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Seroprevalence of African horse sickness virus and equine encephalosis virus in equids and abundance of *Culicoides* midges in Namaqualand, South Africa

BY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF VETERINARY TROPICAL DISEASES, UNIVERSITY OF PRETORIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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Declaration

I, *Christine Schütte*, do hereby declare that during the course of this study Dr. Alison Lubisi and her staff at the serology section of the ARC - Onderstepoort Veterinary Institute, did the serology on collected sera. Me. K. Labuschagne at the Entomology section of the ARC - Onderstepoort Veterinary Institute, did the midge identification. Except where acknowledgements indicate otherwise and for the advice from my supervisors, this dissertation represents my own original work. Neither the full dissertation nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree of Magister Scientiae (Veterinary Tropical Diseases) in the Department of Veterinary Tropical Diseases, University of Pretoria.

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Date: 30 November 2012

Abstract

Seroprevalence of African horse sickness virus and equine encephalosis virus in equids and abundance of *Culicoides* midges in Namaqualand, South Africa

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The purpose of this work was to determine the seroprevalence of African horse sickness virus (AHSV) and equine encephalosis virus (EEV) in Namaqualand, South Africa, as well as the abundance of potential vectors *i.e. Culicoides* midges in the area.

Namaqualand is located in the arid north-west of South Africa. The area represent a unique biogeographical region. There are very phew studies on the seroprevalence of AHSV and EEV in the area. These diseases are unknown in the area and horse owners normally do not vaccinate their animals against these diseases. Information regarding the occurrence of *Culicoides* midges in this area are relative scares.

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Blood samples collected from equids throughout the region were analysed to establish the seroprevalence of the two viruses. ELISA results indicate that 4.4% and 30.9% of 874 equines assayed in Namaqualand have antibodies against AHSV and EEV respectively.

Midges were collected weekly over one year with light traps at three sites in the area. The six most common *Culicoides* species to be collected were: *Culicoides ravus* (29.7%), *Culicoides bedfordi* (25.2%), Culicoides #89 (9.6%), *Culicoides subschultzei* (7.4%), *Culicoides herero* (7.1%) and *Culicoides nivosus* (6.8%). *Culicoides imicola* represented 0.9% and *Culicoides bolitinos* represented 1.5% of the total catches.

Namaqualand is a relativeley low risk area for AHS and EEV. The known vectors for these two diseases are present in low numbers.

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List of Abbreviations

AHS	African horse sickness
AHSV	African horse sickness virus
ARC-OVI	Agricultural Research Council - Onderstepoort Veterinary Institute
BT	Bluetongue
BTV	Bluetongue virus
EE	Equine encephalosis
EEV	Equine encephalosis virus
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
GIS	Geographic Information System
H_2SO_4	Sulphuric acid
MAP	Mean annual precipitation
m.a.s.l	Meter above sea level
NaCl	Sodium Chloride
NDVI	Normalized difference vegetation index

OIE *Office International de Epizootics* World Organisation for Animal Health

rVP7 Recombinant viral protein 7

TST Tris-Saline-Tween

RNA Ribonucleic Acid

Chapter 1

GENERAL INTRODUCTION

Background

African horse sickness (AHS) is a non-contagious viral disease of equids. The causative agent African horse sickness virus (AHSV) is double stranded RNA virus, within the genus *Orbivirus* of the family *Reoviridae*, that is biological transmitted by certain species of *Culicoides* midges (Diptera: Ceratopogonidae) (Coetzer & Erasmus 1994a). The disease is enzootic in sub-Saharan Africa, with occasional spread further north, extending to the extreme south-west of Europe and the Middle East (Rodríguez, Hooghuis & Castaño 1992; Australian Government 2006). AHS can have a disastrous effect as shown with the epizootic that started in 1959 in the Middle East and South West Asia when more than 300 000 animals died (Howell 1960; Rodríguez *et al.* 1992; Mellor 1993; Australian Government 2006).

AHS occurs every summer in the northern parts of South Africa and then spread southwards. The extent of this southward spread is influenced by the abundance of *Culicoides* midges and therefore by the extent of favourable climatic conditions for the breeding and survival of *Culicoides* midges. Conditions are favourable for epidemics when there are early and heavy rains followed by warm, dry spells (Coetzer & Erasmus 1994a).

Equine encephalosis (EE) is also a non-contagious viral disease of equids caused by a double stranded RNA virus, within the genus *Orbivirus*. The vectors of this virus are also certain *Culicoides* midges (Howell, Guthrie & Coetzer 2004). There is no vaccine available against EE, so the prevalence of natural infection will correlate to seroconversion (Howell *et al*. 2004). EE is endemic in most parts of South Africa with sporadic localised outbreaks (Howell, Groenewald, Visage, Bosman, Coetzer & Guthrie 2002).

Namaqualand is situated in the arid north-west of South Africa. It forms a distinct biogeographical area within the larger Succulent Karoo biome (Desmet 2007). The low average rainfall (106 mm per year) (SA Explorers 2011), renders the area as not ideal for the occurrence of *Culicoides* midges. AHS and EE are mostly unknown under the local population and very few horse owners vaccinate their animals against AHS. From January 1993 to February 2011, no outbreaks of AHS were reported in this area (Department of Agriculture, Forestry and Fisheries 2011).

This study aims to determine the seroprevalence of AHSV, EEV and the abundance of *Culicoides* midges in Namaqualand. Findings of this study can be used to identify an area with low risk of AHSV and its vectors.

Objectives of the study

1. To determine the seroprevalence of AHSV in equids in Namaqualand.
2. To determine the seroprevalence of EEV in equids in Namaqualand.
3. To determine the abundance of the known and potential vectors of AHSV in Namaqualand.

Chapter 2

REVIEW OF THE LITERATURE

African Horse Sickness

African horse sickness is caused by the African horse sickness virus, which is classified in the genus *Orbivirus* in the family Reoviridae (Calisher & Mertens 1998). The virus is transmitted biologically by certain species of midges in the genus *Culicoides* (Mellor & Hamblin 2004). Nine serotypes have been identified (Howell 1962). The virus is highly pathogenic for horses, with a mortality of 90 to 95% for serotypes 1 to 8 (Coetzer & Erasmus 1994a). The mortality for serotype 9 is slightly less at 70% (Coetzer & Erasmus 1994a). The virus is less pathogenic for mules (mortality 50 – 70%) and donkeys and zebras are very resistant (Coetzer & Erasmus 1994a).

The World Organisation for Animal Health (OIE) lists AHS as a notifiable disease, as this is a transmissible disease that has the potential for very serious and rapid spread (World Organisation for Animal Health). The emergence of bluetongue virus (BTV) (also an *Orbivirus* and an OIE listed disease) in Europe (MacLachlan & Guthrie 2010), as well as the resent emergence of Schmallenberg virus (*Orthobunyavirus*) demonstrates the disastrous effect these viruses can have when

they emerge in new areas. The efforts to regulate and control these vector-borne viruses will increase in the future.

AHS has a major impact on the equine industry in South Africa. To facilitate trade with the European Union (EU), South Africa declared AHS a controlled animal disease on 6 February 1997. This regulation declared a part of Western Cape Province as a controlled area for AHS. The controlled area is divided into three zones, the first zone is the AHS free zone, which is approximately 140 km² and comprises part of the Metropolitan Cape Town (Figure 1). The second is the Surveillance zone (Figure 1) with a minimum radius of 50 km that surrounds the Free zone and the third is the Protection zone (Figure 1) that has a minimum radius of 100 km (Government Gazette of South Africa 6 February 1997; Guthrie 1999).

Horses have to spend 20 days in the free zone followed by 40 days in quarantine in an insect free stable before they can be exported to European Union (EU) countries (European Union). Since the declaration of this regulation in 1997, there were four recorded outbreaks in the AHS Controlled Area. These outbreaks occurred in 1999, 2004, 2006 and 2011 (Racing South Africa 2011). Each of these outbreaks resulted in a temporary ban on exports of horses (Sinclair, Bührmann & Gummow 2006).

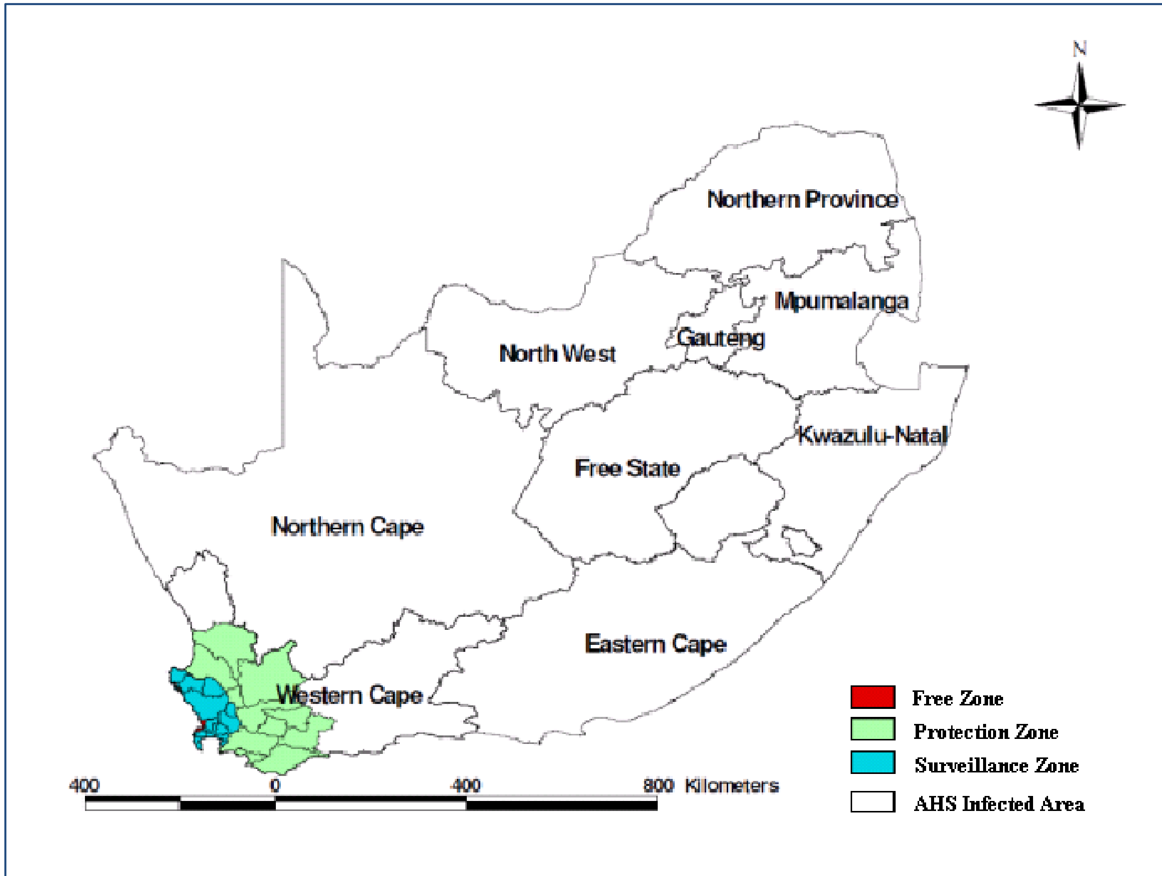

Figure 1 The designated control areas for AHS in the south Western Cape Province of South Africa (Racing South Africa 2011).

The disease also has a financial impact on the local industry. The 2004 embargo alone resulted in an estimated R250 million loss in revenue for South Africa (Racing South Africa 2011). All equines (except in the AHS free zone and Surveillance zone) are required by law to be vaccinated. This is done at the ages of six to nine months and must be repeated between the ages of 12 and 15 months, and once every year thereafter (Government Gazette of South Africa 6 February 1997). The control of

horse movement within these areas is difficult due to high numbers of horses participating in equestrian events. The numbers of donkeys and horses owned by the informal communities and small scale farmers also make it difficult to enforce horse movement control. The increase in game farming with the resulting increase in game translocation, including zebra, can also interfere with the control of AHS. Apart from the vaccination and certification costs, the disease can also lead to mortalities of valuable horses. AHS also impacts on the informal sector were horses are used for transport and as draught animals. There is also an emotional impact with the loss of a companion animal.

In South Africa AHS is more prevalent in the warm coastal regions and in low-lying, moist inland regions of the summer rainfall area. The first outbreaks usually occur at the beginning of February with most cases in March and April. Outbreaks of the disease usually disappear with the first frost at the end of May (Coetzer & Erasmus 1994a).

By law, all cases of AHS must be reported to the State Veterinary Services. There are 1 443 outbreaks reported on the website of the Department of Agriculture, Forestry and Fisheries for the period 1997 to 2010 (Table 1) (Department of Agriculture, Forestry and Fisheries 2011).

Table 1 A summary of the duration, number of outbreaks and number of cases of AHS between 1997 and 2010 in South Africa (Department of Agriculture, Forestry and Fisheries 2011)

Year	Number of outbreaks	First Case	Last Case	Total cases
1997/1998	32	October 1997	July 1998	146
1998/1999	88	September 1998	August 1999	462
1999/2000	126	November 1999	August 2000	708
2000/2001	79	January 2001	July 2001	2 224
2001/2002	103	September 2001	August 2002	600
2002/2003	69	January 2003	June 2003	1 018
2003/2004	131	October 2003	June 2004	672
2004/2005	106	January 2005	July 2005	1 072
2005/2006	176	September 2005	June 2006	1 118
2006/2007	44	September 2006	July 2007	164
2007/2008	211	December 2007	July 2008	1 798
2008/2009	186	October 2008	June 2009	874
2009/2010	92	August 2009	August 2010	161

The first outbreaks are usually recorded in September to November (Table 1). The last cases are usually reported in June to August (Table 1). The largest number of cases (2 224) were reported in 2000/2001 (Table 1). No outbreaks of AHS were reported in the Namaqualand region.

An extensive survey conducted to determine the prevalence of antibodies against AHSV and EEV in donkeys in South Africa during 1983-1985 and 1993-1995 indicated that the seroprevalence of AHSV in the Namaqualand magisterial district varied between 15% and 50% (Venter, Paweska, Williams & Nevill 1999b). During these surveys, an attempt was made to collect serum samples from at least 30 donkeys from each magisterial district of South Africa. This study reported the seroprevalence of AHSV in 4 891 donkey sera as 30.1% (Venter *et al.* 1999b).

Zebra can be viraemic for as long as six weeks before they develop AHSV specific antibodies (Barnard 1998). The minimum herd size to maintain a permanent focus of infection is unknown (Barnard 1998). Donkeys can be viraemic, and act as a potential source of virus for the insect vectors, for at least 12 days (Hamblin, Salt, Mellor, Graham, Smith & Wohlsein 1998). Donkeys and zebras are both considered as cyclic hosts of AHSV, as they develop antibodies against the virus. A reservoir host act as a long-term source of virus, and none have been identified as of yet for AHSV (Mellor 1994).

Overwintering occurs when there are interruptions in normal transmission through adverse climatic conditions. These conditions are unsuitable for the emergence or activity of *Culicoides* adults or temperatures may be too low for virus replications. AHSV is able to persist these periods without the observation of new cases (Wilson, Mellor, Szmaragd & Mertens 2009).

There is no evidence of vertical transmission, and studies suggest that the virus is unable to infect the ovaries or penetrate the membrane surrounding the eggs (Wilson *et al.* 2009). Bluetongue virus nucleic acid have been detected in larval pools of field-collected *Culicoides* midges. (White, Wilson, Blair & Beaty 2005). This supports the hypothesis that vertical transmission might be possible, but further studies are needed to determine the likelihood, validity and relevance of this finding (White *et al*. 2005). Nunamaker *et al* (1990) has shown that, although BTV antigens were present in the oocytes, the progeny did not contain infectious BTV.

In the absence of a long-term reservoir and transovarial transmission, the survival of AHSV is dependent on the continuous and uninterrupted cycle of transmission. The transmission cycle will only be interrupted if the vector-free periods are longer than the maximum period of viraemia in the local susceptible vertebrate population, as the last infected vertebrate host will have died or recovered before new vectors arrive (Mellor 1994).

EQUINE ENCEPHALOSIS

Equine encephalosis virus (EEV) has similar epidemiological features to AHSV with respect to its natural hosts, vectors and environmental conditions under which transmission occurs (Howell *et al*. 2004). Equine Encephalosis (EE) occurs during late summer and autumn (Coetzer & Erasmus 1994b). EE was first described in 1967 (Erasmus, Adelaar, Smit, Lecatsas & Toms 1970). Serological studies showed that there were widespread infections of horses during the late summer of 1967 and that EEV had not occurred during the preceding 10 years (Erasmus *et al.* 1970; Viljoen & Huismans 1989).

There are seven serotypes of EEV and they are transmitted by certain species of *Culicoides* midges (Venter, Groenewald, Paweska, Venter & Howell 1999a). It was shown that *C. imicola* and *C. bolitinos* can become infected and permit replication of EEV and that both these midges might transmit the virus to susceptible hosts (Venter *et al*. 1999a; Venter, Groenewald, Venter, Hermanides & Howell 2002).

The clinical signs associated with EEV in horses are less severe than for AHSV with 90% of horses presenting no or only mild clinical signs (Coetzer & Erasmus 1994b). The mortality is less than 5% (Coetzer & Erasmus 1994b). Equine encephalosis is endemic in most of South Africa with sporadic localised outbreaks (Howell *et al*. 2002). In 2008/2009, there was an outbreak of EE spanning more than 60 equine premises reported in Israel (Oura, Batten, Ivens, Balcha, Alhassan, Gizaw, Elharrak,

Jallow, Sahle, Maan, Mertens & Maan 2012). EEV is also present in Ethiopia, Ghana and The Gambia, but not in Morocco, north of the Sahara desert (Oura *et al*. 2012). Howell *et al* (2008) showed a variable annual seroprevalence of EEV, ranging from 34.7% (2002) to 3.6% (2004), in Thoroughbred yearlings in South Africa.

Paweska *et al* (2004) found a seroprevalence of 77% to EEV in South Africa, with a seroprevalence of 83.9% in the Northern Cape Province. This study was done on horse sera collected between 1999 and 2001 (Paweska & Venter 2004). An extensive survey conducted during 1983-1985 and 1993-1995 where donkey sera were tested for AHSV and EEV, the prevalence of EEV from the total number of tested sera (4 875) was reported to be 49.3%. In the Namaqualand area, the prevalence of EEV was 0% (Venter *et al*. 1999b). Antibodies against EEV have also been shown in zebra (Barnard & Paweska 1993).

The transmission patterns of AHSV and EEV can be derived from the prevalence patterns in the different age classes of the vertebrate hosts. In an endemic area, there is a steady increase in the prevalence with age. Where there are periodic epidemics, there are sharp increases in the prevalence at ages that corresponds to each epidemic. In areas where there is infrequent introductions of the virus or where there is insufficient vectors or hosts to maintain an epidemic, there is no apparent pattern of the prevalence in the different age classes (Lord, Venter, Mellor, Paweska & Woolhouse 2002).

CULICOIDES MIDGES

AHSV and EEV are arboviruses. The geographical distribution and seasonal incidence of AHSV and EEV are determined by the availability of the virus, susceptible vertebrate hosts and the presence of competent arthropod vectors. Both these viruses are transmitted almost exclusively by certain species of *Culicoides* biting midges (Mellor, Boorman & Baylis 2000).

Culicoides species are small biting flies. There are more than 1 400 species, of which 96% are obligated blood feeders. The primary method for species identification is by their respective wing patterns (Meiswinkel, Venter & Nevill 2004).

Susceptible *Culicoides* females become infected with AHSV while feeding on viremic vertebrate hosts and therefore the virus must be present in the peripheral blood vessels or in the skin tissues of the host (Wilson *et al.* 2009). The development of the virus from ingestion by the midge to the time that it is able to transmit to another vertebrate host is known as extrinsic incubation. During this time, the virus penetrates and replicates in the cells of the gut wall. From there the virus spreads through the haemocoel to infect the salivary glands. The virus can then be transmitted from the arthropod to the vertebrate host during subsequent blood feeding.

The extrinsic incubation period is dependent on the ambient temperature experienced by the vector (Wilson *et al.* 2009). Virogenesis slows down as the lower temperature affects the activity of viral RNA polymerase and effect the ability of the vector to modulate viral replication in cells (Welby, Baylis, Rawlings & Mellor 1996; Paweska, Venter & Mellor 2002). The lower threshold for AHSV replication within a midge is between 11.4 ºC and 13.3 ºC (Carpenter, Wilson, Barbar, Veronesi, Mellor, Venter & Gubbins 2011). A higher environmental temperature also influences virogenesis within insects because it disrupts gut barriers to replication and dissemination within the insect. Therefore, insects reared at a higher environmental temperature may have a higher level of competence as vectors (Wittmann, Mellor & Baylis 2002; Wilson *et al.* 2009, Carpenter *et al.* 2011). This “leaky gut” phenomenon might result of *Culicoides* species that are not normally considered as vectors, to be able to transmit the virus (Mellor *et al.* 2000).Temperature also affects the infection rates, so at higher temperatures the infection rate is higher, the virogenesis is faster and the transmission is sooner. The temperature also affects the midges, and they survive for shorter periods (Mellor *et al.* 2000). The survival rate of *Culicoides sonorensis* was three times longer at 15 ºC than at 30 ºC (Wittmann *et al.* 2002). The vector competence or susceptibility to infection in the midges is also under genetic control (Tabachnick 1991).

After infection of the vertebrate host, the virus multiplies in the regional lymph nodes and then spread to the pulmonary microvascular endothelial cells. After this primary

viraemia the virus infects a range of secondary organs. Replication in these organs produces a secondary viraemia (Wilson *et al.* 2009).

Before an arthropod species can be considered a proven vector of a specific virus, it must fulfil all of the following four criteria as stipulated by the WHO (Walton 2004):

1. The virus must be isolated from field-collected insects.
2. Insects must become infected after feeding upon a viraemic host.
3. Insects must be able to transmit the virus by bite.
4. There must be field evidence that the infected insects are associated with the vertebra population in which the infection is occurring.

Up until now *Culicoides imicola* is considered as the only vector that has fulfilled all four criteria for AHSV (Meiswinkel 1998; Meiswinkel *et al.* 2004). *Culicoides imicola, C. bolitinos, C. bedfordi, Culicoides dutoiti, Cculicoides engubandei, Culicoides magnus, Culicoides pycnostictus, Culicoides zuluensis and Culicoides gulbenkiani* have been shown to be orally susceptible to infection with AHSV in the laboratory (Paweska, Prinsloo & Venter 2003; Venter & Paweska 2007). *Culicoides variipennis sonorensis*, a midge that occur in North America, is also a highly efficient vector of AHSV in the laboratory (Mellor *et al*. 2000). Several species within the subgenera *Avaritia* and *Remmia* met some of the above-mentioned criteria and should therefore be considered as potential vectors. Members of the subgenus *Remmia* can be abundant in arid to semi-arid areas (Meiswinkel *et al.* 2004). Until now AHSV can not be recovered from *Culicoides coarctatus, Culicoides enderleini, Culicoides*

huambensis, Culicoides leucostictus, Culicoides neavei, Culicoides nevilli, C. nivosus, Culicoides onderstepoortensis, and *C. subschultzei* 10 days after feeding a virus infected blood meal in the laboratory (Venter & Paweska 2007). *Culicoides imicola* is the most abundant *Culicoides* species associated with livestock in the summer rainfall region of South Africa and outbreaks of AHS are usually linked with big numbers of *C. imicola* (Venter, Nevill & Van der Linde 1996).

Culicoides imicola and C*. bolitinos* as well as three other species (non-Avaritia Old World species) *C. leucostictus*, *C. magnus* and *C. zuluensis,* have been shown to be orally susceptible to EEV (Paweska & Venter 2004).

Venter *et al* (2002) has reported that *C. onderstepoortensis, C. magnus, C. bedfordi* and *C. pycnostictus* do not play an important role as vectors of EEV.

There are fourteen species of *Culicoides (C. bedfordi, C. enderleini, C. engubandei, Culicoides expectator, C. dutoiti, C. gulbenkiani, C. huambensis, Culicoides milnei, C. neavei, C. nevilli, C. nivosus, C. onderstepoortensis, C. pycnostictus* and *C. subschultzei*) that, up to date, were not susceptible to oral infection with EEV in the laboratory (Paweska & Venter 2004).

Other blood feeding arthropods, for example mosquitoes and *Hyalomma dromadarii* ticks, have been incriminated as possible vectors, but none have been shown to play a role under natural conditions (Mellor *et al.* 2000; Wilson *et al.* 2009).

Species richness refers to the number of species. Species diversity is an expression or index of the relation between the number of species and the number of individuals (Spellerberg & Fedor 2003). High species richness and diversity in an area will complicate the epidemiology of arthropod borne diseases in an area.

Environmental factors such as temperature, rainfall and the availability of a larval habitat influence the distribution and abundance of *Culicoides* species. The basic requirements for the larval habitat are moisture and a medium containing organic matter. There are four main types of larval habitats: surface water and soil interface, dung pats of large animals, tree-holes, plant and rock cavities and rotting fruits and plants (Meiswinkel *et al.* 2004).

The immature stages of *C. imicola* occur in organically enriched grassy-margins of drainage furrows and on irrigated pastures. Unlike the pupa of most *Culicoides,* species the pupa of *C. imicola* are unable to float on water and are therefore susceptible to drowning (Meiswinkel *et al.* 2004). *Culicoides imicola* is absent in nutrient-poor, quick-draining sandy soils (Meiswinkel 1998). The following factors have been shown to influence the distribution and abundance of *C. imicola:* extreme cold, aridity, topographic slope, soil type and soil fertility (Meiswinkel *et al.* 2004).

Wind speed and the minimum Normalized Difference Vegetation Index ($NDVI_{min}$) are critical factors in the ecology of *C. imicola.* There is a negative correlation

between the abundance of *C. imicola* and wind speed, and a positive correlation with the $NDVI_{min}$ (Baylis & Rawlings 1998).

The larval habitat of *C. bolitinos* differs widely from that of *C. imicola*. The immature stages inhabit exclusively the dung of large herbivores, including cattle, buffalo and blue wildebeest and the larval stages are therefore less dependent on the topographic slope, soil moisture or soil quality (Meiswinkel 1989). They can achieve great abundance in dung deposits on sandy soils (Meiswinkel 1997).

Temperature, however, affects seasonal abundance and colder climatic conditions coincide with the time when *Culicoides* midges disappear from light-trap collections (Meiswinkel *et al.* 2004). Low winter temperatures (>0 ºC) do not kill any of the stages in the life-cycle, but merely slows down the development (Meiswinkel *et al.* 2004). It is also shown that *Culicoides* species can harbour BTV at low temperatures for relative long periods and when the temperature increases, the virus can replicate to transmittable levels (Paweska *et al.* 2002).

Rawlings *et al* (2003) reported on an extensive study to determine the occurrence of *Culicoides* midges in South Africa. Entomological field data were collected over two years from September 1996 to September 1998. At three collection sites in the Northern Cape (Alexander Bay, Springbok and Calvinia) 28 species with an average of 19 species per site were collected. Six species (*C. bolitinos, C. imicola, C. leucostictus, C. nivosus, C. pycnostictus* and *C. subschultzei*), with some

exceptions, were found at all three the sites. *Culicoides bolitinos* was absent at Alexander Bay and *C. leucostictus* was absent at Springbok. A quarter of the captured species were yet un-described *Culicoides* species. There were another six species found at all three sites, three of which were unnamed, namely C. #89, #90, and #119. The most commonly captured species at Springbok was C. #89 and *C. subschultzei*.

Since both *C. bolitinos* (0.0008% of captures) and *C. imicola* (0.0194%) were remarkably uncommon in Namaqualand it was concluded that the area from the Orange River frontier with Namibia, south as far as Calvinia, appears to be the most promising location for a vector- and virus-free quarantine zone (Rawlings *et al*. 2003).

In a country wide light trap survey, published 15 years ago, the largest light trap collections were made at Eiland in the north of the country (*n* = 44 364) and Hluhluwe (*n* = 26 041) (Venter *et al.* 1996). These authors showed that the mean catch size was smaller in arid areas with severe winters, in Upington (Karakul) the average catch size was 133. There were also a significant difference between the average catch size at Karakul (133) and Veekos (1 091), which is less than 10 km apart. Veekos is within 1 km of the Orange River and irrigated. These factors with the dense vegetation and higher relative humidity probably create a microclimate conducting to *Culicoides* midge breeding and survival (Venter *et al.* 1996). The high numbers of *Culicoides* midges, especially that of *C. imicola*, coincide with the high

prevalence of AHSV in the area. This emphasize the role that *C. imicola* play in the epidemiology of this disease.

Chapter 3

MATERIALS AND METHODS

Study area

Namaqualand is situated in the arid north-west of South Africa. It lies between 16º 27’ and 19º 0’ east; 28º 0’, and 31º 10’ south. It forms a distinct biogeographical area within the larger Succulent Karoo biome (Desmet 2007). The study area extends from Pofadder in the east to the Atlantic Ocean in the west (Figure 2). The southern border is the border between the Northern and the Western Cape Province, and the northern border is the border between South Africa and Namibia (Figure 2). Namaqualand is sparsely populated with about 77 000 inhabitants in the region. The main agricultural activities are small stock farming. Namaqualand can be divided into seven broad bioregions based on the physical environment, climate and flora (Desmet 2007). Serum samples were collected from equids resident in six of the seven bioregions (Bushmanland, East Gariep, Hardeveld, Kamiesberg, Richtersveld and Sandveld). Midge collection was done in the Kamiesberg bioregion.

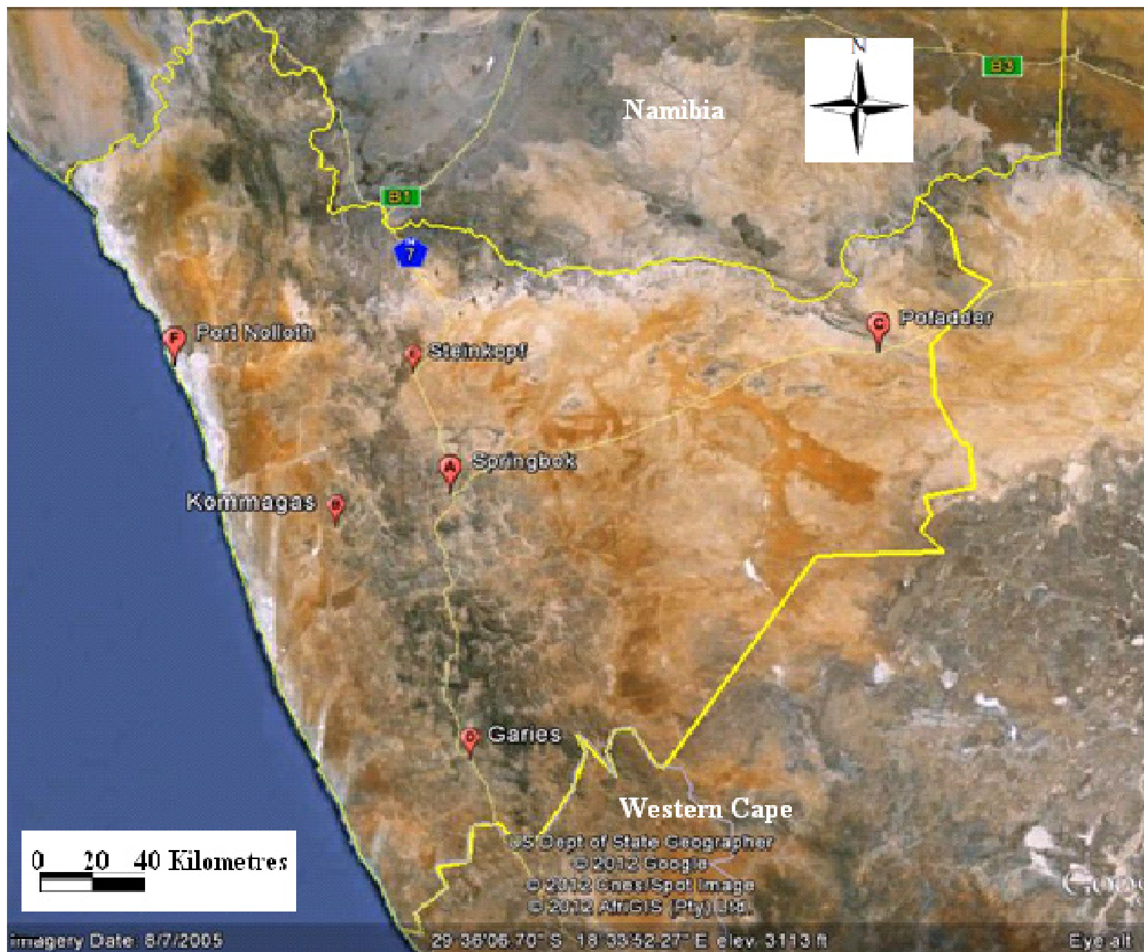

Figure 2 A satellite image depicting the Namaqualand region. The borders of the region is indicated with the yellow line. Indicated with red icons (A-F) are some of the bigger towns in Namaqualand. A: Springbok; B: Kommagas; C: Pofadder; D: Garies; E: Steinkopf; F: Port Nolloth.

Namaqualand is classified as a semi-arid winter rainfall region, but the climate is not uniform (Mackellar, Hewitson & Tadross 2007). There are two precipitation

gradients. The first is the gradual latitudinal aridity with a decrease in annual precipitation towards the north. The second gradient is perpendicular to the first. This is a longitudinal seasonal gradient with winter-rain in the west along the coast, to summer rain in the interior (Desmet 2007). The mean annual precipitation (MAP) ranges from 50 mm in the north-west to over 400 mm in the Kamiesberge (Mackellar *et al*. 2007). Although the mean annual precipitation in this area is low, it is very consistent (Desmet 2007). Due to the cold Atlantic Ocean, the region is a relatively cold desert with mean maximum summer temperatures below 30 °C (Desmet 2007).

Stock owners were requested to bring their animals to a central point for sampling. These collection points varied from settlement to settlement, but it was usually at the local dip tank or animal holding facility. The various collection points were identified by GIS coordinates (Figure 3). Most of the samples were collected from around Springbok and the area north from Springbok heading towards and including Steinkopf (Figure 3).

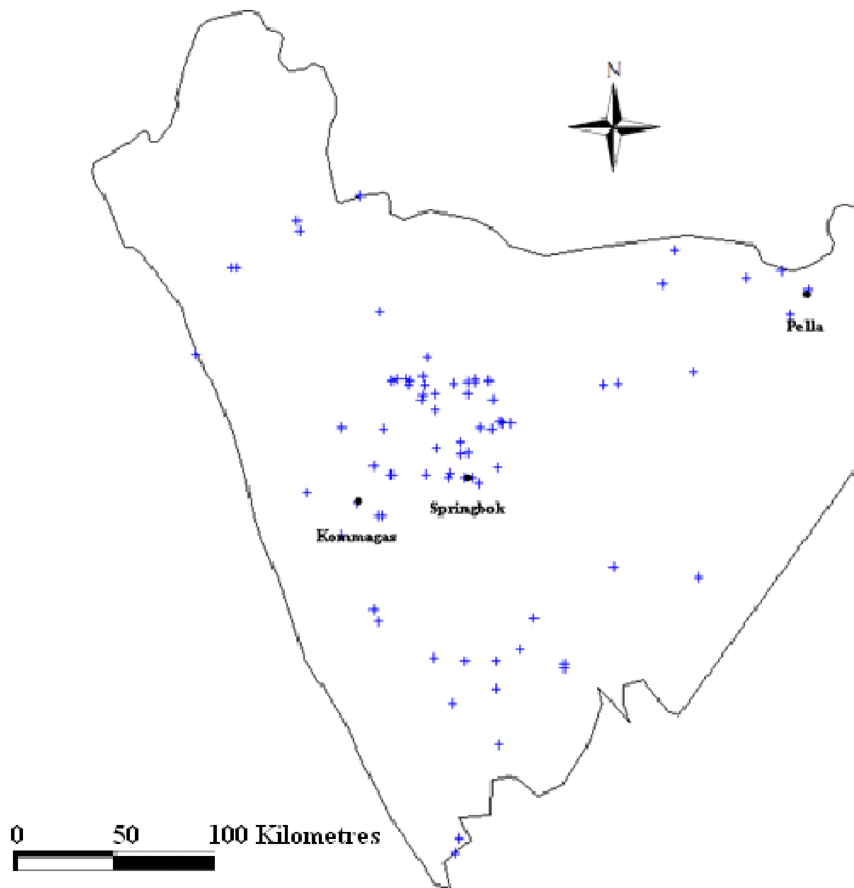

Figure 3 The collection sites in Namaqualand where blood samples were collected from equids to determine the seroprevalence of AHSV and EEV in the area.

Study population

According to the Northern Cape Veterinary Services' census of 2006, there were 830 equids (horses, donkeys and mules) in the study area.

A questionnaire was used to determine the origin and vaccination status of animals before serum collection. Only un-vaccinated animals that originated from the study area were selected. The questionnaire was relatively short with only the details of the owner (Name, Address and Contact numbers), the details of the animals (Name and age) and the three questions (Where was the animal born; Did it ever move out of Namaqualand; Was it vaccinated against AHS). The questionnaire was in Afrikaans, as this is the predominant spoken language of the area. An example of the questionnaire is included as Appendix A.

The study was done under approved protocol (V042/09) from the Research committee and Animal Use and Care Committee of the University of Pretoria. Attached to the questionnaire was also the required consent form of the Animal Use and Care Committee of the University of Pretoria.

Sampling methods

Serum collection

Blood were collected in two 5 ml BD Vacutainer® SST™ Tubes containing silica gel. The blood was centrifuged and inspected for proper separation of serum, gel and cells before it was cooled to 4 ºC and shipped to the laboratory. One tube was sent to the OIE reference laboratory for AHS and Bluetongue (BT) at the ARC-Onderstepoort Veterinary Institute (ARC-OVI). The second tube was sent to the Department of Veterinary Tropical Disease, Faculty of Veterinary Science, Onderstepoort. Samples were aliquoted and stored at -20 ºC until tested.

Midge collection

Midge collections were done with combination 12/220V Onderstepoort light traps (Figure 4).

Figure 4 Midge collection was done with a Onderstepoort light trap. These robust traps have a 2 mm mesh netting around the 8 W blacklight. Collections were made in 200 ml of tap water with Savlon®.

These metal traps are robust and quite heavy (4 kg). They consist of a 220 V down-draught suction fan and a 24 cm 8 W blacklight tube. There is polyester netting, with

a mesh size of 2 mm, around the light source to exclude moths and bigger insects (Venter, Labuschagne, Hermanides, Boikanyo, Majatladi & Morey 2009).

As there was no 220 V electricity available at the collection points, 12 V batteries were used. After each collection these batteries were charged. The batteries were replaced with new batteries after seven to eight months.

Light trap collections were made from 30 June 2008 to 30 July 2009. The traps were operated once a week, starting at dusk for approximately 14-16 hours. The collections were recovered the following day and minimum and maximum temperatures during the collection period were recorded. Midges were collected in 200 ml tap water to which 5 ml of Savlon® antiseptic was added. The collected insects were transferred to 70% alcohol and sent to the ARC-OVI for identification.

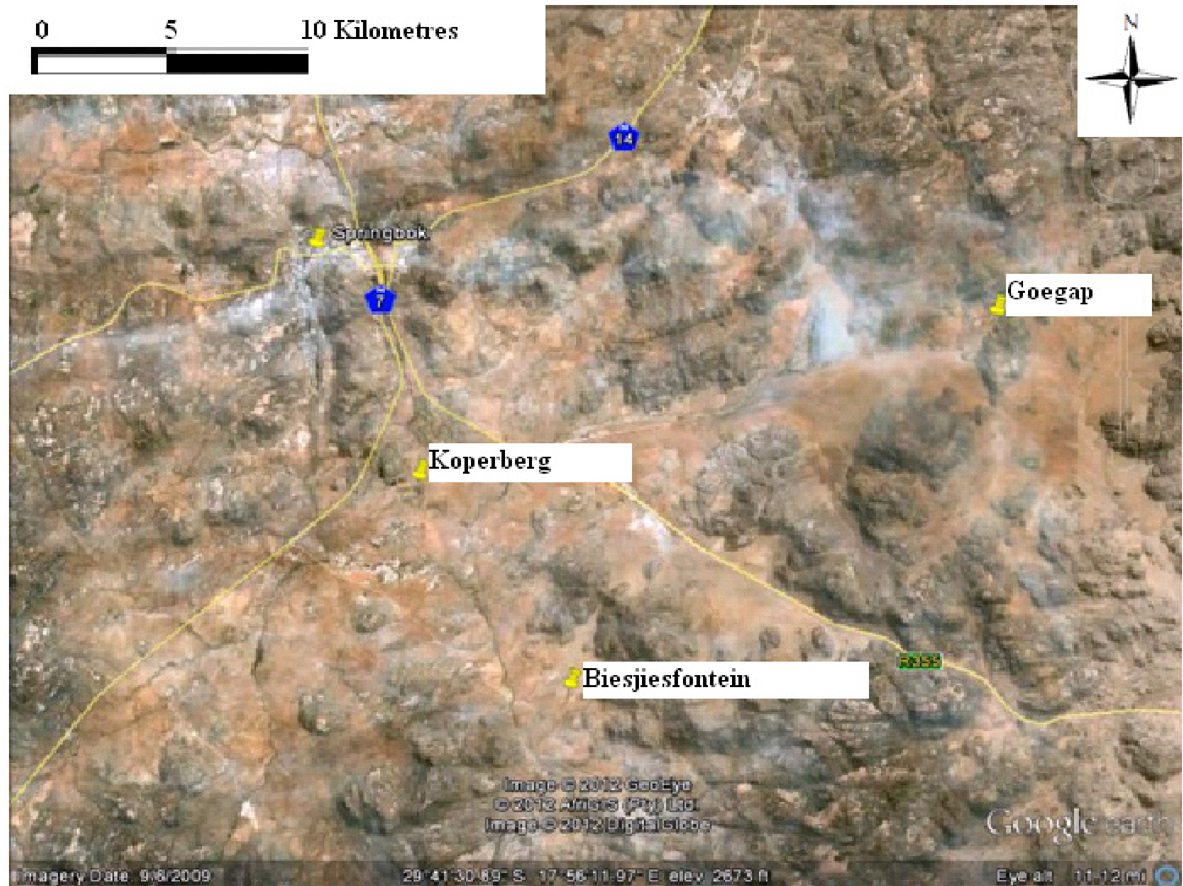

Figure 5 A satellite image of the positions of the three light traps for midge collections. Light trap collections were made at Goegap, Koperberg and Biesjiesfontein. Springbok is marked as a reference point.

Goegap

The first trap was in Goegap Nature Reserve (29º 39’ 57.6” S, 18º 0’ 7.2” E, 1 096 m.a.s.l), about 15 km southeast of Springbok (Figure 5). This site was selected as there are both horses and zebra on the Reserve.

The trap deployed at a height of 1.7 m attached to the edge of a reed canopy at the stables. The surrounding habitat comprised of typical granite hills and sandy plains. There were two horses in the immediate vicinity of the trap. There was no irrigation in the area.

Koperberg

The second trap was placed on the farm, Koperberg (29º 41’ 60” S, 17º 54’ 21.6” E, 950 m.a.s.l) about 7 km south of Springbok (Figure 5). This site was chosen because there were about 10 horses in the vicinity of the trap, making it the second highest population of horses in the area. Secondly, this property used sewage water to irrigate their pastures, and this site was in close proximity of the sewage works. The trap was attached at a height of 1.7 m to the wall of the feed store. There was no overhang to this building. The immediate surrounding area was an irrigated pasture and the stables. At times, depending on the pasture, there were also some sheep in the vicinity of the traps.

Biesjiesfontein

The third trap was placed at the riding school on Biesjiesfontein (29º 43’ 22.8” S, 17º 56’ 9.6” E, 800 m.a.s.l), about 18 km south southeast from Springbok (Figure 5). The 30 horses on this property, gives it the highest population density of horses in Namaqualand. The trap was fixed at a height of 1 m in a natural bush, about 10 m from the 30 horses. The immediate surrounding area consisted of the stables, the manure heap and natural vegetation.

Detection of antibody to AHSV and EEV

An AHSV recombinant VP7 indirect enzyme-linked immunosorbent assay (ELISA) (Maree & Paweska 2005) was used to detect AHSV antibodies in the serum samples.

Briefly, purified AHSV rVP7 antigen was diluted in carbonate-bicarbonate buffer and then passively adsorbed overnight. The plates were washed three times with a TST buffer (0.8 M Tris; 0.15 M NaCl; 0.05% Tween-20 pH8.0). The coated plates were blocked with 3% fat-free milk powder in TST. The plates were washed, and the test-samples were added. The plates were incubated for an hour and washed again. Recombinant protein G conjugated with horseradish peroxidise was added to the wells. The plates were incubated for an hour and washed. Tetra-methylbenzidine was added to the wells and the plates incubated for 10 min. The reaction was stopped by adding H_2SO_4. Optical densities were measured at 450 and 690 nm reference filters (Maree & Paweska 2005). The cut-off values for positive samples where determined through the standard formulas based on the optical densities. Positive samples were determined as samples with titres ≥600. The ELISA has been shown to be superior to the complement fixation test, both in sensitivity and specificity (Williams 1987).

The EEV antibody titres were determined with an indirect ELISA, similar as the ELISA used for the determination of the AHSV-titres. Positive samples were

determined as samples with titres ≥1 200 (A. Rakgwale, personal communication 2012).

Midge identification

After collection, the midges were stored until identified and counted. Large collections were subsampled (Van Ark & Meiswinkel 1992). *Culicoides* midges were counted, sexed and sorted to species level. Females were classified according to the abdominal pigmentation method (Dyce 1969) into unpigmented (nulliparous), pigmented (parous), gravid (with eggs visible in the abdomen) or freshly blood-fed. The numbers of males collected were also recorded. Representative samples of this survey were added to the *Culicoides* reference collections at the ARC-OVI.

Statistical analysis

Data was analysed with Epi Info Version 3.5.1. The GIS maps were also created through Epi Info Version 3.5.1. The Chi-square test was used to establish statistical significant difference between the seroprevalence of AHSV and EEV at the different sites, as well as between the seroprevalence of AHS and EEV and the midge collections. Microsoft Office Excel 2007 was used for the analyzing of the data of the midge collections, as well as for the creation of graphs. The Shannon-Wiener Index, species richness and species evenness were calculated through the following website: http://lbsite.zxq.net/programs/diversity.html.

Chapter 4

RESULTS

A total of 874 serum samples from equids were collected between August 2009 and February 2010 of which 842 were included in this study. Thirty-one animals were excluded on the basis of their AHS vaccination history, or that they originated from outside of the region. One animal was excluded due to an incomplete questionnaire.

Study population

The distribution of samples collected during the study is represented in Table 2.

Table 2 A summary of the sample locations and the number of samples that were collected at each location.

Sample point	Number	Sample point	Number	Sample point	Number
Aggeneys	11	Karos	8	Pella	66
Agterkraal	1	Kharkams	7	Pepperbosbank	5
Annakop	14	Kheis	16	Pienaarsbult	1
Brakdam	2	Khoerkam	19	Potvlei	3
Brakkie	2	Klein Besonderheid	40	Port Nolloth	2
Buffelsrivier	11	Klein Pella	1	Remhoogte	1
Bulletrap	11	Klipfontein	32	Rooifontein	23
Concordia	5	Komaggas	82	Rooipoort	8
Doringbank	8	Kotzehoop	14	Soebatsfontein	8
Eenriet	18	Leliefontein	31	Spoegrivier	3
Eksteenfontein	22	Lepelfontein	6	Springbok	12
Eyams	7	Mik	2	Steinkopf	87
Garies	2	Nababeep	20	Taaibosmond	27
Gladkop	8	Nigramoep	3	Tweerivier	36
Grasvlak	25	Nourivier	17	Uitspan	4
Ikosis	12	Okiep	4	Vredeklip	4
Jakkalsvlei	7	Onderste Eyams	3	Wildehondspoort	6
Kamassies	2	Opdam	14	Witbank	20
Karootjie	1	Paulshoek	7	Witklipmond	26
				Wolfberg	5

Seroprevalence of AHSV

Antibodies to AHSV were detected in 37 of 842 animals tested (Table 3). This gave a seroprevalence of 4.4% (37/842) (Table 3). Antibodies to AHSV were found at 18 of the 58 sites sampled. The seroprevalence varied from 0% to 100% with a significant difference(P<0.001) between sites.

A list of the animals that were positive for antibody to AHSV is included in Appendix B.

Positive samples were distributed throughout the Namaqualand area (Figure 6). The highest number of AHSV antibody positive samples (n = 9) were collected in Pella. There were three areas with no positive animals (Figure 6). The first was around Grasvlak and Uitspan (Area 1). The second area was around Kommagas. (Area 2). The third was around Leliefontein and Tweerivier. (Area 3).

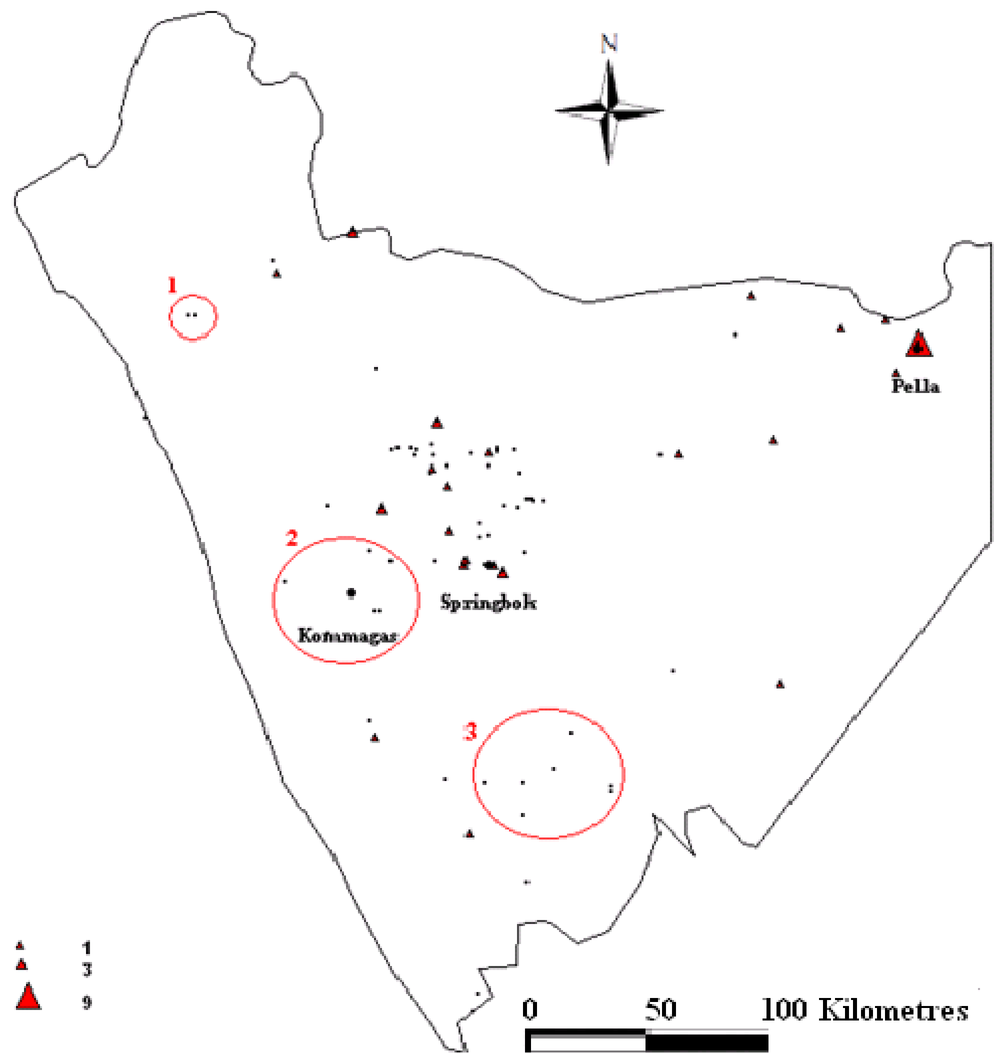

Figure 6 A map indicating the sample points (small black dots) as well as the locations where antibody to AHSV (red triangles) were detected. The size of the triangles indicate the number of positive samples collected. The areas marked 1, 2 and 3 are the areas where there were no AHSV antibody detected.

The ages, ranging from <1 to 49 years, of the animals that were sampled are shown in Figure 7. The age distribution within the sampled population followed a typical pyramid structure with the age group <1 to 3 years at the base and 49 years at the top of the pyramid. The largest group of animals (n = 98) were 3 years. The animals that were positive to AHSV antibody were almost evenly distributed amongst all the age groups (Figure 7).

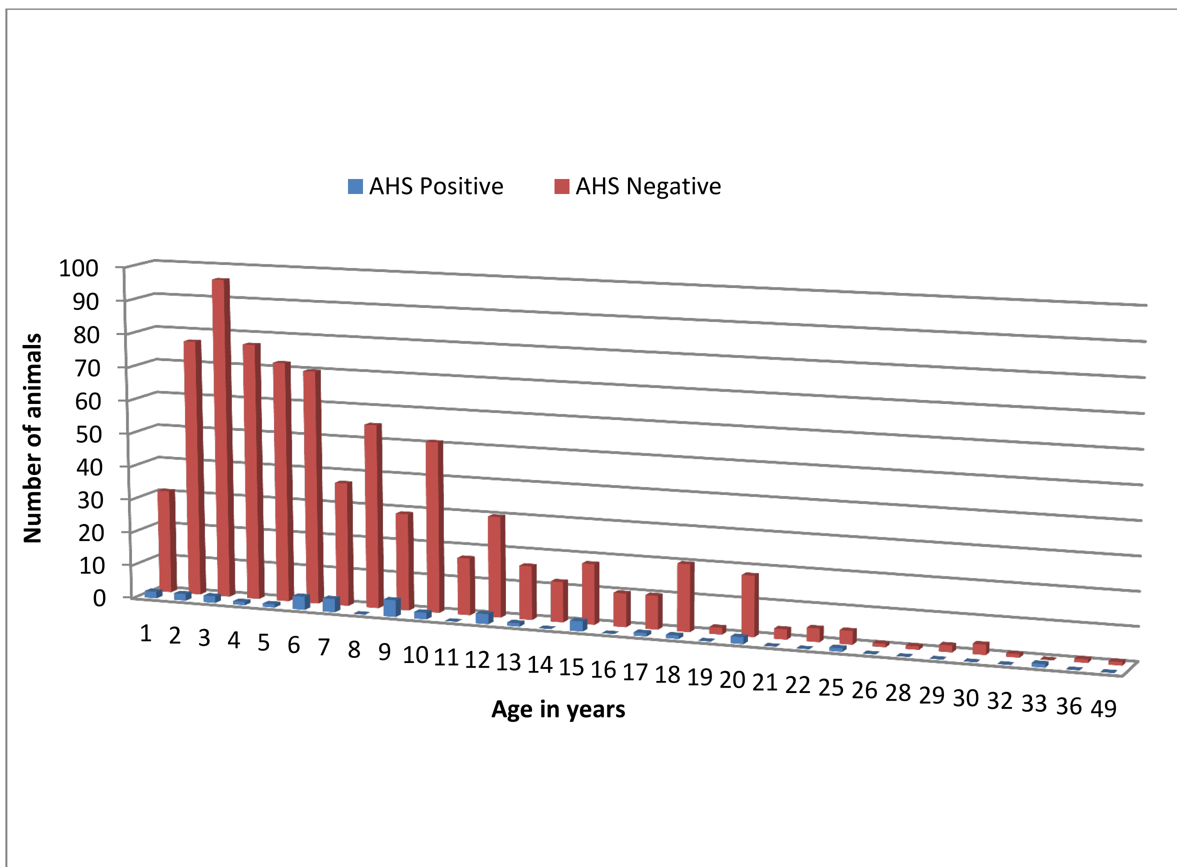

Figure 7 The different ages of animals that tested positive and negative respectively in Namaqualand.

Seroprevalence of EEV

Two hundred and sixty serum samples of the 842 samples tested positive for antibody to EEV in the ELISA (Table 3). This gave a seroprevalence of 30.9% (260/842) (Table 3). Antibody to EEV were detected at 44 of the 58 sites sampled. The seroprevalence varied from 0% to 100%. The seroprevalence differed significantly between sites (P<0.001). The EEV positive samples were distributed throughout Namaqualand (Figure 8) and no areas free of antibody to EEV could be identified.

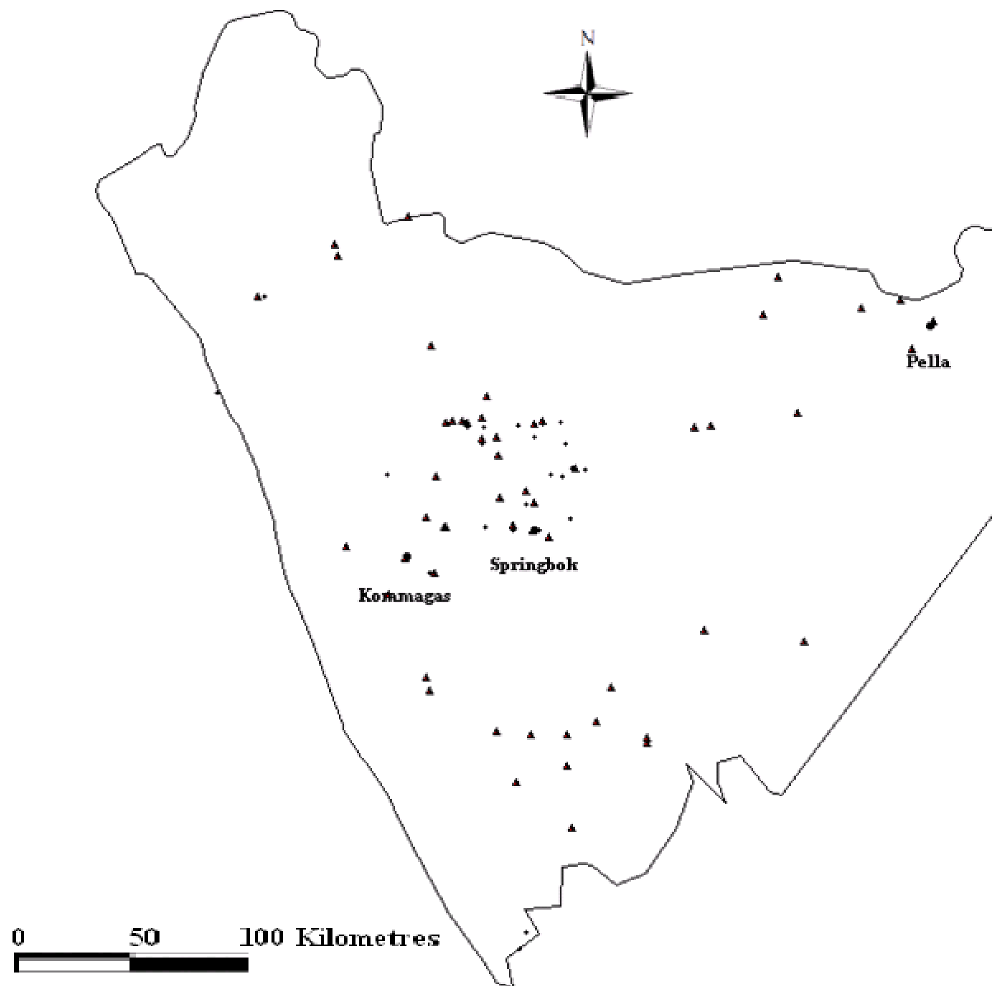

Figure 8 This map indicates the sample points (small black dots) and the locations where EEV antibody positive samples were detected (triangles).

Table 3 Seroprevalence for AHSV and EEV at collection points where 20 or more serum samples were collected.

Sample point	Number of samples collected	Number of ELISA positive samples (prevalence)	
		AHSV	EEV
Steinkopf	87	4 (4.6%)	29 (33.3%)
Komaggas	82	0 (0%)	42 (51.2%)
Pella	66	9 (13.6%)	32 (48.5%)
Klein Besonderheid	40	0 (0%)	4 (10%)
Tweerivier	36	0 (0%)	3 (8.3%)
Klipfontein	32	1 (3.1%)	2 (6.25%)
Leliefontein	31	0 (0%)	2 (6.5%)
Taaibosmond	27	1 (3.7%)	9 (33.3%)
Witklipmond	26	0 (0%)	10 (38.5%)
Grasvlak	25	0 (0%)	1 (4%)
Rooifontein	23	1 (4.3%)	15 (65.2%)
Eksteenfontein	22	1 (4.5%)	4 (18.2%)
Nababeep	20	1 (5%)	2 (10%)
Witbank	20	1 (5%)	11 (55%)
Total for 14 sites were >20 animals were sampled	537	19 (3.5%)	156 (29%)
Total	842	37 (4.4%)	260 (30.8%)

The prevalence of AHSV was significantly higher than that of EEV (P<0.001). AHSV antibodies where found at 18 of the sample sites, while antibodies against EEV where found at 44 of the 58 sample sites. Twenty-four animals were positive for antibody to both AHSV and EEV.

Midges

A total of 134 light trap collections were made during the period 30 June 2008 to 30 July 2009. 132 866 midges were collected. The data are summarised in Appendix C.

Goegap

A total of 46 collections were made at Goegap between 30 June 2008 and 09 July 2009. During these collections 6 253 midges were collected. The mean number of midges collected was 135.9. The largest collection (n = 1 155) was made on 06 March 2009. There were 16 collections were no midges were collected. There were two peaks in the average number of midges collected per month. The first peak was in November and December, and the second was in March (Table 4).

The three most abundant species at Goegap were: *C. bedfordi* (n = 1 699), *C. ravus* (n = 1 239) and *C. subschultzei* (n = 1 268). A total of 20 species were collected. The Shannon-Wiener Index for Goegap was 1.97 and the species evenness 0.66.

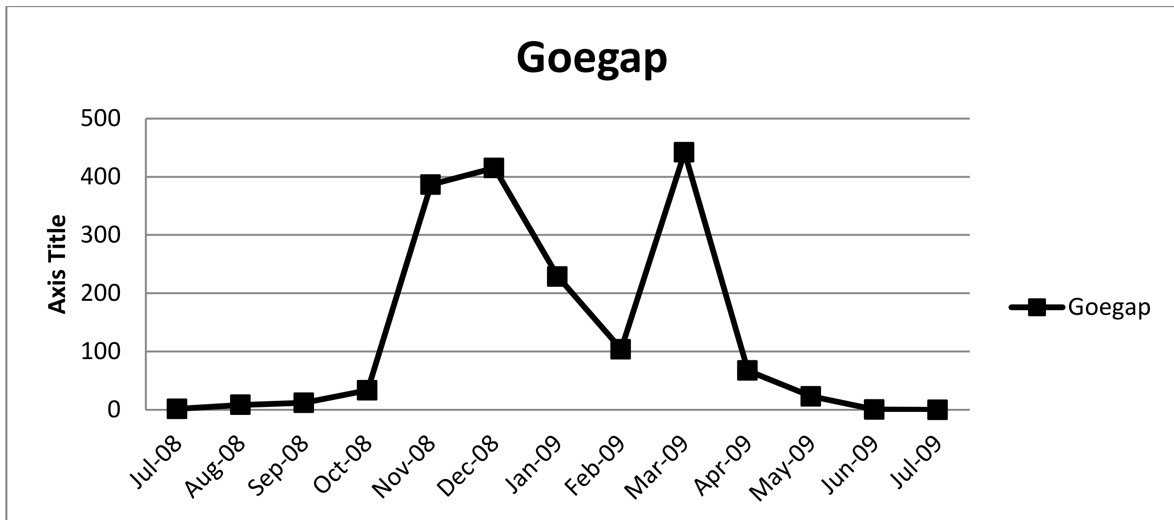

Figure 9 Mean numbers of midges collected per month with light traps at Goegap, Namaqualand.

Biesjiesfontein

A total of 49 collections were made between 30 June 2008 and 23 July 2009. During these collections 20 307 midges were collected. The mean number of midges collected was 414.4. The largest collection (n = 4 256) was made on 25 November 2008. There were two peaks in the average collection size per month. The first peak was in November (Figure 10), the second peak was in February to April, with slightly lower number of midges collected in March. There were 11 collections were no midges were collected.

A total of 26 species were collected and the three most abundant species were *C. ravus* (n = 4 472), *C. bedfordi* (n = 3 844) and *C. herero* (n = 3 647) (Table 4). The Shannon-Wiener Index for Biesjiesfontein was 2.05 and the species evenness 0.63.

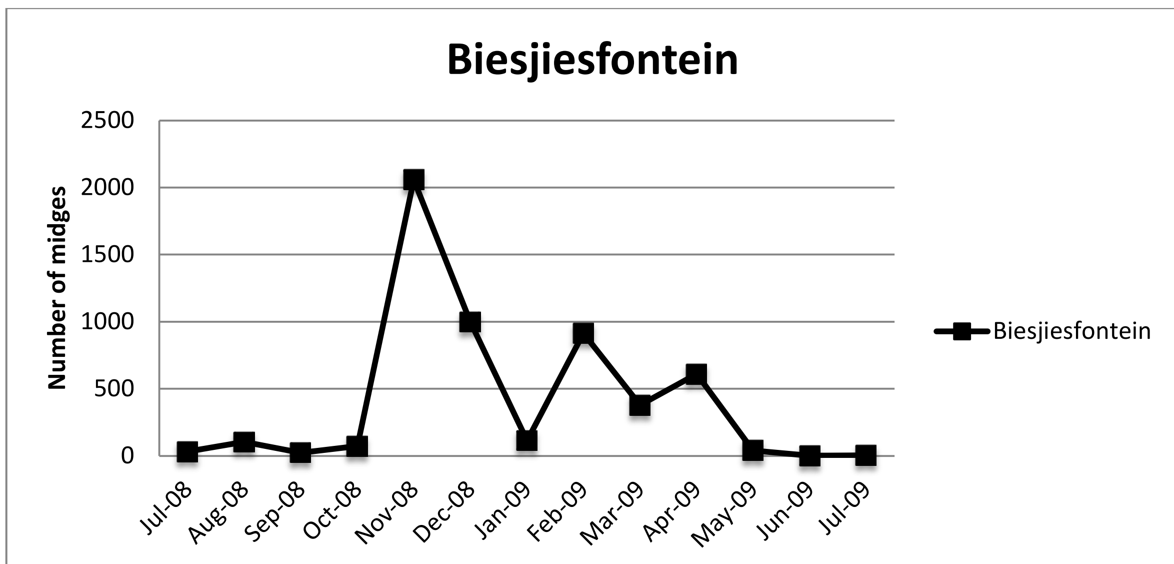

Figure 10 Mean numbers of midges collected per month with light traps at Biesjiesfontein, Namaqualand.

Koperberg

A total of 39 collections were made at Koperberg between 11 August 2008 and 30 July 2009 and 106 306 midges were collected. The mean number of midges collected was 2 725.8. The largest collection (n = 15 861) was made on 18 November 2008. There were also two peaks in the average number of midges caught per month. The first peak was, as at Goegap and Biesjiesfontein in November, and the second in April (Figure 11). There were 6 collections were no midges were collected.

A total of 23 species were collected (Table 4). The three most abundant species were: *C. ravus* (n = 33 765) *C. bedfordi* (n = 27 996) and *C*. #89 (n = 8 717). The Shannon-Wiener Index for Koperberg was 2.01 and the species evenness 0.63.

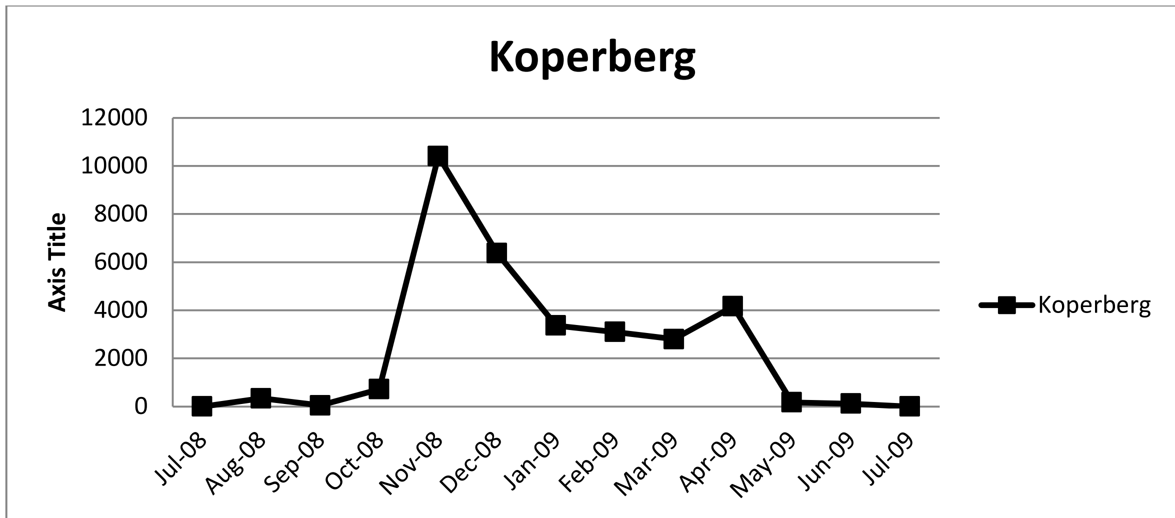

Figure 11 Mean numbers of midges collected per month with light traps at Koperberg, Namaqualand.

There was a significant difference in the number of midges caught between the three sites ($P<0.001$).

There was a sharp drop in the number of midges collected on 11 November 2008 as well as on 18 March 2009, which corresponded with a similar drop in temperature.

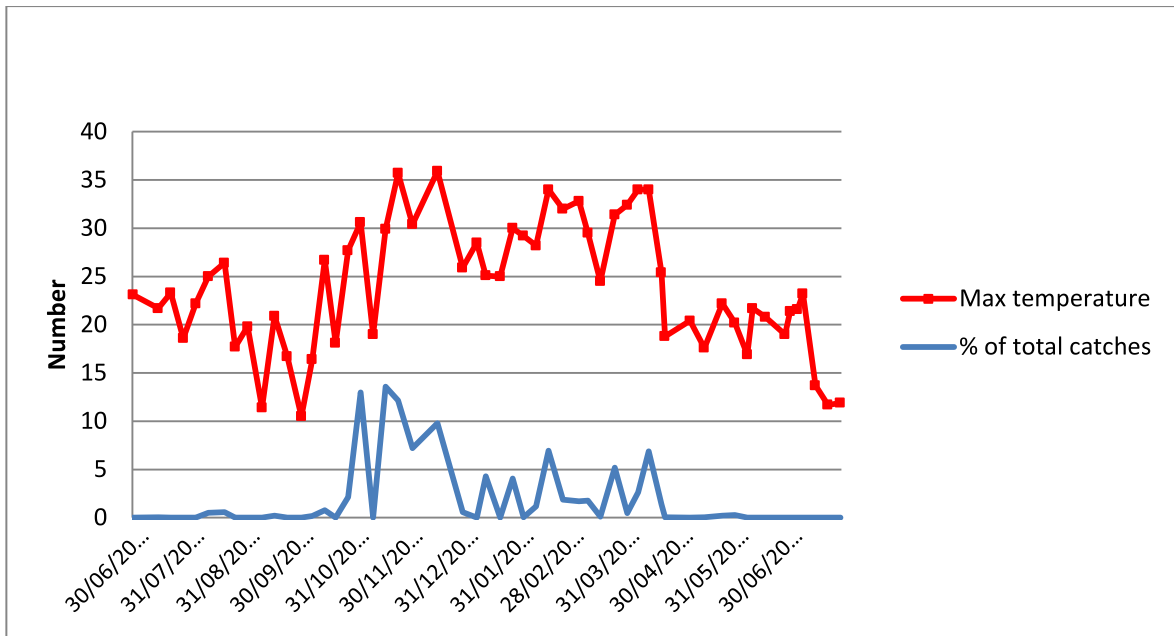

Figure 12 The number of midges collected per collection expressed as a percentage of the total number of midges collected. The red series indicates the maximum temperatures as recorded by the South African Weather Bureau.

In Table 4 there is a summary of the species collected per collection site. In total there were 27 species collected. The Shannon-Weiner Index for the three traps was 2.07 and the species evenness was 0.63.

The six most abundant *Culicoides* species collected were: *C. ravus* (29.7%), *C. bedfordi* (25.2%), C. #89 (9.6%), *C. subschultzei* (7.4%), *C. herero* (7.1%) and *C.*

nivosus (6.8%). The first two species, *C. ravus* and *C. bedfordi* constitute the majority (55.0%) of the total collected (Table 4).

From the potential AHSV vectors, (*C. imicola, C. bolitinos, C. bedfordi, C. dutoiti, C. engubandei, C. magnus, C. pycnostictus, C. zuluensis* and *C. gulbenkiani*) five, namely *C. imicola* (0.9% of the total number of midges collected), *C. bolitinos* (1.5%), *C. bedfordi* (25.2%), *C. engubandei* (<0.1%) and *C. pycnostictus* (3.2%), were present in Namaqualand (Table 4).

From the potential EEV vectors *(C. imicola*, C*. bolitinos*, *C. leucostictus*, and *C. zuluensis)*, three were present - *C. imicola* (0.9%)*, C. bolitinos* (1.5%) and *C. leucostictus* (0.002%) (Table 4).

Table 4 is a summary of the species collected per site. For each site the number collected, the mean and the standard deviation per species are indicated. The last column represents the total for each species, as well as the prevalence for each species

Collection site	Goegap			Biesjiesfontein			Koperberg			Total
Collection period	30/06/2008 to 09/07/2009			30/06/2008 to 23/07/2009			11/08/2008 to 30/07/2009			
Number of collections	46			49			39			134
Species name	Number	Mean	Standard deviation	Number	Mean	Standard deviation	Number	Mean	Standard deviation	Total (%)
C. ravus	1 239	54	62.4	4 472	213	279.7	33 765	1 251	1 477.7	39 476 (29.7%)
C. bedfordi	1 699	85	129.2	3 844	160	238	27 996	1 120	1 624.9	33 539 (25.2%)
C. #89	577	24	42.5	3 490	116	209.4	8 717	349	435.6	12 784 (9.6%)
C. subschultzei	1 268	45	91	2 133	82	105	6 390	256	330.4	9 791 (7.4%)
C. herero	502	30	33	3 647	152	253.7	5 348	223	274.5	9 497 (7.1%)
C. nivosus	22	2	1	437	19	18.6	8 573	306	451	9 032 (6.8%)
C. #119	51	5	10	395	30	51.3	3 901	177	332	4 347 (3.3%)
C. pycnostictus	264	13	24.4	474	17	21.3	3 575	149	168.7	4 313 (3.2%)
C. #90	17	3	2.3	358	20	33.7	2 760	131	223.6	3 135 (2.4%)
C. bolitinos				2	2	0	2 011	2 011	0	2 013 (1.6%)
C. macintoshi	27	4	3.8	100	8	8.9	1 380	63	65.8	1 507 (1.1%)
C. imicola	389	43	95.1	456	33	48.6	339	34	64.5	1 184 (0.9%)
C. #94	146	7	7.4	226	9	17.6	348	17	33	720 (0.5%)
C. remerki	11	4	0.5	19	9	6.5	321	107	98.9	351 (0.3%)
C. huambensis	3	1	0	155	14	17.7	113	16	15	271 (0.2%)
C. exspectator	3	3	0	1	1	0	257	257	0	261 (0.2%)
C. #33	8	3	2	48	4	4.0	204	20	20.5	260 (0.2%)
C. nigeriae				2	2	0	169	169	0	171 (0.1%)
C. schultzei	2	1	0	11	4	3.6	55	18	19.5	68 (0.1%)
C. eriodendroni				22	22	0	46	24	17	68 (0.1%)

Collection site	Goegap			Biesjiesfontein			Koperberg			Total
Species name	Number	Mean	Standard deviation	Number	Mean	Standard deviation	Number	Mean	Standard deviation	Total (%)
C. engubandei	8	8	0	1	1	0	22	22	0	31 (<0.1%)
C. brucei	8	8	0	4	4	0	14	14	0	26 (<0.1%)
C. loxodontis	10	10	0							10 (<0.1%)
C. #54 (d/f)				8	8	0				8 (<0.1%)
C. onderstepoortensis				2	1	0	1	1	0	3 (<0.1%)
C. leucostictus				2	2	0				2 (<0.1%)
C. similis				1	1	0				1 (<0.1%)
Total	6 253	135.9	263.5	20 307	414.4	860.3	106 306	2 725.8	4 373.4	132 866
Species Richness	20			26			23			27
Shannon-Weiner Index	1.97			2.05			2.01			2.07
Species Evenness	0.66			0.63			0.64			0.63

Since transovarial or vertical transmission is has not been confirmed in the genus *Culicoides*, the number of parous females in a population will play an important role in determining the risk in an area (Wilson *et al* 2009). The highest number of parous *C. imicola* were collected at Goegap (n = 92) on 06 March 2009 (Figure 13). There were no parous *C. imicola* present before 17 February 2009 (Figure 13). The peak numbers from all three collection sites occurred during late February to early March (Figure 13). There were still parous midges present up to June 2009 (Figure 13).

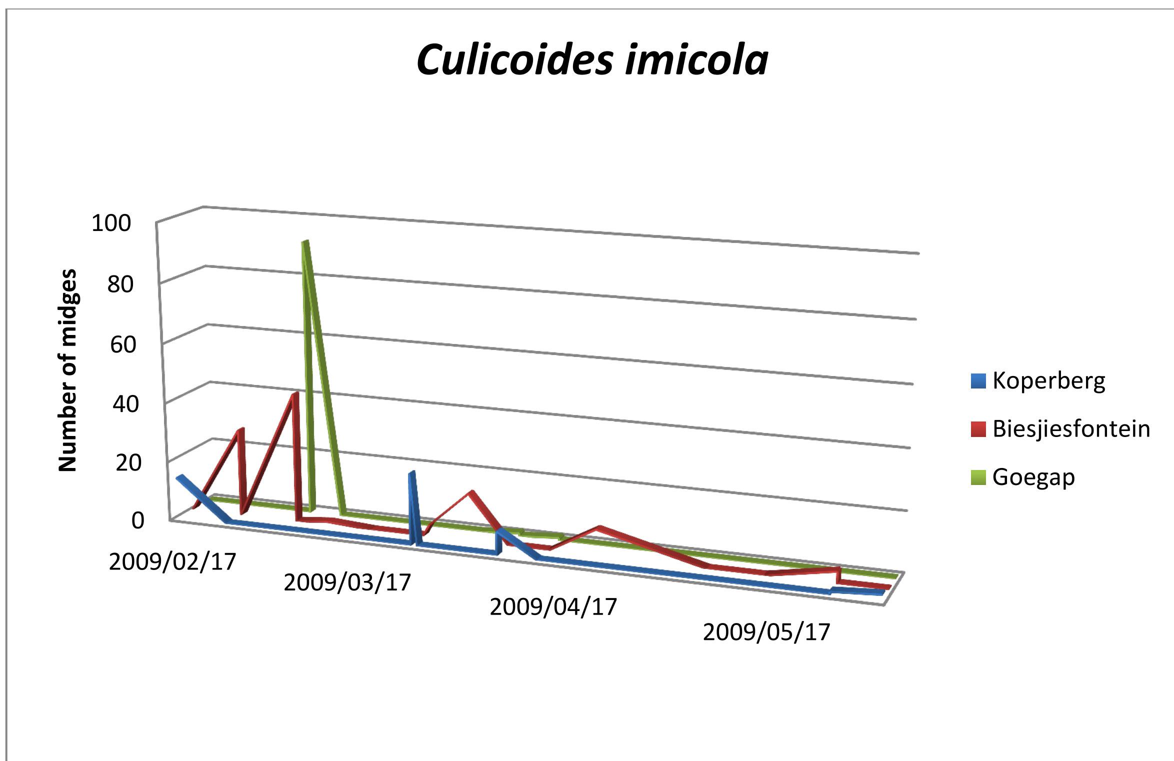

Figure 13 This graph represents the *C. imicola* parous midges that were collected.

Chapter 5

DISCUSSION

Questionnaire

The questionnaire was easy to use. A problem was that a large number of owners and representatives were illiterate. To overcome this challenge all the owners were gathered at the start of the day of serum collection. During the meeting the aim of the study, the procedures and any possible complications were explained to them. Another problem was that there were instances that the owner of the animal was not present, but only a representative. In most of these cases, the owner was unreachable. The information that was given on the questionnaire was therefore not very reliable in these cases.

In retrospect, there were two significant omissions on the questionnaire. The first being failure to record whether the animal was a donkey, mule or horse and the second whether the animal had any previous owner. Some of the positive AHS cases might have been the result of vaccination by the previous owner.

Serum collection

The serum samples (n = 874) collected were in agreement with the 830 predicted samples according to the Northern Cape Veterinary Services' census of 2006.

Namaqualand is a very arid, inhospitable region. To facilitate the collection of the samples notices were distributed to central points. The communities were requested to bring their animals on a specific date to a central collection point. In some areas, this worked very well, and up to 100 animals were sampled on one day. However, in other areas just one or two animals came to the collection point. This was very disappointing, as great distances were travelled to reach them.

The majority of the animals were easy to handle. In some cases the lip twitch had to be used. The animals were generally in a very good condition and well looked after.

Midge collection

The midge collection was successful. There were, however, a few instances where the collection bottle was dislodged by the wind. There were also two instances at Koperberg where some of the horses were left in the camp where the trap was situated and they managed to remove the collection bottles. There were also minimum/maximum thermometers at each of the collection points, but after six to seven months, the thermometers were not reliable anymore and the data from these thermometers were not used, but the data from the South African Weather Bureau was used instead.

Only a small percentage (<0.0001%) of the active blood-seeking females are intercepted by light traps (Meiswinkel *et al.* 2004) and these collections do not

always reflect the true biting rate on animals (Scheffer, Venter, Labuschagne, Page, Mullens, Maclachlan, Osterrieder & Guthrie 2011).

Seroprevalence of AHSV

There is a very low prevalence (4.4%) of antibody to AHSV in Namaqualand. This seems to be a true reflection of the prevalence of the disease. Although the questionnaire did not cover the occurrence of the disease in the area, it was discussed during the meetings at the collection points. African Horse Sickness and EE are unknown among the local population, and they do not vaccinate their horses against AHS.

According to the database of the South African Department of Agriculture, Forestry and Fishery, there were no outbreaks of AHS in Namaqualand from January 1993 to December 2007. However, according to the World Organisation for Animal Health (OIE) publication of 2004, a single case of AHSV serotype 1 was reported in 2001/2. According to the Springbok State Veterinarian Office Yearly Report for 2002 these samples were from the Karasburg district in Namibia. It was therefore wrongfully reported to be from Namaqualand. Despite the fact that Namibia can also be classified as a relative arid region, outbreaks of AHSV are not uncommon and seems to have increased during the last decade (Scaccia, Lelli, Peccio, Di Mattia, Mbulu, Hager, Monaco, Savini & Pini, 2009).

This study found that the prevalence for antibody to AHSV in Namaqualand was 4.4%. One of the problems with this study was that some horses were excluded based on the history known to the current owner, and that some of them could have been vaccinated by their previous owners. It should be mentioned that the inclusion of these possible vaccinated animals implies that the prevalence could be lower than the reported 4.4%.

The sample point with the highest prevalence of 13.6% (9/66) was Pella. Pella is situated close to the Orange River, and there is a possibility that *Culicoides* midge abundance might be higher than in the rest of Namaqualand. Pella was also the collection point that was the furthest east.

There were three areas sampled in the Namaqualand region where none of the equids were positive for antibody to AHSV (Figure 6). The first area included collection points Grasvlak and Uitspan in the Richtersveld region where blood was collected from a total of 29 equids. The second area was around Komaggas. This area included Komaggas, Buffelsrivier, Khoerkam, Potvlei, Agterkraal and Karootjie. In total 117 equids were sampled within this area. The third area was around Leliefontein and included Kheis, Kharkams, Paulshoek, Tweerivier, Leliefontein and Nourivier. A total of 114 samples were collected from this area.

The identification of three areas where all the sampled animals were negative to AHSV-antibodies warrants further investigation. Firstly, it would be interesting to see

if the animals in these areas remain free of antibodies to AHSV. Secondly, it would be interesting to know why antibodies against EEV was present in all three the areas. Could there be competent vectors for EEV, but not for AHSV in these specific areas. Thirdly, the occurrence and abundance of *Culicoides* midges in these specific areas still need to be determined, as these areas are in different bioregions than Springbok.

Namaqualand appears to be an area with a relative low risk of AHS. Although both the virus and vectors of this disease are present, there are no reported outbreaks in Namaqualand. There was no apparent pattern in the prevalence of antibody to AHSV amongst the different age groups. This means that there are infrequent introductions of the virus or that there are insufficient vectors or hosts to maintain an epidemic. Namaqualand can also be used as a source of sero-negative animals for use in future studies on AHSV.

Seroprevalence of EEV

The seroprevalence for EEV in Namaqualand was 30.9%. This was significantly higher than the seroprevalence for AHSV (4.4%), but lower than the reported prevalence (77%) in the rest of South Africa. It is also lower than the prevalence (83.9%) that was reported for the Northern Cape (Paweska & Venter 2004). The difference in vectors and mortality rate in horses can possibly attribute to the difference in the prevalence of these two diseases.

Areas of future research are virus detection and bloodmeal identification on the midges from this area. Serum virus neutralisation can also be used to identify the serotypes of both AHSV and EEV present in this area.

Midges

The survey reported on by Rawlings *et al.* (2003) on the occurrence of *Culicoides* in South Africa provides a good platform for comparing our data. This study was done 10 years ago, and there is a possibility that as a result of climate change, a change in the number of animals and farming practices, the disease situation might have changed.

Firstly, they found that six species (*C. bolitinos, C. imicola, C. leucostictus, C. nivosus, C. pycnostictus and C. subschultzei)* were present at all their collection sites, with the exception that *C. leucostictus* was not present at Springbok *(*Rawlings *et al.* 2003*)*. This study did find *C. leucostictus* at Springbok, but in very low numbers. Only two were collected during one collection at Biesjiesfontein, and this constituted 0.002% of the total midge catches. The other five species are still present in Namaqualand.

Secondly, Rawlings *et al. (*2003) found that the most commonly captured species at Springbok was C. #89 in association with *C. subschultzei*. During this study C. #89 made up 9.6% of the total catches and *C. subschultzei* constituted 7.4% of the total catches and were not the most commonly captured species.

Thirdly, Rawlings *et al.* (2003) found that *C. bolitinos* (0.0008%) and *C. imicola* (0.0194%) were remarkably uncommon in Namaqualand. This study also found that these midges are relative uncommon, but they did constitute a larger part of all the captures, *C. imicola* (0.9%) and *C. bolitinos* (1.5%).

It has been shown that the *Culicoides* species and especially *C. imicola* is abundant in the surveillance zone of the Western Cape (Venter, Koekemoer, Paweska 2006). The vectors were present in numbers equal to those in the traditional AHS endemic areas. The AHS-free zone can easily be compromised, as seen by the AHS outbreaks between 1999 and 2011, and the designation of the AHS-free zone in the Western Cape remains controversial (Venter *et al.* 2006). This study has confirmed that the known vectors for AHS, especially *C. imicola,* are present in very low numbers. This makes Namaqualand possibly the ideal area to establish an additional AHS-free zone, especially during times when there is an outbreak in the Western Cape.

The number of *Culicoides* in Namaqualand is far less than in the north of the country. The total number of midges caught over the year was less than the number collected during one night in the north of the country.

The difference between the number of midges caught at Koperberg and those caught at Biesjiesfontein and Goegap, could be as a result of a microclimate that was created by the irrigation at Koperberg.

This study has only collected midges from one of the bioregions of Namaqualand and further work is necessary to look at the other, especially the areas with no seroprevalence to AHSV. It would be worthwhile to determine *Culicoides* presence and abundance in these areas. This can then be used to identify a truly AHS free zone in South Africa.

Conclusion

This study showed that the seroprevalence of AHSV in Namaqualand was 4.4%. The seroprevalence of EEV in Namaqualand was determined as 30.9%. The six *Culicoides* species that where most abundant were: *C. ravus* (29.7%), *C. bedfordi* (25.2%), C. #89 (9.6%), *C. subschultzei* (7.4%), *C. herero* (7.1%) and *C. nivosus* (6.8%). *C. imicola* represented 0.9% and *C. bolitinos* represented 1.5% of the total catches.

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Appendices

Appendix A: Questionnaire

Vraelys: AHS en EE Navorsing

Eienaar:

Naam: …………………………………………………………………….

Adres: …………………………………………………………………….

…………………………………………………………………….

Tel nr: ……………………………. Sel nr: ……………………………

Diere besonderhede:

Naam: …………………………………………………………………….

Ouderdom: ……………………………………………………………….

Geboorteplek: ……………………………………………………………

Geënt teen AHS: Ja Nee

Gereis buite Namakwaland: Nee

Ja – Besonderhede: ………..…………………………………………..

Naam: …………………………………………………………………….

Ouderdom: ……………………………………………………………….

Geboorteplek: ……………………………………………………………

Geënt teen AHS: Ja Nee

Gereis buite Namakwaland: Nee

Ja – Besonderhede: ………..…………………………………………..

ANIMAL USE AND CARE COMMITTEE

INGELIGTE TOESTEMMING

Ek, die ondergetekende, gee hiermee toestemming dat die dier(e), soos hieronder gespesifiseer, deur die navorser(s), soos hieronder gespesifiseer, gebruik mag word in die prosedure(s) soos hieronder verduidelik.

1. Navorser(s)

- NAAM VAN DIE NAVORSER(S):

Dr. Christine Schütte

- NAAM VAN DIE NAVORSINGS PROJEK:

The seroprevalence of African Horsesickness and Equine Encephalosis in Namaqualand.

- DOEL VAN DIE NAVORSINGS PROJEK:

Om die seroprevalensie van AHSV en EEV in die Namakwaland-area te bepaal.

- BESONDERHEDE VAN DIE PROSEDURE(S):

Bloed word vanaf die nekaar (Jugularis) getrek deur standaard tegnieke.

Twee buisies bloed word van elke dier getrek. BD Vacutainer® SST™ buisies en 20G naald word gebruik.

- RISIKO'(S) VERBONDE AAN DIE PROSEDURE:

Daar is min/geen risiko verbonde aan die prosedure. Die dier mag dalk 'n klein bloedvint (hematoom) of blou kol kry waar die bloed getrek word.

- IDENTIFIKASIE VAN DIE DIER WAT GEBRUIK WORD:

Soos op bladsy 1 aangedui.

- ONMISKENBARE ONDERSKEIBARE BESKRYWING VAN DIE DIER WAT GEBRUIK WORD:

2. Moet deur die eienaar van die dier of deur 'n gemagtigde persoon voltooi word:

- NAAM VAN DIE EIENAAR:

Soos op bladsy 1 aangedui.

- HET JY VOLLEDIGE INLIGTING GEKRY OOR DIE STUDIE?

JA NEE ☐ ☐

- IS AL DIE RISIKO'S VERBONDE AAN DIE PROSEDURE AAN JOU VERDUIDELIK EN VERSTAAN JY HIERDIE RISIKO'S?

JA NEE ☐ ☐

- GEE JY VOLLE TOESTEMMING VIR DIE PROSEDURE?

JA NEE ☐ ☐

3. Die ondertekende partye kom ooreen dat daar geen kompensasie betaalbaar is aan die eienaar van die dier of enige iemand anders en dat alle koste verbonde aan die navorsing deur die navorser(s) gedra word.

4. Die ondertekende partye kom verder ooreen dat die vorm die Universiteit van Pretoria en die ondertekende navorser(s) ten volle vrystel van enige eise as gevolg van die gespesifiseerde prosedure deur of namens die eienaar van die dier.

5. Die ondertekende partye kom ooreen dat geen materiaal van enige soort, insluitend data en navorsingsbevindings, aan enige derde party gegee of vir enige ander doel as wat in hierdie vorm gespesifiseer is, gebruik sal word nie, behalwe met die skriftelike toestemming van die ondergetekende eienaar van die dier.

____________________________ ____________________________

HANDTEKENING NAVORSER(S) HANDTEKENING EIENAAR

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HANDTEKENING GETUIE

DATUM: ______________________________

Appendix B:

This table gives the information of the animals that tested positive to AHSV in the ELISA test.

Animal ID Number	Age in years	Place of birth	Sample point	AHSV antibody titer
3	20	Pofadder	Springbok	1700
4	12	Springbok	Springbok	20000
5	1	Springbok	Springbok	12300
7	1	Springbok	Springbok	5500
24		Nababeep	Nababeep	2500
28	17	Namakwaland	Springbok	7600
39	6	Bulletrap	Bulletrap	2000
88	3	Klipfontein	Klipfontein	3200
255	9	Alexanderbaai	Steinkopf	1900
275	7	Steinkopf	Steinkopf	3000
282	7	Alexanderbaai	Steinkopf	9000
319	15	Buffelsrivier	Nigramoep	3800
320	9	Buffelsrivier	Nigramoep	3000
321	15	Buffelsrivier	Nigramoep	5100
322	20	Eksteenfontein	Eksteenfontein	3200
475	9	Rooifontein	Rooifontein	13000
493	13	Kleinsee	Soebatsfontein	10000
510	6	Rooiwal	Kotzehoop	9700
519	2	Springbok	Kotzehoop	26000
520	2	Rooiwal	Kotzehoop	23000
534	33	Gladkop	Taaibosmond	20000
553	12	Witbank	Witbank	4600
581	12	Blesfontein	Steinkopf	2200
643	4	Steinkopf	Wolfberg	12000
662	5	Pella	Pella	3500

669	10	Pella	Pella	14000
671	9	Pella	Pella	1200
672	9	Pella	Pella	15000
698	15	Pella	Mik	10000
699	3	Pella	Klein Pella	27000
819	18	Annakop	Annakop	11000
825	6	Pella	Pella	5000
829	7	Pella	Pella	20000
830	6	Pella	Pella	7600
831	7	Pella	Pella	5000
833	10	Pella	Pella	5500
838	25	Pofadder	Aggeneys	2200

Appendix C:

In the first column is the date of the collection. In the next three columns are the total midges that were collected per collection site. An asterix indicates that there was no collection made on that date. A zero indicates that the trap was set, but no midges were collected. In the following six columns are the weather information as supplied by the South African Weather Bureau from the weather station set in Springbok (29.6710S 17.8870E Height above sea level:1 006 m).

Date	Goegap	Biesjes-fontein	Koper-berg	Min °C	Max °C	Wind Speed at 20:00	Wind direction at 20:00	Rainfall	Humidity at 20:00
30/06/2008	6	9	*	11.7	23.1	3.5	N	0	31
14/07/2008	0	73	*	11.3	21.7	3.7	WNW	0	50
21/07/2008	0	17	*	13.1	23.3	0	0	0	62
28/07/2008	0	26	*	7.5	18.6	6.8	NNE	0	52
04/08/2008	0	23	*	12.7	22.2	0	0	0	55
11/08/2008	0	211	473	12.7	25	2.5	NNW	0	19
20/08/2008	34	180	547	12.4	26.4	4.4	W	0	60
26/08/2008	0	0	0	2.1	17.7	2.7	NE	0	36
02/09/2008	0	4	4	5.7	19.8	3.1	SSW	0	93
10/09/2008	3	0	2	5.1	11.4	5.1	S	0.2	91
17/09/2008	40	83	167	4.6	20.9	4.4	NW	0	35
24/09/2008	5	6	1	3.3	16.7	1.2	SSE	0	83
02/10/2008	0	3	0	5.5	10.5	4.2	S	2.6	100
08/10/2008	0	4	238	8.7	16.4	6.4	SW	0	100
15/10/2008	150	255	629	12.6	26.7	4.6	SSW	0	59

Date	Goegap	Biesjes-fontein	Koper-berg	Min °C	Max °C	Wind Speed at 20:00	Wind direction at 20:00	Rainfall	Humidity at 20:00
21/10/2008	0	0	1	7.7	18.1	6.2	SSW	0	81
28/10/2008	17	100	2728	10.6	27.7	5	W	0	76
04/11/2008	1008	2040	14196	13.2	30.6	4.3	WSW	0	20
11/11/2008	0	0	7	8	19	5.3	SW	0	97
18/11/2008	223	1937	15861	13.1	29.9	5.4	S	0	43
25/11/2008	315	4256	11564	19.9	35.7	3.5	SSW	0	32
03/12/2008	511	655	8400	15.4	30.4	4.7	SW	0	49
17/12/2008	734	2299	9963	22.1	35.9	5.4	SSW	0	30
31/12/2008	0	38	741	13	25.9	5.7	WSW	0	68
08/01/2009	*	11	19	13.1	28.5	6.7	W	0	66
13/01/2009	430	332	4920	12.9	25.1	7.2	SSW	0	90
21/01/2009	0	0	*	13.8	25	6.2	WSW	0	84
28/01/2009	256	*	5160	14.9	30	4.4	SW	0	45
03/02/2009	54	8	0	13.7	29.2	6.6	S	0	42
10/02/2009	121	68	1394	19.1	28.2	6.4	SSW	0	57
17/02/2009	57	1263	7890	24.2	34	6.1	W	0	20
25/02/2009	183	2319	*	19.7	32	2.6	S	1.6	62
06/03/2009	1155	1091	*	22.9	32.8	3.3	WNW	0.6	53
11/03/2009	16	125	2224	20.7	29.5	3.8	SW	0	60
18/03/2009	55	4	106	12.3	24.5	5.6	SSW	0	49
26/03/2009	541	292	6060	15.8	31.4	2.7	SW	0	47
02/04/2009	22	612	*	23.3	32.4	5.7	NNE	0.2	47
08/04/2009	217	18	3278	22.7	34	1.6	WSW	0	47
14/04/2009	29	0	9128	22.5	34	2.2	W	0	25
21/04/2009	1	1808	*	13	25.4	3.3	NE	0	34

Date	Goegap	Biesjes-fontein	Koper-berg	Min °C	Max °C	Wind Speed at 20:00	Wind direction at 20:00	Rainfall	Humidity at 20:00
23/04/2009	*	*	76	10.2	18.8	3.6	W	7.6	91
07/05/2009	25	0	*	9.7	20.4	3.8	SSW	0	89
15/05/2009	35	7	*	8.8	17.6	4.6	W	0	96
25/05/2009	9	112	170	10.2	22.2	1.7	SE	0	64
01/06/2009	0	0	355	10	20.2	4.4	W	0	64
08/06/2009	1	*	1	7.6	16.9	6.8	ESE	0	56
11/06/2009	*	0	*	10.5	21.7	0	0	0	63
18/06/2009	0	1	*	10.7	20.8	4.6	NNE	0	40
29/06/2009	*	*	2	11.5	19	5.5	N	0	40
02/07/2009	*	7	*	11.9	21.4	1.9	WNW	0	66
06/07/2009	*	*	1	11.7	21.6	0	0	0	54
09/07/2009	*	10	*	14.1	23.2	3.4	WNW	0	40
16/07/2009	*	0	*	6.7	13.7	7.5	NNE	0	43
23/07/2009	*	0	*	3	11.7	6.1	E	0	44
30/07/2009	*	*	0	6.1	11.9	4.1	WSW	0.4	100