A multidisciplinary assessment of the distribution of African horse sickness in Namibia

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To my parents, thank you for always believing in me, and to my daughter, Jolinca, one day you will understand and hopefully be proud.

"A dog might be man's best friend but the horse wrote history" (Unknown)

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PREFACE

The research discussed in this thesis was conducted from January 2013 to April 2015 in the Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus, Potchefstroom, South Africa.

The research conducted and presented in this thesis represents original work undertaken by the author and has not been previously submitted for degree purposes to any university. Where use was made of the work of other researchers, it is duly acknowledged in the text. The reference style used in this thesis is according to the specifications given by the NWU Harvard Referencing Guide.

Any opinions, findings and conclusions or recommendations expressed in this material are those of the author and therefore the NRF does not accept any liability in regard thereto.

SUMMARY

African horse sickness (AHS) is the most lethal infectious, non-contagious, vector-borne disease of equids and accordingly has been declared notifiable by the OIE – World Animal Health Organisation. African horse sickness virus (AHSV) is transmitted via *Culicoides* midges and the disease has a seasonal occurrence that is influenced by environmental conditions that favour the breeding of *Culicoides* midges. Studies on the interactions between the virus, its vector and host require knowledge of the epidemiology of AHSV, the environment as well as anthropogenic factors influencing its occurrence. In an effort to manage AHS, this study addresses the need for a multidisciplinary assessment of criteria in order to characterise the distribution of AHS for the development of a risk assessment tool in Namibia. Contrary to expectations that the arid conditions of Namibia would limit the outbreaks of AHS, on-going and escalating outbreaks caused a renewed interest in the vectors and the distribution of the disease. The first part of the study investigated the historical perspectives on the prevalence and distribution of AHS in southern Africa. The most important observations made during this investigation were the underreporting of AHS in Namibia, as well as the distribution across the districts. The importance of the effects of AHS on historical events is highlighted, with the limited movement of horses during the AHS seasons being an imperative historical precaution. The *Culicoides* species composition and environmental factors influencing AHS occurrence were measured for two years at three sites in Namibia. A total of 79142 *Culicoides* individuals were identified with 48 different species collected. The dominance of the proven AHSV vector varied from 42.7% in Okahandja (high incidence) to 6.8% in Aus (low incidence). A precipitation event is one of the most important environmental parameters, with a significant increase in the number of *Culicoides* collected the week after an event. When comparing the effect of modelled climatic variables on the distribution of AHS in South Africa and Namibia, precipitation was found to have the most significant effect in Namibia and temperature in South Africa. The pattern of AHS occurrence has always been thought to coincide in Namibia and South Africa. However, this seems not to be the case. It was found that although the same climatic parameters in both countries are the drivers for the disease, the combination of the parameters had a different effect on the occurrence of AHS in the respective countries. A social survey was conducted across Namibia and South Africa to assess the relationship between social parameters and the occurrence of AHS outbreaks. Movement of horses was indicated as a major factor in AHS distribution. Areas with higher movement correlated with higher AHS incidence. It was also evident that the process of reporting was unknown to horse-owners and that traditional precautionary measures such as stabling during dawn and dusk was the most popular. Integrating the results obtained during this study, the following parameters were classified according to their importance as drivers of AHS: precipitation > movement status > temperature and humidity relationship > Normalised Difference Vegetation Index (NDVI) > soil type. The last section of the thesis comprises the application of a risk analysis and the development of a qualitative risk tool from which the AHS risk of a site can be estimated. With the application of the risk matrix, Luderitz was found to be the appropriate area to apply for AHS recognition status as a possible equine export station in Namibia. Ultimately, determining the distribution of AHS is a complex process that should involve a variety of scientific fields for a combination of techniques and/or approaches to achieve a comprehensive and applicable risk assessment tool. Significant contributions made by this investigation include the identification of parameters critical for AHS distribution and the development of a risk matrix tool to estimate the risk of the occurrence of AHS outbreaks in Namibia. **Keywords:** anthropogenic effects, *Culicoides*, humidity, precipitation, temperature, qualitative risk matrix.

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LIST OF NUMBERED EQUATIONS

LIST OF ABBREVIATIONS

AHS	African horse sickness
AHSV	African horse sickness virus
ANN	artificial neural network
ANOVA	univariate two-way analysis of variance
BTV	bluetongue virus
EIP	extrinsic incubation period
ELISA	enzyme-linked immunosorbent assay
ERA	European Reanalysis
MODIS	Moderate Resolution Imaging Spectroradiometers
NDVI	Normalised Difference Vegetation Index
OBP	Onderstepoort Biological Products
OIE	World Organisation for Animal Health
OVI	Onderstepoort 220 V UV-light trap
PCA	principal component analysis
PCR	polymerase chain reaction
RDA	redundancy analysis
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RT-qPCR	quantitative real-time reverse transcription polymerase chain reaction
USA	United States of America

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND ON AHS

African horse sickness (AHS) is a devastating, non-contagious, infectious, insect borne disease of equids caused by the African horse sickness virus (AHSV) (Coetzer & Guthrie, 2004). African horse sickness virus (AHSV) is usually transmitted by adult female *Culicoides* midges (Diptera: Ceratopogonidae) with *Culicoides imicola* and *Culicoides bolitinos* as identified vectors (Meiswinkel *et al*., 2004). The aetiological agent AHSV belongs to the Orbivirus genus within the family Reoviridae. There are nine immunologically distinct AHSV serotypes (Howell, 1962). AHS is endemic to sub-Saharan Africa with outbreaks particularly frequent and severe in South Africa (Baylis *et al*., 1999a) and Namibia (Schneider, 1994). It is considered as one of the most lethal horse diseases with mortality rates exceeding 80% in susceptible hosts (Mellor & Wellby, 1998). It has accordingly been declared notifiable by the OIE (World Organisation for Animal Health). This means that it has the potential for very serious and rapid spreading, irrespective of national borders, and for serious socio-economic or public health consequences that are of major importance in the international trade of animals and animal products (OIE, 2012). The mortality risk in the Equidae family is the highest in horses. Mules are considered less susceptible to AHS and deaths in donkeys are seldom recorded (Coetzer & Guthrie, 2004). The indigenous African equid, the zebra (*Equus burchellii*), does not show any clinical signs but is believed to be the primary reservoir of the virus (Barnard, 1993; Barnard & Paweska, 1993; Meiswinkel & Paweska, 2003).

1.1.1. Distribution

The Sahara desert seems to act as a geographical barrier which prevents the establishment of the disease in the northern parts of Africa (Howell, 1962). Until the late 20th century it was believed that AHSV could not be sustained outside sub-Saharan Africa for more than 2 years, except for occasional excursions into northern Africa. Outbreaks resulting in considerable loss to the equestrian industry have occurred in Morocco, the Middle East and Europe (Rodriguez *et al.*, 1992). During 1959-1961 AHSV spread to Saudi Arabia, Syria, Lebanon, Jordan, Iraq, Turkey, Cyprus, Iran, Afghanistan and India (Mellor & Hamblin, 2004). By the end of 1961, the outbreak of the disease was controlled and it was eliminated completely in Asia after the loss of 300 000 equids. The eradication of the disease was achieved through a massive vaccination campaign (Mellor & Hamblin, 2004). During 1965, AHSV once again spread beyond its endemic

zones and made its appearance in Morocco, spreading to Algeria and Tunisia, crossing the Straits of Gibraltar into Spain in October 1966. Spain successfully eliminated the virus following a vaccination and slaughter policy; however, the virus persisted for 2 years within North Africa (Mellor & Hamblin, 2004). In July 1987, an outbreak of AHS was reported in central Spain which was presumed to be caused by the importation of subclinical infected zebras from Namibia (Lubroth, 1988; Mellor *et al*., 1990). The epidemic, having lasted for 4 months, came to an end and it was assumed to be the end of the tragic story. However, this was not the case and more severe outbreaks occurred in Spain during 1988, 1989 and 1990; in Portugal in 1989 and in Morocco in 1989, 1990 and 1991 (Mellor & Hamblin, 2004). These outbreaks where all due to the AHSV serotype 4 which has never before been reported outside of southern Africa (Mellor & Hamblin, 2004). A more recent outbreak in 2007 occurred in Kenya and Senegal which was the first time that AHSV serotype 2 and serotype 7 had been detected in West Africa. The virus was also reported in Nigeria, Ghana, Mali and Mauritania during 2007 (Wilson *et al*., 2009).

1.1.2. Aetiology

AHSV is morphologically similar to other orbiviruses such as bluetongue virus (BTV) and equine encephalitis virus (Coetzer & Guthrie, 2004). The virion is an unenveloped particle about 70 nm in diameter and is made up of a two layered icosahedral capsid, which is composed of 32 capsomeres. The genome comprises 10 double stranded RNA segments, encoding 10 proteins, of which seven are classified as structural proteins (VP1-VP7) and four as non-structural proteins (NS1, NS2, NS3 and NS3A) (Manole *et al.,* 2012). The genome is enclosed within the core particle that comprises two major proteins, VP3 and VP7, which are highly conserved among the serotypes (Mellor & Hamblin, 2004; Maree & Paweska, 2005; Manole *et al.,* 2012). The innermost sub core layer consists of 120 copies of the VP3 protein associated with minor structural proteins VP1, VP4 and VP6 (Mellor & Hamblin, 2004; Manole *et al.,* 2012). The outer surface of the core is composed of 780 copies of VP7 for stability. The core particle is surrounded by the outer capsid composed of two protein trimers, VP2 and VP5 (Mellor & Hamblin, 2004; Maree & Paweska, 2005; Wilson *et al*., 2009; Manole *et al*., 2012). The virus is inactivated at pH values <6 and >12, but remains stable at more alkaline conditions. It is resistant to lipid solvents and relatively heat resilient (Aiello & Mays, 1998; Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004).

1.1.3. Pathogenesis

AHS is an acute or subacute, febrile, seasonal, infectious disease of Equidae. It is characterised by oedema of the subcutaneous tissues and lungs, haemorrhages in some of the internal organs and the accumulation of serous fluids in the body-cavities (Coetzer & Guthrie, 2004). The outcome of infection in horses, including the incubation period and severity, depends largely on the virulence of the virus and susceptibility of the animal (Coetzer & Guthrie, 2004).

AHSV can cause 4 forms of disease which were first described by Theiler (1921): (1) horse sickness fever, (2) cardiac form ("Dikkop"), (3) mixed form and (4) pulmonary form ("Dunkop") (Henning, 1956).

1) Horse sickness fever: This is a mild form causing only mild to moderate fever and oedema of the supraorbital fossae. The incubation period varies from 4-14 days with an expected increase in body temperature of 39-40°C, with fever more prevalent in the afternoons. Additional clinical signs may include congested mucous membranes, anorexia and depression. Almost all animals affected with this form recover (Henning, 1956; Aiello & Mays, 1998; Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004). This form is usually observed in partially immune animals such as the donkey and zebra (Coetzer & Guthrie, 2004; OIE, 2008).

2) Cardiac form ("Dikkop"): Is characterised by subcutaneous oedema, particularly of the head, neck, chest and of the supraorbital fossae associated with an infection of the heart. This subacute form has a longer incubation period and more prolonged course than the acute respiratory form. The cardiac form varies from 7-14 days, and the onset of clinical disease is marked by a febrile reaction (39-41°C) that lasts for 3-6 days (OIE, 2008). Conjunctivae may be congested, petechial haemorrhages may be seen in the eyes and acchymotic haemorrhages may be seen on the surface of the tongue. Colic often features during the course of the disease and a mortality rate of 50-70% is observed (Henning, 1956; Aiello & Mays, 1998; Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004). Other common complications associated with "Dikkop" include paralysis of the oesophagus, especially in cases which involved severe oedematous swelling of the head and biliary fever or equine babesiosis (Coetzer & Guthrie, 2004).

3) Mixed form: This is most common and is a combination of the cardiac and pulmonary forms of the disease. Horses affected by this form may show signs either of respiratory distress followed by oedematous swelling or symptoms of the "Dikkop" form before suddenly developing respiratory distress (Coetzer & Guthrie, 2004). Mortality rate exceeds 70% with death usually occurring 3-6 days after the onset of fever (Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004).

4) Pulmonary form ("Dunkop"): This form may develop so rapidly that an animal can die without previous indication of illness. The incubation period is short, 3-5 days, with a high fever of 40-42°C. Symptoms include interlobular oedema, spasmodic coughing, severe dyspnoea and dilated nostrils; the animal stands with its legs apart and head extended and suffer severe respiratory distress. The conjunctivae are congested and the supraorbital fossae may be swollen. There may be periods of recumbence and terminally, quantities of frothy fluid may be discharged from the nose (Henning, 1956; Aiello & Mays, 1998; Coetzer & Guthrie, 2004; Mellor & Hamblin, 2004). Death usually occurs within a few hours after the first clinical signs are observed (Coetzer & Guthrie, 2004; OIE, 2008). Prognosis for horses suffering from this form is grave with a mortality rate exceeding 95% (Henning, 1956; Aiello & Mays, 1998; Coetzer &

Guthrie, 2004; Mellor & Hamblin, 2004). This is also the form that occurs in dogs (Coetzer & Guthrie, 2004; OIE, 2008).

1.1.4. Vaccine development

Initial development of a vaccine against AHS was initiated in 1905 by Sir Arnold Theiler (Henning, 1956). The first vaccine involved injecting horses simultaneously with a virulent strain of AHSV and an immune serum directed against the same strain (van Dijk, 1998). This method of immunisation proved to be very expensive and immunity was unsatisfactory and unreliable (Henning, 1956). The discovery by Alexander (1935) that AHSV can be attenuated by serial intracerebral passage in mice, represented a significant breakthrough in the understanding of AHSV vaccine development. A prophylactic bivalent live AHS vaccine was developed in South Africa in 1933 (Alexander & Du Toit, 1934), followed by field trials with a quadrivalent vaccine in 1935 (Alexander *et al*., 1936). These neurotropic vaccines which incorporated 6 of the 9 serotypes have been used in South Africa as well as Namibia for decades to immunise horses (Erasmus, 1963). However, the poor immunogenic properties of some of the vaccine strains and the post vaccination encephalitis observed in equids in Midddle Eastern countries following immunisation with the polyvalent neutropic vaccine, led to the discontinuation of the use of the vaccine (van Dijk, 1998). This in turn led to the development of tissue culture-attenuated vaccines by Erasmus (1963). This cell culture-produced vaccine was commercially produced in South Africa until 1990 after which it was discontinued due to safety concerns (van Dijk, 1998). This vaccine was composed of 2 polyvalent vaccine combinations each containing 4 live attenuated virus serotypes. Three of the cell cultured attenuated large plaque strains were incorporated while the remaining serotypes were those that were originally derived from intracerebral passages in mice. After the discontinuance of the vaccine, the current cell culture-attenuated vaccine was introduced. All strains were replaced with new cell culture-attenuated vaccine strains. This vaccine is commercially available and produced by Onderstepoort Biological products (OBP), and contains 7 serotypes - serotypes 5 and 9 are not included. According to van Dijk (1998), the attenuation of serotype 5 was still in progress and serotype 9 is cross protected by serotype 6 and rarely occurs in South Africa (Mellor & Hamblin, 2004).

Several concerns remain with the use of live vaccines, especially in areas where AHS is not endemic. These include teratogenic effects and re-assortment that could occur between the live vaccine and wild type virus (Mellor & Hamblin, 2004). The disadvantages of live-attenuated vaccines have prompted the development of inactivated vaccines. However, although some promising results have been reported, immunisation with the live attenuated vaccine remains the only registered vaccine available against AHS (Paweska *et al.*, 2003; MacLachlan *et al.,* 2007). Despite the availability of live attenuated vaccines, AHS still causes extreme challenges for the veterinary sciences. There is for example no information available on the possibility of

whether *Culicoides* midges could acquire AHSV from a horse vaccinated with the live vaccine and the reversion of virulence of the vaccine virus strains (Paweska *et al.*, 2003; European Commission, 2013).

1.1.5. Other hosts of AHSV

Zebra species are considered to be the natural vertebrate host, but rarely display clinical symptoms of AHSV (Lubroth, 1991). Although zebra represent important reservoir hosts for maintaining the virus in the field, they are not an essential part of the virus replication and transmission cycles (Wilson *et al*., 2009). In a recent study by Becker (2012) in Namibia the Hartmann's mountain zebra was implicated as a possible cycling host of AHSV. The host spectrum of AHSV is known to include mammals other than equids. Camels, bovids, African elephants (Lubroth, 1991), black and white rhinoceroses (Coetzer & Guthrie, 2004), and domestic dogs have been found to test positive for AHSV (Alexander *et al.*, 1995; Coetzer & Guthrie, 2004). In their quest to find a solution for AHS, early researchers such as McIntosh and Theiler determined that goats, ferrets, mice and guinea pigs were also susceptible to AHSV (Henning, 1956). Experimental and natural transmission of AHS to dogs has also been reported through ingestion of infected horse meat as early as 1907 by Theiler (Henning 1956; OIE, 2008). However, Van Sittert *et al*. (2013) found that contrary to what was previously believed, AHS in dogs can be contracted by natural infection via a non-oral route. The role of these hosts is not well understood and their capacity to spread the disease has been previously dismissed (Alexander *et al.*, 1995). However, recent studies by Lo Iacono *et al*. (2014) indicate that even though a host is non-susceptible, its role in the epidemiology of a disease cannot be disregarded. One of their most important findings was the clarification of the role of non-susceptible vertebrate hosts. The risk of a disease occurring in the presence of many hosts (susceptible and non-susceptible) is determined by two factors: the abundance of vectors (that depends on host density) and the differential feeding preference of vectors among animal species (Lo Iacono *et al.*, 2014).

Research on the host preferences of *Culicoides* spp. has strongly focused on the welfare of livestock and *C. imicola* is known to feed on cattle, horses, sheep, goats, pigs and poultry (Scheffer, 2011). However, due to the difficulty with collection and identification of *Culicoides* on a specific host (especially wildlife), most vertebrates are assumed to be possible hosts (Meiswinkel *et al.*, 2004). Some *Culicoides* spp. have even been known to feed on birds when their primary source of blood is scarce (Meiswinkel *et al.*, 2004). Bellis (2013) compiled a record of hosts for Australian *Culicoides* spp., which ranged from mosquitoes to flying foxes and buffalo. A recent study by Martínez-de la Puente *et al*. (2015) determined the feeding preferences of *Culicoides* spp. in Europe. According to their results the feeding preferences of female *Culicoides* differed widely among species which could result in possible amplification

and transmission of pathogens between reservoirs and susceptible species. Studies on the feeding preferences for African *Culicoides* spp. have focused on *C. imicola* (Scheffer, 2011) with studies on other species still lacking.

1.1.6. Overwintering of AHSV

AHSV has a seasonal occurrence determined by the suitability of the climate for vector activity and viral replication. However, in some regions conditions are not suitable for year round vector activity; this poses the question as to where and how the virus survives during the winter. Possible overwintering mechanisms that have been investigated include: (1) survival in the vector either through transovarial transmission that has yet to be identified or virus retention in adults which survive the winter drop in temperature. This mechanism was implicated during the outbreak of AHSV in Spain (Thompson *et al*., 2012). (2) The duration of vireamia is longer in zebras than in the other equids and AHSV seroconversion was found in every month of the year in zebra in the northeast of South Africa (Mellor, 1993; Thompson *et al.,* 2012). This creates the possibility of a continuous and uninterrupted cycle of transmission between vertebrate and invertebrate hosts. Donkeys also "fit the bill" – typically displaying vireamia for up to 4 weeks and low mortalities around 10% (Wilson *et al.*, 2009). (3) Another more recent possibility is vertical transmission within the host; however, this mechanism has not been investigated for AHSV (Thompson *et al*., 2012). (4) According to recent studies by Becker *et al*. (2012) in Namibia, Windhoek district and Venter *et al*. (2014) in South Africa, Onderstepoort, *Culicoides* midges occurred throughout the winter months. The absence of AHSV can be ascribed to several factors but the potential exists for it to remain in midges all year long and an outbreak can commence as soon as the conditions become more optimal for population growth and virus replication (Venter *et al*., 2014). (5) Another possible overwintering mechanism is the survival in an unknown vector or host. Further research is required to evaluate which vectors, if any, could play a role (Mellor, 1993; Thompson *et al.*, 2012). Few of the possible overwintering mechanisms have been conclusively demonstrated and it is also possible that the mechanisms responsible vary between regions and serotypes (Wilson *et al*., 2009).

1.2. VECTORS OF AHS AND THEIR IMPORTANCE

The exact cause of AHSV in early days was very speculative. According to Bruce (1905:329), *"Some people thought that it was due to eating poisonous herbs, others, to some peculiarity or state of the night atmosphere; others to eating grass covered with dew; and still others, to the eating of spiders webs which may be seen on the grass in the morning."*

The possibility that AHS may be transmitted by biting insects was first investigated by Pitchford & Theiler (1903). Their results indicated that horses could be protected against infections when housed in mosquito-proof enclosures (Coetzer & Guthrie, 2004). *Culicoides imicola* was implicated by Du Toit (1944) more than 50 years ago as the main vector of AHS, but it wasn't until 1998 that Meiswinkel & Paweska (2003) discovered a second vector, *Culicoides bolitinos.* Today, the biting midges *C. imicola* and *C. bolitinos* are the recognised principal vectors of AHS in southern Africa (Baylis *et al*., 1999a). These two midge species are widely distributed in sub-Saharan Africa. They are among the world's smallest haematophagous flies measuring from 1 to 3 mm in size. More than 1400 species have been identified across the globe with more than 110 confirmed *Culicoides* spp. in southern Africa of which 1/3 is still undescribed (Mellor *et al.*, 2000; Venter, 2014). Of these, more than 20 species are regularly collected around livestock (Venter, 2014). *C. imicola* is by far the most important vector due to its abundance and extensive distribution range extending from the most southern tip of Africa northwards into southern Europe and eastwards as far as India, Laos, Vietnam and southern China (Meiswinkel *et al.*, 2004). In AHSV endemic areas such as South Africa, *C. imicola* accounts for >90% of the *Culicoides* midges collected during surveillance (Wilson *et al.*, 2009). Despite its importance, very little is still known about the breeding habitat of *C. imicola* (Nevill *et al.*, 2007; Veronesi *et al.*, 2009). The larval habitat of *C. bolitinos* differs markedly from that of *C. imicola* and likely accounts for different distribution ranges and patterns (Nevill *et al.*, 2007). The life cycle of *Culicoides* includes the egg stage, the larval stage with four larval instars, pupa stage and adult midge stage (Mellor *et al.*, 2000). Almost all *Culicoides* require moisture rich habitats and the availability of these environments is a key distribution determinant, influencing abundance and seasonal occurrence (Carpenter *et al*., 2013). The breeding habitat of *C. bolitinos* is bovine dung, which makes it less susceptible to environmental fluctuations such as precipitation and temperature, enabling its presence in cooler areas (Verhoef *et al*., 2014). *C. bolitinos* abundance is directly related to the amount of cattle dung available, which in turn is determined by animal biomass per unit area (Meiswinkel & Paweska, 2003). *C. imicola* is less inclined to enter buildings, whereas *C. bolitinos* prefers the indoors (Meiswinkel *et al.*, 2000). The duration of the life cycle of *C. imicola* varies from 7 days in the tropics to 7 months in temperate regions depending on the climatic conditions (Wittmann & Baylis, 2000).

AHSV is transmitted primarily through the bites of adult female *Culicoides* midge spp., which feed on blood to provide a protein source for egg production. *Culicoides* is able to transmit the virus with a single bite due to “saliva activated transmission” (Wilson *et al.*, 2009). Fig. 1.1 illustrates an integrated representation of the life cycle of *C. imicola* and transmission cycle of AHSV. Factors that affect the various stages of each of these cycles will be discussed in more detail under the heading of each of the known important factors from literature.

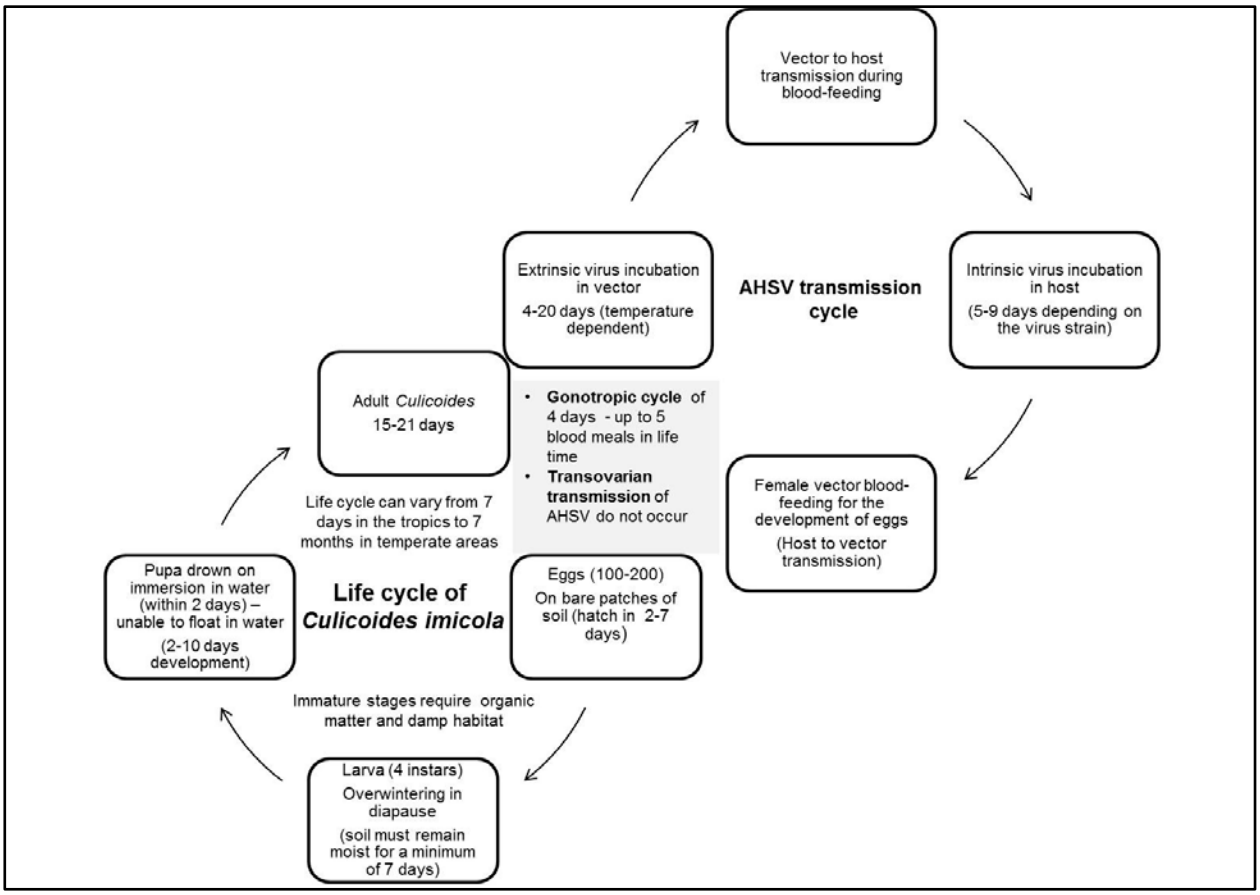


Figure 1.1: Integrated illustration of the life cycle of *Culicoides imicola* and the AHSV transmission cycle from sources: Meiswinkel *et al*., (2004); Purse *et al.* (2005a); Purse *et al.* (2005b); Wilson *et al*. (2009); Venter (2014).

1.2.1. Other possible vectors

Schuberg & Kuhn (1912) showed that *Stomoxys calcitrans* (stable fly) is capable of transmitting AHSV mechanically without replication of the virus in the vector. Nieschulz *et al*. (1934) and Nieschultz & Du Toit (1937) concluded that mosquitos are not vectors of AHSV. However, Ozawa & Nakata (1965) and Ozawa *et al*. (1966) recorded the successful transmission of AHSV to horses via the bites of artificially infected *Anopheles stephensi, Culex pipiens* and *Aedes aegypti,* but they are generally considered to be of minor significance in the field (Wilson *et al*., 2009). According to studies performed by Salama *et al*. (1979) and Awad *et al*. (1981), transmission of AHSV is also possible via the bites of the tick species *Hyalomma dromadarii* and *Rhipicephalus sanguineus* (dog tick). Since ticks have a relatively long lifespan compared to *Culicoides* and it has been suggested that they could provide an effective reservoir for AHSV. The role of ticks in the epidemiology of AHSV remains uncertain (Wilson *et al.,* 2009). *Culicoides sonorensis* is a proven competent vector of AHSV in experimental settings (Wittmann *et al.*, 2002). During the 1987-1990 outbreaks in Spain and Portugal, isolations of AHSV were also made from pools of *Culicoides obsoletus* and *Culicoides pulicaris* (Mellor & Hamblin, 2004). The presence of these vectors in Europe has been cited as the main reason that BTV was able to penetrate into large areas of Europe and because BTV and AHSV utilise the same species of *Culicoides* vectors, it is probable that AHSV can spread into these areas (Mellor & Hamblin, 2004). Previous studies from Venter *et al*. (2009c) indicate a multi-vector potential of AHSV transmission. This aspect will be discussed in more detail in Chapter 6.

1.3. FACTORS INFLUENCING THE DISTRIBUTION OF AHS

1.3.1. Influence of climatic parameters

1.3.1.1. Rainfall

C. imicola occur in regions in Africa where annual rainfall varies between 300- and 700 mm (Meiswinkel & Baylis, 1998). It is found consistently in wet, organically enriched soil or muddy habitats devoid of surface water (Foxi & Delrio, 2010). Water content in soil is one of the most important factors determining habitat suitability for larval development. However, little research has been conducted to elucidate these relationships (Meiswinkel *et al.*, 1994; Mellor *et al.*, 2000; Nevill *et al.*, 2007). Adult females oviposit in enriched, muddy substrates (Foxi & Delrio, 2010) usually on bare patches of soil or low vegetation cover (Fig. 1.1). Eggs are vulnerable to desiccation and hatch within 2-7 days under favourable conditions (Mellor *et al.*, 2000). *C. imicola* midges prefer relatively dry habitats for pupation. Unlike other *Culicoides* spp., the pupae of *C. imicola* drown on immersion in water (Nevill, 1967; Veronesi *et al.*, 2009). The immature stage requires moisture (Mellor *et al.*, 2000) and organic matter (Meiswinkel *et al.*,

1994) for growth and development. The upper layer of soil must remain moist for a minimum of seven days for the larvae of *C. imicola* to complete its cycle (Meiswinkel *et al.,* 2004). Rainfall influences soil moisture and the rate of decomposition of organic matter, therefore directly and indirectly influencing breeding site availability (Gonzalez *et al.,* 2013).

Rainfall (even a light drizzle) inhibits the flight activity of adult midges (Mellor *et al*., 2000). Major outbreaks of AHS in South Africa are strongly associated with heavy rains, preceded by droughts that can significantly increase the abundance of adult *Culicoides* (Baylis *et al.*, 1999b; Wilson *et al*., 2009). This relationship correlates with historical observations that horse sickness appears in seasons with abnormally high rainfall (Meiswinkel & Paweska, 2003). These weather patterns are more common during the El Niño phase of the El Niño – Southern Oscillation (ENSO) (Baylis *et al*., 1999b). According to Nevill (1971), the abundance of *C. imicola* is directly related to the amount of rainfall in the preceding month. *C. imicola* numbers increase more than 200-fold during above-average rainfall seasons and comprise more than 90% of collected catches, with totals reaching more than 10^6 individuals per light trap collection per night (Meiswinkel *et al.*, 2004).

1.3.1.2. Temperature and relative humidity

Various factors affect vector capacity (the ability of the vector population to transmit a pathogen) but none is more influential than temperature. Temperature and humidity are the driving factors for immature vector developmental rates, ultimately influencing adult population size (Mullens *et al*., 2004; Sellers, 1980; Wittmann & Baylis, 2000). The duration of the different developmental stages of *Culicoides* spp. varies with ambient temperature (Kitaoka 1982, Bishop *et al.,* 1996, Mellor *et al*., 2000, Wittmann & Baylis, 2000). The mean developmental period of *C. imicola* recorded during a study by Veronesi *et al.* (2009) was 24 days at 21-24°C, which compares closely with results obtained by Nevill (1967). Significant limitations on the studies of *Culicoides* ecology are due to their small size and fragility which prevents laboratory colonisation of vector species (Carpenter *et al.*, 2013). Larvae are vermiform and the duration of the fourth larval stage varies with ambient temperature (Mellor *et al*., 2000). In countries such as South Africa and Namibia, larval stages may be considerably prolonged because most species overwinter as fourth-instar larvae with colder temperatures being one of the major factors triggering diapause (Wittmann & Baylis, 2000). Intense solar illumination of the larval habitat coupled with high night time temperatures accelerates larval development (Conte *et al.*, 2007). Low temperatures tend to be more significant than higher temperatures as distribution determinants of *Culicoides* species (Gates, 1993; Verhoef *et al.*, 2014). A warmer climate will translate to a shorter life cycle and greater number of generations produced in one season (Kitaoka, 1982; Bishop *et al*., 1996; Wittmann & Baylis, 2000). According to Wittmann & Baylis (2000) the life cycle can vary from 7 days in the tropics to 7 months in temperate regions.

Females feed on blood for the development of eggs (gonotrophic cycle) (Fig. 1.1). Biting rate is considered as a critical parameter, largely because it influences transmission from both host to vector and vector to host (Gubbins *et al.*, 2008). The frequency of feeding is linked to egg development which depends on the ambient temperature. It has been estimated that, with a gonotrophic cycle of four days, *C. imicola* females might take five blood meals during their lifetime, and that the average period between blood meals is estimated at 3.3 to 4.6 days (Meiswinkel *et al*., 2004). Increases in ambient temperature may lead to increased feeding frequency (Wittmann & Baylis, 2000). High temperatures also affect adult longevity and adults are particularly susceptible to desiccation due to their small size (Wittmann *et al*., 2002). Relative humidity affects adult midge survival at different temperatures. Low humidity at low temperatures as well as high humidity at high temperatures are detrimental to survival rates of midges (Murray, 1991; Wittmann *et al.*, 2002). It was found that the longevity of adult *Culicoides* at 30ºC was three times shorter than that at 15ºC (Wittmann & Baylis, 2000). In arid environments, peak activity levels may occur at dawn when the saturation deficit is minimised. It has been suggested that the nocturnal activity of *Culicoides* is in fact an adaptation to exploit the lower risk of desiccation that results from the combination of low temperature and high relative humidity at night (Mellor *et al.,* 2000). Adults can only survive during winter in areas where the daily maximum temperature during the coldest month of the year is ≥12.5°C (Sellers & Mellor, 1993). In related studies, *C. imicola* were found to be active in some areas at temperatures well below 3°C (Sellers & Mellor, 1993; Venter *et al.*, 2014). A study by Verhoef *et al.* (2014) determined that thermal limits and temperature tolerance of closely related *Culicoides* spp. such as *C. imicola* and *C. bolitinos* varies and that this might play a role in the presence or absence of species.

Ambient temperature also affects the rate at which AHSV is able to replicate to transmissible levels following ingestion. The extrinsic incubation period (EIP) takes about 10 days at 25°C (Carpenter *et al.*, 2011). The EIP involves the entry of the virus into the midgut of the *Culicoides* vector, dissemination through the haemocoel and subsequently the infection of the salivary glands (Purse *et al.*, 2005b; Wilson *et al.*, 2009). High temperatures decrease the longevity of adult midges and the duration of the EIP, compensating for the lower adult survivorship (Wittmann & Baylis, 2000). At elevated temperatures, infection rate is higher and rates of virogenesis and transmission are faster. As temperature decreases, virus replication slows down, with the lower threshold at approximately 15°C (Carpenter *et al.*, 2011). Infection and virogenesis rates are proportional to the time spent at optimal temperatures, (a temperature >15°C; <20°C) and the total time spent at these temperatures is a major factor influencing transmission rate (Mellor & Wellby, 1998; Wittmann & Baylis, 2000). AHSV is unable to develop in *Culicoides* midges at temperatures below 15ºC (Carpenter *et al*., 2011) but it may persist in the vector at undetectable levels and when the temperature later rises to permissive levels,

virus replication will be able to recommence and transmission may then be possible (Mellor & Wellby, 1998; Mellor *et al*., 2000).

1.3.3.3. Wind

Culicoides midges can be spread with air currents carrying midges for distances of up to 700 km at heights up to 1.5 km (Johnson, 1969; Sellers, 1980; Meiswinkel *et al*., 2004). This was considered the method by which BTV was distributed across countries around the Mediterranean Sea (Coetzer & Guthrie, 2004). It has also been suggested that infected *Culicoides* midges transported by wind were responsible for the distribution of AHS from Senegal to the Cape Verde Islands in 1943, from Turkey to Cyprus in 1960, and from Morocco to Spain in 1966 (Sellers *et al.*, 1977; Coetzer & Guthrie, 2004). Furthermore, negative correlations have been reported between adult activity and wind speed. Almost all *Culicoides* midge activity is supressed at wind speeds greater than 3 m/s due to their small size (Mellor *et al*., 2000). The dominance of continental anticyclone high pressure systems, characterised by low wind speeds, over southern Africa for many months of the year (up to 80%) (Tyson *et al.*, 1996) contributes to favourable conditions for the midges. At higher wind speeds the mortality rate of *C. imicola* increases which affects abundance (Mellor *et al.*, 2000). *Culicoides* movement occurs over very short distances – usually a few hundred metres, but can be up to 2 km from their breeding site depending on conditions (Johnson, 1969; Mellor *et al*., 2000).

1.3.2. Soil and vegetation characteristics

Immature stages reside in moist, organically enriched, clayey soils which are unvegetated or covered in short grass such as kikuyu (Meiswinkel & Paweska, 2003). Very little data exist on the physical and chemical characteristics of *Culicoides* breeding sites (Foxi & Delrio, 2010). *Culicoides* spp. can breed in a variety of soils if they provide enough moisture and organic matter for the development of the immature stages. The large range of breeding sites can be grouped into four principal categories according to Meiswinkel *et al*. (2004): (a) the water-saturated soil interface between aquatic and terrestrial habitats – In southern Africa most of the major livestock-associated species will be found in this category with a variation in composition of water content and soil type. This includes *C. imicola, C. zuluensis, C. magnus, C. schultzei* group, *C. pycnostictus, C. leucostictus* and *C. nivosus.* (b) Dung pats (fresh dung) – *C. bolitinos*. (c) Tree-holes, plant and rock cavities – These larval habitats vary from deep to shallow water-filled holes containing various amounts of water, decomposing leaf litter and sediment. About 15% of African *Culicoides* spp. including: *C. accraensis, C. clarkei, C. olyslageri, C. eriodendroni, C. punctithorax* and *C. nigripennis* are known to, or suspected to breed in these habitats. (d) Rotting fruit and plants – These larval habitats still need to be investigated in more detail but some *Culicoides* spp. have been reared from rotting fruit.

The developmental stages of *Culicoides* spp. survive in the surface of soil layers (up to 8 cm) (González *et al*., 2013) with *C. imicola* activity only in the upper centimetre (Meiswinkel, 1998). It was found that soil type plays an influential role in the distribution of *C. imicola* (Meiswinkel *et al*., 2004). Large populations were found in areas with clayey, moisture retentive soils and none in sandy and quick draining soils. Meiswinkel (1998) concluded that *C. imicola* would be unable to establish itself in both sandy and arid areas where moisture in the upper layer of the soil drains and dries out rapidly. On clayey soils, intermittent rain (or irrigation) is sufficient to keep the soil saturated for longer periods, and so enables *C. imicola* to become abundant. The general accumulation of clay in valleys and bottom-lands would be ideal breeding habitats for *C. imicola*. This explains why the early colonists noticed that low-lying areas were more prone to AHS outbreaks and therefore moved their horses to higher ground during the summer rainfall season in which the midges thrive in southern Africa. Meiswinkel (1998) described the relationship between soil type, number of *C. imicola* and the number of AHS cases during the 1996 outbreak in South Africa (Fig. 1.2). Studies on the seasonal abundance and prevalence of *C. imicola* have indicated that other than extreme cold and aridity, the degree of slope (inducing water run-off), soil type (whether drainage is slow or rapid) and soil fertility are additional important factors affecting the distribution and abundance of *C. imicola* (Meiswinkel *et al.*, 2004).

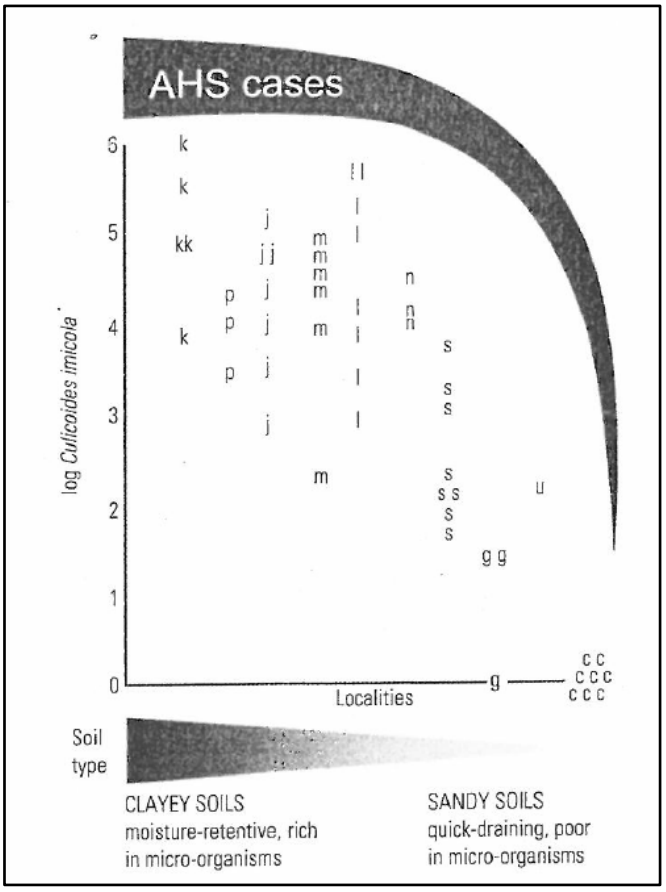


Figure 1.2: A schematic representation from Meiswinkel (1998) of the proposed relationship between soil type, numbers of *Culicoides imicola* and the number of AHS cases during the 1996 outbreak in South Africa based on 52 insect collections at 47 sites. Sites were allocated to 10 region-groups which, moving from left to right, show a decrease in AHS cases and an increase in soil sand content. Regional groups are: Kaalplaas farm (k); Pretoria (p); Johannesburg (j); south-central Mpumalanga (m); eastern Mpumalanga lowveld (l)); Natal (n); Free-state and north-eastern Cape (s); Graaff-Reinet area of central Cape (g); Uitenhage, 30 km inland from the southern coast (u); and south-eastern Cape coast (c).

Previous studies indicate that soil pH has an influence on the presence and abundance of *Culicoides* spp. (Smith, 1966; Smith & Varnell, 1967). Blackwell *et al*. (1999) and Magnon *et al*. (1990) respectively, noted a relationship between *Culicoides* larval distribution and pH in Scottish bog and USA salt marsh habitats (González *et al*., 2013). Schmidtmann *et al.* (2000) found that the relationship between the level of boron in the soil and *Culicoides variipennis* complex can possibly establish predictions for the presence or absence of BTV vector populations. It was also found that *C. obsoletes* was more prevalent in soil samples with high carbon:nitrogen ratios, which reflects the level of mineralisation and decomposition of the organic material (González *et al*., 2013). Unfortunately physical and chemical soil studies related to *C. imicola's* and other African *Culicoides* species' breeding habitats are still lacking due to the difficulty of establishing a colony and their small size.

The abundance of *C. imicola* has been related to the annual minimum Normalised Difference Vegetation Index (NDVI) (Baylis & Rawlings, 1998; Baylis *et al*., 1999a; Conte *et al*., 2007). The

NDVI is widely used within the field of remote sensing and is specifically a measure of chlorophyll abundance, but is also correlated with soil moisture, rainfall, vegetation biomass, coverage, and productivity (Tatem *et al*., 2003; Baylis & Rawlings, 1998). NDVI is calculated from measured brightness values. These values are based on the absorption, transmittance and reflectance of energy by vegetation in the visible red and near-infrared wavelengths of the electromagnetic spectrum (Ward, 2009). The NDVI has been used to classify habitat suitability for disease vectors such as Lyme disease, Malaria and BTV (Ward, 2009). NDVI potentially correlates with the presence of breeding sites (Mellor & Hamblin, 2004). According to Conte *et al*. (2007) the presence of *C. imicola* ranged between NDVI values of 0.27 and 0.31. Previous studies have shown that the inclusion of the NDVI into AHS prediction models proved to be the most important parameter and showed the strongest association with vector occurrence (Baylis *et al*., 1998; Wittmann *et al.*, 2001; Mellor & Hamblin 2004; Purse *et al*., 2007). From these studies it was concluded that *C. imicola* breeds predominantly in habitats that are open to sunlight (Conte *et al.*, 2007). Advanced satellite remote sensing techniques increase the potential for vector monitoring, however, due to the complexity of vector and pathogen interactions the application thereof can be difficult (De la Rocque *et al*., 2004).

1.3.3. Anthropogenic effect

Research on climate change has linked anthropogenic activities to the distribution and occurrence of vector-borne diseases (Sutherst, 2004). In arid and semi-arid environments, increasing anthropogenic activities can lead to severe ecological changes and economical losses (Chen *et al.,* 2014). Previous studies in Namibia indicate that homesteads generally support a greater abundance of *Culicoides* midges, creating favourable “islands” which support their development (Becker *et al*., 2013). The influence of anthropogenic activities is closely linked to animal husbandry practices. *C. imicola* can become superabundant where livestock are kept on irrigated pastures, especially if these pastures occur on clayey, moisture retentive soils (Baylis *et al*., 1999a). It was found consistently in muddy habitats without surface water such as those found adjacent to leaking watering troughs, along pond margins contaminated with animal manure (Foxi & Delrio, 2010), in and around animal pens, at the margins of animal drainage canals and around leaking irrigation pipes (Conte *et al.*, 2007). In recent studies by Becker *et al.* (2013) and Guichard *et al.* (2014), results highlight the importance of irrigation in the occurrence of *C. imicola* in arid regions. Another possibility that has been suggested is that *Culicoides* may be moved over long distances in enclosed horse transport floats (Meiswinkel *et al.*, 2004; Page *et al.,* 2014). International equine trade legislation is extremely strict on the significant risk of the introduction of AHSV into non-endemic countries (van Dijk, 1998). The recent spread of AHSV to West Africa has further increased the risk of its introduction into Europe (Wilson *et al.*, 2009). However, research on the effect of anthropogenic activities on the distribution of AHS is scarce.

1.4. RISK ASSESSMENT AND MODELLING OF AHS

Increased globalisation contributes to rapid and wide geographical spread of diseases that not only impact animal health but also affect international trade (de Vos *et al*., 2010). Quantitative risk assessments usually use mathematical models to describe relationships between vectors and environmental parameters (Astles *et al*., 2006). Research on models and predictions of vector borne disease are numerous, with a random success rate of predicting AHS and its vectors. Trapping data combined with remote sensing imagery were used to develop models for predicting the presence and abundance of *C. imicola* in several countries (Baylis & Rawlings, 1998; Baylis *et al.*, 1999a; Tatem *et al.*, 2003). With the spread of BTV into the northern parts of the world, countries (especially those with large equine populations) are fearful of the introduction of AHS and several risk assessments have been done and import policies are in place (OIE, 2010; Thompson *et al*., 2012). Reliable maps of infectious diseases require an understanding of whether models developed for one location can be applied to another, because the environmental factors that influence disease transmission are unlikely to be uniform over large geographical areas (Baylis *et al*., 1999a). Most models for AHS focus on climate change and the assessment of environmental parameters (Baylis *et al.*, 1999a; Eksteen & Breetzke, 2011; Guichard *et al*., 2014). Risk assessments include factors such as the movement of horses but only in an import and export scenario and not for movements of equids within the borders of a country (Backer & Nodelijk, 2011; Thompson *et al*., 2012). Lo Iacono *et al*. (2014) studied the risk of AHS transmission in Great Britain with the movement of horse owners and the influence of non-susceptible hosts as a transmission factor. Several guidelines are available for risk assessment of plant, animal and human diseases and are usually based on the guidelines given by the OIE (OIE, 2010). Assessment of the risk of introduction, establishment and spread of vector-borne diseases requires a multidisciplinary approach with knowledge of epidemiology, virology, entomology, ecology, climatology and economy (de Vos *et al*., 2010). Risk assessments should focus on looking for combinations of factors that may directly or indirectly affect a risk (Gale *et al.,* 2009). In a data-scarce environment, qualitative risk assessments have often been carried out to address animal health related questions and have proved to be a useful tool (Wieland *et al*., 2011).

A framework for risk assessment of emerging vector-borne livestock diseases was developed by De Vos *et al*. (2010) and provides a toolbox for risk assessments. The specific characteristics of vector-borne infections such as the effect of seasonality on vector biology and vector-pathogen interactions were included. In order to determine the risk probability, the assessment process estimates the level of exposure and the effect of a stressor coming into contact with organisms or the physical environment. Assessing risk depends on a number of factors which include anthropogenic stressors and receptors found in the environment (Wiegers

et al., 1997). This framework needs a lot of data and for an environment such as Namibia its application is limited. Current methodologies for risk assessment focus on predicting the likelihood of movement of known diseases to a new location. Various authors have suggested utilising the biological, ecological, environmental and/or societal factors associated with disease emergence as a way to improve prediction (Bridges *et al*., 2007). Interactions among these factors can be complex, making modelling difficult (Linthicum *et al.*, 1987; Myers *et al.*, 2000). Compared to quantitative risk models, where probabilities of the unwanted events are estimated quantitatively, a qualitative approach uses a certain number of subjective risk levels to describe the probability of the unwanted event to occur (Wieland *et al*., 2011). Cox (2008) concluded that this results in a limited overall resolution with the potential to overestimate the risk. However, qualitative risk assessments prevent an overconfident interpretation of outcomes where little or no data are available (Wieland *et al*., 2011). Qualitative risk assessments are often utilised in models for fisheries, the marine ecosystem and human health industries as they are easy to understand and the decision criteria transparent and logical (Astles *et al.*, 2006).

1.5. PROBLEM STATEMENT, AIM AND OUTLINE OF THESIS

1.5.1. Problem statement

AHS is a RNA, non-contagious, vector borne disease principally affecting equids (horses, donkeys, mules and zebras) and transmitted by *Culicoides* midge spp. Despite early records of an outbreak of the disease in Yemen in 1327, the virus appears to have originated in Africa and was first recognised as a distinct disease with the exploration of Africa. The first account of AHS in southern Africa was at the time of the occupation of the Cape of Good Hope by the Dutch East India Company at the beginning of the 18th century (Mellor, 1993). Following the pandemic spread of AHS-9 through the Middle East in the late 1950s and in the early 60s, exports from South Africa (including Namibia) of Equidae were virtually banned until the late 1990s (Wilson *et al*., 2009). Several aspects of the epidemiology of AHS indicate that it represents a significant risk to Europe and North America, and with the spread of BTV into Europe, international trade legislation for equines became extremely strict (Wilson *et al.,* 2009). A new regulation was adopted during the OIE's general assembly in May 2012 where AHS is now one of four animal diseases for which countries can request an AHS recognition status (Wits Health Consortium, 2014). Namibia is an exporter of pedigree horses and AHS has a significant economic impact (Scacchia *et al*., 2009). It is therefore important to find suitable methods to assess risk and improve the prevention of AHS.

Data on the occurrence of *Culicoides* midges in arid environments are limited, which is critical in the determination of whether AHS can be supported in the area (Conte *et al*., 2007). AHS is considered endemic to Namibia where it is thought to persist due to the presence of zebra acting as a permanent virus reservoir host (Venter *et al*., 1999; de Vos *et al*., 2012; Becker, 2012). In 1908 Enderlein (Cornet & Brunhes, 1994) described the first sub Saharan *Culicoides* spp. in Namibia. Other work that has been performed on AHS in Namibia includes the description by Scacchia *et al*. (2009) of the pathogenesis of outbreaks in 2006. A more recent study by Becker (2012), focused on the occurrence of AHSV in zebra and *Culicoides* in the Khomas area (Windhoek district). However, limited data on *Culicoides* distribution and activities in Namibia are available (Becker, 2012). This information is critical in the determination of the distribution of AHS in arid areas, of which currently very little is known. Detailed mapping of the distribution and abundance of *Culicoides* for a whole country such as Namibia is impractical and a better approach is to attempt to understand the causes of the geographical variation in AHS abundance (Baylis *et al.*, 1999a). The distribution of AHS is difficult to measure because *Culicoides* is distributed across southern Africa and cannot be readily assessed by a correlation with a single environmental parameter. The sheer abundance and prevalence of *Culicoides* makes the prospect of effective control a mere distant dream and research must therefore concentrate on the development of methods to predict when and where disease outbreaks are likely to occur (Mellor *et al.*, 2000; Acevedo *et al.*, 2010). Understanding how spatial variation in environmental conditions affects the demography and population dynamics of *Culicoides* spp. is critical for effective management (Searle *et al.*, 2013). In 2011 Namibia lost approximately 1000 of its horse population to AHS (Kazondovi, 2011) causing a renewed interest in the distribution of the disease, its vectors and the widely used vaccination program. On-going and escalating outbreaks in Namibia highlight the need to clarify the factors that determine the presence and spread of the virus.

In this study a multidisciplinary approach was applied to facilitate the development of a risk assessment tool for AHS in Namibia. During this investigation, three sites in three different districts across Namibia were characterised in terms of a variety of quantitative and qualitative parameters. Furthermore, the current information on AHS and *Culicoides* midges in arid environments is expanded. These findings will facilitate a better comprehension of the distribution of AHS in Namibia and identify the social and environmental risks associated with AHS. To the author's knowledge there has been no attempt to develop a risk assessment for AHS in Namibia. Furthermore, *Culicoides* surveillance, collections and identification of this magnitude have not been carried out in Namibia before.

1.5.2. Aim and objectives

The aim of this study was to evaluate a selection of multidisciplinary criteria in order to characterise the distribution of AHS for the development of a risk assessment tool in Namibia.

Specific objectives:

- An assessment of historical data on the occurrence of AHS in Namibia over the past 100 years.
- The determination of the *Culicoides* spp. composition and assessment of environmental parameters influencing the distribution of AHS at three sites in Namibia.
- The comparison of modelled climatic parameters, possibly influencing the distribution of AHS in Namibia and South Africa.
- An assessment of the relationship between AHS occurrence and anthropogenic parameters; including the movement of horses, preventative measures and knowledge about AHS reporting.
- Integration of multivariate data to determine the most prominent drivers influencing the distribution and incidence of AHS across Namibia.
- Development of a risk assessment tool from which the AHS status of a specific site can be predicted.

1.5.3. Outline of thesis

The materials and methods along with the results and discussion from the stated objectives are presented in the following chapters:

Chapter 2 contains a description of the historical distribution of AHS in Namibia over the past 100 years and a discussion on historical events that have influenced the distribution of AHS in Namibia.

Chapter 3 provides of a description of the three sites across Namibia, sampling methods and conversion of the OVI 220V trap to operate on a 12V solar system. The most prominent parameters influencing the distribution of AHS in Namibia are identified as well as the most abundant *Culicoide*s spp.

Chapter 4 includes the results obtained for the effects of modelled climatic variables on the distribution of AHS in Namibia and South Africa over a period of 19 years (1993-2011).

Chapter 5 provides a discussion of the results of a social survey of the distribution of AHS between Namibia and South Africa and assesses the anthropogenic contributions to the distribution of the disease. The knowledge of horse owners on the reporting process was also assessed and results are discussed here.

Chapter 6 includes a risk assessment model for the occurrence of AHS outbreaks in Namibia based on the 2 year surveillance, historical influential parameters and anthropogenic contributions.

Chapter 7 provides a conclusion of all the stated objectives.

CHAPTER 2

HISTORICAL PERSPECTIVE ON THE PREVALENCE AND DISTRIBUTION OF AHS IN SOUTHERN AFRICA

2.1. INTRODUCTION

Horses are not native to Africa and were first introduced into southern Africa by Jan van Riebeeck and the Dutch settlers in 1652. Horses could not have been introduced overland from Northern Africa due to the physical barrier of the Sahara desert. The presence of AHS and trypanosomiasis, presented a pathogenic hindrance (Law, 1976; Swart, 2010). Van Riebeeck requested horses from the Dutch East India Company on several occasions, believing that horses would prove invaluable for transportation. Their draught power would transform the physical environment and allow for the exploration of the hinterland (Swart, 2010). Van Riebeeck judged horses to be his greatest and principal need and argued that horses would accelerate the settling process, making settlers independent from the local tribes. His requests were granted, and by July 1655, van Riebeeck had 6 Javanese ponies – the first horses to set their hooves on South African soil (Grobbelaar, 2007). Despite several setbacks and the harsh environmental conditions, the horse stock increased in southern Africa and played an integral role in the transformation and colonisation of the African continent. Horses endowed their owners with enhanced military capabilities, hunting aptitude and transport capacity. They not only represented the grasping of power but also the performance of that power in key rituals of society (Swart, 2010). Progress was ultimately carried on the horse's back!

Horses, foreign to southern Africa, were faced with a multitude of diseases that showed southern Africa to be one of the worst environments in the world for equids. It proved that establishing an equine settlement was more difficult than a human settlement (Swart, 2010). Two of the most menacing livestock diseases that settlers and travellers had to face were African animal trypanosomiasis and AHS. According to Moulê (1896), the first historical reference to a disease which can be regarded as AHS, was found in an Arabian document "Le Kitâb El-Akouâ Wa El Chafiâh." Another very early reference to the disease in Africa was made by Father Monclaro in his account of a journey to East Africa in 1569 (Henning, 1956). One of the first incidences of AHS was reported in the Cape Colony in 1719 when 1700 horses died, more than 60% of the horse population (Schneider, 1994; Grobbelaar, 2007). The disease was soon found to be endemic to southern Africa and had a profound influence on the development of Africa (Henning, 1956). Particularly severe outbreaks were recorded during the years 1780, 1801, 1839, 1845, 1862 and 1891. The outbreak of 1854/5 was considered the most virulent.

Mortalities amounted to nearly 70 000, comprising more than 40% of the horse population in the Cape at that time (Henning, 1956). AHS mortality had such a devastating effect that most travellers mentioned it in their notes and diaries. James Backhouse, a Quaker evangelist wrote in 1838: "*One of our horses exhibited symptoms of a fatal disease called in this Colony 'The Sickness'. His eyelids were swollen and the blood-vessels of his mouth and tongue were in a state of congestion. He appeared to be in perfect health last night when tied to the wagon wheel to secure him from Hyenas which are numerous here. This disease usually comes on suddenly and runs its course quickly... He was bled without delay and dosed with Calomel and Tartarised Antimony... He soon rose again and began to eat but quickly lay down and then struggled and died. His death took place about an hour after the symptoms of 'The Sickness' were first noticed. Before night his carcass was nearly consumed by vultures and by the dogs of the Hottentots. Thus quickly a horse is finished in Africa!"* (Gutsche, 1979:6). In the reports of AHS to the horse guard war office in England, the devastating effect of AHS was described in the narrative of Dr Scherzer "*During our residence in the Cape Colony (1857), severe depression existed among the agricultural inhabitants of the western and eastern districts, in consequence of an epidemic which, within two years, had carried off 64,850 horses of the value of 525,000 sterling. Many landowners, in consequence, entirely gave up rearing horses and turned their attention almost exclusively to the breeding of sheep.*" (Nunn, 1888; Swart, 2010). In dealing with this terrifying disease, horse owners had to concoct a mixture of local knowledge from various traditions of healing. Settlers learned from indigenous herdsman about using smoky fires to discourage flies. Travellers also noted that Khoikhoi would relocate their cattle if they manifested illness and implemented the same guidelines against AHS (Swart, 2010). The only useful ways of preventing AHS proved to be moving the horses to higher ground and stabling during dawn and dusk (Schneider, 1994; Swart, 2010). New settlers established themselves in places where horses could survive. This desire to reach horse sickness free zones determined the range of the settlement (Swart, 2010).

At first AHS was confused with diseases like anthrax, human malaria and biliary fever (Henning, 1956). The first scientific research on AHS was carried out by Alexander Edington (1892) who was appointed as the first government bacteriologist of the Cape Colony. With his research he established AHS as a separate disease. His research unfortunately lost credibility when he announced that AHS was caused by a fungus, which he failed to prove (Verwoerd, 2012). The filterability of the AHS virus was demonstrated in 1900 by M'Fadyean in London, proving the viral nature of the agent (Henning, 1956; Verwoerd, 2012). M'Fadyean's findings were independently confirmed by Theiler (1901), Nocard (1901) and Sieber (1911), concluding that the disease was caused by a virus (Henning, 1956). Sir Arnold Theiler is considered the father of veterinary research in South Africa, with a variety of ground breaking experiments on AHS. He found that some horses and mules were immune to experimental infection and he stated on

several occasions that he was unable to produce fatal symptoms in donkeys. He was also the first to show in 1907 that dogs were susceptible to AHS and also realised that AHS is not contagious (Henning, 1956; Koekemoer, 2004).

The first inland expedition from the Cape to cross the Orange River into southern Namibia appears in the records of the hunting expedition of Jacobus Coetse Jansz in 1760. In his journals he writes about how he was able to chase down and kill a giraffe with the use of his horse (Goldbeck *et al*., 2011). European hunters, traders, missionaries and scientists penetrated the interior of Namibia in increasing numbers at the beginning of the 19th century. With horses, greater freedom of movement was achieved and thus greater trade networks were established. Oorlam groups exerted the most significant influence on trade between the Cape and Great Namaqualand (Wallace & Kinahan, 2011). With the establishment of mission stations and trading centres, more horses were imported from the Cape and Europe and distributed across southern Africa, with the first horses reaching central Namibia in 1820 (Schneider, 1994). A further influx of horses occurred at the onset of the diamond rush, as well as the large influx of Cape horses during the time of World War 1 (Goldbeck *et al.*, 2011). As horses moved further into southern Africa, some tribesmen noted that certain areas were at all times disease free. As early as 1843, Jonker Afrikaner was looking for AHS free areas in the vicinity of the Erongo Mountains (Schneider, 1994). During the AHS season, horses would be sent to these areas which were usually at higher elevations in the mountain ranges. These sanctuaries or “Sterbeplätze” were well known by horse owners (Fig. 2.1) (Schneider, 1994). Chief Tseib of Hoachanas area made a living in 1842 by allowing horses from other tribes to graze on the known AHS-free area (Keetmanshoop) during the rainy season, demanding high prices for this privilege (Nunn, 1888; Schneider, 1994).

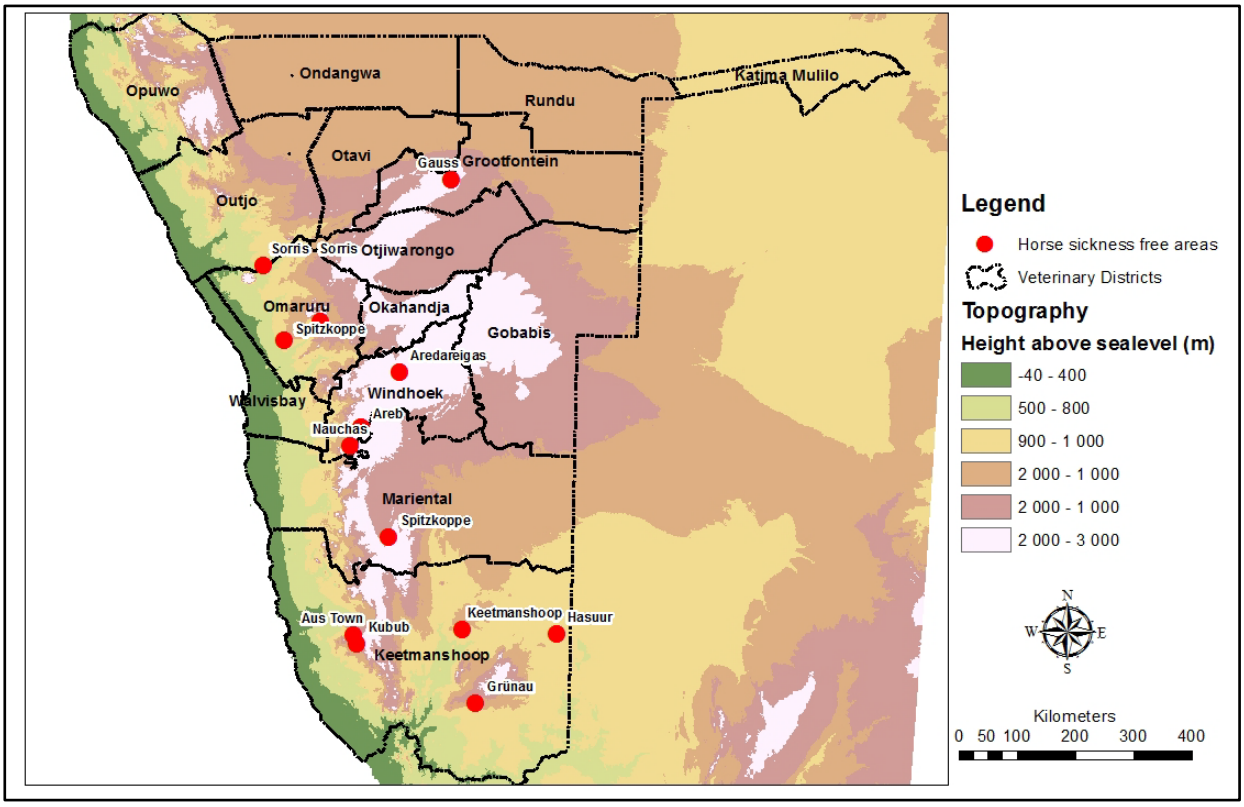


Figure 2.1: The topography of Namibia, showing the veterinary districts and areas which were believed to be AHS free during the Pre-colonial period (Schneider, 1994).

The general mode of transport was riding-oxen while horses were only used to a limited extent due to the presence of AHS (Schneider, 1994). Even Livingstone's exploration was complicated by the inability to use horses due to AHS and he had to use oxen instead (Long & Guthrie, 2013). Horses that recovered from AHS – salted horses, were believed to be immune to future attacks from the dreadful disease and were therefore highly desired, trading at high prices. Salted horses were marked with a slip in their ear and in 1878 travellers paid up to a £100 for such a horse (Schneider, 1994).

The first examinations in respect of animal diseases in Namibia and especially AHS were undertaken by Dr Sander, a retired naval medical officer with a veterinary background that mistook AHS for anthrax (Schneider, 2012). However, the foundation of veterinary research in Namibia was laid by Dr Rickmann who regarded AHS as a disease related to the malaria of man (Henning, 1956). He arrived in Namibia in June 1895 as the first veterinarian. At that time, AHS was one of the most prevalent and important animal diseases. In response to the devastating effects of this disease, the Imperial Bacteriological Institute Gammams was established. The Institute was responsible for the production of vaccines and research on the epidemiology of AHS. The Schutztruppe (German soldiers) employed a large number of veterinarians, and by 1914 each district in Namibia had its own veterinary officer. However, during World War 1, the veterinary structure crumbled as most veterinarians were called for military service. During the South African administration period (1920–1977) veterinary

autonomy was lost and Namibia became a region within the Department of Agricultural Technical Services of South Africa. A total of six veterinarians were appointed during this period and were mostly stationed in the central districts of Namibia. Some responsibilities, such as the control of sheep scab, were placed in the hands of the South-West African Police due to the lack of available veterinary manpower. After the elections of 1980 the central veterinary authority was transferred back to Namibia (Schneider, 2012).

AHS had a direct effect on tribal wars, military operations and the colonisation of Namibia. Any military action was brought to an almost complete standstill during the AHS season. In 1890, close to a thousand horses died of AHS, almost 50% of the total horse population of Namibia. Epidemics of AHS causing high mortalities were reported in the years 1903, 1905, 1907, 1909, 1917 and 1939 (Schneider, 1994).

The objective of this chapter was to assess the historical distribution of AHS in Namibia over the past 100 years and discuss historical events that influenced the distribution of AHS in Namibia.

2.2. MATERIALS AND METHODS

A comprehensive literature review was conducted using historical data on AHS from the Windhoek archives as well as annual reports of AHS incidence from the Directorate of Veterinary services. Namibia had several of names in history, however for the purpose of this thesis we will only use the name “Namibia” as a reference to the country regardless of the political rule. Even though the research methodology of this study is encapsulated within the quantitative paradigm, the scope of this chapter necessitates the use of a qualitative lens in order to contextualise the socio-economic and historic influence on AHS in Namibia.

Data were extracted for the periods 1916-1934 and 1990-2011 from the annual published veterinary reports. Data from 1935-1989 were descriptive and therefore tagged as missing for the analysis. Descriptive data of the annual veterinary reports from 1971-1987 is presented in Table 2.1. Average annual AHS cases were calculated for each district of Namibia. District division of the state vet boundaries were used for the analysis. Historical AHS outbreak occurrence data as reported in the annual reports were analysed to determine differences in horse mortality due to AHS between districts using Chi-Square contingency analysis. The problem with converting data to percentages or only averaging the data was that the sample size is ignored and therefore distorted the results (Fowler & Cohen, 1995). Chi-square contingency analysis, allows the determination of an expected value of AHS outbreak occurrence relative to the national AHS incidence. Cramer’s V test was also performed to support the Chi-Square results by adjusting the significance to factor out sample size. Horse census data as published in the Annual agricultural and veterinary reports were used for the

1916-1934 period, the 1929 census and for the 1990-2011 period, the 2000 census. Number of horses per district is an indication of the introduction of horses to Namibia, and the growth in horse numbers per district in the two time periods.

A dependent t-test and Wilcoxon matched pair test were performed to determine statistically significant differences between the two time periods (1916-1934 and 1990-2011) of the occurrence of AHS outbreaks. Results are illustrated in GIS based maps with the use of ArcGIS 10 (ESRI, 2011).

Very high resolution interpolated monthly precipitation data at a 1 km spatial resolution for a 50 year period (1950–2000) (Hijmans *et al*., 2005) were used as historical descriptive data to support the distribution of AHS outbreak occurrence in Namibia.

2.3. RESULTS AND DISCUSSION

The historical distribution of AHS in Namibia for the period 1916-1934 is illustrated in Fig. 2.2. Low incidence districts were confined to the southern part of the country with medium incidence in the northern districts and high incidence restricted to the central districts of Namibia. AHS free areas are also indicated on the map, with most of the areas located in the southern districts and at higher altitudes. According to the Chi-square analysis and Cramer's V test there was a significant statistical difference ($p<0.001$) in the occurrence of AHS between the districts. Although AHS cannot be linked to a single parameter, the only available historical data was climate data (Hijmans *et al.*, 2005). This included monthly precipitation and temperature data. Comparing the precipitation of the 50 years (1950-2000) with the occurrence of AHS outbreaks, the occurrence of AHS outbreaks corresponded with areas with high precipitation in central Namibia (Fig. 2.3). Higher precipitation contributes to more favourable conditions for vector breeding, thereby indirectly increasing the likelihood of the occurrence of AHS outbreaks (Meiswinkel, 1998). *Culicoides imicola* occurs in areas with annual precipitation of between 300- and 700 mm (Meiswinkel & Baylis, 1998).

Previous studies in Namibia conducted by Becker *et al.* (2013), collected *C. imicola* across a rainfall gradient from east to west in the Windhoek district with an average annual precipitation of between 150 mm in the west and 350 mm in the eastern area of the district. The southern districts of the country are extremely dry with precipitation ranging between 0 and 60 mm, which could account for the low incidence of AHS in these areas. Northern parts of the country are classified as medium incidence although favourable conditions do occur in these districts. However, the number of horses in these districts is low, ranging from 0 to 172, therefore the distribution and occurrence of the disease might not have been well documented. Descriptive data on the occurrence and distribution of AHS from 1971 to 1987 is presented in Table 2.1.

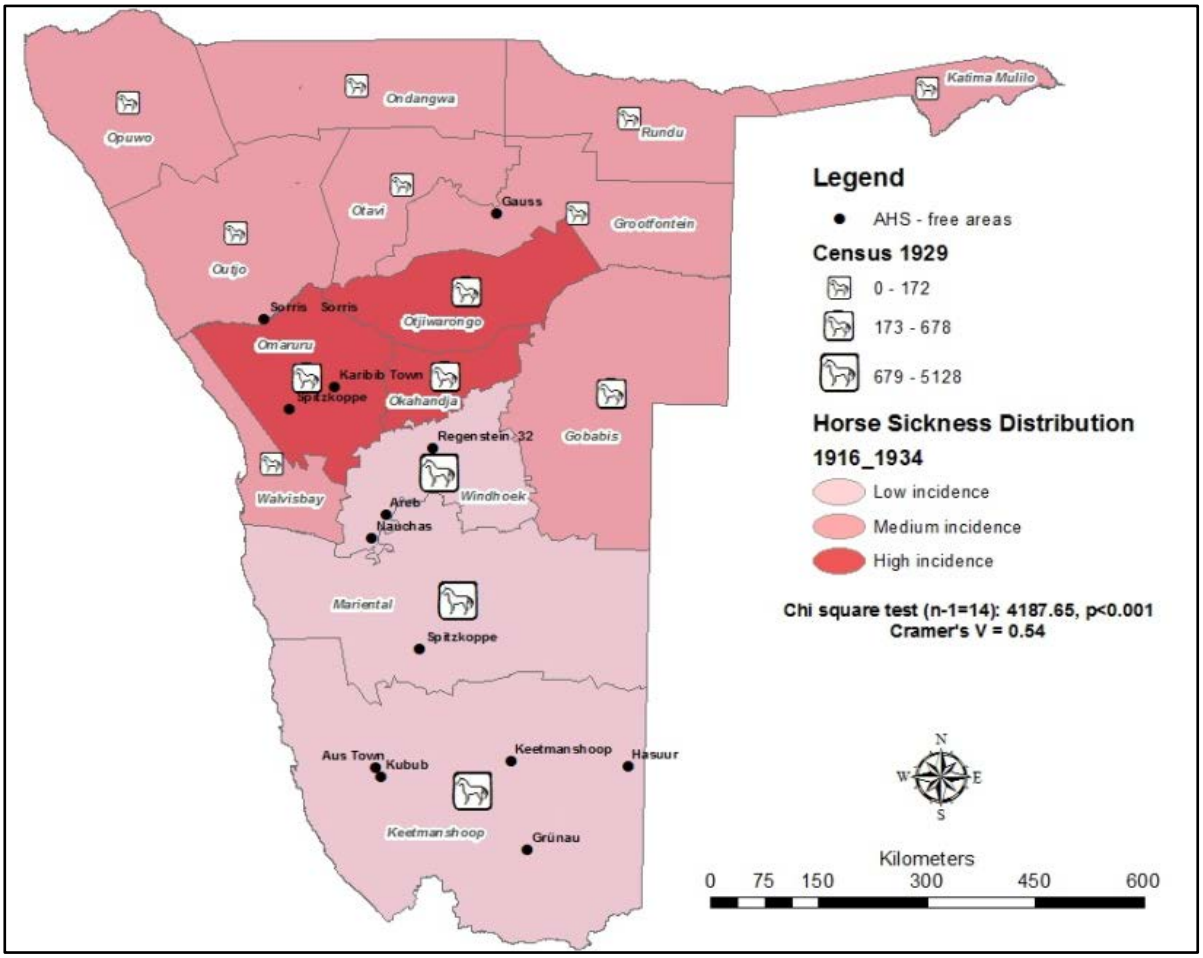


Figure 2.2: Distribution map of AHS in Namibia for 1916-1934 indicating low, medium and high incidence areas. Areas that were believed to be free of AHS during the Pre-colonial and Colonial (German rule) periods are also indicated. The census indicates number of horses per district in 1929. Sources: Annual reports 1916-1934, Directorate of Veterinary Services; Ministry of Agriculture, Water and Forestry, Namibia.

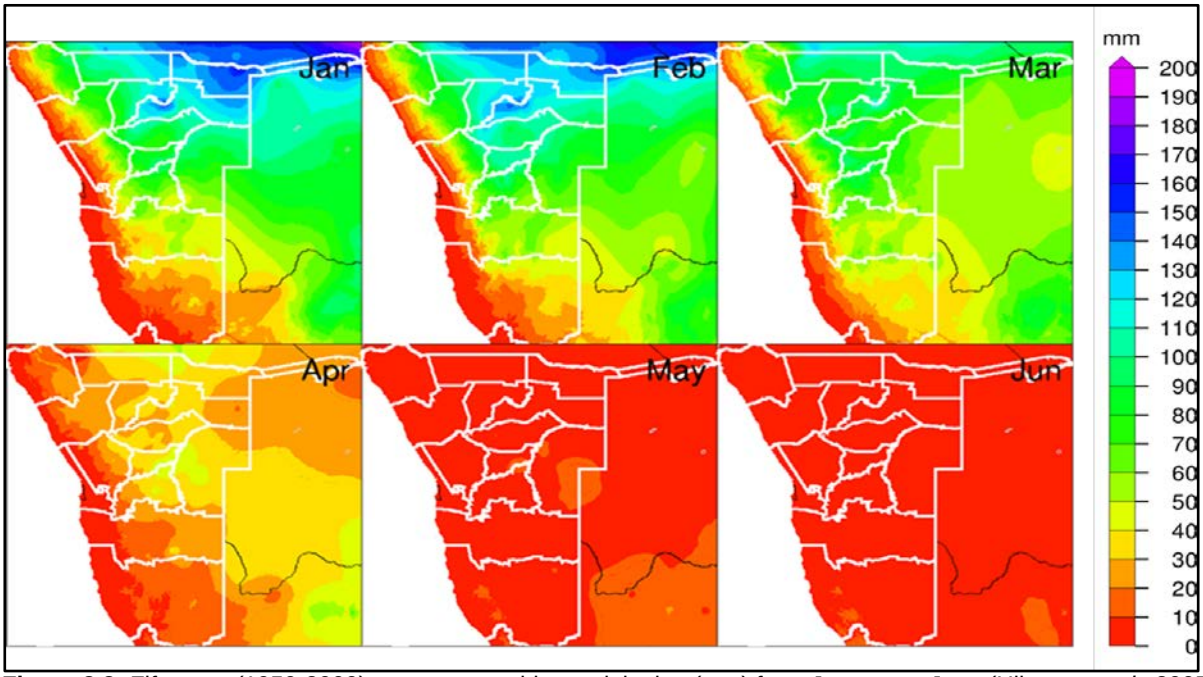


Figure 2.3: Fifty year (1950-2000) average monthly precipitation (mm) from January to June (Hijmans *et al.*, 2005) according to veterinary districts across Namibia.

Table 2.1: Descriptive data on the occurrence and distribution of AHS outbreaks in Namibia for 1971-1987 as published in the Annual reports 1971-1987, Directorate of Veterinary Services; Ministry of Agriculture, Water and Forestry, Namibia.

Year	Description	Other comments
1971-72	More cases than usual - which can be linked to a history of not vaccinating.	
1972-73	A few cases were seen in the Gobabis and Omaruru districts.	
1973-74	Due to the favourable conditions for vectors of the virus, losses reached massive proportions across Namibia.	Losses were also recorded in animals that were vaccinated due to the outbreak of serotype 4.
1974-75	No deaths were reported during this year.	
1975-76	Due to the good rains, the distribution of the disease occurred wide-spread across Namibia.	Both Dikkop and Dunkop cases were diagnosed. There is an increase in demand for the vaccine.
1976-77	A few cases occurred throughout the country. In Grootfontein district a few adult horses died.	The following virus types were isolated: Type 1, 5 and 9. The vaccine is used fairly extensively.
1977-78	AHS was severe during this year with deaths occurring even in vaccinated horses. The increase can be ascribed to the good rains and thus the increase in vectors of the disease.	Cases reported on single farms in the following districts: Keetmanshoop, 36; Mariental, 30; Okahandja, 30.
1979-80	A few cases of Dikkop were reported in the following districts: Gobabis, Keetmanshoop, Namaland, Okahandja and Omaruru.	
1983	Farmers in general made use of the vaccine. Cases were diagnosed in Mariental (3) and Omaruru (1).	
1984	A few cases were reported in the eastern districts of the country: Mariental, Grootfontein, Gobabis	
1985	Rarely diagnosed - 3 outbreaks: 2 in Windhoek and 1 in Grootfontein.	
1987	During the year, 5 outbreaks were reported in the Keetmanshoop, Outjo and Omaruru districts as well as in the Kavango.	One outbreak in the Bondelswarts Reservation in the Karasburg area involved 110 donkeys of which only 10 survived. Although the symptoms and post mortal lesions did resemble those of horse sickness the diagnosis could never be confirmed. Vaccination of horses was practised on a wide scale, especially in the commercial areas.

The AHS distribution pattern for the period 1990-2011 is shown in Fig. 2.4 and is more scattered than for the 1916-1934 period (Fig. 2.2). Horse numbers doubled from the 1934 time period and areas that were previously identified as low incidence districts, now categorised into a higher category. There was a positive correlation between number of horses and AHS cases ($R^2 = 0.508$). According to the Chi-square analysis there was a statistically significant difference ($p<0.01$) between the different districts. However, with the Cramer's V test which adjusts the Chi-square significance to factor out sample size, there was no significant difference (0.069) between the districts. In relation to the precipitation distribution map (Fig. 2.3) precipitation did not visually correspond with the occurrence of AHS for the period 1990-2011 to the same degree as for the 1916-1934 period. This could be due to several societal reasons, with underreporting and the use of a vaccine being the principal suspected causes. The Walvis Bay district changed from a low incidence area in the 1916-1934 period to a high incidence area in the 1990-2011 period with no significant increase in horse numbers. This seems rather strange as this area is mostly desert with little rainfall. This effect is researched further in Chapters 4, 5 and 6. In the northern areas, the Ondangwa district changed from a medium incidence district in the 1916-1934 period to a low incidence district in the 1990-2011 period. However, the horse population increased significantly. It is doubtful that this is a true representation of the history of AHS incidence in the area. This could again be attributed to underreporting or vaccination because favourable temperatures and precipitation ranges do exist in these areas to sustain *Culicoides* spp. This needs to be researched further.

The results of the dependent t-test and the Wilcoxon matched pair test is shown in Table 2.2. According to the dependent t-test ($p>0.14$) and the Wilcoxon matched pair test ($p> 0.24$) there was no significant difference in the occurrence of AHS outbreaks between the two time periods (1916-1934 and 1990-2011). However, underreporting and societal influences must also be taken into consideration. Therefore, when compared based on the visual representation of the occurrence of AHS outbreaks in Fig 2.2 and Fig 2.4, a distinct difference can be observed in the pattern of distribution of AHS between the two periods. The cause of the difference is investigated further in this thesis.

Table 2.2: Results of the dependent t-test and Wilcoxon matched pair test between the two time periods (1916-1934 and 1990-2011) for the occurrence of AHS outbreaks in Namibia, indicating the mean AHS occurrence, standard deviation and p-values.

			p-value	
Time period	Mean AHS occurrence	Standard Deviation	Dependent t-test	Wilcoxon
1916-1934	0.075	0.144	0.1436	0.2489
1990-2011	0.0098	0.020		

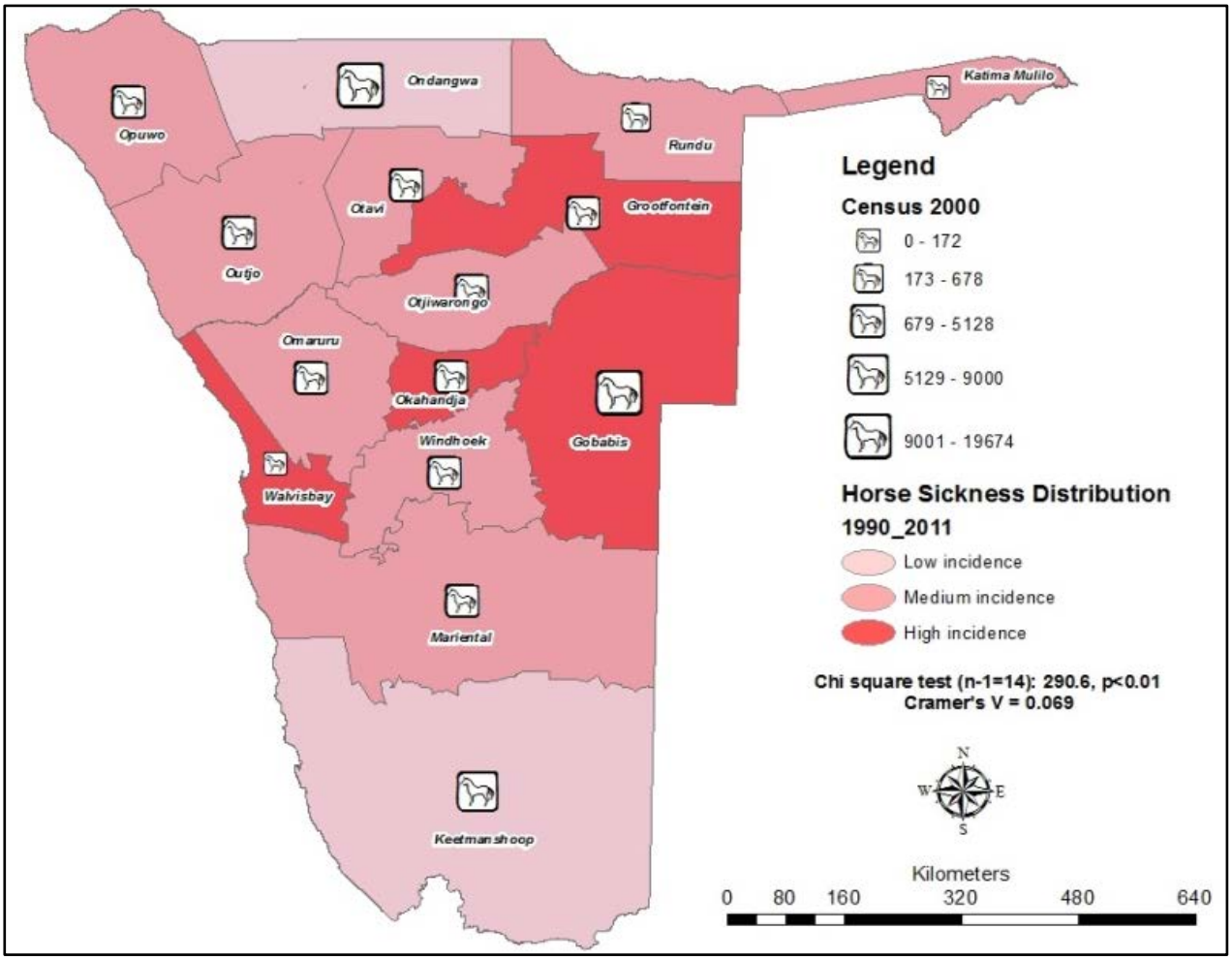


Figure 2.4: Distribution map of AHS in Namibia for 1990-2011, indicating low, medium and high incidence areas. The census indicates number of horses per district in 2000. Sources: Annual reports 1990-2011, Directorate of Veterinary Services; Ministry of Agriculture, Water and Forestry, Namibia.

The most relevant historical events and their influence on the distribution of AHS in Namibia are summarised in Table 2.3. This timeline was constructed from a combination of various literature sources (Henning, 1956; Schneider, 1994; Grobbelaar, 2007; Van den Berg, 2009; Swart, 2010; Goldbeck *et al.*, 2011; Schneider, 2012; Becker, 2012; Verwoerd, 2012). It places events in chronological sequence from the arrival of horses in southern Africa in 1655 up to the 2011 epidemic outbreak of AHS in Namibia. AHS played a major role in the history and development of southern Africa both in times of peace and war. Epidemics of AHS resulted in major transportation impairments (Erasmus, 2009) and losses were also very disruptive to agriculture, mining and military operations (Coetzer & Guthrie, 2004). During the 1923 outbreak, one farmer in the Prieska area lost 53 out of his 57 horses (Henning, 1956). With such a great loss, farm work must have come to a gruelling halt, affecting farmers not only financially but also emotionally.

As discussed in the introduction of this chapter, the Oorlam traders had a significant effect in the initial introduction of horses into Namibia. They were well known for their frequent possession of

rifles, ox-wagons and horses (Kienetz, 1977). Oorlam and Herero groups were opponents for grazing land and therefore trade between the two cultural groups were at a minimum. According to Dugard (1973), the possession of firearms and horses by the Nama and Oorlam groups lead to the defeat of Herero's in several battles, indicating that at that stage Herero's did indeed not have horses. This could have led to the initial lower number of horses in the northern areas (Fig. 2.2) of Namibia as these areas were where the Herero's were settled. The southern areas were mostly inhabited by Nama and Oorlam groups who valued the horse for transportation and military aptitude. Areas free of AHS such as Keetmanshoop were known as early as 1842 by local occupants of Namibia (Schneider, 1994).

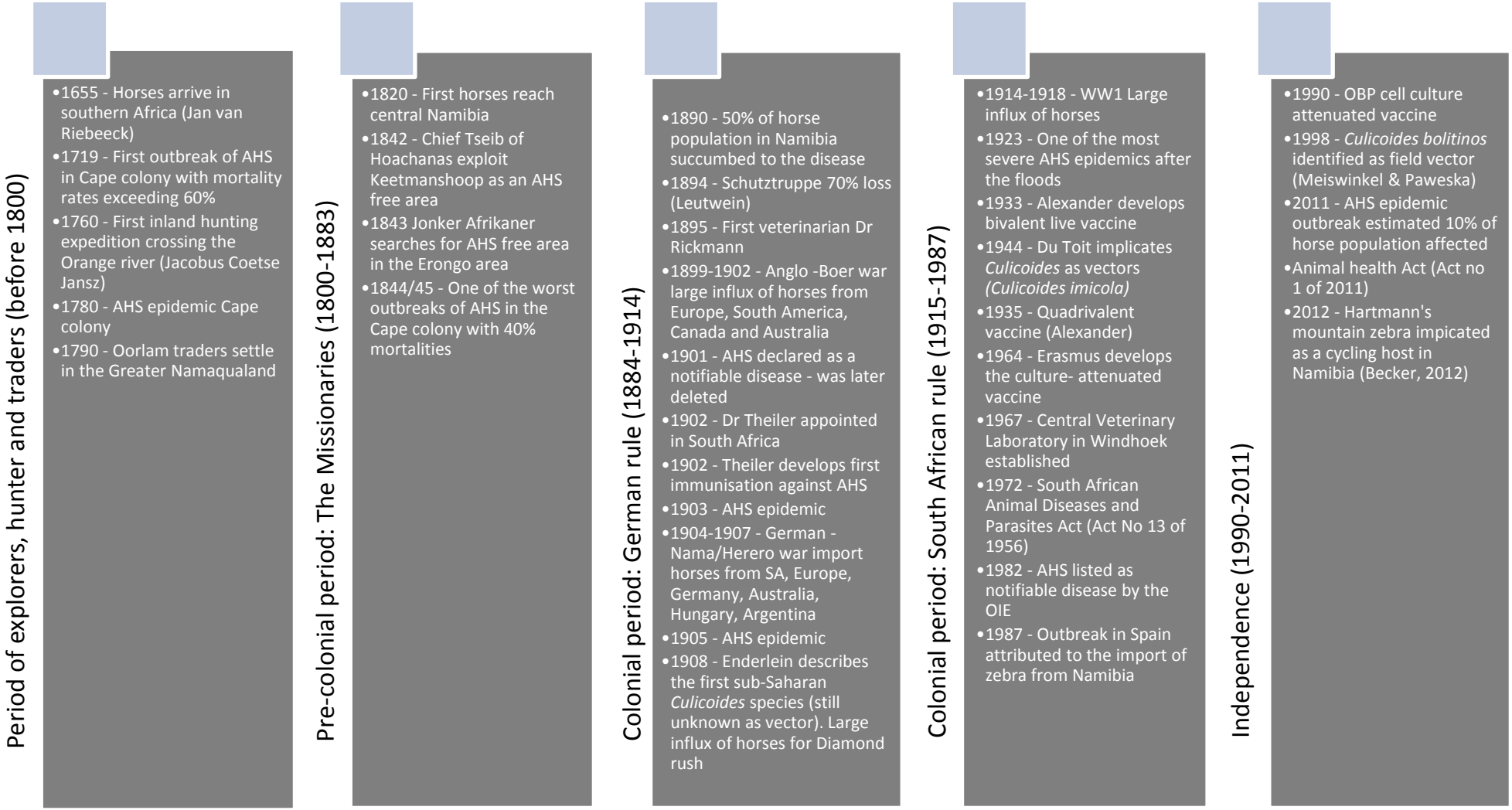
With every large influx of horses into Namibia an outbreak of endemic proportion were soon to follow when conditions were favourable for the increase in *Culicoides* populations. This corresponds with previous results that there is a positive correlation between AHS cases and number of horses. The inflow of horses from the Cape was often hampered due to the outbreak of AHS. These large influxes of horses were mostly during wars where horses were considered as one of the most valuable assets. It was during the Anglo-Boer war that AHS was first declared notifiable and that Dr Theiler was appointed in South Africa with AHS as one of his first priorities. Another aspect that has to be taken into consideration is the conditions that these horses were in. As Swart (2010) mentioned, due to the pressing need for horses during the wars, horses arrived dehydrated, malnourished and with their immune systems severely compromised, making them more susceptible to diseases such as AHS. During these epidemic outbreaks one could only speculate on the effect that it had on the outcomes of battles. Governor Leutwein of the Schutztruppe in 1894 stated that no military excursion takes place during the AHS season unless absolutely necessary (Schneider, 1994).

One example where AHS had a direct effect on military actions was at the well-known Sterbeplatz outside of Windhoek. The Schutztruppe kept their horses on the farm Regenstein in the Auas Mountains during the German colonial times (Fig 2.5) as this area was believed to be free of AHS (Heywood & Lau, 1993). Thus Regenstein had considerable strategic importance for the military mounted units. This was also recognised by the Nama leader, Hendrik Witbooi, who effectively immobilised the Shutztruppe by seizing all of the remaining horses at Regenstein as well as the newly purchased horses on route to Regenstein (Heywood & Lau, 1993).

Research on AHS and the development of an effective vaccine was driven by the need for horses and as this need declined with the introduction of automobiles, new technology and increased political stability, research on the topic went down to a dribble with the last update, the vaccine development in 1990.

Figure 2.5: Photo of the ruins of the Schutztruppe horse camp at Regenstein where horses were kept during the AHS season as a precaution against the disease (June, 2014).

Table 2.3: Timeline depicting the most relevant historical events of AHS and its influence on the distribution in Namibia. Sources: Henning, 1956; Schneider, 1994; Grobbelaar, 2007; Van den Berg, 2009; Swart, 2010; Goldbeck *et al.*, 2011; Schneider, 2012; Becker, 2012; Verwoerd, 2012.



CHAPTER 3

THE *CULICOIDES* SPECIES COMPOSITION AND ENVIRONMENTAL FACTORS INFLUENCING AHS DISTRIBUTION AT THREE SITES IN NAMIBIA

3.1. INTRODUCTION

The first studies on sub-Saharan *Culicoides* spp. date back to 1908 when Enderlein (Cornet & Brunhes, 1994) described two species from Namibia. It was not until 36 years later that a species of *Culicoides* was shown to be the vector of AHS. *Culicoides imicola* was discovered as the most important Old World vector of AHS, with *Culicoides bolitinos* only recently implicated (Meiswinkel & Paweska, 2003). The successful transmission of an insect-borne virus from an infected to a susceptible host depends on the complex relationship between the virus, its insect vector and the vertebrate host, with each being influenced by particular environmental conditions (Wittmann & Baylis, 2000; Wittmann *et al*., 2002; Paweska *et al.*, 2002; Meiswinkel *et al*., 2004). The geographic distribution and seasonal incidence of AHSV depend not only on the presence of the virus and susceptible equines but also on the presence and abundance of competent vectors (Mellor *et al*., 2000).

To qualify as a vector species, certain criteria must be met:

- the virus must be recoverable from field-collected arthropods whose abdomens are free of fresh blood;
- the ability of the arthropod to become systemically infected by feeding on a viraemic host or an artificial substitute must be demonstrated;
- similarly, its ability to transmit the infection biologically through biting must be demonstrated; and
- there should be field evidence confirming the association of infected arthropods with diseased infected vertebrates (Meiswinkel *et al*., 2004; Venter, 2014).

Although *C. imicola* and *C. bolitinos* are the only two species to qualify as field vectors of AHS so far, several other species have tested positive in oral susceptibility experiments (Table 3.1). These studies found that AHSV could persist for at least 10 days in 13 South African livestock associated *Culicoides* spp. This confirms that AHSV is not restricted to a certain *Culicoides* sp.

in the subgenus *Avaritia* but is widespread in the genus *Culicoides* (Mellor & Wellby, 1998; Paweska *et al.*, 2003; Venter *et al.*, 2009c).

Table 3.1: *Culicoides* species implicated as vectors of AHSV (adapted from Bellis, 2013).

***Culicoides* species**	***Culicoides* subgenus**	**Reference**
C. bedfordi Ingram & Macfie 1923	*Synhelea*	Paweska *et al.* (2003)
C. bolitinos Meiswinkel 1989	*Avaritia*	Venter *et al.* (2009c)
C. brucei Austen 1909	*Culicoides*	Venter *et al.* (2009c)
C. enderleini Cornet & Brunhes 1994	*Remmia*	Venter *et al.* (2009c)
C. engubandei de Meillon 1937	*Pontoculicoides*	Venter *et al.* (2009c)
C. exspectator Clastier 1959	*Synhelea*	Venter *et al.* (2009c)
C. gulbenkiani Caeiro 1959	*Avaritia*	Venter *et al.* (2009c)
C. imicola Kieffer 1913	*Avaritia*	Baylis *et al.* (1997); Blackburn *et al.* (1985); Meiswinkel (1997); Meiswinkel (1998); Mellor *et al.* (1990); Venter *et al.* (2009c)
C. leucostictus Kieffer 1911	*Meijerehelea*	Venter *et al.* (2009c)
C. magnus Colaco 1946	*Culicoides*	Venter *et al.* (2009c)
C. pycnostictus Ingram & Macfie 1925	*Meikerehelea*	Venter *et al.* (2009c)
C. sonorensis Khalaf 1956	*Monoculicoides*	Boorman *et al.* (1975); Welby *et al.* (1996)
C. zuluensis de Meillon 1936	Milnei group	Venter *et al.* (2009c)

In an effort to quantify the intricate relationship between hosts, vectors, and the virus, *Culicoides* researchers applied the equation developed for the control of malaria mosquitoes from MacDonald (1957) to *Culicoides* with the addition of vector competence. According to Venter (2014) and Mellor & Hamblin (2004), certain changes as indicated with arrows (↓/↑) in the equation below (Eq. 3.1) can be expected in the vector capacity of a *Culicoides* population with an increase in temperature. Vector density, the probability of feeding on a host and vector competence, will increase with an increase in temperature. The duration of the life cycle and the probability that the vector will survive through 1 day will decrease together with a shortened EIP with an increase in temperature.

Vector capacity: The ability of a *Culicoides* population to transmit virus to a vertebrate host can be determined according to the equation (MacDonald, 1957; Venter, 2014):

Equation 3.1:

$$V = \frac{ma^2 p^n b}{-\log_e p}$$

Where:

m = vector density (↑)

a = probability of a vector feeding on a host in 1 day (↑)

b = vector competence (the ability of a vector to support virus infection and replication and/or dissemination) (↑)

p = probability of vector surviving through 1 day (↓)

n = extrinsic incubation period (EIP) (↓)

$-\log_e p$ = duration of vector's life in days, after surviving EIP (↓)

A competent vector may have a low vector capacity because of low biting rates or survivorship, whereas a vector with low competence may be more efficient in virus transmission (Mullens *et al*., 2004; Venter *et al*., 2009c). Vector competence is genetically determined (Tabachnick, 1991) but it is also influenced by environmental factors which already begin in the habitats of immature stages (Venter *et al.*, 2009c). Several environmental parameters have been identified as drivers of AHS outbreak occurrence and affect the abundance and distribution of *Culicoides* spp. In Chapter 1 these parameters were discussed in detail. Table 3.2 summarises the effect of the different parameters: Rainfall, temperature, humidity, evaporation, wind speed, NDVI and soil type. The direct as well as indirect effects (positive or negative) in both immature and adult life stages are included. A positive effect will lead to the increase of *Culicoides* populations, whereas a negative effect will lead to decreased *Culicoides* population growth or adult activity.

Table 3.2: Positive (pos) and negative (neg) influences of environmental parameters on the developmental stages of *Culicoides* midges.

Environmental parameter:	**Immature stages**		**Adults**	
Rainfall				
• soil moisture content	Pos and Neg	Development of larvae depends on soil moisture content (Meiswinkel *et al*., 1994; Mellor *et al*., 2000; Nevill *et al*., 2007). Pupae drown on immersion in water (Nevill, 1967; Veronesi *et al.*, 2009).		
• rainfall event	Pos	Increasing soil water content – creates favourable breeding habitats.	Neg	Due to their size even a light drizzle inhibits flying (Mellor *et al*., 2000).
• seasonal occurrence	Pos	Previous month rainfall directly related to *Culicoides* abundance (Nevill, 1971) – thus creating more favourable breeding habitats and *Culicoides* populations increase significantly (González *et al.,* 2013).		
Humidity	Pos	Relative humidity is directly proportional to evaporation rate. The higher the humidity the lower the evaporation rate.Therefore, relative humidity will indirectly affect soil moisture content.	Pos and Neg	Relative humidity affects adult midge survival at different temperatures. Low humidity at low temperatures as well as high temperatures and high humidity are detrimental to survival rates of midges (Wittmann *et al.*, 2002).
Temperature	Pos	High night time temperatures accelerate larval development (Conte *et al.*, 2007). Low temperatures induce diapause (Wittmann & Baylis, 2000).	Pos and Neg	High temperatures lead to desiccation due to their small size (Wittmann *et al*., 2002). Influence on EIP rate (Carpenter *et al.*, 2011).
Wind speed			Neg	Due to their small size midge activity are supressed at wind speeds greater than 3 m/s (Sellers, 1980).
Evaporation	Neg	Desiccation of breeding habitats - the upper layer of soil must remain moist for a minimum of seven days for the larvae of *C. imicola* to complete its cycle (Meiswinkel *et al*., 2004).		
NDVI	Pos	Non-vegetative or short grass pastures are the ideal breeding habitat for *C imicola* (Baylis & Rawlings, 1998; Baylis *et al*., 1999a; Conte *et al*., 2007).		
Soil type	Pos	Moisture retentive clayey soils – ideal breeding habitat with a longer water holding capacity (Meiswinkel, 1998).		

AHS is endemic in Namibia but detailed studies of *Culicoides* communities and influencing environmental parameters are limited. In 2011, Namibia lost an estimated 10% of its horse population to AHS and areas that were once thought to be free of this disease were riddled with sick and dying horses. According to Conte *et al*. (2007) and Guichard *et al*. (2014), aridity acts as a limiting factor on the distribution of *Culicoides* midges, yet this no longer appears to be the case. Data on the occurrence of *Culicoides* midges in arid areas are limited – information which is critical to determine whether or not AHS can persist in these areas. Previous studies focused on the Windhoek (Khomas) district (Becker *et al.* 2013) the Walvisbay district near the Brandberg area (Kirk-Spriggs & Meiswinkel, 2014) and in a 400 km perimeter around Windhoek in 2011 (Goffredo *et al.,* 2015). There is no available data on the *Culicoides* spp. in the Aus area. This chapter aims to determine the *Culicoides* spp. composition in Namibia at three different sites and to assess environmental parameters influencing the distribution of AHS. The specific objectives of this chapter were:

- to determine the *Culicoides* spp. composition and abundance at three sites in Namibia.
- to determine of absence or presence of AHSV in the *Culicoides imicola* complex collected at all three sites.
- to determine the most prominent parameters influencing the distribution of AHS.

3.2. MATERIALS AND METHODS

3.2.1. *Culicoides* composition and abundance

3.2.1.1 Installation of UV-light traps

Weekly collections were made during the AHS peak season from January to May (20 weeks) during 2013 and 2014 using the Onderstepoort 220 V UV-light trap (OVI) (Fig 3.1) as described by Venter *et al.* (2009a). This metal trap weighs approximately 4 kg, and is equipped with a 30 cm, 8 W ultraviolet fluorescence tube (Venter *et al*., 2009a). The trap operates by a suction mechanism caused by a suction fan with a mean air flow displacement capacity of 204.5±9.47 m^3/min (del Rio *et al*., 2013). Polyester netting placed around the entrance portals of the trap avoids the collection of bigger insects. At night midges are attracted to the UV light, and are then sucked through the gauze with 2 mm aperture and blown into the 500 ml collection beaker (Venter *et al.*, 2009a). This trap has been used in South Africa since 1970 with great success (del Rio *et al*., 2013). Four traps were installed

per site. Traps were set up in the exact same position during both sampling years. The first trap was set up as close as possible to the horses stables in order to collect the highest possible numbers of *Culicoides*. This trap was used for AHSV identification only and was labelled as TRAP 1– A: Aus; W: Windhoek (Seeis) and O: Okahandja (Table 3.3). The other three traps (TRAPS 2, 3 & 4) were set up at each site exposed at 360° to the natural environment for *Culicoides* spp. identification. Traps were set up as far away as possible from the homestead – taking electrical supply and possible theft of traps into account. According to Venter *et al*. (2012), the attraction range between two traps will be between 2 m and 4 m, with the area covered by a single OVI trap not exceeding 50 m^2. The objective was to evaluate natural breeding sites for the occurrence of *Culicoides* and not the anthropogenic effect on population numbers. Twelve traps were therefore set up at the three different sites. Traps were installed at the same height above the ground - approximately 1.5 m (Venter *et al.*, 2009b).

Even though light-traps are the most common method to monitor *Culicoides* populations, there are some factors that should be taken into consideration: 1) the proportion of the population collected per light trap represents an estimated 1% of the total *Culicoides* population (all collected *Culicoides* individuals) within the active catching area (approximately 50 m^2 depending on site conditions) (del Rio *et al*., 2013); 2) traps do not collect adult males and are biased towards the collection of blood-fed females; 3) light traps only reflect that a species was collected in that direct vicinity and does not prove its involvement in epidemiology (Venter *et al*., 2012); and 4) light trap collections cannot be used to determine biting rate (Viennet *et al.*, 2011). Collections were made in 30% ethylene glycol solution for sample preservation. Samples were transferred and preserved into 70% ethanol for *Culicoides* identification (Goffredo & Meiswinkel, 2004). *Culicoides* spp. are diurnal, preferring to fly under warm, calm conditions (Meiswinkel *et al.*, 2004) and the traps were therefore operated from sunset to sunrise. The traps were regulated by a Troptronic® programmable time switch (model TDDT7). The timer switch synchronised all the traps to switch on at 17h00 and off at 7h00. Traps were emptied once per week (all traps on the same day of the week across Namibia) by farmers on whose properties the traps were installed. During the last 9 weeks of collection in 2013 and the whole 2014 season, a field assistant was appointed to collect the samples and for general maintenance of traps. It was noticed that wear and tear of the gauze positioned over the UV-fluorescent tube did occur, causing larger insects also being collected, thereby making the identification process more laborious and difficult.

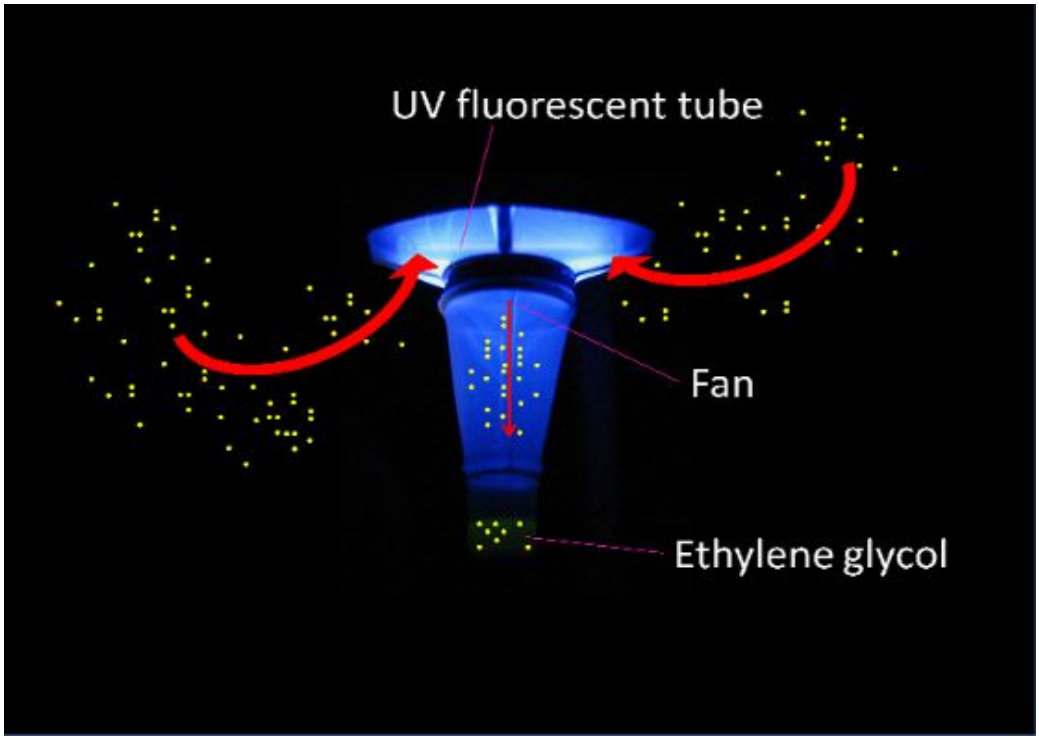


Figure 3.1: Diagrammatic presentation of the operation of the Onderstepoort 220 V suction UV-light trap (Used with permission from: Becker, 2012).

3.2.1.2. Limitations of the OVI 220 V trap

During the 2013 sampling season, unreliable electrical power supply resulted in undependable traps. The unreliable power was a result of distance to the closest power source, power cuts during rain storms and critters gnawing through the electrical cable. To overcome this problem it was decided to convert the 220 V OVI traps to solar powered 12 V traps. The solar systems had their own limitations, of which theft was the major problem. Another problem was that the system could only operate for 6 hours per day, therefore timers had to be set in order for the traps to work during the most active time of day, namely sunrise and sunset. Traps were monitored daily by farm owners and all trap timers were reset every few weeks to allow for change of season. In a pilot study, the OVI electrical traps were compared to the solar system trap and results indicated no significant differences in the number of collected *Culicoides* collected between the two traps. Although the solar system also had limitations, it proved to be successful in collecting *Culicoides* midges and can be used in areas where there is no power supply.

3.2.1.3. Identification of Culicoides midge species

For accurate identification of adult *Culicoides* spp. the variations in the grey and white wing patterns were used using the wing picture atlas of Afrotropical *Culicoides* (Meiswinkel, 1996). *Culicoides* spp. were separated from other insects and identified according to the

subsampling procedure of Van Ark & Meiswinkel (1992). In collections with less than 800 *Culicoides*, each specimen was identified and counted per species. Identification between subgenera must be performed by a specialist due to the difficulty in identification based on the wing patterns (Nevill *et al*., 2007). Therefore species identification was done by a specialist (Karien Labuschagne) from ARC-Onderstepoort Veterinary Institute. Out of 397 collections made, 102 samples (All the trap 1 collections) were analysed for AHSV presence or absence with quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR). A total of 295 collections were analysed for total *Culicoides* (all collected *Culicoides* individuals) and 75% of these samples were examined for more in-depth species identification.

3.2.2. Site selection and description

Sites for the assessment of the present AHS distribution in Namibia were selected according to the historical profile of AHS outbreak occurrence and status of the district (Chapter 2). Namibia is classified as an arid environment with 95% of the country receiving rainfall of less than 500 mm/year (Sweet & Burke, 2000). Characteristics of the three sites: GPS coordinates of traps, altitude, vegetation type and number of collections is given in Table 3.3. Fig 3.6 illustrates some of the traps and site characteristics in all sites across Namibia. The samples were collected weekly during the periods January to May 2013 and January to May 2014. Sampling sites, towns, veterinary districts and topography of Namibia are indicated on the map in Fig. 3.2. The travelling distance between Okahandja and Aus was approximately 650 km and between Okahandja and Windhoek approximately 100 km.

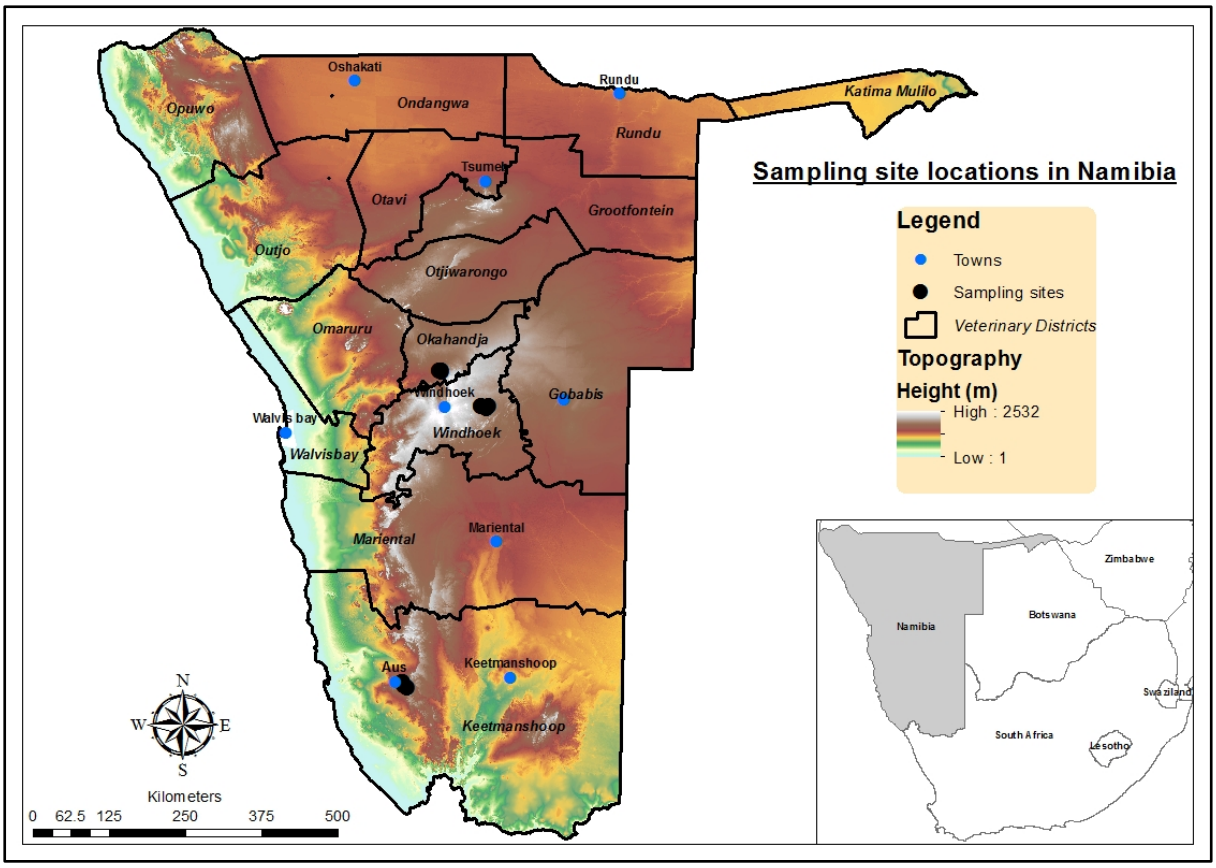


Figure 3.2: Map illustrating the sampling sites, towns, veterinary districts and topography of Namibia.

3.2.2.1. AUS – Keetmanshoop district (Low incidence)

Aus is located in the Keetmanshoop district which is historically a low AHS incidence area. This specific site was chosen as it is close (approx. 20 km) to the well-known feral horses of Namibia site and there has been no record of AHS in these parts of the district over the past 100 years.

Due to the low incidence of AHS in this area, horses are free-roaming on the farm, but an average of 2 horses was in the close proximity to the sampling area. The grazing capacity of the area is very low, therefore grazing camps comprise of hundreds of hectares. Since the farm borders the Sperrgebiet nature reserve, other possible hosts in and around the study area included kudu, gemsbok and other small wildlife such as klipspringers. Zebras do not occur in these areas. Domestic animals included goats and sheep that graze in the communal land next to the study area. Host preferences of *Culicoides* were discussed in Chapter 1.

A site area layout of the traps is shown in Fig. 3.3. Fig 3.6c illustrates the solar system TRAP 3 and site characteristics of the Aus site. The catches made in Trap A1 was used for virus identification. There are no stables for animals because of the low occurrence of outbreaks

of the diseases. The trap location was therefore near the only available natural water source which was presumed to be the best breeding habitat for *Culicoides*. There is a dry river bed running through the site which only has water during flooding. There is a small patch of irrigated garden on the site but traps were set up more than 20 m away from this area. One of the horses was vaccinated in March 2013 with the registered OBP vaccine to be able to compete in endurance races.

Figure 3.3: Site layout of Aus indicating the traps A1-A4, homesteads, irrigated garden and the ephemeral river location.

3.2.2.2. WINDHOEK (SEEIS) – Windhoek district (Medium incidence)

Seeis is a Warmblood stud located in the Windhoek district with 150 horses at stud. Historically, this is a medium incidence area. AHS is considered a problem on the farm with 10% of the horses contracting the disease during the past 5 years. There are approximately 25 horses stabled in the yard which varies with the training regime of the stud. The rest of the horses are free-roaming on the farm. Stables are mostly open paddocks with about 10 closed stables. Horses are vaccinated annually with the registered OBP vaccine during the winter months. A site area layout of the traps is shown in Fig. 3.4. Fig 3.6b illustrates the site characteristics and solar system TRAP 2 near the ephemeral river and horse camp. In addition to the horses, there were some goats and chickens near TRAPS 3 and 4. Otherwise, there were no other hosts in the area except for some small wildlife. There are no zebras on the farm.

There is an ephemeral river bordering the site which will only have water during the rainy summer months. There is an irrigated garden near the homestead, but traps were more than 20 m away from this area.

Figure 3.4: Site layout of Windhoek (Seeis) indicating the traps W1-W4, homesteads, stables, irrigated garden and the ephemeral river location.

3.2.2.3. OKAHANDJA – Okahandja district (High incidence)

The site is in a high incidence area and is situated east from the town of Okahandja. The site is on an Arabian horse stud farm and training centre. There are approximately 25-30 horses at this stable yard, depending on the training regime. All horses are stabled in a semi-open warehouse with structured stables. AHS is considered to be a problem on the farm with mortalities reported every second year in the past 5 years. Horses are vaccinated annually with the registered OBP vaccine. These horses are endurance race horses and are therefore only vaccinated after the endurance season in January. Neighbouring farms all have cattle and there are approximately 20 goats on the site, 1 camel, 2 gemsbok, 1 hand reared zebra (which is stabled with the horses) and other small wildlife. Zebra also occur naturally in the Okahandja district. The site borders an ephemeral river which only has water during the summer rainy season. There is an irrigated patch of lucerne and traps were placed as far away as possible from this patch – more than 10 m. A site area layout of the traps is shown in Fig. 3.5. The solar system TRAP 1 and stable complex at the Okahandja site are shown in Fig 3.6d.

Figure 3.5: Site layout of Okahandja indicating the traps O1-O4, homesteads, stables, irrigated garden and the ephemeral river location.

Table 3.3: Characteristics of the three sites in the entomological surveillance for *Culicoides* in Namibia during 2013 (January-May) and 2014 (January-May). (Vegetation type source: http://www.nnf.org.na/RARESPECIES/InfoSys/GeneralInfo/ListMaps).

Site	Trap ID	GPS coordinates	Altitude (m)	Vegetation type	Number of collections
Aus	A1	S26°39.375' E016°14.840'	1408	Desert/dwarf shrub transition	24
	A2	S26°39.060' E016°14.532'	1388		33
	A3	S26°39.211' E016°14.617'	1387		32
	A4	S26°39.375' E016°14.588'	1390		34
Seeis (Windhoek)	W1	S22°26.647' E017°35.628'	1627	Highland shrubland	38
	W2	S22°26.560' E017°35.712'	1623		34
	W3	S22°26.567' E017°35.320'	1630		30
	W4	S22°26.567' E017°35.271'	1630		17
Okahandja	O1	S21°58.363' E016°55.300'	1348	Thornbush shrubland	40
	O2	S21°58.341' E016°55.290'	1348		40
	O3	S21°58.374' E016°55.295'	1346		40
	O4	S21°58.364' E016°55.411'	1349		35

3.2.3. Weather data

A Vaisala multisensor wonder (WXT520) weather station was installed at each site according to the manufacturer's instructions, operating from a solar system. Fig. 3.6a shows the installation at the Aus site. Due to shipping problems, the weather stations were only installed for the last 6 weeks (April – May) of sampling during 2013, but were operational for the complete 20 weeks (January – May) during the 2014 sampling period. The weather stations took measurements of the following variables every 10 min at each site:

- Precipitation
- Atmospheric pressure
- Wind speed and direction
- Air temperature
- Humidity

For each site data were averaged weekly as well as fortnightly for all of the above mentioned parameters.

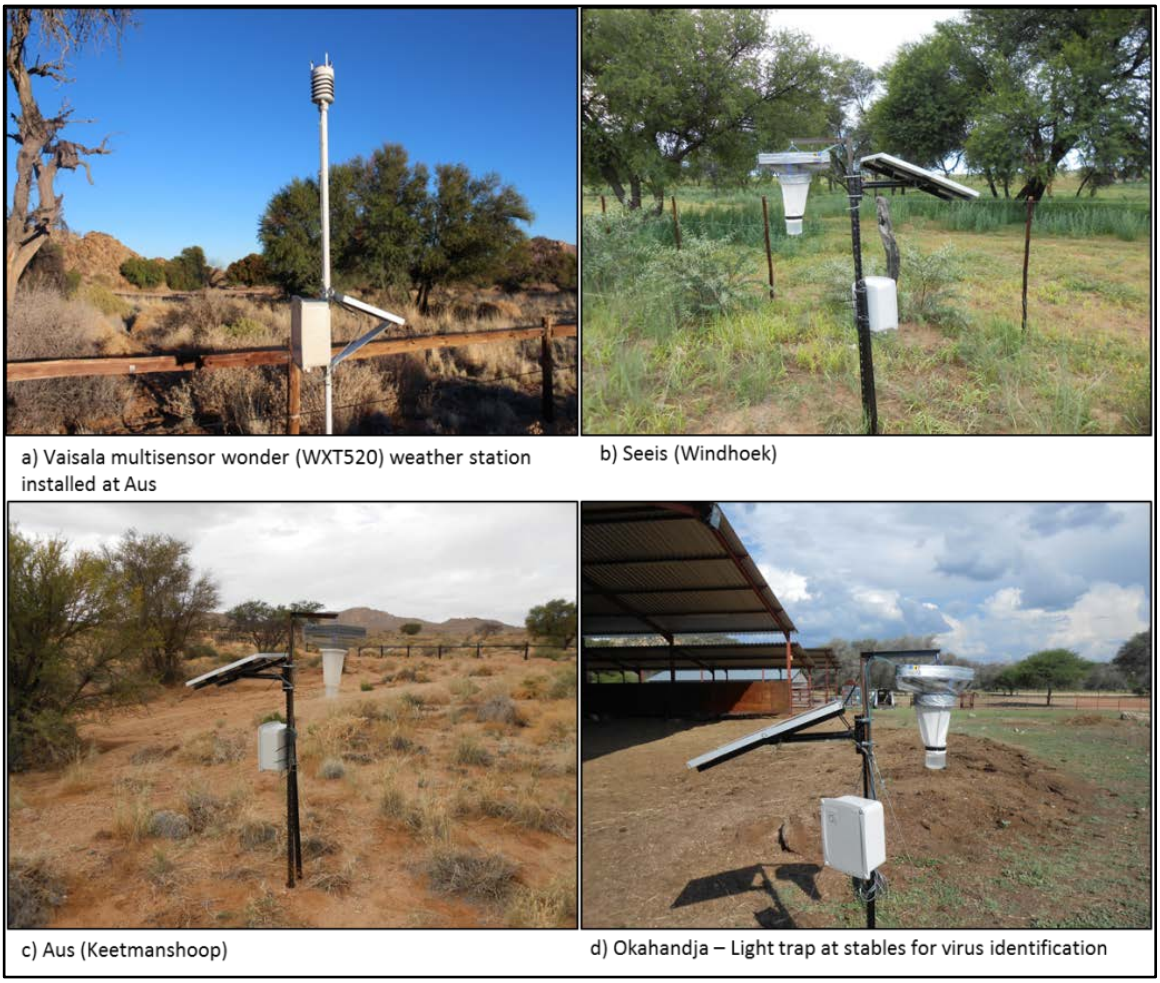


Figure 3.6: Solar operated systems at sampling areas, also showing site characteristics at each site: a) Vaisala weather station at Aus operating from a solar system; b) Trap nr 2 at Seeis stud farm in the Windhoek district near the ephemeral river and horse camp; c) Trap nr 2 at Aus in the Keetmanshoop district; and d) Trap nr 1 for virus identification next to the stable complex in Okahandja.

3.2.4. Normalised difference vegetation index (NDVI)

Remote sensing data from satellites provide area-wide coverage of environmental conditions over long periods of time which have been used to monitor vector presence in various vector-borne disease studies (Baylis & Rawlings, 1998; Tatem *et al*., 2003). NDVI has been shown to be important because *Culicoides* breeds in damp or wet soil. Therefore, NDVI has the potential to indicate the presence of breeding sites (Mellor & Hamblin, 2004). The Moderate Resolution Imaging Spectroradiometers (MODIS) are mounted on Sun Synchronis satellites (Terra and Aqua satellites) that allow geophysical parameters to be measured at a spatial resolution of between 250 and 1000 m (Klingseisen *et al*., 2013). Global coverage of NDVI data are obtained every 1 to 2 days (Xie *et al.*, 2008). MODIS 250 m resolution NDVI data were obtained for the three sites from the ARC-Institute for Soil, Climate and Water for the study period and was available at 16-day composite intervals. All the other data were therefore averaged for every fortnight to facilitate comparison. Average NDVI data were extracted for the study period for all

three sites according to the GPS coordinates of the traps. Similar values were obtained for traps situated within the same grid.

3.2.5. Physical and chemical soil properties

Soil moisture and availability of organic matter are important factors that impact on the survival rate of *Culicoides* spp. (especially *C. imicola*) during the larval stages of their lifecycle (Meiswinkel, 1998; Meiswinkel & Paweska, 2003). These factors need to be taken into consideration in determining the distribution of AHS. Soil properties such as particle-size distribution play an important role in determining soil moisture content. Four composite samples of soil were collected using a random sampling design for each year. Samples were collected around each light trap stationed at the site. Samples were clearly marked with GPS co-ordinates of the point where it was sampled and the date.

Physical and chemical analyses of soil samples were conducted by an independent laboratory according to standard procedures. Quantification of the particle-size distribution of soil samples was conducted according to the procedures advocated by the American Society for Testing and Materials (ASTM, 1961). Organic carbon was determined according to the loss on ignition procedure as described by Donkin (1991).

3.2.6. The presence or absence of AHSV

Several techniques are available for AHSV identification. The indirect sandwich enzyme-linked immunosorbent assay (ELISA), the polymerase chain reaction (PCR) or cell culture and inoculation of newborn mice (OIE, 2008). However, these techniques have the disadvantage of prolonged processing in the laboratory (Scheffer, 2011; Bachanek-Bankowska *et al*., 2014). AHSV can be identified directly in whole blood and other tissues using molecular probes and real-time reverse transcription polymerase chain reaction (RT-PCR) using group specific primers. A number of RT-PCR procedures have been described for AHSV (Mizukosi *et al.*, 1994; Koekemoer & van Dijk, 2004; Agüero *et al.*, 2008; Rodriguez-Sanchez *et al*., 2008; Quan *et al*., 2010). RT-PCR has proved to be an improved rapid technique regarding sensitivity and specificity (Scheffer, 2011). The application of an adapted RT-qPCR for *Culicoides* was first described by Scheffer *et al.* (2011) to quantify viral loads in *Culicoides* midge pools.

Culicoides imicola complex females collected and identified from TRAP 1 were used for virus identification at each site. Female *Culicoides* can be differentiated by Dyce's method (Dyce, 1969), which is based on the presence of pigmentation of the abdomen of parous individuals. According to Braverman & Mumcuoglu (2009) Dyce's method is inaccurate for determining nulliparous and parous females. However, for lack of an alternative method, Dyce's method was utilised and parous, gravid and blood engorged females were used. *Culicoides* samples were

divided into pool sizes to determine the level of detection (De Waal, 2015). Fortnightly collected *Culicoides* were pooled together and divided into three pools of 30 *Culicoides* each. Each pool was tested in triplicate.

A standard procedure for RT-qPCR for *Culicoides* as described in De Waal (2015) was performed. The standard curve was used to determine the efficiency of the AHSV qRT-PCR reaction. The sensitivity of the AHSV assay was analyzed with 10-fold dilution of AHSV RNA. The correlation coefficient (R^2) was 0.997 and the efficiency of the PCR reaction was 108.3% (De Waal, 2015). The samples were homogenised in TRIzol® LS reagent using 3 mm stainless steel beads for 2 minutes in a TissueLyser (Qiagen). RNA isolation was done with the Qiagen Rneasy® MinElute® Cleanup Kit according to the manufacturer's instructions. RNA samples were concentrated to a final volume of 10 µL.

AHSV RNA was detected by a one-step Real-time quantitative reverse transcription PCR, targeting the NS1 segment (AHSV NS1 forward primer: 5'-CgCAATCTTCggATgTAAgC-3', AHSV NS1 reverse primer: 5'-gCACATACCTTggATCTCTg-3' and 6FAM-TCgCCA+TCC+TCA+TCATCg--BBQ AHSV NS1 Taqman LNA). The LightCycler® 480 RNA Master Hydrolysis Probes, Roche and the Bio-Rad CFX96™ Real-Time PCR Detection System were applied according to the manufacturer's instructions. Each sample was analysed in triplicate. The cycling conditions were: i) 98.0°C for 30 seconds, ii) 61.0°C for 10 minutes, iii) 95.0°C for 30 seconds, iv) 95.0°C for five seconds, v) 61.0°C for 30 seconds, vi) followed by 44 cycles of steps iv) and v). The positive controls were 10^7 dilutions of the AHSV4 and a non-template control (NTC) was also included.

3.2.7. Statistical analyses

Statistical analyses were performed using CANOCO for Windows 4.5 (Ter Braak & Šmilauer, 1998) and SPSS (IBM SPSS Statistics for Windows, Version 22.0, 2013).

Multivariate statistics using CANOCO enables researchers to analyse data and summarise patterns of the main characteristics in an easy-to-understand form and to test for statistical significance and predict changes in communities (Lepš & Šmilauer, 2003). Multivariate statistics were performed to investigate the relationship between different variables using principal component analysis (PCA) and redundancy analysis (RDA) (CANOCO for Windows 4.5). Both PCA and RDA analyses are linear methods that explain relationships between variables. A PCA was performed for the Aus dataset, as CANOCO needs at least two response variables to be able to perform an RDA. There were no positive AHSV samples found and therefore only total *Culicoides* (all collected individuals of the *Culicoides* genus) was used as response variable. With only a single response variable available for Aus, the PCA was used to summarise the distributional properties of total *Culicoides* with environmental variables. This is an

unconstrained method that searches for any variable that best explains the distribution of the data (Lepš & Šmilauer, 2003). For Windhoek and Okahandja as well as for the dataset as a whole, an RDA was performed. This is a constrained PCA. There was a set of response variables (AHSV and total *Culicoides*) together with environmental variables where the relationships can be summarised with weighed sums of the environmental variables on the ordination axes (Lepš & Šmilauer, 2003). For the data as a whole, a co-variable descriptor was included to specify the province of origin since the data comprised datasets from three districts across Namibia. This enables CANOCO to factor in differences based on the three districts and then construct an ordination model looking at the data as a whole set. The length of the arrows in the RDA ordination indicates the importance of the specific parameter.

To determine if there was an autocorrelation in total *Culicoides* over time, a Durbin-Watson analysis was performed indicating no time dependency. Therefore, data from all three traps were averaged for further multivariate analyses in CANOCO. A univariate two-way analysis of variance (ANOVA) was performed to determine whether total *Culicoides* collected, differed significantly between sites and years, with $p<0.05$ indicating a significant difference. Spearman rank correlations as well as Pearson correlations (Hauke & Kossowski, 2011) were performed to determine correlations between different soil and climatic variables and total *Culicoides* collected.

ANOVA was also performed on the 2014 dataset of total *Culicoides* to determine if weekly temperature and relative humidity had a significant influence on total *Culicoides*. Temperature and relative humidity were divided into categories as fixed factors (Table 3.4) and total *Culicoides* as the dependent variable.

Table 3.4: Categories of temperature and relative humidity for ANOVA.

Category	Temperature (°C)	Relative humidity (%)
1	<18	<40
2	18-23	40-60
3	>23	>60

Due to the complicated nature of the interactions between environmental variables and the occurrence of *Culicoides,* descriptive statistics were implemented to support the ANOVA results on the interaction between temperature and relative humidity. Weekly temperature and relative humidity data were divided into categories (Table 3.5) and the average (of all three traps) weekly total *Culicoides* collected was noted for each combination of categories.

Table 3.5: Categories of temperature and relative humidity for descriptive statistics.

Temperature category	Temperature (°C)	Relative humidity category	Relative humidity (%)
A	12-15	1	20-30
B	15-17	2	30-40
C	17-19	3	40-50
D	19-21	4	50-60
E	21-23	5	60-70
F	23-25	6	>70
G	>25		

3.3. RESULTS AND DISCUSSION

3.3.1. *Culicoides* species composition

A total of 224 (75%) of the 295 samples collected (79142 midges) were analysed for species identification with 48 different *Culicoides* spp. collected (Table 3.6). *C. imicola* was the dominant species with a total of 29.9% with *C. subschultzei, C. expectator* and *C. ravus* each contributing more than 10% to the species composition. No time dependence was found with Durbin-Watson analysis, probably due to the large number of factors influencing *Culicoides* occurrence. The two-way ANOVA for total *Culicoides* with year and site yielded no statistically significant interaction between year and site ($p=0.552$). However, the main effects (effect of a single factor) of year and site were both highly statistically significant ($p<0.001$ and $p=0.005$ respectively). During 2013, significantly more total *Culicoides* were collected than in 2014. Sidak's post-hoc test indicated that there was a highly significant difference in total number of *Culicoides* individuals collected between Okahandja and Aus ($p=0.004$). However, there was no significant difference in total *Culicoides* collected between Windhoek and Okahandja ($p>0.1$) or Windhoek and Aus ($p>0.05$).

Aus – The lowest number of *Culicoides* was collected at Aus (9980) which accounted for 12.6% of the total number of *Culicoides* collected across all three sites. Eighty three samples (84%) were analysed for species identification. Twenty one different species were identified (Table 3.6). *C. ravus* (45.9%) and *C. herero* (31.5%) were the dominant species with *C. imicola* only comprising 6.8% of the total *Culicoides* collected.

Windhoek (Seeis) – A total of 21819 *Culicoides* was collected at Windhoek which accounted for 27.5% of the total number of *Culicoides* collected across all three sites. Fifty nine samples (72%) were analysed for species identification with 36 different species identified (Table 3.6). *C. subschultzei* (28.4%) was the dominant species, followed by *C. imicola* (12.7%) and *C. herero* (12.5%).

Okahandja – The highest number of *Culicoides* was collected at Okahandja and accounted for 59.8% of the total number of *Culicoides* collected across all three sites. A total of 47343 *Culicoides* were collected with 82 samples (71%) analysed for species identification. Forty one different species of *Culicoides* were identified (Table 3.6). *C. imicola* (42.7%) was the dominant species, followed by *C. expectator* (17.9%) and *C. subschultzei* (14.7%).

The numbers of total *Culicoides* collected (all collected *Culicoides* individuals) as well as *C. imicola* collected (Fig. 3.7) correspond to the AHS outbreak occurrence classification for the districts indicated in Chapter 2: Aus – Low incidence, 31903 *Culicoides,* 6.8% *C. imicola;* Windhoek – Medium incidence, 48871 *Culicoides,* 12.7% *C. imicola;* Okahandja – High incidence, 82249 *Culicoides,* 42.7% *C. imicola.*

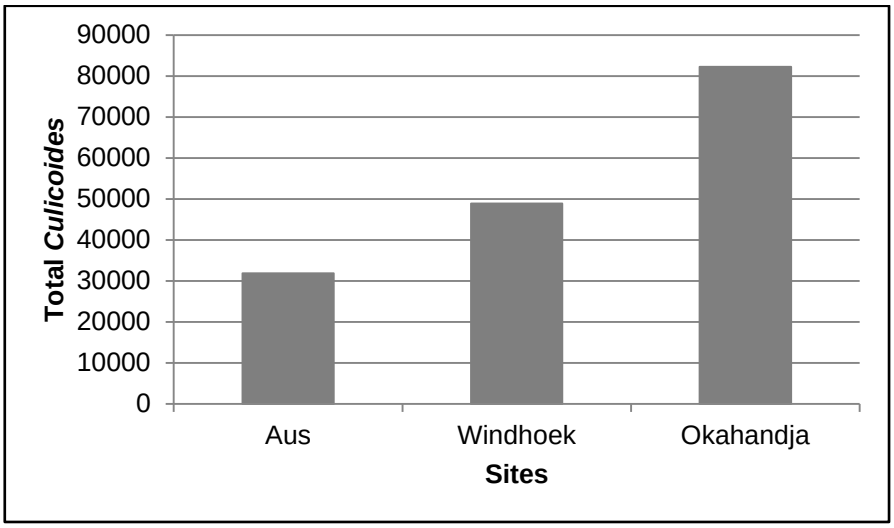


Figure 3.7: Total *Culicoides* collected from all three sampling sites in Namibia for week 1-20, (January – May), 2013 and week 1-20 (January – May), 2014.

Table 3.6: The *Culicoides* species composition from collections at Aus, Okahandja and Windhoek in Namibia, from January-May 2013 and January-May 2014. Shaded cells indicate highest percentage collections of an identified species made at a specific site. Numbered species (#) indicate that this species is yet to be described. *Culicoides* species comprising less than 0.1% of the site and of total collection are indicated with asterisks (*). The total column indicates the percentage of the identified species collected in total for all three sites.

Site	**Aus**	**Windhoek**	**Okahandja**	**Total**
No. of collections identified	83	59	82	224
No. of species	21	36	41	48
Species	**% of site total**	**% of site total**	**% of site total**	**% of grand total**
C. imicola	6.8	12.7	42.7	29.9
C. subschultzei		28.4	14.7	16.6
C. expectator	*	8.9	17.9	13.2
C. ravus	45.9	11.4	4.4	11.5
C. herero	31.5	12.5	3.0	9.2
C. tropicalis	*	11.9	0.6	3.6

C. pycnostictus	0.1	5.2	2.3	2.8
C. leucostictus	0.2	1.8	3.3	2.5
C. nivosus	0.7	1.9	2.0	1.8
C. schultzei	*	0.4	2.1	1.4
C. pretoriensis	0.1	1.3	1.3	1.2
C. similis	5.3	0.7	0.4	1.1
C. enderleini	0.1	0.1	1.3	0.8
C. bedfordi	3.8	0.3	0.3	0.8
#89	4.9	*	*	0.6
#61		0.9	0.4	0.5
C. tuttifrutti (#30)	0.1	0.1	0.7	0.5
Accraensis group		0.1	0.6	0.4
C. punctithorax	0.1	*	0.5	0.3
C. tororoensis		1.0		0.3
C. albopunctatus		0.1	0.3	0.2
C. bolitinos		0.1	0.3	0.2
C. coarctatus		*	0.2	0.1
C. eriodendroni		*	0.1	0.1
C. neavei		*	0.1	0.1
C. brucei	0.2	*	*	0.1
#54 (d/f)		*	0.1	*
Nigripennis group		*	0.1	*
C. cornutus		0.1	*	*
C. macintoshi	0.2	*		*
#94	*		*	*
C. distinctipennis			*	*
#62		*		*
C. miombo		*	*	*
C. kanagai			*	*
C. rhizophorensis			*	*
#50		*	*	*
#54 (p/f)			*	*
#33	*	*		*
#107			*	*
C. glabripennis		*		*
C. loxodontis			*	*
C. nevilli			*	*
C. olyslageri			*	*
C. ovalis			*	*
C. dekeyseri	0.1			*
C. trifasciellus		*		*
#69			*	*
Total no. individuals collected	9980	21819	47343	79142

* *Culicoides* species comprising less than 0.1% of the site and of total collection

C. imicola was the dominant species collected at the three sites, with the highest percentage collected at the Okahandja site. This site had the highest density of possible hosts, which has an effect on the occurrence of *C. imicola* (Meiswinkel *et al.*, 2004). Compared to South Africa where *C. imicola* comprises more than 90% of the collections made (Meiswinkel *et al.*, 2004), its contribution in Namibia was below 30%. Species that have tested positive for AHSV in oral susceptibility experiments (Table 3.1) comprised 49.5% of the total *Culicoides* collected during this survey: *C. imicola, C. expectator, C. pycnostictus, C. leucostictus, C. enderleini, C. bedfordi, C. bolitinos* and *C. brucei.* Although *C. ravus and C. expectator* where some of the most abundant species, it is believed they feed on birds and therefore their role in the transmission of AHS is probably insignificant (Meiswinkel, 1996). Not much research has been done on *C. subschultzei* that was the second most abundant species with no information available on its ability to transmit viruses (Meiswinkel, 1996). It is essential that oral susceptibility experiments be performed on other dominant species in Namibia such as *C. herero* and *C. subschultzei*.

Of the 48 *Culicoides* spp. collected, only 17 species were collected at all three sites (*C. imicola, C. expectator, C. ravus, C. herero, C. tropicalis, C. pycnostictus, C. leucostictus, C. nivosus, C. schultzei, C. pretoriensis, C. similis, C. enderleini, C. bedfordi, #89, C. tuttifrutti, C. punctithorax* and *C. brucei*). Another 15 species were only found at one of the sites - Aus (*C. dekeyseri*); Okahandja (*C. distinctipennis, C. kanagai, C. rhizophorensis, #54 p/f, #107, C. loxodontis, C. nevilli, C. olyslageri, C. ovalis* and *#69*) and Windhoek (*C. tororoensis*, #62, *C. glabripennis*, and *C. trifasciellus*). The other 16 species were only collected in Windhoek and Okahandja. It is possible that species that were not collected in Aus might be sensitive to the more arid climate of Aus.

3.3.2. Absence or presence of AHSV

There is currently an in-depth study underway on the identification and serotyping techniques of AHSV in *Culicoides* midges in Namibia (De Waal, 2015). Therefore, only absence or presence results will be discussed for this study, since serotyping is not part of the scope of this thesis. During the 2013 sampling year, AHSV positive *Culicoides imicola* complex was found at all three sites (Table 3.7). Samples were tested in fortnightly batches. Samples from week 1 and 2 tested positive in Aus and Windhoek. It is suspected that the positive result in Aus was due to vaccination. A horse was vaccinated with the OBP live vaccine in this period on the site. The positive result in Windhoek cannot be due to the vaccine since the horses are vaccinated during winter months. Samples from week 9 and 10 tested positive at Windhoek site, this was also the week with the highest number of *Culicoides* collected. The farmer did report some suspected cases but no mortalities were recorded at the Windhoek site for 2013. In Okahandja, samples from week 11 and 12 tested positive and this also corresponded with the farmer reporting a suspected AHS mortality on the farm.

No positive results were found in Aus during 2014. Samples from week 9 to 12 tested positive in Windhoek and corresponded with suspected AHS mortality on the farm. In Okahandja, samples from week 7 and 8 tested positive – fortunately no cases were reported at the site by the farmer. However, this can also be a vaccine strain as these horses are vaccinated very late in the season due to competitions and travelling.

Table 3.7: Absence (-) / presence (+) of AHSV in field collected *Culicoides imicola* complex from TRAP 1 at three sites in Namibia (Aus, Windhoek and Okahandja), January-May 2013 and January-May 2014. Average fortnightly total *Culicoides* collected from the other three traps over all the three sites are also presented. Shaded cells indicate samples positive for AHSV.

2013	**Aus**	**Average Total *Culicoides***	**Windhoek**	**Average Total *Culicoides***	**Okahandja**	**Average Total *Culicoides***
Week 1+2	+	1952.667	+	925.167	-	1305.667
Week 3+4	-	552.333	-	142.500	-	1077.167
Week 5+6	-	399.200	-	968.000	-	1124.167
Week 7+8	-	728.500	-	1161.500	-	1317.833
Week 9+10	-	804.000	+	11115.000	-	1950.000
Week 11+12	-	37.200	-	1580.750	+	801.333
Week 13+14	-	83.667	-	1014.333	-	706.000
Week 15+16	-	83.667	-	26.333	-	1264.333
Week 17+18	-	19.333	-	69.167	-	309.000
Week 19+20	-	19.000	-	342.833	-	301.833
2014						
Week 1+2	-	437.667	-	145.667	-	424.333
Week 3+4	-	263.833	-	130.000	-	930.833
Week 5+6	-	93.500	-	396.333	-	719.167
Week 7+8	-	240.400	-	102.500	+	804.833
Week 9+10	-	48.500	+	331.250	-	265.333
Week 11+12	-	14.667	+	320.750	-	173.000
Week 13+14	-	9.500	-	349.000	-	95.500
Week 15+16	-	32.400	-	774.000	-	85.200
Week 17+18	-	13.000	-	32.500	-	27.400
Week 19+20	-	6.333	-	185.500	-	108.250

3.3.3. Multivariate statistical analyses per site

3.3.3.1. Results for Aus

The relationship between weather parameters and the weekly total *Culicoides* collected as determined by means of a PCA, is illustrated in Fig. 3.8. A PCA was applicable for the Aus data with only one response variable in the dataset (2013: Week 14-20, April–May and 2014: Week 1-20, January–May). Individual parameters and total *Culicoides* for 2013 and 2014 are represented with bar graphs in Appendix A.1. According to the PCA and the Pearson's correlation ($p<0.01$), the most prominent factor influencing the occurrence of *Culicoides* at the site was temperature. Weeks with high *Culicoides* numbers and weeks of precipitation group together in the right of the graph. Temperatures for these weeks were all above 20°C. The other weeks form a grouping to the left of the graph, during these weeks the conditions were not favourable for the increase of *Culicoides* numbers - with temperatures below 20°C and no

precipitation events. The highest single weekly *Culicoides* collection at Aus corresponds with the highest weekly average temperature of 25.8°C. Descriptive statistics (Table 3.8) showed a tendency that the most *Culicoides* at Aus for 2014 were collected at temperatures between 23-25°C and humidity between 20-40%. However, an increase in *Culicoides* numbers will only be seen with these conditions combined with a precipitation event in the preceding week.

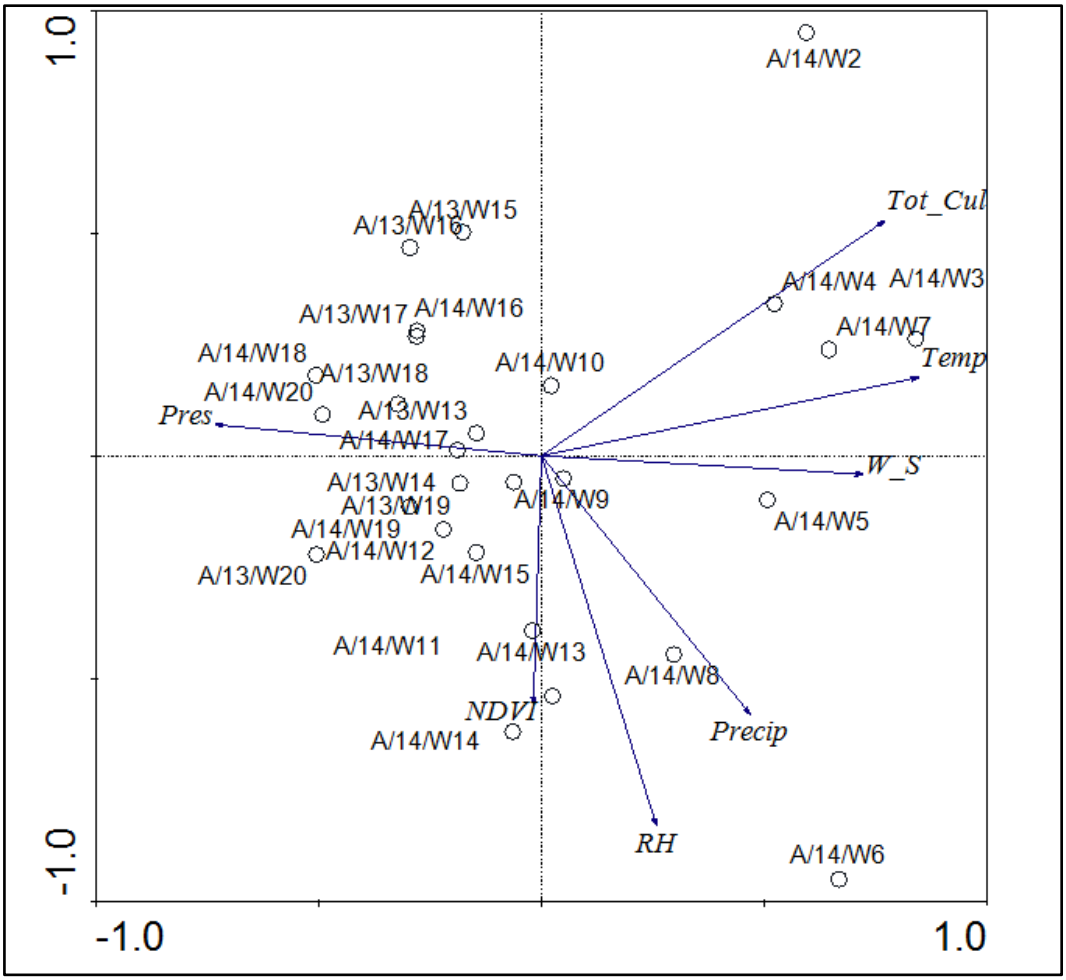


Figure 3.8: PCA ordination diagram illustrating the weekly results for Aus (Week 14 -20, April-May 2013 and Week 1-20, January-May 2014). Eigenvalues for the first two axes were 0.380 and 0.236 respectively. Key to abbreviations: PRECIP: precipitation; RH: relative humidity; NDVI: normalised difference vegetation index; TEMP: temperature; WINDSPE: wind speed; PRES: atmospheric pressure; Tot Cul: total Culicoides; Key to sample abbreviation: Aus/year/week.

Table 3.8: Descriptive statistics indicating the interaction ranges between weekly temperature and relative humidity (Table 3.5) and the occurrence of average weekly total catches of *Culicoides* at Aus from week 14-20, April–May 2013 and week 1-20, January–May 2014.

Temperature category	**Humidity category**	**Total *Culicoides* count**	**Temperature category**	**Humidity category**	**Total *Culicoides* count**
A	1	-	**E**	1	36.00
	2	6.00		2	3.00
	3	2.00		3	75.00
	4	-		4	-
	5	-		5	-
	6	-		6	-
	Total	8.00		**Total**	144.00
B	1	30.50	**F**	1	61.00
	2	107.30		2	991.01
	3	-		3	74.33
	4	-		4	-
	5	-		5	-
	6	-		6	-
	Total	137.86		**Total**	1126.00
C	1		**G**	1	437.67*
	2	148.00		2	-
	3	21.17		3	-
	4	-		4	-
	5	-		5	-
	6	-		6	-
	Total	169.17		**Total**	437.67
D	1	131.00	**Totals in each Humidity category**		
	2	-	1 – 696.10		
	3	28.00	2 – 1255.35		
	4	-	3 – 200.00		
	5	-	4 – 0.00		
	6	-	5 – 0.00		
	Total	159.00	6 – 0.00		

* In combination with a precipitation event

Fig. 3.9 illustrates the relationship between precipitation and total *Culicoides* for week 2-20, (January–May) 2014. Pearson's correlation indicated that a precipitation event in the preceding week has a statistically significant correlation with the total *Culicoides* abundance in the following week (r=0.432; p=0.001). During January 2013 (Week 1 and 2), a positive sample for AHSV was collected. This was also the week were the most *Culicoides* were collected during this survey at Aus. Unfortunately there is no weather data available for January 2013. However, in personal communication with the farm owner, Willem Swiegers (Swiegers, 2013), it was determined that flooding occurred on the farm during this time, probably resulting in the high *Culicoides* numbers.

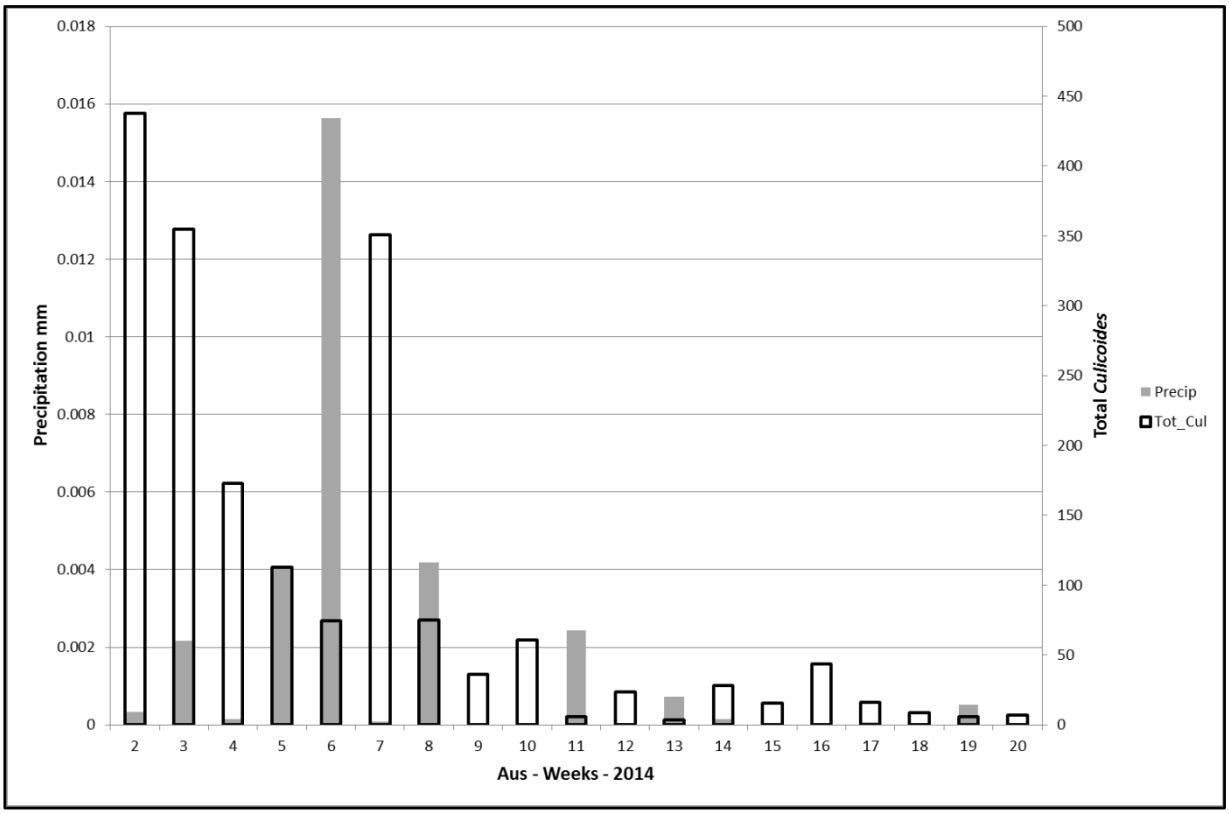


Figure 3.9: Relationship between precipitation (mm) and total *Culicoides* catches for Aus from week 2 – 20 (January-May), 2014.

3.3.3.2. Results for Windhoek (Seeis)

The relationship between fortnightly environmental parameters, total *Culicoides* and AHSV occurrence based on an RDA is illustrated in Fig. 3.10 (2013: Week 14-20, April-May and 2014: Week 1-20, January-May). Individual parameters and total *Culicoides* for 2013 and 2014 are represented with bar graphs in Appendix A.2. The most prominent parameters influencing AHSV occurrence and total *Culicoides* according to the RDA were NDVI, relative humidity and temperature. However, there were no statistically significant correlations according to the Pearson's correlation or Spearman rank correlation between the measured parameters and total *Culicoides* collected. Descriptive statistics (Table 3.9) showed a tendency of high

Culicoides numbers at temperatures above 15°C and below 23°C, with relative humidity ranging between 40% and 75%. The NDVI is in closest relationship with the occurrence of AHSV in the RDA. Weeks with samples that tested positive had the highest NDVI value for the Windhoek site. According to the Pearson's correlation, precipitation did not have a statistically significant correlation (r=0.174; p=0.427) with the occurrence of total *Culicoides* and the same trend as in Aus was not observed.

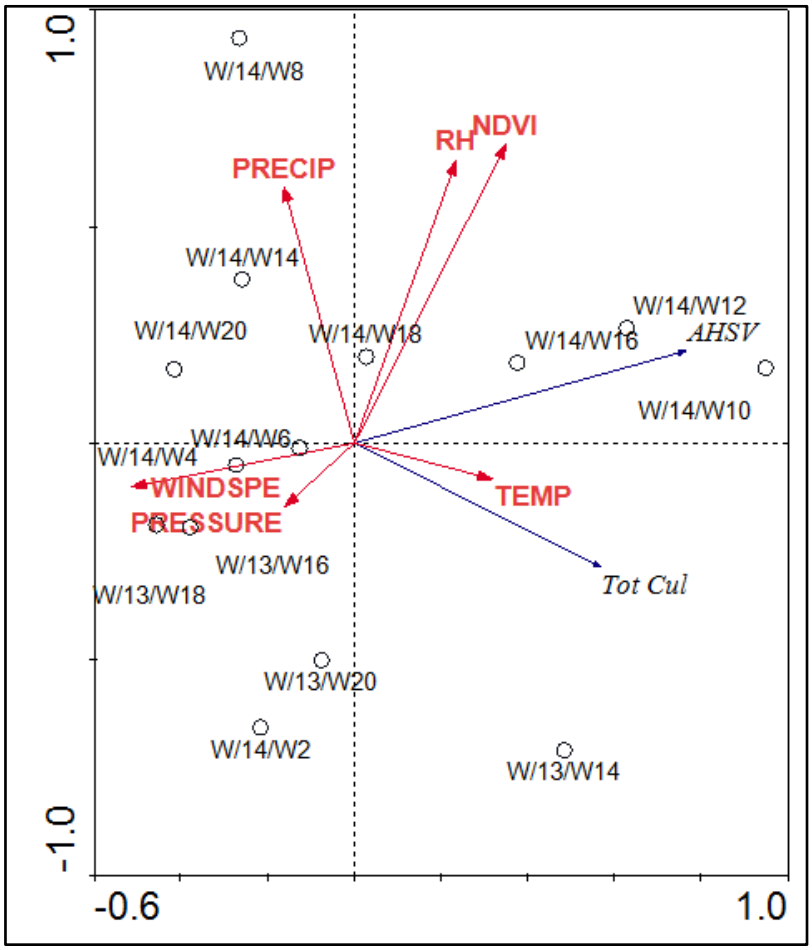


Figure 3.10: RDA ordination diagram illustrating the fortnightly results for Windhoek (Week 14-20, April-May 2013 and Week 1-20, January-May 2014). Red vectors represent the environmental parameters and blue vectors AHSV presence and total Culicoides. Eigenvalues for the first two axes were 0.455 and 0.064 respectively. Key to abbreviations: PRECIP: precipitation; RH: relative humidity; NDVI: normalised difference vegetation index; TEMP: temperature; WINDSPE: wind speed; PRES: atmospheric pressure; Tot Cul: total Culicoides; Key to sample abbreviation: Windhoek/year/week.

Table 3.9: Descriptive statistics indicating the interaction ranges between weekly temperature and relative humidity (Table 3.5) and the occurrence of average weekly total catches of *Culicoides* at Windhoek (Seeis) from week 14-20, April–May 2013 and week 1-20, January–May 2014.

Temperature category	**Humidity category**	**Total *Culicoides* count**	**Temperature category**	**Humidity category**	**Total *Culicoides* count**
A	1	-	**E**	1	-
	2	154.33		2	-
	3	52.67		3	-
	4	403.50		4	290.33
	5	-		5	150.50
	6	-		6	-
	Total	610.50		**Total**	440.80
B	1	-	**F**	1	-
	2	-		2	-
	3	669.67		3	-
	4	-		4	-
	5	1100.00		5	-
	6	395.00		6	-
	Total	2164.67		**Total**	-
C	1	-	**G**	1	-
	2	-		2	-
	3	-		3	-
	4	-		4	-
	5	188.00		5	-
	6	453.50		6	-
	Total	641.50		**Total**	-
D	1	-	**Total *Culicoides* in each Humidity category**		
	2	-	1 – 0.00		
	3	1339.50	2 – 154.33		
	4	-	3 – 2061.84		
	5	372.17	4 – 693.83		
	6	466.00	5 – 1810.67		
	Total	2177.67	6 – 1314.00		

3.3.3.3. Results for Okahandja

The relationship between fortnightly environmental parameters, total *Culicoides* and AHSV occurrence based on an RDA is illustrated in Fig. 3.11 (2013: Week 14-20, April–May and 2014: Week 1-20, January–May). Individual parameters and total *Culicoides* for 2013 and 2014 are represented with bar graphs in Appendix A.3. According to the RDA, the most prominent parameters influencing AHSV occurrence and total *Culicoides* were precipitation, relative humidity, NDVI and temperature. Precipitation is indicated as the most important parameter for the occurrence of AHSV. According to the Pearson's correlation the same trend as in Aus was observed, where a precipitation event had a significant influence on the number of *Culicoides* in the weeks thereafter (r=0.432; p=0.031). Higher precipitation leads to more favourable breeding conditions and therefore higher *Culicoides* numbers. There were no other statistically significant correlations according to the Pearson's correlation or Spearman rank correlation between the measured parameters and total *Culicoides* collected. Wind speed positively associate with total *Culicoides* in the RDA ordination. This was unexpected, as wind speed decreases midge

activity as discussed in Chapter 1. However, wind speeds never increased above 1.6 m/s (Appendix A.3) which is still within the 'active' range of the midges. The positive association might be due to *Culicoides* that are distributed from other areas and that individuals actually fly faster (Johnson, 1969). According to Johnson (1969), Bidlingmayer's data showed that densities of *Culicoides furens* are inversely correlated with the speed of the wind. However, further research is needed to confirm this. Descriptive statistics (Table 3.10) showed a tendency that temperatures ranged above 18°C and below 23° for high *Culicoides* numbers and humidity between 20-65%.

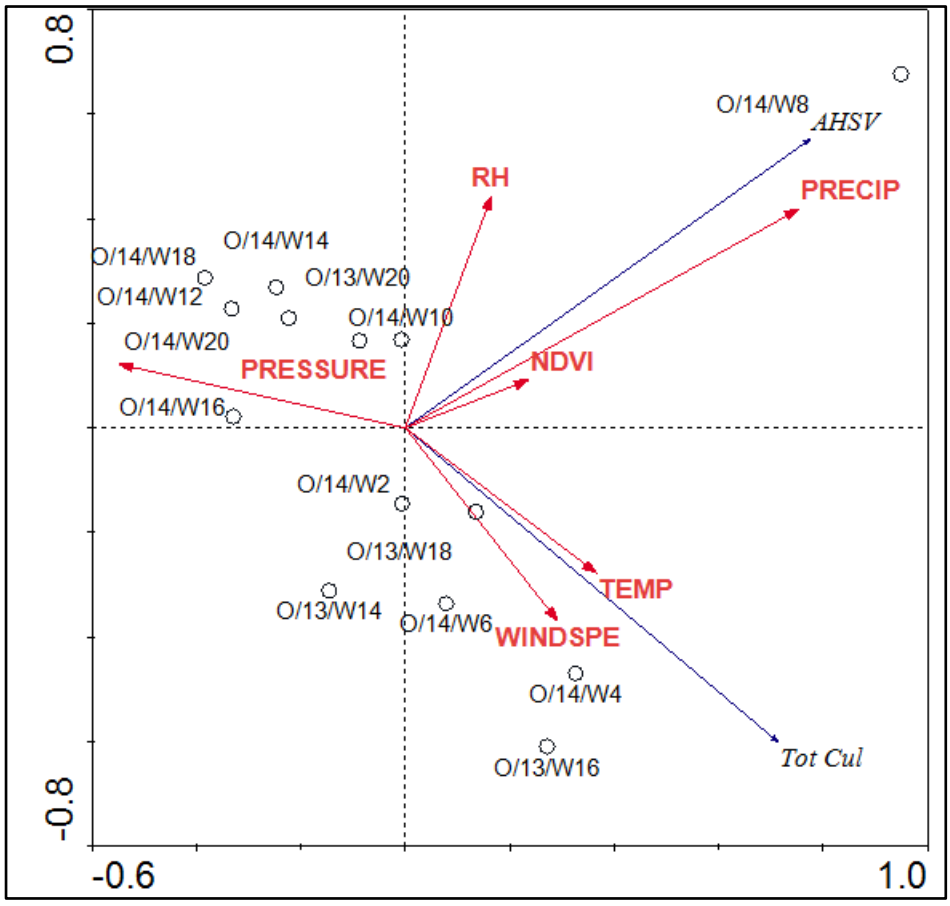


Figure 3.11: RDA ordination diagram illustrating the fortnightly results for Okahandja (Week 14-20, April-May 2013 and Week 1-20, January-May 2014). Red vectors represent the environmental parameters and blue vectors AHSV presence and total Culicoides. Eigenvalues for the first two axes were 0.557 and 0.333 respectively. Key to abbreviations: PRECIP: precipitation; RH: relative humidity; NDVI: normalised difference vegetation index; TEMP: temperature; WINDSPE: wind speed; PRES: atmospheric pressure; Tot Cul: total Culicoides; Key to sample abbreviation: Okahandja/year/week.

Table 3.10: Descriptive statistics indicating the interaction ranges between weekly temperature and relative humidity (Table 3.5) and the occurrence of average weekly total catches of *Culicoides* at Okahandja from week 14-20, April–May 2013 and week 1-20, January–May 2014.

Temperature category	**Humidity category**	**Total *Culicoides* count**	**Temperature category**	**Humidity category**	**Total *Culicoides* count**
A	1	-	**E**	1	-
	2	-		2	-
	3	-		3	940.67
	4	-		4	414.00
	5	-		5	1409.00
	6	-		6	371.33
	Total	-		**Total**	3135.00
B	1	442.67	**F**	1	-
	2	-		2	-
	3	216.50		3	-
	4	26.50		4	2301.00
	5	-		5	-
	6	-		6	-
	Total	685.67		**Total**	2301.00
C	1	1946.00	**G**	1	-
	2	-		2	-
	3	-		3	-
	4	207.67		4	438.67*
	5	67.67		5	-
	6	-		6	-
	Total	2221.34		**Total**	438.67
D	1	901.33	**Total *Culicoides* in each Humidity category**		
	2	460.33	1 – 3290.00		
	3	-	2 – 460.33		
	4	277.00	3 – 1157.11		
	5	1582.00	4 – 3664.87		
	6	-	5 – 3058.67		
	Total	3220.33	6 – 371.00		

* In combination with a precipitation event

3.3.4 Multivariate statistical analyses for Namibia

Multivariate statistical analyses were performed on all data from the three sites to determine the most prominent parameters influencing the distribution of AHS in Namibia. The relationship between the different parameters and the different sites in Namibia based on an RDA is illustrated in Fig. 3.12. The most prominent positive factors influencing distribution and occurrence of AHSV and total *Culicoides* according to the RDA included relative humidity, precipitation, NDVI, clay composition and temperature. Suspected AHS cases during 2014 were much higher than during 2013, which correspond to the higher precipitation events during the year. These suspected cases were reported by personal communication to the researcher, as the Annual Veterinary reports by the Directorate of Veterinary services for Namibia for 2013 and 2014 have not been published to date. There is a strong relationship between the occurrence of AHSV and precipitation, NDVI and relative humidity, as well as the association between total *Culicoides,* temperature and clayey soil. Ranking the different parameters in order of

importance for the occurrence of AHS according to the RDA: precipitation > temperature > relative humidity > clay > NDVI. According to the Pearson's correlation, temperature (p=0.015), NDVI (p=0.005) and precipitation in the previous week (p=0.0001) had a statistically significant positive correlation with total *Culicoides*. The Spearman rank correlation indicated that temperature (p=0.0003), relative humidity (p=0.004), precipitation (0.002), precipitation in the previous week (p=0.0004), NDVI (p=0.001) and wind speed (p=0.028) had a statistically significant positive correlation with total *Culicoides*. The effect of wind speed on *Culicoides* needs further investigation as discussed in the results of Okahandja.

There was no significant difference in the soil particle size distribution between the different sites according to the Pearson's correlation, [r] ranged between 0.057 and 0.33 (p>0.05). Although not statistically significant, the clay composition showed a tendency to correlate positively (r=0.33) in practice with total *Culicoides*. This corresponds with the results of Meiswinkel (1998) and must be researched with more data to determine if it is statistically significant. All the sites had a high percentage sand composition (<2 mm) above 80%. Aus was the only site with particle sizes greater than 2 mm. Particle size influences the water holding capacity in the soil and thus the suitability of breeding habitats. In South Africa, areas where *C. imicola* is absent such as the Port Elizabeth sandy dunes, *C. bolitinos* were implicated as a vector of AHSV (Meiswinkel & Paweska, 2003). In Aus, *C. bolitinos* (breeding in dung) were not collected during this survey, thus also accounting for the low incidence of AHSV. Factors other than the availability of hosts (cattle) and sandy soil must determine the distribution range of *C. bolitinos* since both were present at Aus*.* Verhoef *et al.* (2014) investigated the thermal limits of *C. imicola* and *C. bolitinos* and found that the thermal limits of the larval stage might play a role in the abundance and distribution range of *C. bolitinos* since the adult midge is quite hardy. They suggested that in warmer climates, the exothermic decomposition of dung temperatures are too high for larvae to survive. In section 3.2.2.1 it is mentioned that at the Aus site animals are not kept in stables due to the low grazing capacity of the area; this also applies to other livestock on the farm. This management style therefore does not create heaps of manure where *C. bolitinos* can breed.

It has been shown that in dryer areas such as Sudan, the seasonality of *Culicoides* is largely determined by the timing of the rainy season (Mellor *et al*., 2000). This also seems to be the case in Namibia, with precipitation having the greatest influence according to the RDA. Annual disappearance of *Culicoides* may occur for a part of the year when it is too dry for the midges to breed (Mellor *et al*., 2000). From these results it has been shown that the amount of precipitation does not matter in such dry conditions, as soon as there is a precipitation event (ultimately leading to an increase in soil moisture) *Culicoides* becomes more abundant. It is important to note that areas with higher precipitation, such as Okahandja, will have a higher baseline of *Culicoides* abundance due to more favourable conditions for breeding sites.

However, the Windhoek site was more driven by NDVI (that is also a factor of soil moisture) than precipitation itself. Other possible reasons for the difference in occurrence of *Culicoides* between the three sites are researched further in Chapter 4 & 5 and an integrated conclusion is discussed in Chapter 7.

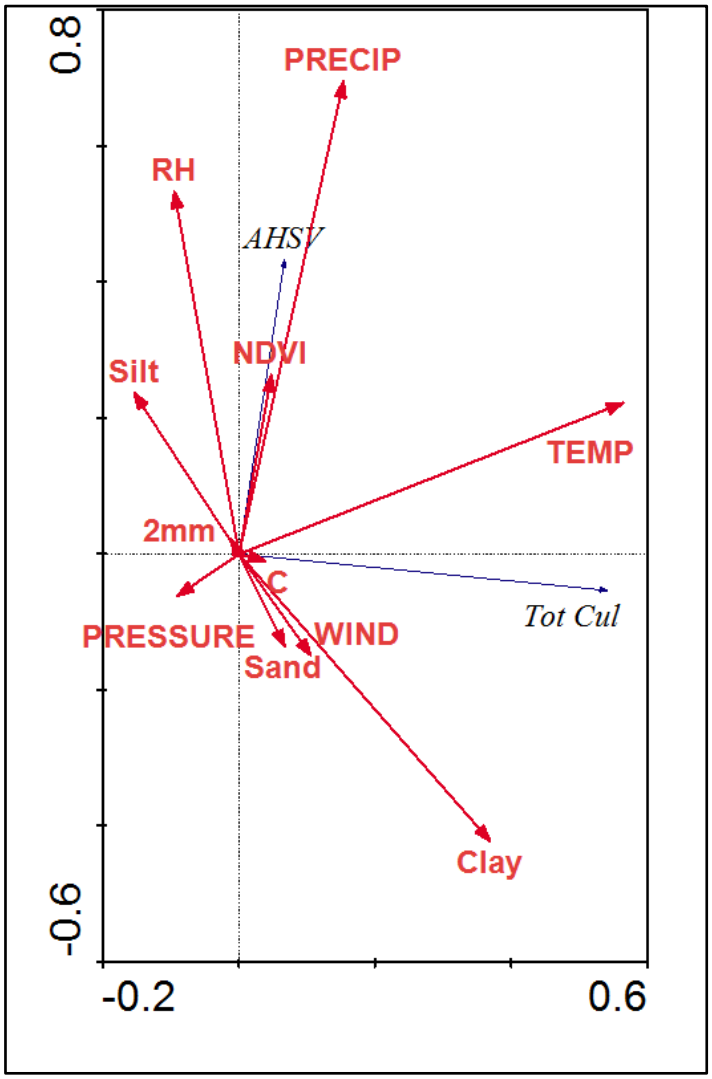


Figure 3.12: RDA ordination diagram illustrating the fortnightly results for all three sites across Namibia (Week 14-20, April-May 2013 and Week 1-20, January-May 2014). A co-variable descriptor to specify the site origin was included. Red vectors represent the environmental parameters and blue vectors AHSV presence and total Culicoides. Eigenvalues for the first two axes were 0.149 and 0.095 respectively. Key to abbreviations: PRECIP: precipitation; RH: relative humidity; NDVI: normalised difference vegetation index; TEMP: temperature; WIND: wind speed; PRESSURE: atmospheric pressure; Tot Cul: total Culicoides; C: percentage organic carbon; 2 mm: soil particles bigger than 2 mm.

The relationship between temperature and humidity has been implicated as one of the determining factors of adult midge activity (Wittmann *et al.*, 2002) that also influences biting rate (Carpenter *et al.*, 2011). The two-way ANOVA on 2014 weekly data indicated no statistically significant effect (p=0.631) of temperature and humidity on total *Culicoides* occurrence. However, the main effect (effect of a single factor) of temperature and relative humidity were

both statistically significant (p=0.003 and 0.002 respectively). Although the interaction effect was not statistically significant, the following trend between total *Culicoides* and temperature and relative humidity was observed but needs to be further investigated. Fig. 3.13 illustrates the interaction between temperature categories and relative humidity categories (Table 3.4) in relation to total *Culicoides*.

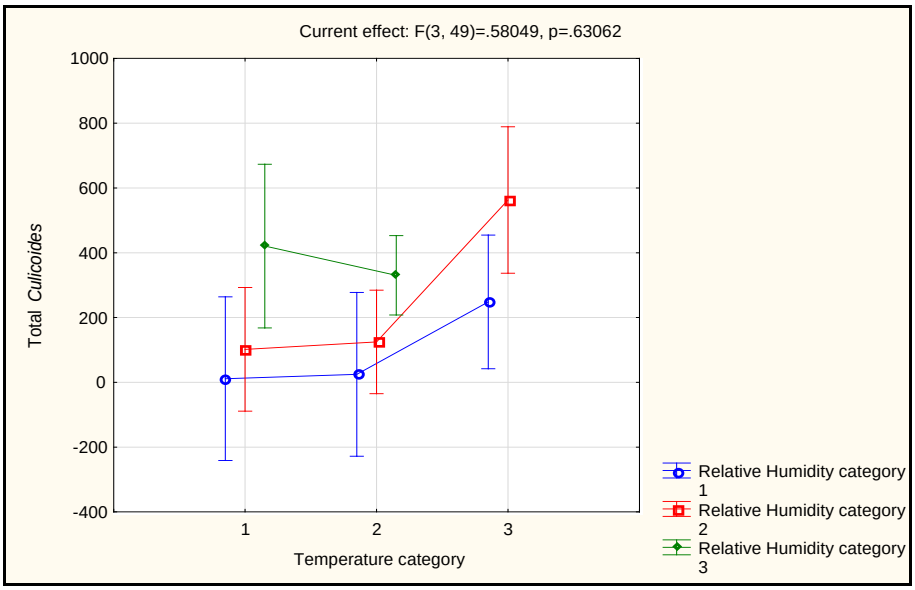


Figure 3.13: Results from the two-way ANOVA to illustrate the relationship between temperature and humidity and the number of total Culicoides. Temperature categories: 1) <18°C; 2) 18-23°C; and 3) >23°C. Humidity categories: 1) <40%; 2) 40-60%; and 3)>60%.

At low temperatures (<18°C) and low humidity (<40%) the mean *Culicoides* numbers were estimated below 100. With temperatures below 18°C and with increasing humidity, higher numbers of *Culicoides* were estimated. At temperatures between 18 and 23°C the estimated number of *Culicoides* increased with increasing humidity. At temperatures higher than 23°C and humidity higher than 60% no *Culicoides* were observed. *Culicoides* were only collected at temperatures higher than 25°C when combined with a precipitation event. At temperatures above 23°C in combination with lower humidity, higher *Culicoides* were estimated than at lower temperatures (<18°C) combined with low humidity (<40%). The most *Culicoides* were estimated at humidity between 40 and 60%. Descriptive statistics (Tables 3.8-3.10) showed a tendency that the most favourable ranges for Namibia were temperatures between 18 and 23°C combined with humidity of 40 to 70%.

CHAPTER 4

THE EFFECT OF MODELLED CLIMATIC VARIABLES ON THE DISTRIBUTION OF AHS OUTBREAKS IN SOUTH AFRICA AND NAMIBIA

4.1. INTRODUCTION

Climate can influence the vector capacity of a *Culicoides* population both through changes in the overall size of the adult population and the proportion of adults within the population capable of transmitting the virus (Wittmann & Baylis, 2000; Lo Iacono *et al.*, 2014). With climate change, there is a possibility of vectors spreading further north and also a potential increase in the vector capacity of northern hemisphere *Culicoides* populations (Wittmann & Baylis, 2000; Purse *et al.*, 2015). Climatic variables are interactive and affect the occurrence of AHS outbreaks either directly, such as temperature, or indirectly through their influence on breeding site formation as discussed in Chapter 1 (Fig 4.1).

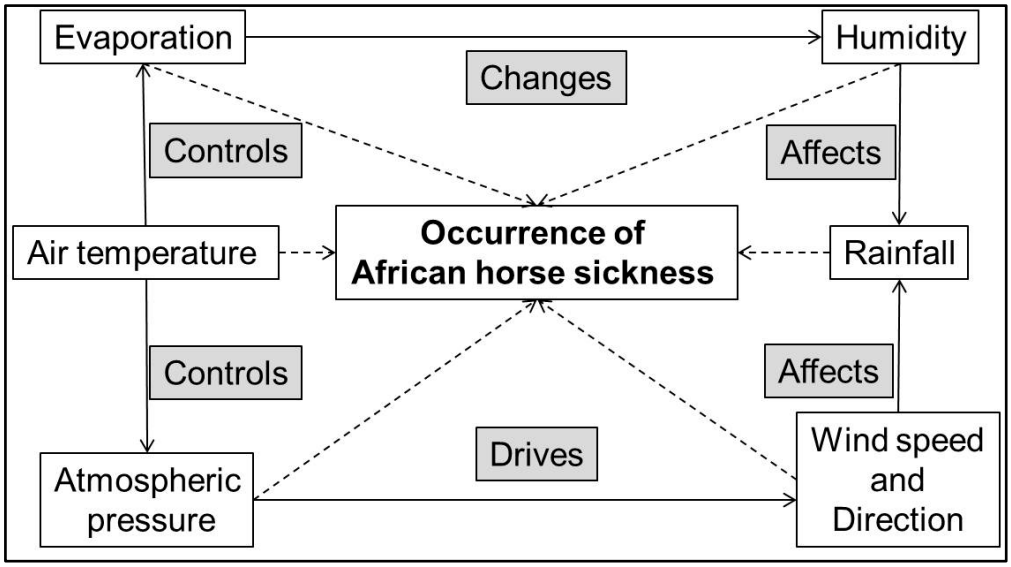


Figure 4.1: Interactions of climatic variables affecting the occurrence of AHS outbreaks. Adapted from Valsson & Bharat (2011).

The sheer pattern of AHS outbreak occurrence across different countries, makes it difficult to predict disease prevalence (Mellor *et al*., 2000). In Morocco, Israel and the winter rainfall region of South Africa, AHS occurs in abundance at the end of summer, coinciding with, or shortly after, the hottest time of the year but months after the last significant rainfall. In Nigeria, the annual peak in AHS prevalence occurs shortly after the rainy season and during the coldest part of the year. In Sudan and the summer rainfall regions of South Africa, the peak AHS season

coincides with the end of the hot and rainy season (Mellor *et al*., 2000). In order to identify risk periods for AHS transmission and to enable more effective targeting of control measures, it is essential to have a better understanding of the relationship between climatic variables and virus transmission by *Culicoides* midges (Mellor *et al*., 2000; Mellor & Hamblin, 2004).

The pattern of AHS outbreak occurrence has always been thought to coincide between Namibia and South Africa. Even when Namibia was part of South Africa and thereafter, the application of control measures, policies and risk assessments of AHS were implemented regardless of the differences in climate. The aim of this chapter was to compare and evaluate the relationship of various modelled climatic variables with the geographical distribution and abundance of AHS in South Africa and Namibia. Specific objectives were:

- to assess the geographical distribution of AHS in South Africa and Namibia from 1993 to 2011.
- to compare the relationship of modelled climatic variables with the distribution and abundance of AHS between South Africa and Namibia on a country- and province/district level.
- to systematically evaluate the importance of modelled climatic variables contributing to the distribution of AHS.

4.2. MATERIALS AND METHODS

4.2.1. Study area

Although the occurrence of AHS cannot be correlated with a single environmental parameter as discussed in Chapter 2 & 3, the only available long-term data is climate data. There is a significant relationship between the occurrence of *Culicoides* midges and climate zones as described by the Köppen-Geiger climate classification system (Brugger & Rubel, 2013). The study was conducted in South Africa and Namibia. Data was analysed at a regional scale – that is per province in South Africa or district in Namibia. According to this classification system (Kottek *et al*., 2006), South Africa is classified as a warm temperate country with warm summers and mild winters with a moisture gradient from east to west (Peel *et al.*, 2007). Namibia is classified as a dry (arid) country where potential evaporation and transpiration exceeds precipitation. The variability in climate types is less in Namibia than in South Africa (Peel *et al.*, 2007).

4.2.2. Historical reported data of the occurrence of outbreaks of AHS

A comprehensive literature review of the historical AHS reported data collected from the Windhoek archives as well as annual reports from the Directorate of Veterinary services in

Namibia was conducted as discussed in Chapter 2. South African AHS reported data was collected from the South African Department of Agriculture, Forestry and Fisheries as published in the annual reports.

Historical AHS reported data for both countries were extracted for the period 1993-2011 from the annual veterinary reports. The average number of reported AHS cases per annum were calculated for each district/province. Namibian data for 2002, 2003 and 2007 were found to be descriptive and therefore tagged as missing. Underreporting needs to be taken into consideration as it was stated in several reports that unreported cases were suspected and this is one of the main conclusions of Chapter 2. Nevertheless, the same sources used by Baylis *et al*. (1999b) in successfully establishing the relationship of the El Niño – Southern Oscillation with the occurrence of AHS were used in this study to investigate the historical patterns of AHS.

4.2.3. Climate data

Due to the fact that measured climate data is sparse in South Africa and Namibia, and to be able to compare results, it was decided to utilise modelled data for this analysis. European Reanalysis (ERA)-Interim data were used for correlations with the historical AHS data. ERA-Interim reanalysis data are global atmospheric reanalysis 0.75°x0.75° (T255) Gaussian gridded climatic data that included a large variety of 6 hourly surface parameters describing climate (Dee *et al*., 2011). Climate data were available from 1 January 1979, produced by the "European Centre for Medium Range Weather Forecasts" (ECMWF) (Berrisford *et al*., 2011).

Daily data were extracted for the period 1993-2011 for Namibia and South Africa. The gridded data were averaged per district/province per year and per month to calculate relationships with the AHS dataset. Climatic parameters were: temperature, soil temperature, atmospheric pressure, relative humidity (calculated from temperature and dew point), dew point temperature, wind speed (calculated from U and V wind components at 10m), evaporation, precipitation, minimum temperature and cloud cover.

4.2.4. Statistical analysis

4.2.4.1. Distribution of AHS in South Africa and Namibia

Historical AHS outbreak occurrence data as reported in annual reports were analysed to determine statistically significant differences in horse mortality due to AHS between countries and districts/provinces using Chi-Square contingency analysis as discussed in the Materials and Methods of Chapter 2. According to the most recent agricultural census of 2004 (Directorate Statistics and Economic analysis, 2004), South Africa had a total of 469 208 horses and Namibia (Directorate of Veterinary Services, 2000) a total of 61 902 in the year 2000.

4.2.4.2. Relationship between climate and the distribution of AHS

Multivariate statistical analyses were performed in Statistica (STATISTICA 12) (StatSoft Inc., 2014) and CANOCO (CANOCO for Windows 4.5) (Ter Braak & Šmilauer, 1998) to investigate the relationship between different modelled climatic factors and the occurrence of AHS outbreaks using a principal component analysis (PCA) multivariate ordination technique. Data were centred and standardised by factors.

The Kruskal Wallis test was performed to determine statistically significant differences regarding the effect of modelled climate variables on the distribution of AHS between the two countries.

4.2.4.3. Temperature and relative humidity relationship with AHS outbreak occurrence

Hierarchical linear modelling was performed in SPSS (IBM SPSS Statistics for Windows, Version 22.0, 2013) on the monthly modelled temperature and humidity data of South Africa (Dee *et al.,* 2011). Monthly AHS reported data for Namibia was not available and therefore the analysis was not performed on the Namibian dataset. Provinces were set as primary units of measurement with temperature and relative humidity divided into categories as fixed factors (Table 4.1) and AHS occurrence as the dependent variable.

Table 4.1: Categories of temperature and relative humidity for hierarchical linear modelling.

Category	Mean monthly temperature (°C)	Mean monthly relative humidity (%)
1	< 20	18-40
2	20-22	40-50
3	22-24	50-60
4	24-26	60-70
5	>26	>70

4.2.4.4. The influence of anthropogenic activities on AHS distribution

Anthropogenic activities have been implicated in various studies as having an influence on the distribution of vector-borne diseases (Sutherst, 2004). These anthropogenic activities can be quantified by including human population size (number of humans per province) and density (humans per square kilometre) in the analysis. Higher human population densities and urbanisation have been found to have a profound effect on the transmission potential of diseases in particular areas (Sutherst, 2004). Artificial neural network (ANN) analysis, Forecaster XL (Alyuda Research LLC, 2012) was used to evaluate the importance of modelled climatic factors and anthropogenic activities on the occurrence of AHS outbreaks. ANN analysis was chosen due to its ability to map non-linear relationships and to accommodate unknown relationships between variables (Eksteen & Breetzke, 2011). Annual average South African historical AHS reported data together with the annual averaged ERA-Interim reanalysis climate data were used for the analysis. Only three years of census data for horses and humans were

available for South Africa. No census data was available for Namibia and therefore this analysis was not done for the Namibian dataset. Social data are provided in Table 5.2. Parameters were: Density Humans – humans per square km; Humans – number of humans per province; Density Horses – horses per square km; Horses – number of horses per province; Min Temp – mean minimum temperature per year; VSWL – mean volumetric soil water layer per year; Precip- mean precipitationper year; Winds – mean wind speed per year; Evap – mean evaporation per year; Temp – mean temperature per year.

4.3. RESULTS AND DISCUSSION

4.3.1. Comparing the relationship of modelled climatic variables with the distribution and abundance of AHS between South Africa and Namibia on a country- and province/district level

Distribution of AHS cases in Namibia and South Africa are provided in Table 4.2. Distribution was categorised into three groups according to the Chi-square values 1) high incidence, 2) medium incidence and 3) low incidence areas. The highest incidence in SA occurred in Eastern Cape followed by KwaZulu-Natal and Gauteng provinces. In Namibia, Gobabis was the district with the highest incidence of AHS followed by Grootfontein, Okahandja, Walvisbay and Omaruru.

Table 4.2: Chi-square values and average values of the occurrence of AHS outbreaks in South Africa and Namibia from 1993-2011 to illustrate the distribution of AHS across provinces/districts. Provinces/districts are listed from the highest incidence to the lowest with the different shaded rows of grey indicating the different incidence groups, 1) high incidence, 2) medium incidence and 3) low incidence.

South Africa provinces	Chi-square value	Average AHS cases	Namibia districts	Chi-square value	Average AHS cases
Eastern Cape	73.44	174	Gobabis	4.014	17.0
KwaZulu-Natal	44.161	73	Grootfontein	2.970	3.6
Gauteng	10.56	42	Okahandja	2.443	3.9
Mpumalanga	2.882	20	Walvisbay	1.977	0.1
Western Cape	-7.391	12	Omaruru	1.266	2.3
Northern Cape	-12.64	18	Katima Mulilo	0.989	0.1
North-West	-31.053	30	Outjo	0.265	2.1
Limpopo	-35.451	12	Windhoek	0.164	3.5
Freestate	-44.501	8	Otjiwarongo	0.010	1.9
			Otavi	-0.091	0.5
			Opuwo	-0.357	0.3
			Rundu	-0.358	0.0
			Mariental	-0.984	2.2
			Keetmanshoop	-3.736	0.3
			Ondangwa	-8.606	0.5

A PCA of the complete dataset is provided in Figure 4.2. This indicated a significant statistical difference between Namibia and South Africa regarding the grouping between the various modelled climate variables and the occurrence of AHS outbreaks. The Kruskal Wallis test also

indicated a significant statistical difference among modelled climatic parameters for the two countries ($p < 0.05$). The pattern of AHS distribution in Namibia and South Africa was thought to coincide with similar climatic parameters acting as drivers for AHS outbreaks. However, this seemed not to be the case, with a diverse split between the countries in the occurrence of AHS outbreaks and modelled climatic parameters. Namibia has a lower humidity, minimum temperature, soil water content and precipitation and higher wind speed and evaporation rate than South Africa. This corresponds with previous studies (Baylis *et al.*, 1999b; Mellor *et al*., 2000; Wilson *et al*., 2009) as discussed in Chapter 1. These factors all have an influence on the occurrence of *Culicoides* midges and contribute to the lower incidence of AHS in Namibia.

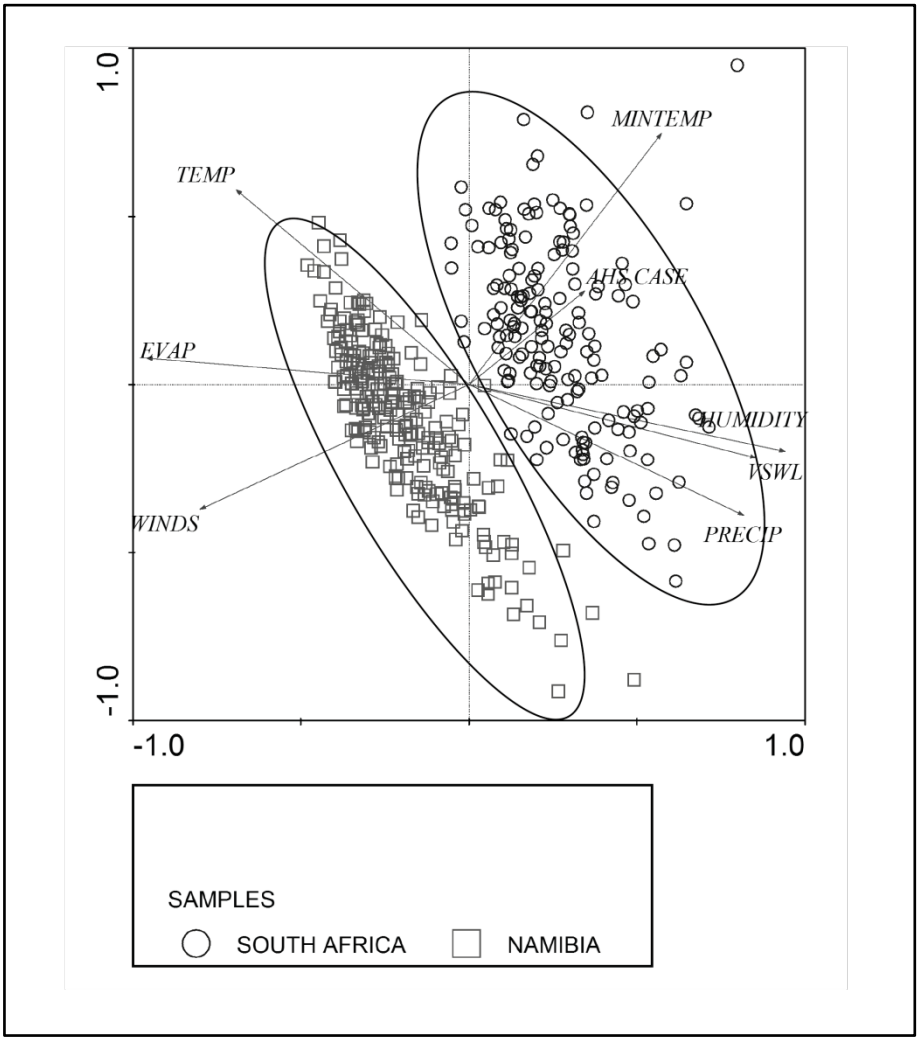


Figure 4.2: PCA ordination diagram of the occurrence of AHS outbreaks in Namibia and South Africa. Squares represent Namibian cases and circles South African cases of AHS. Eigenvalues for the first two axes were 0.603 and 77.4 respectively. Key to abbreviations: MIN TEMP – minimum temperature; AHS CASE – African horse sickness reported cases; VSWL – volumetric soil water layer; PRECIP- precipitation; WINDS – wind speed; EVAP – evaporation; TEMP – temperature.

A PCA was done separately for each country to evaluate whether a difference could be detected given the variation in climate classification between the two countries.

The PCA in CANOCO for Namibia per district (Fig. 4.3) demonstrates a grouping of modelled climatic variables and AHS outbreak occurrence in Okahandja, Gobabis and Grootfontein. Although Windhoek and Walvisbay are medium and high incidence areas, respectively, these two districts do not group with modelled climatic parameters that have an influence on the disease distribution. According to the PCA in STATISTICA, the most significant modelled parameters driving the distribution of AHS were (factor-loadings based on correlations are included in brackets after each variable): humidity (0.967), precipitation (0.922), volumetric soil water layer (0.906); minimum temperature (0.753), evaporation (-0.906) and wind speed (0.718).

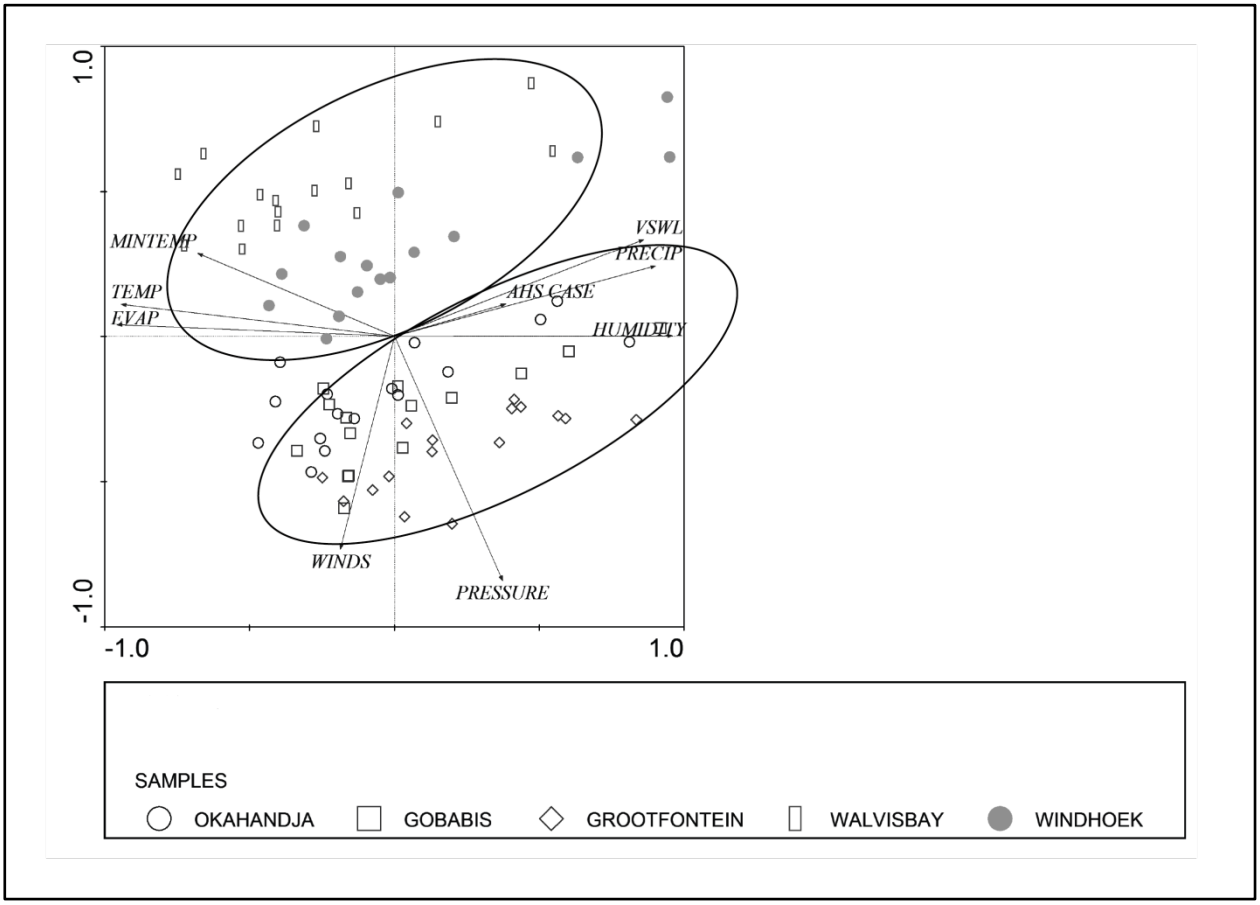


Figure 4.3: PCA ordination diagram illustrating the relationship between modelled climatic variables and high AHS incidence provinces in Namibia. Eigenvalues for the first two axes were 0.507 and 0.194 respectively. Key to abbreviations: MIN TEMP – minimum temperature; AHS CASE – African horse sickness reported cases; VSWL – volumetric soil water layer; PRECIP- precipitation; WINDS – wind speed; EVAP – evaporation; TEMP – temperature.

The PCA in CANOCO for South Africa per province is shown in Fig 4.4. It indicates a grouping of modelled climatic parameters and the occurrence of AHS outbreaks in the Western Cape and Eastern Cape with temperature, minimum temperature, precipitation and volumetric soil water layer. Interesting to note is that the massive outbreak in 1854 started in the Eastern Cape and was preceded with abnormally high precipitation (Henning, 1956). In contrast, no grouping is demonstrated with any modelled climatic variables and AHS in Gauteng and KwaZulu-Natal.

According to the PCA in STATISTICA, the most significant modelled parameters driving the distribution of AHS were (factor-loadings based on correlations are included in brackets after each variable): minimum temperature (0.930); humidity (0.905), precipitation (0.873), volumetric soil water layer (0.844), and evaporation (-0.907) which was significantly negatively correlated.

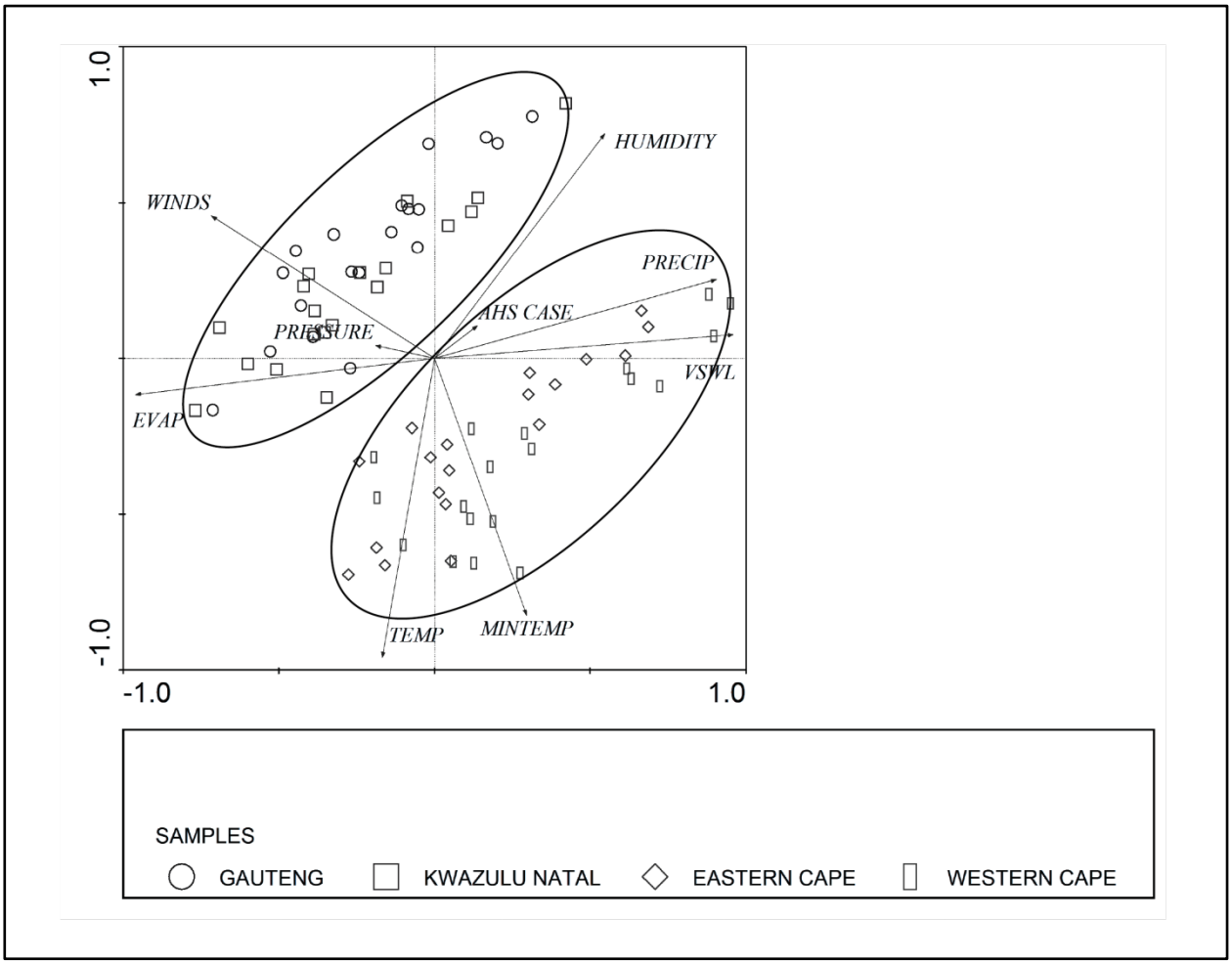


Figure 4.4: PCA ordination diagram illustrating the relationship between modelled climatic variables and high AHS incidence provinces in South Africa. Eigenvalues for the first two axes were 0.397 and 0.292 respectively. Key to abbreviations: MIN TEMP – minimum temperature; AHS CASE – African horse sickness reported cases; VSWL – volumetric soil water layer; PRECIP- precipitation; WINDS – wind speed; EVAP – evaporation; TEMP – temperature.

On a provincial and district level the following provinces/districts grouped with some modelled climatic variables (Fig. 4.3 & 4.4): South Africa: Eastern Cape and Western Cape and in Namibia: Okahandja, Gobabis and Grootfontein.

The incidence of AHS in these regions could be attributed to favourable climatic conditions for *Culicoides* midges as described in Chapter 1. Precipitation, humidity and temperature were once again implicated as the main drivers of AHS outbreak occurrence. It is important to take note that very few studies define ranges of precipitation, temperature and humidity in which *Culicoides* is active. This is due to the difficulty of establishing a *Culicoides* culture for ecological studies in the laboratory and the variety of factors influencing *Culicoides* abundance. It is not so

much that the climatic drivers differ between the countries, but that the combination of the drivers has a different influence on the occurrence of AHS outbreaks. In South Africa, temperature had the most significant effect on the occurrence of AHS outbreaks whereas in Namibia, humidity and precipitation were the main drivers. Precipitation was more influential in Namibia than in South Africa. This can be due to the aridity of the country and therefore precipitation events might be the initial trigger for outbreaks. The importance of precipitation was also highlighted in Chapter 3 as the most important climatic parameter.

4.3.2. Temperature and relative humidity relationship with occurrence of AHS outbreaks in South Africa

The hierarchical linear modelling in Table 4.3 of the monthly South African temperature, relative humidity and AHS data indicates a pattern of AHS outbreaks driven by the relationship between modelled temperature and humidity factors. Monthly AHS outbreaks occurrence data were not available for Namibia and therefore Namibian data were not included in the dataset. However, a similar analysis together with descriptive statistics was performed on available data for Namibia in Chapter 3. The temperature range within which most of the AHS cases occurred in South Africa during the 19 year period (1993 – 2011), was between 20 and 26°C. The maximum AHS cases were reported with temperatures between 20 and 22°C related to humidity between 50-70% (Table 4.3). This relationship is supported by laboratory results as reported by Wittmann *et al*. (2002). Low humidity (40%) and low temperatures (15°C) as well as high temperatures (30°C) and high humidity (85%) are unfavourable for the survival rates of midges (Wittmann *et al.*, 2002). This relationship is also seen in the analysis of AHS occurrence across the 19 year study. AHS incidence was low at low temperatures (<20°C) and low humidity (<40%) as well as high temperature (>26°C) and high humidity (>60%).

Table 4.3: Hierarchical linear modelling of the monthly South African temperature, relative humidity and AHS data. Shaded cells indicate the highest estimated AHS incidence in the relationship between modelled temperature and humidity factors (Table 4.1). Key to abbreviations: Temp_cat – Temperature category; Hum_cat – Humidity category; Std Error – Standard Error.

Temp_cat	Hum_cat	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	0.692	3.428	-6.030	7.415
	2	2.189	2.429	-2.574	6.952
	3	5.549	2.972	-.278	11.377
	4	6.852	5.004	-2.962	16.666
	5	7.692	10.840	-13.567	28.951
2	1	7.250	4.370	-1.320	15.820
	2	18.971	6.606	6.015	31.928
	3	16.444	9.212	-1.622	34.511
	4	28.583	7.978	12.937	44.229
	5	20.068	5.892	8.513	31.624
3	1	.101	4.397	-8.523	8.725
	2	7.156	5.826	-4.271	18.582
	3	16.923	7.665	1.891	31.955
	4	43.111	5.826	31.685	54.537
	5	9.836	3.727	2.528	17.145
4	1	1.247	4.343	-7.270	9.764
	2	5.260	4.574	-3.711	14.231
	3	16.561	3.948	8.818	24.304
	4	1.323	3.428	-5.400	8.046
	5	1.043	8.150	-14.939	17.026
5	1	1.336	3.268	-5.074	7.745
	2	2.103	2.881	-3.547	7.754
	3	6.000	3.160	-.197	12.197
	4	0.720	7.817	-14.610	16.050
	5	.[b]			

4.3.3. The influence of anthropogenic activities on the occurrence of AHS outbreaks in South Africa

An ANN analysis for South African data to determine the effects of various parameters on the occurrence (presence) of AHS outbreaks was conducted. No census data were available for Namibia and therefore the analysis was not done for the Namibian dataset. Social data for Namibia and South Africa are provided in Table 5.1. The analysis had a prediction accuracy of 55%. Results from the three years of available data in South Africa (Fig 4.5) indicated that the parameter with the greatest influence on AHS incidence was annual temperature (22.39%), followed by human population (number of humans per province) (15.67%) and thereafter horse population (number of horses per province)(14.81%) and annual precipitation (10.12%).

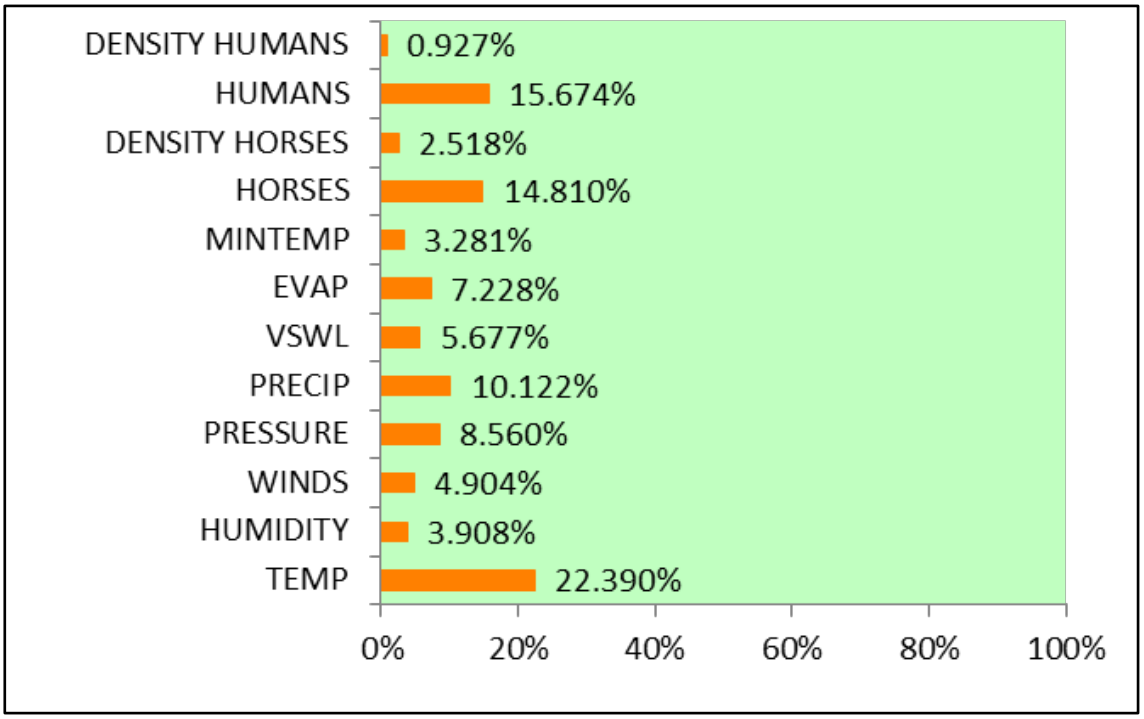


Figure 4.5: Contribution of various parameters in the ANN on the occurrence of AHS performed on the South African data. Correlation and the fit for the ANN was >50%. Key to abbreviations: DENSITY HUMANS – humans per square km; HUMANS – amount of humans per province; DENSITY HORSES – horses per square km; HORSES – amount of horses per province; MIN TEMP – mean minimum temperature/year; VSWL – mean volumetric soil water layer/year; PRECIP – mean precipitation/year; WINDS – mean wind speed/year; EVAP – mean evaporation/year; TEMP – mean temperature/year.

Results from the PCA per country on district and provincial level suggest that there are other factors, besides climatic factors, contributing to the distribution of AHS in South Africa and Namibia. The probability that the AHS occurrence of outbreaks in provinces such as Gauteng and Kwa-Zulu Natal could be due to increased anthropogenic activities is supported by the ANN. There may be a threshold where anthropogenic influences become greater towards the occurrence of outbreaks and distribution of AHS than climatic variables. This anthropogenic effect is investigated further in Chapter 5 for Namibia and South Africa. Even in the historical perspective, the influence of South Africa on AHS distribution in Namibia was showed, therefore the interactions between the two countries remain essential. AHS distribution has no political boundaries.

CHAPTER 5

A SOCIAL SURVEY OF AHS IN NAMIBIA AND SOUTH AFRICA

5.1. INTRODUCTION

Although the current spread of AHS is not completely understood, movement of equines has been regarded as the most likely and biggest threat for the distribution of the disease across borders (Coetzer & Gurthrie, 2004; De Vos *et al*., 2012). According to Coetzer & Guthrie (2004) AHS can be distributed over great distances if equids incubating the disease are translocated by land, air or sea. As a result, several restrictions and policies have been put in place to protect the equine populations of other countries. The AHS outbreak during 1987 in central Spain was believed to have originated by the importation of 10 zebra from Namibia (Mellor, 1993). The outbreak of 1965 in Northern Africa was initiated by the movement of infected donkeys across the Sahara from West Africa (De Vos *et al*., 2012). In an effort to keep southern Africa in the horse export trade, the OIE code of 1995 allowed the creation of an AHS free zone. Movement control was put in place and a control area was established in the Western Cape (Bosman *et al*., 1995). However, for the rest of the country as well as in Namibia, it was business as usual, with no movement restrictions during an AHS outbreak or within the peak season.

By way of the Animal Disease Act 35 of 1984, AHS was declared a notifiable and controlled disease and horse owners are required to notify their local state veterinarian of any suspected cases. The Act also requires that all horses must be vaccinated annually with a registered vaccine, except in the AHS free and surveillance area in the Western Cape (South Africa).

For horses to be exported from Namibia they must be moved to the controlled area in the Western Cape (South Africa) and remain in a vector-protected establishment for several months (OIE, 2014). Unfortunately, with the outbreak of 2014 within the controlled area in South Africa, all exports were stopped and at present horses can only be exported via Mauritius after a 90 day residency. This is an extremely stressful and long process for any horse and without exercise the horse must have several months in its new country to train and regain strength (Wits Health Consortium, 2014). From 1997 until 2010, approximately 1000 horses per year have been exported from South Africa to the European Union. In 2010 only 126 horses in 2011, 144 horses and in 2012 only 68 horses were exported (European Commission, 2013). This is a severe decrease in the contribution to the gross domestic product (GDP) of South Africa, compared to the early 1950's when there were no quarantine limitations and up 350 000 horses were exported (Guthrie, 2014; Anon, 2012). According to David Thiselton from Gold circle, "SA

horseracing and other equestrian sports have the potential to multiply their economic contribution if export protocols are relaxed". Needless to say, export of horses from southern Africa is down to a minimum and an alternative export strategy is urgently needed.

A new regulation was adopted during the OIE's general assembly in May 2012 where AHS is now one of four animal diseases for which countries can request an AHS-free recognition status. Changes also included a substantially shortened pre-export quarantine period which expedites the export of horses (OIE, 2013). However, the establishment of an AHS free zone, as developed in the Western Cape, was not successful due to several outbreaks in the controlled area (Guthrie, 2014), with the most recent outbreak in 2014. Export of equines from southern Africa is therefore again under international scrutiny.

The effect of diseases in livestock on socio-economic factors is well researched (Sutherst, 2004; Thompson, 2012). However, the effect of socio-economic factors on the distribution of the disease is poorly understood. The purpose of the current investigation was to assess the relationship between AHS occurrence and social parameters, including factors such as the movement of horses, preventative measures and knowledge about AHS reporting.

5.2. MATERIALS AND METHODS

5.2.1. Study area

The study area included Namibia and South Africa on a regional scale as per district (Namibia) or province (South Africa). Both countries were included in this study due to the movement of horses between the two countries for competitions and also because South Africa is one of the biggest export markets for Namibian horses. During the 2011 AHS outbreak, all exports of horses were suspended from Namibia and South Africa costing breeders millions of Rands (Kazondovi, 2011). Also Namibia do not export/import horses directly, but use the facilities in the controlled area of South Africa – This will be discussed in more detail in Chapter 6.

Distribution data of AHS (1993-2011) in South Africa and Namibia, as discussed in Chapter 3, were used. Human census of 2011 for South Africa (Statistics South Africa, 2012) and Namibia (National Planning Commission, 2011) were used as a measure of anthropogenic activities. According to the most recent agricultural census of 2004 (Directorate Statistics and Economic Analysis, 2004), South Africa had a total of 469 208 horses and Namibia (Directorate of Veterinary Services, 2000) a total of 61 902 in the year 2000 (Table 5.1).

5.2.2. Questionnaire survey

A cross-sectional study was conducted to collect information from horse owners in Namibia and South Africa. Ethical clearance for the study was obtained from the North-West University,

Potchefstroom Campus ethical committee (ethical approval number NWU-00041-14-S3). Completion of the questionnaire was anonymous and voluntary from all participants. The complete questionnaire is provided in Appendix B. Detailed surveys are expensive, time consuming and questionnaire surveys are often perceived as intrusive by some members of society (Heiervang & Goodman, 2011). The use of web-based surveys and response rate is a topic on which several studies have been conducted with both disadvantages and advantages to web-based versus traditional questionnaires (Heiervang & Goodman, 2011; van Gelder *et al*., 2010). A web-based survey was chosen for this study due to the extent of the area to be surveyed. Some demographic bias is expected in participation, with an under-representation of poorer families because of their lower access to the Internet. 'Fluid survey' was used for the development of the survey, and although this was only available in English, participants were given the option to answer in Afrikaans as well. The survey was launched on the 6th of May 2014 on Facebook and the link was shared to horse related groups in South Africa and Namibia. Facebook © (2015) is an online social network, connecting friends, family and colleagues. Once a link is shared on a group, the members of that group can share the link to other groups and post it on their timelines. Groups were found by using the Facebook search function with keywords such as: equine, horses and AHS. The link to the questionnaire was posted on two groups, whereafter it was shared to another five groups and reposted on member's timelines. The researcher's email and contact details were supplied to participants in case there were any questions. The post was continuously "bumped up the thread" so that it remained at the top of the Facebook pages for the duration of the study. The survey was closed at the end of June 2014 when a decline in the response rate was observed. The questionnaire consisted of four sections: 1) demographic information (compulsory); 2) human/equine interaction (voluntary); 3) knowledge of AHS (voluntary); and 4) notification of AHS (compulsory). In an effort to negate respondent frustration with questions he or she deemed unimportant, not all sections were compulsory.

5.2.3. Statistical analysis

Gephi 0.8.2.B was used to illustrate the movement of horses between countries and districts/provinces. Gephi is an interactive visualisation and exploration platform for different kinds of networks and complex systems (Bastian *et al.*, 2009). Network analyses are often applied in epidemiological studies where countries are represented as nodes and animal movements as links (effect) in the network. It has been used in a range of applications in veterinary medicine, including: epidemic analysis, disease distribution patterns, predictive modelling, risk analysis, the efficacy of surveillance systems and the impact of movement regulations (Grisi-Filho *et al*., 2013). A Fruchterman Reingold layout, which is a force directed algorithm, was applied to the horse movement data. The ranking of the nodes (districts/provinces) was done according to eigenvector centrality which is a measure of the

importance of a node in a network based on its connections. Depending on the AHS status of the node (province/district) as described in Table 5.1, a numerical attribute was also allocated to each node. High incidence areas were allocated 3, medium incidence, 2 and low incidence 1. Thus, the network analysis had 18 nodes (districts/provinces), with 1084 effects – movement of horses (90 out-degree effects - movement to other provinces and 994 in-degree effects - movement only within the province). Annual movements of equines between January and December were included in the analysis due to the possibility of AHSV overwintering in vectors in certain provinces if favourable climatic conditions persisted in the new location (Paweska *et al.*, 2002; Thompson *et al*., 2012).

5.3. RESULTS AND DISCUSSION

5.3.1. Distribution of AHS in South Africa and Namibia

Distribution data of AHS in South Africa and Namibia with applicable social data is provided in Table 5.1.

Table 5.1: Percentage incidence of AHS in South Africa and Namibia from 1993-2011 together with number of horses per district/province, number of humans per district/province, density of humans per km^2 , density of horses per km^2 and surface area of the district/province. Shaded rows indicate the high incidence provinces/districts.

South Africa (Provinces)	**% AHS incidence**	**Number of Humans 2011**	**Density of Humans / km^2**	**Number of Horses 2004**	**Density of Horses / km^2**	**Surface area km^2**
Eastern Cape	0.212	6 562 053	39	34375	0.203	169580
Kwazulu-Natal	0.144	10 267 300	111	120936	1.313	92100
Gauteng	0.111	12 272 263	721	38300	2.252	17010
Mpumalanga	0.097	4 039 939	51	20495	0.258	79490
Western Cape	0.052	5 822 734	45	23826	0.184	129370
Northern Cape	0.049	3 509 953	30	37006	0.318	116320
North-West	0.041	1 145 861	3	73672	0.204	361830
Limpopo	0.021	5 404 868	44	56999	0.460	123910
Free State	0.013	2 745 590	21	63599	0.491	129480
Total		**51 770 561**		**469 208**		
Namibia (Districts)	**% AHS incidence**	**Number of Humans 2011**	**Density of Humans / km^2**	**Number of Horses 2000**	**Density of Horses / km^2**	**Surface area km^2**
Katima Mulilo	5.882	90100	5.019	17	0.001	17951
Walvisbay	5.714	107100	4.926	35	0.002	21741
Grootfontein	0.390	34500	0.407	916	0.011	84760
Okahandja	0.179	41600	2.354	2170	0.123	17672
Omaruru	0.147	43300	1.036	1567	0.038	41772
Gobabis	0.086	70800	1.372	19674	0.381	51598
Outjo	0.076	42400	0.727	2705	0.046	58297
Windhoek	0.069	340900	9.208	5092	0.138	37019
Otjiwarongo	0.066	53800	1.484	2787	0.077	36233
Otavi	0.055	32200	0.998	819	0.025	32251
Mariental	0.046	79000	0.719	4824	0.044	109737
Opuwa	0.030	45900	0.745	995	0.016	61573
Keetmanshoop	0.004	76000	0.471	6039	0.037	161339
Ondangwa	0.003	824800	17.199	13720	0.286	47955
Rundu	0	117400	2.591	542	0.012	45302
Total		**1999800**		**61902**		

*% AHS incidence - % of the total number of horses that were reported with AHS from 1993-2011

5.3.2. Demographic information of survey respondents

A total of 508 responses were collected during the survey period. From the 417 completed questionnaires received, 73% of respondents were aged 20-49, 20% 50 and older and 8% younger than 20. Respondents were 84% female and 16% male. Groups included both South African (78%, 323) as well as Namibian (23%, 94) residents. All nine provinces of South Africa were represented with 35% (109) from the Gauteng province. This is expected as this is the province with the highest human population and horse density in South Africa. In Namibia, the northern districts were less represented but 41% (38) of responses were from the Windhoek district which is the district with the second highest density of humans in the country. Of the respondents, 69% (269) have a tertiary qualification. Most of the respondents (78%) had a full-time occupation and only spent time with their horses after working hours.

5.3.3. Human/Equine interactions

The participants were mostly social riders, at 71%, with 29% being professional riders. All of the participants owned one or more horses with only a few of the other equines (donkeys, mules and zebras) owned. Participants (97%) attach an emotional value rather than economic value to their equines. Although horses are mostly used for recreation or performance purposes many have a considerable monetary value (Coetzer & Guthrie, 2004). Previous studies indicate that approximately 80% of horse riders in the Western world are female, due to a typically emotional relationship with horses. This is a topic of increasing interest on its own and there are studies on the rise of female equestrianism (Savvides, 2011). Horse owners (67%) indicated that they travel with their horses on a regular basis to competitions.

According to the network analysis presented in Fig 5.1, the ranking of horse movement intensity in South Africa is: Gauteng > Free State > KwaZulu-Natal > Mpumalanga > Western Cape > North-West > Limpopo > Northern Cape > Eastern Cape. In Namibia the ranking is: Okahandja > Windhoek > Walvis Bay > Otjiwarongo > Omaruru > Gobabis > Grootfontein > Keetmanshoop > Mariental. Overall, Gauteng (RSA) is the node with the most connections to other nodes in the network as well as within the province itself. In Chapter 3 it was shown that climatic parameters were not the principal drivers for the occurrence of AHS in Gauteng and anthropogenic activities were implicated. Movement of horses can be classified as an anthropogenic effect, since the transport of horses is prompted by owners for competitions. These results support this finding, indicating a great deal of movement of horses occurring into and out of the province. The Free State province (RSA) was ranked second with KwaZulu-Natal (RSA), Okahandja (Namibia), Windhoek (Namibia), and Walvisbay (Namibia) following respectively. The high intensity horse movement nodes were all medium to high AHS incidence provinces/districts. Movement for Okahandja and KwaZulu-Natal was limited to within the respective countries. Free State and Windhoek were ranked high with concentrated movement of horses between provinces as well

as countries. These were also the provinces/districts with the second highest number of horses in their respective countries. Low AHS intensity provinces such as Limpopo (RSA) and Keetmanshoop district (Namibia) had correspondingly low horse movement. Eastern Cape (RSA) was the highest AHS incidence area but was ranked relatively low, with movement mostly within the province. This was one of the provinces where climatic parameters were implicated as key drivers of AHS distribution in Chapter 3. The Western Cape (RSA) was in the medium rankings of horse movement intensity, with most of the movement within the province. However, there was still movement of horses within the peak AHS season from high incidence areas into this province. Movement control measures are only activated after an outbreak has occurred which is especially problematic for export of horses. These results indicate that areas with the highest movement of horses corresponded to the areas with a high occurrence of AHS. Movement of horses has to be regarded as a key contributing factor to the distribution of AHS.

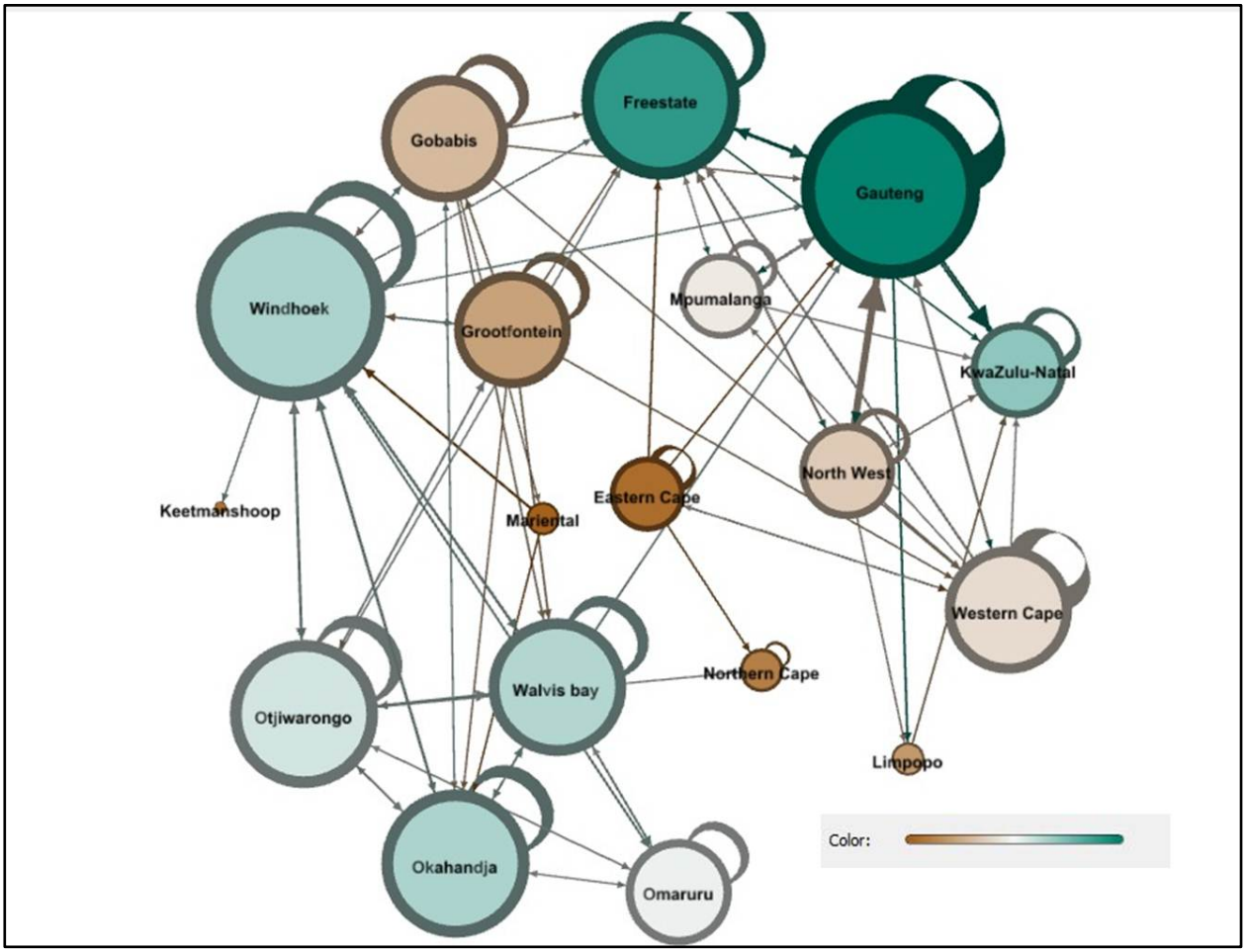


Figure 5.1: Network analysis for the movement of horses in South Africa and Namibia. Provinces and districts are presented as nodes (circles) and edges (arrows) indicate movement of horses. Edges indicate the direction of movement between nodes as well as the intensity (thickness of arrows). Half circles around the nodes indicate the intensity (thickness) of movement within the node. The colour intensity (as indicted with the colour bar from dark caramel (low importance) to dark turquoise (high importance) and size of the node indicate the importance of the node within the network.

5.3.4. Precautionary measures, treatment plans and restrictions regarding AHS

This section of the questionnaire focused on determining precautionary measures, treatment strategies and restrictions experienced by horse owners. Precautionary measures taken against AHS included a variety of methods (Table 5.2). This was the response of an open-ended question where respondents could select more than one answer. The most popular precautionary measures used were chemical repellents for *Culicoides* applied on horses (64%) and stabling of horses during dusk and dawn (59%).

Table 5.2: Percentages of respondents indicating their use of precautionary measures toward AHS in Namibia and South Africa.

Precautionary measure	Percentage (%)
Chemical repellent	64%
Stabling during dusk and dawn	59%
Immunity booster	51%
Natural repellent	46%
Fly sheets	28%
Other, please specify...	15%
Fans in stables	7%

Of the 341 responses received in this section, 83% indicated that they vaccinated their horses with the registered vaccine supplied by OBP. Horse owners that have had experience with AHS were 47% of the respondents, with 88% of these indicating that the affected horses had been vaccinated. Most of the participants vaccinate all their horses according to the manufacturer's instructions between September and December. According to the South African Animal Health Act 35 of 1984, it is compulsory since 2009 that all horses outside of the free and protected zone in the Western Cape must be vaccinated annually. However, in a notice dated 26 March 2015, the Department of Agriculture, Forestry and Fisheries (South Africa) placed a restriction on the AHS vaccination period in South Africa. AHS vaccination periods have been restricted from 1 June to 31 October in the AHS controlled area, with recommendations that the same policy is adopted for the rest of the country. It is suspected that the change in policy was based on results indicating the possible involvement of the vaccine in the AHS outbreak in the controlled area in 2014 (Anonymous, 2015). Namibia used the same legislation as South Africa until 2011 when their new Animal Health Act of 2011 came into effect. The new Namibian Animal Health Act of 2011 does not incorporate the policy of AHS vaccination. However, according to the horse owners societies' regulations, horses must be vaccinated in order to compete at local or international competitions. One can only hope that a similar or the same notice with regards to AHS vaccination restrictions will soon follow in Namibia.

5.3.5. Notification of AHS

The last section of the questionnaire focused on the knowledge of participants on the reporting of AHS. One of the most pertinent conclusions from previous chapters is that AHS was being underreported and underestimated by all parties involved (European Commission, 2013). This section intended to shed some light on the reason for this.

Although 93% of the participants were aware that AHS is a notifiable and controlled disease, with 89% accepting the responsibility as their own for reporting cases, the process and efficiency of reporting is unknown. Respondents (78%) considered it the responsibility of a private veterinarian to report cases and 71% indicated it to be responsibility of the state veterinarian. More than 50% of participants indicated that cases should be reported when there is one suspected case in the area in order to notify other horse owners to take extra precautions. From responses, it became evident that there is a fair amount of confusion on where and when to report a case. This correlates with the conclusions from the European Commission in May 2013 that the current weak passive surveillance and reporting system prevents the obtaining of an accurate picture of the situation as a whole. "*The AHS control and regionalisation system suffers from the unclear attribution of responsibilities, insufficient cooperation and staff performing controls."* (European Commission, 2013).

CHAPTER 6

QUALITATIVE RISK ANALYSIS OF THE DISTRIBUTION OF AHS IN NAMIBIA

6.1. INTRODUCTION

Current methodologies for risk assessment focus on predicting the likelihood of movement of diseases to new locations (Bridges *et al.*, 2007). The import risk analysis developed by the OIE (2010) calculates the risk of the importation of a disease into a country to which the disease is not endemic. However, to be able to prevent or decrease the frequency of disease occurrence, it is important to understand the intra-country spread. This is extremely important for diseases such as AHS in countries where the disease is endemic and where strict exporting protocols of equines are implemented. Namibia is an exporter of pedigree horses to countries such as South Africa, Europe and the Arabian Peninsula (Caporale *et al*., 2009; Scacchia *et al.*, 2009). However, due to the risk of the introduction of AHS, strict exportation policies are set in place by the OIE and importing countries. The OIE policy released in May 2012, enable countries to apply for AHS free zones from where exporting policies are less restrictive. If able to comply with all the requirements, this new policy can provide Namibia with an AHS-free zone from which export of equines can commence. Specific objectives for this chapter were:

- the application of a risk analysis to characterise the risk of the occurrence of AHS outbreaks in Namibia.
- the development of a risk assessment tool to determine the AHS risk in specific areas in Namibia.
- to investigate possible areas for use in equine export through the application of the risk matrix.

6.2. MATERIALS AND METHODS

In a data scarce environment, qualitative risk assessment frameworks have proved useful to assess risk associated with animal health diseases (Wieland *et al*., 2011). Risk is the probability of a hazard to produce severe consequences (Astles *et al.*, 2006). Qualitative risk analysis is an interactive process consisting of four main stages (Fig. 6.1) (OIE, 2010):

- hazard identification,
- risk assessment,
- risk management and
- risk communication.

De Vos *et al*. (2010) developed a risk assessment framework for emerging vector-borne livestock diseases. The framework was developed for livestock diseases transmitted by mosquitoes, midges and ticks. However, transmission dynamics will differ between these vectors due to lifespans, biting rates and stricter requirements for their ecological niche. These applications are mostly relevant in an export and import scenario where there is a likelihood of the introduction of a disease to a non-endemic country, for example the export of equines from Namibia. For the risk assessment in Namibia, principles of the OIE risk analysis were incorporated in combination with an ecological qualitative risk analysis often used in aquatic environments (O'Brien & Wepener, 2012), as well as the framework developed by De Vos *et al*. (2010) (Fig. 6.1).

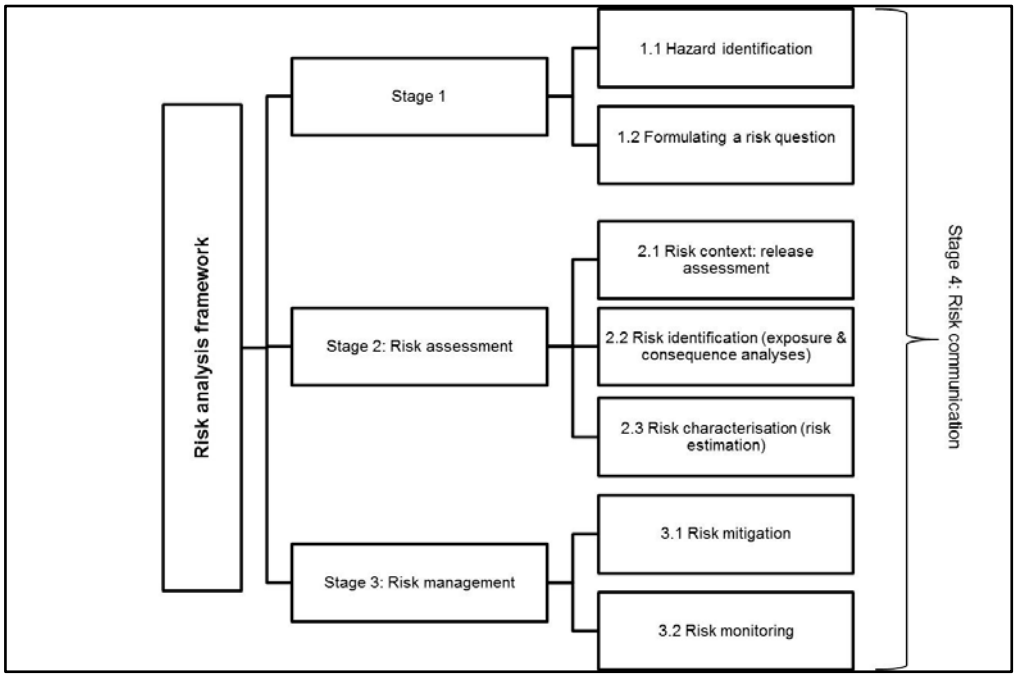


Figure 6.1: Proposed framework for the four stages of qualitative risk analysis to define the intra-country likelihood of an AHS outbreak occurring (Astles *et al*., 2006; OIE, 2010; O'Brien & Wepener, 2012).

Please take note that due to the qualitative nature of this chapter, some of the materials and methods are discussed as part of the steps within the results and discussion section in order to better understand the chronological sequence of the risk analysis.

Hazard identification is a categorisation step that identifies biological agents dichotomously as a potential hazard or not. It is necessary to identify where the hazard is present in the country, and whether it is a notifiable disease or is subject to control or eradication (OIE, 2010). It is also during this stage that a risk question is formulated.

Risk assessment provides insights about sources and levels of risk and their potential impacts. This stage can also be termed as the analysis phase (U.S Risk assessment forum, 1992). According to the OIE import risk analysis, this stage will be categorised into four different steps: release assessment, exposure assessment, consequence assessment and risk estimation (OIE, 2010). However, for the use of this study the following three steps within the risk assessment stage will be utilised (Astles *et al.*, 2006): **First step**: risk context – this provides the spatial and temporal extent, and a point of reference for the next step. For the purpose of the AHS status of a district, the following terminology will apply (OIE, 2004): Low – rare but does occur; Medium – occurs regularly; High – occurs very often. **Second step**: risk identification – this identifies which components are at risk and why, by generating a conceptual model that delineates the potential relationships between sources, stressors, habitats and endpoints (O'Brien & Wepener, 2012). Relevant terminology: factors influencing the occurrence of AHS are termed as 'stressors'. 'Endpoints' refer to management goals, or an expression of goals that the analyst wants to achieve by undertaking the risk analysis. 'Habitats' refer to the physical environment that integrates the effects of stressors impacting on the system. 'Source' is an entity or activity that releases the stressor into an environment (O'Brien & Wepener, 2012). Risk of transmission refers to the risk that AHSV is able to spread to hosts, implying that a competent vector is present at a specific time and environmental conditions are suitable for virus replication and spread. The likelihood of the occurrence of AHS outbreaks refers to the probability that AHSV can spread from vector to host and vice versa given the conditions of introduction into a district. The **third step** is risk characterisation – this aims to estimate the likelihood that the various sources of risk will cause the undesirable event as identified in the risk content.

Risk management, the third stage in the risk analysis framework, takes mitigated action against identified risks and monitors whether the actions are effective. Risk management contains two parts, risk mitigation and risk monitoring (Astles *et al*., 2006). *Risk mitigation* aims to minimise the risk of an undesirable event defined in the risk context. This can be achieved by the evaluation and implementation of management responses. *Risk monitoring* is the second part of risk management and aims to collect information to determine whether the initiatives implemented in the strategy minimised the risk.

Risk communication, the last stage, actually takes place as part of all stages between stakeholders, the community, management and researchers (Astles *et al.*, 2006; OIE, 2010).

6.3. RESULTS AND DISCUSSION

6.3.1. The application of a qualitative risk analysis on the occurrence of AHS outbreaks in Namibia

6.3.1.1. Stage 1: Hazard identification and formulating the risk question

The qualitative risk assessment considers the likelihood of the occurrence of outbreaks of AHS in Namibia between different districts via various pathways. The assessment builds on previous chapters where (Fig. 6.2): 1) the historical distribution of AHS in Namibia was investigated; 2) surveillance of multidisciplinary data were used to identify drivers of AHS for Namibia; 3) climatic drivers between South Africa and Namibia were compared utilising modelled climatic data; and 4) multivariate analyses were applied to illustrate relationships between the occurrence of AHS and multidisciplinary parameters.

AHS is endemic to Namibia and classified as a controlled, notifiable disease as described by the OIE and incorporated in the Namibian Animal Health Act of 2011. Although several risk assessments and prediction models have been developed for South Africa, research on AHS in Namibia and arid areas is limited. As mentioned in Chapter 4, the climatic drivers for South Africa and Namibia are the same but the combination of the parameters has a different effect in each country. Therefore, risk assessment analyses for South Africa cannot be directly applied to Namibia.

Risk question: What is the seasonal likelihood (which is an outcome of multidisciplinary parameters) of the occurrence of outbreaks of AHS within Namibia?

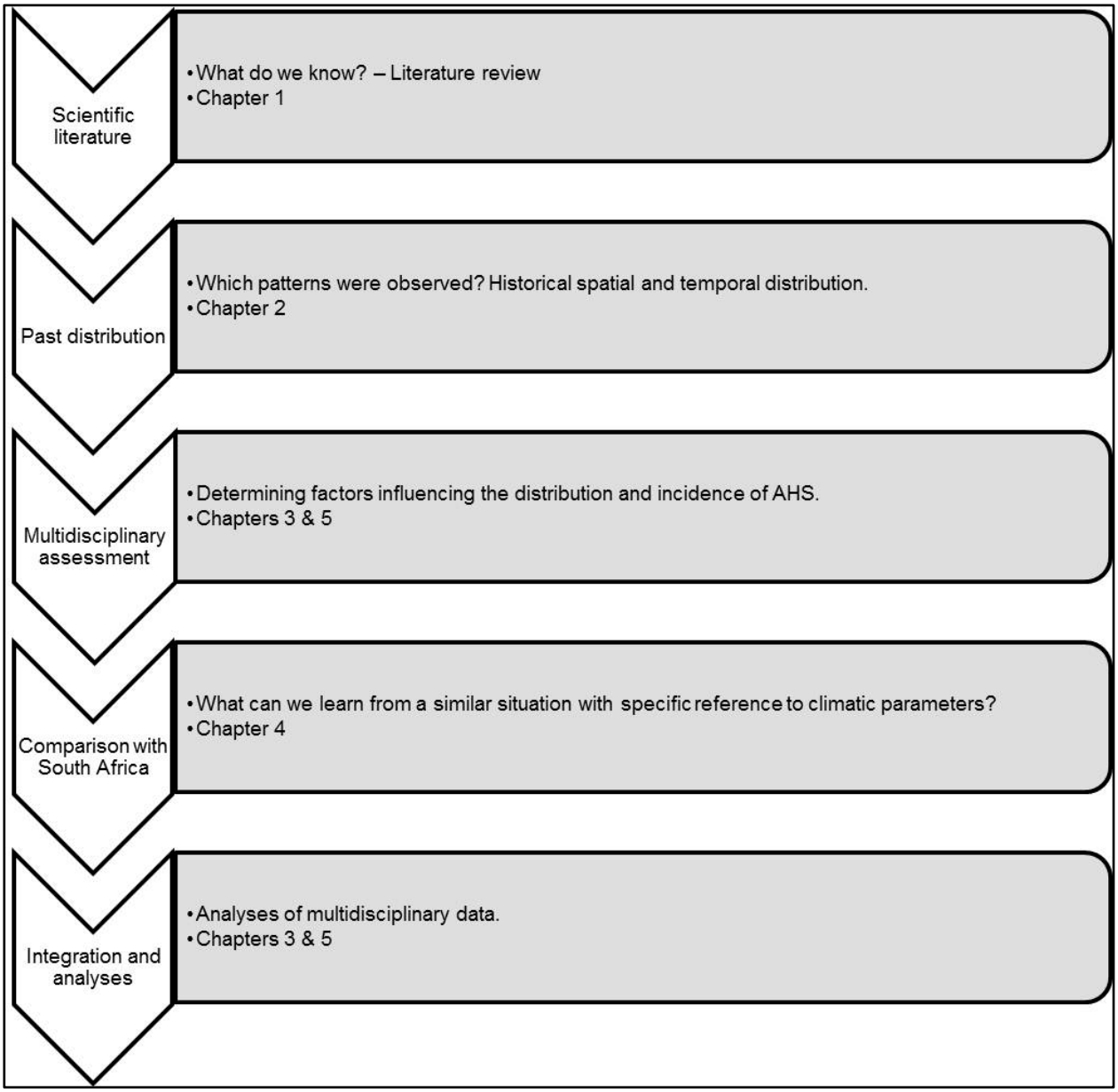


Figure 6.2: A concise overview of the supporting evidence used for conceptualisation of the risk assessment on the distribution of AHS in Namibia.

6.3.1.2. Stage 2: Risk assessment

Step 1 - Risk context

The probability of distribution (release assessment) (Fig. 6.1) describes the pathways by which AHS can be introduced into Namibia. Two major groupings of pathways were identified, namely imports (legal/illegal) from i) endemic and ii) non-endemic areas (Fig. 6.3). Requirements for equine movement from neighbouring countries such as South Africa or Botswana depend on whether it is permanent or temporary. Requirements that have relevance to AHS include a valid passport and health certificate (Directorate of Veterinary Services, 2007). The focus for this assessment was on intra-community movement (structured) as well as imports (temporary/permanent) from South Africa as described in Chapter 5. According to the

Directorate of Veterinary Service (2007), import of horses from third party countries (endemic and non-endemic) may only take place through South Africa.

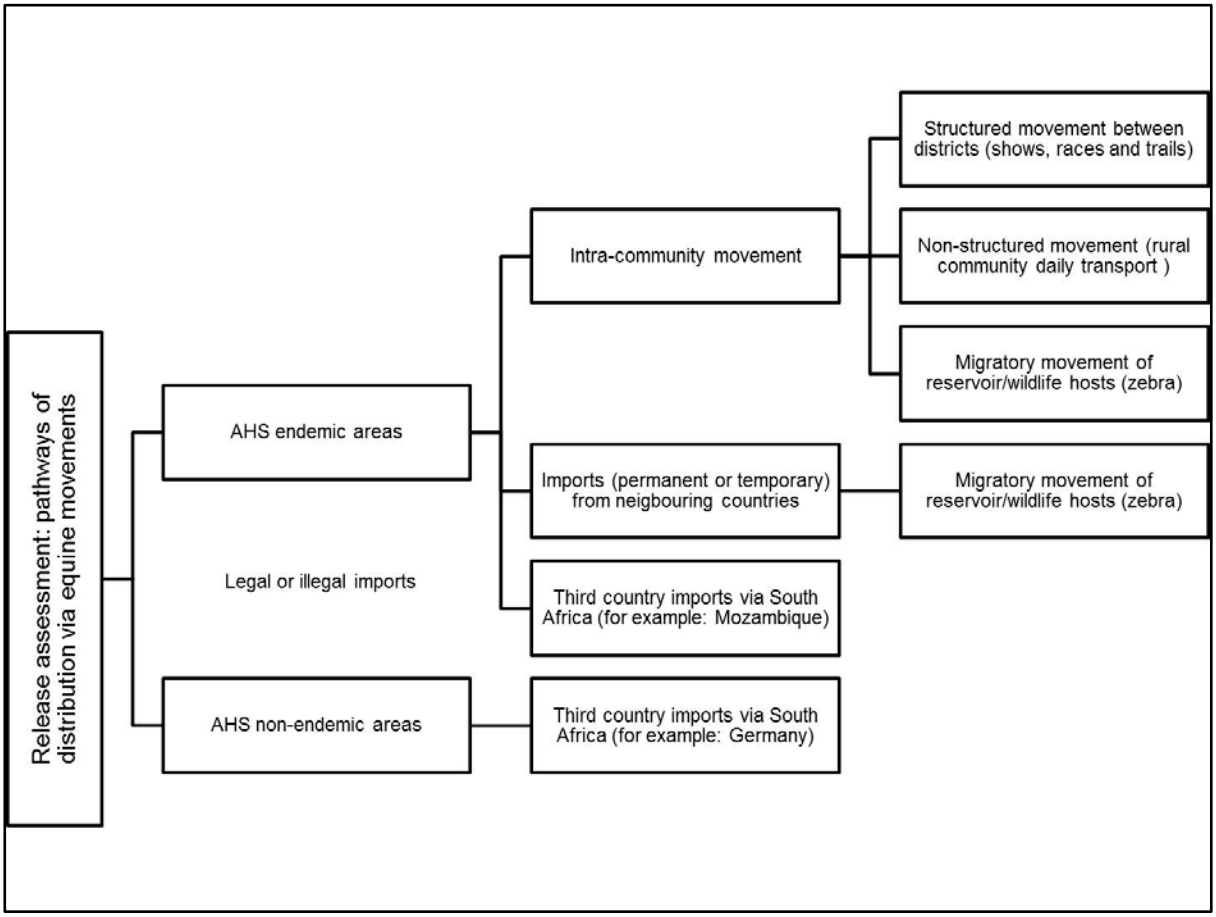


Figure 6.3: Release assessment: pathways of distribution of AHS via equine movements in Namibia.

The second phase in the risk context step is to provide the spatial and temporal extent of the risk. The spatial and temporal scales for the historical distribution of AHS in Namibia are described in Chapter 2. Districts were classified according to their historical AHS incidence relative to the national AHS incidence and horse population: low, medium and high. According to the distribution of AHS, the southern districts are low incidence areas, with the high incidence areas located within the central districts and medium incidence in the northern districts. There was a definite change in AHS distribution from the 1916-1934 period (Fig 2.2) to the 1993-2011 period (Fig 2.4). This can be attributed to societal effects, vaccination and underreporting. These effects must be addressed during the risk management stage.

Step 2 - Risk identification

The risk identification process (Fig. 6.1) involves a conceptual model of the relationships between all the parameters involved that influence the distribution of AHS. In a classic risk identification step, risk sources will be identified and they will be classified according to their level of risk (Astles *et al.*, 2006). In an AHS endemic area such as Namibia, the risk is not so

much driven by the importation of infected animals, but by the conditions that drive the persistence and the levels of incidence of the disease. Temperature could not be identified as a source or a risk factor. However, the increase of temperatures together with the interaction of other variables such as humidity and precipitation can lead to more favourable conditions and subsequent seasonality of AHS outbreaks as discussed in Chapters 3 and 4. The development of breeding habitats and *Culicoides* activity can increase the risk of the occurrence of outbreaks of the disease.

A conceptual model (Fig. 6.4) was constructed which describes the relationships of stressors involved in the distribution of AHS. These parameters must not at any time be considered exclusive of each other, since several parameters influence one another. This conceptual model (Fig. 6.4) serves as the exposure analysis as described by the OIE and transmission dynamics (De Vos *et al*., 2010). The probability of **transmission** and **exposure** of AHSV is defined by the presence of competent vectors, hosts and climatic and regional factors (*Sources*). Being dependent on the rate of recruitment from breeding sites*, Culicoides* population sizes are highly heterogeneous at a local scale and will vary in response to host breeding-site factors in addition to climatic factors (*Stressors*) (Meiswinkel *et al.*, 2004). This is likely to be reflected in the significant variation in the risk of AHSV transmission at a local scale. The size and quality of breeding sites depend on soil type, slope of terrain, rate of dung removal from animal holdings, urbanisation and availability of water sources (*Habitats*) (Meiswinkel, 1998). Only if competent vectors and susceptible hosts are present in sufficient numbers, is the **distribution** of AHS possible (De Vos et al., 2012). The disease will **establish** in an area when certain parameters are favourable for AHSV replication and transmission with a high vector-host ratio. Host variables that affect biting rates include the number and type of livestock and ease of access to them (Meiswinkel *et al*., 2004; Purse *et al*., 2007). *Endpoints* refer to the ultimate management goals and will be discussed in the risk management section.

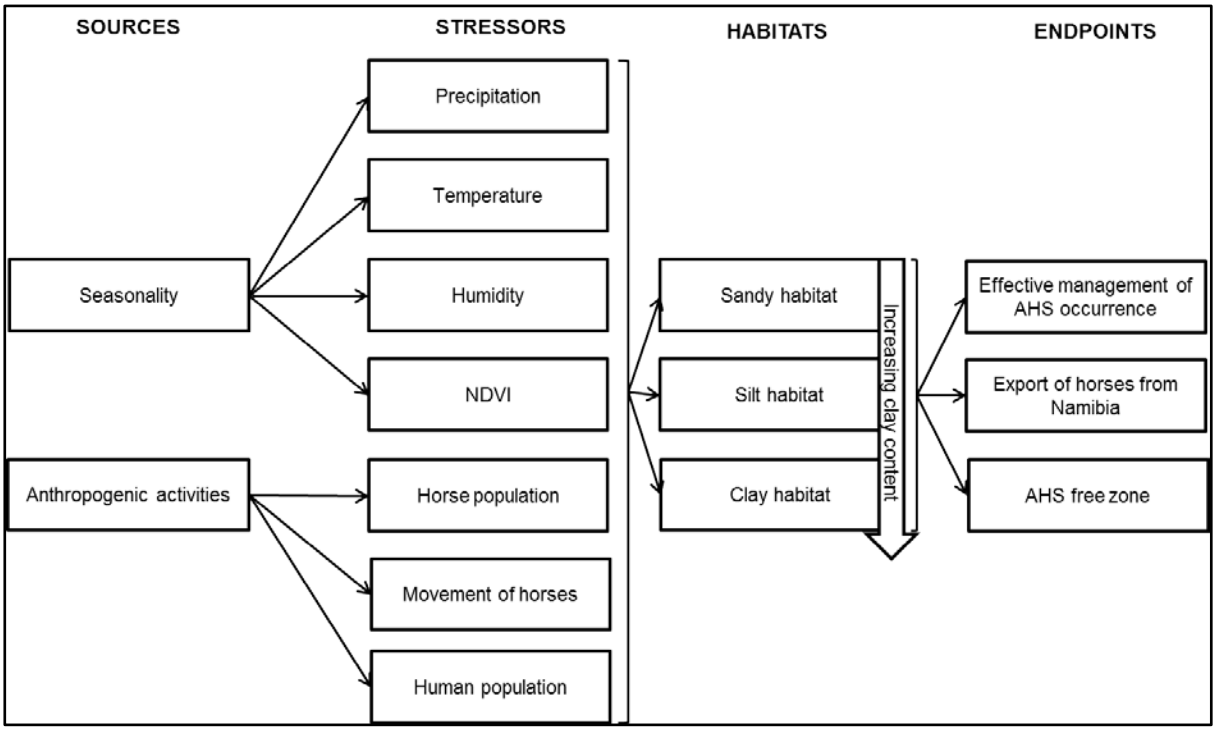


Figure 6.4: Conceptual model presenting the possible relationships between identified sources, stressors, habitats and endpoints in the assessment of the distribution of AHS.

Step 3 - Risk characterisation

The third step of the risk assessment stage (Fig.6.1) is where the results from the risk context and risk identification steps are used to analyse and evaluate the risk of an AHS outbreak occurring (U.S Risk assessment forum, 1992). This is a multi-stage process that begins with the broad components and identifies what the general risks to the outbreak of AHS are. In the second stage, each of the components is assessed in detail.

Interactions between the different stressors on the vector-pathogen-host epidemiological cycle are illustrated in Fig. 6.5. A breakdown of the components involved in the different stages of AHS transmission is given in Table 6.1, identifying the general risks of the occurrence of AHS outbreak. The indicated shaded component is regarded as the highest risk area namely the *Culicoides* immature stages. Unfortunately, little is known about the exact breeding habitats of *Culicoides* and their immature stages as discussed in Chapter 1. Control of this stage is nearly impossible (Miranda, 2014, Venter, 2014).

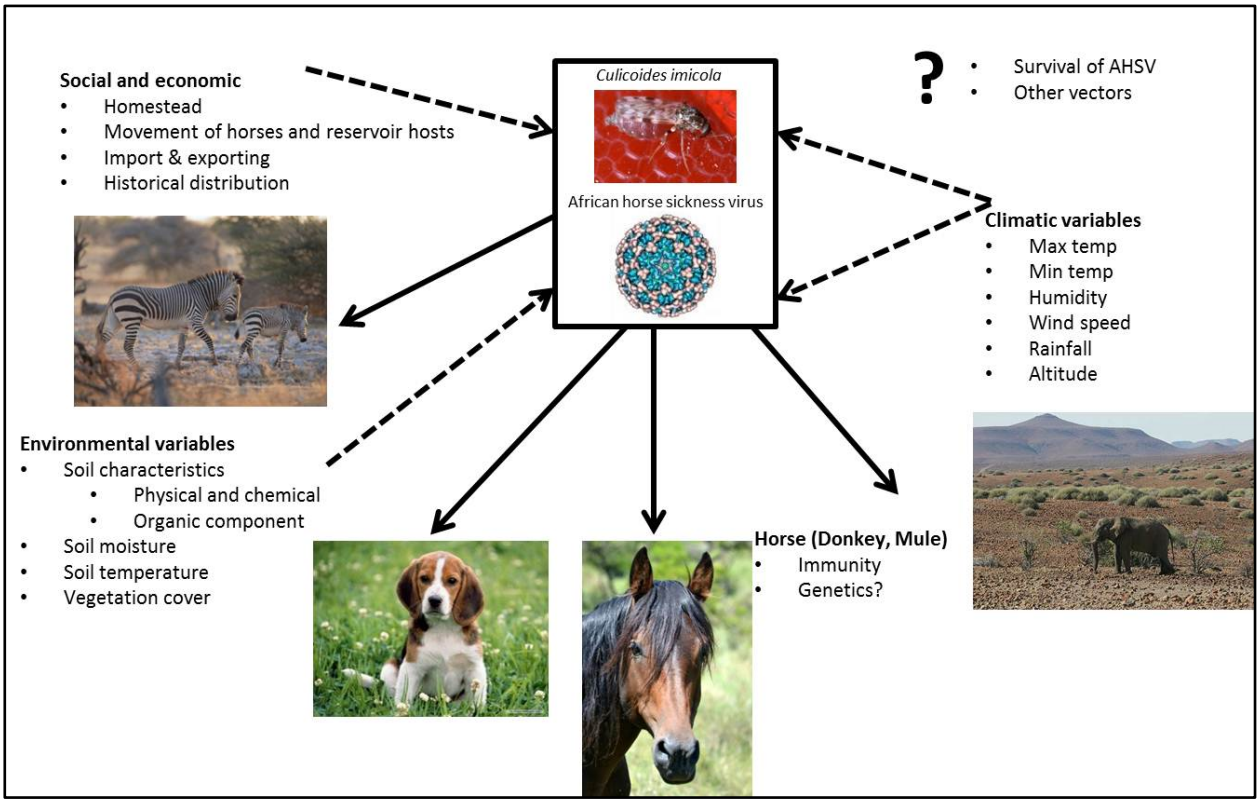


Figure 6.5: Interactions between selected stressors with effects on the vector-pathogen-host epidemiological cycle. Broken lines indicate the effects of the stressor on *Culicoides imicola* and/or AHSV and solid lines the effect on hosts. The question mark indicates the uncertainty component Selected references: Barnard, 1993; Wittmann & Baylis, 2000; Meiswinkel *et al*., 2004; Mellor & Hamblin, 2004; Wilson *et al*., 2009; van Sittert *et al*., 2013.

Table 6.1: Broad components of different developmental stages of *Culicoides* contributing to the occurrence of AHS outbreaks in Namibia and identification of the different levels of general risks for the occurrence of AHS outbreaks. H= high risk; I= intermediate risk; L= low risk; U= unknown

COMPONENTS	**CLIMATE**			**ENVIRONMENTAL**			**ANTHROPOGENIC**			
	Temperature	Precipitation	Humidity	NDVI	Soil type	Organic Carbon	Population Humans	Population Horses	Vaccination	Movement of horses
Availability of breeding sites	-	H	-	H	H	I	H	I	-	-
Immature stages	H	H	I	H	I	H	U	I	-	-
Adult *Culicoides*	H	L	H	L	-	-	L	H	L	-
AHSV replication (gonotrophic cycle)	H	-	-	-	-	-	-	-	U	H

The stressors that can be identified and measured from this study as those that influence the outbreak of AHS, are given in Table 6.2. The level of risk was determined from the analyses performed in the previous chapters (Chapter 3, 4 & 5) as well as scientific literature. Each of these levels of risk was given a ranking according to the results to enable the quantification of the risk of an outbreak in an area. Weighting for risk was done conservatively. Factors that contribute to a low risk will be ranked as one, intermediate risk as two and high risk as three. Parameters indicated in previous chapters and literature as having the highest contribution to risk of the occurrence of AHS outbreaks were given a double weight (x 2). Weighing was allocated so that with a double ranking, risk will resort in a higher risk category.

- Due to the qualitative nature of the data, the risks of anthropogenic effects are not highlighted enough. Results from the ANN in Chapter 4 implicated the role of human populations as one of the main drivers of AHS outbreaks. In addition, movement of horses per district were identified to have an influence on the occurrence of AHS outbreaks in Chapter 5 with the Gephi analysis (Fig. 5.1).The location mentioned only represent 'for the moment' an indication of movement numbers of horses. Therefore, anthropogenic influences will contribute a "double ranking" when ranked as high due to the uncertainty related to its impact.
- Precipitation was considered as the initial trigger of AHS in Namibia as discussed in Chapter 3 and 4 and will therefore also constitute a "double ranking" in the intermediate and high ranking.
- Temperature and humidity are illustrated together as it was determined that the relationship between these parameters play an important role in *Culicoides* activity (Chapters 3, 4 and Wittman *et al*. (2002)). However, there can be different combinations of these factors and therefore each was allocated their own score. Weekly or monthly temperature and humidity data can be used, as results from Chapters 3 and 4 indicated the effect remains the same regardless of the data used.

The risk of a district according to these factors can be determined by adding the rankings of all six parameters.

Uncertainty component that influences the occurrence of AHS outbreaks is rather large and requires a significant amount of future research (Fig. 6.5). Uncertainty component that influences the distribution of the disease, such as the overwintering of the disease, other possible hosts (susceptible as well as non-susceptible) and vectors, migration of the zebra population, the role of equine genetics and immune status of susceptible host populations were not included in the risk analysis. Assigned levels of risk may be incorrect because to these uncertainty components. This uncertainty is due to a lack of understanding of the

nature of AHS outbreaks, *Culicoides* vectors and the environment in which it operates. In a recent study by Bessel *et al*. (2014) on Schmallenberg virus, feeding preferences of *Culicoides* were shown to have implications for the transmission of the disease. Some species were found to be 5 times more likely to feed on cattle than on sheep. The African *Culicoides* species feeding preferences is something that needs urgent attention for the epidemiology of livestock diseases. Risk factors included in the assessment were those that were identifiable, measurable and significant. Furthermore, the complex interaction of the stressors, not only with the vectors but also with the virus, hinders the quantification of AHS risk. An example of how risk can possibly be estimated, in spite of these shortcomings, will be discussed in more detail in combination with a qualitative risk matrix (Fig. 6.7) where the rankings as described in Table 6.2, are visually presented.

Table 6.2: Overview of the occurrence of AHS outbreak risk ranking scheme indicating ranks allocated to stressors and habitat variables to estimate and quantify the risk of an area. Ranks were determined from integration of previous chapters' results. Low risk = 1; Intermediate risk = 2 and High risk = 3.

STRESSORS	**RANKING FOR QUANTIFICATION OF RISK**		
	Low (1)	**Intermediate (2)**	**High (3)**
Precipitation	No precipitation	More than 100 mm/annum (x2)	More than 300 mm/annum. Any above average precipitation event (x2)
Temperature (weekly/monthly)	< 15°C and > 25°C	15-18°C	18-25°C
Humidity (weekly/monthly)	> 70%	20-70%	40-70%
NDVI (function of soil moisture and vegetation cover)	< 0.2	0.2-0.35	>0.35
Movement status	Keetmanshoop Mariental	Gobabis Grootfontein Omaruru	Walvis Bay Okahandja Windhoek } x2
HABITAT			
Soil type	Sandy – low organic content	Silt – medium organic content	Clayey – high organic content

Another aspect for risk characterisation that has to be considered is the sensitivity of the *Culicoides* sp. towards stressors Sensitivity refers to the degree to which a vector responds to an external perturbation, such as temperature (Sutherst, 2004). A conceptual model of the geographic distribution of a vector species related to its climate envelope was described by Sutherst (2000, 2004) (Fig. 6.6). This is also believed to be true for *Culicoides* vectors and the occurrence of AHS. A change in the suitability of an environment within the current geographical distribution of the disease will alter the development, survival and reproductive rates of vectors. This will in turn affect the intensity of disease transmission and the exposure of the disease to the population (Sutherst, 2004). Populations within the core of distribution A (Fig. 6.6) have minimal stresses and are relatively insensitive to change in temperature or moisture. However, populations in marginal parts of distribution B (Fig.6.6)

are more sensitive to change. At or near the edges of the distribution there is a greater potential for high variability in the occurrence of limiting conditions (Sutherst, 2004). The underlying pressures driving transmission dynamics of AHS will vary more around the edges of the area of endemicity in response to climate variability. The *Culicoides* spp. in Okahandja and Windhoek will be mostly associated with population (A) and the Aus population will more likely correspond to the position of population (B). Sensitivity of *Culicoides* spp. reflects uncertainty towards stressors. However, due to complicated nature of the relationship between *Culicoides* – AHSV – and the environment (anthropogenic and natural) it was not included in the risk matrix tool (Fig 6.7).

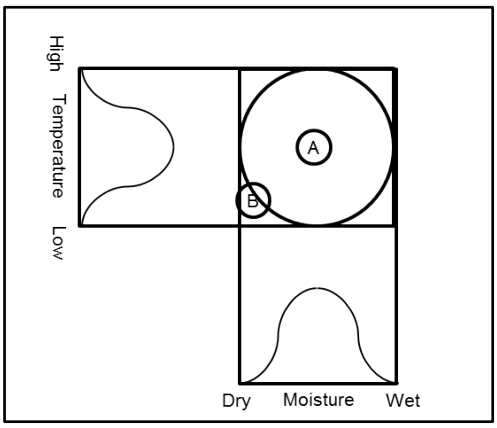


Figure 6.6: Conceptual model of the geographical distribution of a vector species (*Culicoides*) related to its climatic envelope. Population (A) near the centre of the climatic envelope will be less sensitive to variations in temperature and moisture than population (B) near the edge of the envelope (Sutherst, 2004).

6.3.1.3. Stage 3: Risk management

Risk management is one of the major stages of risk analysis. The aim of risk management is to address the high risk issues raised in the risk assessment and to ensure that there is no increase in risk (Astles *et al*., 2006).

There are four ways in which the qualitative risk analysis contributes to the development of management plans of AHS:

- The factors contributing to high risk are clearly identified.
- The link between risk levels and stressors contributes to better monitoring of *Culicoides* and AHS.
- It identifies which activities are increasing the risk. This gives direction to the management plan as to which activities require change.

- The qualitative risk analysis reveals significant knowledge gaps in our understanding of AHS, its vectors and the interactions with environmental parameters.

However, the question is how to manage something that cannot be controlled? Environmental and climatic parameters are not manageable - the environment is either favourable for the *Culicoides* midges or not. From this study, it is evident that there are very few environments that are unsuitable for *Culicoides* to occur; although some areas are more favourable than others and this will influence the risk of a specific area. This is where the sensitivity of *Culicoides* populations will contribute to risk estimation. The occurrence of AHS is a numbers game since it depends on the sensitivity, competence, numbers and fitness of the *Culicoides* population to enable transmittance of AHSV to another host. From a risk assessment perspective there are certain risks that we cannot manage in an AHS endemic area and thus the focus should be on risks that can be managed.

As discussed in Chapters 4 and 5, the effects of anthropogenic and social activities are usually not taken into consideration in the management and evaluation of intra-community distribution of AHS - an aspect that is manageable. The occurrence of horse events are at its peak during the AHS season. Results from the social survey indicated that districts with more horse movement are higher incidence areas. In Chapter 4, the ANN results implicate human population as one of the largest contributing factors. Anthropogenic activities (such as gardening – homestead effect, and activities associated with animal husbandry), especially in arid areas, increase suitable breeding habitats, thereby influencing the probability of an AHS outbreak.

The goal will be to reduce the likelihood of an outbreak or to contain an outbreak, since the elimination of the disease in southern Africa is impossible. Integrated control strategies are recommended to maximise efficiency and acceptability from an animal health and environmental standpoint. Risk mitigating practices that can be added to the current preventative measures include:

- movement control during outbreaks,
- fewer horse events during the AHS season,
- policies on AHS reporting, and
- education of owners.

Namibia has no official policy in place regarding AHS and movement of equines within the country. The development of a policy with regards to equine movement will not only advance the understanding of AHS, but also of other equine diseases such as West Nile virus, Equine

encephalitis virus, Dourine and Strangles. This will include the large population of rural donkeys and mules. This movement policy can include actions such as:

- as soon as AHS is confirmed in an area, all equine movement from that site and within a 150 km radius from that site must be limited;
- for equine imports (temporary/permanent) from South Africa, the vaccination record should be checked not only for AHS vaccination but also the period during which the equine was vaccinated.

If there is horse movement from South Africa to Namibia during the peak AHS season, conditions of travelling such as vector protected vehicles, treating of animals with chemical repellents, as well as the transport route and stopover points should be known before entry is allowed. Horses travelling through or from Gauteng pose a greater risk to the introduction of the disease into Namibia than horses travelling from the Northern Cape Province. One of the biggest problems associated with epidemic outbreaks of diseases such as AHS, is movement of infected hosts and vectors. With policies in place to prevent host movement, epidemic outbreaks could be managed.

Continued vector surveillance is required in high risk areas (as described by the OIE) for a better understanding of the presence and competence of these vectors in Namibia. The development of a field test kit for AHSV would assist in reducing underreporting and is something that is currently lacking and under researched. A user-friendly reporting interface is another aspect that needs careful consideration and attention. A certain trend has been experienced in South Africa where suspected cases of AHS are reported on Facebook pages to give warning to other horse owners. This has not been taken up by the Namibian horse owners and although it has its benefits, the disadvantages must also be investigated.

Some mitigation actions are discussed in Fig. 6.7 that follow a particular risk score. The focus is mainly on reducing the biting rate and monitoring horse health. Traditional methods, such as stabling of horses remain important actions that should be taken, especially in areas of high risk. The entering of *Culicoides* into buildings may be dependent on temperature (Meiswinkel *et al.*, 2004). In high risk areas, such as Windhoek and Okahandja, additional covering of stables with 80% shade cloth is recommended. Although ceiling fans had no suppressant effect according to Meiswinkel *et al.* (2000), with all ventilation closed off with shade cloth it will promote air circulation in the stables.

Vaccination during winter months (low vector numbers) is essential. Even though AHS vaccination is not defined in the Namibian Animal Health Act of 2011, most horse owners do vaccinate their animals to comply with competition requirements. Winter months are

unfortunately considered as a high competitive season. Show presenting bodies could start planning horse events taking AHS vaccination periods and AHS peak seasons into consideration.

Another action suggested is stable yard management. This includes vector control. In the risk characterisation step, the vector immature stage was deemed to pose the highest risk. Although management of this stage is nearly impossible, some actions can be taken. *Culicoides* population size was found to be one of the most important factors determining whether or not an outbreak occurs as well as influencing the intensity of the outbreak (Lo Iacono *et al*., 2014). Stable yards provide ideal breeding habitats with leaking water troughs and overgrazed paddocks. Management strategies would benefit from ensuring that there is no leaking water around stables and in paddocks. If a "water bucket system" is employed it is recommended that excess water from the buckets is emptied in an area where water will quickly evaporate.

Furthermore, grazing horses with cattle will increase the risk of AHS by increasing potential breeding habitats. Therefore, removal of cattle dung pats is imperative, especially in high risk areas. Furthermore, the effect of non-susceptible hosts is still unknown in Africa and can also contribute to the transmission of the disease.

6.3.1.4. Stage 4: Risk communication

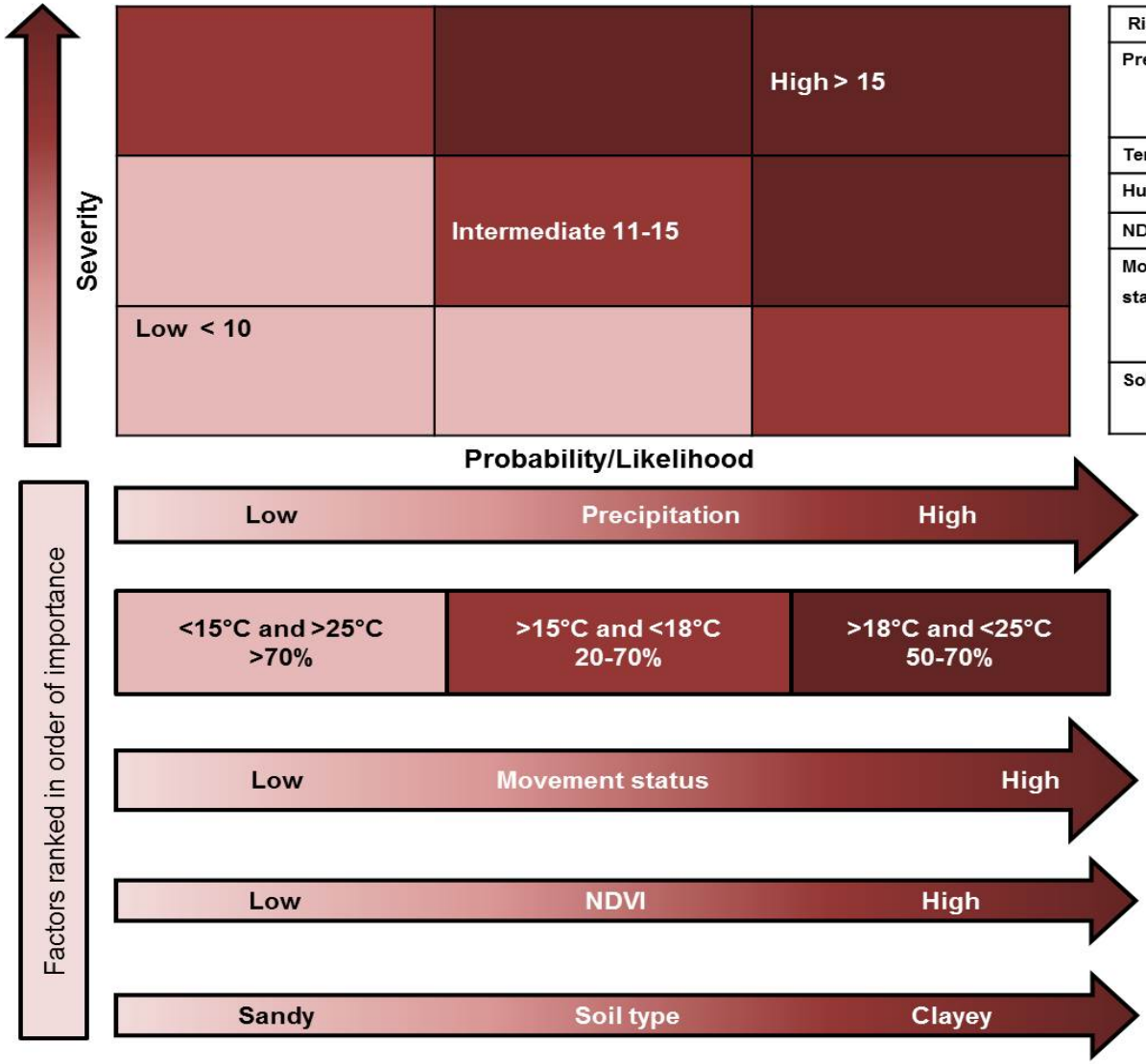
The purpose of risk communication is to provide information and feedback for decision-making and better understanding (Astles *et al.*, 2006). Horse owners are likely to be more alert and cooperative when they are better informed about the risk in their district. Despite its importance, risk communication has not been given adequate attention in AHS risk analysis. Astles *et al*. (2006) described areas of communication that needs to be planned for effective risk communication as follows: WHO is doing the communicating (credibility)?; WHAT is the purpose?; TO WHOM is the communication directed?; WHAT (relevance) is to be communicated?; HOW is it to be communicated?; and lastly. Does the reception of the communication increase understanding?

6.3.2 Development of a qualitative risk matrix for the occurrence of AHS outbreaks in Namibia

The risk level from a specific factor can be determined using a qualitative risk matrix (Astles *et al.*, 2006). Qualitative risk matrices provide a pictorial way of determining levels of risk. It is composed of two axes, which describe the overriding factors that determine the likelihood of an event occurring (Astles *et al.*, 2006). The most critical aspect of developing a risk

matrix is choosing appropriate factors for the axes. The two factors chosen to determine the distribution risk of AHS in Namibia were: 1) the probability/likelihood of an outbreak and 2) the severity of an outbreak (Fig. 6.7). The *likelihood* increases according to different increasing factors (precipitation, temperature and humidity, anthropogenic, NDVI and soil type), whereas increased *severity* is a factor of increased *Culicoides* numbers and AHSV presence. The two axes formed a 3 x 3 matrix (9 cells), which was divided, into 3 equal levels of risk. The arrangement of the factors was greatly influenced by the complexity of the relationships that drive AHS occurrence – motivation for ranking was deduced from results of the previous chapters as discussed in 6.3.1.2. Uncertainty was ignored in the matrix. Assigned level of risk might be incorrect due to uncertainty and might even "spread" over several cells. Therefore, risk levels was given in ranges of risk. High areas of risk correspond with high likelihood and high severity. From this risk matrix, the risk status of specific areas can be determined in order to manage the risk more effectively. The likelihood of an AHS outbreak depends on seasonal parameters, with a precipitation event acting as the initial trigger. Severity cannot be quantified and needs further research. However, parameters will influence the severity of AHS occurrence by increasing the number of *Culicoides* in an area and thereby increasing the risk of the occurrence of AHSV. For this reason risk is mostly perceived and described from the likelihood dimension which adequately represent the thee levels of risk.

The matrix is one of the first attempts to estimate the AHS occurrence risk of an endemic AHS area. The social component (anthropogenic effect) is included in the matrix - important to note is that this component can contribute to an increased risk but at the same time, its effect is independent of any of the other parameters. As mentioned in the risk characterisation step, the risk can be calculated by adding the ranking of the six different parameters (Table 6.2). The rationale for double scoring and weight allocation were described in the Risk characterisation step 6.3.2.1. Scores are related to the likelihood dimension of the matrix. High movement status and above average precipitation contribute double their ranking due to the importance of these parameters in the contribution to the distribution of AHS. Therefore, in areas such as Walvis Bay, Okahandja and Windhoek, the anthropogenic effect will be the highest contributor. In these areas the risk must be considered high during the AHS season, especially in the rainy season and all mitigating measures must be in place. In other areas with a lower movement status (as determined in Chapter 5), precipitation acts as the initial trigger whereafter the risk will increase. In low risk areas an extreme precipitation event (such as flooding) will rank the risk during that period in a higher category. Mitigating actions are suggested as part of the risk tool that automatically follows a particular score in the risk matrix.



Risk	Low (1)	Intermediate (2)	High (3)
Precipitation	No precipitation	More than 100 mm/annual (x2)	More than 300 mm/annual (x2)
Temperature	< 15°C & > 25°C	15-18°C	18-25°C
Humidity	> 70%	20-70%	40-70%
NDVI	< 0.2	0.2-0.35	>0.35
Movement status	Keetmanshoop Mariental	Gobabis Grootfontein Omaruru	Walvis Bay Okahandja Windhoek (x2)
Soil type	Sandy – low organic content	Silt – medium organic content	Clayey – high organic content

Mitigating actions

Low	Intermediate	High
Rare but does occur	Occurs regularly	Occurs very often
Vaccinate during WINTER	Vaccinate during WINTER	Vaccinate during WINTER
Stable yard management	Stable yard management	Stable yard management
Communication - report outbreaks	Communication - report outbreaks	Communication - report outbreaks
Reduce biting rate (fly sheets, fly repellents and stable horses)	Reduce biting rate (fly sheets, fly repellents and stable horses)	Reduce biting rate (fly sheets, fly repellents and stable horses)
	Monitor horse health daily	Monitor horse health daily
		Movement restrictions around outbreaks

Figure 6.7: A qualitative risk matrix incorporating the most prominent factors that influence the occurrence of AHS outbreaks in Namibia. Key to abbreviation: NDVI: Normalised difference vegetation index.

6.3.3. Application of the qualitative risk matrix using independent data

An objective of this study was to determine whether the risk matrix can be applied to identify AHS-free zones in Namibia for possible exports of equines. The logical areas from where exports are most likely to occur in Namibia are Walvis Bay, Windhoek and Luderitz, since these areas have some of the needed infrastructure. However, historically only the Luderitz area has been free of AHS (as reported annually by the Ministry of Agriculture, Water and Forestry – Directorate of Veterinary Services). Windhoek and Walvis Bay experienced increasing AHS outbreaks since 1990 (Chapter 2). The risk matrix developed during this study was tested by applying it to independent data to compare the different areas.

Publicly available data was used:

(Project Atlas): http://www.uni-koeln.de/sfb389/e/e1/download/atlas_namibia/e1_download_living_resources_e.htm#biomes_vegetationand ; and for weather data: http://weather.namsearch.com/monthly_reports

Table 6.3: The application of the qualitative risk matrix, comparing Luderitz, Walvis Bay and Windhoek as possible ports of export of equines from Namibia.

January-May	**Luderitz**	**Walvis Bay**	**Windhoek**
Average monthly temperature	17°C (Low = 1)	18°C (Low = 1)	20°C (High = 3)
Average monthly humidity	79% (Low = 1)	81% (Low = 1)	45% (Intermediate = 2)
Precipitation	Low = 1	Low = 1	Intermediate = 2 (x2)
Soil type	Low = 1	Low = 1	Intermediate = 2
Movement status	Low = 1	High = 3 (x2)	High = 3 (x2)
Vegetation type – vegetation structure – biomass production	Namib desert – Namib grassland – bare ground (Low = 1)	Namib desert – Namib grassland – bare ground (Low = 1)	Tree and shrub savanna – dense shrubland – very high green vegetation biomass (Intermediate = 2)
Total ranking	6 (Low)	11 (Intermediate)	19 (High)
Additional supporting data			
Human population (2011 census)	13700	107100	340900
Horse population (2000 census)	0	<100	>5000

The AHS risk according to the risk matrix (Table 6.3) classifies Luderitz as a low risk area, Walvis Bay as intermediate and Windhoek as high. NDVI data was not available for the different areas. However, vegetation data indicates that Windhoek has a higher biomass production than the other two areas. The relatively high average monthly temperature in combination with humidity can potentially increase the risk of high *Culicoides* numbers in Windhoek. Together with the high movement status, the distribution of AHS within and from this area is a high risk. In Walvis Bay there is an average of 2 days precipitation per month during the AHS season.

Moisture requirements for the establishment of *Culicoides* spp. in Namibia are low as (Chapter 3) and this implies a higher risk in this area than in Luderitz. The anthropogenic component in the Walvis Bay area also contributes to the higher risk in this area, with horse movement from across Namibia as well as from South Africa (Chapter 5). Based on these results, Luderitz would be the recommended area to apply for AHS free zoning as stipulated in the OIE policy. Currently there are no horses at Luderitz. The closest horses are the famous Namib feral horses approximately 120 km east of Luderitz, near Aus. The isolation of the Luderitz desert area is ideal for an exporting scenario and anthropogenic activities can be correctly managed. This area could also be used for South African horse exports if the participating countries can come to a general consensus on the movement of horses. However, before any exporting scenario from Luderitz can be considered, a risk assessment together with surveillance for vectors of AHS and AHSV according to the OIE Terrestrial Animal Health Code (2014) must be performed from Keetmanshoop through to Luderitz.

Another factor that has to be taken in to consideration is the feral horse population at Garub which is on route to Luderitz near Aus. The horse population was at an estimated 190 horses at the end of 2013 and can increase to approximately 300 in favourable years. From personal communication with Dr Telane Greyling, specialist on feral horse dynamics (Greyling, 2013), it was evident that these horses had no antibodies against AHSV when they were tested a few years earlier. The introduction of AHSV will cause devastation to these horses which are a well-known tourist attraction and international symbol of Namibia. In Chapter 3, a two year study of *Culicoides* performed in Aus is discussed. During this study, <10 000 *Culicoides* were collected with *C. imicola* making up approximately 7% of these collections. However, after flooding in January 2013 there was a significant increase in the numbers of *Culicoides* collected and AHSV was detected in the collected *C. imicola* complex. This stresses the importance of how carefully the management plan must be developed.

CHAPTER 7

CONCLUSION

One of the most critical times for any horse owner in southern Africa is during the AHS season. This dreadful disease has caused losses of great economic and emotional value for as long as horses have been on the African continent. From several previous studies on AHS, it is evident that a more holistic and ecosystem approach had to be followed in order to achieve the best possible solutions towards the objectives put forward for this study. There had to be effective interaction between scientific analysis, horse-owner's innovation and the development of principles and tools to make sure the recommendations that emerged from this study are based on the best knowledge and outcomes from the study. In order to characterise the distribution of AHS for the development of a risk assessment tool in Namibia, six objectives were set. A concise conclusion on the outcome of these objectives is given below.

i) An assessment of historical data on the occurrence of AHS in Namibia over the past 100 years (Chapter 2)

During a desktop study on the available archive data of reported AHS cases in Namibia, it was found that several major historical events played a role in the introduction of horses to southern Africa with AHS influencing the outcomes of some of these events. The influence of horses and AHS on the history of Namibia is not well recorded and sometimes hidden in soldiers' reports and missionaries' diaries. AHS and the harsh conditions of Namibia were mentioned in several letters as one of the reasons that enemies could not be pursued and one can only wonder whether the outcome of battles would have been different if AHSV had not occurred in southern Africa. AHS outbreaks have been largely underreported and underestimated in Namibia and thus the full impact on the economy and horse industry cannot be fully assessed. Results indicate a variation in the distribution of AHS in past (Fig. 2.2) and present (Fig 2.4) time periods, but there was no statistically significant difference. The variation in the difference of the distribution pattern can be due to several reasons, one of which can be the use of the OBP vaccine and the increase in the movement of horses over long distances. It is concluded from these results that the central districts of Namibia were the highest incidence areas with lower incidence in the southern districts. One of the most important perspectives gathered from this historical assessment of AHS, is that even during times of war, horse movement were suspended during the rainy season.

ii) The determination of the *Culicoides* species composition, environmental factors influencing *Culicoides* and its impact on AHS distribution at three different sites across Namibia (Chapter 3)

Surveys were conducted over a two-year period at three different sites based on historical AHS status across Namibia. Various parameters (soil properties, vegetation characteristics, temperature, relative humidity, precipitation wind speed and direction) were measured in relation to *Culicoides* numbers. *C. imicola* was the dominant species in the highest incidence site, Okahandja. *C. ravus* and *C. subschultzei* were the dominant species in Aus and Windhoek, respectively. Not much research has been done on *C. subschultzei,* which was the second most abundant species, with no information available on its ability to transmit viruses. It is essential that oral susceptibility experiments must be performed on other dominant species in Namibia to determine their competence in the transmission of AHSV. Of the 48 *Culicoides* spp. collected (Table 3.6), only 17 species were collected at all three sites with another 15 species only found at one of the sites. The other 16 species were only collected in Windhoek and Okahandja. It is possible that species that were not collected in Aus might be sensitive to the more arid climate of Aus.

A positive relationship was found between the number of *Culicoides* collected and the incidence of AHS in the area as described in the historical overview. AHSV was present at all three sites during 2013 but only in Windhoek and Okahandja during 2014 (Table 3.7). The greatest number of positive AHSV samples was detected in Windhoek (4) and Okahandja (2) during the survey. Weather variables are interdependent of each other and have a complex interaction with *Culicoides* abundance. A precipitation event is one of the most important parameters, with a significant increase in the number of *Culicoides* collected the week after an event. Okahandja and Aus are at the two opposite ends of precipitation ranges, and the above trend in *Culicoides* numbers was observed at both sites. Areas with higher rainfall such as Okahandja would have higher baseline *Culicoides* numbers, with the creation of more favourable breeding sites.

iii) Comparison of the effect of modelled climatic parameters on the distribution of AHS outbreaks in South Africa and Namibia (Chapter 4)

Modelled ERA-Interim data and AHS reported data were used to compare the effect of climatic variables on the distribution of AHS outbreaks between South Africa and Namibia. The Eastern Cape in South Africa and Gobabis in Namibia were the areas with the highest AHS incidence over the 19 year survey period (1993-2011). The most significant modelled climatic parameters influencing AHS outbreaks were: humidity, temperature, soil water content, precipitation and evaporation. Parameters implicated as the main drivers for the distribution of AHS using ERA-Interim modelled data and measured data for a two year (2013 & 2014) survey were the same regardless of which data was used. This indicates that for the prediction of AHS outbreaks, satellite data such as the ERA interim data set is effective. In South Africa temperature had the most significant effect on the occurrence of AHS outbreaks whereas in Namibia, humidity and precipitation were the main drivers. For the South African data, the temperature and humidity

ranges were determined as well as the effect of anthropogenic influences on the distribution of the disease. The pattern of AHS outbreaks has always been thought to coincide between Namibia and South Africa. However, this seems not to be the case. It was found that although the same parameters in both countries are drivers for the disease, the combination of the parameters had a different effect on the occurrence of AHS outbreaks. Although a lot can be learned from previous research in the South African content, Namibia needs to develop its own policies and regulations regarding AHS management.

iv) An assessment of the relationship between AHS occurrence and social parameters, including the movement of horses; preventative measures and knowledge about AHS reporting (Chapter 5)

A social survey was conducted to determine the effect of movement of horses on the distribution of AHS. The survey also assessed the knowledge of horse owners with regards to reporting of the disease. The present role of horses in Namibia regarding transportation in the rural areas, the economic importance of the horses exported for international competitions and also the emotional value of horses for owners, add up to a value that cannot be quantified. It was evident that the process of reporting was unknown to owners - this was not due to the lack of willingness from respondents but due to a surveillance system that is failing horse owners. The most popular precautionary measures were chemical repellents and stabling of horses during dusk and dawn. Movement of horses was implicated as a major factor in AHS distribution. Areas with higher horse movement correlated with higher AHS incidence (Fig. 5.1). Until better movement control and reporting policies are set in place during outbreaks for South Africa and Namibia, AHS will not be successfully contained or managed.

v) Integration of multidisciplinary data to determine the most prominent drivers influencing the distribution and incidence of AHS across Namibia (Chapter 3)

Multivariate analyses of data from the two year survey (2013 & 2014) indicate the environmental parameters in order of importance for the distribution of AHS as (Fig. 3.12): precipitation > temperature > clay > relative humidity > NDVI. Soil particle size distribution also played a role, with a moderate significant relationship between clay/organic carbon and total *Culicoides* collected. At the Windhoek site (Fig. 3.10), NDVI had a clear positive relationship with the occurrence of *Culicoides*. The importance of NDVI was also significant in the RDA (Fig. 3.12) when assessing the combination of all data. The relationship between temperature and humidity had an important influence on adult midge activity. Temperature and humidity ranges in areas with the highest *Culicoides* activity were determined at temperatures of 18-23°C combined with humidity of 40-70%. During the social survey, movement of horses was implicated as a driver of the disease. Integration of the results with the long-term modelled climatic data ranked the importance of the factors in the incidence and distribution of AHS as: precipitation > movement

status > temperature and humidity combination > NDVI > soil type. These results were combined to form part of the qualitative risk assessment discussed in Chapter 6.

vi) Development of an AHS risk assessment tool (Chapter 6)

A risk analysis was performed to characterise the risk of the occurrence of AHS outbreaks in Namibia. The immature stages of *Culicoides* development were estimated to contribute the most to the category of high risk in an area. However, research on the effective control of the immature stage - *Culicoides* larvae as well as their environment - is still needed. A qualitative risk matrix was developed with the integration of the results from the previous chapters to estimate the risk of the seasonal likelihood of the occurrence of AHS outbreaks within Namibia. This is the first matrix to incorporate anthropogenic activities and soil characteristics as stressors to determine the risk of the occurrence of AHS outbreaks in Namibia. The risk matrix ignores uncertainty, which can result in incorrect scores calculated for an area. With the application of the risk matrix (Fig. 6.7), the occurrence of AHS outbreak risk in a district can be estimated as low, intermediate or high and mitigating actions are suggested to be taken accordingly. The risk matrix was applied to identify potential areas that could serve as an AHS free export stations for horses from Namibia. Luderitz was found to be such an area with the most favourable conditions for export of equines. With the relevant data and a few adjustments to the risk matrix, it could be applied in other countries where it is necessary to determine the intra-country spread of AHS.

The results obtained from this study show that a multidisciplinary approach is imperative for the management of AHS. Significant contributions made by this investigation include the identification of parameters critical for AHS distribution and the development of a risk matrix tool to estimate the risk of the occurrence of AHS outbreaks in Namibia. Although it is clear that limitations in the current risk matrix still exist, this investigation is of great importance for the development of an applicable tool for AHS management. There is also a better understanding of *Culicoides* spp. composition and abundance in areas where no surveillance has been done before. This is also the first study of AHS that included an anthropogenic component as a stressor that affect inter-country distribution in an AHS endemic area. The final future goal would be to establish a reliable and applicable risk tool, as well as a user-friendly AHS reporting interface for Namibia. Continuous surveillance of the distribution of AHS is an essential management aspect that must be taken into consideration, especially if Namibia wants to establish an AHS-free zone for equine export. Ultimately, determining the distribution of AHS is a complex process that should involve a variety of scientific disciplines and a combination of techniques and/or approaches to develop a comprehensive and applicable risk assessment tool. In this regard, the current investigation serves to elucidate several competencies and weaknesses of the existing approach.

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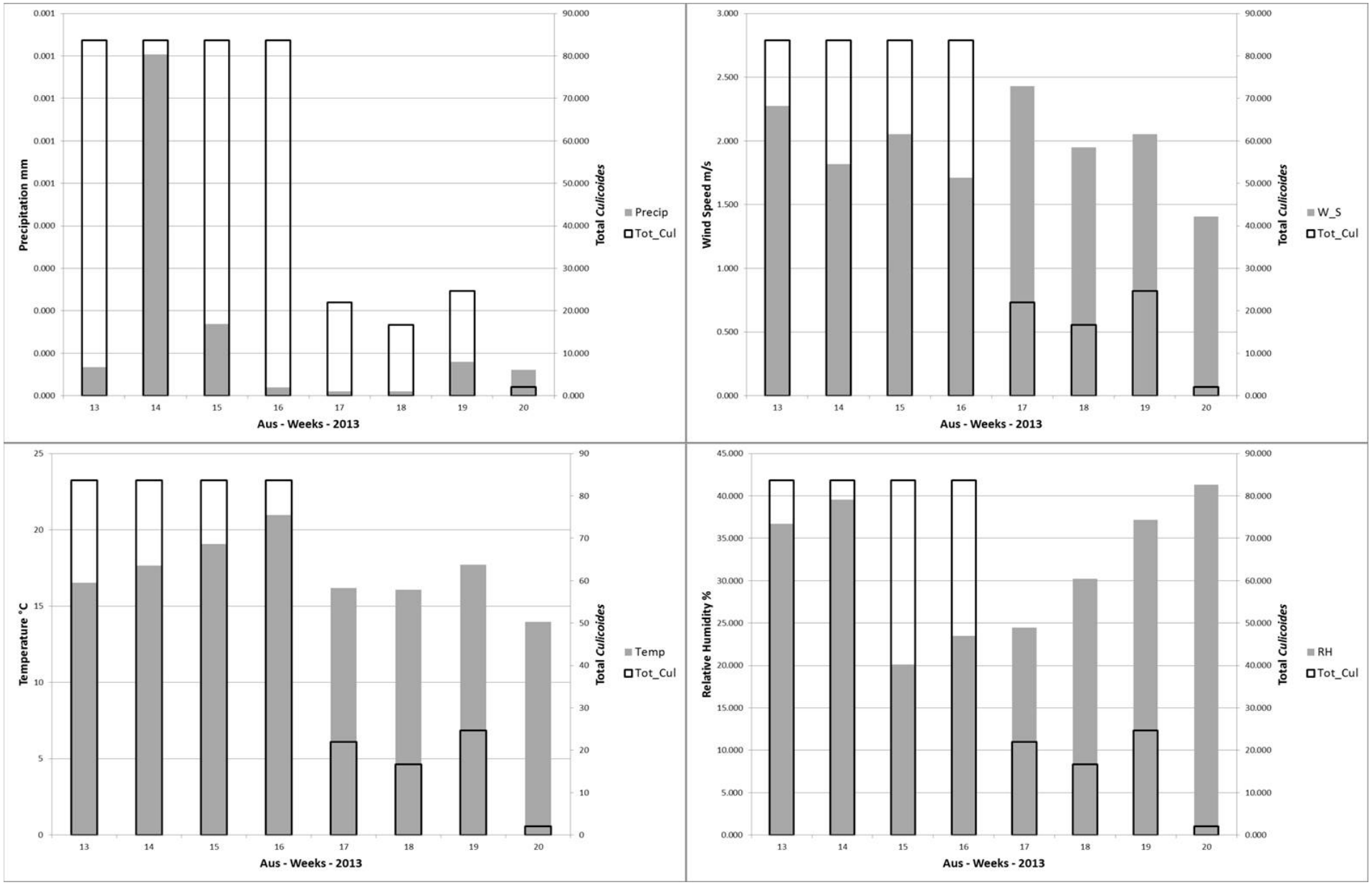
APPENDIX A: RESULTS PER SITE

APPENDIX A.1. AUS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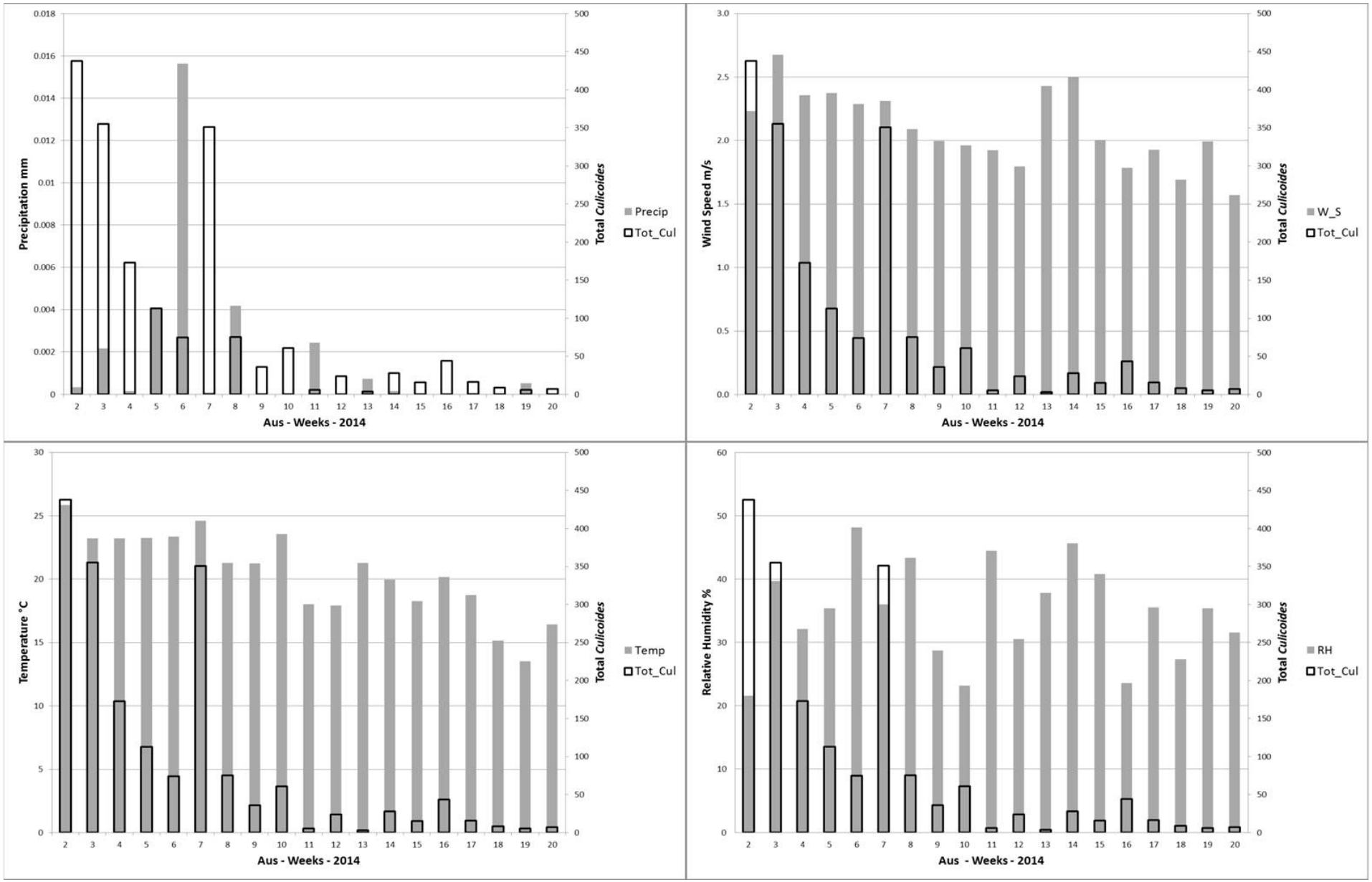
Precip
Tot_Cul
Aus - Weeks - 2013
Wind Speed m/s
W_S
Temperature °C
Temp
Relative Humidity %
RH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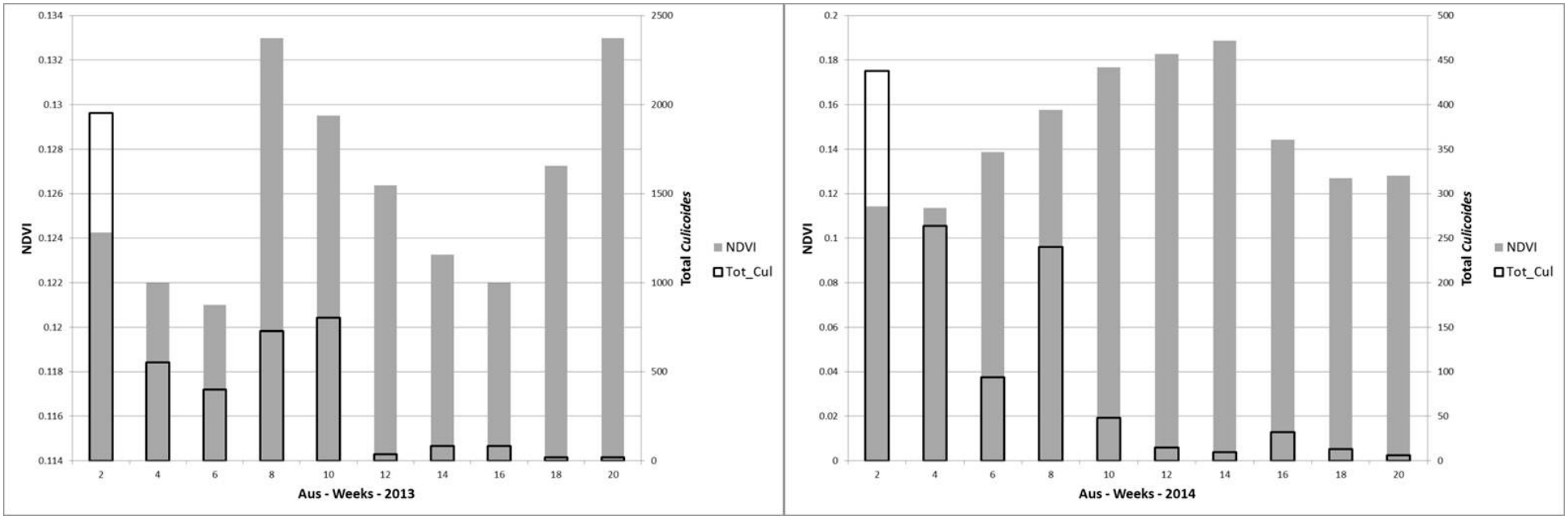
Precip
Tot_Cul
Aus - Weeks - 2014
Wind Speed m/s
W_S
Temperature °C
Temp
Relative Humidity %
RH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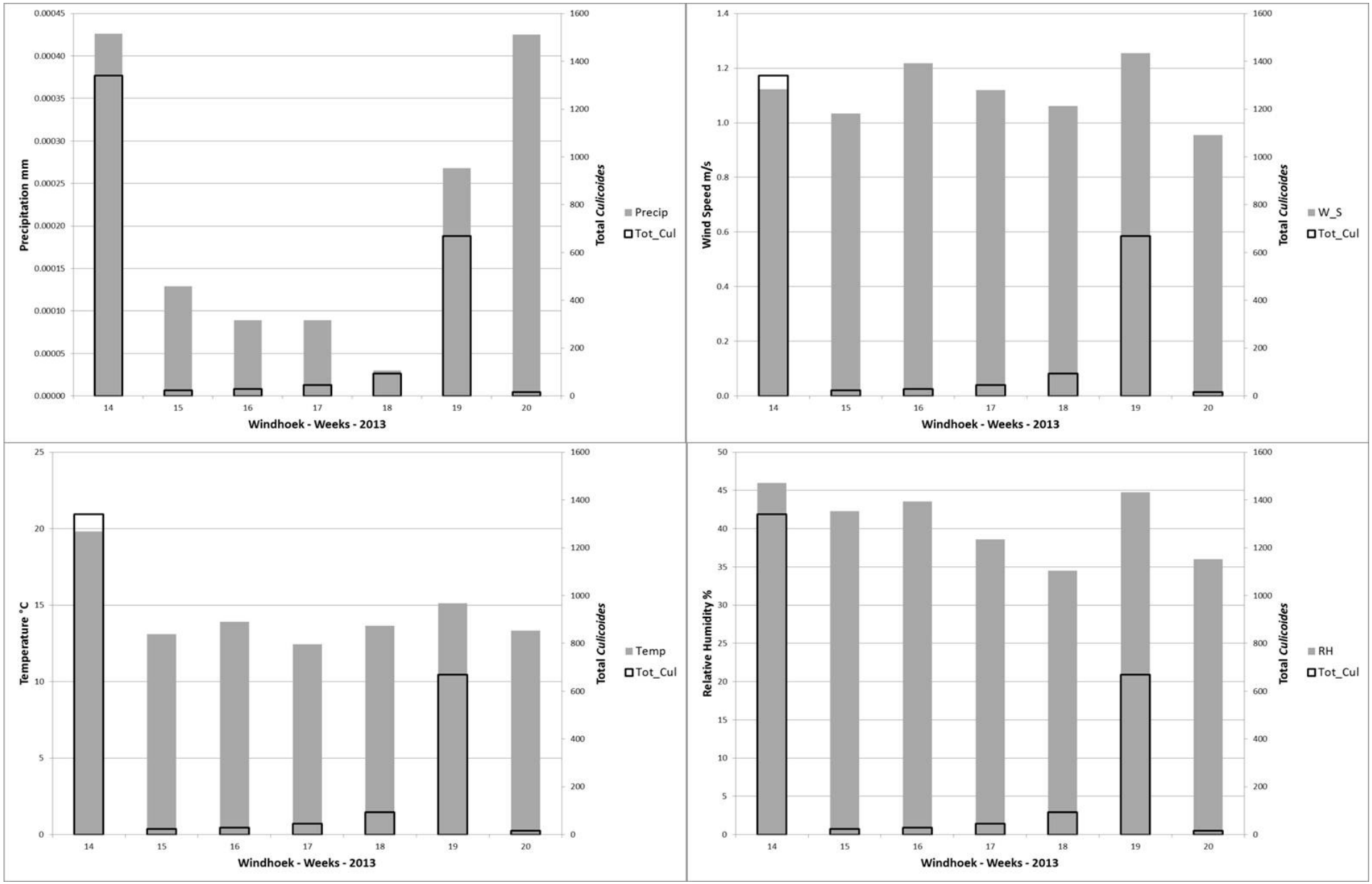
Aus - Weeks - 2014

APPENDIX A.2. WINDHOEK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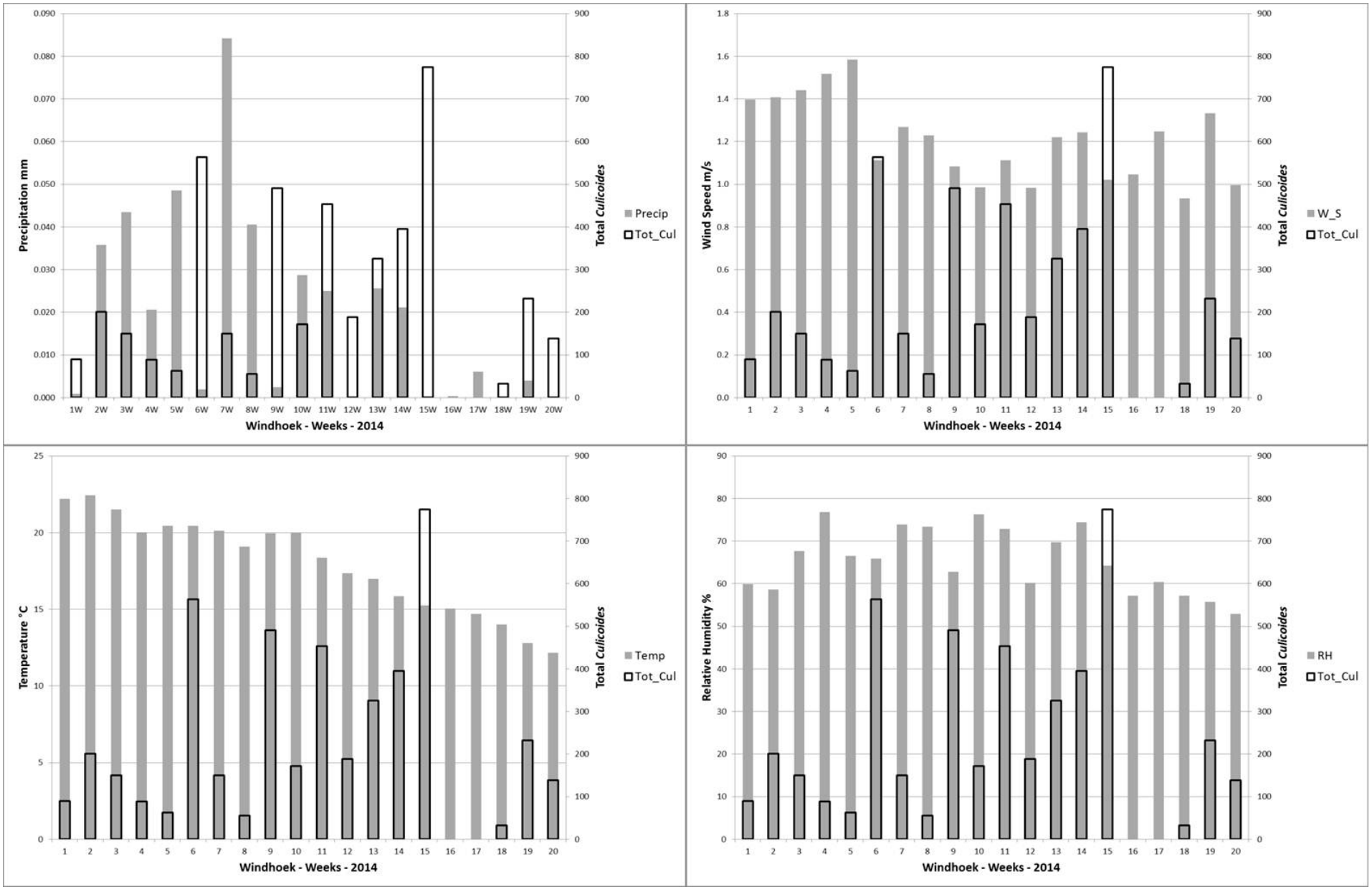
Precip
Tot_Cul
Windhoek - Weeks - 2013
Wind Speed m/s
W_S
Temperature °C
Temp
Relative Humidity %
RH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Precip
Tot_Cul
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Windhoek - Weeks - 2014
Wind Speed m/s
Total Culicoides
W_S
Tot_Cul
Windhoek - Weeks - 2014
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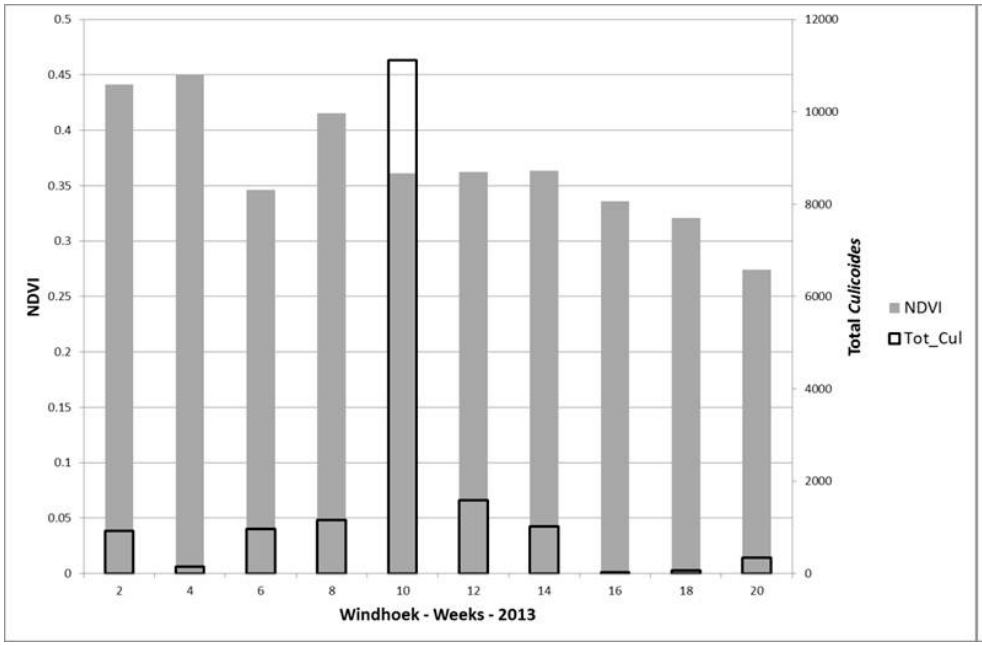
Windhoek - Weeks - 2014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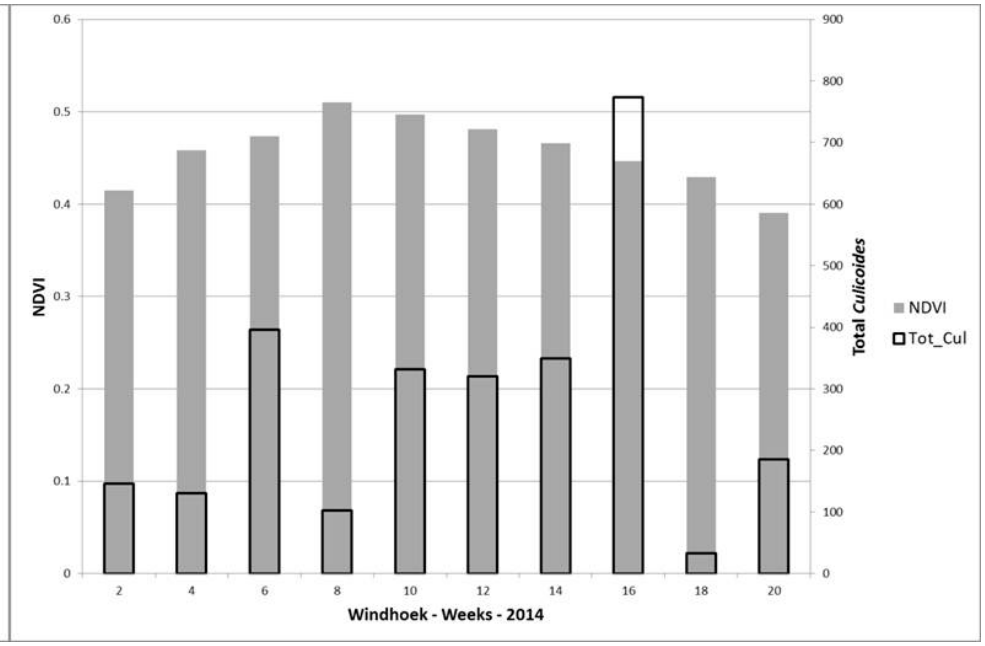
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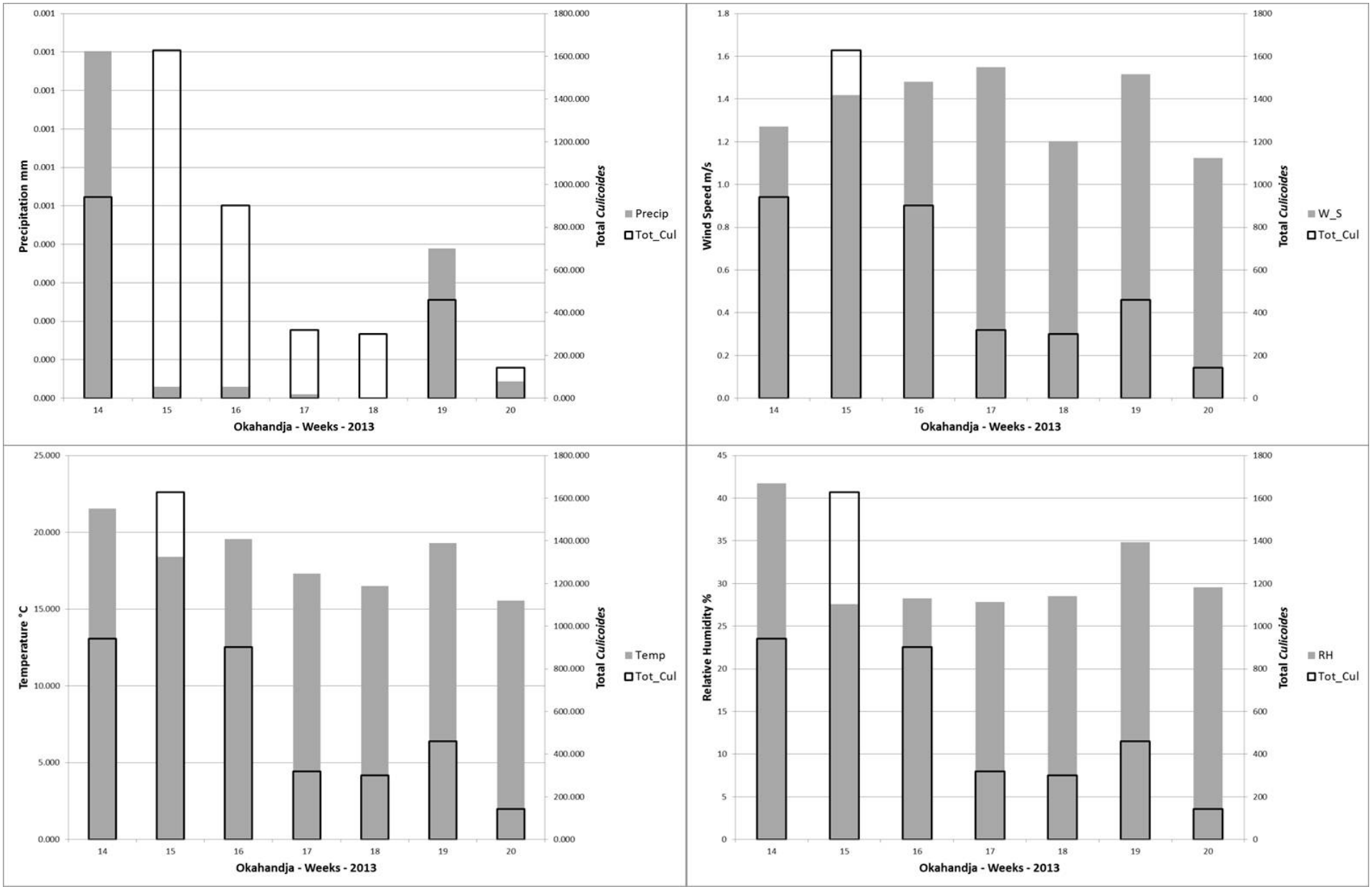
NDVI
Tot_Cul
Windhoek - Weeks - 2014

APPENDIX A.3. OKAHANDJA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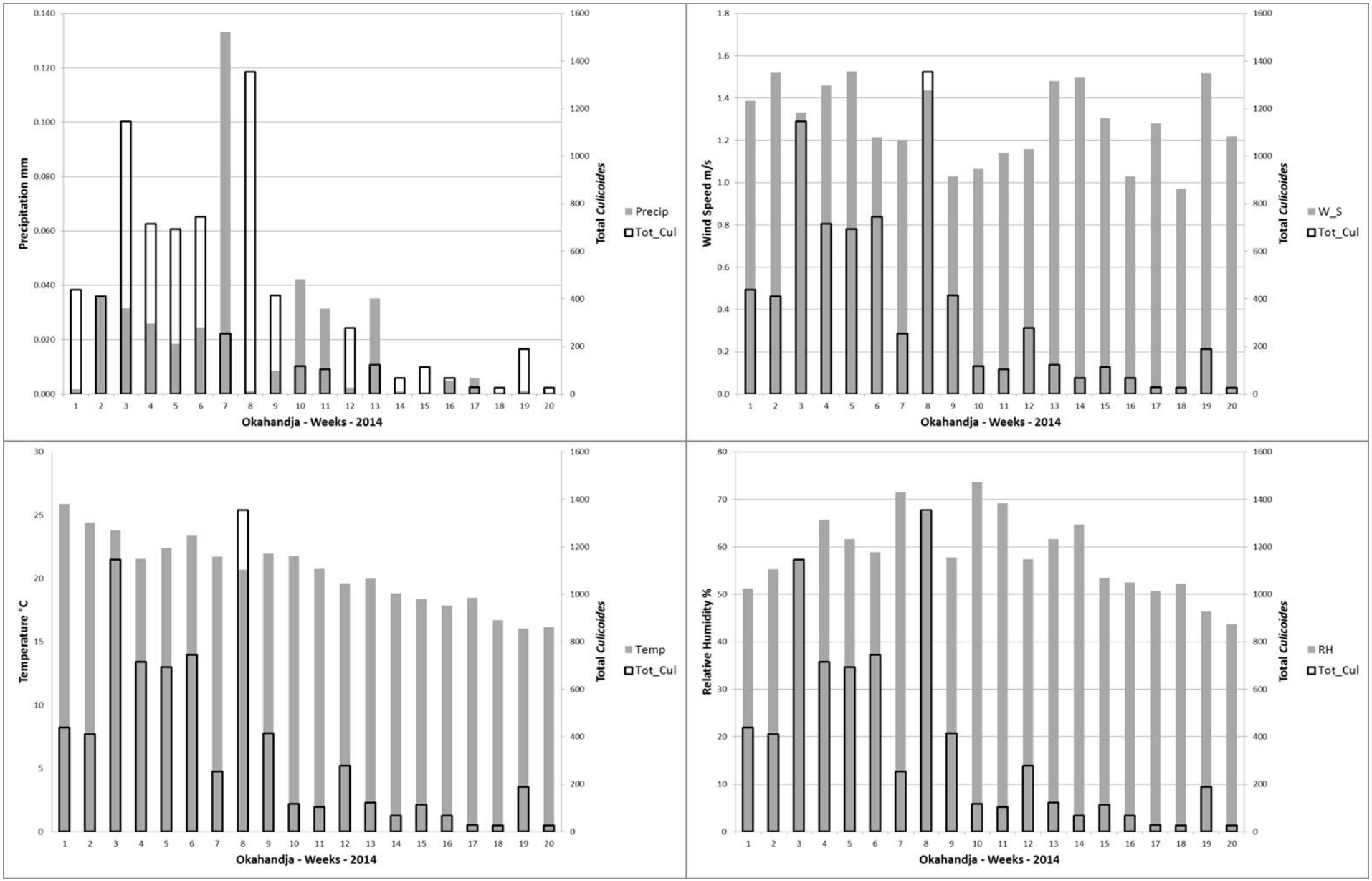
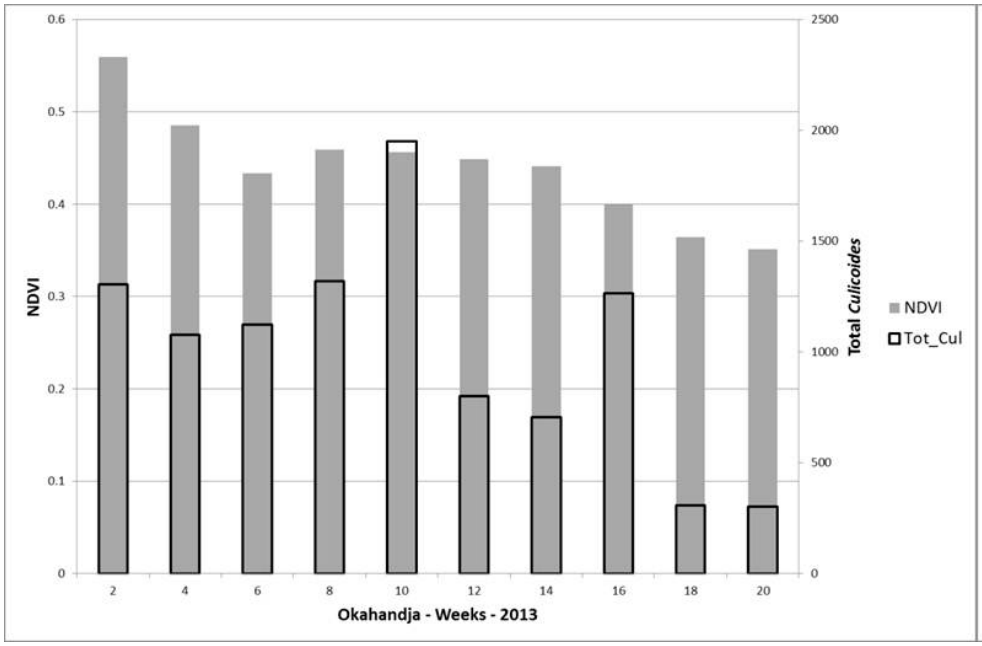
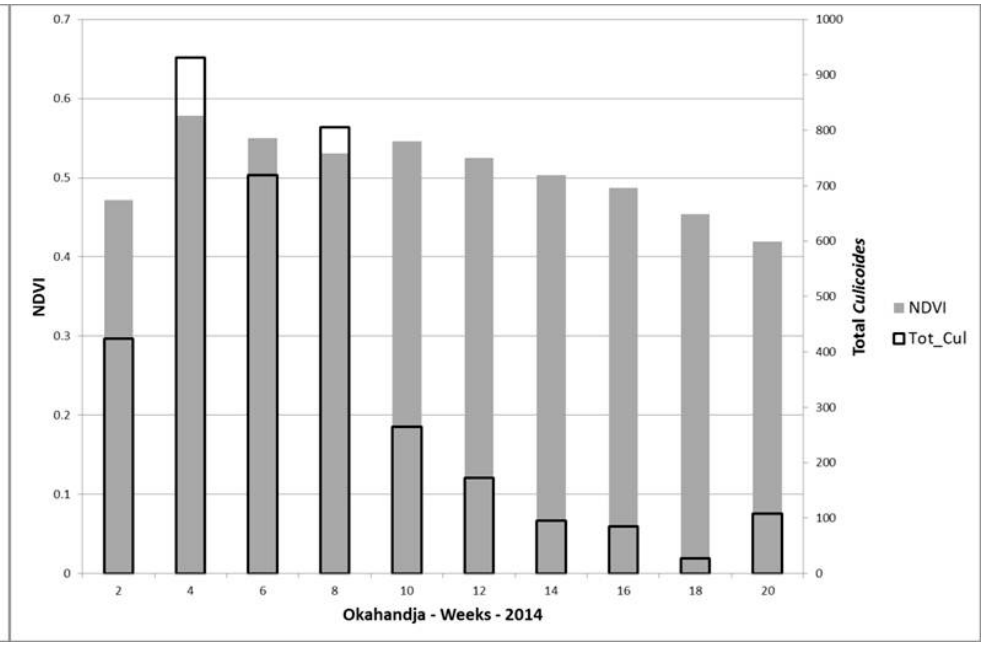
Temp
Relative Humidity %
RH


Precipitation mm
Total Culicoides
Precip
Tot_Cul
Okahandja - Weeks - 2014
Wind Speed m/s
W_S
Temperature °C
Temp
Relative Humidity %
RH


NDVI
Total Culicoides
NDVI
Tot_Cul
Okahandja - Weeks - 2013


NDVI
Total Culicoides
NDVI
Tot_Cul
Okahandja - Weeks - 2014

APPENDIX B: SOCIAL SURVEY QUESTIONNAIRE

Questionnaire for horse owners, stud farms and riders in South Africa and Namibia

Section 1: Demographic information

a) Age

b) Gender:

1. Male
2. Female

c) Population group:

1. Asian
2. Black
3. Coloured
4. White
5. Other (please specify)

d) Home Language:

1. Afrikaans
2. English
3. Other (please specify)

e) Country:

1. South African
2. Namibian

f) GPS location:

f) Province:

1. Mpumulanga		10. Grootfontein	
2. North-West		11. Gobabis	
3. KwaZulu-Natal		12. Keetmanshoop	
4. Gauteng		13. Katima Mulilo	
5. Eastern Cape		14. Mariental	
6. Western Cape		15. Ondangwa	
7. Northern Cape		16. Okahandja	
8. Freestate		17. Omaruru	
9. Limpopo		18. Opuwa	
		19. Otjiwarongo	
		20. Outjo	
		21. Otavi	
		22. Rundu	
		23. Windhoek	
		24. Walvisbay	

g) Level of education:

1. None	
2. Primary	
3. Some secondary	
4. Completed secondary	
5. Tertiary	
6. Other (please specify)	

h) Occupation

1. Full time horse related job	

2. Other occupation (please specify how many hours per day is allocated towards horses)

Section 2: Human/ Equine interaction

a) For what purpose do you keep equines?

1. Aesthetical value	
2. Rider: Professional	
3. Rider: Social	
4. Work	
5. Stud farm	
6. Other (please specify)	

b) What type, quantity and breed do you have?

Type	**Quantity**	**Breed (eg. Arab, Friesian)**
1. Horse		
2. Donkey		
3. Mule		
4. Zebra		

c) What type of value do you attach to your equines?

1. Emotional	
2. Economical	

d) What monetary value do you personally ascribe per equine?

Type	**Monetary value R/Nam$**
1. Horse	
2. Donkey	
3. Mule	
4. Zebra	

e) How often do you travel with your equines?

1. Weekly	
2. Monthly	
3. Once every 3 months	
4. Once every 6 months	
5. Once a year	
6. Other (please specify)	

f) During what time of year do you travel most often with your equines and to where do you travel?

Month:	**Destination (eg. Gauteng, Kyalami)**
1. January	
2. February	
3. March	
4. April	
5. May	
6. June	
7. July	
8. August	
9. September	
10. October	
11. November	
12. December	
13. Other (please specify)	

Section 3: African horse sickness (AHS)

a) Which precautionary measures do you take against AHS?

1. Stabling during dusk and dawn	
2. Fans in stables	
3. Chemical repellent	
4. Natural repellent	
5. Immunity booster	
6. Fly sheets	
7. Other (please specify)	

b) Do you vaccinate your horses with the OBP vaccine?

1. Yes	
2. No	
3. Other (please specify)	

c) Do you vaccinate (OBP vaccine) any of your other equines against AHS?

Type	**Vaccinate Yes/No**
1. Donkey	
2. Mule	
3. Zebra	

d) Which horses do you vaccinate and during what time of the year?

Horses	**Month**
1. All the horses	
2. Only broodmares	
3. All stud animals	
4. Only horses going to shows/that travel	
5. Other (please specify)	

e) Have you ever experienced AHS?

1. Yes	
2. No	

f) Where, when and how many of which equines were affected with AHS. Were the animals vaccinated and when was the last date of vaccination? (please specify for the last 5 years or as far back as related)

Month & Year	**District & Province**	**Quantity**	**Horse/Mule/Donkey/ Zebra**	**Vaccinated Yes/No**	**Date of the last vaccination**

**If there is not enough space feel free to add an attachment with the specifications.

g) Please explain what happened and how you experienced it?

h) How did you handle the situation?

1. Didn't take any action
2. Self medicate (eg. Boereraad)
3. Alternative treatment
4. Called the vet
   - a) Private vet
   - b) State vet
5. Other (please specify)

i) What is the distance to your nearest vet?

1. Less than 5km
2. 5-10km
3. 10-25km
4. 25-50km
5. 50- 100km
6. More than 100km
7. Other (please specify)

i) What was the nature of the diagnosis?

1. Suspicion (horse showing symptoms, not confirmed by vet)
2. Clinical (AHS symptoms – confirmed by vet)
3. Post Mortem (confirmed by vet)
4. Laboratory (blood and tissue samples were sent for analysis)
5. Other (please specify)

If laboratory diagnostics were used, please answer the following questions:

i) Who took the samples for the diagnostic test?

1. Owner	
2. Private vet	
3. State vet	
4. Other (please specify)	

ii) Do you know which diagnostic test was used

1. Yes (please specify)	
2. No	

iii) Who paid for the diagnostic tests?

1. AHS trust	
2. Owner	
3. State	
4. Other (please specify)	

Section 4: Notification of African horse sickness

a) Are you aware that AHS is a notifiable disease and that it must be reported to the authorities according to the law?

1. Yes	
2. No	

b) Do you think it is necessary to report AHS and why?

c) Whose responsibility is it to report an AHS outbreak?

1. Nobody	
2. Horse owner	
3. Private vet	
4. State vet	
5. Other (please specify)	

d) Where do you report an AHS case?

1. I don't know	
2. Private vet	
3. State vet	
4. AHS trust	
5. Department of animal health	
6. Other (please specify)	

e) When do you report an AHS outbreak?

1. Don’t report
2. When there is one suspected case in the area
3. Clinical signs appear among several horses in the area
4. Mortality of several horses in the area
5. Only when diagnostic test indicate AHS
6. Other (please specify)

f) Briefly describe the reporting process in your own words.

g) Are there any other aspects of AHS that you feel the researcher should take into account that we did not mention?
