dry winter season. This was not confirmed. Seropositivity for BoHV-1, BRSV, and PI-3 was found to be independent of sampling time. However, a significant low seropositivity for BVDV was observed in May ($P = 0.0026$) and a significant high seropositivity of BAV-3 was observed in September ($P = 0.0137$). The lower seropositivity for BVDV observed in May at a level of 20.4% was lower than the average prevalence of 35.0% in this study. High seroprevalence was recorded in winter (Hodgins et al., 2002; Scott et al., 2011) among feedlot cattle and cattle that are housed during winter where susceptible animals come into close contact with infected cattle.

6. Conclusion

Livestock production plays a big role in the agricultural sector in South Africa (Simela, 2012) with 70% of the South African land area suitable for grazing. However, livestock production is challenged by many constraints including livestock diseases. In the Mnisi communal area, the perceived major constraints to livestock production are nutritional problems, diseases, access to water, drought, ticks and lack of knowledge of disease control by communal farmers (Van Rooyen, 2011). Nutritional problems could be a result of limited grazing land for their livestock because of overstocking linked with lack of sufficient rain and hence, insufficient grazing to last through the dry season. An additional limiting factor to livestock production specifically in Mnisi, is the endemicity of foot and mouth disease (FMD) in the area adjacent Kruger National Park limiting the marketing of cattle to the control zone surrounding the Park. The prevalence of respiratory infections in cattle may have a negative impact on the livelihood of the Mnisi farmers, hence the need to obtain relevant data to address this challenge.

The results of this study confirmed the endemicity of five respiratory tract pathogens in the Mnisi community. Exposure to BRSV and BAV-3 were especially high when compared to BoHV-1, BVDV and PI-3. Sudden changes in weather conditions coupled with stress may predispose cattle to respiratory infections (Taylor et al., 2010). The five respiratory tract viruses included in this study 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. Mixing and dipping of cattle may exert anxiety sufficient enough to stress animals and suppress body defence mechanisms rendering cattle susceptible to infections. Mixing of larger numbers of cattle from multiple sources or rather different herds may be responsible for the increased risk of respiratory infections (Taylor et al., 2010). Equally, herd size can play a role in the spread of respiratory infections potentially resulting in an increased seroprevalence. BRSV and BAV-3 were found to be more prevalent in cattle between one and two years old. Older animals were more likely to be seropositive compared to young animals. This is likely due to the fact that they have had longer duration of exposure,

hence more time to acquire antibodies. Possibilities of older animals making contact with infected material and infected cattle several times are higher than in younger animals.

The location of dip tanks were not risk factors for most of the viruses because frequent dipping caused frequent contact between different herds which may have increased the chances of spreading pathogens. Furthermore, it is possible that the stress incurred during dipping may have had a suppressing effect on their immunity.

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8. Appendices

Table 8.1 Example of optical density (OD_{raw}) readings generated by a spectrophotometer

BoHV-1	BVDV	BRSV	PI-3	BAV-3	Negative Control	BoHV -1	BVDV	BRSV	PI-3	BAV-3	Negative Control
2.556	2.323	2.8	2.101	2.21	0.439	2.082	1.68	2.583	1.177	1.257	0.653
2.949	1.529	1.312	1.689	1.963	0.675	1.696	0.427	1.705	0.526	1.091	0.337
0.931	0.686	2.104	1.463	1.89	0.758	1.622	0.532	1.595	1.073	2.454	0.401
0.481	0.352	1.486	0.706	2.274	0.304	0.365	0.358	1.496	0.548	1.297	0.366
2.225	1.869	1.454	0.93	0.772	0.655	1.218	1.021	0.896	0.938	1.667	0.731
3.049	1.098	1.553	1.227	1.373	0.773	2.506	1.486	2.392	1.524	1.51	0.791
2.562	1.587	1.36	1.003	0.816	0.581	1.216	0.39	0.871	0.497	1.965	0.488
0.567	2.478	1.467	0.634	1.225	0.324	1.278	2.098	1.855	0.998	1.11	0.601

Table 8.2 Raw data was calculated by taking in optical density values for each sample to generate the IOD for each pathogen

BoHV -1	BVDV	BRSV	PI3	BAV-3	-ve Control	BoHV -1	BVDV	BRSV	PI3	BAV-3	-ve Control
2.117	1.884	2.361	1.662	1.771		1.429	1.027	1.93	0.524	0.604	
2.274	0.854	0.637	1.014	1.288		1.359	0.09	1.368	0.189	0.754	
0.173	-0.072	1.346	0.705	1.132		1.221	0.131	1.194	0.672	2.053	
0.177	0.048	1.182	0.402	1.97		-0.001	-0.008	1.13	0.182	0.931	
1.57	1.214	0.799	0.275	0.117		0.487	0.29	0.165	0.207	0.936	
2.276	0.325	0.78	0.454	0.6		1.715	0.695	1.601	0.733	0.719	
1.981	1.006	0.779	0.422	0.235		0.728	-0.098	0.383	0.009	1.477	
0.243	2.154	1.143	0.31	0.901		0.677	1.497	1.254	0.397	0.509	

Table 8.3 Example of sample prevalence and degree of positivity for each of the pathogen investigated as were calculated

BoHV-1 positivity	BoHV-1 degree of positivity	BVDV positivity	BVDV degree of positivity	BRSV positivity	BRSV degree of positivity	PI3 positivity	PI3 degree of positivity	BAV-3 positivity	BAV-3 degree of positivity
107.4162	3	45.32909	2	26.98009	1	61.01083	3	72.72727	3
8.171941	0	-3.82166	0	57.00974	2	42.41877	2	63.91869	3
8.360888	0	2.547771	0	50.06353	2	24.18773	1	111.2366	5
74.16155	2	64.43737	3	33.84159	1	16.54633	0	6.606437	0
107.5106	3	17.25053	0	33.03685	1	27.31649	1	33.87916	2
93.57581	2	53.39703	2	32.99449	1	25.3911	1	13.26934	1
11.47851	0	114.3312	5	48.41169	2	18.65223	0	50.87521	2
67.50118	2	54.51168	2	81.74502	4	31.52828	1	34.10503	2
64.19462	1	4.77707	0	57.94155	2	11.37184	0	42.57482	2
57.67596	1	6.953291	0	50.57179	2	40.43321	2	115.9232	5
-0.04724	0	-0.42463	0	47.86108	2	10.95066	0	52.56917	2
23.00425	0	15.39278	0	6.988564	0	12.45487	0	52.8515	2
81.01086	2	36.8896	1	67.81025	3	44.10349	2	40.59853	2
34.38829	1	-5.2017	0	16.22194	0	0.541516	0	83.39921	4
31.97922	1	79.4586	3	53.11309	2	23.88688	1	28.74082	1

Table 8.4 BoHV-1 degree of positivity, number of positive animals and prevalence by location

Name of location	Degree of positivity				Number positive	Total samples	Prevalence %
	1	2	3	4			
Clare A	5	9	2	1	17	39	43.6
Clare B	9	7	2		18	46	39.1
Dixie	5	2	2		9	16	56.3
Gottenburg	4	6	6	1	17	38	44.7
Hlalakahle	3	6	4		13	36	36.1
Seville A	6	4	5		15	38	39.5
Seville B	5	4	9	2	20	39	51.3
Tlhavekisa	3	5	6	1	15	31	48.4
Utha A	5	6	3		14	36	38.9
Welverdiend A	11	9	7	2	29	60	48.3
Welverdiend B	7	7	1	1	16	44	36.4
Grand Total	**63**	**65**	**47**	**8**	**183**	**423**	43.3

Table 8.5 BVDV-1 degree of positivity, number of positive animals and prevalence by location

Name of location	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Clare A	2	5	2			9	39	23.1
Clare B	3	4	1	2	1	11	46	23.9
Dixie		1	1			2	16	12.5
Gottenburg	3	4	4	4	3	18	38	47.4
Hlalakahle	4	5		1	4	14	36	38.9
Seville A	2	1	1			4	38	10.5
Seville B	5	3	2	1	6	17	39	43.6
Tlhavekisa	5	3	3	2	1	14	31	45.2
Utha A	6	4	4	1		15	36	41.7
Welverdiend A	6	7	3	4	3	23	60	38.3
Welverdiend B	1	1				2	44	4.6
Grand Total	**37**	**38**	**21**	**15**	**18**	**129**	**423**	**30.5**

Table 8.6 BRSV degree of positivity, number of positive animals and prevalence by location

Name of location	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Clare A	12	10	11	1		34	39	87.2
Clare B	11	10	12	6		39	46	84.8
Dixie	5	6	1			12	16	75.0
Gottenburg	6	15	8	3		32	38	84.2
Hlalakahle	13	6	7	4		30	36	83.3
Seville A	16	9	5			30	38	79.0
Seville B	14	11	9	1		35	39	89.7
Tlhavekisa	4	10	10	3		27	31	87.1
Utha A	13	10	7			30	36	83.3
Welverdiend A	15	15	13	2	1	46	60	76.7
Welverdiend B	11	15	6	2		34	44	77.3
Grand Total	**120**	**117**	**89**	**22**	**1**	**349**	**423**	**82.5**

Table 8.7 PI-3 degree of positivity, number of positive animals and prevalence by location

Name of location	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Clare A	9	4	4	1	1	19	39	48.7
Clare B	7	5	3	3	1	19	46	41.3
Dixie	4	2	1	2		9	16	56.3
Gottenburg	5	4	5	2	2	18	38	47.4
Hlalakahle	7	6	1	1	3	18	36	50.0
Seville A	9	2	2		1	14	38	36.8
Seville B	6	9	6	2		23	39	59.0
Tlhavekisa	5	8	2		1	16	31	51.6
Utha A	7	4	2		1	14	36	38.9
Welverdiend A	13	9	4	1	1	28	60	46.7
Welverdiend B	6	3	1			10	44	22.7
Grand Total	**78**	**56**	**31**	**12**	**11**	**188**	**423**	**44.4**

Table 8.8 BAV-3 degree of positivity, number of positive animals and prevalence by location

Name of location	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Clare A	11	11	2	5	3	32	39	82.1
Clare B	11	7	5	5	7	35	46	76.1
Dixie	3	6	1	1	4	15	16	93.8
Gottenburg	11	10	6	5	3	35	38	92.1
Hlalakahle	10	10	4	5	2	31	36	86.1
Seville A	11	8	5	2	3	29	38	76.3
Seville B	6	8	8	8	5	35	39	89.7
Tlhavekisa	5	2	6	8	6	27	31	87.1
Utha A	8	6	5	6	4	29	36	80.6
Welverdiend A	6	14	7	10	11	48	60	80.0
Welverdiend B	18	10	5	1	2	36	44	81.8
Grand Total	**100**	**92**	**54**	**56**	**50**	**352**	**423**	**83.2**

Table 8.9 BoHV-1 degree of positivity, number of positive animals and prevalence by location

Time of sampling (month)	Degree of positivity				Number positive	Total samples	Prevalence %
	1	2	3	4			
April	10	9	7	1	27	54	50.0
May	14	15	6	2	37	108	34.3
June	14	19	8	2	43	84	51.2
July	5	6	11	1	23	58	39.7
August	18	13	10	1	42	98	42.9
September	2	3	5	1	11	21	52.4
Grand Total	**63**	**65**	**47**	**8**	**183**	**423**	**43.3**

Table 8.10 BVDV degree of positivity, number of positive animals and prevalence by location

Time of sampling (month)	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
April	6	7	3	3	3	22	54	40.7
May	7	7	1	3	4	22	108	20.4
June	8	11	4	2	1	26	84	31.0
July	3	5	6	5	4	23	58	39.7
August	9	7	5	2	2	25	98	25.5
September	4	1	2		4	11	21	52.4
Grand Total	**37**	**38**	**21**	**15**	**18**	**129**	**423**	**30.5**

Table 8.11 BRSV degree of positivity, number of positive animals and prevalence by location

Time of sampling (month)	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
April	13	14	12	1	1	41	54	75.9
May	30	27	21	10		88	108	81.5
June	21	21	23	6		71	84	84.5
July	11	24	13	4		52	58	89.7
August	37	26	15	1		79	98	80.6
September	8	5	5			18	21	85.7
Grand Total	**120**	**117**	**89**	**22**	**1**	**349**	**423**	**82.5**

Table 8.12 PI-3 degree of positivity, number of positive animals and prevalence by location

Time of sampling (month)	Level of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
04//2013	12	9	3	1	1	26	54	48.15
05//2013	18	11	5	2	4	40	108	37.04
06//2013	17	13	7	3	1	41	84	48.81
07//2013	7	9	6	2	3	27	58	46.55
08//2013	23	8	6	3	2	42	98	42.86
09//2013	1	6	4	1		12	21	57.14
Grand Total	**78**	**56**	**31**	**12**	**11**	**188**	**423**	**44.44**

Table 8.13 BAV-3 degree of positivity, number of positive animals and prevalence by location

Time of sampling (month)	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
04//2013	5	13	7	10	10	45	54	83.3
05//2013	35	24	13	6	9	87	108	80.6
06//2013	20	17	5	16	11	69	84	82.1
07//2013	15	12	10	10	5	52	58	89.7
08//2013	22	19	16	10	12	79	98	80.6
09//2013	3	7	3	4	3	20	21	95.2
Grand Total	**100**	**92**	**54**	**56**	**50**	**352**	**423**	**83.2**

Table 8.14 BoHV-1 degree of positivity, number of positive animals and prevalence by sex

Sex	Degree of positivity				Number positive	Total samples	Prevalence %
	1	2	3	4			
Female	35	30	23	4	92	219	42.0
Male	28	35	24	4	91	204	44.6
Grand Total	**63**	**65**	**47**	**8**	**183**	**423**	**43.3**

Table 8.15 BVDV degree of positivity, number of positive animals and prevalence by sex

Sex	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Female	20	19	12	10	7	68	219	31.1
Male	17	19	9	5	11	61	204	29.9
Grand Total	**37**	**38**	**21**	**15**	**18**	**129**	**423**	**30.5**

Table 8.16 BRSV degree of positivity, number of positive animals and prevalence by sex

Sex	Degree of positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Female	64	57	47	14	1	183	219	83.6
Male	56	60	42	8		166	204	81.4
Grand Total	**120**	**117**	**89**	**22**	**1**	**349**	**423**	**82.5**

Table 8.17 PI-3 degree of positivity, number of positive animals and prevalence by sex

Sex	Degree of Positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Female	41	33	16	8	10	108	219	49.3
Male	37	23	15	4	1	80	204	39.2
Grand Total	**78**	**56**	**31**	**12**	**11**	**188**	**423**	**44.4**

Table 8.18 BAV-3 degree of positivity, number of positive animals and prevalence by sex

Sex	Degree of Positivity					Number positive	Total samples	Prevalence %
	1	2	3	4	5			
Female	39	47	29	34	34	183	219	83.6
Male	61	45	25	22	16	169	204	82.8
Grand Total	**100**	**92**	**54**	**56**	**50**	**352**	**423**	**83.2**

Table 8.19 BoHV-1 statistical and numerical summary results by geographic location Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	8.51
Degrees of freedom	10
P-value	0.579

Location	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Clare A	0	0	0	0	2	3	4	0.8	1.1	1.3	0.5	1.2	39	0
Clare B	0	0	0	0	1	2	3	0.6	0.9	1.4	0.4	0.9	46	0
Dixie	0	0	0	1	1.2	3	3	0.9	1.1	1.1	0.4	1.5	16	0
Gottenburg	0	0	0	0	2	3	4	1	1.3	1.3	0.6	1.4	38	0
Hlalakahle	0	0	0	0	2	3	3	0.8	1.1	1.5	0.4	1.1	36	0
Seville A	0	0	0	0	1	3	3	0.8	1.1	1.4	0.4	1.1	38	0
Seville B	0	0	0	1	3	3.1	4	1.2	1.4	1.1	0.8	1.7	39	0
Tlhavekisa	0	0	0	0	2	3	4	1.1	1.3	1.2	0.7	1.6	31	0
Utha A	0	0	0	0	1.2	3	3	0.7	1	1.4	0.4	1.1	36	0
Welverdiend A	0	0	0	0	2	3	4	1	1.2	1.2	0.7	1.3	60	0
Welverdiend B	0	0	0	0	1	2	4	0.6	1	1.6	0.3	0.9	44	0
All	**0**	**0**	**0**	**0**	**2**	**3**	**4**	**0.9**	**1.1**	**1.3**	**0.8**	**1**	**423**	**0**

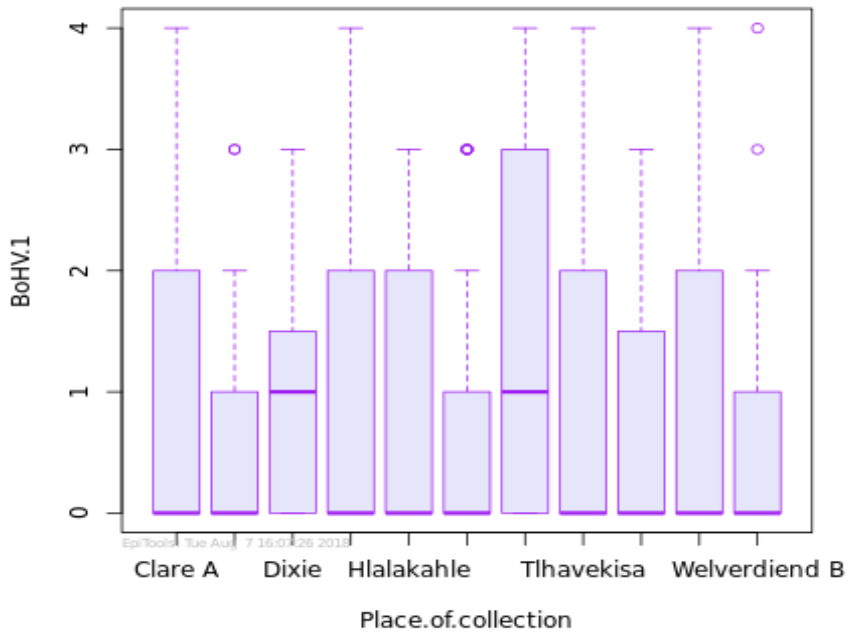

Figure 8.1 BoHV-1 seroprevalence in cattle by geographic location

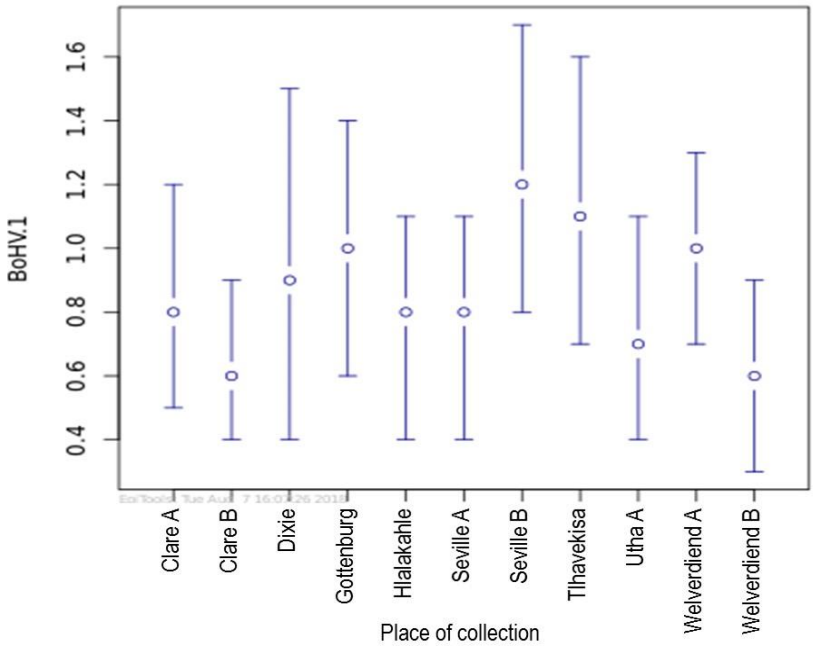

Figure 8.2 BoHV-1 mean antibody titre level in cattle by geographic location

Table 8.20 BVDV statistical and numerical summary results by geographic location Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	43.71
Degrees of freedom	10
P-value	< 0.0001

Location	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Clare A	0	0	0	0	0	2.1	3	0.5	0.9	2	0.2	0.7	39	0
Clare B	0	0	0	0	0	3.8	5	0.6	1.2	2.1	0.2	0.9	46	0
Dixie	0	0	0	0	0	2.2	3	0.3	0.9	2.8	-0.1	0.7	16	0
Gottenburg	0	0	0	0	3	5	5	1.4	1.8	1.3	0.9	2	38	0
Hlalakahle	0	0	0	0	2	5	5	1.1	1.7	1.6	0.5	1.6	36	0
Seville A	0	0	0	0	0	1.1	3	0.2	0.6	3.3	0	0.4	38	0
Seville B	0	0	0	0	2	5	5	1.3	1.9	1.4	0.7	1.9	39	0
Tlhavekisa	0	0	0	0	2	4	5	1.1	1.5	1.4	0.5	1.6	31	0
Utha A	0	0	0	0	1.2	3	4	0.8	1.2	1.4	0.4	1.2	36	0
Welverdiend A	0	0	0	0	2	4	5	1	1.5	1.5	0.6	1.4	60	0
Welverdiend B	0	0	0	0	0	0	2	0.1	0.3	4.9	0	0.2	44	0
All	**0**	**0**	**0**	**0**	**1**	**4**	**5**	**0.8**	**1.4**	**1.8**	**0.6**	**0.9**	**423**	**0**

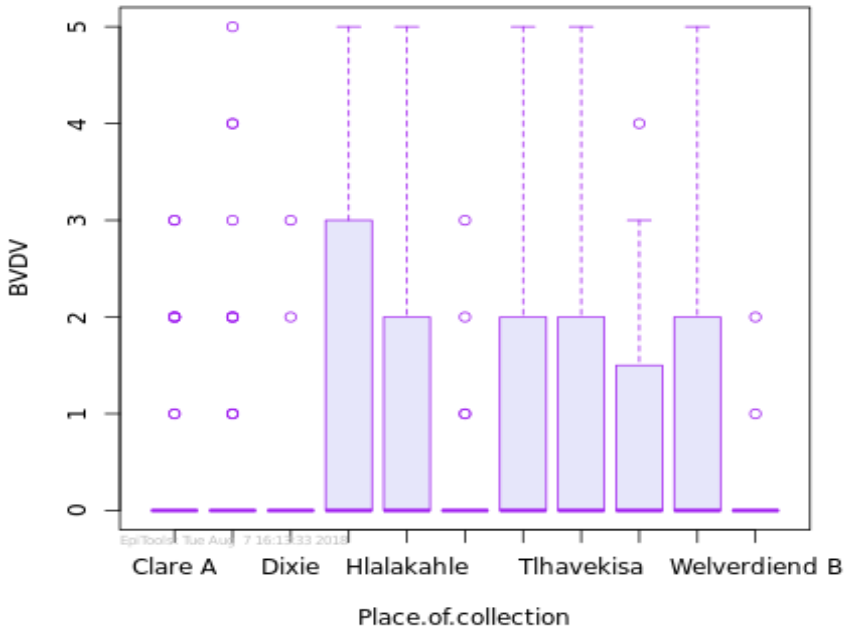

Figure 8.3 BVDV seroprevalence in cattle by geographic location

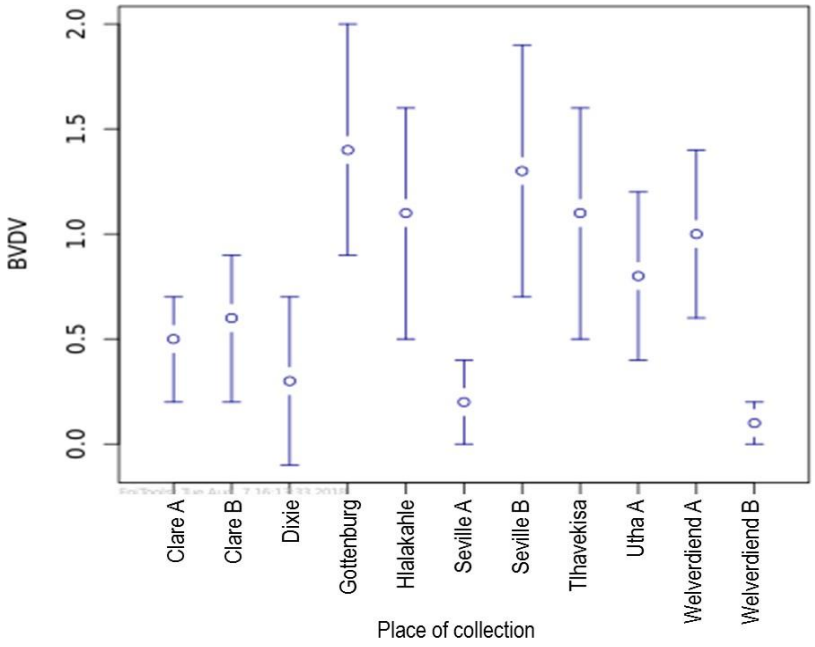

Figure 8.4 BVDV mean antibody titre level in cattle by geographic location

Table 8.21 BRSV statistical and numerical summary results by geographic location Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	17.47
Degrees of freedom	10
P-value	0.0645

Location	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Clare A	0	0	1	2	3	3	4	1.8	1.1	0.6	1.4	2.1	39	0
Clare B	0	0	1	2	3	4	4	2	1.3	0.7	1.6	2.4	46	0
Dixie	0	0	0.8	1	2	2.2	3	1.2	0.9	0.7	0.8	1.7	16	0
Gottenburg	0	0	1	2	3	4	4	1.9	1.2	0.6	1.5	2.3	38	0
Hlalakahle	0	0	1	1	3	4	4	1.7	1.3	0.7	1.3	2.1	36	0
Seville A	0	0	1	1	2	3	3	1.3	1	0.7	1	1.6	38	0
Seville B	0	0	1	2	2.5	3	4	1.7	1	0.6	1.4	2	39	0
Tlhavekisa	0	0	1.5	2	3	4	4	2.1	1.2	0.6	1.7	2.5	31	0
Utha A	0	0	1	1	2	3	3	1.5	1	0.7	1.2	1.8	36	0
Welverdiend A	0	0	1	2	3	3	5	1.6	1.2	0.8	1.3	1.9	60	0
Welverdiend B	0	0	1	2	2	3	4	1.5	1.1	0.7	1.2	1.9	44	0
All	**0**	**0**	**1**	**2**	**3**	**4**	**5**	**1.7**	**1.2**	**0.7**	**1.6**	**1.8**	**423**	**0**

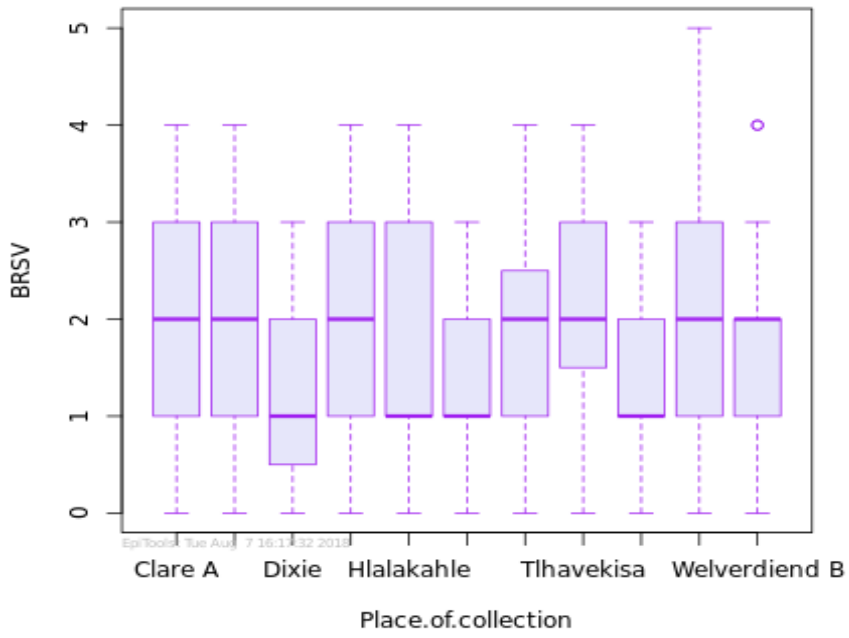

Figure 8.5 BRSV seroprevalence in cattle by geographic location

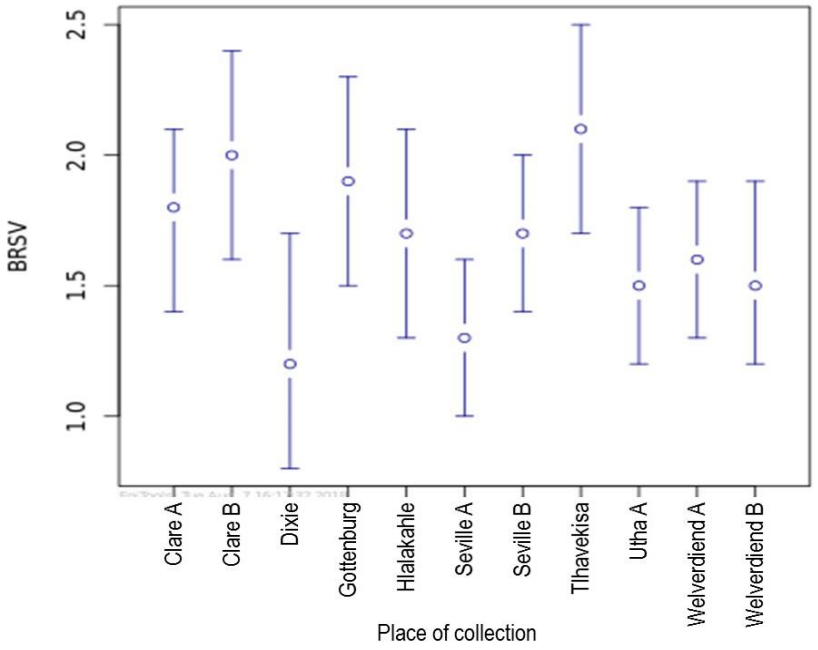

Figure 8.6 BRSV mean antibody titre level in cattle by geographic location

Table 8.22 PI-3 statistical and numerical summary results by geographic location Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	19.47
Degrees of freedom	10
P-value	0.0347

Location	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Clare A	0	0	0	0	1.5	3.1	5	1	1.3	1.3	0.6	1.4	39	0
Clare B	0	0	0	0	1.8	4	5	0.9	1.4	1.5	0.5	1.3	46	0
Dixie	0	0	0	1	2	4	4	1.2	1.4	1.2	0.5	1.9	16	0
Gottenburg	0	0	0	0	2	4.1	5	1.2	1.6	1.3	0.7	1.7	38	0
Hlalakahle	0	0	0	0.5	2	5	5	1.1	1.6	1.4	0.6	1.6	36	0
Seville A	0	0	0	0	1	3	5	0.6	1.1	1.7	0.3	1	38	0
Seville B	0	0	0	1	2	3.1	4	1.3	1.3	1	0.9	1.7	39	0
Tlhavekisa	0	0	0	1	2	3	5	1	1.3	1.2	0.6	1.5	31	0
Utha A	0	0	0	0	1	3	5	0.7	1.2	1.6	0.3	1.1	36	0
Welverdiend A	0	0	0	0	1.2	3	5	0.9	1.2	1.4	0.6	1.2	60	0
Welverdiend B	0	0	0	0	0	2	3	0.3	0.7	2.1	0.1	0.6	44	0
All	**0**	**0**	**0**	**0**	**2**	**4**	**5**	**0.9**	**1.3**	**1.4**	**0.8**	**1**	**423**	**0**

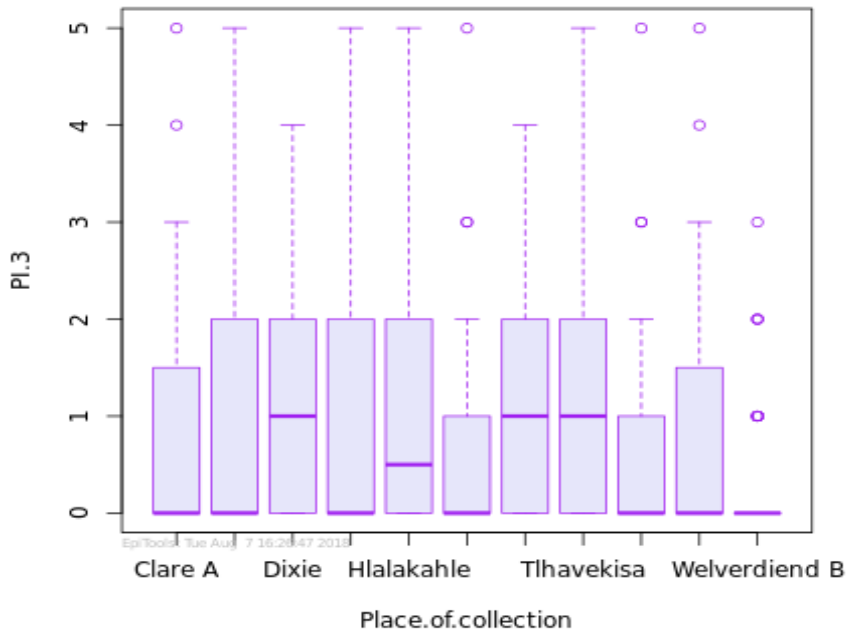

Figure 8.7 PI-3 seroprevalence in cattle by geographic location

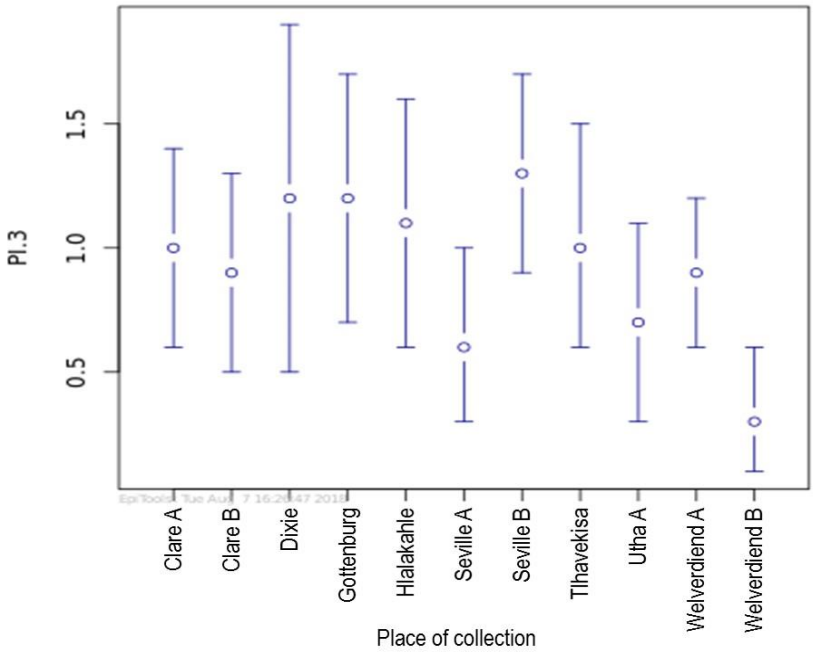

Figure 8.8 PI-3 mean antibody titre level in cattle by geographic location

Table 8.23 BAV-3 statistical and numerical summary results by geographic location Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	24
Degrees of freedom	10
P-value	0.0076

Location	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Clare A	0	0	1	2	2.5	5	5	1.9	1.5	0.8	1.4	2.4	39	0
Clare B	0	0	1	2	3.8	5	5	2.1	1.8	0.9	1.6	2.6	46	0
Dixie	0	0.8	1.8	2	4.2	5	5	2.6	1.7	0.6	1.8	3.4	16	0
Gottenburg	0	0	1	2	3	5	5	2.2	1.4	0.6	1.8	2.7	38	0
Hlalakahle	0	0	1	2	3	4.2	5	2	1.4	0.7	1.5	2.5	36	0
Seville A	0	0	1	1	2.8	5	5	1.7	1.5	0.9	1.2	2.2	38	0
Seville B	0	0	1.5	3	4	5	5	2.6	1.5	0.6	2.2	3.1	39	0
Tlhavekisa	0	0	1	3	4	5	5	2.9	1.7	0.6	2.3	3.5	31	0
Utha A	0	0	1	2	4	5	5	2.2	1.7	0.8	1.6	2.7	36	0
Welverdiend A	0	0	1	2	4	5	5	2.5	1.8	0.7	2.1	2.9	60	0
Welverdiend B	0	0	1	1	2	3.9	5	1.5	1.2	0.8	1.2	1.9	44	0
All	**0**	**0**	**1**	**2**	**3.5**	**5**	**5**	**2.2**	**1.6**	**0.7**	**2**	**2.3**	**423**	**0**

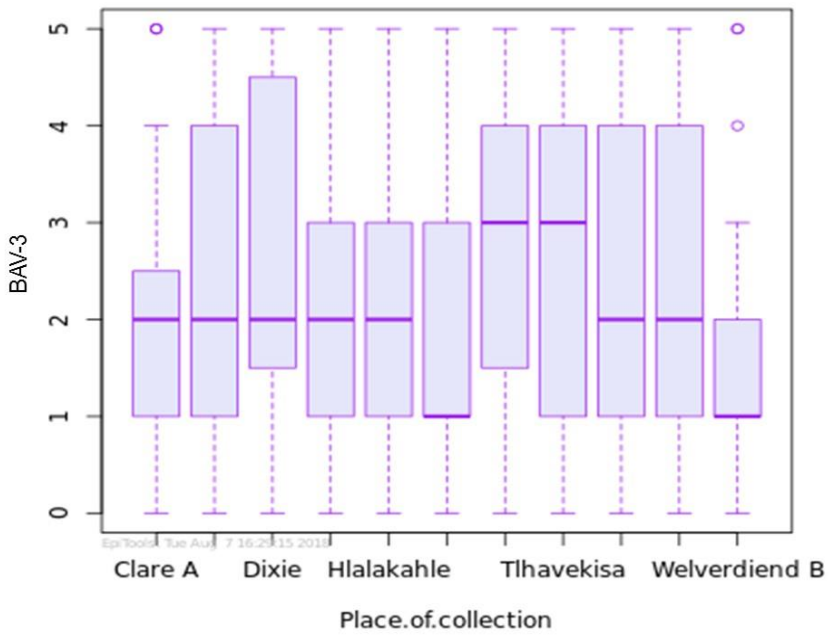

Figure 8.9 BAV-3 seroprevalence in cattle by geographic location

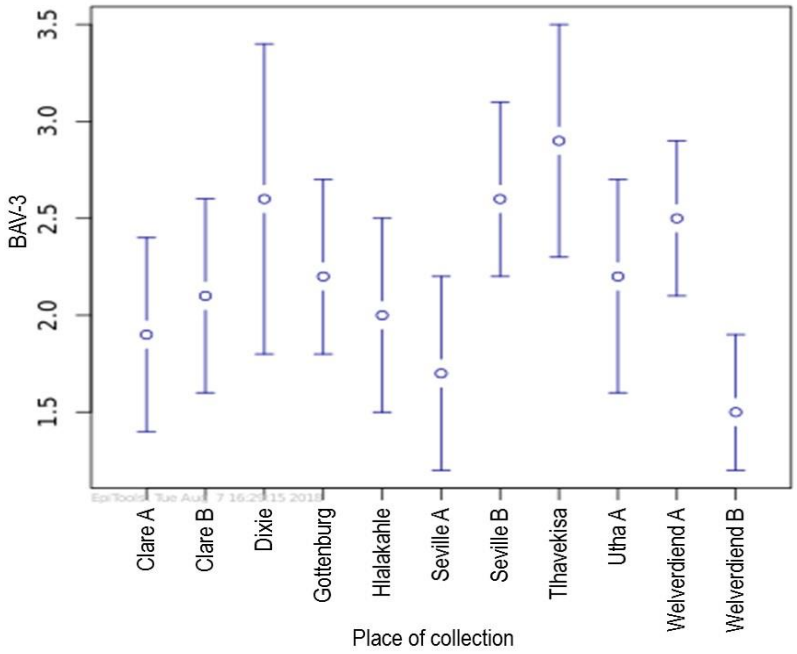

Figure 8.10 BAV-3 mean antibody titre level in cattle by geographic location

Table 8.24 BoHV-1 statistical and numerical summary results for BoHV-1 by Age Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	133.27
Degrees of freedom	2
P-value	< 0.0001

Age group	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Age group-A	0	0	0	0	0	2	4	0.3	0.7	2.4	0.2	0.4	210	0
Age group-B	0	0	0	0	1	3	4	0.8	1.1	1.4	0.5	1.1	65	0
Age group-C	0	0	1	2	3	3	4	1.7	1.2	0.7	1.5	1.9	148	0
All	**0**	**0**	**0**	**0**	**2**	**3**	**4**	**0.9**	**1.1**	**1.3**	**0.8**	**1**	**423**	**0**

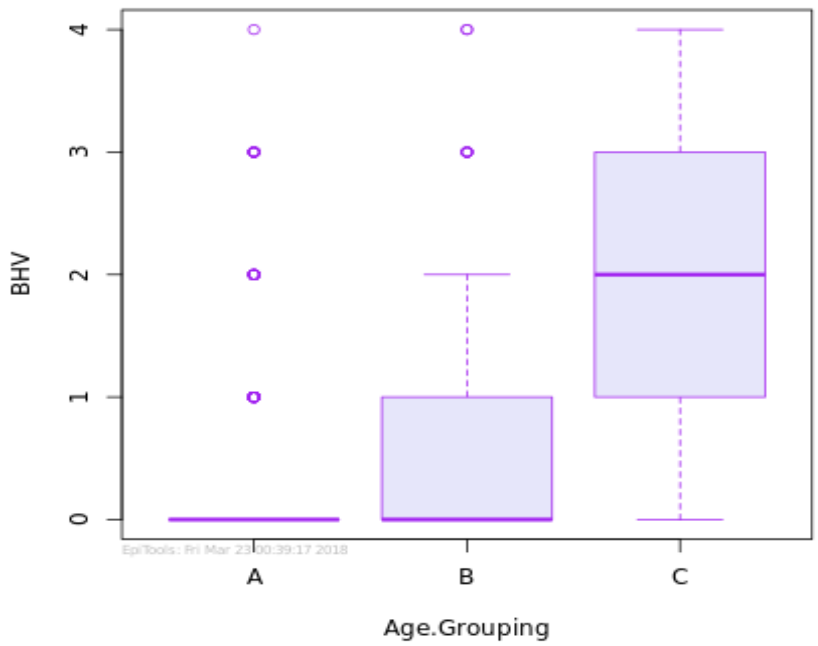

Figure 8.11 BoHV-1 seroprevalence in cattle by age group

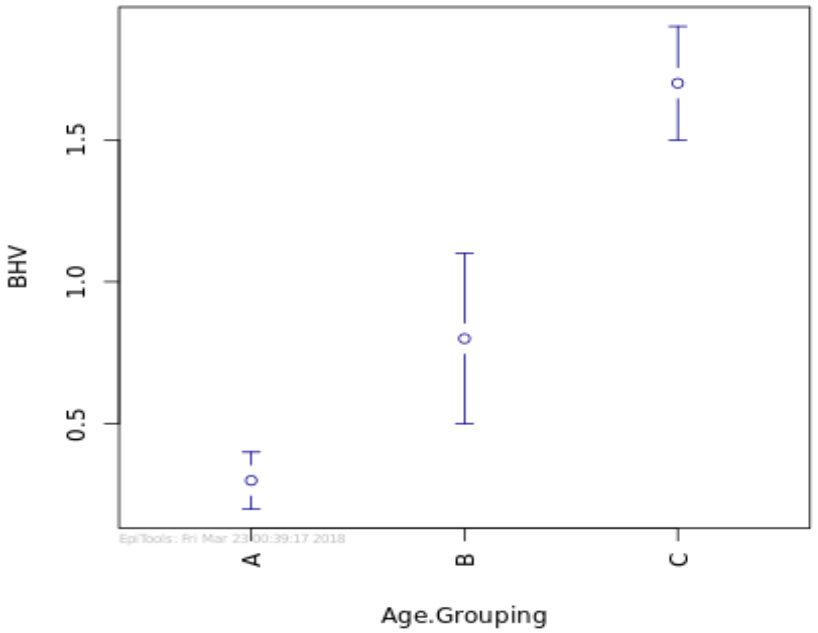

Figure 8.12 BoHV-1 mean antibody titre level in cattle by age group

Table 8.25 BVDV statistical and numerical summary results by age group Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	31.51
Degrees of freedom	2
P-value	< 0.0001

Age grouping	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Age group-A	0	0	0	0	0	4	5	0.6	1.4	2.3	0.4	0.8	210	0
Age group-B	0	0	0	0	0	4.6	5	0.6	1.4	2.1	0.3	1	65	0
Age group-C	0	0	0	0.5	2	4	5	1.1	1.4	1.3	0.9	1.3	148	0
All	**0**	**0**	**0**	**0**	**1**	**4**	**5**	**0.8**	**1.4**	**1.8**	**0.6**	**0.9**	**423**	**0**

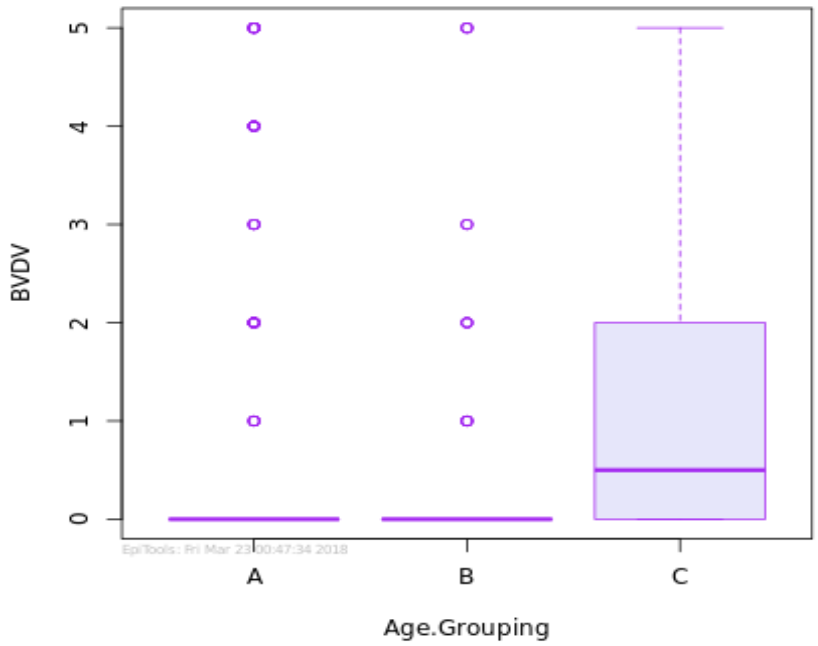

Figure 8.13 BVDV seroprevalence in cattle by age group

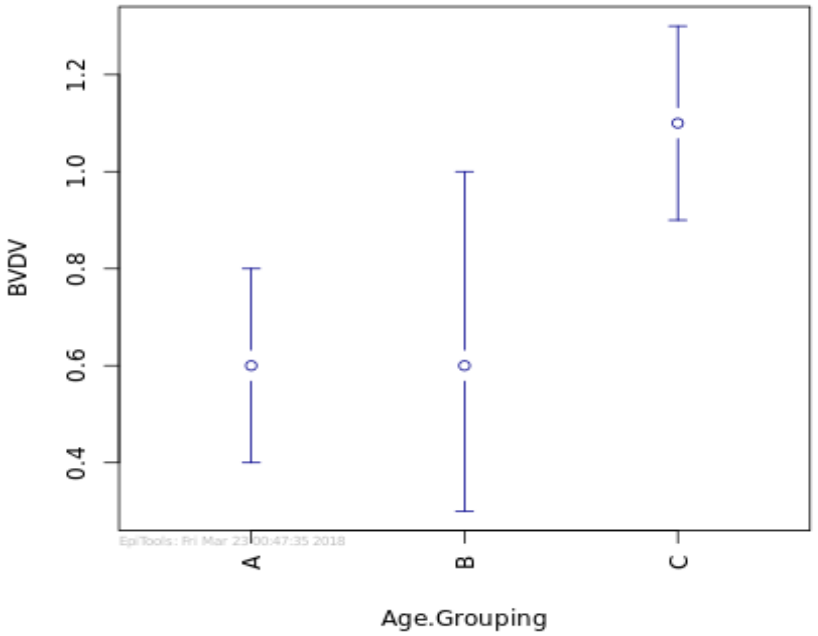

Figure 8.14 BVDV mean antibody titre level by age group

Table 8.26 BRSV statistical numerical summary results for BRSV by Age group Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	43.16
Degrees of freedom	2
P-value	< 0.0001

Age group	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Age group-A	0	0	0	1	2	3	4	1.3	1.1	0.9	1.2	1.5	210	0
Age group-B	0	0	1	2	3	4	4	2.2	1.1	0.5	1.9	2.4	65	0
Age group-C	0	1	1	2	3	3.7	5	2	1	0.5	1.8	2.2	148	0
All	**0**	**0**	**1**	**2**	**3**	**4**	**5**	**1.7**	**1.2**	**0.7**	**1.6**	**1.8**	**423**	**0**

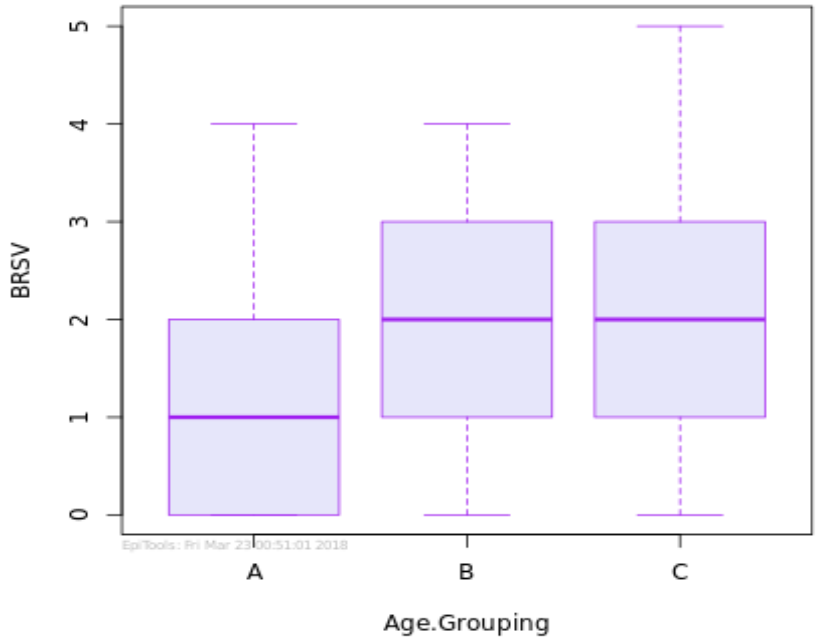

Figure 8.15 BRSV seroprevalence in cattle by age group

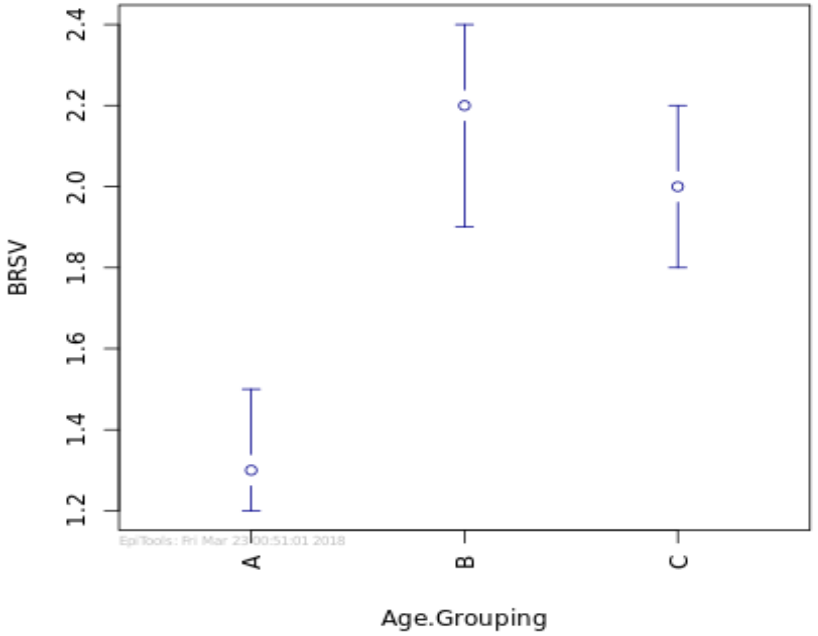

Figure 8.16 BRSV mean antibody titre level in cattle by age group

Table 8.27 PI-3 statistical and numerical summary results for PI3 by age group Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	87.22
Degrees of freedom	2
P-value	<0.0001

Age group	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Age group-A	0	0	0	0	0	3	5	0.5	1	2.2	0.3	0.6	210	0
Age group-B	0	0	0	0	1	3	4	0.8	1.1	1.4	0.5	1	65	0
Age group-C	0	0	0	1	2	4.7	5	1.6	1.4	0.9	1.4	1.8	148	0
All	**0**	**0**	**0**	**0**	**2**	**4**	**5**	**0.9**	**1.3**	**1.4**	**0.8**	**1**	**423**	**0**

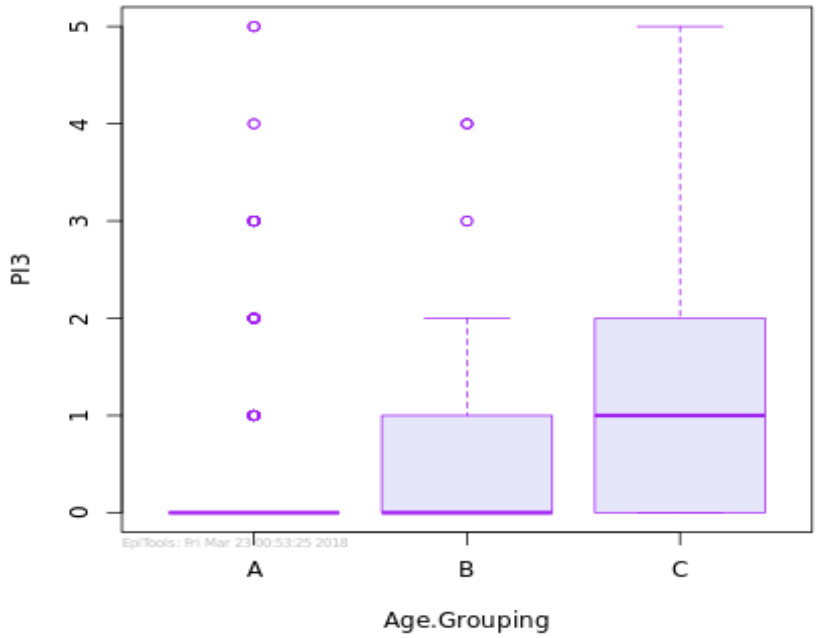

Figure 8.17 PI-3 seroprevalence in cattle by age group

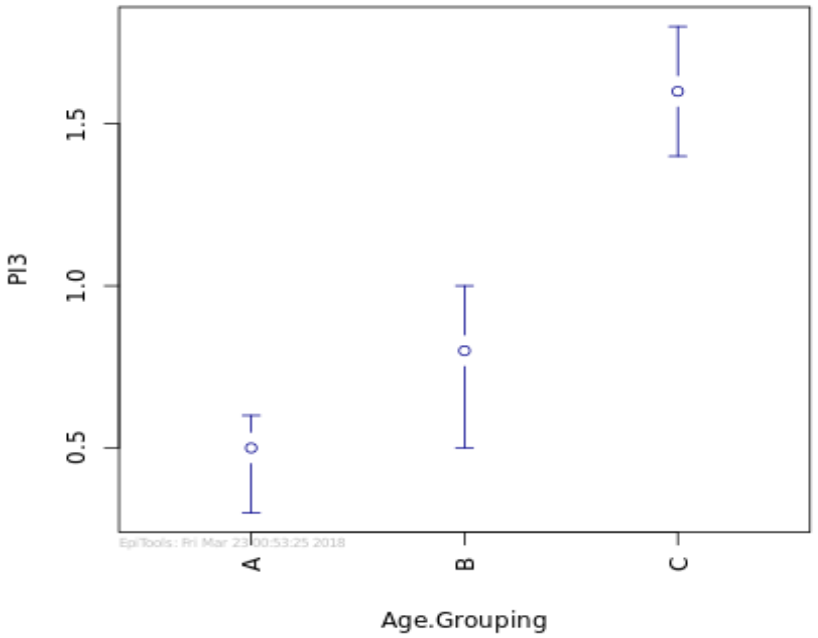

Figure 8.18 PI-3 mean antibody titre level in cattle by age group

Table 8.28 BAV-3 statistical and numerical summary results by Age group Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	35.45
Degrees of freedom	2
P-value	<0.0001

Age group	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Age group-A	0	0	0	1	3	5	5	1.8	1.7	0.9	1.6	2	210	0
Age group-B	0	0	1	2	3	4.8	5	2.1	1.3	0.6	1.7	2.4	65	0
Age group-C	0	1	2	3	4	5	5	2.8	1.5	0.5	2.5	3	148	0
All	**0**	**0**	**1**	**2**	**3.5**	**5**	**5**	**2.2**	**1.6**	**0.7**	**2**	**2.3**	**423**	**0**

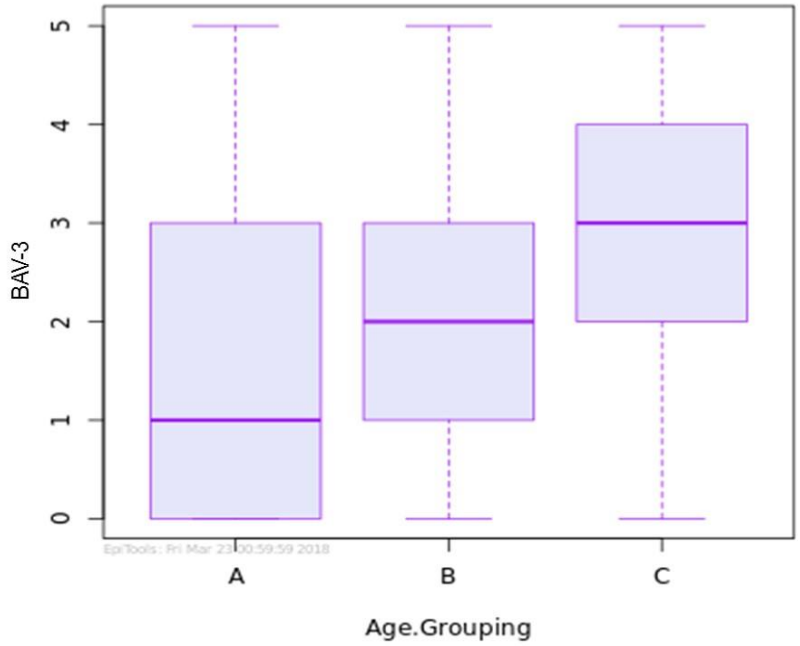

Figure 8.19 BAV-3 seroprevalence in cattle by age group

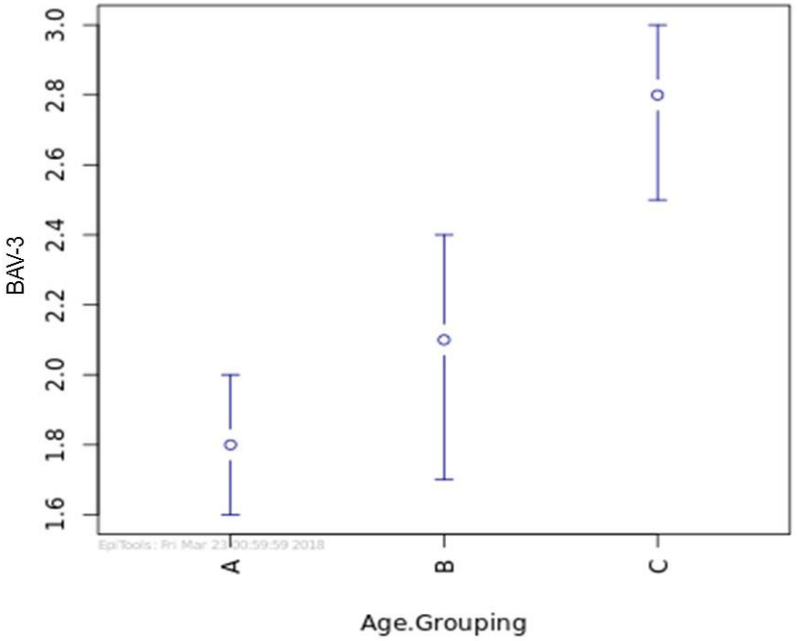

Figure 8.20 BAV-3 mean antibody titre level in cattle by age group

Table 8.29 BoHV-1 statistical and numerical summary results by Sex Wilcoxon rank sum test

Wilcoxon rank sum	21507.5
P-value	0.4627
Estimated difference	0
95% confidence interval for difference	0-0

Sex	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Female	0	0	0	0	2	3	4	0.8	1.1	1.4	0.7	1	219	0
Male	0	0	0	0	2	3	4	0.9	1.2	1.3	0.8	1.1	204	0
All	**0**	**0**	**0**	**0**	**2**	**3**	**4**	**0.9**	**1.1**	**1.3**	**0.8**	**1**	**423**	**0**

Frequency table of results

BoHV-1 degree of positivity	Female	Male	Sum
BoHV-1-0	127	113	**240**
BoHV-1-1	35	28	**63**
BoHV-1-2	30	35	**65**
BoHV-1-3	23	24	**47**
BoHV-1-4	4	4	**8**
Sum	**219**	**204**	**423**

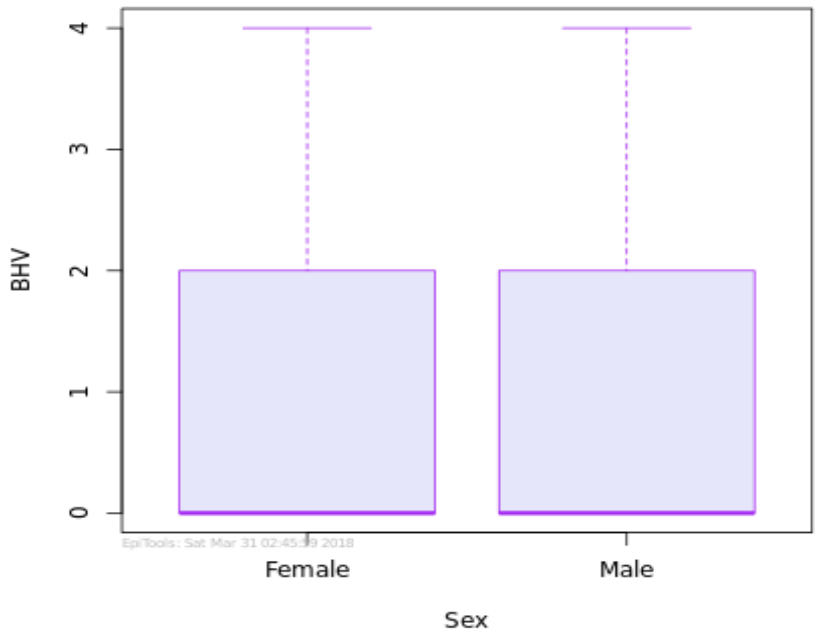

Figure 8.21 BoHV-1 seroprevalence in cattle by sex

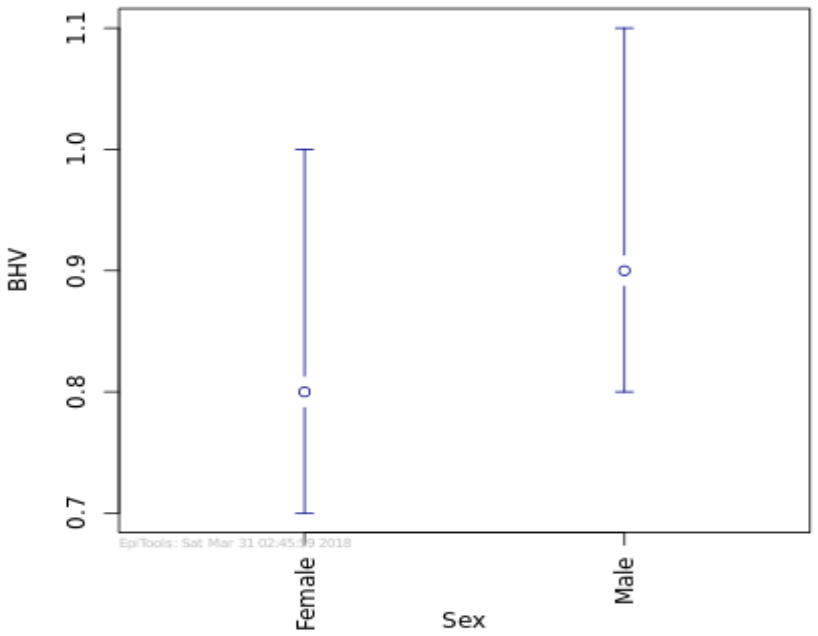

Figure 8.22 BoHV-1 mean antibody titre level in cattle by sex

Table 8.30 BVDV statistical and numerical summary results by Sex Wilcoxon rank sum test

Wilcoxon rank sum	22543.5
P-value	0.8411
Estimated difference	0
95% confidence interval for difference	0-0

Sex	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Female	0	0	0	0	1	4	5	0.8	1.4	1.8	0.6	1	219	0
Male	0	0	0	0	1	4.8	5	0.8	1.4	1.8	0.6	1	204	0
All	**0**	**0**	**0**	**0**	**1**	**4**	**5**	**0.8**	**1.4**	**1.8**	**0.6**	**0.9**	**423**	**0**

Frequency table of results

BVDV degree of positivity	Female	Male	Sum
BVDV-0	151	143	**294**
BVDV-1	20	17	**37**
BVDV-2	19	19	**38**
BVDV-3	12	9	**21**
BVDV-4	10	5	**15**
BVDV-5	7	11	**18**
Sum	**219**	**204**	**423**

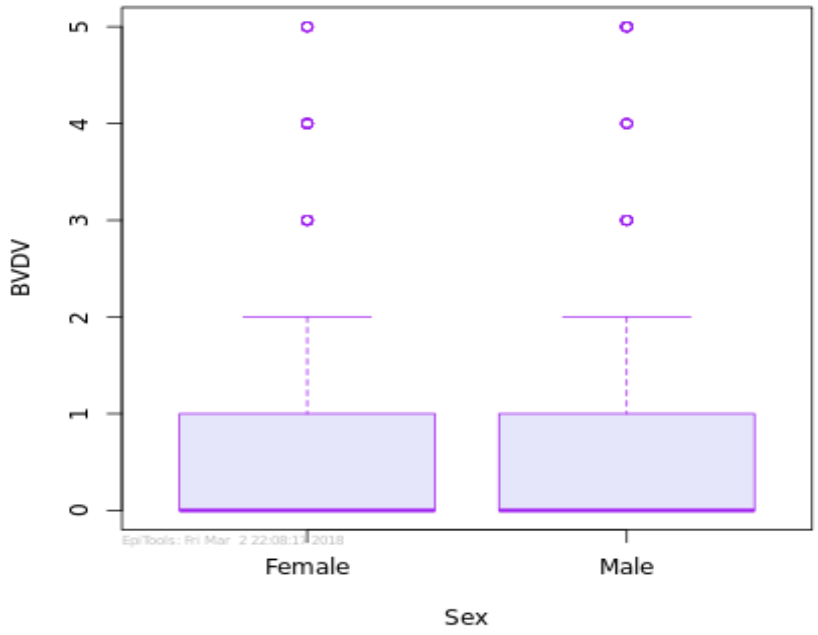

Figure 8.23 BVDV seroprevalence in cattle by sex

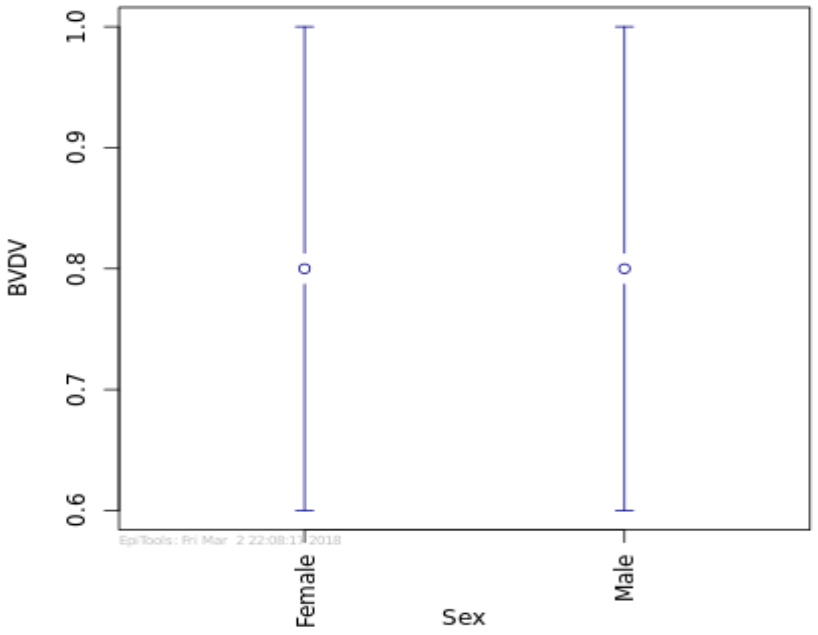

Figure 8.24 BVDV mean antibody titre level in cattle by sex

Table 8.31 BRSV statistical and numerical summary results by Sex Wilcoxon rank sum test

Wilcoxon rank sum	23205
P-value	0.4772
Estimated difference	0
95% confidence interval for difference	0-0

Sex	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Female	0	0	1	2	3	4	5	1.7	1.2	0.7	1.6	1.9	219	0
Male	0	0	1	2	2	3	4	1.6	1.1	0.7	1.5	1.8	204	0
All	**0**	**0**	**1**	**2**	**3**	**4**	**5**	**1.7**	**1.2**	**0.7**	**1.6**	**1.8**	**423**	**0**

Frequency table of results

BRSV degree of positivity	Female	Male	Sum
BRSV-0	36	38	**74**
BRSV-1	64	56	**120**
BRSV-2	57	60	**117**
BRSV-3	47	42	**89**
BRSV-4	14	8	**22**
BRSV-5	1	0	**1**
Sum	**219**	**204**	**423**

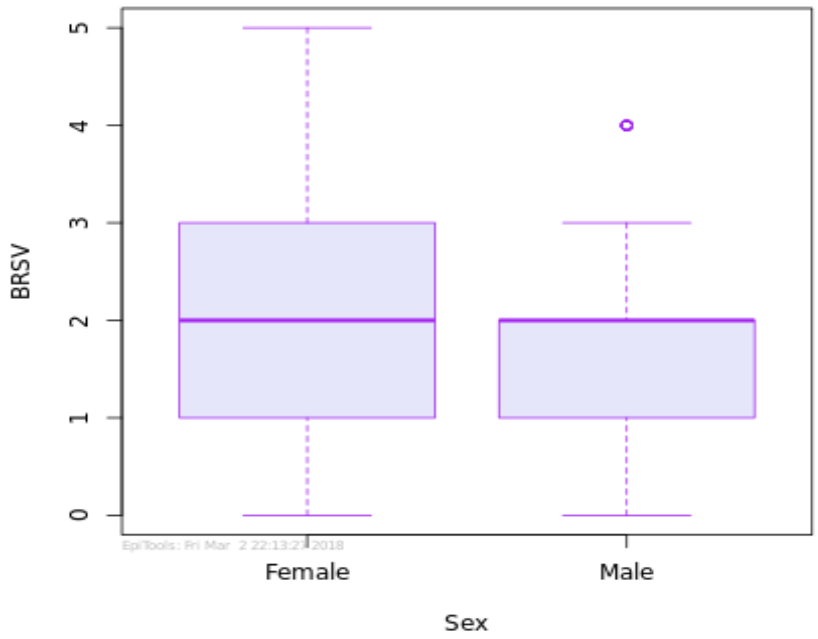

Figure 8.25 BRSV seroprevalence in cattle by sex

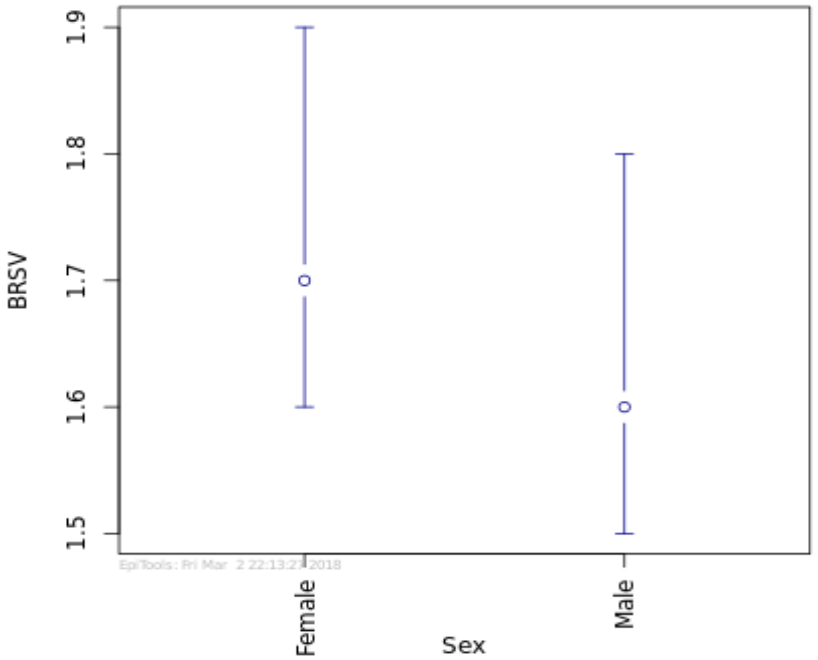

Figure 8.26 BRSV mean antibody titre level in cattle by sex

Table 8.32 PI-3 statistical and numerical summary results by Sex Wilcoxon rank sum test

Wilcoxon rank sum	25124
P-value	0.0143
Estimated difference	0
95% confidence interval for difference	0-0

Sex	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Female	0	0	0	0	2	4	5	1.1	1.4	1.3	0.9	1.3	219	0
Male	0	0	0	0	1	3	5	0.7	1.1	1.5	0.6	0.9	204	0
All	**0**	**0**	**0**	**0**	**2**	**4**	**5**	**0.9**	**1.3**	**1.4**	**0.8**	**1**	**423**	**0**

Frequency table of results

PI-3 degree of positivity	Female	Male	Sum
PI-3-0	111	124	**235**
PI-3-1	41	37	**78**
PI-3-2	33	23	**56**
PI-3-3	16	15	**31**
PI-3-4	8	4	**12**
PI-3-5	10	1	**11**
Sum	**219**	**204**	**423**

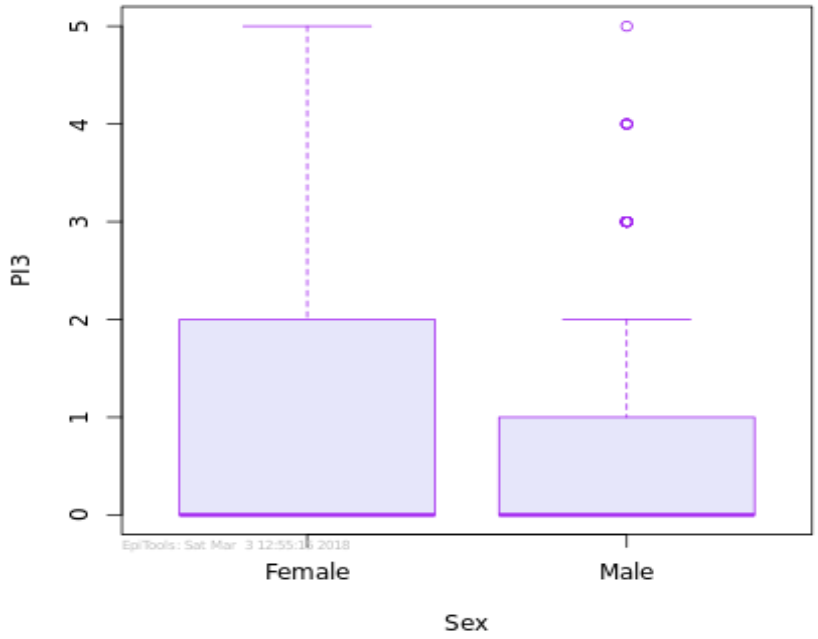

Figure 8.27 PI-3 seroprevalence in cattle by sex

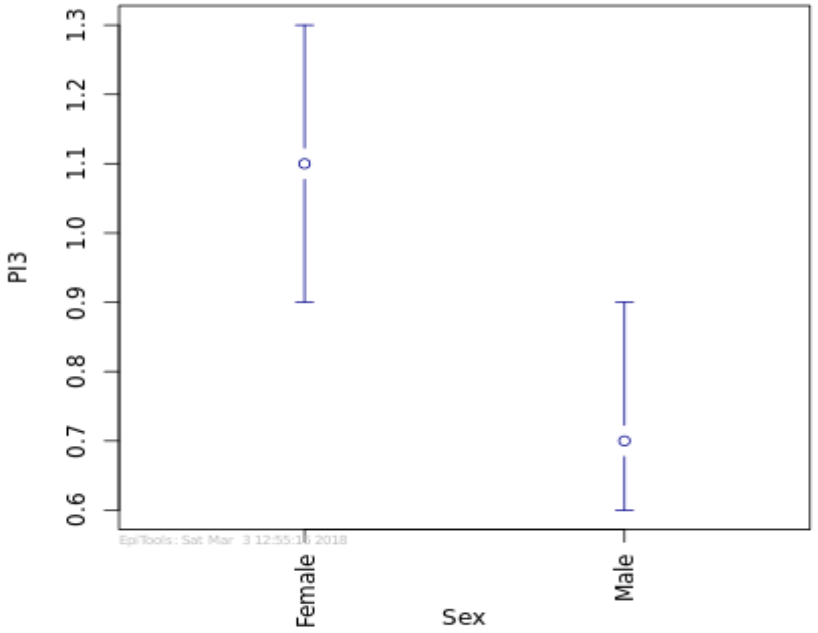

Figure 8.28 PI-3 mean antibody titre level in cattle by sex

Table 8.33 BAV-3 statistical and numerical summary results by Sex Wilcoxon rank sum test

Wilcoxon rank sum	24583.5
P-value	0.068
Estimated difference	0
95% confidence interval for difference	0-1

Sex	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
Female	0	0	1	2	4	5	5	2.4	1.7	0.7	2.2	2.6	219	0
Male	0	0	1	2	3	5	5	1.9	1.5	0.8	1.7	2.1	204	0
All	**0**	**0**	**1**	**2**	**3.5**	**5**	**5**	**2.2**	**1.6**	**0.7**	**2**	**2.3**	**423**	**0**

Frequency table of results

BAV-3 degree of positivity	Female	Male	Sum
BAV-3-0	36	35	**71**
BAV-3-1	39	61	**100**
BAV-3-2	47	45	**92**
BAV-3-3	29	25	**54**
BAV-3-4	34	22	**56**
BAV-3-5	34	16	**50**
Sum	**219**	**204**	**423**

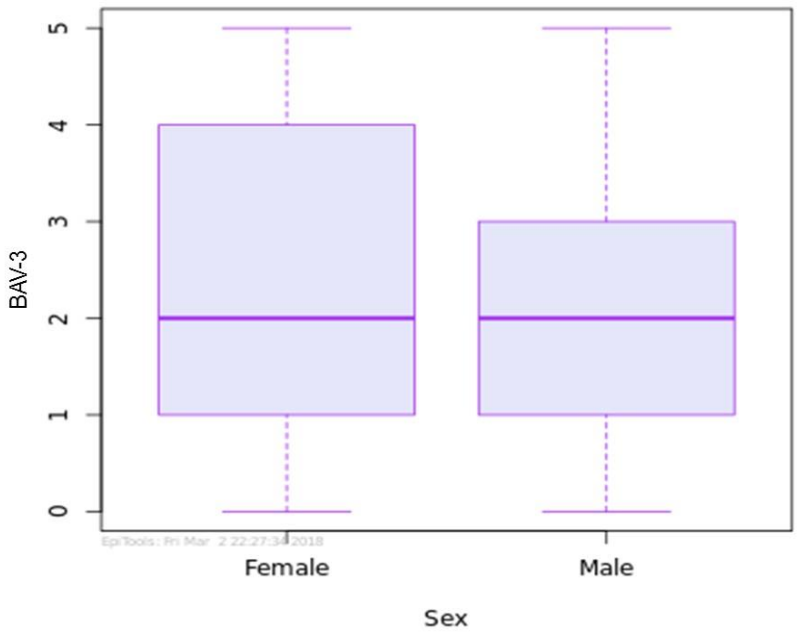

Figure 8.29 BAV-3 seroprevalence in cattle by sex

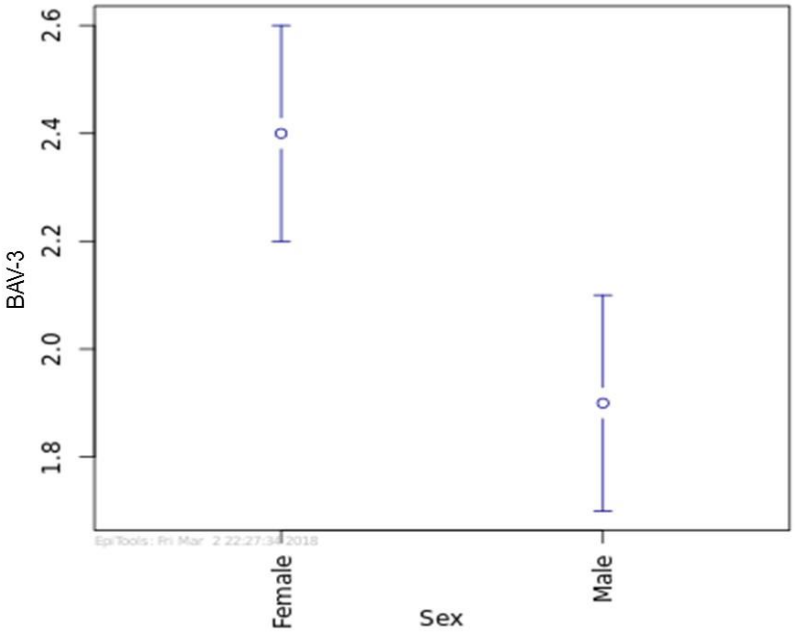

Figure 8.30 BAV-3 mean antibody titre level in cattle by sex

Table 8.34 BoHV-1 Statistical and numerical summary results by month Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	8.5
Degrees of freedom	5
P-value	0.131

Time of samples collection (month)	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
April	0	0	0	0.5	2	3	4	1	1.2	1.2	0.7	1.3	54	0
May	0	0	0	0	1	3	4	0.6	1	1.6	0.5	0.8	108	0
June	0	0	0	1	2	3	4	1	1.2	1.2	0.8	1.2	84	0
July	0	0	0	0	2	3	4	0.9	1.3	1.4	0.6	1.3	58	0
August	0	0	0	0	1	3	4	0.8	1.1	1.4	0.6	1	98	0
September	0	0	0	1	3	3	4	1.3	1.4	1.1	0.7	1.9	21	0
All	**0**	**0**	**0**	**0**	**2**	**3**	**4**	**0.9**	**1.1**	**1.3**	**0.8**	**1**	**423**	**0**

BoHV-1and degree of positivity	April	May	June	July	August	September	Sum
BoHV-1-0	27	71	41	35	56	10	**240**
BoHV-1-1	10	14	14	5	18	2	**63**
BoHV-1-2	9	15	19	6	13	3	**65**
BoHV-1-3	7	6	8	11	10	5	**47**
BoHV-1-4	1	2	2	1	1	1	**8**
All	**54**	**108**	**84**	**58**	**98**	**21**	**423**

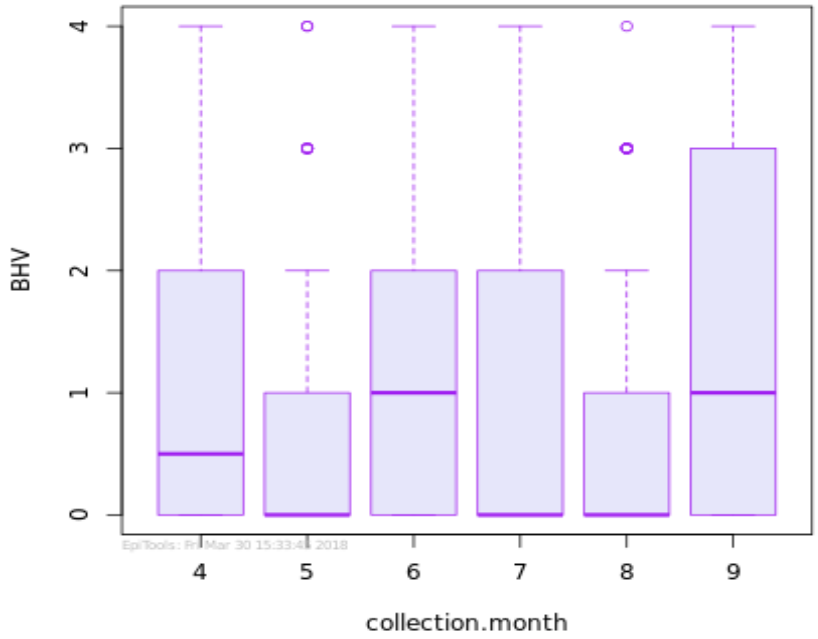

Figure 8.31 BoHV-1 seroprevalence in cattle by month of sampling

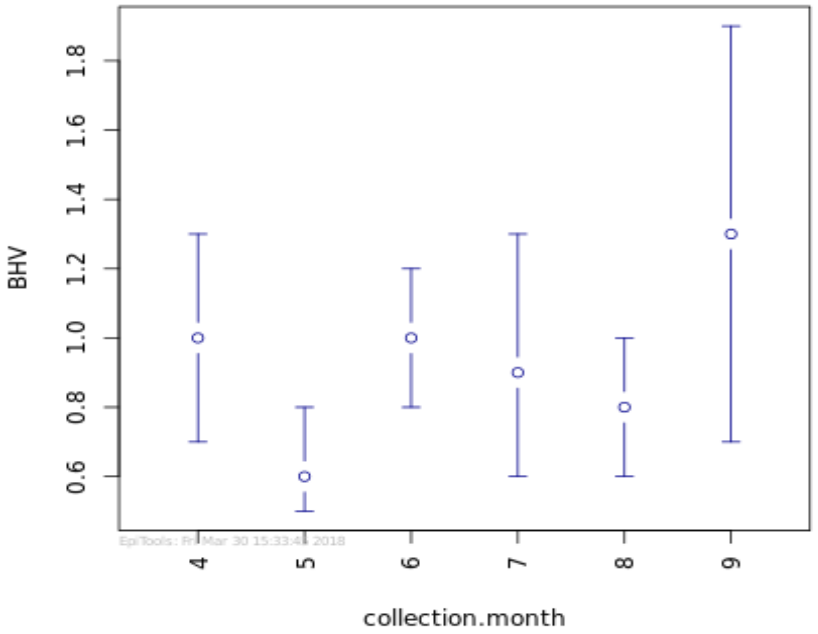

Figure 8.32 BoHV-1 mean antibody titre level in cattle by month of sampling

Table 8.35 BVDV statistical and numerical summary results by month Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	18.33
Degrees of freedom	5
P-value	0.0026

Time of samples collection (month)	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
April	0	0	0	0	2	4.3	5	1	1.5	1.5	0.6	1.4	54	0
May	0	0	0	0	0	4	5	0.5	1.2	2.4	0.3	0.8	108	0
June	0	0	0	0	1	3	5	0.7	1.1	1.8	0.4	0.9	84	0
July	0	0	0	0	2.8	5	5	1.2	1.7	1.4	0.8	1.7	58	0
August	0	0	0	0	0.8	3	5	0.6	1.2	2	0.3	0.8	98	0
September	0	0	0	1	3	5	5	1.5	2	1.3	0.7	2.4	21	0
All	**0**	**0**	**0**	**0**	**1**	**4**	**5**	**0.8**	**1.4**	**1.8**	**0.6**	**0.9**	**423**	**0**

Pathogen and degree of positivity	April	May	June	July	August	September	Sum
BVDV-0	32	86	58	35	73	10	**294**
BVDV-1	6	7	8	3	9	4	**37**
BVDV-2	7	7	11	5	7	1	**38**
BVDV-3	3	1	4	6	5	2	**21**
BVDV-4	3	3	2	5	2	0	**15**
BVDV-5	3	4	1	4	2	4	**18**
All	**54**	**108**	**84**	**58**	**98**	**21**	**423**

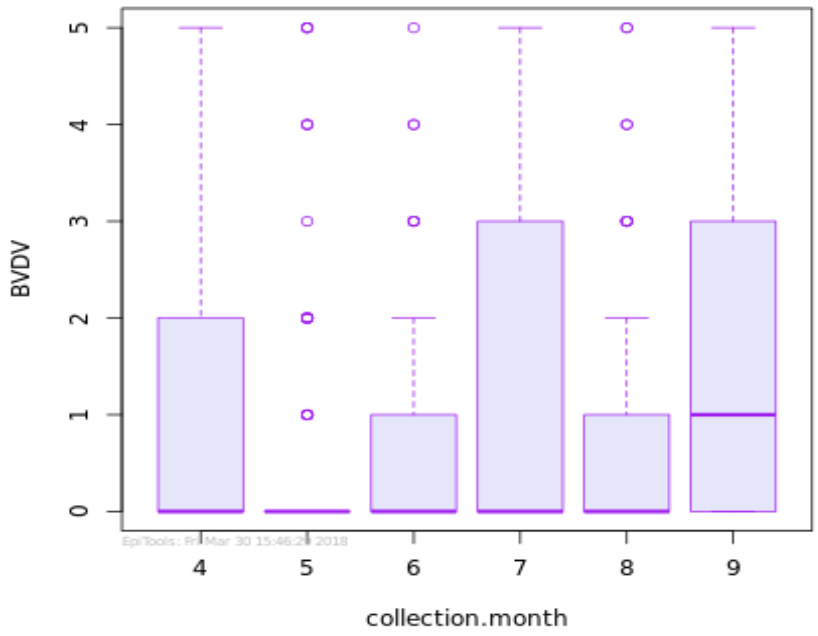

Figure 8.33 BVDV seroprevalence in cattle by month of sampling

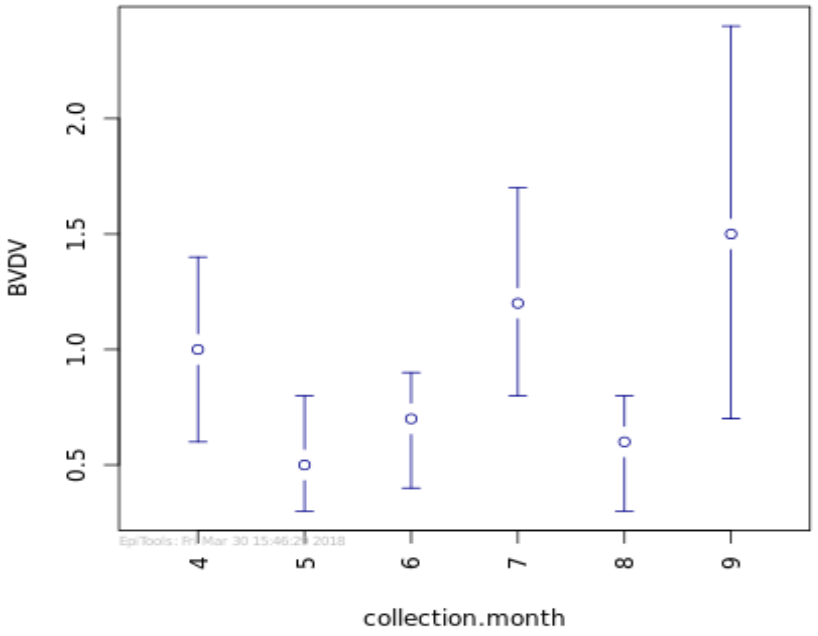

Figure 8.34 BVDV mean antibody titre level in cattle by month of sampling

Table 8.36 BRSV statistical and numerical summary results by month Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	11.77
Degrees of freedom	5
P-value	0.0381

Time of samples collection (month)	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
April	0	0	1	2	2.8	3	5	1.6	1.2	0.8	1.3	1.9	54	0
May	0	0	1	2	3	4	4	1.7	1.2	0.7	1.5	2	108	0
June	0	0	1	2	3	4	4	1.9	1.2	0.6	1.6	2.1	84	0
July	0	0	1	2	3	4	4	2	1.1	0.5	1.7	2.2	58	0
August	0	0	1	1	2	3	4	1.4	1	0.7	1.2	1.6	98	0
September	0	0	1	1	2	3	3	1.6	1	0.7	1.1	2	21	0
All	**0**	**0**	**1**	**2**	**3**	**4**	**5**	**1.7**	**1.2**	**0.7**	**1.6**	**1.8**	**423**	**0**

BRSV degree of positivity	April	May	June	July	August	September	Sum
BRSV-0	13	20	13	6	19	3	**74**
BRSV-1	13	30	21	11	37	8	**120**
BRSV-2	14	27	21	24	26	5	**117**
BRSV-3	12	21	23	13	15	5	**89**
BRSV-4	1	10	6	4	1	0	**22**
BRSV-5	1	0	0	0	0	0	**1**
All	**54**	**108**	**84**	**58**	**98**	**21**	**423**

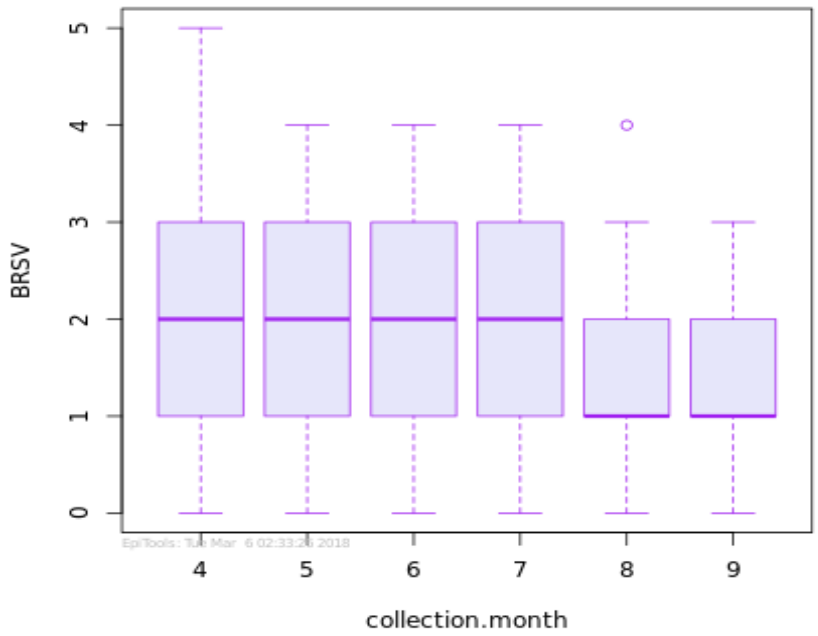

Figure 8.35 BRSV seroprevalence in cattle by month of sampling

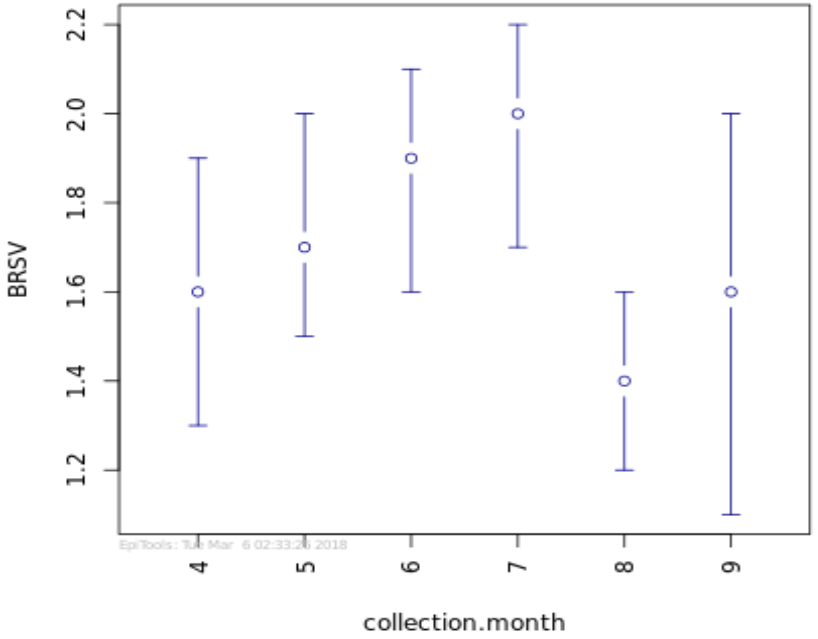

Figure 8.36 BRSV mean antibody titre level by month of sampling

Table 8.37 PI-3 statistical and numerical summary and frequency results by month Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	7.14
Degrees of freedom	5
P-value	0.2101

Time of samples collection (month)	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
April	0	0	0	0	1.8	3	5	0.9	1.2	1.3	0.6	1.2	54	0
May	0	0	0	0	1	3.6	5	0.8	1.3	1.7	0.5	1	108	0
June	0	0	0	0	2	3	5	1	1.2	1.3	0.7	1.2	84	0
July	0	0	0	0	2	4.1	5	1.1	1.5	1.3	0.8	1.5	58	0
August	0	0	0	0	1	3.1	5	0.8	1.2	1.5	0.6	1	98	0
September	0	0	0	2	2	3	4	1.4	1.4	1	0.8	2	21	0
All	**0**	**0**	**0**	**0**	**2**	**4**	**5**	**0.9**	**1.3**	**1.4**	**0.8**	**1**	**423**	**0**

PI-3 degree of positivity	April	May	June	July	August	September	Sum
PI-3-0	28	68	43	31	56	9	**235**
PI-3-1	12	18	17	7	23	1	**78**
PI-3-2	9	11	13	9	8	6	**56**
PI-3-3	3	5	7	6	6	4	**31**
PI-3-4	1	2	3	2	3	1	**12**
PI-3-5	1	4	1	3	2	0	**11**
All	**54**	**108**	**84**	**58**	**98**	**21**	**423**

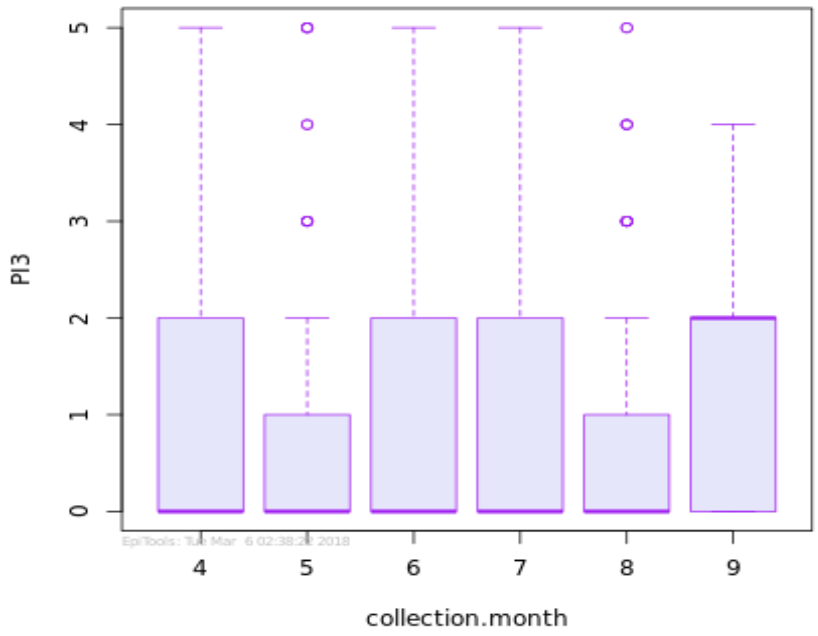

Figure 8.37 PI-3 seroprevalence in cattle by month of sampling

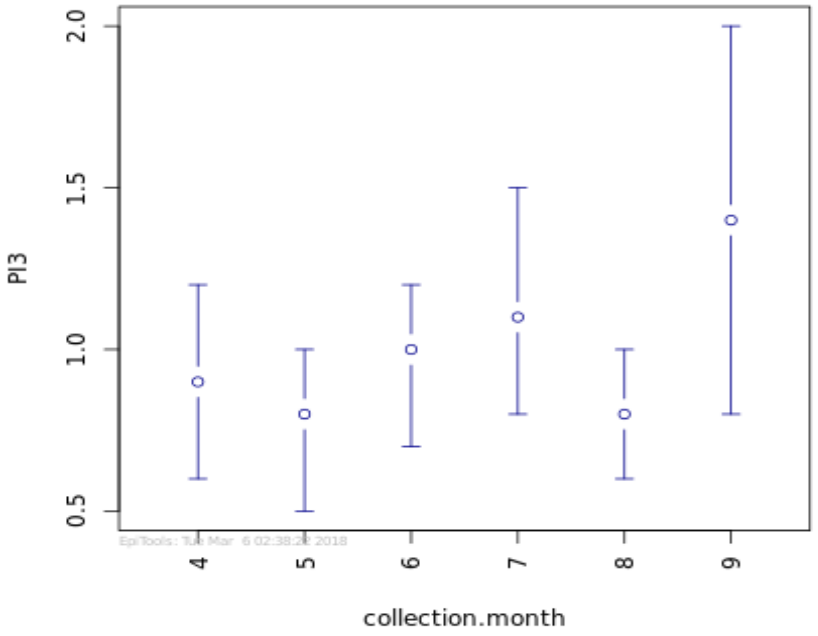

Figure 8.38 PI-3 mean antibody titre level by month of sampling

Table 8.38 BAV-3 statistical and numerical summary and frequency results by month Kruskal-Wallis rank sum test

Kruskal-Wallis rank sum	14.33
Degrees of freedom	5
P-value	0.0137

Time of samples collection (month)	Min	5%	25%	50%	75%	95%	Max	Mean	Std dev	CoV	Lower 95% CL	Upper 95% CL	Count	NA
April	0	0	1.2	2.5	4	5	5	2.6	1.7	0.7	2.2	3.1	54	0
May	0	0	1	1	3	5	5	1.8	1.5	0.8	1.5	2	108	0
June	0	0	1	2	4	5	5	2.2	1.7	0.8	1.9	2.6	84	0
July	0	0	1	2	3.8	5	5	2.3	1.5	0.6	1.9	2.7	58	0
August	0	0	1	2	3	5	5	2.1	1.6	0.8	1.8	2.4	98	0
September	0	1	2	2	4	5	5	2.7	1.5	0.5	2.1	3.3	21	0
All	**0**	**0**	**1**	**2**	**3.5**	**5**	**5**	**2.2**	**1.6**	**0.7**	**2**	**2.3**	**423**	**0**

BAV-3 degree of positivity	April	May	June	July	August	September	Sum
BAV-3-0	9	21	15	6	19	1	**71**
BAV-3-1	5	35	20	15	22	3	**100**
BAV-3-2	13	24	17	12	19	7	**92**
BAV-3-3	7	13	5	10	16	3	**54**
BAV-3-4	10	6	16	10	10	4	**56**
BAV-3-5	10	9	11	5	12	3	**50**
All	**54**	**108**	**84**	**58**	**98**	**21**	**423**

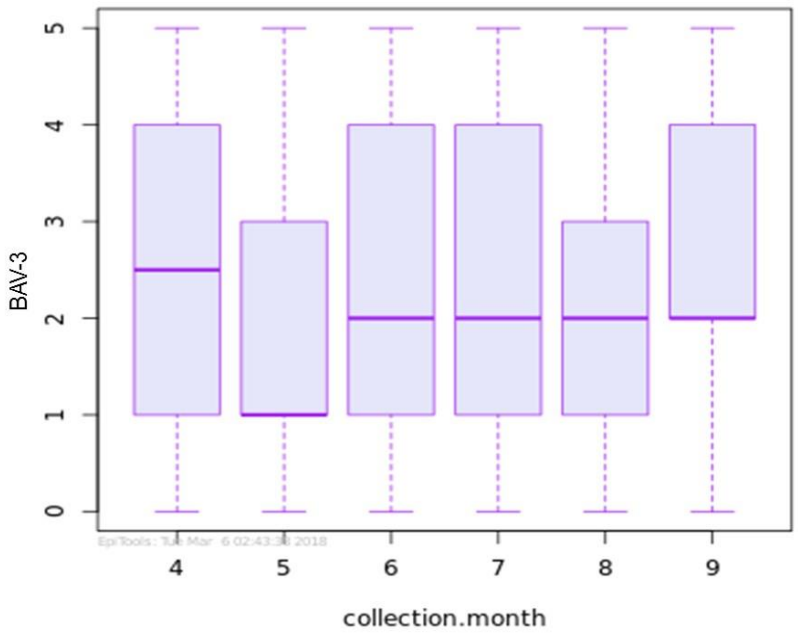

Figure 8.39 BAV-3 seroprevalence in cattle by month of sampling

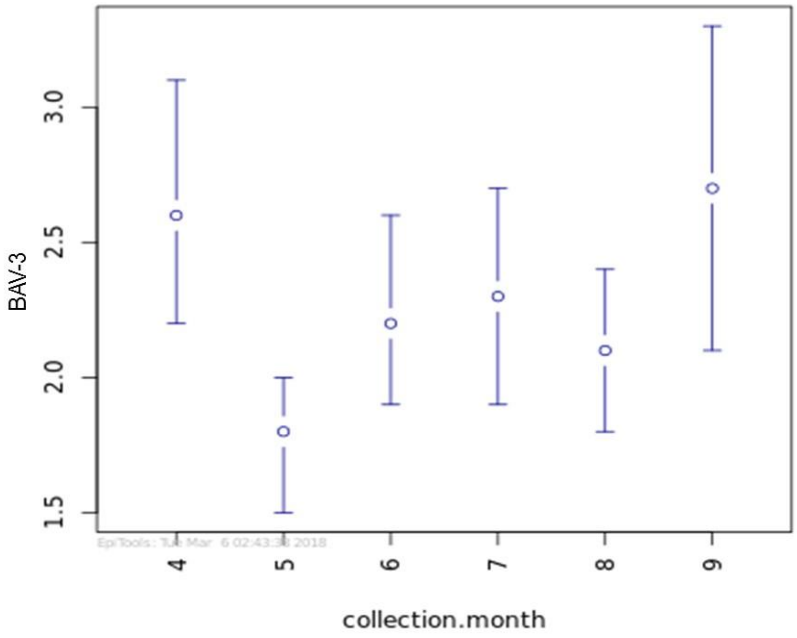

Figure 8.40 BAV-3 mean antibody titre level by month of sampling

9. Animal ethics committee approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Spatial serosurvey of respiratory tract viral infections in bovine cattle at the wildlife-livestock interface in the Mnisi farming community in South Africa**
PROJECT NUMBER	**V099-14 B**
RESEARCHER/PRINCIPAL INVESTIGATOR	**RN Athingo**

STUDENT NUMBER (where applicable)	**UP_14338158**
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

APPROVED	Date 14 June 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

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