# Evaluation of African horse sickness surveillance in the controlled area of South Africa

John Duncan Grewar

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Department of Production Animal Studies

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Supervisor: Prof. Peter N. Thompson

Co-supervisor: Dr. Thibaud Porphyre

## Author statement

I, John Duncan Grewar, declare that the thesis, which I hereby submit for the degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution

## Ethics statement

I, John Duncan Grewar, have obtained, for the research described in this work, the applicable research ethics approval. I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for researchers and the policy guidelines for responsible research.

## Acknowledgments

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My family has grown over the past three years! My beautiful wife Marli has not only supported me in the process but has given birth to two wonderful daughters, Aili and Lulu, and this has provided some perspective in my life. To the rest of my family, both blood and wine, and to each one of my friends who have asked: "*how are the studies going?"* I thank you – it's gone well.

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## List of Abbreviations

| | |
|---|---|
| AHS | African horse sickness |
| AHSV | African horse sickness virus |
| ALOP | Appropriate level of protection |
| ASF | African swine fever |
| CEM | Contagious equine metritis |
| CI | Confidence interval |
| Cq | Quantitation cycle |
| CSF | Classical swine fever |
| DAFF | Department of Agriculture, Forestry and Fisheries (South African Government) |
| DIVA | Differentiating infected from vaccinated animals |
| EC | European Commission |
| EDTA | Ethylenediaminetetraacetic acid |
| EE | Equine encephalosis |
| EEV | Equine encephalosis virus |
| EHF | Equine Health Fund |
| ELISA | Enzyme-linked immunosorbent assay |
| EU | European Union |
| EVA | Evaluation of surveillance components (see RiskSUR) |
| FFD | Freedom from disease |
| FMD | Foot and mouth disease |
| FVO | Food and Veterinary Office (see EU) |
| FZ | Free zone (AHS controlled area of South Africa) |
| FZSZ_CZ | That part of the AHS free zone and surveillance zone intersecting with the 2016 AHS outbreak containment zone |
| FZSZ_NonCZ | That part of the AHS free zone and surveillance zone not intersecting with the 2016 AHS outbreak containment zone |
| HHP | High health – high performance |
| HPAI | Highly pathogenic avian influenza |
| ID | Identification |

| | |
|---|---|
| i-ELISA | Indirect ELISA (see ELISA) |
| LAV | Live attenuated vaccine |
| NA | Not applicable |
| NPC | Non-profit company |
| OASIS | Outil d'analyse des systèmes de surveillance |
| OIE | World Organisation for Animal Health |
| OVR | Onderstepoort Veterinary Research (previously Onderstepoort Veterinary Institute) |
| PCR | Polymerase chain reaction |
| PI | Probability interval |
| POSC | Post-outbreak surveillance component |
| PRRS | Porcine reproductive and respiratory syndrome |
| PSC | Passive surveillance component |
| PZ | Protection zone (AHS controlled area of South Africa) |
| RiskSUR | Risk-based surveillance (EU funded project FP7) |
| RNA | Ribonucleic acid |
| RT-qPCR | Real-time quantitative PCR (see PCR) |
| RVF | Rift Valley Fever |
| SERVAL | SuRveillance EVALuation framework |
| SI | Swine influenza |
| SNV | Single-nucleotide variants |
| SSC | Sentinel surveillance component |
| SurF | Surveillance evaluation Framework |
| SZ | Surveillance zone (AHS controlled area of South Africa) |
| USD | Currency – United States dollar |
| VI | Virus isolation |
| WCDOA | Western Cape Department of Agriculture |

## Summary

**Evaluation of African horse sickness surveillance in the controlled area of South Africa**

by

John Duncan Grewar

Supervisor: Prof. P.N. Thompson (University of Pretoria)
Co-supervisor: Dr. T. Porphyre (University of Edinburgh)
Department: Production Animal Studies
Degree: Doctor of Philosophy

Surveillance is one of the core components of freedom from disease status declarations made by countries with regards to African horse sickness (AHS). This is especially true for South Africa which has a controlled area defined specifically for trade purposes. Three AHS surveillance activities are evaluated in this thesis: the surveillance during the 2016 Paarl AHS outbreak; the stand-alone freedom from disease survey undertaken in 2017; and a two-year surveillance sensitivity and probability of freedom analysis based on multiple surveillance components (passive surveillance, active sentinel surveillance and the 2017 survey) within three distinct zones in the AHS controlled area.

Outbreak surveillance in 2016 established affected population proportions and these results were included as within and between-herd estimates for evaluation of surveillance in the post-outbreak period. The stand-alone 2017 survey established that the point in time probability of freedom ranged between 73.1% and 100% in March 2017. Scenario tree analysis showed that, at a design prevalence of 1 animal in 1% of herds, the median posterior probability of freedom from AHS in the AHS controlled area after the 24-month post-outbreak period was between 98.3% - 99.8%. The final median probability of freedom had been realised by the 9th month after the 2016 outbreak had been resolved. The inclusion of active surveillance provided minimal additional confidence in surveillance outcomes.

Freedom from AHS was achieved fairly soon after the outbreak concluded in 2016 and this freedom was driven by the passive surveillance component. Surveillance challenges arise, in the South African context, as a result of high numbers of vaccinated animals within the population at risk, the seasonality of AHS and limitations of the DIVA (differentiating infected from vaccinated animals) capabilities of existing routine laboratory tests. Current global standards require a two-year post-incursion period of AHS freedom before re-evaluation of free zone status. Our findings show that the length of this period could be decreased if adequately sensitive surveillance is performed. In order to comply with international standards, active surveillance will remain a component of AHS surveillance in South Africa. Passive surveillance, however, can provide substantial evidence supporting AHS freedom status declarations, and further investment in this surveillance activity would be beneficial.

# Chapter 1

## Introduction

## Impact of African horse sickness on equine health and trade

African horse sickness (AHS) has had a substantial impact on the trade of live horses from South Africa. This is especially so where the final destination of these equids is a non-African country (Grewar, 2016; Sergeant, Grewar, Weyer, & Guthrie, 2016). The modernised protocol describing the requirements for direct live-equine trade between South Africa and the European Union came into force in January 1997 (EC, 1997). Between then and December 2018 South Africa has only been able to export live equids directly to the EU for only 44% of the time (Figure 1). The suspension of trade during this 22 year period has been directly as a result of AHS outbreaks in the controlled zones of South Africa and the resulting European-legislated two-year trade suspension (EC, 2010) in the post-outbreak periods. In recent years direct trade has to date not occurred between South Africa and the EU since the suspension of trade in 2011 as a result of the AHS outbreak near the town of Mamre in the Malmesbury district, approximately 50 km from Cape Town (Grewar et al., 2013). While outbreaks have occurred since 2011 (in 2014 and 2016) the ongoing suspension of trade has also been associated with the failure of South Africa to comply with the required controls to ensure the disease-free status, and particularly that of AHS, of horses due for export (EC, 2013). Figure 1 is an infographic showing the past 22-year trade period between South Africa and the EU and the periods of suspension referencing the outbreaks and decisions that have impacted trade.

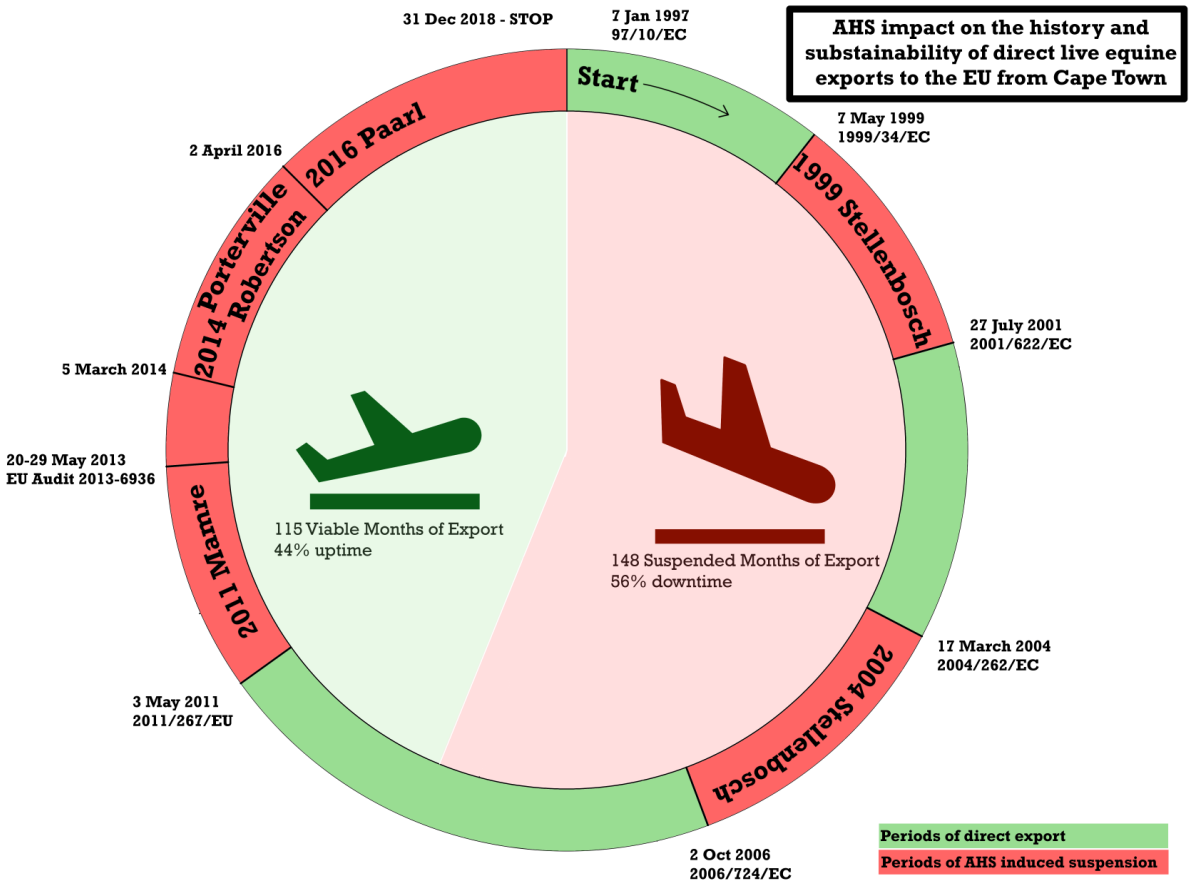


**Figure 1**: Infographic depicting the impact that African horse sickness (AHS) has made on the ability of South Africa to export horses directly to the European Union between January 1997 and December 2018. Year and location labels indicate outbreaks of AHS within the AHS controlled area of South Africa.

## General AHS surveillance

Surveillance for AHS plays a pivotal role in establishing the disease (or freedom thereof) status of equines within the South African AHS controlled area in the Western Cape Province. South Africa is in a unique situation where the freedom classification is in the context of a country where: 1) AHS is endemic in large parts of the country; 2) there is an AHS controlled area but the disease has occurred there; 3) control includes the vaccination of horses with a live attenuated vaccine and; 4) it has been shown that this vaccine has the potential to revert to virulence or to re-assort and result in outbreaks (Weyer et al., 2016). Surveillance, therefore, does not only have a very specific spatial resolution (individual animal in quarantine through to entire zonal freedom), but also a temporal resolution where surveillance is performed prior to, during and after the outbreak period. Inadequate surveillance activity, both prior to outbreaks and in the post-outbreak period, was highlighted in the major findings of

the audit that is responsible for the current suspended status of live equid trade to the EU (EC, 2013).

## Background to thesis

From a personal perspective, this thesis reflects the work that I have performed over the past ten years. My initial MSc work was on body temperature trends of Thoroughbred foals in the AHS surveillance zone. I then worked for 7 years as an epidemiologist for the Western Cape Department of Agriculture (WCDOA), during which major outbreaks of AHS occurred in 2011 and 2014 and when South Africa applied to the World Organisation for Animal Health (OIE) for official AHS free status. Finally, since 2016, I've worked for the industry based team tasked with establishing controls to account and improve the inadequate systems identified by the 2013 FVO (European Food and Veterinary Office) audit. My industry based role has been both technical and non-technical, with exposure to international trade protocol development and negotiation as well as full systems development and integration for all matters related to AHS control in South Africa. While mine has not been a particularly academic career I believe it has given me the unique opportunity to view the intricacies of a challenging disease like AHS from both a health impact and a trade perspective, and also it has given me exposure to the impact of AHS on the local, regional and global scale.

In 2016, two days after leaving the Western Cape Government and starting my industry based role, an outbreak of AHS occurred in the controlled zone of South Africa, near Paarl in the Western Cape. I was part of a team meeting with the South African Department of Agriculture Forestry and Fisheries (DAFF) reviewing the 2013 EU audit findings when the laboratory reported the first confirmed case. At the time we had the goal of requesting another audit from the EU as soon as two-years had elapsed since the 2014 AHS outbreak in the controlled area. Needless to say, the 2016 outbreak put paid to those plans. As outbreaks of controlled diseases often do, the 2016 AHS outbreak provided the impetus to develop integrated systems with regards to movement control (primarily through developing a permit system), horse farm and individual animal registry within the controlled area, dealing with the vaccination and permission to vaccinate against AHS and finally to integrate the sentinel surveillance

programme. One of the findings of the EU in 2013 was that the post-outbreak contingency was inadequate, and so in 2017, we undertook a stand-alone freedom from disease survey in the 2016 outbreak area to provide evidence of cessation of 2016 AHS virus circulation. The freedom from disease survey was undertaken with assistance from Dr Evan Sergeant, then working for AusVet (Pty) Ltd, and, after the sampling phase, I visited Evan in Australia to learn how to evaluate surveillance systems quantitatively. Their team have spent time on numerous projects developing the methodology for sample size and post-surveillance evaluation for freedom from disease surveys. During this visit, the components of this thesis began to take shape and the scope of the thesis was developed, initially with Evan's insight, and then formalised with Professor Peter Thompson, my primary supervisor.

The final goal of this thesis was to keep an applied outlook in mind at all times. South Africa is in a unique position where globally important diseases such as AHS, Rift Valley fever (RVF), highly pathogenic avian influenza (HPAI), African swine fever (ASF), foot and mouth disease (FMD), porcine reproductive and respiratory syndrome (PRRS) and classical swine fever (CSF) are prevalent or have sporadically occurred. As such, outbreak investigation, surveillance and surveillance evaluation are distinct processes that many local field epidemiologists have to deal with, and the outcomes of which are critical for both food safety and sustainable trade flows. I hope that the methodology that we have used here, which is not mathematically or programmatically complicated, can be replicated in other South African, and African, outbreak contexts and hopefully this will be a benefit in future.

## Scope of the thesis

The methodology for evaluation of surveillance programs is well described: the scope of this thesis is less methodology based and more the practical application of known methods in the South African context, with the added complexity of AHS being a vector-borne disease. The 2016 outbreak and its description forms the first primary chapter (Chapter 3), and does not have a specific focus on the surveillance component of the outbreak. To provide additional context of the outbreak to this thesis, surveillance-specific aspects of the outbreak are discussed in the final general discussion (Chapter 6). Outbreaks set the foundation of AHS surveillance in South

Africa's controlled area. Without them post-outbreak surveillance and required evaluation would not be necessary. Outbreaks further provide sampling frames and highlight surveillance constraints, such as a high proportion of vaccinated equines in the AHS controlled area or the seasonal aspect of AHS having real impacts on post-outbreak surveillance. These aspects are dealt with in the second primary chapter of the thesis (Chapter 4) where a stand-alone freedom from disease survey is described in the year following the 2016 Paarl AHS outbreak. This chapter's methodology of quantitatively evaluating an active surveillance event can be used in other South African contexts. For the same reason, the coding required to produce the manuscript outcomes is published in a reproducible manner in the annexes of this thesis (Annexures 9 and 10 for Chapters 4 and 5 respectively). The final primary chapter shows a stochastic model that was developed to establish the monthly posterior probability of freedom for AHS in the AHS controlled area for three separate surveillance components (Chapter 5). These components included passive surveillance, active monthly sentinel surveillance and a stand-alone freedom from disease survey during the two years following the 2016 outbreak. The outcomes established which surveillance programme provides what level of confidence of freedom, how soon does that confidence of freedom reach a trade resumption level (or at least a perceived trade acceptable level) and finally whether any of the current surveillance activities are contributing enough to the overall surveillance to warrant their inclusion given the expense of active surveillance.

The primary elements of AHS surveillance that are covered within this thesis can be summarised within the framework suggested by the OIE (Cameron et al., 2014) and are summarised in Table 1 below.

**Table 1**: World Organisation for Animal Health surveillance framework with relevant components focussed on in this thesis

| Framework component | Description | Notes relevant to this thesis |
|---|---|---|
| Objective | Promoting the re-establishment of trade post-AHS outbreak in the controlled area of South Africa | |
| Health event: case definitions | Confirmed AHS case as per the OIE case definition for infection with African horse sickness virus (OIE, 2016, para. 12.1.1) | |
| Surveillance activities | Outbreak surveillance: the surveillance for active circulation during an outbreak of AHS in the surveillance zone in 2016 | |
| | Passive surveillance: the ongoing surveillance within the AHS controlled area by horse owners and veterinarians detecting horses that are showing clinical signs that may be associated with AHS infection and where investigation takes place to an end point of testing for AHSV, primarily using PCR based techniques | Evaluation between July 2016 and June 2018 (2-year period) in monthly time-steps. |
| | Sentinel surveillance: the ongoing monthly sentinel surveillance within the AHS free and surveillance zone detecting AHS infection through the testing of approximately 150 horses using PCR and including 60 previously unvaccinated sero-sentinels using ELISA. Target animals are spatially proportionate to the underlying equine population in the target zones | |
| | Once-off post-outbreak freedom from AHS survey after the 2016 outbreak – limited to the outbreak containment zone | |
| Logistics | Outbreak surveillance: undertaken by passive and active means in the immediate area surrounding the index case of the 2016 outbreak. Purposive sampling based on location with increased sampling on AHS positive holdings | |
| | Passive surveillance: Scenario tree analysis based on final probability of freedom from AHS infection using expert opinion for branch probabilities where potentially infected horses are detected by owners/managers; these observations are reported to a veterinarian; the veterinarian investigates the situation with an end point of sampling potentially infected horses and having samples tested for AHSV | Monthly time-steps evaluated with posterior probability of freedom defined by probability of freedom based on surveillance activity and modulated by the probability of introduction during the month based on prior outbreak frequency. Analysis geographically orientated to three distinct areas within the AHS controlled area i.e. the AHS free and surveillance zone outside of the 2016 outbreak secondary containment zone; the AHS surveillance zone contained by the 2016 outbreak secondary containment zone and; the AHS protection zone |
| | Sentinel surveillance: monthly laboratory based testing data linked to individual sentinels and the holding they were sampled on | |
| | Once-off post-outbreak freedom from AHS survey | |
| Stakeholders, authority and responsibilities | The stakeholders are primarily the South African Equine industry, represented in the 2016-2018 period by the Equine Health Fund (Wits Health Consortium – University of the Witwatersrand) and the South African Equine Health and Protocols NPC; the South African Government represented in the AHS controlled area by the Western Cape Department of Agriculture (WCDOA - Veterinary Services) and in the country by the Department of Agriculture Forestry and Fisheries (DAFF - Animal Health). Equine veterinarians in the AHS controlled area play an integral role in surveillance, mainly in the passive surveillance components, and were important components of the expert option obtained for the scenario tree analysis of the passive surveillance programme | |
| Relevant regulations | AHS is a controlled disease in South Africa; the country is zoned by legislation (Animal Diseases Act (Act No.35, 1984)). Surveillance activity was initially formulated by the Western Cape Veterinary Services (WCDOA, 2012) and in July 2018 the surveillance plan for AHS was implemented into DAFF policy (DAFF, 2018) | |

| | | |
|---|---|---|
| Estimated costs | Outbreak surveillance: R 3.1 million | Extrapolated from costs published from 2011 AHS outbreak (Grewar et al., 2013) |
| | Passive surveillance: Unknown – estimates difficult since testing data specifically for this reason are not available | |
| | Sentinel surveillance: R 1.476 million per annum (Grewar et al., 2017) | |
| | Once off post-outbreak freedom from AHS survey: R210,000 – Chapter 4 | |

## Problem statement

AHS has a substantial impact on the trade of live equids from South Africa. Surveillance for the disease, particularly in the AHS controlled part of the country, forms part of the control measures that allow trade to occur. While extensive knowledge of the epidemiology of AHS is known, the evaluation of AHS surveillance has not, to date, been undertaken. In particular, the quantification of passive surveillance has not been defined, nor the relative benefit that active surveillance activities add to the overall probability of freedom from the disease. Global standards, set by the OIE and EU, restrict trade for at least a two year period after AHS incursions have occurred in a free area. The scientific justification of the length of this period is not evident. Quantification of the probability of freedom from AHS in the post-outbreak period and the length of time it takes to achieve a trade acceptable level will provide a basis for reviewing of this two-year restriction period.

## Final thoughts

The political component of trade cannot be overlooked at this juncture as non-EU countries are particularly cognisant of the status of the South African – EU trade status for live equids. Progress towards establishing new and re-opening previously established trade routes with non-EU countries, such as Hong Kong, South Korea and Singapore has been hampered by the lack of ongoing trade with the EU, and the EU-South African status acts as a pivot point in all non-African country trade of live equids.

While the politics of trade remains a constant uncertainty this project provides a basis for the technical compliance with specific components of the trade protocols, both in place and in development. As in clinical practice, the importance of evidence-based trade and disease control policy cannot be understated.

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# Chapter 2

## Literature review

## Introduction

This thesis is primarily comprised of published or ready to be published chapters and the pertinent literature reviewed for each chapter is included in the introduction, materials and methods and discussion components of the three primary chapters (Chapters 3-5). The primary aim of this project was to quantitatively evaluate AHS surveillance in the post-outbreak period in the AHS controlled area of South Africa. More specifically it was to establish the sensitivity of surveillance and the associated probability of freedom for both active and passive surveillance activities. Surveillance evaluation has been extensively reviewed, particular so in developing frameworks to evaluate surveillance programs. In this review pertinent components of these frameworks that have relevance and focus on similar analysis for AHS and for other arboviral diseases in the African context are highlighted. This literature review is not exhaustive, and the review is not focussed on all components of surveillance evaluation, but rather those about the matter at hand.

Freedom from disease surveillance is an important component of veterinary epidemiology. This component is particularly important outside of the academic, farm and/or outbreak level implementation of epidemiological methods as it is used by regional authorities and Governments to form the basis of risk assessments. Freedom from disease surveillance methods have influenced the global movement of horses from South Africa through formal protocols for trade between it and the European Union, a major target of trade for South Africa (EU, 2008).

Benefits of surveillance analysis can be broadly classified; applicable in this project's scope is where evaluation can assist in determining freedom from disease for trade purposes. Evaluation may also provide a basis for the economic and logistical justification of surveillance activities.

## African horse sickness

AHS is a globally important orbiviral disease that impacts trade but also has the potential to result in substantial loss of equine life and has serious economic consequences (Coetzer & Guthrie, 2004; Grewar, 2016b). This would account for the numerous and thorough reviews of the disease and its epidemiology (Coetzer &

Guthrie, 2004; Crafford et al., 2003; Gould, Higgs, Buckley, & Gritsun, 2006; MacLachlan & Guthrie, 2010; Thompson, Jess, & Murchie, 2012; Zientara, Weyer, & Lecollinet, 2015). Furthermore, AHS is one of currently six (and the only equine disease) where countries can apply for official OIE freedom for the disease (http://www.oie.int/animal-health-in-the-world/official-disease-status/). A dedicated chapter is included in both the OIE Terrestrial Animal Health Code and the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2016, 2019). In this focussed literature review those epidemiologic components of AHS that have a direct impact on the choices and decisions made during the surveillance activities associated with the disease in South Africa are considered. These surveillance activities take place during outbreaks and in the post-outbreak period. They include formal freedom from disease surveillance as well as passive surveillance activities.

### *Incubation and infectious period*

Incubation period is defined as the duration between infection of an individual by a pathogen and the clinical manifestation of the disease this pathogen causes (Merriam-Webster's collegiate dictionary, 2003). The incubation period for AHS is composed of two distinct periods: 1) an intrinsic incubation period within the equine host lasting between 2-21 days although generally considered to be less than 7 days (Sergeant, Grewar, Weyer, & Guthrie, 2016) and 2) a temperature-dependent extrinsic incubation period lasting between 7-10 days in the *Culicoides* vector (Lubroth, 1988; Mellor & Boorman, 1995). The extrinsic incubation period is more specifically the time taken from infection of the midge vector to when it becomes infective to the equine host, this after virus multiplication has taken place within the midge. This extended total incubation period has the potential to impact the surveillance activities undertaken during outbreaks; particularly if those outbreaks have a major sub-clinical component where the false-negative classification of affected animals may occur. The latter potentially occurs when repeat sampling of affected properties is not undertaken and a lack of clinical signs decreases the sensitivity of passive surveillance. Although it has been found experimentally that the detection of viral RNA can occur during the incubation period and prior to clinical manifestation of the infection (Quan, Lourens, MacLachlan, Gardner, & Guthrie, 2010), the use of the incubation period as a proxy for

the period between infection and detection has been used in developing testing protocols. For example, at least 14 days between AHS PCR testing was proposed by Sergeant et al. (2016) to ensure that serial testing would detect positive horses. Two post-vaccination (live attenuated vaccine (LAV)) studies performed in South Africa showed that the majority of first time AHS vaccinated horses that returned positive PCR results did so in the first 3 weeks after vaccination, with the majority in the second-week post-vaccination (Burger, 2016; Weyer, 2016).

The infectious period for AHSV infection is considered as 40 days by the OIE (OIE, 2019). AHSV RNA has, however, been detected in the post-infection period for up to between 97 - 130 days (Quan et al., 2010; Weyer et al., 2013) for clinically affected horses while subclinical or mildly clinical horses had detectable AHSV RNA for up to 40 days post-infection (Weyer et al., 2013). As Weyer et al. discuss, the detection of RNA does not imply the presence of viable AHSV, and experimentally AHSV has been isolated in the post-infection period for up to 21 days, albeit in previously naïve animals (Coetzer & Guthrie, 2004). In the post-vaccination period AHSV RNA has been detected using RT-qPCR for up to at least 12 weeks post-administration (Burger, 2016; Weyer, 2016). This impacts the ability to perform post-outbreak freedom from disease surveillance if DIVA (Differentiating Infected from Vaccinated Animals) tests are not available and vaccination is likely to take place in the area where such surveillance is considered. This is further discussed in Chapter 4.

### *Clinical signs associated with African horse sickness*

The clinical presentation of AHS infection has been well described, and signs are associated with circulatory and/or pulmonary dysfunction. Pathognomonic signs are however not evident. The section below is based on the review by Coetzer and Guthrie (2004).

The disease has been associated with four forms namely: 1) pulmonary; 2) cardiac; 3) mixed and 4) horse sickness fever. The pulmonary form is an acute form with a short term fever and severe pulmonary distress and results in a high case fatality rate. The frothy discharge from the nostrils seen most often *post mortem* is an image commonly used to depict AHS cases (see Figure 2). This clinical sign, associated with the

proteinaceous fluid transfer into the pulmonary system which, when mixed with air, forms a frothy foam-like material, is however not seen in every case of AHS, irrespective of the associated form.

**Figure 2**: Frothy nasal exudate typically seen in the pulmonary form of AHS. This photo was taken during the 2011 AHS outbreak in the Malmesbury district and is courtesy of Camilla Weyer

The cardiac form of the disease is characterised by subcutaneous oedema of primarily the head and neck and classically seen with swelling in the supra-orbital fossae (Figure 3).

**Figure 3**: Subcutaneous oedema, evident by swollen supra-orbital fossae, the only outwardly evident clinical sign (other than death) in an acute death in an AHS case in the 2011 outbreak in the Malmesbury district. In this case, no frothy nasal exudate was evident and this is an example is of the cardiac form of the disease. Photo was taken by the author.

The mixed form of the disease is a mixture between the pulmonary and cardiac forms of the disease and is likely the most prevailing form. It is however not specifically diagnosed as such since the clinical signs will progress from one form to the next in many cases. This form is evident at *post mortem* where evidence of both the cardiac and pulmonary form will be clear macro-pathologically. Horse sickness fever is a milder form of the disease and is evident where underlying protection, whether through prior vaccination or exposure, is present.

The basis of the passive surveillance component of AHS surveillance is the underlying probability that horses will show clinical signs of the disease. While it was long assumed that zebra and donkeys do not show overt signs of infection (Barnard, 1998; Coetzer & Guthrie, 2004) recent evidence has shown that domestic horses can also exhibit sub-clinical infection (Weyer et al., 2013). This has been shown to occur during outbreaks in the AHS controlled area where laboratory-based testing has detected cases which would otherwise have gone unnoticed (Table 2). Even though there are no pathognomonic clinical signs of disease, the passive surveillance for the disease has been pivotal in detecting outbreaks in the controlled area of South Africa, with all

outbreaks documented since 1997 having been detected through this surveillance mechanism.

### *Transmission*

AHS is a vector-borne disease, transmitted primarily by *Culicoides* spp. midges, with *C. imicola* and *C.bolitinos* considered proven vectors of the disease. Numerous other species have been identified during vector surveillance undertaken during outbreaks of AHS (Meiswinkel, Venter, & Nevill, 2004) and competent vectors of the disease are present in the AHS controlled area of South Africa. The vector component of the epidemiology of the disease has a direct impact on the incubation period as there is a within-midge cycle which results in an extrinsic incubation period (see *Incubation and infectious period* above). The surveillance of midges is recommended by the OIE as a component of overall AHS surveillance. The purpose, however, is not to detect the presence of circulating virus; in fact, this is not recommended as the prevalence of vector infection is very low and animals act as a much better option for this (OIE, 2016). Vector surveillance is aimed at either confirming the absence of vectors or establishing baseline levels of associated species and numbers to generate trends for what can be expected.

The vector-borne nature of the disease also impacts on the choice of disease surveillance strategy. The choice of a single or two-stage surveillance strategy relates to the potential for diseases to cluster, with clustered diseases best approached with a two-stage strategy where groups and then animals within groups are randomly selected (Cameron & Baldock, 1998). Vector-borne disease may still cluster within herds since distribution of vectors are related to the hosts they feed on. Aerial vectors, such as *Culicoides* midges, are not, however, generally affected by quarantine stand-still of animal movements on or off-farm. The local spread of infection is therefore unlikely to be hampered by on-farm control unless such control includes extensive vector control. This method of control has not been described in AHS outbreaks in South Africa. These parameters impacted the surveillance design as described in the active freedom from disease survey described in Chapter 4.

### *Case definition and diagnostic tests*

The case definition for a targeted disease is a common thread that runs through any evaluation of the prevalence/incidence components of the epidemiology of the disease and therefore plays a critical role in the surveillance for disease. The OIE recommends the use of a clear case definition for animal health surveillance and standardising a case definition improves the specificity and comparability of surveillance between surveillance components (Cameron et al., 2014). The internationally accepted *Infection with AHSV* case definition is published by the OIE (OIE, 2016). For the purpose of this thesis, cases of AHSV are defined by this standard which reads:

> *"1) AHSV has been isolated and identified from an equid or a product derived from that equid; or*
>
> *2) antigen or ribonucleic acid specific to AHSV has been identified in samples from an equid showing clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed case; or*
>
> *3) serological evidence of active infection with AHSV by detection of seroconversion with production of antibodies against structural or non-structural proteins of AHSV that are not a consequence of vaccination have been identified in an equid that either shows clinical signs consistent with AHS, or is epidemiologically linked to a suspected or confirmed case"*

Because of the widespread vaccination practised in South Africa and the current serological tests that preclude the ability to differentiate between infected and vaccinated animals, the third definition is unlikely to play a large role during AHS outbreak investigation. For the purposes of the serological sentinel surveillance undertaken in the AHS free and surveillance zone, it does, however, serve a purpose (Grewar & Weyer, 2016). The second OIE case definition option for confirming cases relates in the context of this project to the use of the RT-qPCR used commonly in South Africa (Guthrie et al., 2013). PCR testing has played an important role in establishing cases (including subclinical cases) in the outbreaks that have occurred in the AHS controlled area of South Africa since its development (Grewar et al., 2013, 2018). The PCR forms the bedrock of AHS investigation and this has primarily to do

with the fact that it is a test that has been recognised by the OIE to be a suitable method for establishing the prevalence of infection, confirmation of clinical cases and the individual animal freedom from infection prior to movement (OIE, 2019). While the OIE Terrestrial Manual indicates that the PCR may have limitations for population freedom from disease, the South African context, where widespread vaccination against AHS is undertaken, makes it the only practical test available.

## The South African context with regards to African horse sickness

### *The African horse sickness controlled area*

The AHS controlled area in South Africa is a legislated area defining the AHS free, surveillance and protection zones in relation to the rest of South Africa, the latter being considered an infected zone (Figure 4). This regionalisation was based on work done by Bosman et al. (Bosman, Brückner, & Faul, 1995) and was legislated into the Animal Disease Act (Act 35 of 1984) on 2 February 1997 by Government Notice R.254. Primary control associated with regionalisation consists of movement and vaccination control. In short: for movement to a zone of higher control a pre-movement health check by a veterinarian is required within 48 hours of movement. Prior vaccination against AHS must have occurred and more than 40 days before movement but within two years of the movement. The exception to this rule involves movements from the surveillance into the free zone where pre-movement vaccination is not required. This is due to vaccination rules within the country. By law, all horses are required to be annually vaccinated against AHS, except those within the AHS free and surveillance zones where vaccination is prohibited unless derogation from Veterinary services. For horses moving and originating in the AHS infected zone of the country a Government movement permit is required. Weyer et al. (2016) established that outbreaks of AHS in the controlled area have been generally linked to either a re-assortment or reversion to virulence of the live attenuated virus vaccine currently registered for use in South Africa. As a result of this work, seasonal permission to vaccinate was instituted (Grewar, 2015) where authorisation to vaccinate would only be considered in the low-vector period of the year.

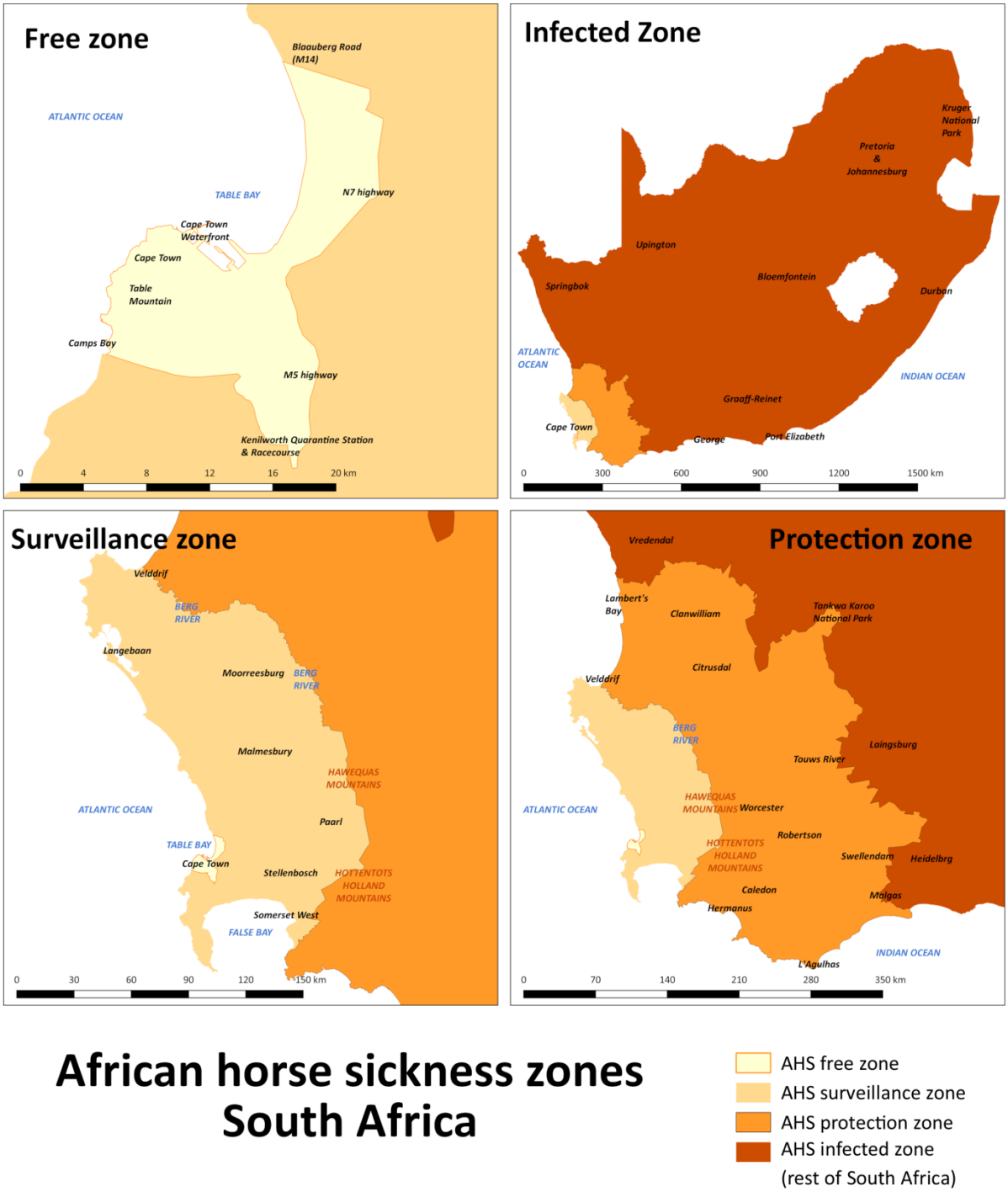


**Figure 4**: African horse sickness controlled zones in South Africa

### *Outbreaks in the controlled area*

Outbreaks of AHS have occurred over the past two decades, providing important information on the epidemiology of AHS, in both a general and controlled area-specific sense. Table 2 shows the underlying population at risk and the animal and herd level case totals for the confirmed outbreaks between 1997 and 2018. These data

have been critical for this project since they have provided information on the design prevalence for active surveillance activities as well as establishing subclinical probabilities which are used in the scenario tree evaluation in Chapter 5.

In addition, four cases of AHS were reported to the OIE by South Africa in 2013. These were located in the AHS surveillance zone (Melkbosstrand) but were considered atypical for AHS since it was believed to be due to the detection of residual vaccine RNA from the previous year's vaccination (Grewar, 2013). For the purposes of this thesis, the 2013 suspect cases are not considered further.

**Table 2**: Case totals (horse and herd) with underlying populations at risk of outbreaks in the African horse sickness controlled area between 1997 and 2018

| Outbreak | Cases | Sub-clinical cases | Positive herds | Population at risk | | |
|---|---|---|---|---|---|---|
| | | | | All horses | Horses in positive herds | All herds |
| 1999 Stellenbosch | 54 [1†] | - | 18 [2] | 485 [2] | 112 [‡] | 76 [‡] |
| 2004 Stellenbosch | 23 [3] | 0 [3] | 8 [3] | 4289 [4] | 201 [4] | 603 [5] |
| 2006 Robertson | 32 [6] | - | 8 [‡] | 844 [‡] | 774 [‡] | 26 [‡] |
| 2011 Mamre | 84 [3] | 15 [3] | 47 [3] | 447 [7] | 228 [‡] | 81 [‡] |
| 2014 Porterville | 89 [3] | 52 [3] | 31 [3] | 868 [8] | 250 [‡] | 118 [8] |
| 2014 Robertson | 22 [3] | 17 [3] | 8 [3] | 839 [8] | 680 [‡] | 25 [8] |
| 2016 Paarl | 21 [9] | 14 [9] | 8 [9] | 1817 [9] | 296 [9] | 118 [9] |

†The initial OIE reported case total was adjusted by the 2014 case-fatality rate since this outbreak case definition was primarily based on death
‡WCDOA – unpublished outbreak data
1. (Sergeant et al., 2016)
2. (OIE, 1999)
3. (Weyer et al., 2016)
4. (Sinclair, 2007)
5. (Sinclair, Bührmann, & Gummow, 2006)
6. (Bührmann & Hon, 2006)
7. (Grewar et al., 2013)
8. (Grewar, 2016a)
9. (Grewar et al., 2019)

The spatial distribution of outbreaks is shown in Figure 5 and the temporal and source components of the outbreaks are shown in Figure 6. Data sources for these figures are in Table 2 and this depiction was used for outbreaks prior to 2016 by Weyer et al. (2016). Outbreaks have been primarily associated with the AHS surveillance zone, with the Porterville 2014 outbreak intersecting the boundary between the protection and surveillance zone, and the two outbreaks in Robertson (2006 and 2014) occurring in the Protection zone exclusively. No cases to date have occurred within the AHS free zone since its inception.

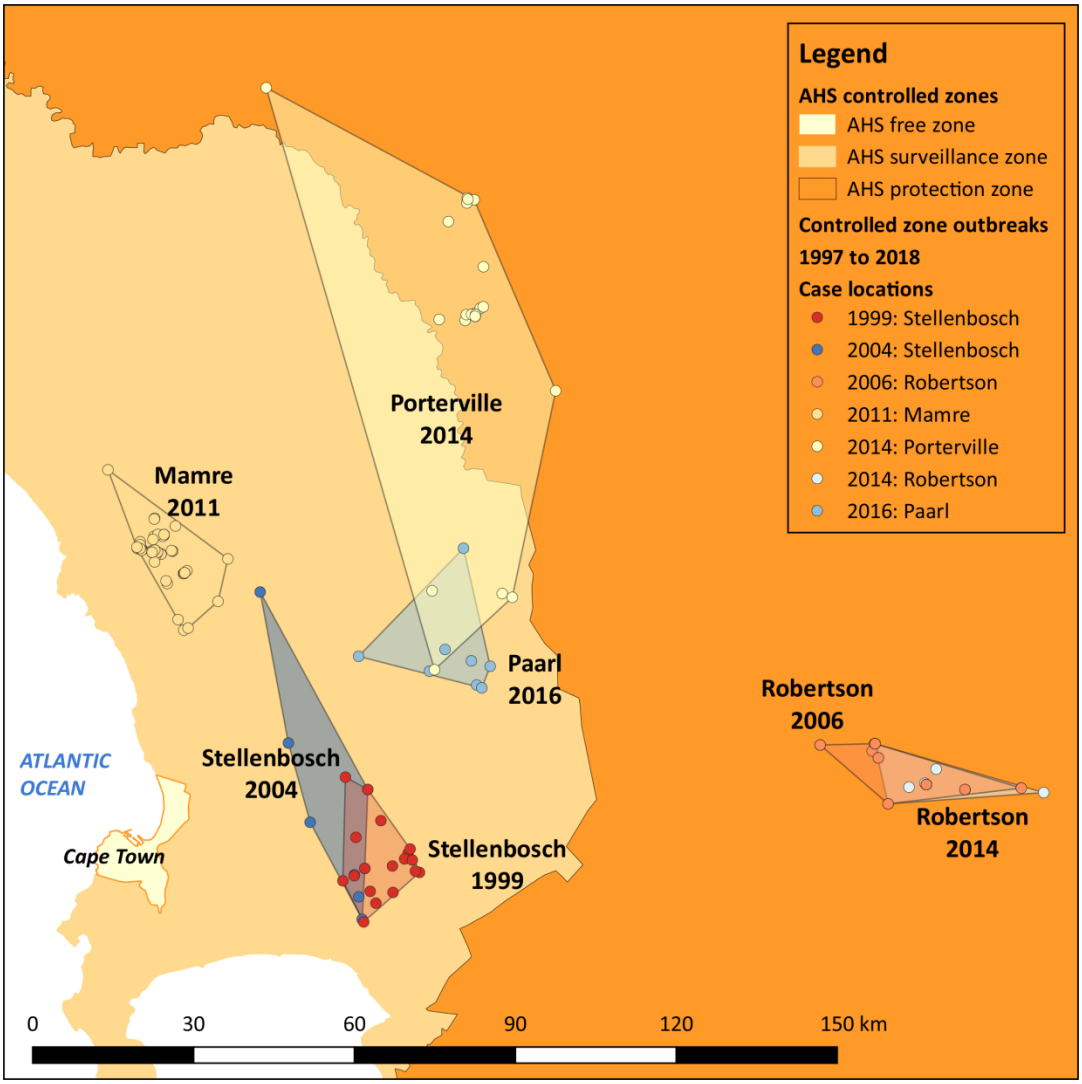


**Figure 5**: Spatial locations of African horse sickness outbreaks in the AHS controlled area of South Africa between 1997 and 2018 with convex hull geometries surrounding case locations for each outbreak.

The seasonal trends associated with controlled area cases have been predominantly in the late summer and autumn, and this is consistent with AHS cases in the rest of South Africa. Between 1992 and 2016 the majority of cases reported by Provincial Veterinary Services to the national Department of Agriculture, Forestry and Fisheries (DAFF) have been between February and May (Final 2016 African Horse Sickness Season Report (Amended), 2016). Outbreaks have lasted between 32 and 83 days, with an average outbreak period of 58 days and a median period of 57 days. Research identified the source of outbreaks (the majority of all outbreaks and all AHS-type 1 outbreaks) in the controlled area since 1997 have been as a result of re-assortment or reversion to virulence of the live attenuated vaccine in use in South Africa (Weyer et

al., 2016). The non-AHS type 1 outbreaks that have occurred (type 7 in 1999 and type 5 in 2006) were as a result of illegal movement of an infected animal from the AHS infected zone of South Africa.

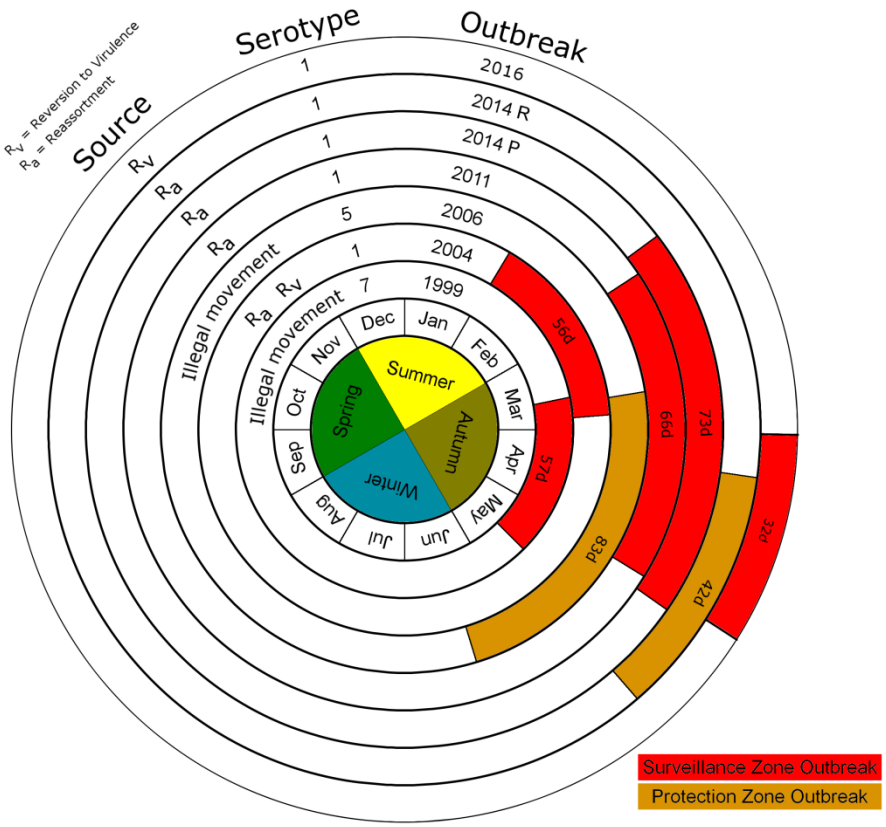


**Figure 6**: Temporal and source of African horse sickness outbreaks in the controlled zones of South Africa between 1997 and 2018. Red bands indicate outbreaks in the surveillance zone while gold bands indicate the two outbreaks that occurred in the AHS protection zone – in Robertson in 2006 and 2014. Note that 2014 P refers to the Porterville outbreak that year, while 2014 R refers to the Robertson outbreak that year

## Surveillance evaluation and analysis

Hoinville et al. (2013) describe surveillance as:

> *"The systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and -welfare data from defined populations. These data are essential for describing health-hazard occurrence and to contribute to the planning, implementation, and evaluation of risk-mitigation actions".*

They further note that:

> *"The term 'occurrence' is used here to mean the prevalence or incidence of health hazard, whether prevalence or incidence is appropriate will depend on the purpose of the surveillance."*

This comprehensive description implies that the evaluation of surveillance programs requires a framework upon which consistent/standardised measures and information can be reported. Four well described frameworks for animal health surveillance evaluation are the: 1) EVA (evaluation of surveillance components) tools within the RiskSUR (Risk-based animal health surveillance systems) project (Calba et al., 2013); 2) the published OIE guidelines for terrestrial animal health surveillance (Cameron et al., 2014); 3) SERVAL (SuRveillance EVALuation framework - Drewe et al., 2015) and the work carried out by Hoinville et al. over the years who developed proposed surveillance terms and concepts to standardise surveillance evaluation terminology (Hoinville et al., 2013); 4) the SurF framework (SURveillance evaluation Framework - Muellner et al., 2018) which is cross-sectorial and extends beyond just animal health. The OASIS framework (Hendrikx et al., 2011) is based on well-used frameworks but documentation regarding its detail could not found with, to the best of my knowledge, the online repository of documentation no longer maintained. In essence, one or more of the following four objectives form the target of surveillance activity: 1) the early detection of new or emerging diseases; 2) freedom from disease; 3) case detection and 4) monitoring of disease prevalence/incidence.

The analytical investigation into the sensitivity of surveillance and the final estimates of the probability of freedom, which is the primary focus of this work, forms just a part of surveillance evaluation. Evaluation explores the underlying context of the disease; surveillance planning relating to activities prior to, during and following actual data collection; resource (financial, personnel and equipment) availability and allocation prioritisation within the surveillance scope; and reporting on surveillance activities. In this work, the components of surveillance that have an impact on the analysis of results are discussed but the comprehensive evaluation of the entire system is not performed.

### *Quantification of surveillance activities*

The quantification of surveillance component results allows a standardised evaluation across varying surveillance activities such as passive, risk-based freedom from disease and random freedom from disease surveillance. Examples of the analysis of surveillance outcomes using techniques similar to those used in this project are widespread and vary between settings within the animal health context. Selected examples to illustrate this are shown in Table 3.

**Table 3**: Examples of surveillance analysis using techniques similar to those used in this project

| Disease and country | Primary goal | Notes | Reference |
|---|---|---|---|
| Bovine brucellosis in New Zealand | Establish adequate future options for surveillance in the freedom from disease setting | This publication was one of the first to evaluate surveillance programs incorporating varying test sensitivity and also based component sensitivity on the hypergeometric approximation rather than the binomial distribution. Already then in the discussion it was evident that the international legislation relating to recognition of freedom needed to be evaluated | (MacDiarmid, 1988) |
| Bovine Johne's disease in Australia | Value of historical and ongoing freedom from disease surveillance | This publication considers the time interval between separate activities and also incorporated stochastic scenario tree analysis into each surveillance component | (Martin, 2008) |
| Tuberculosis in farmed deer in Sweden | Establish freedom from disease likelihood after potential introduction and associated control | A stochastic scenario tree approach was used to establish system surveillance sensitivity to establish freedom from disease – includes discussion that this method is appropriate for passive surveillance sensitivity | (Wahlström et al., 2010) |
| Bluetongue in ruminants in Switzerland | Develop evidence based surveillance | A stochastic scenario tree modelling approach was used to establish a scientifically and economically justifiable surveillance programme for this vector-borne disease. The economic analysis used here also supports an effort for sustainable and relevant surveillance, something that is particularly important for resource stressed environments such as South Africa | (Hadorn, Racloz, Schwermer, & Stärk, 2009) |
| Viral haemorrhagic septicaemia in salmon in Norway | Evaluate routine clinical surveillance and establishing effectiveness of risk-based surveillance | A stochastic scenario tree approach was again used and a sensitivity analysis identified those probabilities that had the highest impact on the sensitivity and freedom from disease outcome of the surveillance. This is also an example of how proposed methods are used in a challenging surveillance environment such as that of aquatic animal disease | (Lyngstad et al., 2016) |
| PRRS in pigs in Sweden | Establish post-incursion freedom from disease | Surveillance components included outbreak based testing, routine abattoir surveillance and passive clinical surveillance | (Frössling, Ågren, Eliasson-Selling, & Lewerin, 2009) |

Included in Table 3 are examples where freedom from disease surveillance evaluation also establishes the resource allocation and justification thereof for different surveillance activities. Other examples of this include the evaluation of different abattoir based surveillance techniques and its impact on bovine tuberculosis freedom and the probability of freedom over time (Calvo-Artavia, Alban, & Nielsen, 2013). *Trichinella* surveillance evaluation in Denmark established that a risk-based process

could provide the same assurance of freedom while decreasing costs significantly (Alban, Boes, Kreiner, Petersen, & Willeberg, 2008).

### *Evaluation and analysis of South African freedom from disease surveys*

Requirements for the monitoring for controlled diseases are the basis for surveillance activity in South Africa. The South African Government set the requirements for the control and surveillance of controlled diseases and often industry stakeholders involved play a role in supporting (financially and logistically) these surveillance activities. Examples of these can be found in the surveillance required for the movement of buffalo in the country where FMD, brucellosis, tuberculosis and corridor disease are tested pre-movement in individual animals (DAFF, 2017). The ostrich industry has an extensive surveillance programme for avian influenza and Newcastle disease to prove disease freedom and thus promote trade (DAFF, 2012). In their case, however, the system is census-based with testing prior to and post-movement, pre-slaughter as well as defined six-monthly once-off surveillance within each commercial ostrich farm in South Africa. Animal health surveillance in the pig industry has occurred in South Africa over the past few years: these primarily due to outbreaks of exotic disease (FMD (Bruckner et al., 2002); PRRS (Oosthuizen, 2010) and CSF (Sandvik et al., 2005)) driving the need for countrywide freedom from disease surveillance (De Klerk, 2012).

The equine industry is primarily involved in disease freedom surveillance for two diseases: AHS (DAFF, 2018a) and contagious equine metritis (CEM – DAFF, 2018b). Surveillance for AHS includes pre-movement passive clinical surveillance (for movements into the AHS controlled area) as well as active surveillance in the AHS surveillance zone. The latter activity is structured to provide ongoing provision of freedom status to potential trade partners.

Freedom from disease surveillance programs in South Africa have been evaluated, although the publication of such results is sporadic. The only literature regarding the analysis of surveillance in a quantitative form that could be found was the evaluation of the porcine CSF, PRRS and swine influenza (SI) countrywide freedom from disease surveys as mentioned above (De Klerk, 2012). Zonal freedom for FMD was re-

established in February 2014 for South Africa, but it could not be ascertained whether the sensitivity of the surveillance undertaken was evaluated. Certainly a countrywide, risk-based surveillance programme was undertaken in a freedom from disease format in mid-2012 (Grewar, 2012).

To my knowledge, formal freedom from disease surveillance for AHS has only taken the form of the sentinel surveillance programme in the AHS controlled area of South Africa. Semi-scientific Government publications of the post 2014 freedom from disease investigations (after the Porterville and Robertson outbreaks) have been published by the WCDOA Veterinary Services (Grewar, 2016a). There is no publically available evidence that AHS specific freedom from disease surveys have been undertaken outside of South Africa, although the requirements of the OIE to officially declare countries or zones free from the disease would require that a description of the AHS surveillance system is in place (OIE, 2018, para. 5). It is likely that, while the application document for OIE freedom is not a matter of public knowledge, most countries (or zones within countries) that have received official freedom have made use of passive surveillance (listed by the OIE as clinical surveillance) which is often used as a methodology to detect exotic or emerging animal diseases (Cameron, Njeumi, Chibeu, & Martin, 2014).

### *Surveillance sensitivity and probability of freedom*

The underlying methodologies used in this project are described by Martin et al. (Martin, Cameron, & Greiner, 2007) and the research aims to apply these methods to the South African AHS surveillance system. Martin et al. recognised the need to make use of both structured random surveys (such as Chapter 4 in this thesis as well as the sentinel surveillance programme in the AHS surveillance zone) and non-random surveillance (such as the passive surveillance system) in order to substantiate freedom from disease using multiple sources of evidence.

The methodology is based on the principle of establishing unit and (where two-stage surveillance is used) cluster sensitivity of surveillance which is aggregated to establish overall component sensitivity and then merging the various components sensitivities into an overall system sensitivity. Using this system sensitivity the negative predictive

value for surveillance systems $P(D-|T-)$, i.e. the probability of freedom given a negative surveillance outcome, is established.

The methodology used in the post-outbreak evaluation is based on probability theorem and the primary components of this are based on three main premises:

1. The sensitivity of surveillance or testing in a population can be established given an underlying probability of infection and the number of units tested/surveyed
2. Bayes' theorem can be applied to the surveyed population to establish the probability of a truly negative surveillance activity given a negative surveillance result
3. When surveillance activities are performed in consecutive surveillance periods the prior probability of freedom at each period can be modulated by the previous periods' probability of freedom and the probability that disease was introduced between time-step evaluations

The analytical approach is described in detail in the methodology of Chapter 4 and 5 below but these three components are briefly explained here.

**1. Sensitivity based on surveillance**

Sensitivity ($Se$) of a test or system is the probability that a positive animal/herd/system will test positive if the disease is present – this is, therefore, a conditional probability

$$Se = P(test+|disease+)$$

**2. Bayes' theorem applied to establish the probability of a truly negative surveillance result**

Bayes' theorem applied to the testing of a surveyed unit is described in Figure 7 below. A unit surveyed (animal or herd) is either infected or uninfected with an underlying probability of infection ($P$). The ability to correctly detect that status is determined by the sensitivity ($Se$) and specificity ($Sp$) of the test that is used resulting in four permutations where results are either true (positive or negative) or false (positive or negative). The probability of freedom is defined by the probability that the disease is not present given a negative result – i.e. $P(D-|T-)$. Using Bayes' theorem, and

assuming that the specificity of surveillance is 100% - i.e. all positive results are followed up to their negative conclusion, the probability of freedom can be established based on the prior probability of freedom ($PriorPFree$) and the sensitivity of the surveillance test used using this equation:

$$PFree = \frac{PriorPFree}{1 - Se \times (1 - PriorPFree)}$$

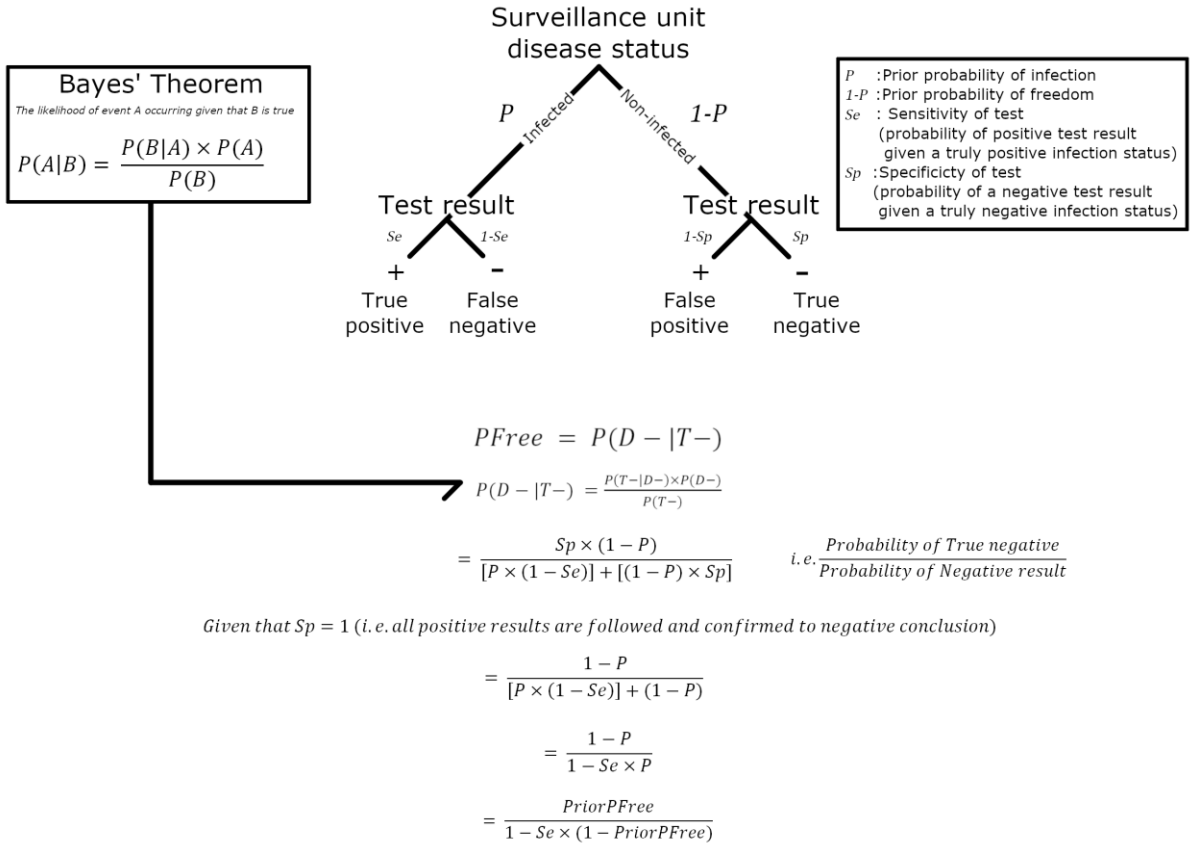


**Figure 7**: Infographic describing Bayes' theorem and its application in freedom from disease probability

### 3. Establish probability of freedom over consecutive surveillance periods

The probability of infection for any given surveillance period is defined by two probabilities – the probability of infection ($PInfection$) carried over from a prior surveillance period and the probability of introduction ($PIntro$) during the surveillance period. Because these events are not mutually exclusive the combination of these probabilities can be established using the sum rule of probabilities so that

$$PInfection_t = PInfection_{t-1} + PIntro_t - (PInfection_{t-1} \times PIntro_t)$$

where $t$ represents the surveillance time period. This can be reformatted focussing on the probability of freedom given that $PInfection = 1 - PFree$ to finally establish the equation which estimates the probability of freedom in consecutive surveillance periods:

$$PriorPFree_t = 1 - [1 - PFree_{t-1} + PIntro_t - ((1 - PFree_{t-1}) \times PIntro_t)]$$

### *Selection of a surveillance period*

In Chapter 5, a time-step of one month was chosen to represent the surveillance periods under review. The primary reason was that the sentinel surveillance programme is defined by monthly sampling events, so monthly time steps fit in perfectly with this surveillance component which is the primary active component required by South Africa's EU counterparts for trade purposes (EU, 2008). Cameron et al. (2014) review the decisions that should be taken when establishing which time period to select. In summary of that discussion, an increase in the time period will increase the sensitivity of the surveillance system since an increase in period correlates to an increase in the number of animals that are surveyed during the period. For the same reason decreasing the period decreases the sensitivity of the surveillance, but considers that historical information is less likely to be of surveillance value the further one moves from a surveillance event.

A monthly surveillance period is similar to what other authors have used for other viral diseases in freedom from disease evaluations/development (Frössling et al., 2009; Goutard et al., 2012; Hadorn et al., 2009; Lyngstad et al., 2016; Martin, Cameron, Barfod, Sergeant, & Greiner, 2007).

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# Chapter 3

## A field investigation of an African horse sickness outbreak in the controlled area of South Africa in 2016

J.D. Grewar[ab], C.T. Weyer[c], G.J. Venter[d], L.S. van Helden[e], P. Burger[b], A.J. Guthrie[c], P. Coetzee[c], K. Labuschagne[d], G. Bührmann[e], B.J. Parker[b], P.N. Thompson[a]

[a] Epidemiology Section, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

[b] Equine Health Fund, Wits Health Consortium, 8 Blackwood Avenue, Parktown, 2193, South Africa

[c] Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

[d] Epidemiology, Parasites and Vectors, Agricultural Research Council - Onderstepoort Veterinary Research, Private Bag X05, Onderstepoort, 0110, South Africa

[e] Western Cape Veterinary Services, Private Bag X01, Elsenburg, 7607, South Africa

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## Summary

An outbreak of African horse sickness (AHS) caused by AHS virus type 1 occurred within the South African AHS surveillance zone during April and May 2016. The index case was detected by a private veterinarian through passive surveillance. There were 21 cases in total, which is relatively low compared to case totals during prior AHS outbreaks in the same region (and of the same AHS virus type) in 2004, 2011 and 2014. The affected proportion of horses on affected properties was 0.07 (95% CI 0.04, 0.11). Weather conditions were conducive to high midge activity immediately prior to the outbreak but midge numbers decreased rapidly with the advent of winter. The outbreak was localised, with 18 of the 21 cases occurring within 8 km of the index property and the three remaining cases on two properties within 21 km of the index property, with direction of spread consistent with wind-borne dispersion of infected midges. Control measures included implementation of a containment zone with movement restrictions on equids. The outbreak was attributed to a reversion to virulence of a live attenuated vaccine used extensively in South Africa. Outbreaks in the AHS control zones have a major detrimental impact on the direct export of horses from South Africa, notably to the European Union.

## Keywords

African horse sickness type 1; Culicoides; Disease outbreak; Horses; South Africa

## Introduction

African horse sickness (AHS) is an arboviral disease of equines (primarily horses) caused by African horse sickness virus (AHSV), an orbivirus belonging to the Reoviridae family, transmitted by *Culicoides* midges (Diptera: Ceratopogonidae). AHS can cause severe morbidity and mortality in susceptible horse populations (Coetzer, & Guthrie, 2004). The export of live horses from South Africa has historically been hampered by AHS (Grewar, 2016) which is enzootic in the country, with differing levels of infection risk as defined by control zones (Bosman, Brückner, & Faul, 1995). The AHS controlled area, located in the most south-western region of the country, is legislated (Animal Diseases Act (Act No.35), 1984) and consists of an AHS free zone surrounded by a surveillance zone and a protection zone. The rest of South Africa is considered endemic.

Previous outbreaks of AHS in the AHS controlled area have been described (Grewar et al., 2013; Sinclair, Bührmann, & Gummow, 2006; Weyer et al., 2016), with recent evidence that the majority of these outbreaks have been due to AHS live attenuated vaccine strain reassortment and/or reversion to virulence (Weyer et al., 2016). We describe the environmental, host, vector and viral patterns and attributes of the recent outbreak of AHS which occurred in the AHS surveillance zone in the Western Cape Province in April and May 2016 as well as the control measures implemented. This information will contribute to the scientific knowledge of the epidemiology and the implementation of integrated control measures for this globally important disease.

## Materials and Methods

### *Initial events and control*

In April 2016 the Equine Research Centre, University of Pretoria, reported a confirmed positive result for AHS using a real-time reverse transcriptase PCR (RT-qPCR) test on a blood sample taken by a private veterinarian from a clinically ill horse on 2 April 2016 in the Paarl region of the Western Cape. A containment zone of 15 to 50 km radius around the index property was established the day after the outbreak was confirmed and was later reduced to a 15 to 25 km radius. All subsequent cases occurred within the revised containment zone which was dissolved on 13 June 2016, 40 days

after the last case on 4 May. All unauthorised movement of horses into, within, through and out of the containment zone was prohibited. Where movements were permitted measures were implemented to protect against the dissemination of AHS infection. These measures included: pre-movement AHSV RNA-based testing; vector protected stabling prior to or post-movement; pre-movement veterinary health examinations and same day movement with departure and return to the property of origin within daylight hours.

### *Case definitions*

The case definition used in this outbreak was based on the World Organisation for Animal Health (OIE) AHS case definition of an infected animal (OIE, 2016). For analysis, a differentiation was made between animals showing clinical signs of AHS or not (Table 4). Negative animals were categorised as laboratory tested for AHS or not. Properties were classified positive if at least one positive AHS case occurred on the property.

**Table 4**: African horse sickness (AHS) case definition categories used during the 2016 outbreak in the controlled area of South Africa, with associated totals of horses associated with each type.

| AHS Status | Code | Description | Total horses associated with each case definition type | Comment |
|---|---|---|---|---|
| Positive | P1 | Clinical and/or *post mortem* signs synonymous with AHS with a positive RT-qPCR and/or virus isolation result | 7 | |
| | P2 | Positive RT-qPCR and/or virus isolation result only | 14 | Subclinical cases |
| | P3 | Clinical and/or *post mortem* signs synonymous with AHS with no AHS positive laboratory confirmation but with epidemiological links to a confirmed case | 0 | None occurred during the outbreak but this forms an important part of the OIE AHS case definition |
| Negative | N1 | Clinical and/or *post mortem* signs synonymous with AHS with confirmation of another cause of disease AND with a negative RT-qPCR | 6 | |
| | N2 | Routine outbreak surveillance with negative RT-qPCR | 757 | |
| | N3 | Clinical surveillance with no reported and/or detected clinical signs synonymous with AHS | 1033 | |

### *Surveillance*

For best estimates of the animal and disease patterns in the outbreak, a 10 km zone around the index case was considered the outbreak epicentre where the most thorough data collection and sampling took place. In addition, sample-based surveillance took place within 5 km of all infected properties. When logistically

possible all horses were sampled from properties where sample-based surveillance was performed. Whole blood in EDTA was collected from live horses for AHSV detection by PCR. Samples from dead horses included lung and splenic tissue. Clinical surveillance on properties in the containment zone was through property visits by officials and owner and/or private veterinarian reporting of suspect clinical cases. Results of the surveillance zone's AHS existing sentinel surveillance programme were also considered.

Two Onderstepoort 220 V suction light traps were set up on the property neighbouring the index property the day after the outbreak was confirmed. This property had a population of 37 horses and a confirmed case of AHS occurred during the course of the outbreak. Midges were collected as previously described (Venter, Koekemoer, & Paweska, 2006). For the first nine weeks of the outbreak, trap catches were pooled on a 3-day basis (mean 3.35 days, range 2-5 days). After the containment zone restrictions were lifted, weekly collections were made. Collections were collated into batches of 200 midges and one batch per trap per sampling period was tested (n = 31) from batches collected during the first 2 months of the outbreak.

### *Laboratory testing*

All *Culicoides* batches, blood and organ samples were tested for AHSV using an RT-qPCR assay which targets the VP7 viral gene (Quan, Lourens, MacLachlan, Gardner, & Guthrie, 2010). This test has a median diagnostic sensitivity and specificity of 97.8% and 99.9% respectively (Guthrie et al., 2013). Positive samples were typed using a combination of three triplex RT-qPCR assays (Weyer et al., 2015). Virus isolation (VI) was performed on selected horse samples on baby hamster kidney cell culture as previously described (Quan, van Vuuren, Howell, Groenewald, & Guthrie, 2008). Genome sequencing and analysis of single-nucleotide variants (SNV) associated with the attenuation of the AHSV modified live vaccine was performed as previously described (Weyer et al., 2016). Serum samples sourced from the AHS sentinel surveillance programme were tested using an i-ELISA with a diagnostic specificity of 100% and sensitivity of 99.4%. (Maree, & Paweska, 2005). Negative to positive transitions are considered as seroconversions in the sentinel programme.

Light trap collections were individually analysed with Culicoides species identification performed using stereo microscopy. All count analysis was aggregated to daily catch totals of only female midges, with the proportion of species also only taking females into account.

### *Climate*

Weather data (maximum temperature ($^0$C), rainfall (mm), wind direction (arc degree) and wind speed (m/s)) were supplied by the Agricultural Research Council of South Africa from a weather station situated within 2 km south of the index property. The time resolution of the data was on an hourly basis and the daily maximum temperature was used as a proxy for general daily temperature.

### *Data capture and analysis*

Field data were either captured on paper forms or on Android-based cellular phones using an OpenDataKit platform (Borriello, 2011) with forms developed in XLSForms (www.xlsform.org). All information was collated on a centralised PostgreSQL (PostgreSQL Global Development Group - www.postgresql.org) database. All statistical analysis and graph preparation was performed using R (R Core Team, 2016) with the following packages: ggplot2 (Wickham, 2009), RPostgreSQL (Conway, Eddelbuettel, Nishiyama, Prayaga, & Tiffin, 2016), plyr (Wickham, 2011), dplyr (Wickham, & Francois, 2015), zoo (Zeileis, & Grothendieck, 2005) and scales (Wickham, 2016). Smooth conditional means for Culicoides count, rainfall and temperature data plotted for the outbreak period were established using local polynomial regression fitting. All map generation through the outbreak and for this report was performed using ArcGIS™ (ESRI®, Redlands, USA). Univariate analysis of associations between animal factors and AHS infection was performed using Fisher's exact test with $p \leq 0.05$ considered significant.

## Results

### *Clinical findings*

Fourteen (67%) of the 21 cases did not exhibit clinical signs associated with AHS and were classified as subclinical cases (Table 4). In the remaining seven cases the clinical signs consisted primarily of fever, anorexia and swollen supraorbital fossae. There

were four cases where death or euthanasia occurred. Individual case clinical presentation, detection, demographic, temporal and concurrent infection information is shown in supplementary table S1 (Annexure 1).

### *Disease, affected proportions, temporal and animal patterns*

Overall frequency data by vaccination status, breed, sex and colour are shown in Table 5. Non-tested, clinically negative horses were considered AHS negative during the outbreak and this may have resulted in an underestimation of the case totals given that subclinical AHS can occur (Weyer et al., 2013). To account for this, affected proportions were established for both overall and tested populations at risk.

**Table 5**: Proportional and frequency data for animal factors and properties during the 2016 African horse sickness outbreak in the controlled area of South Africa. Affected proportions (with 95% binomial exact confidence intervals) and p-values for associations are included where relevant.

| Factor | Category | Epicentre | | | | Overall containment zone | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | Positive | Proportion (95% CI) | p-value‡ | Total | Positive | Proportion (95% CI) | p-value‡ |
| Vaccination status | Vaccinated | 320 | 9 | 0.03 (0.01-0.05) | 0.04 | 1184 | 10 | 0.01 (0-0.02) | 0.10 |
| | Unvaccinated | 73 | 6 | 0.08 (0.03-0.17) | | 408 | 8 | 0.02 (0.01-0.04) | |
| | Unknown status | 155 | 3 | | | 225 | 3 | | |
| Breed† | American Saddlebred | 161 | 1 | 0.01 (0, 0.03) | 0.02 | 167 | 1 | 0.01 (0, 0.03) | 0.04 |
| | Arab | 7 | 1 | 0.14 (0, 0.58) | | 64 | 1 | 0.02 (0, 0.08) | |
| | Boerperd | 19 | 2 | 0.11 (0.01,0.33) | | 37 | 2 | 0.05 (0.01, 0.18) | |
| | Friesian | 20 | 0 | 0 (0, 0.17) | | 32 | 0 | 0 (0, 0.11) | |
| | SA Warmblood | 32 | 1 | 0.03 (0, 0.16) | | 70 | 1 | 0.01 (0, 0.08) | |
| | Thoroughbred | 123 | 2 | 0.02 (0, 0.06) | | 1070 | 5 | 0 (0, 0.01) | |
| | Cross/Other /Unknown | 186 | 11 | | | 377 | 11 | | |
| Sex | Male | 254 | 12 | 0.05 (0.02, 0.08) | 0.15 | 695 | 13 | 0.02 (0.01, 0.03) | 0.12 |
| | Female | 262 | 6 | 0.02 (0.01, 0.05) | | 917 | 8 | 0.01 (0, 0.02) | |
| | Unknown/Not Captured | 32 | 0 | | | 205 | 0 | | |
| Colour† | Bay | 201 | 8 | 0.04 (0.02, 0.07) | 0.16 | 774 | 11 | 0.01 (0.01, 0.03) | 0.25 |
| | Black | 39 | 0 | 0 (0, 0.09) | | 56 | 0 | 0 (0, 0.06) | |
| | Chestnut | 170 | 4 | 0.02 (0.01, 0.06) | | 385 | 4 | 0.01 (0, 0.03) | |
| | Grey | 64 | 5 | 0.08 (0.03, 0.17) | | 153 | 5 | 0.03 (0.01, 0.07) | |
| | Unknown/Other | 74 | 1 | | | 449 | 1 | | |
| Total horses | | 548 | 18 | 0.03 (0.02, 0.05) | | 1817 | 21 | 0.01 (0.01-0.02) | |
| Properties visited | | 48 | 6 | 0.13 (0.05,0.25) | | 118 | 8 | 0.07 (0.03,0.13) | |

†only breeds/colours with at least 30 per category in total were included as separate categories
‡Fisher's exact p; calculations only performed on known factor classifications; unknown/other categories excluded
CI: confidence interval

Seven of the 21 cases and six of the eight positive properties were detected through clinical surveillance by private veterinarians and owners during the outbreak. The remaining 14 cases were detected through active surveillance in response to the outbreak.

An average of 10 horses (median = 3) were sampled per property and in total 784 unique horses from 81 properties were tested. All AHS cases occurred within 5 weeks of the index case (Figure 8). The overall crude AHS affected proportion within the horse population was 0.01 (21/1817; 95% CI: 0.01, 0.02). For laboratory tested horses it was 0.03 (21/784; 95% CI: 0.02, 0.04) overall, 0.05 (18/381; 95% CI: 0.03, 0.07) within the outbreak epicentre and 0.07 (21/296; 95% CI: 0.04, 0.10) on the eight positive properties. Subclinical cases were detected only from late April to early May 2016. The crude property-level AHS affected proportion was 0.10 (8/81; 95% CI 0.04, 0.19) for properties where at least one horse was laboratory tested, while for epicentre properties the crude property-level AHS affected proportion was 0.14 (6/48; 95% CI: 0.05, 0.29).

There was a univariate association between vaccination status and AHS infection in the outbreak epicentre ($p = 0.04$) although this association was not evident in the containment zone as a whole. A univariate association of breed with AHS was also observed, both in the outbreak epicentre and the containment zone as a whole (Table 2). No association could be established between AHS infection and sex or colour (Table 2). Affected horse proportions are shown in supplementary figure S1 (Annexure 2) for the eight affected properties based on laboratory-tested horses. Positive AHS VI results were obtained from four cases on three different properties and were all typed as AHSV type 1.

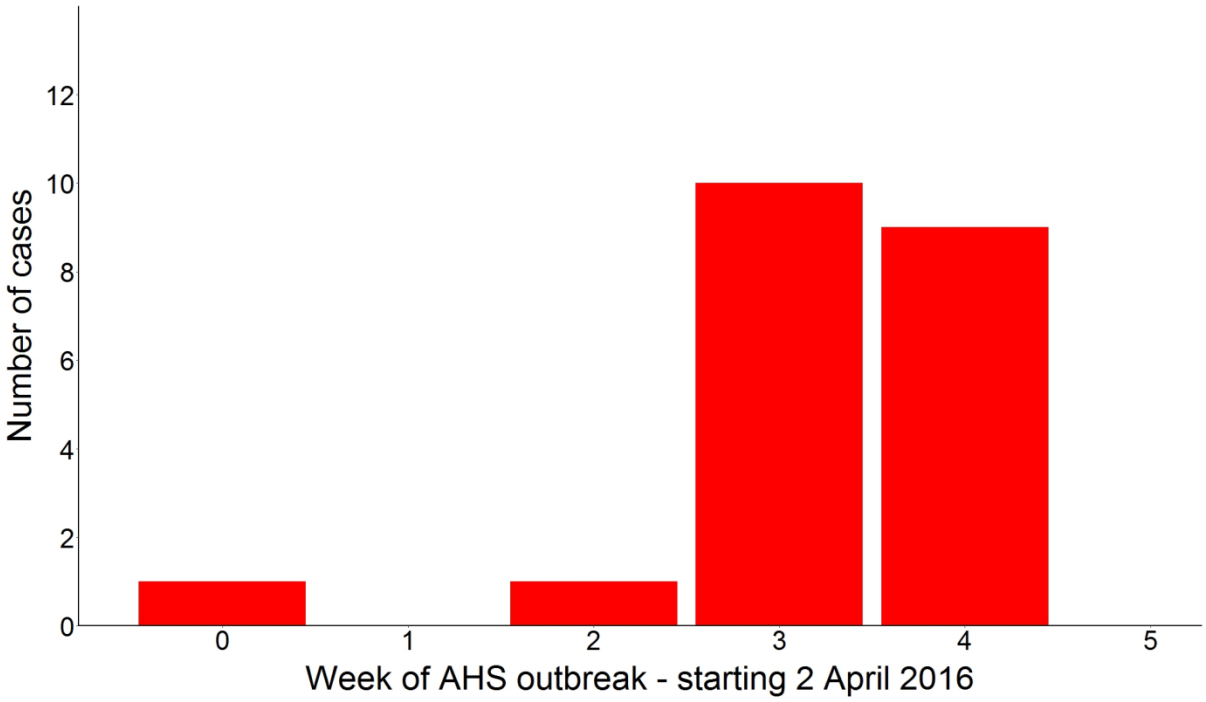


**Figure 8**: The epidemic curve of the 2016 African horse sickness (AHS) outbreak in the controlled area of South Africa. Week zero indicates the first week of the outbreak starting 2 April 2016.

### *Spatial considerations*

Six of the eight AHS infected properties were within 10 km of the index property, which, along with a neighbouring property that later became infected, was the most south-eastern affected property (Figure 9).

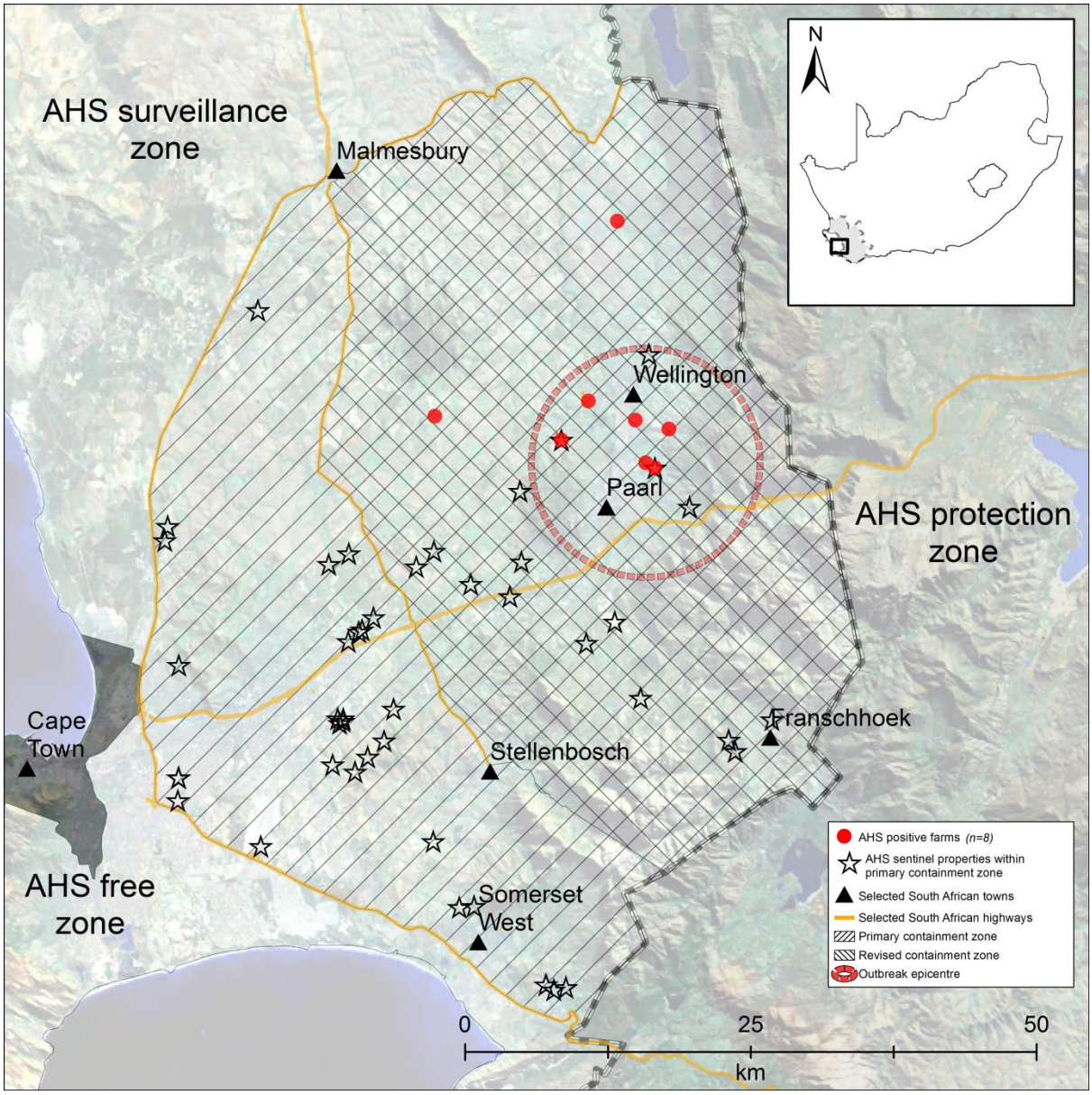


**Figure 9**: A map of the 2016 African horse sickness (AHS) outbreak that occurred in South Africa's controlled area depicting the positive AHS properties as well as the sentinel properties within the outbreak containment zone. The epicentre was defined as a 10 km radius around the index property. Major roadways associated with the outbreak are depicted and the primary and revised containment zones are also shown.

### *Vector and Climate*

The outbreak occurred in mid-autumn in a winter rainfall area of South Africa where the April and May mean long term temperature ranges between 11.1$^0$C - 22.8$^0$C (Schulze et al., 2008). The summer (approximately October to March) in the Western Cape, and South Africa in general, during 2015/2016 was hot and dry because of the El Niño influence of 2015 which extended into 2016. There were consistently high daily temperatures with maximums above 30$^0$C until a rainy period towards the latter part

of the outbreak in late April (Figure 9). This wet period was immediately followed by a spike in temperature with a parallel increase in vector abundance.

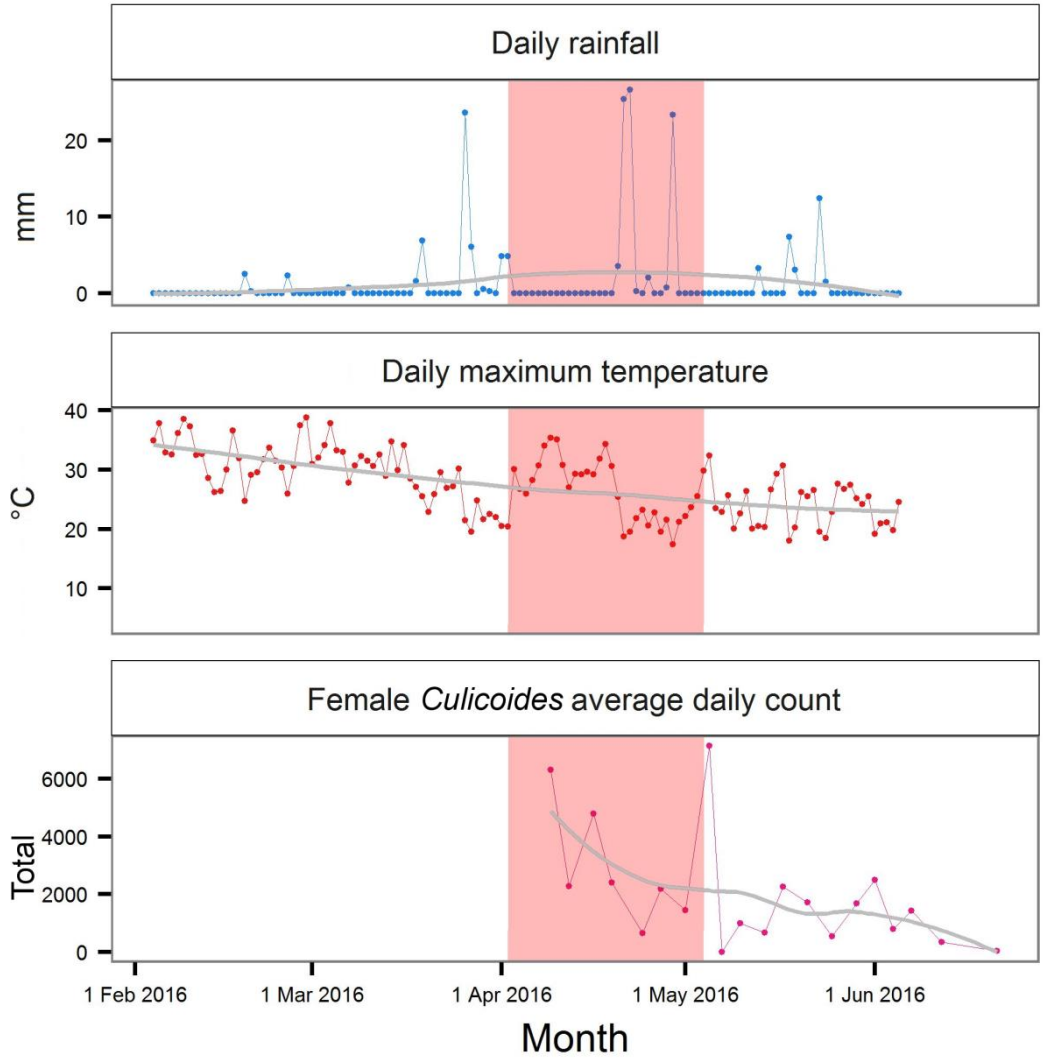


**Figure 10**: Environmental and vector parameters measured prior to, during (shaded pink band) and after the 2016 African horse sickness outbreak period in the controlled area of South Africa (outbreak period considered between first and last detected case date). Midge data is the average catch allocated to the respective median day of the catch period for each sampling event. Midge trapping took place on one of the outbreak positive properties which neighboured the index property. Environmental data was obtained from the closest weather station (within 2 km) to the index property.

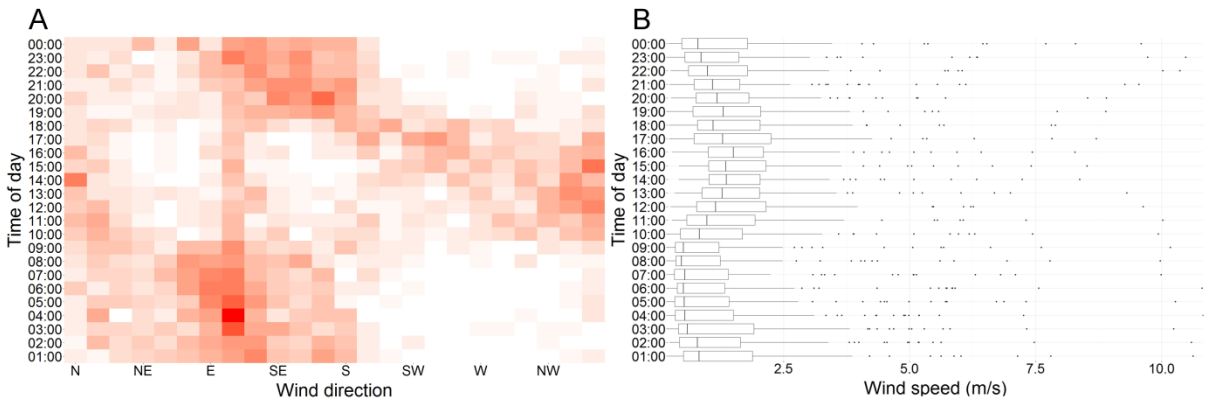


**Figure 11**: Wind direction (A) and speed (B) as measured between 1 March 2016 and 1 June 2016 by the closest weather station (within 2 km) to the index property of the 2016 African horse sickness outbreak in the controlled area of South Africa. Time of day (hourly classification) is represented on the Y-axis with wind direction (in $15^0$ increments and labelled based on the points of the compass) and wind speed (m/s) represented on the x-axis in A and B respectively. The colour range from white to dark red in (A) indicates the amount of time, for that time of day and wind direction, that wind was present with white being seldom present and red indicating often present, all relative to other time/direction combinations. N: north; E: east; S: south; W: west; NE: north-east; SE: south-east; SW: south-west; NW: north-west

Wind patterns present in the outbreak epicentre immediately prior to and during the outbreak are depicted in Figure 11. A diurnal pattern of wind direction was present (Figure 11A), with the majority of wind occurring from the south and south-east between 19:00 and 09:00 and more of a north/north-westerly origin during the daylight hours. Wind speed was highest generally in mid to late afternoon with wind speed tailing off in the evening and during the night (Figure 11B).

The proportional breakdown of *Culicoides* species detected during the outbreak is shown in Table 6. In total 17 species were identified, although >95% were *Culicoides imicola*. The majority (>90%) of the midges collected consisted of nulliparous and parous females. One midge pool, collected over three nights from 11 to 14 April, tested inconclusive for the presence of AHSV RNA (Cq value of 38.05 where the positive cut-off is 37).

**Table 6**: The total and proportion of female *Culicoides* species collected during and immediately after the 2016 African horse sickness outbreak in the controlled area of South Africa. Species that represented less than 0.5 % (n=12) of the total have been grouped.

| ***Culicoides* species** | **Total collected** | **Proportion of total collected** |
|---|---|---|
| *C. imicola* | 244881 | 95.5% |
| *C. subschultzei* | 4159 | 1.6% |
| *C. bolitinos* | 1659 | 0.65% |
| *C. zuluensis* | 1580 | 0.62% |
| *C. nivosus* | 1456 | 0.57% |
| Other species (n = 12) | 2585 | 1.06% |
| Total | 256320 | 100% |

## *Movement control*

A total of 323 movement events involving 903 horses occurred into, out of, through or within the outbreak containment zone. The movement direction and various control measures are summarised in Table 7. The majority of horses moving out of the AHS containment area (309/513; 60%) were bound for destinations in the AHS infected zone of South Africa while the majority of horses moving into the containment zone (129/232; 56%) originated from within the AHS controlled area.

**Table 7**: Counts of movement permits (and counts of associated horses indicated in brackets) issued during the 2016 African horse sickness (AHS) outbreak in the controlled area of South Africa and associated with the outbreak containment zone. Risk mitigation categories and movement direction are used as classifications.

| **Direction of movement (relative to containment zone)** | **Total permits (total horses)** | **Emergency permits** | **Pre-movement AHS testing** | **Same day return** | **Vector protected stabling** | | |
|---|---|---|---|---|---|---|---|
| | | | | | **Pre-move only** | **Post-move only** | **Pre- and post-move** |
| Out of | 134 (513) | 2 (2) | 40 (101) | 38 (125) | 51 (146) | 4 (11) | 0 |
| Into | 97 (232) | 23 (23) | 46(166) | 4 (22) | 8 (33) | 14 (15) | 0 |
| Within | 87 (146) | 21 (24) | 31 (45) | 8 (17) | 3 (4) | 5 (7) | 0 |
| Through | 5 (12) | 0 | 2(7) | 0 | 0 | 0 | 3 (5) |
| Total | 323 (903) | 46 (49) | 119 (319) | 50 (164) | 62 (183) | 23 (33) | 3 (5) |

### *Sentinel findings*

During the outbreak, the AHS sentinel surveillance programme included nine serological and 55 viral RNA evaluations from within the epicentre, with samples derived from 16 horses from four sentinel properties. Two of these properties had at least one case of AHS during the outbreak: one had two sentinels which were confirmed AHS cases (out of 10 cases on the property) while the other had a non-sentinel AHS case. All sentinel cases were detected through PCR testing.

### *Source of the outbreak*

The 2016 Paarl outbreak was shown to be due to a reversion to virulence of the AHSV type 1 strain of the live attenuated vaccine (Guthrie, A.J. Unpublished results). This was based on the comparison of the outbreak viral genome nonsynonymous SNV's to those associated with the original attenuated virus used in the modified live vaccine. An evaluation of the movements into the controlled area from the AHS infected zone did not detect any high-risk movements that could be associated with the outbreak.

## Discussion

The outbreak occurred in April and May 2016 which is consistent with previous AHS outbreaks in the same region since 1999. The case total, overall affected proportion and case fatality proportion for this outbreak compared to previous outbreaks was low, with a comparatively high subclinical proportion (Grewar et al., 2013; Weyer et al., 2016). One of the limitations of the disease proportions established during this outbreak was that case clustering is likely to have occurred on affected properties. To account for this, affected proportions were established for individual properties, the outbreak epicentre and the outbreak containment zone as a whole. The epidemic curve of the outbreak suggests that the index case could have been the primary case, or closely associated with it, given the time period between it and the next reported case, which was followed closely by the bulk of the cases (Figure 8). Herd immunity in the outbreak area is likely to have played a role in preventing further propagation of the outbreak, with 81.4% of horses censused in the containment zone having been previously vaccinated against AHSV.

The association between breed and AHS infection should be viewed with caution, since 10 cases were in mixed breed horses and infection occurred on only eight properties, so clustering potentially played a role in this association. Different breeds may also have varying herd-level vaccination coverage given differences in their use. Breed as a risk factor for AHS has, to the best of our knowledge, not been reported and it is assumed that all breeds are susceptible (Coetzer, & Guthrie, 2004).

There is a relatively long incubation period for AHS given that both an intrinsic (2-21 days) and a temperature dependent extrinsic cycle (7-10 days) need to occur within the mammal host and midge respectively (Mellor, & Boorman, 1995). This will influence the estimates made of the various disease affected proportions during an AHS outbreak with a high percentage of subclinical cases, as negative testing may be followed by a subclinical infection.

Local weather conditions will influence *Culicoides* abundance, and while we have considered temperature and rainfall data two months prior to the outbreak (Figure 9), abundance would have been influenced by climatic conditions preceding this. On a local scale the climatic conditions of high temperature with a spike of rainfall, which was evident both prior to and towards the end of the outbreak, would have promoted increasing vector populations (Meiswinkel, Venter, & Nevill, 2004). The south-easterly wind pattern during this outbreak and general case distribution in a north-westerly direction from the index property makes it plausible that local spread of virus was through the wind dispersal of infected midges. However, the topography of the outbreak area cannot be ignored, since it occurred in a well-defined valley bordered by mountains on its eastern/south-eastern and western edges. This influences horse population distribution, so vector dispersal associated spread of infection could initially only have taken place in a general northerly or southerly direction.

The dominant species of midge collected during the outbreak was *C. imicola*, a species considered a proven vector of AHSV (Meiswinkel et al., 2004). The testing of selected midge pools from the first two months of the outbreak did not result in the detection of AHSV. This is consistent with results found during the 1999 and 2004 AHS

outbreaks in Stellenbosch, approximately 30 km south-west of this outbreak, and indicates very low field infection prevalence in the vector (Venter et al., 2006).

This is not the first time that an AHS live attenuated vaccine virus, with evidence of reversion to virulence, has been the source of an outbreak of AHS within the AHS surveillance zone of South Africa (Weyer et al., 2016), although the SNV's were not identical to those previously described (Guthrie, A.J. Unpublished data). Introduction of the virus into the outbreak area could have occurred in several ways. There could have been circulation of vaccine associated virus in the region; however this is unlikely given that such circulation was not detected through any ongoing sentinel surveillance, passive surveillance by private veterinarians or a post-2014 freedom from disease AHS surveillance study, all of which took place in the 2016 outbreak area (Western Cape Department of Agriculture, Unpublished data). Vaccination in the AHS surveillance and free zone of South Africa is by State authorisation only and may only be done between June and October. The wind dispersal of AHS from the infected area of South Africa is also an unlikely scenario for introduction. The prevailing wind conditions during summer in the south-western Cape region of South Africa: in the 2015/2016 AHS season the closest detected AHS (type unknown) outbreak was 600 km to the east (Final 2016 African Horse Sickness Season Report (Amended), 2016). Future research into wind dispersal of Culicoides midges in South Africa is required to explore the patterns and risk of this dispersal method. The most likely route of introduction of the virus was either via an illegal AHS vaccination within the AHS surveillance zone in the vicinity of the index property or via a horse harbouring a reverted or reverting vaccine strain being moved illegally into the area.

## Conclusion

This is a detailed description of an AHS outbreak in the AHS surveillance zone in South Africa. The outbreak source virus originated from the reversion to virulence of the live attenuated vaccine, presumably introduced through illegal vaccination or movement within the AHS controlled area. This outbreak was detected by a private veterinarian through the existing passive surveillance programme in the AHS surveillance zone of South Africa, highlighting the importance of this form of surveillance in AHS. The climatic conditions promoted the initial transmission of the

virus but a combination of control measures, vaccination status of the exposed population and cooler climatic conditions decreased the case numbers and the local scale impact of the outbreak. The global scale impact remains significant, preventing the direct export of live horses from South Africa to the EU under existing EU legislation for at least two years. This outbreak emphasises the importance of the judicious use of the live attenuated vaccine within the AHS controlled area of South Africa.

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## Conflict of Interest

The Equine Health Fund, a division of Wits Health Consortium (Pty) Ltd, is funded by private donors (http://www.equinehealthfund.co.za/Home.aspx). The Equine Research Centre, University of Pretoria, is supported by South African horse industry funding, partially through the Equine Health Fund. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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# Chapter 4

## Establishing post-outbreak freedom from African horse sickness virus in South Africa's surveillance zone

J.D. Grewar[ab], E.S. Sergeant[c], C.T. Weyer[b], L.S. van Helden[d], B.J. Parker[b], T. Anthony[d], P.N. Thompson[a]

[a] Epidemiology Section, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

[b] Equine Health Fund, Wits Health Consortium, 8 Blackwood Avenue, Parktown, 2193, South Africa

[c] AusVet Animal Health Services, Level 1, 34 Thynne St, Canberra, Australian Capital Territory, 2617, Australia

[d] Western Cape Veterinary Services, Private Bag X01, Elsenburg, 7607, South Africa

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## Summary

An African horse sickness (AHS) outbreak occurred in South Africa's AHS controlled area in autumn 2016. A freedom from disease survey was performed to establish the likelihood of ongoing circulation of the associated virus during the same period the following year. A single-stage surveillance strategy was employed with a population-level design prevalence of 1% to establish a survey population sensitivity of 95% (probability that one or more positive horses would be detected if AHS was present at a prevalence greater than or equal to the design prevalence). In March 2017 a total of 262 randomly selected horses from 51 herds were sampled from the 2016 outbreak containment zone. Three within-herd and herd-level design prevalence scenarios were used in evaluating the post-survey probability of freedom. Depending on the underlying design prevalence scenarios, effectively ranging between 0.8% and 6.4%, and the use of informed or uninformed priors, the probability of freedom derived from this surveillance ranged between 73.1% and 99.9% (uninformed prior) and between 96.6% and 100% (informed prior). Based on the results the authors conclude that it is unlikely that the 2016 AHS virus was still circulating in the autumn of 2017 in the 2016 outbreak containment zone. The ability to perform freedom from disease surveys, and also to include risk-based methods, in the AHS controlled area of South Africa is influenced by the changing underlying population at risk and the high level of vaccination coverage in the horse population. Ongoing census post-outbreak must be undertaken to maintain a valid sampling frame for future surveillance activity. The seasonality of AHS, the restricted AHS vaccination period and the inability to easily differentiate infected from vaccinated animals by laboratory testing impact the ability to perform a freedom from disease survey for AHS in the 12 months following an outbreak in the controlled area.

## Keywords

African horse sickness type 1; Surveillance evaluation; Freedom from disease

## Introduction

African horse sickness virus (AHSV) is an orbivirus causing African horse sickness (AHS) in equids. It is transmitted by *Culicoides* spp. vectors and results in significant clinical disease and equine losses in sub-Saharan Africa (Coetzer & Guthrie, 2004). The disease has impacted the international trade of horses from Southern Africa due to its occurrence within South Africa's AHS controlled area (Grewar, 2016). This controlled area consists of an inner AHS free zone, a surveillance zone and a protection zone (Figure 12), and was established, based on historical risk profiling and the nature of the equine population in the zone, to allow direct trade of equines between South Africa and the European Union (EU) (Bosman, Brückner, & Faul, 1995). Animal health control and regulatory measures relating to AHS are in place in the controlled area and include restrictions on ownership, movement and vaccination of equines associated with the controlled area (Animal Diseases Act (Act No.35, 1984)). Movement control is primarily focussed on horses originating from the AHS infected zone and moving into the AHS controlled area, and prerequisites for movement include: positive identification; pre-movement health and vaccination status attestation by a veterinarian; and the issuing of permits by the Veterinary Services based on a low risk AHS profile of the area from where the horse originates. Vaccination against AHS in the free and surveillance zone is specifically prohibited unless authorised by the State Veterinary Authority, and authorised vaccination is restricted to the June - October period (winter and spring) each year to minimize the risk of vector transmission of live attenuated vaccine virus. The vaccination coverage within the horse population in the AHS free and surveillance zone remains high however (70% of horses surveyed during the 2004 outbreak were previously vaccinated) due to compliance with the movement protocol between AHS control zones in the country (Sinclair, Bührmann, & Gummow, 2006).

Surveillance in the AHS controlled area consists of both active and passive components. The active sentinel surveillance programme targets 150 horses a month with proportional sampling based on the underlying horse distribution in the surveillance and free zones. All sentinels are tested for AHSV RNA, with 60

unvaccinated horses included which are also tested using serology for AHSV group-specific antibodies (Grewar et al. 2017).

Freedom from disease surveys in animal populations are undertaken for a variety of reasons. Global and regional standard-setting organisations such the World Organisation for Animal Health (OIE) and the European Commission institute specific requirements for freedom from disease surveillance, with outcomes ranging from the herd, region, country and ultimately global population level. A freedom from disease status is beneficial to either promote trade of animals and animal products between countries (Vallat, 2006) or to provide confidence in the safety of food, for example, *Trichinella* surveillance in the pig industry in EU (EU, 2015). Beyond categorising populations as free of disease, freedom from disease surveys are also undertaken to allow areas to return to a freedom status after an incursion of a disease. Requirements to return to freedom in the post-AHS outbreak period in the controlled area of South Africa is an example of such a scenario, with these requirements included in the EU legislation regulating the importation of horses from South Africa (EU, 2008).

During April and May 2016, an outbreak of AHSV type 1 occurred in South Africa's AHS surveillance zone near the town of Paarl, extending the already imposed ban of direct trade of horses between South Africa and the EU (Grewar et al., 2019). This study describes the freedom from disease survey undertaken to assist in classifying the AHS status of the AHS controlled area affected by the 2016 AHS outbreak and provide evidence of AHS freedom in order to regain AHS free status and promote resumption of trade. It details the influence that changing equine populations can potentially have on the definition of an appropriate sampling frame and the impact that AHS vaccination has on the timing of surveillance activity, particularly within a disease control area where legislation prescribes a seasonal vaccination protocol. Furthermore, the interpretation of surveillance results and the ability to perform representative sampling from strata of different risk in order to increase the efficiency of the surveillance design are challenging where registered vaccines and available diagnostic tests do not allow for the differentiation between infected and vaccinated animals (DIVA).

## Materials and Methods

### *Ethics approval*

Ethics approval for this study was obtained by the Western Cape Department of Agriculture's (WCDOA) Departmental Ethics Committee for Research on Animals (Reference DS17/119). Informed written consent was obtained from each participating herd owner/manager.

### *Sample size and surveillance strategy*

The sampling frame (1813 horses in 118 herds) was established using population data obtained during the 2016 Paarl AHS outbreak. These data were primarily obtained from the outbreak epicentre and all herds within 5 km of infected herds in the outbreak (Grewar et al., 2019), and as shown in Figure 12, the majority of herds within the sampling frame (and all the herds involved in the final sampling) were found within the secondary containment zone of the 2016 outbreak. The sampling frame was dominated by Thoroughbred horses (59%) with American Saddlebred (9%), South African Warmblood (3.8%), Arab (3.5%), Boerperd (2%) and Friesian (2%) making up the majority of the remaining known purebred horses, while crossbred or unknown breeds made up the remaining horses (20.7%). The outbreak data were collected in April and May 2016 and updated animal-level census data in that area were not available when the freedom from disease survey took place in March 2017.

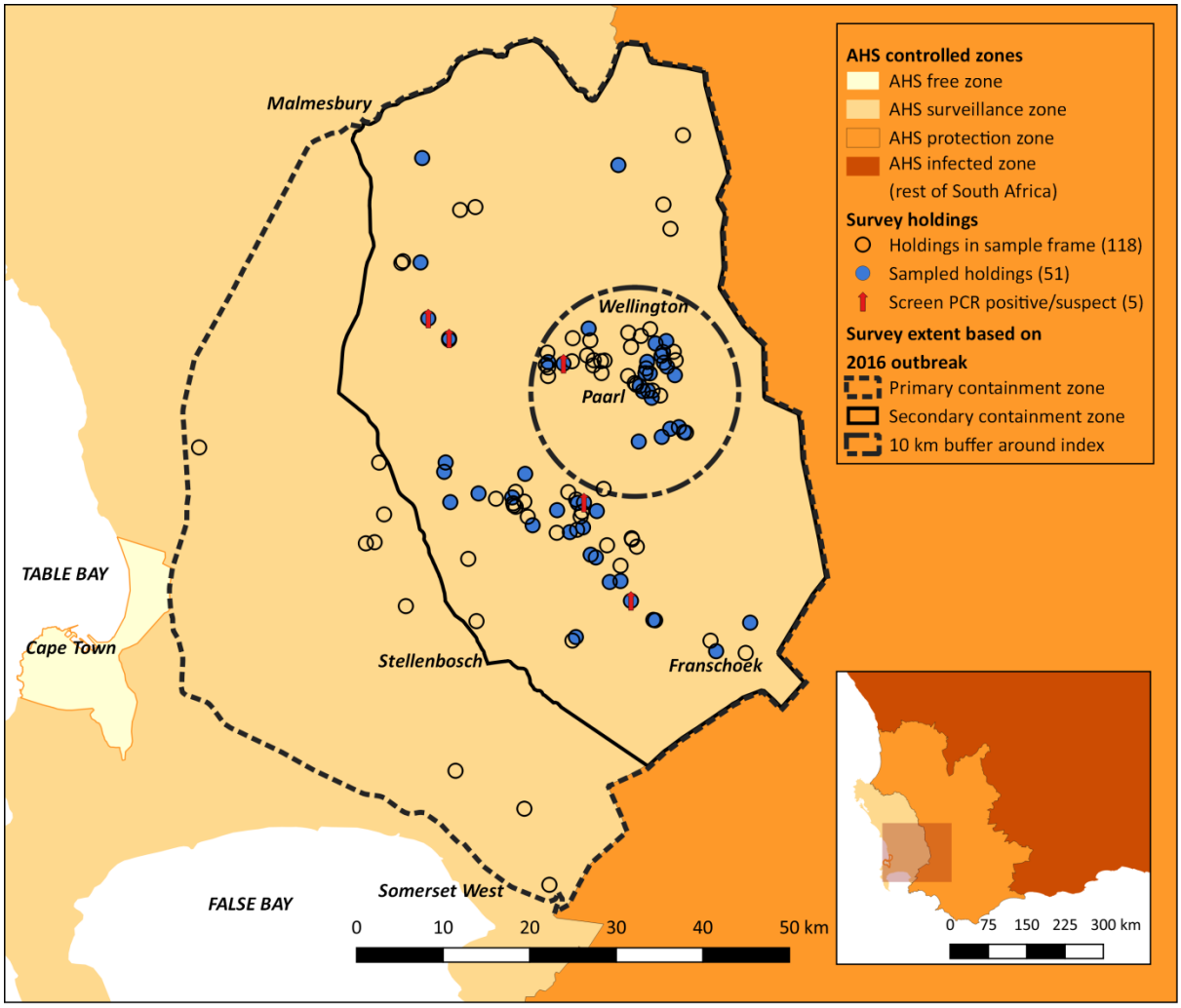


**Figure 12**: Herds associated with the 2017 African horse sickness freedom from disease survey. Black-rimmed circles indicate all herds within the sampling frame, blue-filled circles show herds where sampling took place (n=51). Upward red arrows show the five herds where screening PCR results were suspect or positive and where follow-up investigation was performed. Extents of the 2016 outbreak are shown with the black dashed polygon indicating the primary outbreak containment zone, the solid black line indicating the secondary containment zone and the dash-dot line indicating the area within 10 km of the index case. African horse sickness controlled zones are indicated by varying shades of orange-brown, from the free zone in Cape Town in the south-west, the surveillance zone within which the survey was conducted, and the protection zone which acts as a further buffer from the infected zone which consists of the rest (and majority) of South Africa.

A single-stage surveillance strategy was chosen and calculations for the total number of horses to sample were made using previously described methods (Cameron & Baldock, 1998a) implemented in EpiTools (Ausvet (Pty) Ltd: http://epitools.ausvet.com.au/), using the '*Sample size to achieve specified population level sensitivity*' option. The overall crude AHS affected proportion within the horse population during the 2016 Paarl AHS outbreak was 0.01 (Grewar et al., 2019), and this was used as the design prevalence to be detected through the surveillance. The AHSV real-time reverse transcription quantitative PCR (RT-qPCR) screening assay used in

the survey had an estimated median sensitivity of 0.978 and a median specificity of 0.999 and has been proposed as highly useful for discriminating between AHSV-infected and non-infected horses (Guthrie et al., 2013). Serological testing was not considered an option for screening in the survey, since the goal was a point-in-time estimate of probability of freedom from the previously circulating 2016 AHSV, which the RNA-based testing could provide with a single test rather than the paired testing required for determining seroconversion; the latter would be required since the vaccination coverage in the region is high and there was no DIVA serological test available. Overall specificity of 100% was assumed since follow-up to a final negative endpoint was performed for each horse that tested suspect or positive on RT-qPCR. A type one error rate of 5% was used reflecting a 95% probability of detecting AHS should it exist within the survey parameters. The population size was known (N=1813) and the sample size calculation used the hypergeometric approximation. Based on these parameters a sample size of 271 horses was established, and a random list of horses to be sampled was extracted from the population data, without replacement, using the 'Random sampling from a sampling frame' option in EpiTools. Due to the time period between the 2016 outbreak and the survey in March 2017, during which changes occurred in the equine population, a random replacement list was drawn up for each herd using the population dataset to replace horses selected to be sampled that were unavailable on the day of sampling. A single round of random herd selection was required to replace two herds which were unavailable for the survey. An updated aggregated census was obtained when each sampled herd was visited to allow for accurate post-surveillance evaluation.

The sampling time-frame for whole blood samples stored in EDTA is shown in Figure 13. The RT-qPCR used as a screening test was not DIVA capable and the survey time period was selected in order to decrease the likelihood of false positive RT-qPCR screening results due to recent vaccination in 2016 in the area where the survey was to take place. The latest AHS vaccination date was obtained for each sampled horse. Sampling took place during a similar time of year to when the Paarl 2016 outbreak occurred while still leaving enough time to do follow-up investigations before the start of the next vaccination period which started in June 2017.

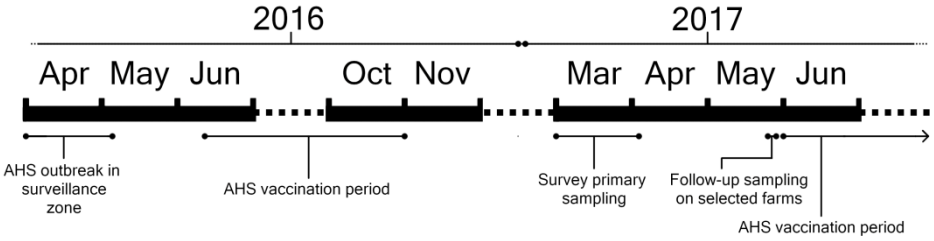


**Figure 13**: Sampling time-frames associated with the 2017 African horse sickness freedom from disease survey. The 2016 section indicates the period in which the 2016 outbreak took place as well as the subsequent vaccination period where African horse sickness vaccination could take place in the AHS controlled area. The primary and follow-up sampling periods for the survey took place prior to the start of the vaccination period in 2017.

### *Surveillance case definition*

The goal of the surveillance was to establish the probability of freedom from the AHSV that was responsible for the Paarl 2016 outbreak. The surveillance case definition used was based on a combination of the isolation and RNA detection clauses of the OIE's AHS case definition (OIE, 2016), specifically:

> *"AHSV has been isolated and identified from an equid or a product derived from that equid; or*
>
> *antigen or ribonucleic acid specific to AHSV has been identified in samples from an equid showing clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed case"*

Screening of primary samples was performed using the RT-qPCR as previously mentioned where the sensitivity of the test influenced the sample size. The lack of DIVA capability and the group-specific nature of the screening PCR resulted in the requirement for further diagnostic testing to establish a final case classification. AHSV typing, using a type-specific RT-qPCR (Weyer et al., 2015), and virus isolation (VI), was performed on all suspect samples. The latter assisted in establishing the likelihood of suspect results originating from live virus circulation rather than residual RNA from prior vaccination. Sequencing of any VI positive cultures was planned in order to genetically link any positive results to the 2016 outbreak; however, there were no VI positives which precluded the use of sequencing.

All suspect or positive RT-qPCR samples were re-extracted and re-tested. In herds where suspect or positive RT-qPCR results were obtained a follow-up sample was collected, from all previously sampled horses, for further RT-qPCR testing.

### *Post-surveillance evaluation*

Population sensitivity and confidence of freedom were calculated using previously described methods (Martin, Cameron, Barfod, Sergeant, & Greiner, 2007) and since the population size was known for each sampled herd the hypergeometric approximation for determining population sensitivity was used (MacDiarmid, 1988; Cameron & Baldock, 1998a). Population sensitivity ($SeP$) was initially calculated assuming the entire population was a single herd as defined in the single-stage surveillance strategy using Eq. 1:

$$SeP = 1 - (1 - SeU \times \frac{n}{N})^d \quad (1)$$

where $SeU$ is the sensitivity of the RT-qPCR assay, $n$ is the number of animals tested, $N$ is the number of animals in the population and $d$ is the number of expected diseased animals, a product of the animal level design prevalence and the number of animals in the population.

Separately $SeP$ was calculated assuming a 2-stage design using herd-level data, for varying animal and herd-level design prevalence values. Population sensitivity was estimated as Eq. 2:

$$SeP = 1 - (1 - \frac{\sum SeH_i}{n} \times \frac{n}{N})^d \quad (2)$$

Here $SeH$ is estimated separately for each herd sampled, using Eq.1. $N$ is the number of herds in the entire population, $n$ is the number of herds tested and $d$ is the number of herds expected to be infected, a product of the herd-level design prevalence and the number of herds in the population.

Three combinations of within-herd and herd-level design prevalence were used to reflect varying scenarios established from: the Paarl 2016 outbreak; AHS outbreaks in the same AHS controlled zone in South Africa in 1999, 2004, 2011, 2014 and 2016

(Grewar et al. 2018; Weyer et al. 2016; Sergeant et al., 2016; Sinclair, Bührmann, & Gummow, 2006; Western Cape Department of Agriculture (WCDOA, unpublished data); and a generic option to reflect the overall design prevalence (0.01) used in the initial sample size calculation (Table 9). Since an effective design prevalence of 0.01 can be obtained through combination of a range of within-herd and herd-level values, a separate evaluation was made of the resulting probability of freedom for combinations of these prevalences. For this analysis, within-herd prevalence values between 0.02 and 0.5 were used, with corresponding herd-level prevalences between 0.5 and 0.02 respectively, such that the product of the two values was fixed at 0.01.

The confidence of freedom estimates ($P\_free$), equivalent to the negative predictive value of the surveillance programme, for both an uninformed (0.5) and informed (0.912) prior confidence of freedom ($PriorP\_free$) were established using Eq. 3:

$$P_{free} = \frac{PriorP_{free}}{(1 - SeP \times (1 - PriorP_{free}))} \quad (3)$$

where population sensitivity ($SeP$) is determined by Eq.1 or Eq.2 for single or two-stage evaluation respectively. The AHS surveillance zone in the Western Cape of South Africa undergoes active monthly sentinel surveillance. An evaluation of the programme between September 2016 and August 2017 showed a final posterior probability of freedom of 95.9%. The posterior probability of freedom at the end of February 2017 from that analysis was 91.2% (Grewar et al., 2017), and this was used as the informed prior estimate of confidence of freedom.

## Results

Of the targeted 271 randomly selected horses to sample, 262 were sampled from 51 herds, of which 166 (63%) were selected in advance from the sampling frame, with the remainder being randomly selected replacement horses (Figure 12). Table S1 (Annexure 3) and S2 (Annexure 4) provides a summary of the demographics of the sampled horses. Overall the prior vaccination status against AHS was 97.5% (n= 237 of 243 participants with a known vaccination history).

Five horses from five different herds tested suspect or positive on RT-qPCR on the primary round of sampling. All previously sampled horses in each of the five herds

were then re-sampled (n=76). After both rounds of sampling, a total of 8 horses from 5 herds had tested suspect or positive on the group-specific RT-qPCR (Table 8). All samples tested negative on VI and this precluded the sequencing of any of these samples. Positive AHSV typing results were found in 3 of the 8 horses. Horse 1883 was AHSV type 1 positive on its screening sample, and it tested negative on the group-specific RT-qPCR on follow-up sampling. Horse 479 was AHSV type 3 positive on both the screening and follow-up sampling rounds. Horse 141 was typed as AHSV type 1 on follow-up sampling after being negative on the initial screening round. None of the 8 suspect horses fulfilled the positive case definition of the surveillance protocol as a result of a combination of their prior AHS vaccination history (all were vaccinated in 2017), the quantitation cycle values of their group-specific screening RT-qPCR results, their AHSV typing and virus isolation results, as well as a lack of any clinical signs associated with AHS detected during sampling.

**Table 8**: Demographic and testing results for all screened and follow-up RT-qPCR suspect and positive horses showing their African horse sickness virus type specific and virus isolation results.

| Herd ID/Horse ID | Age† | Days between positive result and last AHS vaccination | Primary round of surveillance | | | Follow-up round of surveillance | | |
|---|---|---|---|---|---|---|---|---|
| | | | Minimum RT-qPCR Cq Value | AHS type specific RT-qPCR result | VI result | Minimum RT-qPCR Cq Value | AHS type specific RT-qPCR result | VI result |
| 14/141 | 2 | 217 | Negative | N/A | N/A | 35.1 | AHSV1 | Negative |
| 14/307 | 20 | 316 | Negative | N/A | N/A | 36.7 | Negative | Negative |
| 14/316 | 2 | 146 | 35.94 | Negative | Negative | Negative | N/A | N/A |
| 24/479 | 13 | 211 | 31.4 | AHSV 3 | Negative | 33.5 | AHSV 3 | Negative |
| 66/1396 | 9 | 238 | 34.6 | Negative | Negative | Negative | N/A | N/A |
| 149/1869 | 2 | 205 | Negative | N/A | N/A | 34.9 | Negative | Negative |
| 149/1883 | 2 | 128 | 31.42 | AHSV 1 | Negative | Negative | N/A | N/A |
| 6469/6230 | 4 | 219 | 35.44 | Negative | Negative | 37.6 | Negative | Negative |

AHS: African horse sickness
Cq: quantitation cycle
N/A: Not applicable
RT-qPCR: Real-time reverse transcription quantitative PCR
VI: Virus isolation
†Years old– rounded to the nearest year

Evaluation of the system sensitivity and probability of freedom for three different scenarios with respect to within and herd-level design prevalences are shown in Table 9. The graphical surveillance outcomes, obtained from varying combinations of within-herd and herd-level prevalences resulting in an overall effective design

prevalence of 0.01, is shown in Supplementary figure 1 (Annexure 5), with the range of outcomes included in Table 9. For effective design prevalences (the product of the within-herd and herd-level prevalences) ranging between 0.8% and 6.4%, established during the Paarl 2016 outbreak and averaged from prior outbreaks in the AHS controlled area between 1999 and 2016 respectively, the sensitivity of the surveillance system, i.e. its probability of detecting a positive case given the population had been infected, ranged between 63.2% and 99.9%. The confidence of freedom differed when using an uninformed prior compared to an informed prior, ranging between 73.1% and 99.9% in the former and between 96.6% and 100% in the latter.

**Table 9**: Mean herd-level surveillance sensitivity, population surveillance sensitivity and overall confidence of freedom from African horse sickness infection for the population using uninformed and informed priors. Columns reflect the varying design prevalences used as inputs to analyse the surveillance outcomes for both single-stage and two-stage analysis.

| | **Descriptions and values of design prevalences based on varying data sources** | | | |
|---|---|---|---|---|
| | **Design prevalence used in survey design (single-stage analysis)** | **Generic prevalences to reflect an effective overall design prevalence used in survey design (two-stage analysis)†** | **Prevalences from Paarl 2016 outbreak data** | **Prevalences from historical AHS surveillance zone outbreak data** |
| **Input design prevalence** | | | | |
| Within-herd animal level prevalence ($P^*_U$) | 0.01 | 0.2 (0.02 – 0.5) | 0.128 | 0.278 |
| Herd-level prevalence ($P^*_c$) | N/A | 0.05 (0.5 – 0.02) | 0.067 | 0.233 |
| Effective population prevalence ($P^*_U$ x $P^*_c$) | 0.01 | 0.01 | 0.008 | 0.064 |
| **Resulting outcome** | | | | |
| Mean herd-level surveillance sensitivity (*MeanSSH*) | N/A | 0.515 (0.25 – 0.69) | 0.448 | 0.586 |
| Population surveillance sensitivity (*SeP*) | 0.945 | 0.779 (0.632 – 0.999) | 0.821 | 0.999 |
| Confidence of population freedom – uninformed prior (*PFreeU*) | 0.948 | 0.819 (0.731 – 0.999) | 0.848 | 0.999 |
| Confidence of freedom – informed prior (*PFreeI*) | 0.995 | 0.979 (0.966 – 1) | 0.983 | 0.999 |

† The range and resulting outcomes for combinations of design prevalences reflecting an effective population prevalence of 0.01 are included in parentheses

## Discussion

To our knowledge, this is the first published freedom from disease survey for AHS in a post-outbreak scenario. Although freedom from disease surveillance methodologies are well described we found the practical application in the AHS and South African context challenging when designing and evaluating the programme. Challenges arose due to the requirement to perform this surveillance in a zone within an AHS infected country where a high proportion of horses were vaccinated against AHS, with legislation prescribing a seasonal vaccination protocol; moreover the vaccine used was live-attenuated and the routine diagnostic tests available cannot differentiate between infected and vaccinated animals. These factors dictated the time period appropriate to perform the surveillance (early autumn) and, as a result of the seasonal nature of AHS infection, this time period is likely to be the same for future post-outbreak surveillance programs of a similar nature. Challenges were compounded because of the seasonal nature of the majority of horse breeding in the area which changed the equine population between outbreak and survey, making both establishing an animal-level sampling frame and using a risk-based surveillance approach difficult.

Overall, 97.5% of all sampled horses were previously vaccinated, which is substantially higher than the overall vaccination status of all horses in the population at the time of the Paarl 2016 AHS outbreak (74.3% - Grewar et al., 2019). This difference is likely due to a change in the vaccination status of individual horses following the Paarl 2016 outbreak, and the lack of an updated sampling frame in 2017 that included new unvaccinated horses. The well-vaccinated population, combined with the use of a vaccine that did not allow DIVA diagnostics, lowered the appropriate design prevalence of the surveillance system, hence increasing the required sample size and associated cost. It also precluded the use of serological testing as an option for screening or confirmatory testing, and increased the possibility, as experienced in this survey, of detecting false positive reactors most likely due to residual vaccine RNA. These factors resulted in a complex case definition where follow-up strategies required re-sampling, virus isolation, typing assays and/or genome sequencing to confirm the diagnosis for screened suspect cases.

Depending on the chosen underlying design prevalence and the use of informed or uninformed priors, the confidence of freedom from this once off surveillance event ranged between 73.1% and 100%. The evaluation of the surveillance programme in both a single-stage (animal level only) and two-stage fashion (both animal and herd level) provides estimates of the probability of freedom based on the implemented sampling strategy and also accounts for any clustering of infection in herds. This illustrates the importance of reporting disease outbreak prevalences, both at animal and herd level, where freedom from disease surveillance may be contemplated during the post-outbreak period. A two-stage sampling strategy will generally provide less information (lower sensitivity estimates) than a one-stage strategy for equivalent sample sizes. This is evident from the results where the evaluation of the survey in a two-stage manner gave lower sensitivity and probability of freedom estimates for equivalent effective population prevalences. The exception is as shown in Supplementary figure 1, with very high underlying herd-level prevalence in conjunction with low within-herd prevalence (effectively no clustering of infection in herds). The main reason for this effect is that when sampling from a population, each additional animal sampled from a herd that has already been sampled provides progressively less information about population status than an additional animal sampled from a previously unsampled herd. While the OIE is prescriptive in the required design prevalence to establish freedom for certain diseases like brucellosis and bovine spongiform encephalopathy (OIE, 2018a; OIE, 2018b) it is not explicit when describing the required design prevalence for AHS freedom (OIE, 2016). The clearest indication of trade acceptable design prevalence for AHS freedom comes from the surveillance requirements of the EU for AHS in the South African sentinel surveillance programme, where the required sample size corresponds to an animal level design prevalence of 5% (EU, 2008). Animal level design prevalences selected for freedom from disease surveys for other arboviral diseases range between 1% and 5% (Camphor, 2014; Diarmita, 2018; Grigore, 2018; Tratalos et al., 2018) but can be as low as an effective animal level prevalence of 0.5% (Stokes, Baylis, & Duncan, 2016). In our case the design prevalence used to determine the overall sample size assumed that the AHSV associated with the Paarl 2016 outbreak was circulating in 1% of the population; decreasing this below 1% would have been cost-prohibitive. Our choice of a simple

random survey treating the entire population as a single homogenous population stemmed from the fact that we had an individual animal sampling frame, the target population was contained within a relatively small geographic area and the hazard surveyed for was midge-borne, making infection clustering less likely compared to a contact transmitted agent. Since the area surveyed was relatively small, the added cost of sampling additional herds was not considered prohibitive and the advantage of making use of a two-stage survey design, where herd and animal sample sizes can be manipulated to reduce costs while still maintaining appropriate outcomes (Cameron & Baldock, 1998b), was not considered.

The total cost of the survey amounted to R210, 000 with the majority of cost associated with laboratory testing (51%) and personnel time (35%). As a component of total costs associated with disease control and return to freedom this is relatively minor. Not only do outbreaks in the AHS controlled area incur substantial direct costs (Grewar et. al., 2013) but the annual industry-wide revenue loss of a direct export market outside of Africa and ongoing AHS control and surveillance in the controlled area is estimated at R500 million and R6 million per year respectively (A. Todd – South African Equine Health and Protocols NPC, personal communication). Using a lower design prevalence would therefore not substantially inflate the overall cost of control; however, because the impact of AHS outbreaks in the controlled area in South Africa is long lasting (at least two years loss of direct trade opportunity to major trade partners) and due to the high level of compliance required for direct trade, it is difficult to estimate the actual benefit that a single survey, such as the one described here, would have on re-opening trade. Thus the choice of design prevalence was based on likely disease parameters.

It should be considered that the horse population changes over a one year period, and generating a sampling frame in 2017, from 2016 outbreak census data, results in sample selection bias towards horses associated with the outbreak. While we do not expect this made a practical difference to the outcome of the survey, this bias would be mitigated through either maintaining a thorough census after outbreaks or generating an up to date census prior to selecting horses to sample. During the Paarl 2016 outbreak, the risk of unvaccinated horses being diagnosed with AHS was 2.3 times higher than in previously vaccinated horses (WCDOA, unpublished data). A

component of opportunistic risk-based surveillance (separate sampling of unvaccinated horses) was incorporated into the surveillance plan in addition to the single strata design, but the results thereof could not be statistically evaluated along with the non-risk-based data due to a lack of representativeness of the sampling. Demographic data showed a clear bias towards young horses (and hence breeding establishments on a herd level) being more likely to be unvaccinated. The foaling season for Thoroughbred horses, the breed most represented in the sampling frame for this surveillance (59%), runs from August through early December each year (Schulman, Marlow, & Nurton, 2012), so there is a large influx of unvaccinated foals into any potential population at risk in the AHS surveillance zone during this period. This fluctuation in the individual horse level demographic and vaccination status made using risk-based surveillance a logistical challenge, particularly in this high-density population. Risk-based surveillance strategies can improve both the effectiveness and cost implications for freedom from disease surveillance (Stärk et al., 2006); however, for it to be feasible in future surveys of this nature ongoing census, demographic and risk factor data collection would need to take place in the population at risk between the cessation of the outbreak and the freedom from disease survey.

For the described surveillance event the result was not only specific for the virus associated with the prior outbreak, but the sampling frame was based on the prior outbreak controlled area, which was focused primarily on herds surrounding infected herds (Grewar et al., 2019). This limited the geographic extent of the surveillance outcome. A point-in-time freedom from disease survey forms just part of an overall surveillance strategy for a scenario where establishment of zonal freedom is attempted in a country with endemic disease.

## Conclusion

This study showed that it was unlikely that the AHSV responsible for the Paarl 2016 outbreak in the Western Cape was still circulating the following autumn in the area defined by the outbreak containment zone. Post-outbreak capture of census, demographic and risk factor data in populations at risk that will be targeted for freedom from disease surveys is critical to inform future survey design. This is

especially true where factors such as disease seasonality, use of a live attenuated virus vaccine, a seasonal vaccination policy and a lack of available tests with DIVA capabilities preclude the possibility of performing this surveillance immediately after the cessation of an outbreak.

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## Conflict of Interest

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# Chapter 5

## Post-outbreak African horse sickness surveillance: A scenario tree evaluation in South Africa's controlled area

J.D. Grewar[ab], T. Porphyre[c], E.S. Sergeant[d], C.T. Weyer[b,e], P.N. Thompson[a].

[a] Epidemiology Section, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

[b] South African Equine Health & Protocols NPC, Baker Square, Paardevlei, Cape Town, 7110, South Africa

[c] The Roslin Institute, University of Edinburgh, Edinburgh, EH25 9RG, Scotland, United Kingdom

[d] AusVet Animal Health Services, Level 1, 34 Thynne St, Canberra, Australian Capital Territory, 2617, Australia

[e] Department of Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

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## Summary

An African horse sickness (AHS) outbreak occurred in March and April 2016 in the controlled area of South Africa. This extended an existing trade suspension of live equids from South Africa to the European Union. In the post-outbreak period ongoing passive and active surveillance, the latter in the form of monthly sentinel surveillance and a stand-alone freedom from disease survey in March 2017, took place. We describe a stochastic scenario tree analysis of these surveillance components for 24 months, starting July 2016, in three distinct geographic areas of the controlled area. Our results show that, if AHS virus was circulating at a minimum prevalence of 1 infected animal in 1% of herds, the median probability of freedom from AHS in all three areas was between 98.3% - 99.8%. This high level of freedom probability had been attained in all three areas within the first 9 months of the two-year period. The primary driver of surveillance outcomes was the passive surveillance component. Active surveillance components contributed minimally (less than 0.2%) to the final probability of freedom. Sensitivity analysis showed that the probability of infected horses showing clinical signs was an important parameter influencing the system surveillance sensitivity. The monthly probability of disease introduction needed to be increased to 20% and greater to decrease the overall probability of freedom to below 90%. Current global standards require a two-year post-incursion period of AHS freedom before re-evaluation of free zone status. Our findings show that the length of this period could be decreased if adequately sensitive surveillance is performed. In order to comply with international standards, active surveillance will remain a component of AHS surveillance in South Africa. Passive surveillance, however, can provide substantial evidence supporting AHS freedom status declarations, and further investment in this surveillance activity would be beneficial.

## Keywords

African horse sickness; Surveillance evaluation; Freedom from disease; Scenario tree

## Introduction

African horse sickness (AHS) is a disease of equids caused by African horse sickness virus (AHSV), an *Orbivirus* transmitted by *Culicoides* midges (Coetzer & Guthrie, 2004). It is a disease of global importance and is one of six diseases for which official World Organisation for Animal Health (OIE) freedom can be obtained (OIE, 2018). The disease impacts the ability of countries to trade live equids. Notably AHS is one of six equine diseases that require above-standard biosecurity to comply with conditions for the movement of high-health high-performance (HHP) horses within international guidelines (OIE, 2016a, 2016b). There has been recent evidence of the changing distribution of several *Orbiviruses* transmitted by *Culicoides* midges. Recent large scale orbiviral epidemics, such as Bluetongue in Europe, has resulted in sensitisation to the reality that the emergence of these diseases is possible in previously unaffected regions. This is particularly true in regions that have resident vectors (MacLachlan & Guthrie, 2010; Mellor & Leake, 2000).

Historically South Africa's primary export route for live horses has relied on direct export to the European Union (EU) under existing trade protocols based on three primary import standards (EC, 2008, 2010, 2018) or through the use of Mauritius as a stepping stone to Europe (Grewar, 2016). South Africa has not directly traded domestic equines with any non-African country since 2011 as a result of an AHS outbreak in that year (Grewar et al., 2013). South Africa does not have official OIE freedom status from AHS but does have a controlled area that is considered free from the disease which has been developed specifically for trade purposes (Bosman, Brückner, & Faul, 1995; South African Government, 1984). Sporadic outbreaks have however occurred in the controlled area and surveillance plays a crucial role in the ability to adhere to existing trade conditions. The objective of surveillance for AHS in this context is to demonstrate freedom from AHS. In this study, we aim to estimate the sensitivity and probability of freedom in the AHS controlled area throughout the two years following the 2016 outbreak (Grewar et al., 2019). This outbreak was resolved in June 2016 and for this evaluation the first surveillance period is July 2016.

While collectively evaluating three different components of surveillance (passive surveillance, ongoing active sentinel surveillance and a structured stand-alone

freedom from disease survey) we also evaluate them individually to provide a basis for justification of ongoing investment in these components. Furthermore, we provide a basis for discussion regarding the applicability of a two-year suspensive condition for a disease such as AHS in the post-outbreak period, as required by the EU and OIE (EC, 2010; OIE 2016b, 2018), assuming a well-developed surveillance programme is in place.

## Materials and Methods

### *Ethics approval*

While this study was a desktop exercise the data obtained regarding sampled animals originated either from official Government surveillance activity or studies where ethics approval had been granted by the Western Cape Department of Agriculture's (WCDOA) Departmental Ethics Committee for Research on Animals (Reference DS17/119). Ethics approval for expert opinion interviews was obtained from the University of Pretoria's Faculty of Humanities Research Ethics Committee (Reference HUM014/0519).

### *Model overview and general methods*

A stochastic scenario tree model was developed based on the work described by Martin et al. (Martin, Cameron, & Greiner, 2007). Scenario trees in surveillance characterise a population (in this case by geographic location) and sequentially model the infection probabilities and detection occurrences within surveillance components to give realistic estimates of outcomes such as the sensitivity of surveillance and probability of freedom. The methodology of Martin et al. (2017) establishes surveillance component sensitivity and the subsequent probability of freedom from disease accounting for multiple surveillance components. Since a reliable individual animal dataset was available, methods were modified using the hypergeometric approximation for estimating herd and component sensitivities (MacDiarmid, 1988). Sensitivity and probability of freedom outputs are reported as median probabilities with 95% probability intervals (PI) following 10000 iterations. The individual animal was considered the primary surveillance unit and the data were aggregated on a monthly basis for analysis (surveillance period).

All data were managed in a PostgreSQL database (https://postgresql.org) and the model was run in R (R Core Team, 2019) using the following packages: mc2d for management of probability distributions and Monte-Carlo simulations (Pouillot & Delignette-Muller, 2010); RPostgreSQL for data import (Conway, Eddelbuettel, Nishiyama, Prayaga, & Tiffin, 2016); dplyr, tibble and reshape2 for data manipulation (Müller & Wickham, 2018; Wickham, 2007; Wickham & Francois, 2015); functions extracted from the RSurveillance package for posterior probability of freedom calculations (Sergeant, 2016); and ggplot2 for graphical outputs (Wickham, 2009). qGIS (https://qgis.org) and PostGIS (https://postgis.net/) were used for generating spatial outputs.

### *Surveillance evaluation areas*

African horse sickness is a legally controlled disease in South Africa and part of the control is through regionalisation of the country into AHS zones (Bosman et al., 1995; Animal Diseases Act (Act No.35, 1984)). The AHS controlled area consists of three zones – an inner AHS free zone (FZ), middle surveillance zone (SZ) and outer protection zone (PZ) - Figure 14. In practice, the FZ and SZ have the same AHS surveillance policy and they were merged for this evaluation (FZSZ). The 2016 AHS outbreak secondary containment zone, however, delineated the region where a structured freedom from disease survey was performed (Grewar et al., 2019) and the combined FZSZ was separated into that part intersecting with the 2016 AHS secondary containment zone (FZSZ_CZ – A1 in Figure 14) and the remainder (FZSZ_NonCZ – A2 in Figure 14). The AHS PZ is considered the third surveillance area (B in Figure 14).

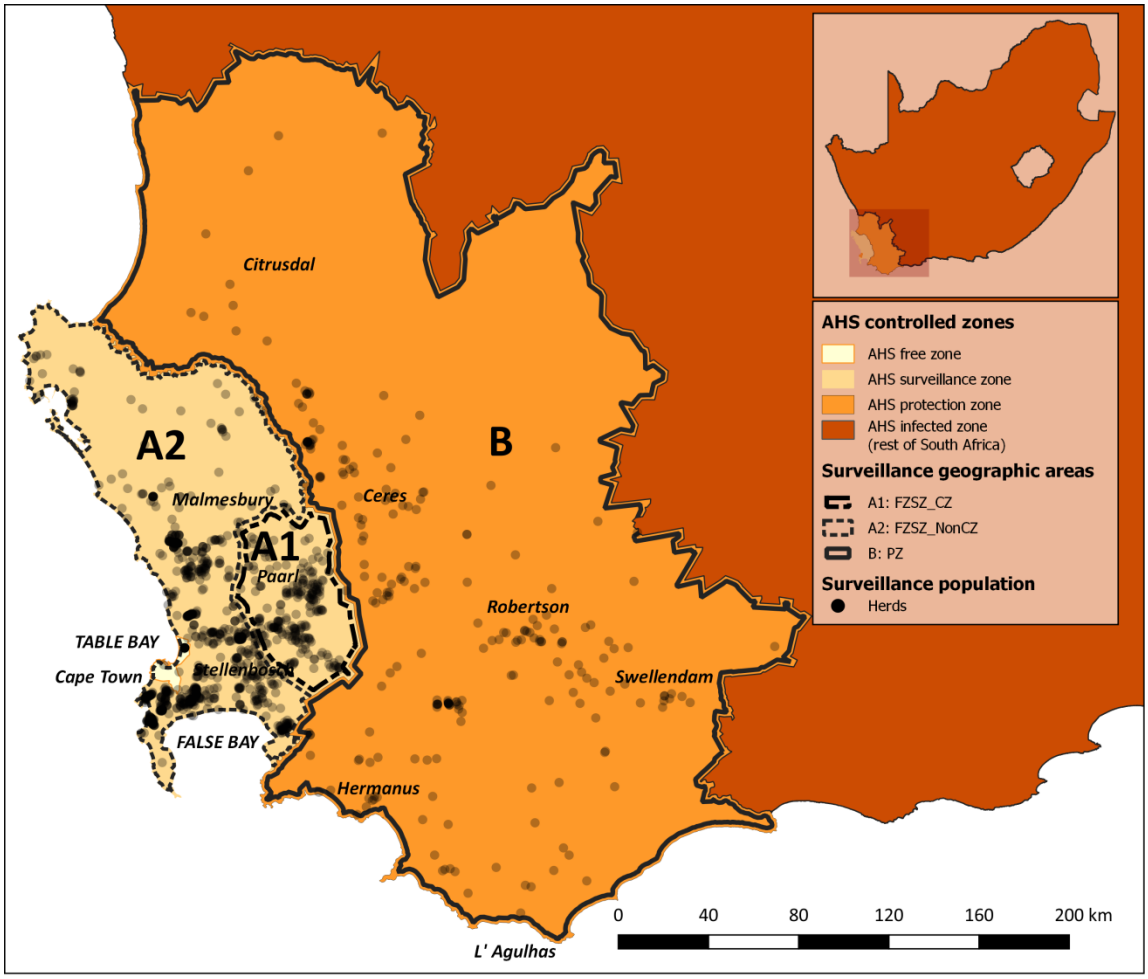


**Figure 14**: Surveillance evaluation areas categorising African horse sickness (AHS) surveillance evaluation. The evaluation areas are superimposed on the current South African AHS controlled zones. Evaluation area A1:FZSZ_CZ refers to the area within the AHS free and surveillance zone that includes the containment zone of the 2016 AHS outbreak (Grewar et al., 2019); A2:FZSZ_NonCZ is that part of the AHS free and surveillance zone excluding the 2016 AHS outbreak containment zone and B: PZ reflects the boundaries of the AHS protection zone. Herds associated with surveillance are shown as black circles.

### *Surveillance component overview and available data*

Surveillance components are defined by the source of data and the methods used for its collection to investigate the occurrence of one or more hazards in a specific population (RISKSUR consortium, 2013). We describe the evaluation of AHS in terms of three components; ongoing passive surveillance (PSC), ongoing monthly sentinel surveillance (SSC) and a stand-alone post-outbreak freedom from AHS disease survey (POSC). The detailed processes of the active components (SSC and POSC) have been described (Grewar et al., 2019; Grewar & Weyer, 2016) and only information pertinent to this quantitative evaluation of the system as a whole are expanded upon below.

## Passive surveillance component (PSC)

Passive surveillance takes place throughout the AHS controlled zone and this component is represented in each surveillance area analysed. The legislative onus on reporting confirmed or suspect AHS cases detected by veterinarians, laboratories or any other person is established in South African law (Animal Diseases Act (Act No.35, 1984)). The PSC in the AHS controlled area of South Africa is explicitly included in South Africa's AHS surveillance strategy. The PSC is primarily reliant on the owners and/or managers of horses detecting suspect cases after clinical signs of the disease are evident, those clinically ill horses being investigated by a veterinarian and samples being taken for AHS diagnosis.

## Sentinel surveillance component (SSC)

The sentinel surveillance component refers to the monthly testing of selected sentinels proportionally sampled based on the underlying equine population within the AHS FZ and SZ. This programme was initially established specifically to provide the active surveillance basis for AHS freedom for trade with the EU (EC, 2008). While the programme does include serological testing of approximately 60 animals per month (previously unvaccinated animals), all animals are tested using a real-time quantitative PCR (RT-qPCR) (Guthrie et al., 2013) with a monthly target of 150 animals. For consistency with other components and our proposed case definition, only the results from the PCR based sentinel testing were considered for this analysis. Full reports regarding the sentinel programme for the period reviewed in this manuscript are available (Grewar & Weyer, 2018; Grewar, Weyer, Burger, Russouw, & Parker, 2016; Grewar et al., 2017).

Results from the sentinel surveillance programme were obtained by permission from the Western Cape Department of Agriculture (WCDOA). The SSC is only relevant in the FZSZ_CZ and FZSZ_NonCZ since sentinel surveillance is not performed in the AHS Protection zone. Table 10 shows the surveillance period, sampled totals and associated herd and horse-level census pertaining to the SSC.

**Table 10**: Sentinel surveillance component – number of sentinel herds and horses tested with underlying census represented by sentinel herds. Counts are split between the two surveillance areas that have sentinel surveillance performed within them

| Surveillance period (months starting 1 July 2016) | Surveillance evaluation area | | | | | |
|---|---|---|---|---|---|---|
| | FZSZ_CZ | | | FZSZ_NonCZ | | |
| | Number of sentinel herds | Number of horses in sentinel herds | Number of sentinels tested | Number of sentinel herds | Number of horses in sentinel herds | Number of sentinels tested |
| 1 | 13 | 430 | 37 | 40 | 723 | 133 |
| 2 | 13 | 430 | 41 | 42 | 735 | 132 |
| 3 | 13 | 448 | 47 | 37 | 611 | 110 |
| 4 | 13 | 448 | 46 | 35 | 604 | 108 |
| 5 | 13 | 448 | 47 | 35 | 598 | 107 |
| 6 | 12 | 418 | 45 | 37 | 611 | 104 |
| 7 | 12 | 418 | 43 | 38 | 614 | 105 |
| 8 | 12 | 418 | 44 | 37 | 611 | 103 |
| 9 | 12 | 418 | 43 | 36 | 597 | 103 |
| 10 | 13 | 420 | 47 | 32 | 553 | 97 |
| 11 | 12 | 418 | 42 | 35 | 573 | 100 |
| 12 | 12 | 370 | 42 | 28 | 458 | 82 |
| 13 | 13 | 420 | 44 | 33 | 575 | 95 |
| 14 | 12 | 410 | 41 | 32 | 532 | 90 |
| 15 | 12 | 410 | 43 | 34 | 570 | 89 |
| 16 | 14 | 448 | 47 | 36 | 606 | 101 |
| 17 | 13 | 430 | 47 | 34 | 596 | 97 |
| 18 | 13 | 413 | 49 | 33 | 514 | 90 |
| 19 | 13 | 412 | 50 | 37 | 520 | 97 |
| 20 | 13 | 412 | 43 | 37 | 596 | 103 |
| 21 | 14 | 419 | 54 | 38 | 599 | 105 |
| 22 | 13 | 371 | 46 | 39 | 646 | 106 |
| 23 | 14 | 419 | 45 | 37 | 594 | 98 |
| 24 | 15 | 539 | 47 | 38 | 600 | 101 |

FZSZ_CZ: AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone

## Post-outbreak freedom from disease survey component (POSC)

A stand-alone freedom from disease survey targeting the containment zone of the 2016 Paarl outbreak was undertaken in March 2017 (Grewar et al., 2019). Data from this study was integrated into the surveillance dataset used in this evaluation. The number of herds and animals within herds differs slightly to the published reference to this component since census data was extracted from the WCDOA in March 2019 for this evaluation, as described below. For this component a total of 262 horses in 51 herds were tested, representing 2235 horses in total. The POSC is only relevant in the FZSZ_CZ and for one surveillance period, namely March 2017 (i.e. surveillance period nine).

### *Population of interest*

Herd location and herd-level census data were provided by the WCDOA and were generated from movement permits, historical outbreak censuses, vaccination authorisation and routine censuses undertaken in the controlled area. The population of interest was limited to domestic horses. The AHS controlled area does contain small populations of zebra (555 animals in 54 herds in the SZ and 1068 animals in 81 herds in the PZ) and donkeys (115 animals). These species do not, however, show overt clinical signs of the disease (Coetzer & Guthrie, 2004) and are therefore not represented in passive surveillance activities. Donkeys were not specifically excluded from active surveillance programs but, because of their low populations, were not represented in either the SSC or the POSC.

The census information used in this evaluation was based on a once-off data extraction in March 2019, and that herd-level population was duplicated for each surveillance period. The total herds and associated horses per surveillance area are shown in Table 11 and the locations of these herds are shown in Figure 14.

**Table 11**: Census information of herds and horses within the African horse sickness (AHS) controlled area of South Africa.

| Surveillance area | Number of herds | Number of horses (mean per herd/median per herd) |
|---|---|---|
| FZSZ_CZ | 234 | 4476 (19/6) |
| FZSZ_NonCZ | 890 | 8386 (9/4) |
| PZ | 233 | 3655 (16/4) |
| **Total** | **1357** | **16517 (12/4)** |

FZSZ_CZ: AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone
PZ: Protection zone

### *Surveillance case definition*

The case definition for all three surveillance components is based on the OIE's case definition for infection of African horse sickness (OIE, 2016b). Given the lack of pathognomonic clinical signs for the disease, however, the end-point of all components' detection nodes is based on laboratory testing. Although investigations into suspect cases of AHS include diagnostic tests other than the RT-qPCR, the group-specific RT-qPCR is the entry-point into the laboratory testing process. No positive case would exclude a positive RT-qPCR test. No cases of AHS were detected or

reported during the surveillance period evaluated. Accurate information on numbers of passive surveillance investigations and negative clinical reporting is not available. In the SSC program a total of 8 horses were investigated to a negative conclusion between July 2016 – June 2018 (Grewar & Weyer, 2018; Grewar, Weyer, Burger, Russouw, & Parker, 2016; Grewar et al., 2017). Details of screening tests and investigations of the POSC have been published (Grewar et al., 2019). We conclude that all suspect cases detected through any of the surveillance programs were followed to their negative end-points. The specificity of each surveillance component (the probability that a negative disease status will have a negative surveillance outcome) is therefore considered as 100%.

### *Scenario tree*

A graphical representation of the scenario tree depicting the evaluation of all three surveillance components is shown in Figure 15 with descriptions of nodes and branch distributions/proportions included in Table 12.

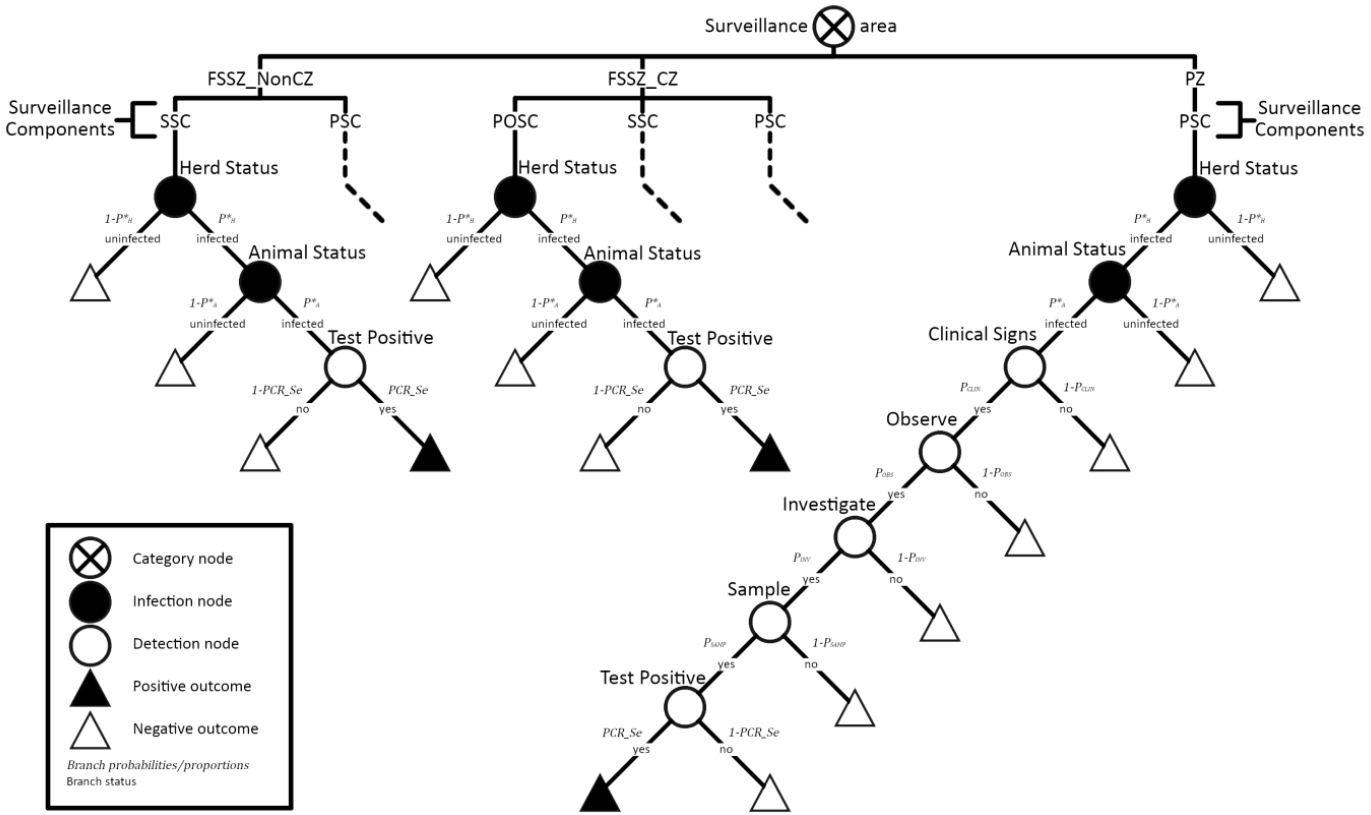


**Figure 15**: Scenario tree depicting the evaluation of three surveillance components within the African horse sickness (AHS) control area of South Africa. Descriptions, values and distributions of branch probabilities and proportions are described in Table 12. Dashed lines indicate relevant surveillance components within the associated surveillance area but that are identical and shown in another surveillance area. Note that in the PZ only the PSC is relevant and no active surveillance programs take place in this area. PSC: Passive surveillance component; SSC: Sentinel surveillance component; POSC: Post-outbreak stand-alone surveillance component; FZSZ_CZ: AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone; FZSZ_NonCZ: AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone; PZ: Protection zone

**Table 12**: Input parameters for the African horse sickness surveillance evaluation model

| Input parameter | Parameter code | Value | Applicable surveillance components | Explanation and source |
|---|---|---|---|---|
| Animal-level design prevalence | $P_A^*$ | 1 animal per herd | All | |
| Herd-level design prevalence | $P_H^*$ | 0.01 | All | Estimate based on herd-level prevalence from outbreak data between 1997 and 2016 |
| Probability of freedom from AHS at surveillance period 1 | $PriorPfree_1$ | 0.5 | All | Initial probability of freedom in surveillance period 1 (July 2016) reflecting an uninformed prior |
| Sensitivity of RT-qPCR | $PCR\_Se$ | $Beta$ (9.65,1.19) | All | Sensitivity of the RT-qPCR used for laboratory testing of AHS derived from a median value of 0.978 (95% interval of 0.708 - 0.9996) (Guthrie et al., 2013; Sergeant, Grewar, Weyer, & Guthrie, 2016) |
| Probability of introduction of AHS in each surveillance period | $Pintro$ | $Pert$ (0.017, 0.033, 0.067) | All | Value is based on the number of outbreaks in the AHS controlled area in the 210 months since 1 January 1999. 1999 was the first year since the regionalisation of South Africa in AHS controlled zones that an outbreak occurred. The Pert distribution accounts for variability in $Pintro$ with half and double the actual outbreak incidence as lower and upper bounds as previously described (Alban, Boes, Kreiner, Petersen, & Willeberg, 2008). |
| Probability of infected animal showing clinical signs | $P_{CLIN}$ | $Beta(c_i + 1, n_i - c_i + 1)$ | PSC | Probability of individual infected animal showing clinical signs of AHS based on the clinical case proportions observed in randomly selected outbreak $i$†. Based on the Bayesian estimate of a population proportion where clinical signs ($c$) are successes of $n$ cases observed in outbreak $i$ (Vose, 2008) |
| Probability of horse owner/manager detecting horse showing clinical signs of AHS | $P_{OBS}$ | $Pert\ (i\_lower, i\_most\ likely, i\_upper)$ | PSC | Randomly selected expert opinion from expert $i$ on the probability that a herd owner/manager will observe an infected animal showing clinical signs of AHS* |
| Probability of horse being investigated by a veterinarian | $P_{INV}$ | $Pert\ (i\_lower, i\_most\ likely, i\_upper)$ | PSC | Randomly selected expert opinion from expert $i$ on the probability that a herd owner/manager will request a veterinarian to investigate an infected animal observed to have been showing clinical signs of AHS* |
| Probability of sample being taken for AHS testing | $P_{SAMP}$ | $Pert\ (i\_lower, i\_most\ likely, i\_upper)$ | PSC | Randomly selected expert opinion from expert $i$ on the probability of a veterinarian obtaining a sample from a horse whose owner requested an investigation for* |

†Only outbreaks where subclinical cases were detected and reported on are included here – namely 2011, 2014 (both outbreaks) and 2016

*Expert opinion is area-based and random selection of an expert for his/her opinion is performed for each calculation based on where the relevant herd is situated. Experts gave a most likely, a lower and an upper estimate for each probability

AHS – African horse sickness

## Herd and animal design prevalence

The probability that a herd is infected ($P_H^*$) was estimated as 1%. This was based on the herd-level prevalence from described AHS outbreaks in the AHS controlled area between 1999 and 2016 assuming an underlying herd population of 1357 herds (Table 11). In this period an average of 18 herds were affected per outbreak (Grewar et al., 2019; Weyer et al., 2016; WCDOA unpublished outbreak data). For animal-level prevalence ($P_A^*$): since the herd size throughout the AHS controlled area is relatively small (Table 11), using a percentage based $P_A^*$ was not meaningful, and the animal detection level was set as an integer value of one infected animal per herd. The design prevalence set for the study was, therefore, one animal in 1% of herds which translates to one infected animal in approximately two infected herds within the FZSZ_CZ and PZ and nine infected herds within the FZSZ_NonCZ.

## Probability of detection

The basis for the probability of detection for individuals across all three surveillance components was underlined by the sensitivity of the RT-qPCR ($PCR\_Se$) used routinely for surveillance and investigation in the AHS controlled area. This was modelled as a $Beta(9.65, 1.19)$ distribution as previously defined (Sergeant et al., 2016). For the SSC and POSC, the animal level sensitivity $SeU$ is equivalent to $PCR\_Se$.

For the PSC four detection nodes define the probability that samples from infected horses were presented for testing for AHSV. The first was the probability of clinical signs being exhibited by an infected horse ($P_{CLIN}$). This probability was modelled as Beta distributions based on clinical case proportions from outbreaks in the AHS controlled area where subclinical cases had been detected – namely the 2011, 2014 (two separate outbreaks) and 2016 outbreaks. The four Beta distributions were based on the Bayesian estimate of a population proportion (Vose, 2008) where cases showing clinical signs ($c$) are successes of $n$ outbreak cases. A random selection from any of the four distributions was made to inform $P_{CLIN}$ for each iteration of the model.

Expert opinion was elicited to establish the likelihood that these infected horses, that are showing clinical signs, will be detected by owners/managers ($P_{OBS}$), investigated by a veterinarian ($P_{INV}$) and sampled for testing for AHS infection ($P_{SAMP}$). Experts were

selected based on the primary investigator's knowledge of equine veterinarians working the AHS controlled area and included both private practitioners (n=9) and regulatory veterinarians (n=3) with experience in the equine field. Opinions were obtained through structured telephonic interviews where responses were independent of other experts. Each expert gave opinion relative to the surveillance area/s in which they confirmed they had a reliable opinion, and each opinion included the expert's minimum, most likely and maximum estimate of the probability described. Expert opinion probabilities were not aggregated but rather an individual opinion was randomly selected, with replacement, for each model iteration from the pool of opinions relative to the underlying surveillance area. The selected opinion was converted into a Pert distribution with the expert's minimum, most likely and maximum correlating to the same values within the Pert distribution, and a random value from this distribution was extracted per iteration. Supplementary table 1 (Annexure 7) gives the raw expert opinion data obtained while Table 13 gives the summarised outcome. The animal-level sensitivity ($SeU$) for the PSC was calculated as the product of $P_{CLIN}$, $P_{OBS}$, $P_{INV}$, $P_{SAMP}$ and $PCR\_Se$.

**Table 13**: Expert opinion summary of the probabilities of the observation of clinically ill horses, the investigation of these horses and the probability of sampling with the goal of testing for African horse sickness. The median and range of probabilities given are shown for the minimum estimate, the most likely estimate and the maximum estimate given by experts. The estimates are categorised by the applicable surveillance area under evaluation.

| Surveillance evaluation area | Number of expert opinions elicited | Model parameter | Median and range of probabilities obtained | | |
|---|---|---|---|---|---|
| | | | Minimum estimate | Most likely estimate | Maximum estimate |
| FZSZ_CZ | 4 | $P_{OBS}$ | 0.625 (0.5-0.8) | 0.8 (0.8-0.94) | 0.93 (0.9-1.0) |
| | | $P_{INV}$ | 0.65 (0.5-0.9) | 0.8 (0.7-0.98) | 0.95 (0.8-1.0) |
| | | $P_{SAMP}$ | 0.825 (0.7-0.9) | 0.95 (0.8-0.95) | 1.0 (0.9-1.0) |
| FZSZ_NonCZ | 6 | $P_{OBS}$ | 0.5 (0.4-0.8) | 0.725 (0.48-0.95) | 0.945 (0.75-1.0) |
| | | $P_{INV}$ | 0.6 (0.4-0.8) | 0.7 (0.6-0.9) | 0.8 (0.8-1.0) |
| | | $P_{SAMP}$ | 0.825 (0.55-0.9) | 0.95(0.6-0.95) | 1.0 (0.9-1.0) |
| B : PZ | 7 | $P_{OBS}$ | 0.7 (0.1-0.8) | 0.8 (0.7-0.94) | 0.96 (0.8-1.0) |
| | | $P_{INV}$ | 0.6 (0.1-0.9) | 0.8 (0.65-0.95) | 1.0 (0.85-1) |
| | | $P_{SAMP}$ | 0.9 (0.5-1.0) | 0.95 (0.6-1.0) | 1.0 (1.0-1.0) |
| All areas | 17 | $P_{OBS}$ | 0.6 (0.1-0.8) | 0.8 (0.48-0.95) | 0.96 (0.75-1.0) |
| | | $P_{INV}$ | 0.6 (0.1-0.9) | 0.7 (0.6-0.98) | 0.95 (0.8-1.0) |
| | | $P_{SAMP}$ | 0.85 (0.5-1.0) | 0.95 (0.6-1.0) | 1.0 (0.9-1.0) |

FZSZ_CZ: AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone
PZ: Protection zone

### *Probability of introduction*

For calculations where the probability of freedom of a surveillance period was determined a probability of introduction ($Pintro$) was required. This value was estimated from the historical number of outbreaks (n=7) detected in the AHS controlled area between 1 January 1999 and the start of the surveillance evaluation, a total of 210 months. Though the probability of introduction calculated in this manner would decrease during the evaluation, to remain conservative the value at the first surveillance period was used throughout. To establish a realistic input distribution for $Pintro$, the periods at risk were both halved and doubled to establish the upper and lower limits of a Pert distribution, $Pert(0.017,0.033,0.067)$, from which a random value per iteration was extracted (Alban et al., 2008).

### *Unadjusted herd sensitivity, component sensitivity and component probability of freedom*

Herd-level sensitivity was estimated based on the equation adapted from (MacDiarmid, 1988) for each surveillance period evaluated using the hypergeometric approximation so that the herd sensitivity for each herd $h$ is:

$$SeH_h = 1 - (1 - SeU \times \frac{n}{N})^d \quad (4)$$

where $n$ is the number of horses screened, $N$ the total number of animals in the herd and $d$ the integer number of infected animals per herd. For herd-level sensitivity $d$ equated to 1.

The unadjusted surveillance component sensitivity is determined through the same equation as the herd sensitivity (Eq. 4) except that, since the sensitivity for each herd varies, $SeU$ is the mean of $SeH$ across all herds and herd level values are used for $n$, $N$ and $d$. For herd-level calculations $d = P_H^* \times N$ rounded up to the next integer and $P_H^*$ is the herd-level design prevalence. As for herd-sensitivity calculations for the PSC, all herds are subject to surveillance so that $n = N$.

The unadjusted probability of freedom for each surveillance component for each surveillance period $t$ was established to estimate the freedom probability each component would result in independent from other components. The probability of

freedom for each surveillance period is dependent on the component sensitivity ($CSe$) and the posterior probability of freedom for the preceding period ($PriorP_{free}$) so that

$$P_{free} = \frac{PriorP_{free}}{1 - CSe_t \times (1 - PriorP_{free})} \quad (5)$$

The prior probability of freedom is revised for each surveillance period to account for the probability of infection exceeding the design prevalence during the surveillance period, through either an increase above the threshold of an undetected existing infection or the introduction of a new infection ($P_{intro}$) so that

$$PriorP_{free_t} = 1 - [1 - Pfree_{t-1} + P_{intro_t} - \left((1 - Pfree_{t-1}) \times P_{intro_t}\right)] \quad (6)$$

For the first surveillance period, an uninformed prior probability of freedom of 0.5 was used.

### *Adjusted overall system sensitivity and overall probability of freedom*

In establishing the overall system sensitivity and probability of freedom we did not assume independence between surveillance components since herds involved in either of the active surveillance programs (SSC and POSC) would be included in the PSC (Martin et al., 2007, para. 5.2). In short: for each surveillance period, we estimated herd-sensitivity (Eq. 4) and the resulting posterior probability of infection ($PostPInf$) for all herds in the PSC, where $PostPInf_h = 1 - Pfree_h$. $Pfree_h$ is calculated using Eq. 5, substituting $SeH_h$ for $CSe$ and $1 - P_H^*$ for $PriorP_{free}$. This process was repeated successively for the SSC and POSC.

The component sensitivity for the PSC ($CSe_{PSC}$) was then estimated in the same manner as previously, assuming independence, while adjusted component sensitivities for the SSC and POSC were estimated substituting mean values of $PostPInf$ for the PSC and SSC, respectively, as shown in Eq. 7.

$$CSe_{SSC,POSC} = 1 - (1 - mean(SeH_h) \times \frac{n}{N})^{mean(PostPInf_h) \times N} \quad (7)$$

The final system sensitivity per surveillance period per surveillance area is calculated by

$$SSe_{adjusted} = 1 - \Pi\,(1 - CSe_i) \quad \textbf{(8)}$$

The system probability of freedom is derived from the system sensitivity similarly to each component (Eq. 5) except the adjusted $SSe$ is used instead of $CSe$. The prior probability of freedom for each period is revised for each time step as in Eq. 6.

### *Sensitivity analysis*

To establish which inputs ($P_{CLIN}$, $P_{OBS}$, $P_{INV}$, $P_{SAMP}$ and $PCR\_Se$) had the largest impact on the system sensitivity ($SSe$), Spearman's correlation coefficients were derived for each combination. Coefficients were depicted in tornado plots (Supplementary figure 1 (Annexure 6)). To evaluate the impact of $P_{intro}$ on the final probability of freedom we estimated the maximum probability of freedom ($Pfree_{equilibrium}$ : equilibrium probability of freedom) from mean $P_{intro}$ and system sensitivity (Watkins, Martin, Kelly, Madin, & Watson, 2009) as

$$Pfree_{equilibrium} = (1 - \frac{P_{intro}}{SSe})/(1 - P_{intro}) \quad \textbf{(9)}$$

where values for $P_{intro}$ and $SSe$ were mean values of the final surveillance period. Permutations of $PFree_{equilibrium}$were established for changing $P_{intro}$values from the simulated mean and for 5% increments between 5-25%.

## Results

### *Probability of freedom*

The final probability of freedom for each surveillance area is shown in Table 14 and is categorised by the overall system and independent component probability of freedom. Figures 16 to 19 show the graphical representation of the changing probability of freedom for both the system and independent components where applicable. Note that the PSC is the only component implemented in the PZ surveillance area; hence the system and component outcomes are equivalent. A median probability of between 98.3 and 99.8% was the final posterior probability of freedom across the controlled area after 24 months. This level had been obtained by the 9$^{th}$, 3$^{rd}$ and 7$^{th}$ period in the FZSZ_CZ, FZSZ_NonCZ and PZ respectively. In general, a plateau of median freedom probability had been obtained throughout by approximately 4 months into the

surveillance. The uncertainty surrounding the median system probability of freedom, as shown in the 95% PI band in Figure 16-19, reached stable levels at approximately the same period as when the final probability of freedom had been achieved.

**Table 14**: Final adjusted system and unadjusted component posterior probability of freedom after 24 months of surveillance after the Paarl 2016 outbreak in the African horse sickness (AHS) controlled area of South Africa

| Surveillance evaluation area | Overall system | | PSC | | SSC | | POSC* | |
|---|---|---|---|---|---|---|---|---|
| | Median | 95% PI | Median | 95% PI | Median | 95% PI | Median | 95% PI |
| FZSZ_CZ | 0.983 | 0.911-0.999 | 0.982 | 0.904-0.999 | 0.271 | 0.171-0.381 | 0.227 | 0.14-0.325 |
| FZSZ_NonCZ | 0.998 | 0.975-1 | 0.998 | 0.972-1 | 0.575 | 0.406-0.716 | NA | |
| PZ | 0.984 | 0.906-1 | 0.984 | 0.906-1 | NA | | NA | |

* The POSC took place in period 9 alone although the value reflects the 24th month posterior probability of freedom
PSC: Passive surveillance component
SSC: Sentinel surveillance component
POSC: Post-outbreak stand-alone surveillance component
PI: Probability interval
FZSZ_CZ: AHS Free and Surveillance zone within 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS Free and Surveillance zone outside of 2016 AHS outbreak containment zone
PZ: Protection zone
NA: Not applicable

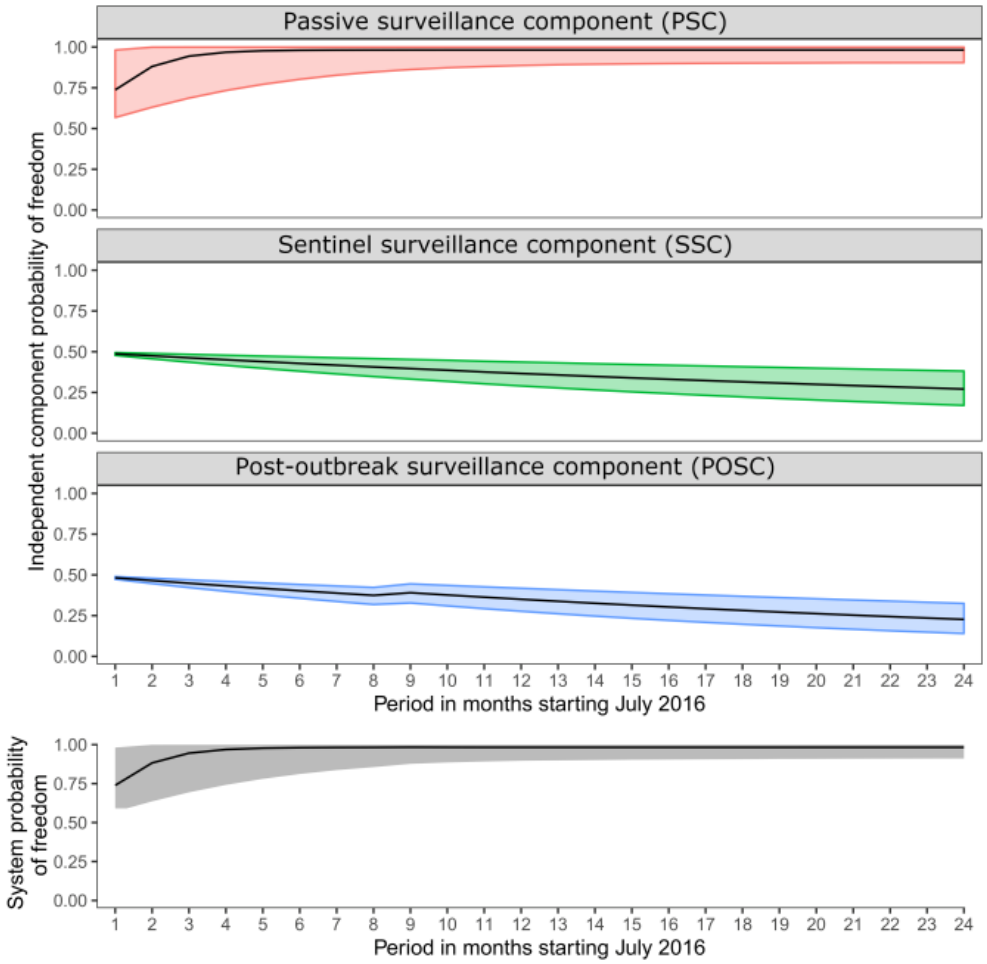


**Figure 16**: Overall system and independent component probability of freedom from African horse sickness in the Free and Surveillance zone within the 2016 AHS outbreak containment zone (FZSZ_CZ) by monthly periods over 24 months starting July 2016. The black line per plot indicates the median probability of freedom with shaded bands indicating the 95% probability interval.

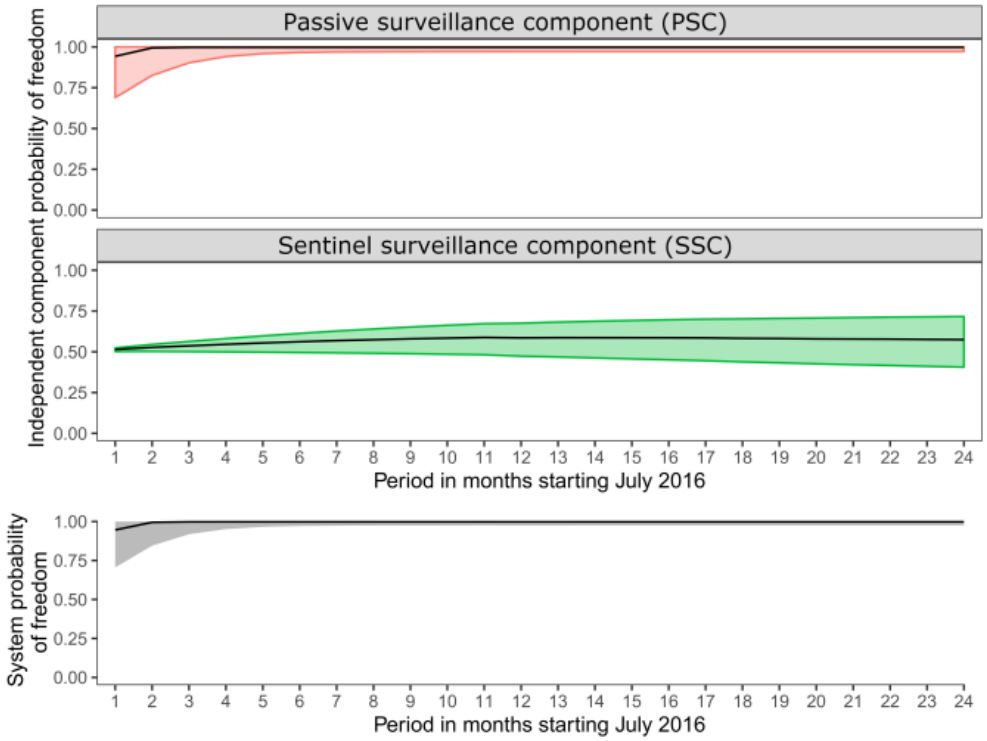


**Figure 17**: Overall system and independent component probability of freedom from African horse sickness (AHS) in the Free and Surveillance zone outside the 2016 AHS outbreak containment zone (FZSZ_NonCZ) by monthly periods over 24 months starting July 2016. The black line per plot indicates the median probability of freedom with shaded bands indicating the 95% probability interval.

The high levels of freedom probability attained by the PSC are reflected in the system outcome, and this component is the driver of the overall system probability of freedom. The SSC independently did not provide a probability of freedom much above the prior probability of freedom of 50% for the FZSZ_NonCZ, and for the FZSZ_CZ this component failed to increase with regards to probability of freedom over time.

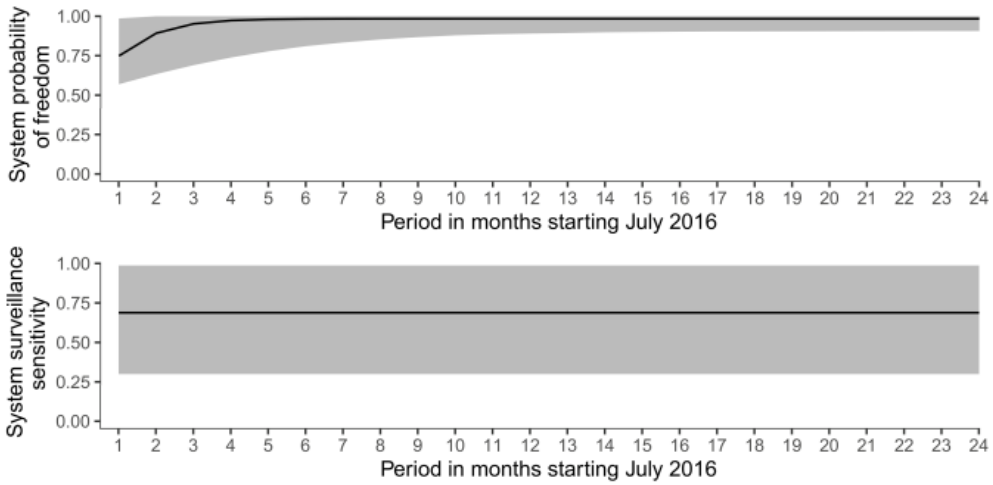


**Figure 18**: Overall system sensitivity and probability of freedom from African horse sickness in the Protection zone (PZ) by monthly periods over 24 months starting July 2016. The black line per plot indicates the median sensitivity and probability of freedom with shaded bands indicating the 95% probability interval.

### *Surveillance sensitivity*

The sensitivity of surveillance for the PSC remains constant in each surveillance period for both the system and independent components, this since the evaluation used a fixed herd-level population throughout. The PSC had consistently higher median surveillance sensitivities when compared to active components in the same area (Figures 19 and 20) and this drives the relatively stable system sensitivities throughout. While the median sensitivity of the SSC was higher for the FZSZ_NonCZ compared to the FZSZ_CZ, the sensitivity of this component, in general, had low sensitivity at levels below 15%. The only perceptible difference that the POSC had on the results was a slight improvement in the 2.5% lower probability level of the system sensitivity of surveillance in the month the survey was performed (Figure 19 period 9).

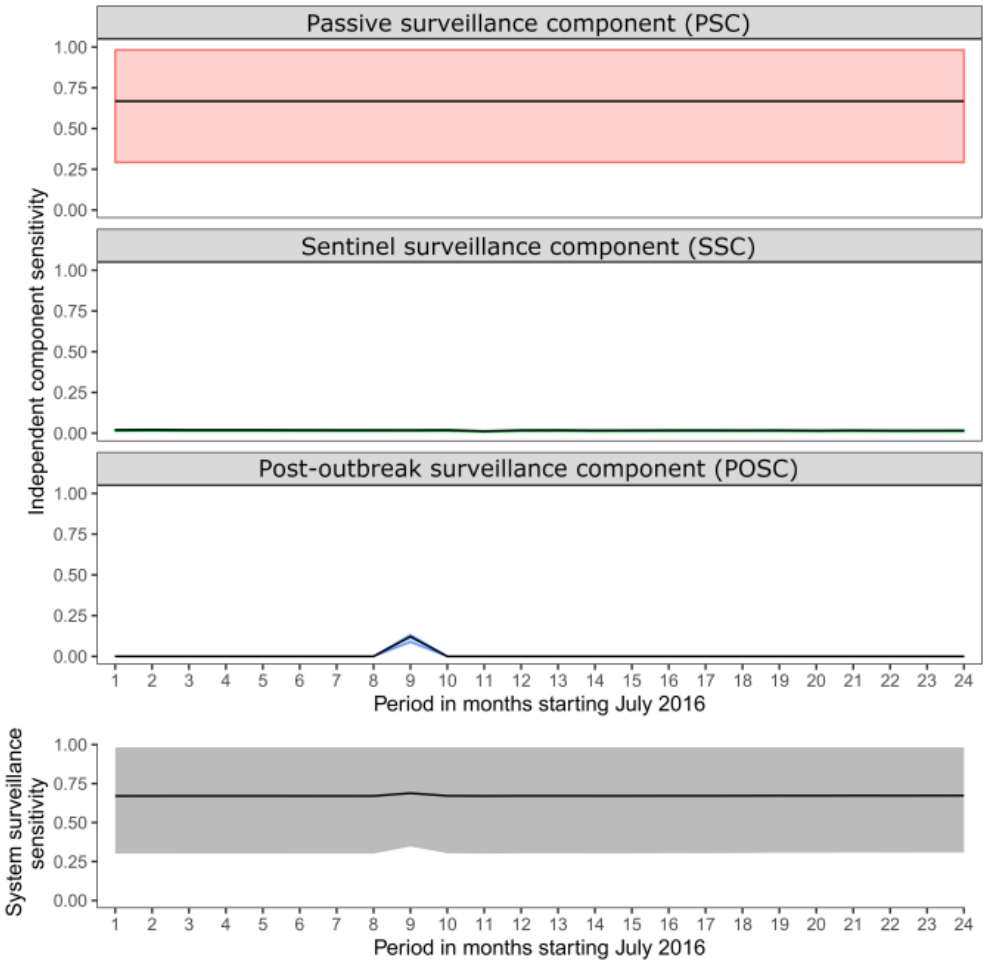


**Figure 19**: Overall system and independent component sensitivity of surveillance in the AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone (FZSZ_CZ) by monthly periods over 24 months starting July 2016. The black line per plot indicates the median sensitivity with shaded bands indicating the 95% probability interval.

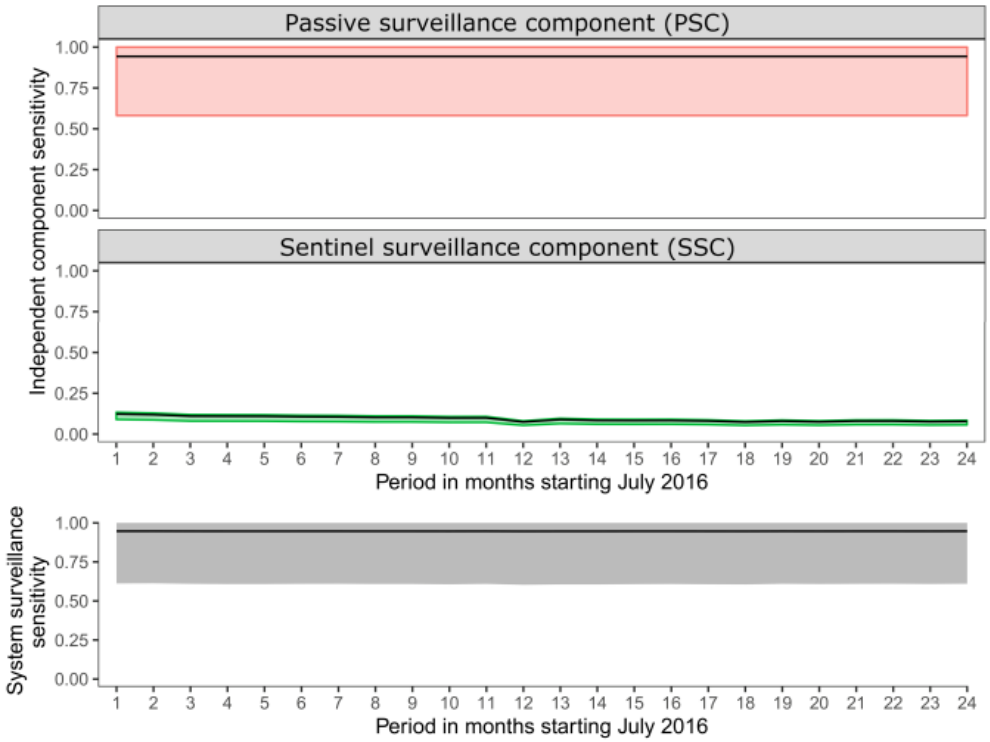


**Figure 20**: Overall system and independent component sensitivity of surveillance in the AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone (FZSZ_NonCZ) by monthly periods over 24 months starting July 2016. The black line per plot indicates the median sensitivity with shaded bands indicating the 95% probability interval.

### *Sensitivity analysis*

Tornado plots of the Spearman's correlation coefficients are depicted in Supplementary figure 1 (Annexure 6). The probability that horses showed clinical signs was the factor that influenced system sensitivity most with coefficients of 0.904, 0.928 and 0.935 for the FZSZ_NonCZ, FZSZ_CZ and PZ respectively. The expected maximum probability of freedom is depicted in Table 15 for each surveillance area and with varying inputs of $P_{intro}$.

**Table 15**: Expected maximum probability of freedom ($PFreeEquil$) in reference to the simulated mean probability of introduction ($Pintro$) and system sensitivity ($SSe$) as well as for changing values of probability of introduction between 5% and 25%

| Surveillance evaluation area | Actual simulation values | | | Evaluation of changing *Pintro* values | | | | |
|---|---|---|---|---|---|---|---|---|
| | mean *SSe* | Mean *Pintro* | *PFreeEquil* | 5% | 10% | 15% | 20% | 25% |
| FZSZ_CZ | 0.679 | 0.036 | 0.982 | 0.975 | 0.947 | 0.917 | 0.882 | 0.842 |
| FZSZ_NonCZ | 0.909 | 0.036 | 0.996 | 0.995 | 0.989 | 0.982 | 0.975 | 0.967 |
| PZ | 0.691 | 0.036 | 0.983 | 0.976 | 0.950 | 0.921 | 0.888 | 0.851 |

FZSZ_CZ: AHS Free and Surveillance zone within the 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS Free and Surveillance zone outside the 2016 AHS outbreak containment zone
PZ: Protection zone

## Discussion

### *Probability of freedom and surveillance sensitivity*

Our model provides simulated results for specific surveillance areas which were defined by different combinations of surveillance components. The estimates show high posterior probabilities of freedom throughout the AHS controlled area in the 24 months succeeding an AHS outbreak. The passive surveillance component drives the high estimates of the system probability of freedom. The practicality of this has been shown through the historical detection of outbreaks in the AHS controlled area where all outbreaks, since 1997, have been detected through passive surveillance. The primary reason passive surveillance has such a high comparative impact on final system outcomes is that every horse and every herd contribute to this component which drives up the herd, component and finally system sensitivity, and hence probability of freedom. The probabilities within the passive component which may decrease its effectiveness are those that influence whether infected horses show clinical signs and whether clinically suspect affected horses are identified, investigated and tested for AHS. Clinical signs of AHS can include fever, pulmonary distress, subcutaneous oedema (primarily of the head and neck) and death in severe cases. Signs are, however, generally not pathognomonic (Coetzer & Guthrie, 2004), but the clinical nature of AHS does make it a disease that is conducive to passive surveillance. The passive surveillance probabilities, based on expert opinion, were generally high. This illustrates the advantage of having a well-defined legislated disease control zone and where a high level of contact occurs between veterinarians, the public and regulatory officials as a result of regulations surrounding AHS vaccination and movement control. The higher estimates of the model in the FZSZ_NonCZ occur as a result of the higher number of horses and herds in this area compared to the other surveillance areas considered.

The active surveillance components generally had low sensitivity and final probability of freedom outcomes. For instance, by the time the POSC survey was performed in period nine in the FZSZ_CZ the median and 95% probability interval of probability of freedom in that area were almost at stable levels. It should be noted that the active components were not designed at the design prevalence evaluated here and both were

designed assuming a single homogenous population (single-stage design). Theoretically one could remove the active components from the surveillance programs for the AHS controlled area and estimates of sensitivity and probability of freedom would represent those of the passive component alone. The resources required for active surveillance are substantial – the POSC cost approximately 15 500 USD (R210 000) while the sentinel surveillance programme costs approximately 105 000 USD (R1.476 Million) per year (Grewar et al., 2017, 2019). If these resources were spent on further improving passive surveillance, and in particular the probability of clinically suspect horses being presented for AHS testing, the surveillance programme would be simplified without losing sensitivity. In general improvements along the passive surveillance scenario pathway would be best achieved through improved communication and education of horse owners, veterinarians and laboratories involved in AHSV testing, with ensuring capacity for testing in the latter also an important consideration. The practical consequences of utilising a passive surveillance program alone would, however, need to be carefully considered and further studied. It is likely that, by simply performing active surveillance, the sensitivity of the passive surveillance program is improved by raising awareness through dissemination of disease and surveillance information and results to stakeholders.

It is likely that indigent communities have limited access to affordable veterinary care and this is likely to decrease the effectiveness of passive surveillance. Two horse sub-populations in communities in the AHS controlled area where this may be evident have been associated with AHS outbreaks in the recent past – Mamre in 2011 (Grewar et al., 2013) and Saron in 2014 (Weyer et al., 2016). In these communities, the Government veterinary service plays an integral role in passive surveillance, through the work of animal health technicians, in order to avoid non-reporting of clinical signs associated with AHS. While the use of probability distributions for the expert opinion detection nodes in the scenario tree accounts for uncertainty of these surveillance events, further investigation of sub-populations of both equines and their owners would provide additional certainty to the evaluation of the passive surveillance component. If specific sub-populations were present these could be included as separate risk categories in the analysis.

The decision to evaluate AHS surveillance for two years was not arbitrary. This period is applicable in both EU and OIE legislation relating to the period of trade suspension or AHS freedom status in the post-outbreak period respectively (EC, 2010 Article 2(f); OIE, 2016, 2018). Our results show that, at least for the probability of freedom based on surveillance, the 24-month posterior probability of freedom is attained well within 12 months during the post-outbreak period. The seasonality of outbreaks does have relevance, however. Outbreaks in the controlled area of South Africa have occurred in late summer and early autumn. This implies that the first few months of the post-outbreak period occurs in winter where the likelihood of transmission of AHSV is decreased due to the impact cold weather has on both vector proliferation, biting rates and virus replication within the vector (Backer & Nodelijk, 2011; Meiswinkel, Venter, & Nevill, 2004).

The sensitivity analysis showed that the probability that a horse shows clinical signs of infection is an important component of the model. The observed variability in $P_{CLIN}$ is due to the variability in the clinical expression of disease in the outbreaks used to model this parameter (which varied considerably). This variability is likely due to the fact that these outbreaks (2011, 2014 (n=2) and 2016) were due to reversion to virulence and/or reassortment of live attenuated vaccine strains (Grewar et al., 2019; Weyer et al., 2016), with variable virulence, depending on the nature of the reversion and/or reassortment. We would expect outbreaks due to wild strains of virus would generally have high values for $P_{CLIN}$, and therefore should be more easily detected. Subclinical infection does not imply that no clinical signs are present but rather that they are below the threshold of normal detection. Public education of the clinical presentation of AHS would lower this threshold. Increasing the probability of introduction of AHS into the different zones only had a substantial effect in the FZSZ_CZ and PZ where the average surveillance sensitivity was 68 and 69% respectively. Still, however, in these areas, an increase in the probability of AHS introduction to 20% and above (from the simulation mean of 3.6%) was required to bring the maximum probability of freedom down to below 90%.

### *Model considerations*

This evaluation considers the domestic horse population in the AHS controlled area and does not include donkeys or wild equids such as zebra. Zebra do exist in the AHS Surveillance and Protection zone and constitute 8.9% of the known equid population in the controlled area. Donkeys, while not explicitly excluded from active surveillance, make up a known total of 0.7% of the equid population. In our opinion, the exclusion of these species does not make a substantial difference to the evaluation. We further believe the domestic horse population is representative enough act as a proxy for any outbreaks occurring in other species where spill-over to the domestic horse population is likely to occur given the vector-borne nature of transmission. Recently it has been shown that the plains zebra (*Equus burchelli*) populations in the Western Cape Province, and in particular within the AHS controlled area, are unlikely to be large enough to allow persistent AHS infection (Porphyre & Grewar, 2019). Surveillance data from these populations would, however, be beneficial to provide a more complete surveillance picture. An analysis of proximity of zebra and/or donkeys to domestic horses would provide further insight into the validity of our assumptions.

The extraction of the underlying population at risk at a single point in time is unlikely to have much impact on overall results. Changes in herd sizes will have no impact on passive surveillance components and only a minor impact on the sentinel surveillance component given that the underlying animal detection prevalence was one infected animal per herd. Changes in the number of herds is also likely to only have a minor impact on any of the components. Based on our personal experience, the demographics of the equine population in the AHS controlled area, both spatially and in terms of numbers of individuals and herds, is unlikely to have changed substantially prior to and during the period analysed.

The choice of the surveillance unit in this study was the individual horse. In research using a similar process, the passive surveillance component is often evaluated at a herd level. Horses are generally not considered a production animal and, even where they are kept for production purposes, such as in the breeding industry, each horse is generally individually identified and their care is very individually intensive. Furthermore, for the active surveillance components, individual horses are considered

the surveillance unit and expert opinion that was obtained for the associated detection nodes of the PSC was elicited on an individual horse basis. Evaluating surveillance at individual animal level assumes independence between horses in the same herd and probabilities do not change where multiple cases occur. Our approach is a conservative one due to the choice of a single horse as the within-herd design prevalence, rendering issues of lack of independence of horses within herds irrelevant.

Both the active surveillance components have a degree of selection bias. The SSC animals are selected based on their prior vaccination status since sentinels are not recruited if they are vaccinated against AHS within the preceding two years. The POSC sampling frame was reliant on the census taken during the 2016 outbreak (Grewar et al., 2019). We do not believe that this selection bias has a substantial influence on the component analysis and since the PSC was the main driver of system outcomes this is not considered an important issue.

Scenario-tree analysis of surveillance activity forms just a part of surveillance evaluation. While the outputs presented provide a quantitative estimate of the surveillance sensitivity and probability of freedom over a period of time, there are other factors which influence the ability of surveillance to detect disease. Well-described frameworks for the evaluation of surveillance activities in animal health have been published (Calba et al., 2013; Cameron et al., 2014; Comin et al., 2019; Drewe et al., 2015; Hoinville et al., 2013; Muellner et al., 2018); the results of this study would be best contextualised within one of these frameworks to provide a more holistic evaluation of AHS surveillance in the controlled area of South Africa.

## Conclusion

Our results show that, if AHSV was circulating at a minimum prevalence of one infected animal in 1% of herds, the median probability of freedom from AHS in the AHS controlled area after the 24-month post-outbreak period was between 98.3% - 99.8%. The final median probability of freedom had been realised by the 9$^{th}$ month after the 2016 outbreak had been resolved, with a plateau in the probability of freedom obtained by approximately the 4$^{th}$ month across the region. The high level of probability of freedom was driven primarily by the passive surveillance component.

A two-year post-AHS outbreak period is the global standard for the lifting of trade suspension or regaining AHS freedom for affected zones or countries. Our work shows that if surveillance is undertaken in a manner that provides realistic estimates of freedom, the two-year period should be reviewed. We would recommend that a re-evaluation of freedom from AHS should be permissible from 6 months after an outbreak has been resolved. Additional confidence in freedom can be provided if a period of low vector abundance has elapsed in the interim.

We have shown that the relative benefit of active surveillance components is minimal if passive surveillance is undertaken in a focussed and measurable manner. We further conclude that, while active surveillance will remain a feature of AHS surveillance and control, resource allocation to activities supporting and developing passive surveillance for the disease would be justified. This would be even more applicable in countries or zones where vaccination is either not permitted or is used in limited areas during outbreaks so that clinical expression of an outbreak is not masked by high herd immunity.

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## Conflict of Interest

South African Equine Health and Protocols NPC is a registered non-profit company in South Africa (registration number 2017/528099/08). It is privately funded and the funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

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# Chapter 6

## General remarks, discussion and conclusions

## Introduction

In this section some surveillance specific remarks and additional results of the first study are presented that were not included in the publication associated with Chapter 3. Further discussion on some of the decisions that were taken when evaluating the surveillance for AHS in the post-outbreak period is also presented. While describing the 2016 AHS outbreak in the AHS controlled area of South Africa and the post-outbreak surveillance undertaken it has become evident that some opportunity exists to do further research in this field and some of these opportunities are highlighted.

## Surveillance during the 2016 outbreak

### *Sampling frequency*

Sampling and surveillance during outbreaks of emerging, re-emerging or exotic diseases are more likely to be focussed on the suspect or positive properties in comparison to negative properties. The surveillance undertaken during the 2016 AHS outbreak illustrates this. During that outbreak, an average of four sample events per affected property was undertaken. The multiple sampling of negative properties (both clinically and sample-based) was not, however, logistically possible. In this circumstance, under-detection of cases during outbreaks is likely to occur. This is particularly true for diseases where subclinical infection is present, such as AHS. While this might not affect the positive/negative status of an affected area, it does impact on the proportions established from the outbreak analysis. As described in this thesis, the animal and herd-level disease proportions that are observed during outbreaks are used in designing and evaluating future surveillance activity, and it is important to estimate them as accurately and realistically as possible.

Active surveillance during outbreak mitigation procedures can theoretically be undertaken to account for the underestimation of the affected proportion. However, for every resource that is spent on non-affected holdings, the ability to understand and mitigate risk from known positive holdings decreases. Figures 21 and 22 show planning maps used during the 2016 AHS outbreak to allocate resources to surveillance during that outbreak (WCDOA – unpublished information). A primary team was allocated to the 3 and 5 km buffers surrounding the primary case (orange and yellow bands in

Figure 21 respectively), while surveillance from the south and north was targeted to known equine holdings working inwards towards the index case. Finally, there was a focus leading away from the index case in a south-westerly direction, also targeting an area of known populations. New surveillance targets needed to be established once cases occurred beyond an 8km zone surrounding the index case (orange lines in Figure 22). While it is not evident in the image, the orange lines follow major secondary roads in the area. As negative properties were confirmed, either through laboratory or clinical surveillance, the surveillance effort invariably moved on to the next holding in the planned surveillance lines. As infection spread, the area that required active surveillance increased substantially, increasing the resource required to survey new holdings rather than previously surveyed holdings.

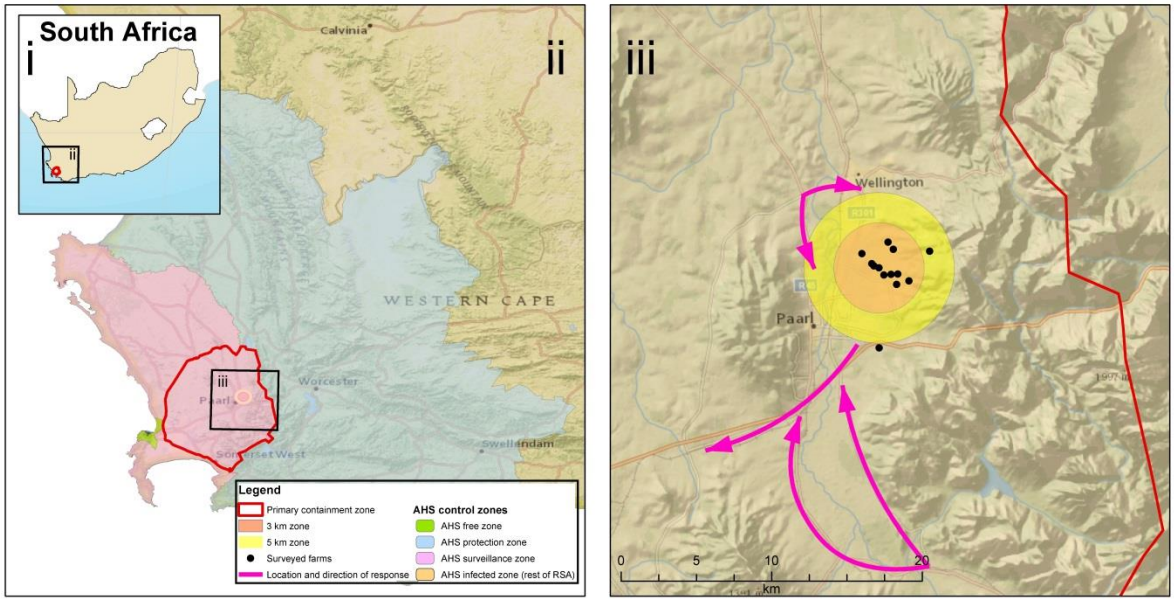


**Figure 21**: Surveillance planning map from the 2016 African horse sickness (AHS) outbreak – 14th April 2016, showing the containment zones and location and direction of response. Source: WCDOA outbreak repository and created by the author

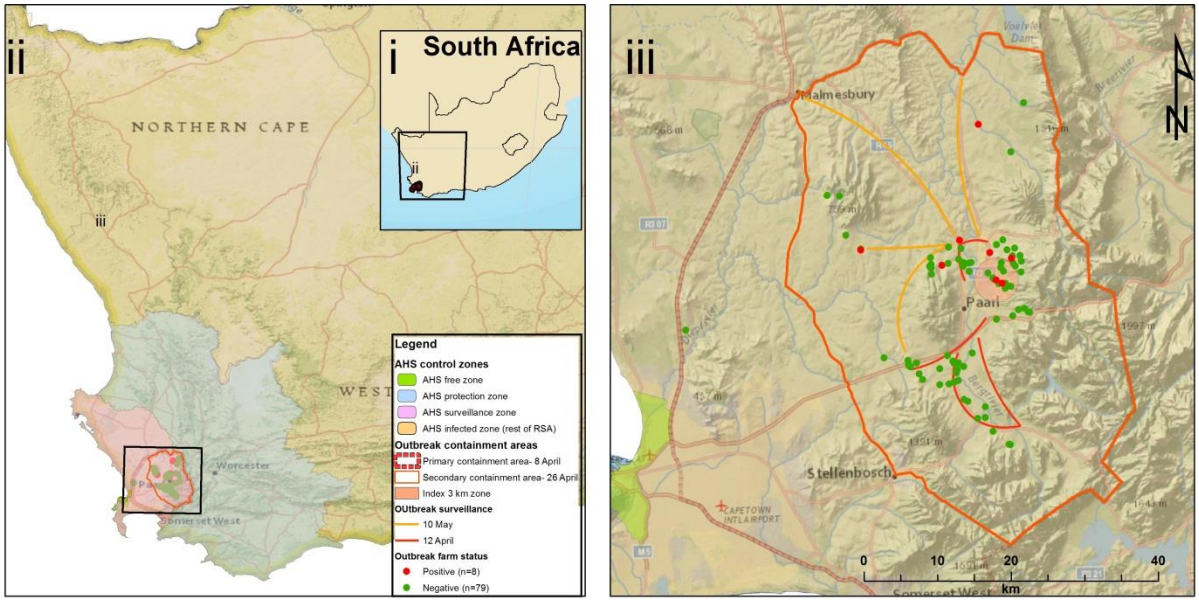


**Figure 22**: Planning map from the 2016 African horse sickness (AHS) outbreak – 8th June2016. Source: WCDOA outbreak repository and created by the author

Surveillance during outbreaks does however include passive surveillance by owners/managers of horse holdings. If some effort is made to ensure that owners and/or managers are aware of an outbreak of AHS and realise the benefit of detecting cases early, this component of surveillance can decrease the under-estimation of case proportions.

### *Equine encephalosis as a surveillance proxy for African horse sickness*

Equine encephalosis (EE) is, like AHS, an arboviral infection of equines with the Orbivirus (EEV) transmitted by *Culicoides* midges. In contrast to AHS, EE is generally a mild disease, with recovery in most cases. Clinical signs may include anorexia, pyrexia, mucous membrane congestion and icterus, and clinically AHS should be considered as a differential diagnosis in horses showing signs of EE. The mortality rate of EE is estimated at 5%. (Coetzer & Guthrie, 2004; Howell, Guthrie, & Coetzer, 2004). EE occurred concurrently to AHS during the 2016 outbreak and this provided an opportunity to evaluate differences in disease epidemiology for these diseases in the same environmental and outbreak setting. This was possible since the laboratory testing for AHS during the outbreak in 2016 (Equine Research Center – University of Pretoria) tested for EEV concurrently with a PCR developed in South Africa (Rathogwa

et al., 2014). AHS sentinel surveillance samples were at the time routinely tested for EEV in the months leading up to the outbreak.

A total of 781 unique horses from the outbreak containment zone were tested for EEV between January 2016 and 1 July 2016. Cases of EE had been detected in the months leading up to the AHS outbreak (Figure 23) with the EE and AHS epidemic curves both spiking in late April and early May. A total of 67 EE cases were detected on 26 properties (Figure 24) out of a tested population of 430 horses on affected properties resulting in a horse-level affected proportion for the first half of 2016 of 0.16 (95% CI: 0.12, 0.19). The crude property-level EE affected proportion was 0.27 (95% CI: 0.18, 0.37). Four properties within the outbreak containment zone had a concurrent infection of both AHS and EE. These four properties accounted for 67% (n = 14) and 37% (n = 25) of AHS and EE cases respectively. Only one horse had a co-infection of both AHS and EE. The majority of EE cases occurred during the same time as the AHS cases occurred (Figure 23), and although all EEV testing for the first half of the year was considered, EE cases only started occurring in early March with the final EE case in early June.

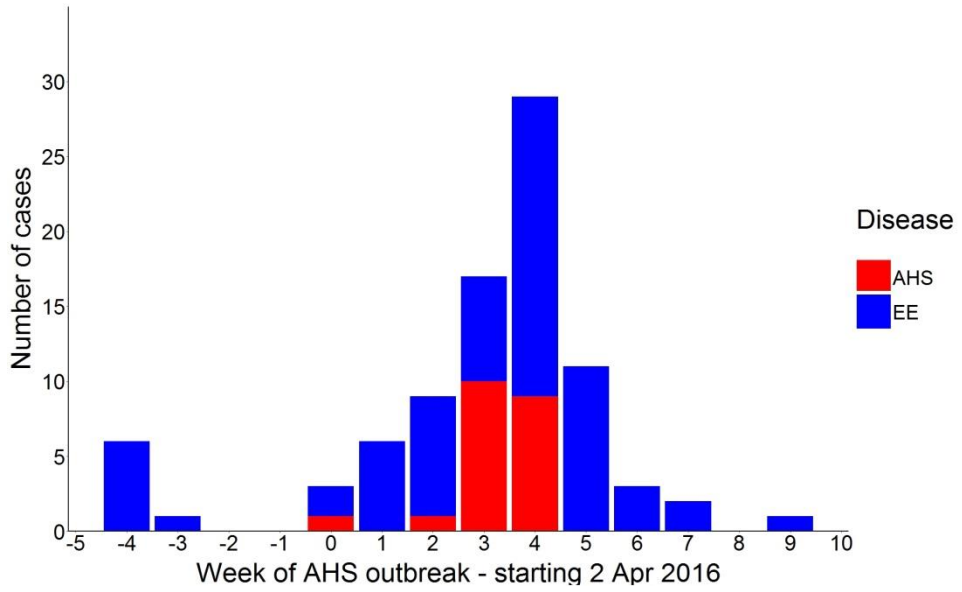


**Figure 23**: Outbreak epidemic curve for both African horse sickness (AHS) and equine encephalosis (EE). Week zero indicates the first week of the AHS outbreak starting 2 April 2016. All cases of EE detected within the AHS outbreak containment zone between 1 January and 1 July 2016 have been included.

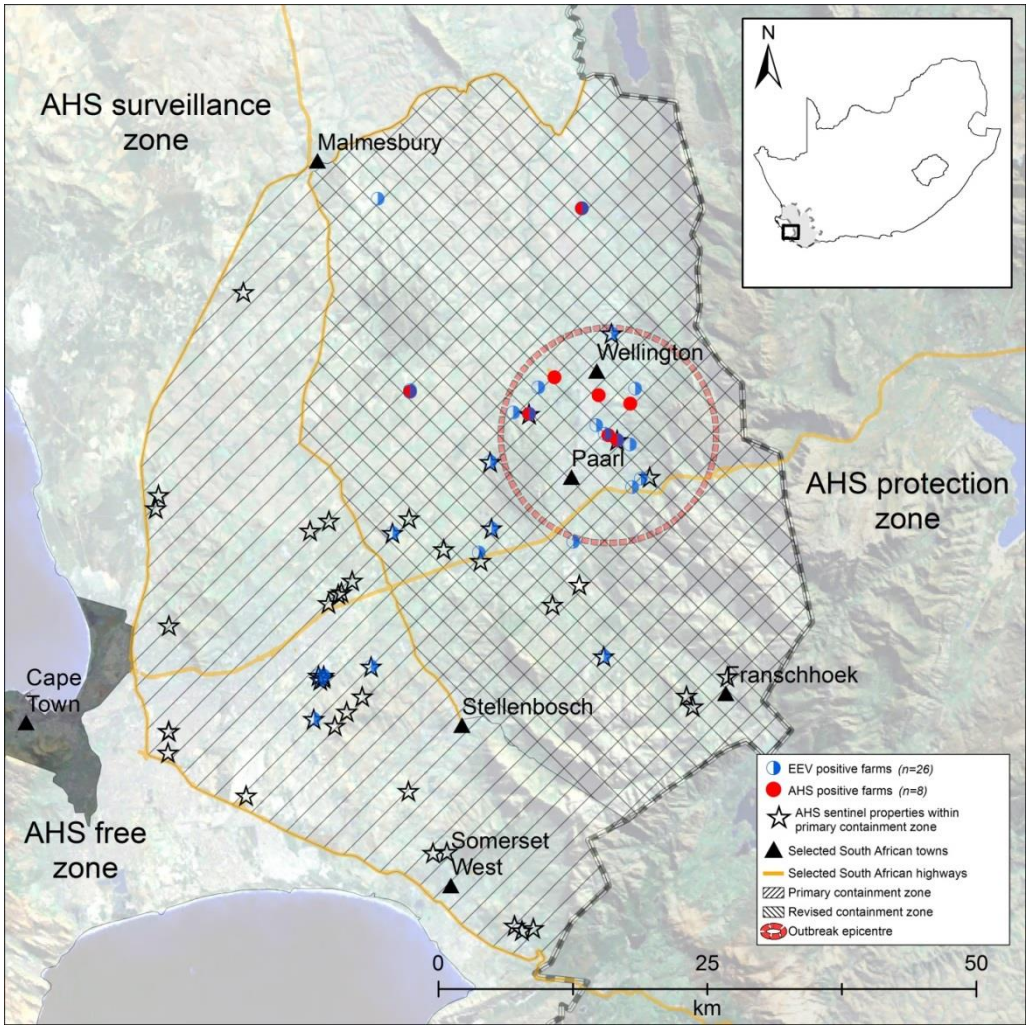


**Figure 24**: A map of the outbreak and surrounding area depicting the positive African horse sickness (AHS) and equine encephalosis virus (EEV) infected properties as well as the sentinel properties within the outbreak containment zone. The epicentre was defined as a 10 km radius around the index property.

There are benefits of performing parallel surveillance of both AHS and EE. The success of case detection for EE provides a control for negative testing of AHS since the epidemiology of these diseases is very similar. An example of this is the detection of positive cases of EE prior to the outbreak (Figure 23) provides evidence that the index case detected of AHS is likely to be close to or the true index case of the outbreak (as discussed in Chapter 3 this is also supported by the time gap seen between the first and second cases of AHS detected during the outbreak). The final AHS case is also more likely to have been accurately identified since surveillance for EE showed cases occurring after the last AHS case. This implies that surveillance activities were successful in their ability to detect a similar virus should it exist, and therefore

provides confidence in closing the outbreak after an OIE appropriate 40 day period after the final case.

The spatial extent of the outbreak defined by the EE cases (Figure 24) provided evidence that the initial containment zone included events with known transmission of a virus showing very similar epidemiology to AHSV infection.

During the outbreak, EE was also included in the testing of the midge pools collected from one of the affected properties. While vector testing for disease is not particularly sensitive, one pool of midges tested positive for EEV RNA which led to an estimate of the infection prevalence of EE in field-collected midges of 0.00016 (95% CI: 0, 0.0008). This provides a basis for what levels are likely for AHSV in similar circumstances.

Finally, the disease proportions established relating to EE were consistently higher for all tested categories compared to AHS. With EE there is an advantage that prior vaccination against the disease does not play a role in the dissemination of the disease. This is because a vaccine is not available for this disease in South Africa. The control measures implemented for AHS during an outbreak will, however, also plausibly mitigate the spread of EEV. Cases of EE are regularly detected within the AHS control area (Grewar, Thompson, Lourens, & Guthrie, 2015; Howell, Nurton, Nel, Lourens, & Guthrie, 2008) so the population is not naïve. The more natural progression of EE does, however, provide a more accurate basis for epidemiologic predictions for future AHS outbreaks in unvaccinated populations.

## Considerations taken for evaluation of AHS surveillance

### *Design prevalence decision*

The setting of surveillance design prevalence for herd and animal-level is one of the key decisions that drive sample size determination for freedom from disease surveillance. Design prevalences were required for both chapters 4 and 5, and in the former, a range was used to evaluate surveillance for a variety of scenarios to assist in providing realistic outputs of the probability of freedom. The choice made prior to that stand-alone surveillance activity was based on the expected prevalence that was determined by the actual prevalence values that were experienced during the Paarl

2016 outbreak. A conservative approach was taken with an overall outbreak prevalence determined by the total outbreak cases as a percentage of the entire population at risk in the controlled area that was surveyed/censused during the outbreak. This resulted in a design prevalence of 1%. During the post-outbreak evaluation, a two-stage process was undertaken which was dependent on both within-herd and between-herd design prevalences. To provide a range of possible surveillance outcomes, three different inputs were used – a generic option that reflected the effective 1% design prevalence (so that herd-level prevalence multiplied by animal –level prevalence in affected herds would equate to 1%); a 2016 outbreak estimate based on actual 2016 outbreak data and then an average of between herd and within-herd prevalences as reported from AHS outbreaks reported between 1997 and 2016 in the AHS controlled area.

For the scenario tree evaluation (Chapter 5) accounting for passive, sentinel and the stand-alone freedom from disease survey, a design prevalence (1 infected animal in 1% of herds) was based on a conservative estimate using prior outbreaks as a guide. In this case, however, the population at risk was all herds in the controlled area. Furthermore, because the animal level prevalence was less than 1% and most herds had less than 100 animals in them, keeping the animal level design prevalence as a proportion was nonsensical. A single animal per herd affected was therefore chosen as the animal level design prevalence. Cameron et al. (Cameron, Njeumi, Chibeu, & Martin, 2014) provide a framework for the process of defining a design prevalence that is acceptable to trade partners considering freedom from disease is often linked to this goal. Their suggestions, in decreasing order of preference, are shown in Table 16, including comments relevant to this study.

**Table 16**: Cameron et al. (Cameron, Njeumi, Chibeu, & Martin, 2014) framework for the process of defining a freedom from disease surveillance design prevalence

| Design prevalence determination approach (in order of preference) | Examples and comments | Study based comments |
|---|---|---|
| Global standards | OIE Terrestrial Animal Health Code – Bovine tuberculosis at 0.2% of herds and 50% within herd prevalence. | The OIE does not prescribe set design prevalences for AHS (OIE, 2016) |
| Regional Standards | EU regulations for EU member states – *Trichinella* surveillances requires an animal prevalence of 0.0001% for exemption from examination for holding/.compartment based freedom | AHS does not currently occur in the EU and countries that require freedom from AHS obtain official OIE freedom thus exempting them from ongoing freedom from disease surveillance. Freedom from disease in a post-outbreak context has not, to the best of my knowledge, occurred outside of South Africa in an officially free country/zone. |
| Trading partner requirements | AHS surveillance for trade between South Africa and the EU is an example here – see comment | With regard to the *Regional Standards* point above the EU directive pertaining to South Africa's requirements for sentinel surveillance indicate an implied (they indicate a sample size of 60 sentinels per month) animal level design prevalence of 5% for the Surveillance and Free zones of the country (EU, 2008) |
| Acceptable level of protection (ALOP) | Here the importing country's acceptable risk level determines the appropriate design prevalence for the country of export. Cameron et al. mention that this is a seldom used method given that few (if any) countries publish an acceptable level of risk for specific diseases | To my knowledge no country publishes an explicit quantitative ALOP for AHS |
| Biology | Biological estimates of spread and infectious potential of diseases allow the estimation of design prevalence. | This approach was used in determining the generic herd and within-herd animal level design prevalence in Chapter 4, and effectively the use of historic outbreak prevalence to establish the design prevalence, as was performed both Chapter 4 and 5. Cameron et.al (21014) mention that this is the most common approach, and as long as the estimates are realistic for the disease, and that it its acceptable for both partners in a trade relationship, then it will work well. |
| Arbitrary choice | If no conclusion can be made based on the above options then an arbitrary, acceptable, design prevalence is justified. Most commonly a 1% herd level and 1%, 5% or 10% within herd animal level prevalence is used. | |

### *Vaccination level influencing surveillance*

The AHS surveillance zone was initially established with the purpose of using both passive and active sentinel surveillance to establish freedom from disease and ensure low-risk trade (EU, 2008). It is clear that it was implied that vaccination against AHS in the surveillance zone would be at low levels. This would ensure that a 'normal' AHS outbreak/incursion would result in an outbreak that would be clinically detectable. Vaccination requirements by the EU are that no *systematic vaccination* takes place: if vaccination takes place it must be performed by derogation and permission of State authorities. It became evident that already by 2004 the function of the surveillance zone was being impaired by vaccination. At the time this was not only by vaccination as required for movement control, but also because outbreak control included

vaccination (Sinclair, Bührmann, & Gummow, 2006). This latter principle was in place until the 2014 outbreak when concerns were raised as to the possibility of re-assortment and reversion to virulence of the live attenuated vaccine in use (WCDOA, 2014). To link with the selection of design prevalence discussed here: the biology and epidemiology of AHS will change in a well-vaccinated population, and the spread and incidence one might expect in a naïve population is less likely to occur in a well-vaccinated population. This drives down design prevalence, as discussed by Cameron et al. (2014), in the vaccinated population as currently present in the South African AHS controlled area.

### *Selection of prior probability of freedom*

When evaluating surveillance over time (Chapter 5) the probability of freedom (and by implication infection) after each surveillance period influences these values in the following time period. A selection of an initial prior (for surveillance period 1) must however be made. An argument exists for choosing a primary prior of 0% or 50% (uninformed prior), or somewhere in between these values. When performing an active freedom from disease survey it would be unrealistic if the initial prior probability of freedom is < 50%, implying that the design team concedes that the probability of freedom is less than 50% before starting out on that surveillance activity. If that were the case then the effort would rather be put into controlling the disease. Using an uninformed prior of 50% is still conservative – conceding that an organisation is prepared to do freedom from disease surveillance while still having a 50% chance of having the disease is certainly conservative. Priors would realistically, even in unknown situations, be higher than 50%. This is particularly true in a disease like AHS where seasonal components of the transmission (due to the vector influenced disease patterns), and ongoing passive surveillance ensures a subjective probability of freedom which justifies attempting freedom from disease surveillance.

For the stand-alone freedom from disease survey evaluation both an uninformed prior of 50% and an informed prior of 91.2% were evaluated, with the latter established by an evaluation of that season's sentinel surveillance programme up to that point. That programme's evaluation, however, started with an initial prior probability of freedom of 50% (Grewar & Weyer, 2018).

### *Scope of surveillance*

One of the standard components of surveillance evaluation pertains to the scope or objectives of the surveillance. The frameworks are briefly reviewed in the literature review, but to reiterate, the following four surveillance objectives form the basis of surveillance objectives:

- the early detection of new or emerging diseases;
- freedom from disease;
- case detection and;
- monitoring of disease prevalence/incidence.

The work undertaken in this thesis touches on each one of these objectives for surveillance for AHS in South Africa's controlled area. During an AHS outbreak surveillance is focussed towards case detection to establish spread and provide an epidemiologic understanding of the temporal dynamics of the outbreak. An example for the latter for instance is where evaluating epidemic curves provides information on index case and potential cessation of the outbreak. Monitoring of disease incidence on infected holdings is also performed, if not through active sampling then through passive detection by holding owners/managers and their associated private veterinarian. Post-outbreak period freedom from disease surveillance provides a basis for the resumption of trade and lifting of any control measures that may have been instituted during a survey. Finally, in the case of AHS in South Africa, this surveillance also can be used to survey for re-emergence of the disease as a result of re-introduction.

It remains important that the scope of each surveillance activity undertaken is considered and reported as such. An example is the freedom from disease survey which was undertaken almost a year after the 2016 outbreak (Chapter 3). Here the primary goal there was not to establish whether there was circulating AHSV in March and April 2017 (the sentinel and passive surveillance account for that), but rather to establish that the AHSV1 that was circulating in 2016 was no longer circulating the following year. Another example is where the sentinel surveillance programme is treated as an early detection activity. While evaluation of the sentinel programme is

important in a temporal context, and any aberrant results are followed up on timeously, the programme is designed to provide confidence of freedom over a retrospective period. The passive surveillance component is in place to attain the early detection surveillance objective. Using the frameworks above will assist both industry and Government stakeholders in defining their surveillance programs and ensuring they are fit for purpose.

### Further research opportunities

It would be very useful to evaluate the entire AHS surveillance effort and not just the analytical outcome of surveillance as described above, using results from this thesis and incorporating a chosen framework for animal health surveillance evaluation. This would provide insight into the non-analytical components of surveillance and may highlight further research needs, establish critical control points and potentially highlight fragility within the system. Given the close relationship between the equine industry and the government, a multi-disciplinary team would be required to perform this work. One of the components that was largely omitted in the evaluation above was the economic component of evaluation where cost-benefit of certain surveillance activities was not quantified. It was certainly alluded to that the passive surveillance programme provides adequate confidence of freedom; however, one would need to take into account the loss of legitimacy, particularly from international stakeholders, if active surveillance components were removed.

Surveillance for AHS in South Africa is not limited to equines in the controlled area. Surveillance is undertaken outside of the controlled area which has an impact on the ability to move horses into the AHS controlled area of South Africa – and evaluating this component would also be useful. Furthermore, as highlighted in the OASIS and RiskSUR evaluation frameworks (Calba et al., 2013; Hendrikx et al., 2011) the vector and wildlife components of AHS could also be considered. Active vector surveillance is undertaken at the Kenilworth Quarantine station for instance, and the OVR entomology section does a fair amount of vector surveillance in South Africa. Wildlife surveillance is opportunistically performed in the controlled area but other sources of wildlife data would be worthwhile establishing. Zebra are considered to be maintenance hosts for the ongoing circulation of AHSV where their populations are

large enough to ensure a continual influx of naïve animals – in the South African context this is assumed to be the case only in the Kruger National Park, in the north-eastern parts of the country (Bosman, Brückner, & Faul, 1995). Recently it has been shown that the plains zebra (*Equus burchelli*) populations in the Western Cape Province, and in particular within the AHS controlled area, are unlikely to be large enough to allow persistent AHS infection (Porphyre & Grewar, 2019). Surveillance data from these populations would, however, be beneficial to provide a more complete surveillance picture.

## Concluding remarks

In one sense the surveillance and control for AHS is made simple by the epidemiologic patterns associated with the disease. There is a short incubation period with no carrier status, so outbreak surveillance has an achievable end-point in areas where endemic disease is not present. The vector aspect of the disease results in seasonal patterns of occurrence which allows seasonal vaccination in the AHS surveillance and free zone. This means that vaccination of horses can continue to be used as a control measure for movements into zones of higher control in the country. In terms of control during an outbreak, vector control, through both chemical and physical barriers separating vectors from hosts, can be fairly simple to implement and is likely to decrease outbreak extent and impact. Also, the inevitable winter that follows outbreaks will reduce the ability of the vector to successfully transmit the virus. While subclinical infection does occur and clinical signs of the disease are not pathognomonic, the clinical presentation of cases which will invariably occur during an outbreak does support the use of passive surveillance for the disease. This has been shown historically to be a successful surveillance mechanism in the controlled area and the simulation results from the scenario model certainly support this. The AHSV is blood associated so sampling for the disease during surveillance activity is not complicated or particularly expensive. Finally, the individual nature of horse ownership/management promotes the ability to develop accurate sampling frames for surveillance, and the value of individual animals' results generally in compliance with regulatory requirements in outbreaks. The caveat on the latter point is that

communication of regulatory measures must result in owners perceiving that such measures implemented are likely to be protective in their situation.

Conversely, the epidemiology of AHS also has the impact of making it a challenging disease to control and survey, and it invariably becomes a non-tariff barrier to trade. The high morbidity and case-fatality rates that have been reported in naïve populations make it a threat to non-endemically affected equine populations. The individualistic nature of horse ownership/management increases the perception of risk since owners see this risk of incursion for animals they have a very close bond with. The fact that subclinical infection occurs in horses, and that this is the standard presentation in wild equids or donkeys, results in the need to consider active surveillance, especially in outbreaks in non-endemic environments and where the outcomes of surveillance will be used to develop future surveillance activity. The use of the live attenuated AHS vaccine in the controlled area has resulted in a high proportion of the horses present being vaccinated. The challenge this presents for surveillance is that serological surveillance is hampered given the lack of DIVA competent tests available. This challenge is offset by the real-time PCR that has been developed allowing for parallel testing of sentinels to enhance active surveillance in the AHS controlled area. The vector-borne nature of AHS also presents a challenge to surveillance – disease spread in an outbreak is difficult to predict (although if wind direction is constant it can be predicted) and the combination of intrinsic and extrinsic incubation periods can result in lag times where false negative surveillance, on a system level, may occur, particularly towards the end of an outbreak.

AHS in South Africa results in a significant challenge to the trade in live horses from sub-Saharan Africa. The results of this work, however, show quantitatively that current surveillance within the control area in the Western Cape Province is adequate to provide a solid basis for freedom from disease declarations, and that adequate levels of freedom are likely to occur well within 12 months after any incursion has been resolved. It is important to finally note that surveillance in itself does not imply freedom – it simply enhances the ability to declare freedom.

### *Main conclusions of the study*

- AHS surveillance challenges arise in the South African controlled area as a result of high numbers of vaccinated animals within the population at risk, the seasonality of AHS and limitations of the DIVA capabilities of existing routine laboratory tests.
- Surveillance for AHS in the controlled zone during outbreaks should be balanced between detecting new cases in order to assist control measures while ensuring that accurate prevalence of infection is obtained, since the latter parameters are used in future freedom from disease surveillance activities
- The stand-alone 2017 survey established that the point-in-time probability of AHS freedom ranged between 73.1% and 100%, thus providing evidence of the cessation of circulation in the containment zone of the AHSV responsible for the 2016 outbreak.
- A scenario tree analysis showed that, at a design prevalence of 1 animal in 1% of herds, the median posterior probability of freedom from AHS in the AHS controlled area after the 24-month post-outbreak period was between 98.3% - 99.8%. The final median probability of freedom had been realised by the 9th month after the 2016 outbreak had been resolved.
- Confidence in freedom from disease was largely ensured by the passive surveillance programme, with active surveillance providing minimal additional confidence.
- The results of the study provide the basis for the discussion on the appropriate length of time after incursions of AHS that should pass prior to evidence-based decisions being taken with regard to opening trade. In the AHS context this period is currently set at two years through OIE and EU legislation. We recommend that a re-evaluation of freedom from AHS should be permissible from 6 months after an outbreak has been resolved if adequate surveillance and control measures are in place.

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# Annexures

# Annexure 1: Table S1 from Chapter 3

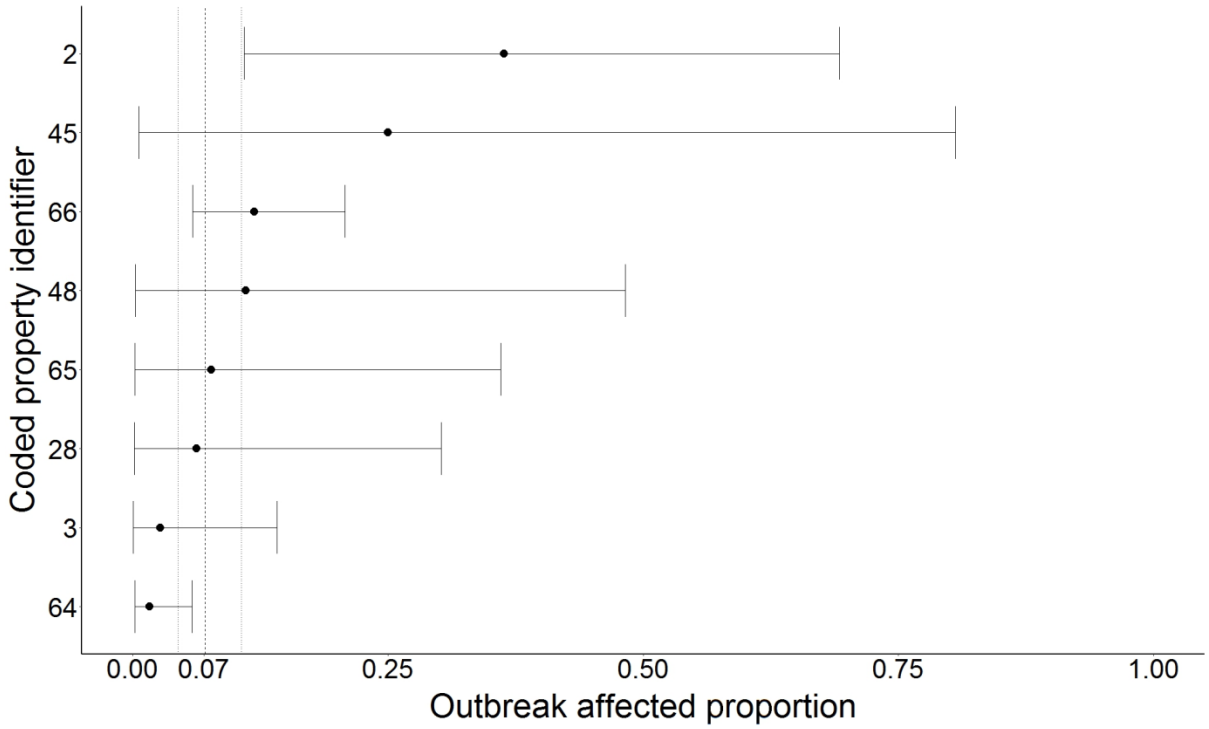
**Supplementary Table 1:** (Table S1 in published manuscript) Individual case clinical picture, detection, demographic, temporal and concurrent infection information for the 21 cases detected during the 2016 AHS outbreak in the controlled area of South Africa.

| Holding ID | Horse ID | Case Date† | Case definition code | Surveillance method used | Approximate age – years unless specified | Sex | Breed | Colour | Death Date† | AHS Vaccination status at start of outbreak | Clinical signs noted by attending veterinarian | Concurrent infection/s |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 4 | 2 April | P1 | Passive clinical surveillance (prior to outbreak) | 2 | Colt | Arabian | Bay | 3 April | Unvaccinated | Pyrexia; congested mucous membranes (oral); swollen supraorbital fossae | Piroplasmosis |
| 3 | 32 | 22 April | P1 | Outbreak clinical surveillance | 11 | Gelding | Crossbreed | Chestnut | | Vaccinated | Pyrexia | Equine encephalosis |
| 2 | 5 | 26 April | P2 | Outbreak response surveillance | 2 | Mare | Crossbreed | Bay | | Unvaccinated | | |
| 2 | 9 | 26 April | P2 | Outbreak response surveillance | 12 | Mare | Crossbreed | Grey | | Vaccinated | | |
| 2 | 11 | 26 April | P2 | Outbreak response surveillance | 4 | Stallion | Warmblood | Bay | | Unvaccinated | | |
| 45 | 554 | 26 April | P2 | Outbreak response surveillance | 25 | Gelding | Thoroughbred | Chestnut | | Vaccinated | | |
| 48 | 566 | 26 April | P2 | Outbreak response surveillance | 10 | Gelding | Crossbreed | Chestnut | | Vaccinated | | |
| 66 | 725 | 27 April | P1 | Outbreak clinical surveillance | 7 | Mare | Crossbreed | Bay | 2 May | Vaccinated | Pyrexia; ataxia, respiratory compromise; subcutaneous oedema | |
| 64 | 708 | 28 April | P1 | Outbreak clinical surveillance | 8 months | Filly | Thoroughbred | Bay | 28 April | Unvaccinated | Colic; pulmonary oedema; rapid disease course with respiratory compromise | Severe intestinal roundworm infestation with associated colic |
| 66 | 1464 | 28 April | P1 | Outbreak clinical surveillance | 5 | Gelding | Crossbreed | Grey | 2 May | Unvaccinated | Pyrexia; ataxia; severe respiratory compromise; subcutaneous oedema | |
| 65 | 710 | 29 April | P1 | Outbreak clinical surveillance | 2 | Colt | Thoroughbred | Bay | | Vaccinated | Swollen supraorbital fossae; mild depression | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 66 | 723 | 29 April | P2 | Outbreak response surveillance and Sentinel surveillance | 10 | Mare | Crossbreed | Chestnut | Unknown | |
| 28 | 450 | 2 May | P1 | Outbreak clinical surveillance | 8 months | Colt | American Saddlebred | Bay | Unvaccinated | Swollen supraorbital fossae |
| 64 | 1039 | 3 May | P2 | Outbreak response surveillance | 4 months | Filly | Thoroughbred | Bay | Unvaccinated | |
| 66 | 1394 | 3 May | P2 | Outbreak response surveillance | 8 | Gelding | Percheron | Grey | Vaccinated | |
| 66 | 1409 | 3 May | P2 | Outbreak response surveillance | 12 | Mare | Boerperd | Bay | Unvaccinated | |
| 66 | 722 | 4 May | P2 | Outbreak response surveillance and Sentinel surveillance | Unknown | Mare | Thoroughbred | Grey | Unknown | |
| 66 | 1411 | 4 May | P2 | Outbreak response surveillance | 6 | Stallion | Crossbreed | Skewbald | Unknown | |
| 66 | 1415 | 4 May | P2 | Outbreak response surveillance | 16 | Gelding | Crossbreed | Bay | Vaccinated | |
| 66 | 1417 | 4 May | P2 | Outbreak response surveillance | 17 | Gelding | Crossbreed | Grey | Vaccinated | |
| 66 | 1431 | 4 May | P2 | Outbreak response surveillance | Unknown | Gelding | Boerperd | Bay | Vaccinated | |

ID: Identification; AHS: African horse sickness
†All dates occurred in 2016

## Annexure 2: Figure S1 from Chapter 3




**Supplementary Figure 1**: (Figure S1 in published manuscript) Forest plot of the horse-level affected proportion (with 95% binomial exact confidence intervals) of African horse sickness found on the 8 positive properties associated with the 2016 outbreak in the controlled area of South Africa. Each property's outbreak affected proportion is indicated by a black circle. The overall horse-level affected proportion on positive properties is shown by the dashed vertical line with its 95% confidence interval depicted as vertical dotted lines.

# Annexure 3: Supplementary Table 1 from Chapter 4

**Supplementary Table 2**: (Supplementary Table 1 in published manuscript) Age and sex demographic information of sampled horses (n= 262) from the 51 herds sampled and categorised by their vaccination status at time of sample. Values in cells represent the number of screened suspect/number of total horses in classification.

| Age (years) | Male | | | Female | | | Sex not indicated | | | Total aggregated by age |
|---|---|---|---|---|---|---|---|---|---|---|
| | Vaccinated | Unvaccinated | Unknown vaccination status | Vaccinated | Unvaccinated | Unknown vaccination status | Vaccinated | Unvaccinated | Unknown vaccination status | |
| <1 | | | | 0/1 | | | | | | **0/1** |
| 1-2 | 4/22 | | 0/2 | 0/14 | 0/1 | 0/2 | 0/11 | | | **4/52** |
| 3-5 | 0/5 | | 0/1 | 1/22 | 0/4 | 0/2 | | | | **1/34** |
| 6-10 | 0/23 | | 0/1 | 1/41 | | 0/1 | | | | **1/66** |
| >10 | 2/30 | 0/1 | | 0/38 | | 0/5 | | | | **2/74** |
| Unknown | 0/3 | | 0/1 | 0/25 | | 0/3 | 0/2 | | 0/1 | **0/35** |
| **Total aggregated by sex and vaccination status** | **6/83** | **0/1** | **0/5** | **2/141** | **0/5** | **0/13** | **0/13** | **0/0** | **0/1** | **8/262** |
| **Total aggregated by sex** | | **6/89** | | | **2/159** | | | **0/14** | | |

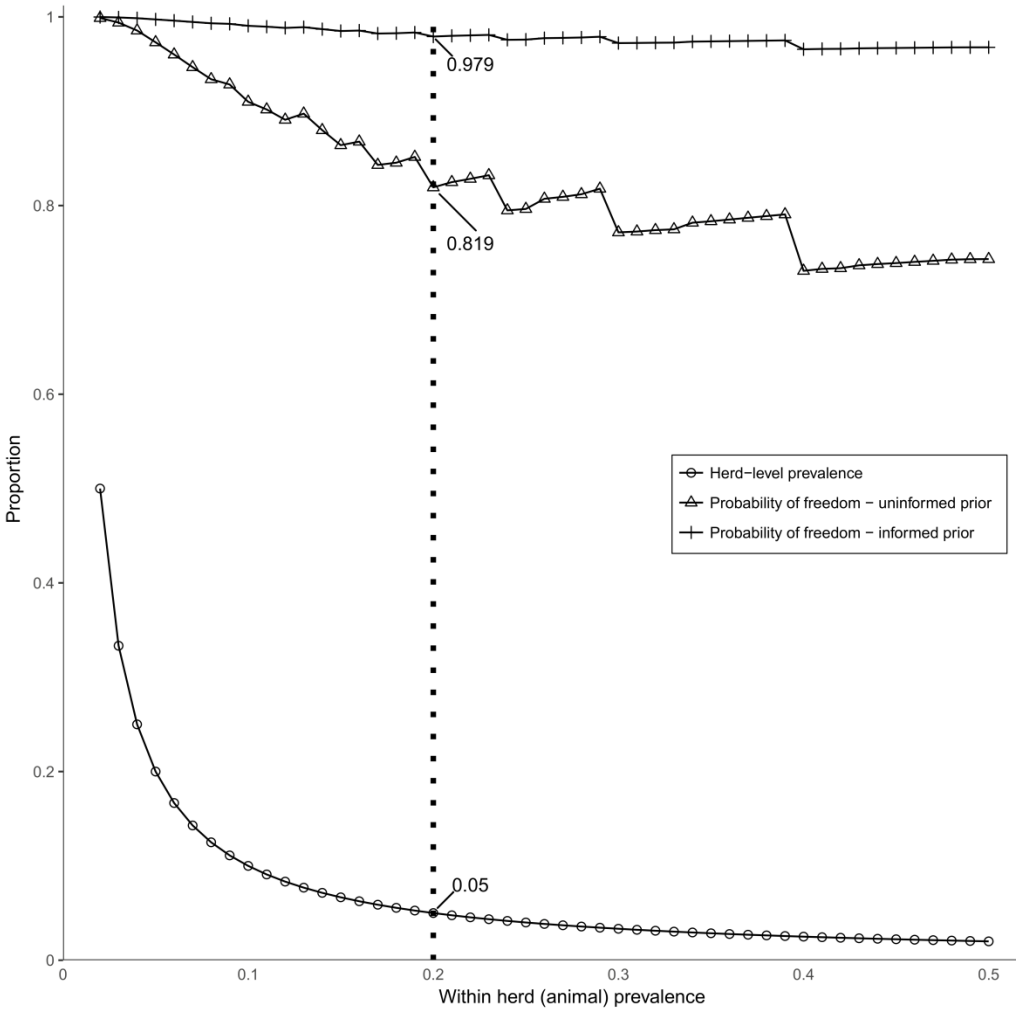
# Annexure 4: Supplementary Table 2 from Chapter 4

**Supplementary Table 3**: (Supplementary Table 2 in published manuscript) Colour, breed and days since last vaccination of sampled horses (n= 262) from the 51 herds sampled and categorised into their screening status.

| Demographic category | Category | Screening Negative | Screen positive | Total |
|---|---|---|---|---|
| Colour | Bay | 123 | 5 | 128 |
| | Chestnut | 51 | 3 | 54 |
| | Grey | 22 | 0 | 22 |
| | Other | 17 | 0 | 17 |
| | Unknown/Not indicated | 41 | 0 | 41 |
| Breed | Thoroughbred | 159 | 7 | 166 |
| | American Saddlebred | 22 | 0 | 22 |
| | Cross Bred | 20 | 1 | 21 |
| | Other | 44 | 0 | 44 |
| | Unknown/Not indicated | 9 | 0 | 9 |
| Days Since Last Vaccination* | *Total evaluated* | 228 | 8 | 236 |
| | Mean (days) | 309 | 210 | |
| | Median (days) | 245 | 214 | |

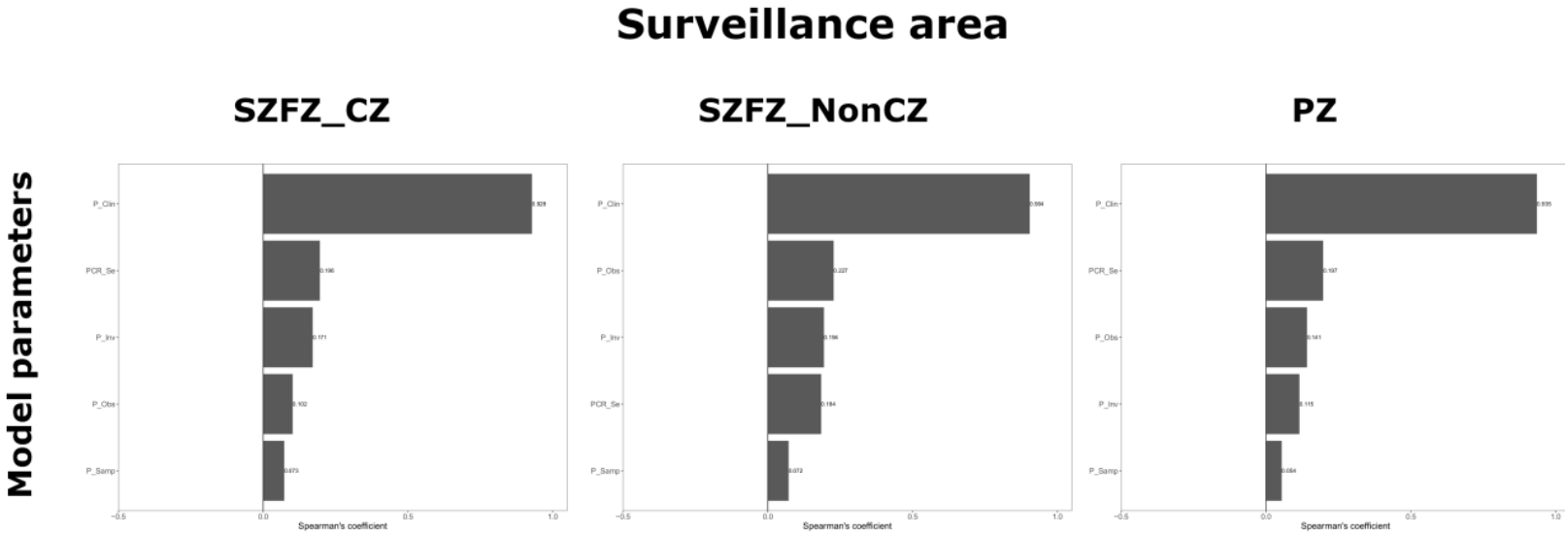
* Only animals with a known date of last African horse sickness vaccination are included in this section (n=236)

## Annexure 5: Supplementary Figure S1 from Chapter 4




**Supplementary Figure 2**: (Supplementary figure 1 in published manuscript) Varying combinations of within- and between herd prevalence, reflecting an effective design prevalence of 1%, and their corresponding probability of freedom from African horse sickness outcomes where both an informed (91.2%) and uninformed (50%) prior probability of freedom was assumed prior to the freedom from disease survey. The dotted line indicates the point prevalences used (within herd prevalence of 20% and between herd prevalence of 5%) for the generic situation referred to within the manuscript.

## Annexure 6: Supplementary Figure 1 from Chapter 5




**Supplementary Figure 3**: (Supplementary figure 1 in submission) Spearman's coefficients for model input parameters where the system surveillance sensitivity is considered as the compared variable. FZSZ, free and surveillance zone; CZ, 2016 AHS outbreak containment zone; PZ, protection zone

# Annexure 7: Supplementary Table 1 from Chapter 5

**Supplementary Table 4**: (Supplementary Table 1 in submitted chapter) Expert opinions of the probabilities of the observation of clinically ill horses, the investigation of these horses and the probability of sampling with the goal of testing for African horse sickness. The values are shown for the minimum estimate, the most likely estimate and the maximum estimate given by experts. The estimates are categorised by the expert and for the applicable surveillance area considered.

| Expert | Surveillance area | Model parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $P_{OBS}$ | | | $P_{INV}$ | | | $P_{SAMP}$ | | |
| | | Min | Most likely | Max | Min | Most likely | Max | Min | Most likely | Max |
| 1 | FZSZ_NonCZ | 0.65 | 0.8 | 0.9 | 0.6 | 0.7 | 0.8 | 0.7 | 0.8 | 0.9 |
| 1 | FZSZ_CZ | 0.65 | 0.8 | 0.9 | 0.6 | 0.7 | 0.8 | 0.7 | 0.8 | 0.9 |
| 2 | PZ | 0.7 | 0.8 | 1 | 0.6 | 0.7 | 1 | 0.9 | 0.95 | 1 |
| 3 | FZSZ_NonCZ | 0.8 | 0.95 | 0.99 | 0.7 | 0.9 | 1 | 0.85 | 0.95 | 0.99 |
| 4 | FZSZ_NonCZ | 0.5 | 0.65 | 1 | 0.6 | 0.7 | 0.8 | 0.8 | 0.95 | 1 |
| 4 | FZSZ_CZ | 0.5 | 0.8 | 1 | 0.7 | 0.9 | 1 | 0.8 | 0.95 | 1 |
| 5 | FZSZ_NonCZ | 0.4 | 0.6 | 0.75 | 0.5 | 0.7 | 0.8 | 0.85 | 0.95 | 1 |
| 5 | FZSZ_CZ | 0.8 | 0.94 | 0.96 | 0.9 | 0.98 | 1 | 0.85 | 0.95 | 1 |
| 5 | PZ | 0.8 | 0.94 | 0.96 | 0.88 | 0.9 | 0.95 | 0.85 | 0.95 | 1 |
| 6 | PZ | 0.5 | 0.8 | 1 | 0.4 | 0.8 | 1 | 1 | 1 | 1 |
| 7 | PZ | 0.7 | 0.9 | 1 | 0.5 | 0.7 | 0.85 | 1 | 1 | 1 |
| 8 | PZ | 0.1 | 0.7 | 0.8 | 0.1 | 0.9 | 1 | 0.6 | 0.75 | 1 |
| 9 | FZSZ_NonCZ | 0.4 | 0.48 | 0.8 | 0.4 | 0.6 | 0.8 | 0.9 | 0.95 | 1 |
| 9 | FZSZ_CZ | 0.6 | 0.8 | 0.9 | 0.5 | 0.7 | 0.9 | 0.9 | 0.95 | 1 |
| 10 | PZ | 0.3 | 0.7 | 0.9 | 0.6 | 0.65 | 0.99 | 0.5 | 0.6 | 1 |
| 11 | PZ | 0.8 | 0.9 | 0.95 | 0.9 | 0.95 | 1 | 0.95 | 0.98 | 1 |
| 12 | FZSZ_NonCZ | 0.5 | 0.8 | 1 | 0.8 | 0.85 | 0.9 | 0.55 | 0.6 | 1 |

FZSZ_CZ: AHS free and surveillance zone within the 2016 AHS outbreak containment zone
FZSZ_NonCZ: AHS free and surveillance zone outside the 2016 AHS outbreak containment zone
PZ: protection zone
Min: Minimum
Max: Maximum

## Annexure 8: List of equations used in thesis body and R code annexures

$$P_{CLIN} = 1 - P_{SUBCLINICAL} \quad (1)$$

$$P_{TESTED} = P_{CLIN} \times P_{OBS} \times P_{INV} \times P_{SAMP} \quad (2)$$

$$SeU_{PSC} = P_{TESTED} \times PCR\_Se \quad (3)$$

$$SeH_h = 1 - (1 - SeU \times \frac{n}{N})^d \quad (4)$$

$$CSe = 1 - (1 - mean(SeH_h) \times \frac{n}{N})^d \quad (5)$$

$$P_{free} = \frac{PriorP_{free}}{1 - CSe_t \times (1 - PriorP_{free})} \quad (6)$$

$$PriorP_{free_t} = 1 - [1 - Pfree_{t-1} + P_{intro_t} - \left((1 - Pfree_{t-1}) \times P_{intro_t}\right)] \quad (7)$$

$$PostPInf_h = 1 - Pfree_h \quad (8)$$

$$Pfree_h = \frac{1 - PInf_h}{1 - SeH_h \times PInf_h} \quad (9)$$

$$PInf_{h(SSC)} = PostPInf_{h(PSC)} \quad (10)$$

$$PInf_{h(POSC)} = PostPInf_{h(SSC)} \quad (11)$$

$$CSe_{SSC,POSC} = 1 - (1 - mean(SeH_h) \times \frac{n}{N})^{mean(PostPInf_h) \times N} \quad (12)$$

$$SSe_{adjusted} = 1 - \Pi (1 - CSe_i) \quad (13)$$

$$P_{free(t)} = \frac{PriorPfree}{(1 - SSe_{adjusted(t)} \times (1 - PriorPfree)} \quad (14)$$

$$PFree_{equilibrium} = (1 - \frac{P_{intro}}{SSe})/(1 - P_{intro}) \quad (15)$$

## Annexure 9: R code for establishing surveillance sensitivity and probability of freedom – stand-alone freedom from AHS survey (Chapter 4)

### *Notes*

At the time of publication the R version used for the evaluation was 3.4.1 (2017-06-30) aka *Single Candle* on a Windows operating system

### *R Libraries required*

| R Code | Comment |
|---|---|
| `require(data.table)` | Required to read in the data from online using the `fread` function |

### *Data and underlying parameters*

| R Code | Comment |
|---|---|
| `surveillancedata<-fread('http://jdata.co.za/datasources/ffdsurveillancedata.csv')` | Obtain data of tested farms |
| `head(surveillancedata)`<br>`str(surveillancedata)` | Surveillance data is the holding based (n=51) that were sampled with the holding ID, census on the holding at time of sampling (`horsetotal`) and the number of horses sampled (and tested negative) during the surveillance |
| `N.horses<-1813` | Number of horses in sampling frame/population |
| `N.holdings<-118` | Number of holdings in sampling frame/population |
| `n.sampled <-sum(surveillancedata$testednegative)` | Total number of horses sampled |
| `n.sampled.herds <-nrow(surveillancedata)` | Total number of herds sampled |
| `pstar.singlestage.unit <-0.01` | Design prevalence for the 1-stage survey - 1% of horses |
| `pstar.twostage.unit.generic<-0.2` | Two stage evaluation - generic option - unit (within-herd) prevalence - 20% of horses in affected herds |
| `pstar.twostage.herd.generic<-0.05` | Two stage evaluation - generic option - herd (between herd) prevalence - 5% of herds affected |
| `pstar.twostage.unit.paarl2016<-0.1283` | Two stage evaluation - Paarl 2016 outbreak information option - unit (within-herd) prevalence - 12.83% of horses in affected herds |
| `pstar.twostage.herd.paarl2016<-8/118` | Two stage evaluation - Paarl 2016 outbreak information option - herd (between herd) prevalence - 6.77% of herds affected |
| `pstar.twostage.unit.historicoutbreaks<-0.278` | Two stage evaluation - Historical outbreak information option - unit (within-herd) prevalence - 27.8% of horses in affected herds |
| `pstar.twostage.herd.historicoutbreaks<-0.233` | Two stage evaluation - Historical outbreak information option - herd (between herd) prevalence - 23.3% of herds affected |
| `testsensitivity<-0.978` | Sensitivity (97.8%) of AHS q-RT PCR used in survey (Guthrie et al., 2013) |
| `unknownprior <- 0.5` | Unknown prior probability of freedom - 50% probability that AHS free at start of survey |
| `knownprior <- 0.912` | Known prior probability of freedom - 91.2% probability that AHS free at start of survey - based on sentinel surveillance data from Western Cape Dept. of Agriculture (Grewar et. al., 2017) |

### *Single stage evaluation*

Establish population sensitivity of surveillance as in Equation 1 in Chapter 4 (equivalent to Equation 4 in Annexure 8 above except this is for the entire population evaluated). Note that the estimation of the number of diseased animals ($d$) should be a whole number and therefore the use of the `ceiling` function to establish $d$ which is

the population at risk multiplied by the design prevalence. This is true for all determination of sensitivity, be it at animal or herd level.

| R Code |
|---|
| `sep.singlestage<-1-(1-testsensitivity*(n.sampled/N.horses))^ceiling(N.horses*pstar.singlestage.unit)` |

Establish the probability of freedom as in Equation 3 of Chapter 4 (equivalent to Equation 9 in Annexure 8 with herd versus population considerations as per Step 1 above)

| R Code | Comment |
|---|---|
| `pfree.singlestage.unknownprior<-unknownprior/(1-sep.singlestage*(1-unknownprior))` | Unknown prior probability of freedom |
| `pfree.singlestage.knownprior<-knownprior/(1-sep.singlestage*(1-knownprior))` | Known prior probability of freedom |

### *Multi-stage evaluation*

#### *Generic within-herd and between-herd design prevalence*

Establish sensitivity of surveillance within each herd using the same method as Single stage evaluation above. Since each herd will return population sensitivity a vector is required to hold these values which will be of length 51 given that 51 herds were tested. A `for-loop` is used to establish each herd's sensitivity. Finally the mean sensitivity for all herds is established.

| R Code | Comment |
|---|---|
| `SeP.herd.generic<-vector()` | Vector to hold herd sensitivities |
| `pfree.singlestage.knownprior<-knownprior/(1-sep.singlestage*(1-knownprior))` | Known prior probability of freedom |
| `for (i in 1:nrow(surveillancedata)){`<br>`  intermin.SeP.herd<-1-(1-testsensitivity*(surveillancedata$testednegative[i]/surveillancedata$horsetotal[i]))^ceiling(surveillancedata$horsetotal[i]*pstar.twostage.unit.generic)`<br>`  SeP.herd.generic <-c(SeP.herd.generic,intermin.SeP.herd)`<br>`}` | Note that `intermin.SeP.herd` is temporary for each `for-loop` since the function runs each time the `for-loop` operates |
| `SeP.herd.generic.mean<-mean(SeP.herd.generic)` | Mean herd sensitivity |

Now that the sensitivity of surveillance is obtained for each herd the overall component sensitivity can be established, this by using the mean $SeP$ as per Equation 2 in Chapter 4 (equivalent to Equation 5 in Annexure 8 substituting $CSe$ for $SeP$). Note

that here the number of expected disease elements refers to the number of herds which is estimated from the herd-level design prevalence.

| R Code |
|---|
| SeP.twostage.generic<-1-(1-<br>SeP.herd.generic.mean*(n.sampled.herds/N.holdings))^ceiling(N.holdings*pstar.twostage.herd.generic) |

Confidence of freedom is obtained in the same way as single-stage evaluation.

| R Code | Comment |
|---|---|
| pfree.twostage.generic.unknownprior<-<br>unknownprior/(1-SeP.twostage.generic*(1-<br>unknownprior)) | Unknown prior probability of freedom |
| pfree.twostage.generic.knownprior<-knownprior/(1-<br>SeP.twostage.generic*(1-knownprior)) | Known prior probability of freedom |

The code for two stage evaluation for different design prevalences as per Table 9 in Chapter 4 is shown below but it is a replica of the generic option above, just with different design prevalences.

### *Paarl 2016 within-herd and between-herd design prevalence*

| R Code |
|---|
| SeP.herd.paarl2016<-vector() |
| for (i in 1:nrow(surveillancedata)){<br>intermin.SeP.herd<-1-(1-<br>testsensitivity*(surveillancedata$testednegative[i]/surveillancedata$horsetotal[i]))^ceiling(surveilla<br>ncedata$horsetotal[i]*pstar.twostage.unit.paarl2016)<br>SeP.herd.paarl2016 <- c(SeP.herd.paarl2016,intermin.SeP.herd)<br>} |
| SeP.herd.paarl2016.mean<-mean(SeP.herd.paarl2016) |
| SeP.twostage.paarl2016<-1-(1-<br>SeP.herd.paarl2016.mean*(n.sampled.herds/N.holdings))^ceiling(N.holdings*pstar.twostage.herd.paarl2016<br>) |
| pfree.twostage.paarl2016.unknownprior<-unknownprior/(1-SeP.twostage.paarl2016*(1-unknownprior)) |
| pfree.twostage.paarl2016.knownprior<-knownprior/(1-SeP.twostage.paarl2016*(1-knownprior)) |

### *Historical outbreaks within-herd and between-herd design prevalence*

| R Code |
|---|
| SeP.herd.historicoutbreaks<-vector() |
| for (i in 1:nrow(surveillancedata)){<br>intermin.SeP.herd<-1-(1-<br>testsensitivity*(surveillancedata$testednegative[i]/surveillancedata$horsetotal[i]))^ceiling(surveilla<br>ncedata$horsetotal[i]*pstar.twostage.unit.historicoutbreaks)<br>SeP.herd.historicoutbreaks <- c(SeP.herd.historicoutbreaks,intermin.SeP.herd)<br>} |
| SeP.herd.historicoutbreaks.mean<-mean(SeP.herd.historicoutbreaks) |
| SeP.twostage.historicoutbreaks<-1-(1-<br>SeP.herd.historicoutbreaks.mean*(n.sampled.herds/N.holdings))^ceiling(N.holdings*pstar.twostage.herd.h<br>istoricoutbreaks) |
| pfree.twostage.historicoutbreaks.unknownprior<-unknownprior/(1-SeP.twostage.historicoutbreaks*(1-<br>unknownprior)) |
| pfree.twostage.historicoutbreaks.knownprior<-knownprior/(1-SeP.twostage.historicoutbreaks*(1-<br>knownprior)) |

### *References*

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## Annexure 10: R code for the scenario tree analysis as described in Chapter 5

### *Introduction*

Annexure 9 above describes in detail the process followed for a simple surveillance evaluation. The code for Chapter 5 is more detailed simply because a stochastic process was followed which entailed 10000 iterations of the model. Also because three different components could potentially be involved in an area, and there were three areas to evaluate, an overall for-loop involved three levels (area, component and surveillance period). In this section the description of certain processes is not explicitly detailed except to highlight important parts of the code or links to specific equations the code is associated with as in Annexure 8.

This code can be fully run given the libraries required are available in R. It is not tabular as in Annexure 9 and the symbol # relates to comments in R code which do not affect the running of code when pasted into an R console like RStudio.

### *Acknowledgements*

Dr Thibaud Porphyre assisted in making the initial code more efficient by using a single for-loop to run the model and his input in this regard is hereby acknowledged.

### *Base scenario tree model*

#### *Note*

All equations referred to below are applicable the list in Annexure 8. For the purposes of brevity the submitted manuscript (Chapter 5) included only the key equations and where equations were similar the differences were indicated in the text. Annexure 8 however includes all equations actually used in the scenario tree model. Note that in the code the following abbreviations are used

- FFD – Freedom from disease survey as described in Chapter 4. Equivalent to POSC (Post-outbreak surveillance component)
- PCS – Passive component of surveillance – equivalent to PSC (Passive surveillance component)
- SSC – Sentinel surveillance component

### *Libraries required*

```
require(mc2d) # manage distributions of inputs
require(RPostgreSQL) # import initial data from cloud database
require(dplyr) # manipulation of dataframes
library(reshape2) # manipulation of dataframes
library(tibble) # manipulation of dataframes
library(ggplot2) # graphical outputs
library(gridExtra) # graphical outputs
```

### *Functions*

The functions used to establish probability of freedom were extracted from the RSurveillance package as referenced in Chapter 5 since the RSurveillance package was no longer available for the R version used.

### *Functions from RSurveillance*

```
discountedprior<-function (prior, p.intro)
{
  prior.disc <- 1 - (1 - prior + p.intro - ((1 - prior) * p.intro)) # Equation 7
  return(prior.disc)
}

pfree.initial <- function (sep, p.intro, prior = 0.5) # Equation 6 – First step
{
  if (length(p.intro) < length(sep))
        p.intro <- rep(p.intro, length(sep))
        prior.disc <- numeric(length(sep))
        pfree <- numeric(length(sep))
        prior.disc <- discountedprior(prior, p.intro)
        pfree <- prior.disc/(1 - sep * (1 - prior.disc))
        return(data.frame(SeP = sep, PIntro = p.intro, PFree = pfree))
}

pfree.final <- function (sep, p.intro, prior = 0.5)  # Equation 6 - Completion
{
  if (length(p.intro) < length(sep))
    p.intro <- rep(p.intro, length(sep))
  prior.disc <- numeric(length(sep))
  pfree <- numeric(length(sep))
  pfree[1] <- pfree.initial(sep[1], p.intro[1], prior[1])[, 3]
  prior.disc[1] <- discountedprior(prior, p.intro[1])
  if (length(sep) > 1) {
    for (p in 2:length(sep)) {
      prior.disc[p] <- discountedprior(pfree[p - 1], p.intro[p])
      pfree[p] <- pfree.initial(sep[p], p.intro[p], pfree[p -
                                                          1])[, 3]
    }
  }
  return(data.frame(Period = 1:length(sep), SeP = sep, PIntro = p.intro,
                    `Discounted prior` = prior.disc, PFree = pfree))
}

pfree.equ_1<-function (sep, p.intro) # Equation 15
{
  pf.equ <- (1 - (p.intro/sep))/(1 - p.intro)
  prior.equ <- 1 - (p.intro/sep)
  return(data.frame(Equ_PFree = pf.equ, Equ_prior = prior.equ))
}
```

### *Created functions*

```
# Create vector of expert opinions for each type (obs, investigate, sample) of length iterations
# Create dataframe of all probabilities in scenario tree (the three expert opinion probabilities and
then the probability of showing clinical signs which is generated per zone)
```

```
zone_start <- function(zoneval, seed.no){

  # Expert opinion: P_Obs
  dfexpertopinion.zone.obs<-dfexpertopinion %>% filter(zone == zoneval & component == 'obs')
  #return a sampled dataset for the number of iterations but leaving only the min mode and max just to
decrease the output
  dfexpertopinion.zone.obs<-dfexpertopinion.zone.obs[sample(nrow(dfexpertopinion.zone.obs),
iterations, replace = TRUE), ][,c("min","mode","max")]
  #create vector of the rpert samples for each row in dataframe which is a length iteration
  set.seed(seed.no)
  P_Obs<-rpert(dfexpertopinion.zone.obs$min, dfexpertopinion.zone.obs$mode,
dfexpertopinion.zone.obs$max)

    # Expert opinion: P_Inv
  dfexpertopinion.zone.investigate<-dfexpertopinion %>% filter(zone == zoneval & component ==
'investigate')
  dfexpertopinion.zone.investigate<-
dfexpertopinion.zone.investigate[sample(nrow(dfexpertopinion.zone.investigate), iterations, replace =
TRUE), ][,c("min","mode","max")]
  set.seed(seed.no)
  P_Inv<-rpert(dfexpertopinion.zone.investigate$min, dfexpertopinion.zone.investigate$mode,
dfexpertopinion.zone.investigate$max)

  # Expert opinion: P_Samp
  dfexpertopinion.zone.sample<-dfexpertopinion %>% filter(zone == zoneval & component == 'sample')
  dfexpertopinion.zone.sample<-dfexpertopinion.zone.sample[sample(nrow(dfexpertopinion.zone.sample),
iterations, replace = TRUE), ][,c("min","mode","max")]
  set.seed(seed.no)
  P_Samp<-rpert(dfexpertopinion.zone.sample$min, dfexpertopinion.zone.sample$mode,
dfexpertopinion.zone.sample$max)

  # Combine the scenario tree nodes from probability of showing Cx through to the final expert opinion
of P_samp
  step.p <- data.frame(cbind(P_Clin, P_Obs, P_Inv, P_Samp))

  # Create final probability of a passive surveillance horse actually getting tested
  P_tested<-step.p$P_Clin*step.p$P_Obs*step.p$P_Inv*step.p$P_Samp # Equation 2

  # subset the surveillance dataset to just those herds within that zone
  dfsurveillance.zone <- dfsurveillance[which(dfsurveillance$zone == zoneval),]

  rval <- list(dfsurveillance.zone = dfsurveillance.zone,
               P_tested = P_tested,
               P_Obs = P_Obs,
               P_Inv = P_Inv,
               P_Samp = P_Samp)

  return(rval)
}

#Sensitivity of surveillance at herd level
SEH_comp <- function(dfsurveillance.period, periodval, zoneval){
  SEHtemp <- expand.grid(1:iterations,dfsurveillance.period$n_census)
  names(SEHtemp) <- c("rowit","n_census")
  SEHtemp$herdID <- sort(rep(1:nrow(dfsurveillance.period),iterations)) # create dummy herd ID across
entire dataset
  SEHtemp$P_tested <- rep(P_tested,nrow(dfsurveillance.period)) # P_tested
  SEHtemp$PCR_Se <- rep(PCR_Se,nrow(dfsurveillance.period)) # PCR_Se
  SEHtemp$Pstar_h <- rep(Pstar_h,nrow(dfsurveillance.period)) # Herd prevalence – design prevalence
  SEHtemp$n_tested_sentinel <- dfsurveillance.period[SEHtemp$herdID,"n_tested_sentinel"] # add number
tested in SSC
  SEHtemp$n_tested_ffd <- dfsurveillance.period[SEHtemp$herdID,"n_tested_ffd"] # add number tested in
POSC

  vecmerge_dat <- SEHtemp %>%
    transmute(SEH_herd_PCS = 1-(1-P_tested*PCR_Se)^1, # Equation 4 accounting for Equation 3 but where
a single animal is infected as the animal level design prevalence
              PostPInf_herd_PCS = 1-(1-Pstar_h)/(1-Pstar_h*SEH_herd_PCS),#Equations 8 and by expansion
9 where Pinfh = PStar_h
              SEH_herd_SSC = 1-(1-PCR_Se*(n_tested_sentinel/n_census))^1,# Equation 4 but were a
single animal is infected
              PostPInf_herd_SSC = 1-(1-PostPInf_herd_PCS)/(1-PostPInf_herd_PCS*SEH_herd_SSC),
#Equation 8, 9 and allowing for 10
```

```
              SEH_herd_POSC = 1-(1-PCR_Se*(n_tested_ffd/n_census))^1,
              PostPInf_herd_POSC = 1-(1-PostPInf_herd_SSC)/(1-PostPInf_herd_SSC*SEH_herd_POSC)
#Equation 8, 9 and allowing for 11
    )

  vecmerge_dat$herdid <- dfsurveillance.period[SEHtemp$herdID,"holdingid"]
  vecmerge_dat$zone <- zoneval
  vecmerge_dat$period <- periodval
  vecmerge_dat$iteration <- rep(1:iterations,nrow(dfsurveillance.period))
  # put all iteration values per herd into a list with every level representative of a herd
  return(vecmerge_dat)
}

calc_unadjCSe_PSC <- function(measure.vars, pstarH_val){
  rval = 1 - ((1 - mean(measure.vars))^ceiling(pstarH_val * length(measure.vars))) # Equation 5 for
PSC where n=N
  return(rval)
}

calc_unadjCSe_SSC <- function(measure.vars, pstarH_val, N_herds){
  rval = 1-((1-mean(measure.vars,na.rm=T) * sum(!is.na(measure.vars)) / N_herds)^ceiling(pstarH_val *
N_herds)) # Equation 5 for SSC and POSC
  return(rval)
}

# For the SSC component specifically
calc_adjCSe_SSC <- function(data){
  ppinf.temp<-with(data, tapply(PostPInf_herd_SSC,period,mean) ) # establish mean of PostPInf_herd_SSC
within a period
  n.temp<-with(data, tapply(PostPInf_herd_SSC,period,length) ) # establish number of values of above
  meanseh.temp<-with(data, tapply(SEH_herd_SSC,period,mean) ) # establish mean of SEH_herd within a
period
  rval <- 1-((1-meanseh.temp*n.temp/N_herds)^(ppinf.temp * N_herds)) # Equation 12 for SSC
specifically
  return(rval)
}
# For the POSC component specifically
calc_adjCSe_POSC <- function(data){
  ppinf.temp<-with(data, tapply(PostPInf_herd_POSC,period,mean) )
  n.temp<-with(data, tapply(PostPInf_herd_POSC,period,length) )
  meanseh.temp<-with(data, tapply(SEH_herd_POSC,period,mean) )
  rval <- 1-((1-meanseh.temp*n.temp/N_herds)^(ppinf.temp * N_herds))  # Equation 12 for POSC
specifically
  return(rval)
}
```

### *Load testing data into R*

```
con<-dbConnect(
  dbDriver("PostgreSQL"),
  dbname="ahssurveillance",
  host="jdatadb.cepdwx8xwoab.eu-west-1.rds.amazonaws.com",
  port=5432,
  user=" phdreadonly",
  password=" grewarphddata")

dfsurveillance<-dbGetQuery(con,"SELECT * FROM surveydata")
dfoutbreakdata<-dbGetQuery(con,"SELECT * FROM outbreakdata")
dfexpertopinion<-dbGetQuery(con,"SELECT * FROM expertopinion")

dbDisconnect(con) #please don't forget this line - run this to prevent queuing of database connections
rownames(dfoutbreakdata) <- dfoutbreakdata$outbreakyear # add rownames based on year to outbreakdata
```

### *Settings and Inputs*

```
# Base
iterations<-10000
surveillanceperiods<-1:24 # number of months from 1 July 2016 to 30 June 2018 - period of interest for
paper
seed.number <- 1234

#Herd level prevalence
```

```
Pstar_h<-rep.int(0.01, iterations)

# PCR_Se
set.seed(seed.number)
PCR_Se<-rbeta(iterations, 9.65, 1.19)

# P_intro
P_intro <- nrow(dfoutbreakdata)/
  (length(seq(as.Date('1999-01-01'), as.Date('2016-07-01'), by='month')) - 1) #number of outbreaks by
number of months at risk

set.seed(seed.number)
P_intro<-rpert(n=iterations, P_intro/2, P_intro, P_intro*2)

# P_Clin based on published data from outbreaks reporting subclinical rates
# here we select the outbreaks with subclinical signs, create a beta distribution for each one of the
iterations, merge all values and sample 10000 values for use in the model

where_sub = ( grepl("weyer",dfoutbreakdata$refherds) | grepl("grewar",dfoutbreakdata$refherds) ) &
!is.na(dfoutbreakdata$subclinical) & dfoutbreakdata$subclinical>0
dfoutbreakdata$outbreakyear[where_sub]
dfoutbreakdata[where_sub]

temppclin<-vector()

for (i in dfoutbreakdata$outbreakyear[where_sub]) {
  set.seed((seed.number))
  temp<-dfoutbreakdata %>% filter(row.names(dfoutbreakdata) %in% c(i))
  temp<-rbeta(iterations,
              temp$cases-temp$subclinical+1, #success + 1
              temp$cases - (temp$cases-temp$subclinical+1) # n-s+1
  )
  temppclin<-c(temppclin,temp)
}

P_Clin<-sample(temppclin,size = 10000,replace = TRUE)

# Zone lists
zone_list <- c("FZSZ_NonCZ","FZSZ_CZ","PZ")
output_zone <- vector("list", length=length(zone_list))
names(output_zone) <- zone_list
```

### *The foundation model*

```
for (z in zone_list){
  #declare vector of outcomes
  outcomes <- vector("list", length=6)
  names(outcomes) <-
c("CSe_unadjusted","CPFree_unadjusted","SSe_adjusted.summ","SSPfree_adjusted.summ","SSe_adjusted.SA","
Equil.SA")

  #Total number of herds per zone
  N_herds<-nrow(dfsurveillance[which(dfsurveillance$period == 1 & dfsurveillance$zone == z),])

  # Create vector of expert opinions for each type (obs, investigate, sample) of length iterations
  surv_rval = zone_start(zoneval = z, seed.no = seed.number)
  dfsurveillance.zone <- surv_rval$dfsurveillance.zone # Testing count data
  P_tested <- surv_rval$P_tested
  P_Obs <- surv_rval$P_Obs
  P_Inv <- surv_rval$P_Inv
  P_Samp <- surv_rval$P_Samp

  # Start of the surveillance period loop
  zone_pertab <- vector()
  for (p in surveillanceperiods){ #for every period - i.e. 1 through 24
    dfsurveillance.period<-dfsurveillance.zone[which(dfsurveillance.zone$period==p),]
    SEH_herd <- SEH_comp(dfsurveillance.period, p, z)
    zone_pertab <- rbind(zone_pertab,SEH_herd)
  } # end of period loop

  # Outcome 1 - Unadjusted herd sensitivity and component sensitivity
  zone_pertab$pstarH_val <- Pstar_h[zone_pertab$iteration]
  zone_pertab$keep.SEH_POSC <- NA
```

```
  zone_pertab$keep.SEH_SSC <- NA
  keep.herd_POSC <- unique(zone_pertab$herdid[zone_pertab$period == 9 & zone_pertab$SEH_herd_POSC !=
0])
  keep.herd_SSC <- unique(zone_pertab$herdid[zone_pertab$period == 1 & zone_pertab$SEH_herd_SSC != 0])
  zone_pertab$keep.SEH_POSC[zone_pertab$herdid %in% keep.herd_POSC] <-
zone_pertab$SEH_herd_POSC[zone_pertab$herdid %in% keep.herd_POSC]
  zone_pertab$keep.SEH_SSC[zone_pertab$herdid %in% keep.herd_SSC] <-
zone_pertab$SEH_herd_SSC[zone_pertab$herdid %in% keep.herd_SSC]

  zone.CSe_unadjusted <- zone_pertab%>%
    group_by(period,iteration)%>%
    summarise(CSe.PCS = calc_unadjCSe_PSC(SEH_herd_PCS,pstarH_val=mean(pstarH_val)),
              CSe.SSC = calc_unadjCSe_SSC(keep.SEH_SSC,pstarH_val=mean(pstarH_val), N_herds),
              CSe.POSC = calc_unadjCSe_SSC(keep.SEH_POSC,pstarH_val=mean(pstarH_val), N_herds)
    )

  # To retrieve the summary for plotting
  melt.CSe_unadjusted <- melt(zone.CSe_unadjusted, measure.vars = c("CSe.PCS","CSe.SSC","CSe.POSC"))

  df_CSe_unadjusted <- melt.CSe_unadjusted %>%
    group_by(period,variable) %>%  #group by the period column
    summarise(median=median(value,na.rm =T),
              lo95=quantile(value,prob=0.025,na.rm =T),
              up95=quantile(value,prob=0.975,na.rm =T))

  # Outcome 2 - Unadjusted probability of freedom not influenced by other components
  zone.CSe_unadjusted$p.intro <- P_intro[zone.CSe_unadjusted$iteration]
  zone.CPFree_unadjusted = zone.CSe_unadjusted %>%
    group_by(iteration)%>%
    mutate(CPFree.PCS = pfree.final(CSe.PCS,p.intro=mean(p.intro),prior=0.5)[,"PFree"],
           CPFree.SSC = pfree.final(CSe.SSC,p.intro=mean(p.intro),prior=0.5)[,"PFree"],
           CPFree.POSC = pfree.final(CSe.POSC,p.intro=mean(p.intro),prior=0.5)[,"PFree"])
  zone.CPFree_unadjusted <-
zone.CPFree_unadjusted[,c("period","iteration","CPFree.PCS","CPFree.SSC","CPFree.POSC")]

  # To retrieve the summary for plotting
  melt.CPFree_unadjusted <- melt(zone.CPFree_unadjusted, measure.vars =
c("CPFree.PCS","CPFree.SSC","CPFree.POSC"))

  df_CPFree_unadjusted <- melt.CPFree_unadjusted %>%
    group_by(period,variable) %>%  #group by the period column
    summarise(median=median(value),
              lo95=quantile(value,prob=0.025,na.rm =T),
              up95=quantile(value,prob=0.975,na.rm =T))

  #Outcome 3: Overall System sensitivity
  # The overall system sensitivity takes into consideration the modulation of components as a result
of prior sensitivity - Equations 8 through 13
  # PostPInf_herd has alereadY been established in the main for-loop - accounts for equations 8 - 11

  #3.1 PCS Seh_herd which is identical since P_star_h is the first probability of infection for the
PCS which is the first component we apply this to
  zone.CSe_adjusted.PCS<-zone.CSe_unadjusted[,c("period","iteration","CSe.PCS")]
  names(zone.CSe_adjusted.PCS) <- c("period","iteration","value")
  zone.CSe_adjusted.PCS$variable <- "CSe_adjusted.PCS"
  zone.CSe_adjusted.PCS$zone <- z

  #3.2 SSC AND POSC adjusted  sensitivity
  zone.SeH_SSC.foroverall <-
zone_pertab[,c("zone","period","iteration","SEH_herd_SSC","PostPInf_herd_SSC")]
  zone.SeH_POSC.foroverall <-
zone_pertab[,c("zone","period","iteration","SEH_herd_POSC","PostPInf_herd_POSC")]

  zone.SeH_SSC.foroverall<-zone.SeH_SSC.foroverall[zone.SeH_SSC.foroverall$SEH_herd_SSC != 0,]  # here
extraction of just the sentinel herds to assist in mean calculations
  temp = split(zone.SeH_SSC.foroverall,zone.SeH_SSC.foroverall$iteration) # get temp data set for
every iteration
  zone.CSe_adjusted.SSC <- as.data.frame(sapply(temp, calc_adjCSe_SSC))

  zone.SeH_POSC.foroverall<-zone.SeH_POSC.foroverall[zone.SeH_POSC.foroverall$SEH_herd_POSC != 0,]
  temp = split(zone.SeH_POSC.foroverall,zone.SeH_POSC.foroverall$iteration)
  zone.CSe_adjusted.POSC <- as.data.frame(sapply(temp, calc_adjCSe_POSC))

  if(length(zone.CSe_adjusted.SSC)>0 & length(zone.CSe_adjusted.POSC)>0){
```

```
    zone.CSe_adjusted.SSC$period <- as.numeric(rownames(zone.CSe_adjusted.SSC))
    zone.CSe_adjusted.SSC_1 <- melt(zone.CSe_adjusted.SSC, measure.vars = as.character(1:iterations))
    names(zone.CSe_adjusted.SSC_1) <- c("period","iteration","value")
    zone.CSe_adjusted.SSC_1$zone <- z
    zone.CSe_adjusted.SSC_1$variable <- "CSe_adjusted.SSC"

    names(zone.CSe_adjusted.POSC) <- "value"
    zone.CSe_adjusted.POSC$period <- 9
    zone.CSe_adjusted.POSC$iteration <- factor(1:iterations, levels =
levels(zone.CSe_adjusted.SSC_1$iteration))
    zone.CSe_adjusted.POSC$zone <- z
    zone.CSe_adjusted.POSC$variable <- "CSe_adjusted.POSC"

    db_SSe_adjusted<-rbind(as.data.frame(zone.CSe_adjusted.PCS),
zone.CSe_adjusted.SSC_1[,names(zone.CSe_adjusted.PCS)],
zone.CSe_adjusted.POSC[,names(zone.CSe_adjusted.PCS)])
  }  else {
    if(length(zone.CSe_adjusted.SSC)>0 & length(zone.CSe_adjusted.POSC)==0){
      zone.CSe_adjusted.SSC$period <- as.numeric(rownames(zone.CSe_adjusted.SSC))
      zone.CSe_adjusted.SSC_1 <- melt(zone.CSe_adjusted.SSC, measure.vars =
as.character(1:iterations))
      names(zone.CSe_adjusted.SSC_1) <- c("period","iteration","value")
      zone.CSe_adjusted.SSC_1$zone <- z
      zone.CSe_adjusted.SSC_1$variable <- "CSe_adjusted.SSC"

      db_SSe_adjusted<-rbind(as.data.frame(zone.CSe_adjusted.PCS),
zone.CSe_adjusted.SSC_1[,names(zone.CSe_adjusted.PCS)])

    } else {
      if(length(zone.CSe_adjusted.SSC)==0 & length(zone.CSe_adjusted.POSC)>0){
        names(zone.CSe_adjusted.POSC) <- "value"
        zone.CSe_adjusted.POSC$period <- 9
        zone.CSe_adjusted.POSC$iteration <- factor(1:iterations, levels =
levels(zone.CSe_adjusted.SSC_1$iteration))
        zone.CSe_adjusted.POSC$zone <- z
        zone.CSe_adjusted.POSC$variable <- "CSe_adjusted.POSC"


        db_SSe_adjusted<-rbind(as.data.frame(zone.CSe_adjusted.PCS),
zone.CSe_adjusted.POSC_1[,names(zone.CSe_adjusted.PCS)])

      } else {
        db_SSe_adjusted<-zone.CSe_adjusted.PCS
      }
    }
  }

  db_SSe_adjusted <- db_SSe_adjusted %>%
    group_by(period, iteration) %>%
    summarise(SSe_adjusted = 1 - prod(1 - value)) # Equation 13

  # For sensitivity analysis
  db_SSe_adjusted.SA<-db_SSe_adjusted
  db_SSe_adjusted.SA$P_Clin <- P_Clin[as.numeric(db_SSe_adjusted.SA$iteration)]
  db_SSe_adjusted.SA$PCR_Se <- PCR_Se[as.numeric(db_SSe_adjusted.SA$iteration)]
  db_SSe_adjusted.SA$P_Obs <- P_Obs[as.numeric(db_SSe_adjusted.SA$iteration)]
  db_SSe_adjusted.SA$P_Inv <- P_Inv[as.numeric(db_SSe_adjusted.SA$iteration)]
  db_SSe_adjusted.SA$P_Samp <- P_Samp[as.numeric(db_SSe_adjusted.SA$iteration)]
  db_SSe_adjusted.SA$P_intro <- P_intro[as.numeric(db_SSe_adjusted.SA$iteration)]

  db_SSPfree_equil.summ <-db_SSe_adjusted.SA %>% ungroup() %>%
    summarise(meansse = mean(SSe_adjusted),
              meanpintro = mean(P_intro),
              equilpf = as.numeric(pfree.equ_1(mean(SSe_adjusted),mean(P_intro))[1]),
              equilpf05 = as.numeric(pfree.equ_1(mean(SSe_adjusted),0.05)[1]),
              equilpf10 = as.numeric(pfree.equ_1(mean(SSe_adjusted),0.1)[1]),
              equilpf15 = as.numeric(pfree.equ_1(mean(SSe_adjusted),0.15)[1]),
              equilpf20 = as.numeric(pfree.equ_1(mean(SSe_adjusted),0.20)[1]),
              equilpf25 = as.numeric(pfree.equ_1(mean(SSe_adjusted),0.25)[1]))

  # Summarise across the iterations
  db_SSe_adjusted.summ <- db_SSe_adjusted %>%
    group_by(period) %>%
    summarise(median = median(SSe_adjusted),
```

```
              lo95 = quantile(SSe_adjusted, prob=0.025),
              up95 = quantile(SSe_adjusted, prob=0.975)
    )
#Outcome 4: Overall Probability of Freedom - adjusted for by interaction between surveillance
components
  #Equation 14
  db_SSe_adjusted$p.intro <- P_intro[as.numeric(db_SSe_adjusted$iteration)]
  db_SSPfree_adjusted = db_SSe_adjusted %>%
    group_by(iteration)%>%
    mutate(SSPfree_adj = pfree.final(SSe_adjusted,p.intro=mean(p.intro),prior=0.5)[,"PFree"])


  db_SSPfree_adjusted.summ<-db_SSPfree_adjusted %>%
    group_by(period)%>%
    summarise(median = median(SSPfree_adj),
              lo95 = quantile(SSPfree_adj,prob=0.025),
              up95 = quantile(SSPfree_adj, prob=0.975))


  outcomes[["CSe_unadjusted"]] <- df_CSe_unadjusted
  outcomes[["CPFree_unadjusted"]] <- df_CPFree_unadjusted
  outcomes[["SSe_adjusted.summ"]] <- db_SSe_adjusted.summ
  outcomes[["SSPfree_adjusted.summ"]] <- db_SSPfree_adjusted.summ
  outcomes[["SSe_adjusted.SA"]] <- db_SSe_adjusted.SA
  outcomes[["Equil.SA"]] <- db_SSPfree_equil.summ

  output_zone[[z]] <- outcomes
}
# Saving the final output allows the transfer of the output to another computer to decrease the amount
of time (and cost) spent on a cloud computer.
save(output_zone,file="phdoutput.RData")
```

### *Outputs from base model*

```
load("phdoutput.RData") # if performing this section on a separate computer (ensure the file is in the
R working directory)
plot_zone <- vector("list", length=length(zone_list))
names(plot_zone) <- zone_list

for(zz in zone_list){
  figure_zone <- vector("list", length=4)
  names(figure_zone) <- paste("fig",1:length(figure_zone),sep="")

#figure 1 – unadjusted sensitivity of surveillance
  figure_zone[["fig1"]] <- ggplot(data = output_zone[[zz]]$CSe_unadjusted,
                                  aes(x=period, y=median,
ymin=lo95,ymax=up95,col=variable,fill=variable)) +
    facet_wrap(~variable,ncol=1)+
    geom_ribbon(alpha=1/3) + geom_line(color = "black")+
    theme_bw() + theme(legend.position = "none",
                       panel.grid = element_blank())+
    ylim(0,1) + scale_x_continuous(breaks = c(1:24),
                                   expand = c(0,0),
                                   limits = c(0.5,24.5)) +
    labs(x = "Period in months starting July 2016",
         y = "Independent component sensitivity")

  #figure 2 – unadjusted probability of freedom
  figure_zone[["fig2"]] <- ggplot(data = output_zone[[zz]]$CPFree_unadjusted,
                                  aes(x=period, y=median,
ymin=lo95,ymax=up95,col=variable,fill=variable)) +
    facet_wrap(~variable,ncol=1)+
    geom_ribbon(alpha=1/3) + geom_line(color = "black")+
    theme_bw() + theme(legend.position = "none",
                       panel.grid = element_blank())+
    ylim(0,1) + scale_x_continuous(breaks = c(1:24),
                                   expand = c(0,0),
                                   limits = c(0.5,24.5)) +
    labs(x = "Period in months starting July 2016",
         y = "Independent component probability of freedom")

#figure 3 – Adjusted system sensitivity of surveillance
```

```
  figure_zone[["fig3"]] <- ggplot(data = output_zone[[zz]]$SSe_adjusted.summ, aes(x=period, y=median,
ymin=lo95,ymax=up95)) +
    geom_ribbon(alpha=1/3) + geom_line(color = "black")+
    theme_bw() + theme(legend.position = "none",
                       panel.grid = element_blank())+
    ylim(0,1) + scale_x_continuous(breaks = c(1:24),
                                   expand = c(0,0),
                                   limits = c(0.5,24.5)) +
    labs(x = "Period in months starting July 2016",
         y = "System surveillance sensitivity")

#figure 4 – Adjusted system probability of freedom
  figure_zone[["fig4"]] <- ggplot(data = output_zone[[zz]]$SSPfree_adjusted.summ, aes(x=period,
y=median, ymin=lo95,ymax=up95)) +
    geom_ribbon(alpha=1/3) + geom_line(color = "black")+
    theme_bw() + theme(legend.position = "none",
                       panel.grid = element_blank())+
    ylim(0,1) + scale_x_continuous(breaks = c(1:24),
                                   expand = c(0,0),
                                   limits = c(0.5,24.5)) +
    labs(x = "Period in months starting July 2016",
         y = "System probability of freedom")
  plot_zone[[zz]] <- figure_zone
}
lay <- rbind(c(1,1,1),
             c(1,1,1),
             c(1,1,1),
             c(2,2,2))

# Save plots to pdf, making a separate file for each plot.
for (i in 1:3) {
  file_name = paste("sensitivity_", i, ".pdf", sep="")
  pdf(file_name)
  print(grid.arrange(plot_zone[[zone_list[i]]]$fig1,
                     plot_zone[[zone_list[i]]]$fig3,
                     layout_matrix = lay))
  dev.off()}

for (i in 1:3) {
  file_name = paste("probfree_", i, ".pdf", sep="")
  pdf(file_name)
  print(grid.arrange(plot_zone[[zone_list[i]]]$fig2,
                     plot_zone[[zone_list[i]]]$fig4,
                     layout_matrix = lay))
  dev.off()}

# some selected tabular outputs used in submission
# Final probability of freedom after 24 months of surveillance
for(zz in zone_list){
  print(zz)
  print(output_zone[[zz]]$CPFree_unadjusted %>% filter(period==24))}

for(zz in zone_list){
  print(zz)
  print(output_zone[[zz]]$SSPfree_adjusted.summ %>% filter(period==24))}

# To see what month the various 24th month final probability of freedom occurs in for components and
for overall
for(zz in zone_list){
  print(zz)
  print(as.data.frame(output_zone[[zz]]$SSPfree_adjusted.summ)
  )}

for(zz in zone_list){
  print(zz)
  print(as.data.frame(output_zone[[zz]]$CPFree_unadjusted %>% filter(variable == "CPFree.PCS"))
)}

for(zz in zone_list){
  print(zz)
  print(as.data.frame(output_zone[[zz]]$CPFree_unadjusted %>% filter(variable == "CPFree.SSC"))
  )}

for(zz in zone_list){
```

```
  print(zz)
  print(as.data.frame(output_zone[[zz]]$CPFree_unadjusted %>% filter(variable == "CPFree.POSC"))
  )}
```

### *Sensitivity analysis*

```
output_zone.sa<- vector("list", length=length(zone_list)) #output shell for sensitivity analysis
names(output_zone.sa) <- zone_list

for (i in zone_list) {
  SA <-output_zone[[i]]$SSe_adjusted.SA %>% filter(period == 24) # only using the 24th period for
evaluation
  cormat<-cor(SA[,c(3:8)], method = "spearman") # correlation co-efficient (rho)

  torplotdata<-cormat %>% as.data.frame %>% cbind(term = row.names(cormat),.)
  torplotdata<-torplotdata[,c(1,2)]
  torplotdata<-torplotdata[-1,]
  torplotdata$term <- factor(torplotdata$term, levels =
torplotdata$term[order(abs(torplotdata$SSe_adjusted))])

  output_zone.sa[[paste(i,"tornadoplot")]]<-ggplot() + # tornado plot
    geom_bar(data = torplotdata, aes(x=term, y=SSe_adjusted), stat = "identity") +
    geom_text(data = torplotdata, aes(x=term, y=SSe_adjusted, label = round(SSe_adjusted,3)),
              hjust = 0, colour = "black", size = 3) +
    theme_bw() + theme(legend.position = "none",
                       panel.grid = element_blank(),
                       axis.text = element_text(size = 12),
                       axis.title = element_text(size = 14))  +
    scale_y_continuous(expand = c(0,0),
                       limits = c(-.5,1.05)) +
    labs(x = "Model parameter",
         y = "Spearman's coefficient",
         title = paste("System sensitivity - ",i)) + coord_flip() +
      geom_hline(yintercept=0, linetype="solid")
}

# Evaluation of P_equilibrium on changing values of P_intro
output_zone.saequil<- vector()

for (i in zone_list) {
  temp<-cbind(data.frame('zone'= i), output_zone[[i]]$Equil.SA)
  output_zone.saequil<-rbind(output_zone.saequil, temp)
}
output_zone.saequil
```

## Annexure 11: University of Pretoria – Animal Ethics Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

**Faculty of Veterinary Science**
**Animal Ethics Committee**

**5 June 2019**

**Approval Certificate with Conditions**
**New Application**

| | |
|---|---|
| **AEC Reference No.:** | REC047-19 |
| **Title:** | Evaluation of freedom from African horse sickness surveillance programs in South Africa |
| **Researcher:** | Dr JD Grewar |
| **Student's Supervisor:** | Prof PN Thompson |

Dear Dr JD Grewar,

The **New Application** as supported by documents received between 2019-03-26 and 2019-05-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2019-05-27.

Please note the following about your ethics approval:

1. The use of species is approved:

| Species and Samples | Number |
|---|---|
| Horses | 336 |

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2020-04-05.
3. Please remember to use your protocol number (REC047-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

**Ethics approval is subject to the following:**

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

**Condition: Section 20 permit from DAFF to be obtained prior to the commencement of the study.**

We wish you the best with your research.

Yours sincerely

**Prof V Naidoo**
**CHAIRMAN: UP-Animal Ethics Committee**

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

## Annexure 12: DAFF Section 20 Approval Exemption

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
**REPUBLIC OF SOUTH AFRICA**

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

**Enquiries:** Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
**Reference:** 12/10

Dr John Grewar
Research and Innovation Manager
SA Equine Health & Protocols
Email: john@saehp.com

Dear Dr Grewar

**RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)**

This project is exempt from Section 20 approval as the samples were legally collected for official AHS surveillance.

The Director: Animal Health has no objection to the data being used for publication purposes.

Kind regards,

Maja.

**DR. MPHO MAJA**
**DIRECTOR OF ANIMAL HEALTH**
**Date:** 2019 -07- 01

## Annexure 13: University of Pretoria – Humanities Research Ethics Committee (Expert opinion data)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Humanities 100.
1919 - 2019

Research Ethics Committee

31 May 2019

Dear Dr Grewar

| | |
|---|---|
| **Project:** | **Evaluation of freedom from African horse sickness surveillance programmes in South Africa** |
| **Researcher:** | **Dr JD Grewar** |
| **Supervisors:** | **Prof PN Thompson and Dr TFC Porphyre** |
| **Department:** | **Veterinary Science** |
| **Reference number:** | **04180194 (HUM014/0519)** |

Thank you for the application that was submitted for ethical consideration.

It appears that data has already been collected and that the research has been completed. The Research Ethics Committee therefore **notes** the project.

The committee requests that you accept full responsibility for any ethical implications that may arise from the research.

Please do not hesitate to contact us for further information or should you require clarification on our decision.

Sincerely

pp

**Prof Maxi Schoeman**
**Deputy Dean: Postgraduate Research and Ethics**
**Faculty of Humanities**
**UNIVERSITY OF PRETORIA**
**e-mail:PGHumanities@up.ac.za**

cc: Prof PN Thompson and Dr TFC Porphyre (Supervisors)

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

**Research Ethics Committee Members: Prof MME Schoeman (Deputy Dean);** Prof KL Harris; Mr A Bizos; Dr L Blokland; Dr K Booyens; Dr A-M de Beer; Ms A dos Santos; Dr R Fasselt; Ms KT Govinder Andrew; Dr E Johnson; Dr W Kelleher; Mr A Mohamed; Dr C Puttergill; Dr D Reyburn; Dr M Soer; Prof E Taljard; Prof V Thebe; Ms B Tsebe; Ms D Mokalapa

*Authors note: The expert opinion data had in fact not been collected prior to the humanities ethics application required for this aspect of the project*

# Annexure 14: University of Pretoria – Faculty of Veterinary Science Research Ethics Committee Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

**Research Ethics Committee**

| | |
|---|---|
| Project Title | **Evaluation of freedom from African horse sickness surveillance programs in South Africa** |
| Project Number | **REC047-19** |
| Researcher / Principal Investigator | **Dr JD Grewar** |

| | |
|---|---|
| Dlissertation / Thesis submitted for | **Doctoral** |

| | |
|---|---|
| Supervisor | **Prof PN Thompson** |

| | |
|---|---|
| **APPROVED** | Date: **2019-05-06** |
| CHAIRMAN: UP Research Ethics Committee | Signature: |

Office of the Chairman: Research Ethics Committee
Room 2-24, Pathology Building, Onderstepoort
University of Pretoria, Private Bag X04
Onderstepoort 0110, South Africa
Tel +27 (0)12 529 8052
Email
www.up.ac.za

## Annexure 15: Western Cape Government: DECRA Animal Ethics Committee Approval

Western Cape Government
Agriculture

Dr Gininda Msiza
Chairperson: DECRA
Email: GinindaM@elsenburg.com
tel: +27 21 808 5001 fax: +27 21 808 7619

Reference: DECRA DS17/119

Dear Dr Grewar

**PROJECT ON FREEDOM OF DISEASE SURVEY – PAARL 2016 AHS OUTBREAK**

The DECRA evaluated the project proposal on Freedom of Disease Survey – Paarl 2016 AHS outbreak, submitted on 6 February 2017 for ethical review.

The project was approved with DECRA reference number: DS17/119 on 15 Feb 2017 for the period 1-31 March 2017.

Please note that annual reports should be submitted to the DECRA secretariat. The report submitted on 23 September 2017 is hereby acknowledged.

Kind regards

Dr Gininda Msiza
**DECRA Chairperson**

Date: 20 March 2018

## Annexure 16: Western Cape Department of Agriculture: approval of data usage from Sentinel Surveillance Programme

Western Cape Government
Agriculture

**Veterinary Services**
**Animal Health**
garyb@elsenburg.com
tel: +27 21 808 5253
fax: +27 21 808 5125
Private Bag X1, Elsenburg, 7607
www.elsenburg.com

Ref: Docs/Research/Mar2019

To whom it may concern,

**RE: Dr John Grewar - Research evaluating the African horse sickness surveillance systems in place in the African horse sickness controlled area in the Western Cape Province of South Africa**

Dr John Grewar is currently researching the African horse sickness surveillance systems in place in the AHS controlled area of the Western Cape Province of South Africa. Part of this evaluation entails a desktop analysis of the active surveillance components of the AHS surveillance system, one of which is the Sentinel Surveillance program in the AHS Free and Surveillance zones.

The Sentinel Surveillance program entails the monthly sampling of horses (approximately 150 per month) from throughout the AHS surveillance zone and has been in place since 1997 in the Western Cape. Over the past 3 years the program logistics have been managed by the Equine Health Fund (Wits Health Consortium) and more recently the South African Equine Health and Protocols NPC: this management is on behalf of and under the oversight of the Western Cape Department of Agriculture: Veterinary Services, and more specifically the State Vet Boland office.

The sentinel surveillance program falls within the scope of the national Department of Agriculture, Forestry and Fisheries (DAFF) AHS surveillance plan and falls within the management mandate of the Western Cape Veterinary Services. We are aware and approve of the access that Dr Grewar has and requires, for the evaluation of the data generated by our Sentinel Surveillance program.

Please do not hesitate to contact me in this regard.

Yours sincerely,

DR. G. BUHRMANN
B.Sc. B.V.Sc.
STAATSVEEARTS/
STATE VETERINARIAN

**Date:** 14/03/2019

REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF AGRICULTURE
OFFICIAL VETERINARIAN
14 MAR 2019
DR. G. BUHRMANN B.Sc B.V.Sc
REG. NO: D83/2276
WESTERN CAPE PROVINCE

**Dr. G. Buhrmann**
Chief State Veterinarian Boland
Western Cape Dept. of Agriculture
Veterinary Services.
**Cell No:** +27 83 642 0602

Department of Agriculture
Veterinary Services: Animal Health
SV Boland
Muldersvlei Road
Private Bag X1, Elsenburg, 7607