PROPHYLACTIC STRATEGIES IN THE CONTROL OF AFRICAN HORSE SICKNESS

Tarryn Lyn Simpkin

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School of Agricultural Sciences and Agribusiness
Faculty of Science and Agriculture
University of KwaZulu-Natal
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I hereby certify that this research is the result of my own investigation. Where use was made of the work of others, it has been duly acknowledged in the text. The results in this dissertation have not been submitted, in whole or in part, for a degree at any other University.

Tarryn Lyn Simpkin
October 2008

I hereby release this thesis for examination in my capacity as supervisor.

Ms MB Young
October 2008

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ABSTRACT

African horse sickness (AHS) is a non-contagious viral disease transmitted by an arthropod vector and is endemic to sub-Saharan Africa. The disease affects all equine species, but is more severe in horses and other equid species not native to Africa. Vaccination is the only demonstrated means of its prevention. The horse-owning public provides much anecdotal evidence of prophylactic strategies, such as repellents, stabling, alternate hosts, traps, paraffin, blankets, smoke or fans.

The present study investigated the relationship of these strategies to the incidence of AHS, and evaluated alternate hosts, wind speed and repellents on the activity of males and females of the different *Culicoides* species.. Cypermethrin and citronella-containing repellents repelled the most female midges. Sheep and cattle offer an alternate blood meal to gravid and nulliparous female midges. Fans are very effective in keeping midges away from horses. Methods are summarised for the horse owner to implement in addition to vaccination to prevent AHS.

Keywords: prevention, African horse sickness, *Culicoides*, midge, repellents, alternate hosts, wind speed

CHAPTER 1 AFRICAN HORSE SICKNESS

1.1 Introduction

African horse sickness (AHS) is a non-contagious viral disease transmitted by an arthropod vector and is endemic to sub-Saharan Africa. The disease affects all equine species, but is more severe in horses and other equid species not native to Africa (European and Asian donkeys) (Lord *et al.*, 1997). AHS is an “A list”disease at the World Organisation for Animal Health (OIE, 2006). It’s a highly infectious and virulent disease and like most other viruses cannot be directly treated for. Although AHS is endemic to Southern Africa there have been outbreaks in other parts of the World including Spain, Portugal, India, Pakistan (Mellor & Hamblin, 2004), with worldwide figures from 1996 to 2004 showing 1659 outbreaks (OIE, 2006). It is therefore crucial that adequate measures are discovered to control and even possibly prevent the habitual reappearance of this viral disease.

In the last ten years South Africa has experienced more than 20 different outbreaks (sudden eruptions of the disease in a specific area) each year, with one year having 196 different outbreaks. From the year 1996 until 2004 there have been more than 2000 equine deaths and around 3094 cases of AHS reported (OIE, 2006) (Figure 1). Data collected for the 2005/2006 season has already shown that there have been 844 cases of AHS and 143 deaths (AHS, 2006). In a country where the horseracing gambling revenues alone are around R800 million (CASA, 2006), let alone other disciplines such as polo, eventing, showjumping and dressage, where horse prices can move into hundreds of thousands, it seems imperative that this disease is controlled before the major economic effects are truly felt. Effects in the second economy are important as well. Thousands of horses and donkeys are used in traction (Pearson, 2006) and in transport in the rural areas. Many people derive a livelihood from the

activities of these animals, and as they are largely unvaccinated, they are decimated in outbreaks.

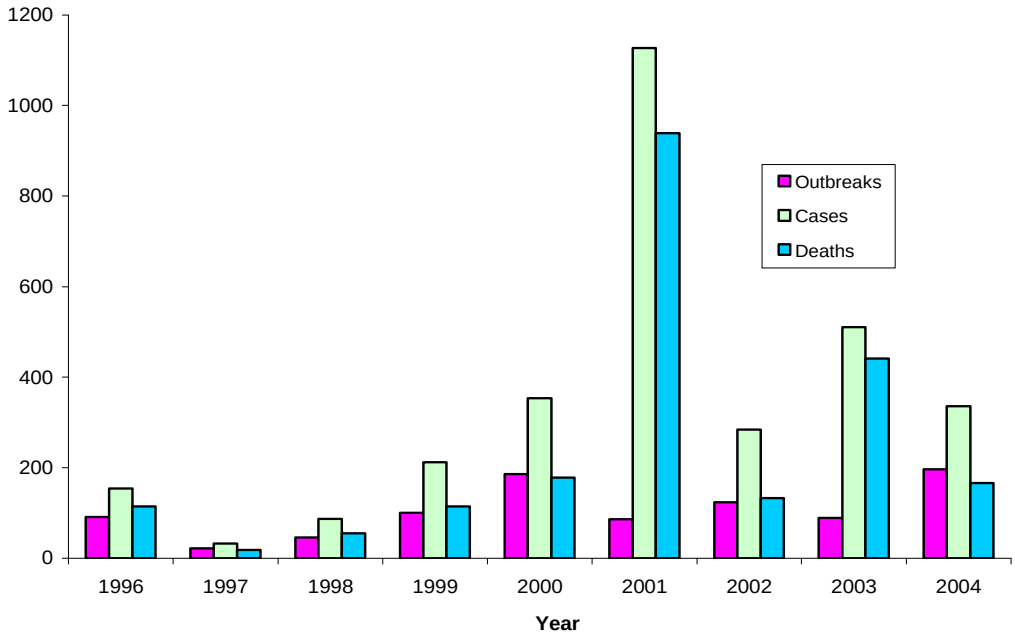

Figure 1 Outbreaks, cases of and deaths due to African horse sickness in South Africa from 1996 to 2004.

Although Gauteng is the main area for outbreaks, KwaZulu-Natal (KZN) is fast becoming a second hotspot for AHS. From 1996 to 2004, KZN had a total of 126 outbreaks spread over the eight years (OIE, 2006), while it ranked second highest in terms of provincial counts in the 2005/2006 AHS season, having 105 horse sickness outbreaks (AHS, 2006). It is clear from these figures that KZN is now a central region for AHS and due to the differences in climate and rainfall between KZN and Gauteng it is crucial that prevention and control methods for this area be investigated.

1.2 Origins

African Horse Sickness was first referenced in conjunction with an epidemic that occurred in the Yemen in 1327 (Moule, 1896, cited by Mellor & Hamblin, 2004). The virus is said to almost certainly

originate from Africa, although explorations documented by Father Monclaro relate how Franciso Baro, who journeyed through East Africa in 1569, witnessed horses that had been brought over from India dying from an unexplained illness (Theiler, 1921). The Dutch settlers who arrived at the Cape of Good Hope in 1652 witnessed this illness (Coetzer & Erasmus, 1994). It was only documented in Southern Africa 60 years after the initial introduction of horses in 1657 (Mellor & Hamblin, 2004). The first major outbreak of the disease known then as 'perrezieke' or 'pardeziekte' occurred in 1719 killing more than 1700 animals (Coetzer & Erasmus, 1994). Major epidemics of the disease in Southern Africa have been reported from 1780 and seem to occur every 20 to 30 years. The most severe historical losses were reported in years 1780, 1801, 1839, 1855, 1862, 1891, 1914, 1918, 1923, 1940, 1946 and 1953. The 1855 outbreak was seen to be the most ruthless with 70 000 horses dying, about 40% of the equine population in the Cape of Good Hope (Theiler, 1921).

In 1855 the disease first appeared in Eastern towns and moved westwards as far as the mountains known as Clanwilliam. The virus was reported throughout South Africa except for the Cape. Reasons for this were attributed to rainfall data that showed the Cape to have less rain than other cities (where rainfall was recorded). This is the first evidence that shows the major link between horse sickness, climate and rainfall patterns (Theiler, 1921).

1.3 **Aetiology**

The causative agent of AHS is the African Horse Sickness virus (AHSV). The AHSV is a viscerotropic virus of the family *Reoviridae* (derived from the phrase "respiratory enteric orphan viruses" because it was initially said to be a virus in search of a disease). AHSV belongs to the genus *Orbivirus* (Roy *et al*., 1994) and there are nine antigenically distinct serotypes (Howell, 1962).

1.3.1 Virus Structure

The AHS virus particles are icosahedral in appearance and measure in the region of 55 to 80nm (Wood, 1973) and are unenveloped (Figure 2).The AHSV consists of 7 structural proteins known as viral proteins (VP). These VP's are organized into two distinct layers in a capsid. The outer capsid layer consists of proteins VP2 and VP5. The core or inner capsid consists of both minor and major proteins. The major proteins are VP3 and VP7 and the minor proteins are VP1, VP2 and VP6 (Roy *et al*., 1994).

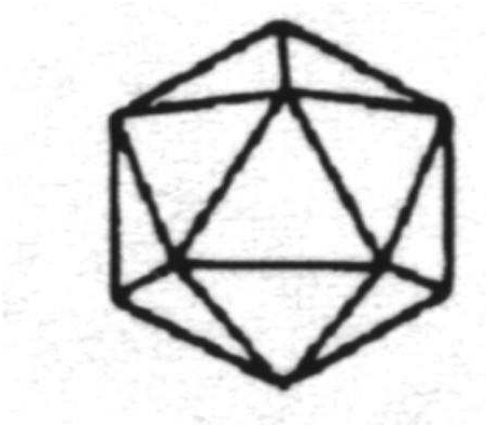

Figure 2 Diagram of the icosahedral shaped virus family, Reoviridae, to which AHS virus belongs.

The virus particles contain only double-stranded RNA (dsRNA) in their genome (Wood, 1973). In the genome core there are ten of these dsRNA segments consisting of three different sizes: large, medium and small (L1-L3, M4-M6 and S7-S10 respectively). Mobility of the virus is determined by how far the fragments move on a SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) and this differs between the nine serotypes of the virus (Roy *et al*., 1994). Besides the seven major structural proteins (VP1- VP7), there are also four non-structural proteins (NS1-N43 and NS4A) (Grubman & Lewis, 1992), conferring characteristics used for the detection of

the virus through different techniques (Groenink; *pers.comm.*). Table 1 shows an example of the coding assignments for each of the genome segments, according to each virus protein, using AHSV serotype 4.

Table 1 AHSV serotype 4 proteins and genome coding assignments, used in viral identification (Grubman & Lewis, 1992)

Viral Proteins	Genome Segment	Location
VP1	1	Core
VP2	2	Outer capsid
VP3	3	Core
VP4	4	Core
VP5	6	Outer capsid
NS1	5	Nonstructural
VP6	6	Core
NS2	8	Nonstructural
VP7	7	Core
NS3	9	Nonstructural
NS4	10	Nonstructural
NS4a	10	Nonstructural

Virus Protein 2 is a major component of the surface of the AHSV. It is also the principle serotype specific antigen for vaccination purposes and therefore also used in serotype detection. VP 5 is less variable between serotypes and VP 3 the most conserved of the inner capsid proteins (Roy *et al*., 1994).

Physico-chemically the AHSV is typical of Orbiviruses (Mellor and Hamblin, 2004). Temperatures of 50^{o}C inactivate the virus after 3 hours exposure or 60^{o}C after 15 minutes exposure. The virus can survive at 37^{o}C for 37 days (OIE, 2002). The virus is stable at alkaline pH values of around 7.0-8.5 (Normal pH of horse blood is 7.42-7.45) (Mellor & Hamblin, 2004).

1.3.2 Mode of Action

The infection cycle of any arthropod-borne virus (arbovirus) starts with the attachment of the virion to the host's cell surface. After that the virus is internalized and the viral attachment proteins interact with complementary receptor sites on the host's cell surface (Roy *et al*., 1994).

After infection the initial multiplication of the AHSV occurs in the regional lymph nodes (Mellor & Hamblin, 2004). Primary viraemia follows with subsequent infection of target organs, specifically the lungs and lymphoid tissues. The virus will then multiply at these sites leading to secondary viraemia (Coetzer & Erasmus, 1994). Secondary viraemia of AHS in horses lasts roughly 4 to 8 days, generally not exceeding 21 days (Coetzer & Erasmus, 1994).

In research, experimentally infected horses were shown to have high concentrations of virus in their spleens, lungs, caecum, pharynx, choroid, plexus and most of their lymph nodes by the second day post infection, followed by the onset of fever or viraemia. By the 3rd day post infection it was shown that the virus was present in most organs (Erasmus, 1972; cited by Coetzer & Erasmus, 1994).

1.3.3 Virus Serotypes

At the present moment, nine different serotypes of the AHSV have been identified (Howell, 1962), each of which are antigenically distinct. Some though are cross neutralized or have some cross relatedness. This relatedness can be seen between AHS serotypes S1 and S2, S3 and S7, S5 and S8 and between serotypes S6 and S9 (Mellor & Hamblin, 2004). Original work referred to a Strain O, which was the first and original strain used extensively by Theiler (Alexander, 1935) and documented in his work in 1921. According to Alexander (1935) two strains were subsequently found in

Onderstepoort in 1932 and 1933 and were named strain 20449 and strain 20464 respectively, but since the date of his publication in 1935 no other strains have been identified. Alexander (1935) also found in his works that strain O and strain 20449 failed to cross neutralize each other while strain O neutralized strain 20464. Howell (1962) in research that identified the final two AHS serotypes gave different identification names for the various serotypes. Strains one to nine were identified as A501, OD, L, 47/58, VH, 114, Karen, 18/60 and 7/60 respectively.

No relationship has been found between certain serotypes and clinical forms of AHS. If certain serotypes could be related to the more acute forms of the disease (especially the pulmonary form where death occurs exceeding quickly), more effective monovalent vaccines could be developed to reduce mortalities.

1.4 Epidemiology

African horse sickness is endemic to tropical and sub-tropical areas of Southern Africa, not restricted to South Africa. The Sahara had seemed to provide a barrier against AHS moving into North Africa (Mellor & Hamblin, 2004), but Ethiopia has had a number of cases over the last ten years (OIE, 2006)

1.4.1 Hosts

It has been shown that the causative virus, AHSV is able to infect most species of Equidae (Lord *et al*., 1996). Zebra are considered to be the natural vertebrate host of the AHSV as they do not exhibit signs of infection but are able to carry the disease (Mellor & Hamblin, 2004). Other species of Equidae; horses, donkeys, mules and other

crossbreeds are susceptible to infection and react to the virus. They are therefore referred to as indicator hosts (Mellor & Hamblin, 2004).

Other wild animals have been surveyed for infectious diseases. In one of these studies on Black and White rhinoceros Fischer-Tenhagen *et al*. (2000) found that rhino from the Kruger National Park (Mpumalanga Province), Mkuzi Game Park (KwaZulu-Natal Province) in South Africa and from three games parks in Kenya tested positive for the AHSV! As this was the first study of its kind on rhinoceros, it is not clear whether the rhino can play host to the virus for any length of time and therefore play any key role in the epidemiology of African Horse Sickness.
It was also shown that dogs are susceptible to the AHSV (Theiler, 1921), but this has been seen to be mostly via experimental infection or after the ingestion of infected horse meat.

Theiler (1921) also made the observation that experimentally infected Angora goats developed typical African horse sickness fever but no other symptoms. Though it has not been documented that sheep and goats are susceptible to African horse sickness, the principle vector for African horse sickness and BTV (Bluetongue virus) that affects sheep and goats is *C. imicola* (Conte *et al*., 2004). Other studies on the host preference of *Culicoides* midges showed that the same species of midge that was found to feed on horses had also fed on cattle and sheep. In this case the species identified was *C. pallidipennis* (Nevill & Anderson, 1972), which was found by Lubenga & Khamala (1976) to be equivalent to *C. imicola*. Another viral disease found to affect cattle (three-day stiff-sickness) is also transmitted by the same insect vector.
Therefore the AHS vector does not exclusively feed on horses and this may in fact be used as a tool to reduce the risk of AHSV infection. It is not known though if these other commercial livestock species harbour the virus for any length of time and then again whether or not they can be implicated in the cycle of AHS. Further

research in this sector could become vital in identifying a possible over-wintering mechanism for the virus.

1.4.2 Vectors and Transmission

Transmission of the African Horse Sickness virus (AHSV) is achieved through the *Culicoides* midge spp.. *Culicoides* spp. (Family Ceratopogonidae) are biting midges and are amongst the most abundant and smallest of all haemotophagous insects being only 1 to 3mm in size with more than 1400 species having been identified (Mellor *et al*., 2000). *Culicoides* spp. are crepuscular (peak activity is between sunset and sunrise) and only the females blood-feed (Mellor *et al*., 2000). Adult vector longevity dropped three fold when temperatures rose from 15℃ to 30℃ (Mellor *et al.,* 2000; Whitman, 2000). Egg development rates within the females increase as temperature rises and so there is a shorter period between batches of eggs and so more batches are laid and thus more blood meals required by the vector females (Lindley 1966; Wittman & Baylis, 2000). This increases the midge's vector potential (Whitman *et al.,* 2002). The insect vector only lives a few weeks, usually 10-20 days but up to 90 days in exceptional cases (Mellor *et al*., 2000). Most *Culicoides* species can survive for long periods as the fourth instar larvae (Whitman & Baylis, 2000, Mullen, 2002).

The rate of viral transmission rises above 25℃ (Welby *et al.,* 1996; Mellor *et al.* 2000; Wittman *et al.* 2002; Capela *et al.* 2003) and stops between 10℃ (Welby *et al.,* 1996) and 15℃ (Mellor *et al.,* 2000). Wittman *et al.* (2002) found that the rate at which AHSV serotype 4 is incubated in *C. sonorensis* was reduced from 18 days at 15ºC to only 5.7 days at 30ºC. Low average annual temperatures stop effective virogenesis above 44ºN (Capela *et al.,* 2003). No transovarial transmission of the virus occurs in *Culicoides* species (Whitman & Baylis, 2000). The virus cannot remain active in the insect because the adults do not live through the four or five winter months (Nevill,

1967), and the overwintering larvae are not infected with the virus until they emerge as adults and get infected by biting a mammal host (Whitman & Baylis, 2000; Mullen, 2002). Only the sick and surviving animals are viraemic (capable of passing on the disease to a vector). Horses are only viraemic for 4-8 days and never more than 21 days (Coëtzer & Erasmus, 1994). Capela *et al.* (2003) postulated that interspecific virus cycling keeps AHSV active from one summer to the next.

Availability of the virus from a reservoir host (still not documented), a susceptible host and the presence of an arthropod vector (Venter *et al*., 2000) as well as the lifecycle and replication of the virus in the midge are therefore all contributing factors in the transmission of the AHSV.

Initially it was proposed that the *Culicoides imicola* Kieffer species was the only field vector involved in the transmission of AHSV (Mellor, 1993). Venter *et al*. (2000) later found that *C. bolitinos* tested positive for carrying AHSV serotypes S3, S5 and S8. Meiswinkel & Paweska (2003) later confirmed that *C. bolitinos* was a new field vector for the AHSV. From these same studies it was also noted that at 14 sites in the eastern Free State *C. bolitinos* was the most abundant species to be found, making up 65% of all *Culicoides* captured (Venter & Meiswinkel, 1994). Meiswinkel & Paweska (2003) also found that 17 other species of *Culicoides* captured during collections in outbreak areas yielded no virus. The conclusion was that *C. imicola* and *C. bolitinos* are exclusive vectors of AHS in South Africa.

1.5 **Diagnosis**

Infection with AHS virus manifests itself in four clinical forms. These four forms vary in their intensity and mortality and have different symptoms and prognoses. African Horse Sickness can be a mis-

diagnosis for other equine diseases, and laboratory diagnosis of blood and tissue samples is necessary to identify the virus correctly.

1.5.1 Subclinical AHS

This form of the disease is often called horse sickness fever and occurs in horses that have some degree of immunity to the AHSV (Coetzer & Erasmus, 1994) and is a very mild form of the disease. This form of the disease is also the only form exhibited in the African donkey and zebra (Mellor & Hamblin, 2004). The only symptom usually exhibited is an elevation in body temperature, usually between 39^{o}C and 40^{o}C. Fever normally lasts about 6 days (Coetzer & Erasmus, 1994). Slight facial oedema may also be present (Mellor & Hamblin, 2004). In some cases secondary symptoms are observed such as drop in appetite, congestion, slight respiratory difficulty and an increased heart rate (Coetzer & Erasmus, 1994). Mortality is rare. AHS fever can often be misdiagnosed as symptoms are very similar to those of equine encephalosis (Mellor & Hamblin, 2004). It is also to be noted that both the equine encephalosis virus (EEV) and AHSV have the same insect vector (Coetzer & Erasmus, 1994), although EEV may also have other as yet unidentified vectors Laboratory diagnosis through molecular techniques or virus neutralization is therefore necessary to avoid differential diagnoses.

1.5.2 Subacute or Cardiac AHS

This form of the disease is also commonly known as ‘Dikkop’ (thick head). It occurs in the late phase of infection and is the most notable symptom of the subacute form. The symptom is a result of subcutaneous swelling of the neck and head, most predominately of the supraorbital fossae (Coetzer & Erasmus, 1994) (Figure 3). Oedema is normally preceded by a fever of about 39-41^{o}C (Mellor & Hamblin, 2004 and OIE, 2002). This form is normally milder than the pulmonary form with mortality rates of approximately 50% (Coetzer &

Erasmus, 1994). In this form of the disease death is usually longer in coming, normally taking a week for the horse to die (OIE, 2002). Colic may also be a feature of this form of AHS as well as difficulty in swallowing when facial oedema is severe (Coetzer & Erasmus, 1994).

Figure 3 A horse displaying supraorbital oedema, which is a common symptom of the "dikkop" form of AHS.

In terms of pathology, a yellowish gelatinous oedema is present in the subcutaneous and intramuscular connective tissue of the forequarter region (Coetzer & Erasmus, 1994). Hydropericardium can also be seen along with haemorrhages on the epicardial and/or endocardial regions (Mellor & Hamblin, 2004). Rarely, pale-grey areas may be seen in the myocardium as well (Coetzer & Erasmus, 1994). Lymph nodes are also swollen and oedematous.

1.5.3 Acute or Respiratory AHS

This form of the disease is also commonly known as the 'Dunkop' (thin head) or pulmonary form AHS. It usually occurs when very susceptible equids are infected, such as foals (Coetzer & Erasmus, 1994) or in serologically naive populations.

Following incubation of the disease, fever (39-41^{o}C) progresses for 1 to 2 days (Mellor & Hamblin, 2004). This is followed by respiratory distress, severe dyspnoea and coughing, sometimes followed by a

frothy discharge from the nostrils (Coetzer & Erasmus, 1994) (Figure 4). This form of the disease develops rapidly and may cause death within a few hours. Very often the only sign of illness is an elevated temperature. In few cases, the disease proceeds long enough to show symptoms such as flared nostrils, protruding tongue, sweating, and extension of the head and neck. Respiratory rates may reach up to 75 breaths per minute (Coetzer & Erasmus, 1994). This form of the disease is extremely fatal and mortality rates usually exceed 95% (Mellor & Hamblin, 2004). Death in this form of the disease usually results from anoxia.

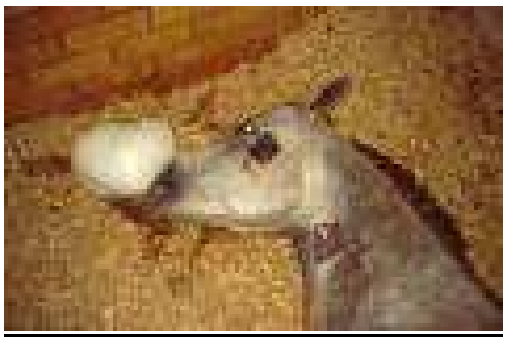

Figure 4 A frothy discharge from the nose usually accompanies a high fever and respiratory distress in horses with pulmonary or acute form of AHS.

Pathology of this form of the disease is characterized by severe oedema of the lungs and hydrothorax. The thoracic cavity is often filled with a pale yellow fluid (Coetzer & Erasmus, 1994). The bronchial tree will usually contain a froth (Mellor & Hamblin, 2004) as well as a serofibrinous fluid that may appear gelatinous (Coetzer & Erasmus, 1994). Lymph nodes may appear slightly enlarged, with bronchial and mediastinal nodes being very swollen and oedematous (Coetzer & Erasmus, 1994)

1.5.4 Mixed Form

The mixed form is the most common form of AHS although it is usually not diagnosed as such. Though both the cardiac and

pulmonary forms are present, one will usually be dominant over the other and thus will be diagnosed as such (Coetzer & Erasmus, 1994). Horses may show signs of respiratory distress characteristic of the peracute form initially, followed by oedema or vice versa (Coetzer & Erasmus, 1994). Mortality rates for this form of the disease are usually around 70% and death will normally occur 3 to 6 days after the initial fever (Mellor & Hamblin, 2004). Pathological signs are characteristic of both forms of the disease.

1.5.5 Differential Diagnosis

Specific AHS symptoms can be confused with symptoms of various other equine diseases such as anthrax, equine infectious anaemia, equine viral arteritis, trypanosomosis, equine encephalosis, piroplasmosis and purpura haemorrhagica (OIE, 2006). The most common differential diagnosis is Equine encephalosis. As the equine encephalosis virus (EEV) is also an *Orbivirus* closely related to the AHSV, many of the symptoms shown by the two viruses are very similar (Mellor & Hamblin, 2004). As both viruses are carried by the same *Culicoides* vector it is also possible to have them occur at the same time and in the same location (Coetzer & Erasmus, 1994). It has also been noted that many cases diagnosed as AHS fever may actually be equine encephalosis, and that AHS may not even be present but that all cases may actually be very similar EEV (Howell; *pers.comm.*). Equine viral arthritis and purpura haemorrhagica show similar oedema and haemorrhages as found in the case of the pulmonary form of AHS (Coetzer & Erasmus, 1994)

1.6 Prevention and Control Methods

As there are no specific treatments for AHS, those animals unfortunate enough to contract the disease have to be treated symptomatically, but due to the virulence of this virus, this is more often than not, inadequate to prevent death. It is critical that the

issues of prevention and control be addressed. There are two major targets in prophylaxis for AHS, vaccination and suppression of the vector. Because of the number of serotypes involved, horses may remain without complete immunity after the first vaccination for AHS (Coetzer & Erasmus, 1994), and even horses vaccinated annually may still be susceptible to the virus after several vaccinations (Mellor & Hamblin, 2004). For this orbivirus, therefore, control of and isolation of horses from the vector is the second part of AHS prophylaxis. There are a vast number of ways that this can be done, many of which will be covered in greater detail in this chapter.

1.6.1 Vaccine

The only vaccine available in South Africa against AHS is a polyvalent, attenuated vaccine which contains AHS serotypes 1,2,3,4,6,7 and 8 (Mellor & Hamblin, 2004). Strains 5 and 9 are not included in the vaccine due to cross relatedness between AHS serotypes S1 and S2, S3 and S7, S5 and S8 and between serotypes S6 and S9 (Mellor & Hamblin, 2004). The virus is live and therefore contains the actual AHS virus. Live vaccines are used because they create a stronger immune response and also because the chances of hypersensitivity are reduced (Tizard, 1992). Because the vaccine is live, it would obviously cause the animal to become infected unless something is done to the virus to reduce its virulence. This is why the AHS vaccine is attenuated. Attenuation is the process of reducing the disease- causing capacity of the virus while still keeping it ‘alive’. A simple method of attenuation may just involve heating the virus until just below the point that would cause death and therefore force it to inactivate some chemicals (Tizard, 1992). The most common and superior way of attenuating a virus is to pass the virus through a laboratory host a number of times (Boyle, 1994). The AHS virus was initially passed through suckling mouse brain. These early vaccines gave good immune responses but sometimes led to side effects (Alexander *et al*., 1936).

If vaccination is to be successful in controlling or preventing outbreaks, then not only does the vaccine itself have to be effective, but there also has to be a good vaccination program that is well managed. In South Africa, there is a basic vaccination guideline supplied by the manufacturer of the vaccine. The current guideline is for the initial vaccine to be given in spring, between August and September (Onderstepoort Biological Products, 2006). This is extremely important because starting a vaccination program after the virus is present will not stop an outbreak from occurring (Lord *et al.*, 1997). Midges start appearing around mid-August to September and then drastically increase in number after the first substantial rainfalls. This usually occurs around November or December in the summer rainfall areas (Meiswinkel *et al.*, 1994). This rise in midge numbers can be correlated to the simultaneous increase in AHS outbreaks,(Figure 5) and it is therefore imperative that by this time of year all animals have sufficient immunity. Immunity only starts to develop two to three weeks after inoculation and immunity against some serotypes may take up to four weeks or longer to develop. One needs also to remember that they need to allow for at least three weeks between the administration of the first and second vaccinations (Onderstepoort Biological Products, 2006). This shows how essential the timing of vaccination is to provide an efficient immunity.

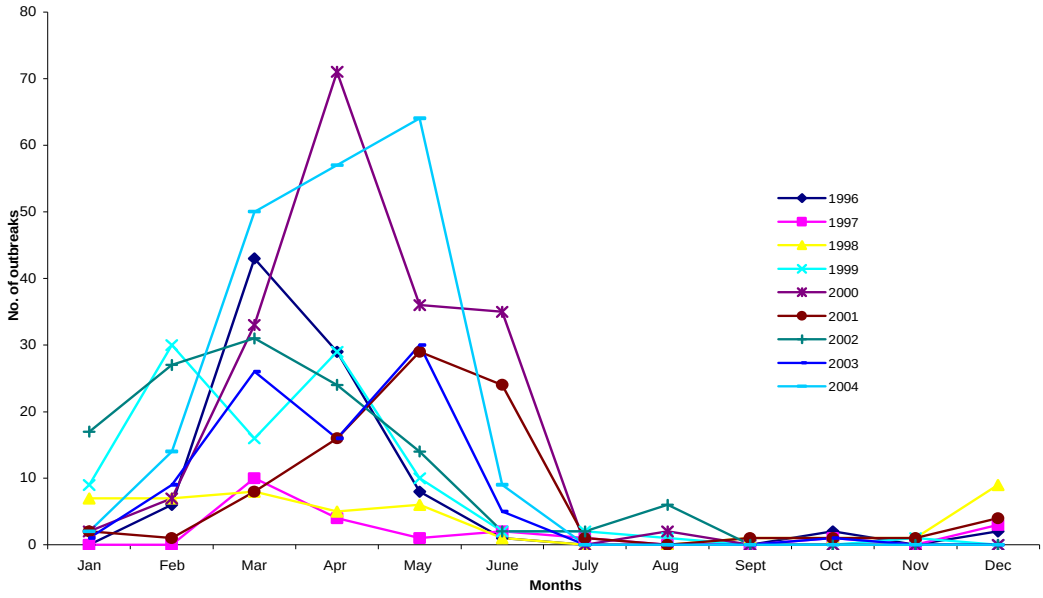

Figure 5 Average monthly outbreaks of African Horse sickness in South Africa between 1996 and 2004.

It is essential that all horses and other equines such as donkeys and mules are also given vaccines. In order to prevent AHS epidemics from occurring in this country, 90% of horses or 75% of horses and donkeys need to be properly vaccinated (Lord et al., 1997). It is therefore essential that an amended vaccination program is developed and that more legislation is put in place to ensure that all equids in South Africa outside the AHS controlled area are vaccinated annually.

1.6.2 Vector Control

This is done to reduce the risk of potential infection by reducing the numbers of the vector involved. It is rare that one is able to destroy an entire population. By controlling numbers one can not only reduce the current threat of infection but also the chances of larger outbreaks or epidemics in the future (Mellor & Hamblin, 2004). There are three major means of targeting a vector that will substantially reduce numbers: habitat alteration, larviciding and adulticiding.

Habitat alteration (removing or destroying the areas in which the vector breeds) can reduce the number of midges and possibly encourage midges to move away from the area to find alternative breeding grounds. The *Culicoides* larvae are usually found in damp areas rich in organic matter (Service, 1971). The two species of *Culicoides* implicated in AHS in South Africa have two different breeding areas. *C. imicola* is usually found in moist clay type soils while *C. bolitinos* reside in the dung of cattle, buffalo and wildebeest (Meiswinkel & Paweska, 2003) and therefore habitat alteration needs to take into account both these areas. It is important therefore to make sure that the farm does not unintentionally provide good breeding sites. Leaking pipes and taps, over-flowing or broken water troughs can all lead to the formation of damp soil areas where *C. imicola* can breed. Though *C. bolitinos* doesn't breed in horse faeces, dung piles can aid in enriching a particular soil and with the wet weather received during the height of the midge season, a perfect breeding site can develop for *C. imicola*. Increases in moisture, permanence of wetness and incident radiation all positively increase midge catches (Jenkins, 2008). Obviously if there are cattle or other bovine species in the same area as the horses then the proper management of all faecal waste is essential. Other areas where midges may breed may not be so easily destroyed or altered and this is where the second step of vector control is used.

Larviciding is a means of chemical control that works by targeting and killing the larval stage of the vector. Initially this was done with the use of oils such as paraffin that spread evenly over water and release toxic hydrocarbons (Service, 1971). Unfortunately due to the nature of the breeding sites of the *Culicoides* this is not effective or safe for livestock. At this time there are many other specific larvicide compounds available that are granulated and can therefore be placed on the soil surface and hence are more effective against *Culicoides* larvae. Some of these compounds are designed to

provide a slow release of insecticide and can be effective for up to 30 days (Holbrook, 1985 cited by Mellor & Hamblin, 2004).

The last method of vector control is adulticiding. This normally takes on the form of fogging (using ultra high voltage sprayers) an area with a fine mist of insecticide. The only problem is that the insecticides do not specifically target the *Culicoides* species and therefore the possibility of destroying other insect populations exists. There are insecticides available for application to horses that will kill the insects when they feed on the animal (Braverman, 1989 cited by Mellor & Hamblin, 2004).

At present not much has been definitively established with regards to biological control, as it has been found that *Culicoides* have very few natural predators or pathogens (Service, 1971).

1.6.3 Managerial

In addition to vector control, one can endeavor to prevent the midges from biting the horse. This managerial aspect in the context of the spread of the AHSV entails controlling animal movement, stabling, and prophylactic interventions in the separation of midge and horse

1.6.3.1 Animal Movement Restrictions

Movement of animals on and off the premises needs to be considered. Zebra and many species of African donkey rarely exhibit any clinical signs of infection (Mellor & Hamblin, 2004). It has also been seen, though not scientifically documented, that some horses may also be sub-clinical for the disease (Welsh, 2006; *pers. comm*.). In these cases, animals will have contracted the virus but will not be showing symptoms, possibly due to being well vaccinated and can therefore inadvertently bring the virus into the stable and increase the resident horses' chances of infection. If at all possible, horses should be tested (via molecular tests, Groenink, *pers.comm.*) for the

presence of the AHSV or a short quarantine period should be observed in case the horse is in the early incubation stage of infection and has not yet shown symptoms. Under most conditions it would normally take less than nine days from time of infection until the point of secondary viraemia, though it has been shown that this may vary from 2 to 21 days (Mellor, 1993). This would help prevent the spread of the disease to uninfected areas. In cases where zebra, mules and donkeys are involved it would be especially important to test for the presence of AHSV as these animals may remain without symptoms throughout their period of infection. Breed of horse may also be a consideration in the duration of viraemia and the possible threat of transmission if the animal is moved.

Movement of animals may also be of concern when horses are moved back and forth between stable yards and/or competition event locations. This is obviously hard to control in terms of continuous testing, but the likelihood of virus infection can be reduced by monitoring virus activity in the area the horse is going to be moved to and taking extra care and precautions when the animal is in an endemic area. Movement of horses into and through the AHS control area in the Western Cape in South Africa is strictly controlled.

1.6.3.2 Stabling

As stated earlier, *Culicoides* midges are crepuscular. Therefore it is traditionally accepted as a preventative method to keep animals stabled between the hours of sunset to sunrise to help reduce the chances that horses may come into contact with the midge vector and therefore the virus. This does reduce the chances that the horse will come into contact with the midge vector, but stable designs in South Africa do not provide a complete barrier against the midge. Barnard (1997) in research conducted at Onderstepoort Veterinary Institute (OVI) showed that although there were fewer midges captured (a ratio of 1 midge inside the stable to 20 midges outside) inside stables; they could not be totally excluded in normal open

fronted stables. Barnard (1997) also showed though that in stables that were not cleaned regularly, there were midge catches of almost double those in clean stables. Jenkins (2008) also demonstrated that *Culicoides imicola* were endophilic, even in winter months. Similar results were also seen by Meiswinkel *et al*. (2000) with about 45% less midges caught inside new stables (built in 1998) and about 25% in older, more open stables. Meiswinkel *et al*. (2000) also looked at differences in midges captured between sealed and unsealed stables in various degrees and found a reduction in midges in sealed stables (Table 2) but again not a total abolition of midges from stables. Due to the minute size of the vector it has seemed almost impossible to exclude them from an area, but stabling can reduce the number of midge encounters. Spray programmes are therefore imperative in and out of peak midge activity (Jenkins, 2008).

Table 2 Number of *Culicoides* midges trapped in various stable treatments (after Meiswinkel *et al.,* 2000).

	Outside	NS/DO	NS/DC	OS/NG	OS/1G	OS/3G
C. bolitinos	35573	6990	1080	9288	838	1579
C. imicola	2620	96	21	40	5	20
Total *Culicoides*	44906	7616	1229	9574	873	1739

NS, new stable; OS, old stable; DO, door open; DC, door closed; NG, no gauze; 1G, one layer of commercially available gauze; 3G, three layers of gauze

Another point to remember when stabling is that all lights inside and near stables should be kept off during hours of darkness as the midges are attracted to the light and will enter the stables, or lights should be placed away from stables. Stabling horses from sunrise to sunset may reduce encounters with midges if the stables are properly sealed but will not totally prevent the possibility of infection. Dung should be removed from stables to reduce microclimates that will attract midges. All gaps and holes in stables should be covered with very fine mesh or gauze, remembering to still allow for adequate

airflow. Closing the top door of a conventional stable would result in decreased air exchange and therefore decrease air quality below acceptable levels (Barnard, 1997). All lights inside and near stables should be switched off at night.

1.6.3.3 Repellents

There are many commercially available repellents as well as 'home made' remedies available to repel the *Culicoides* midge. These repellents fall into two main categories: chemical and natural or plant-derived repellents. In South Africa the majority of insect repellents contain pyrethrums. These are either natural or semi-synthetic. Pyrethrins are a natural insecticide produced by certain species of chrysanthemum while pyrethroids are a semi-synthetic derivative of chrysanthemumic acids. Pyrethrums work by penetrating the insect's nervous system rendering them unable to move or fly away. With natural pyrethrums (pyrethrins) the insects can detoxify and therefore are not killed outright. Pyrethroids are more effective at killing insects but commonly a synergist is used in conjunction with the active ingredient to enhance the properties of the chemical. The most commonly used synergist in South African products is Piperonyl Butoxide. Though products containing pyrethrums are marketed as repellents, their mode of action is more insecticidal than repellent. It is therefore possible even with the application of these products, that midges will be attracted to the horse to bite them, as the repellency of these products is relatively unknown. These products may act more in breaking the cycle of infection by killing midges that are feeding and are therefore in a sense more a vector control method rather than a repellent. Because of the mode of action of the pyrethroids, insects may still partake in a blood meal before they are rendered insensible or are killed.

There are number of other natural oils used in insect repellents, mainly citronella. The insect repellent properties of citronella are still being debated and the question has already arisen about citronella

being an attractant rather than a repellent (Braverman *et al*., 1999) Only sparse research has been carried out on the effectiveness of citronella and even then very little of that research is particular to the *Culicoides* species. Fradin and Day (2002) did carry out a variety of tests on different products containing different active ingredients and these were tested against mosquitoes (*Aedes aegypti*). They found that products containing citronella were greatly inferior in terms of protection to other repellents used, providing protection against mosquitoes for only 20 minutes or less.

The best chemical repellent, known worldwide for its use on humans, is DEET or N,N-diethyl-*m*-toluamide, now called *N,N*-diethyl-3-methylbenzamide (Fradin & Day, 2002). DEET is used as a repellent for a number of biting insects but most commonly against mosquito bites. Its mode of action is not really known but it most likely affects the insect's ability to locate an animal/human to feed on. It possibly disturbs the function of receptors on the antennae (McIver, 1981) but this has only been speculated in relation to mosquitoes. Some work has been done on the effectiveness of DEET against *Culicoides* but no commercially manufactured products containing DEET are available in South Africa for horses. When used on humans to combat mosquito bites it is definitely one of the most superior products available. In studies done on humans it was shown that DEET based products provided significantly longer lasting protection against mosquito bites than any other product used, with effective protection time corresponding to DEET concentration in the product. Protection lasted between 88.4 minutes up to 301.5 minutes with concentration ranging from 4.75% to 23.8% respectively (Fradin & Day, 2002). Studies done with DEET against *Culicoides* have mainly been on humans, using *Culicoides* spp. that have been implicated as pests rather than those known to carry AHSV. Studies have shown that DEET is an effective repellent against some *Culicoides* spp. giving protection of 28 times that of a control, protecting the user for between 276.7 and 352.3 minutes (about 5 hours). If these results

could be extrapolated to species of *Culicoides* that carry AHS, then DEET may be a more effective method to use (Schreck *et al.*, 1979).

1.6.3.4 Wind Speed

Little has been tested on increased wind speed as a form of insect repellent though it is known that the *Culicoides* midge vector is a weak flier with its forward flight in still air only in the order of $0.5ms^{-1}$. Insects can only reach their goal of food source, mate or shelter if their speed is greater than the current air speed otherwise they will be carried downwind by the prevailing faster air speed (Pedgley, 1983). As *Culicoides* are crepuscular feeders they are more active at dusk and dawn when wind speeds often drop. Lessening wind speeds and decreasing wind turbulence at night helps increase the boundary layer in which insects tend to fly (Pedgley, 1983) thus increasing the capability of insects to fly towards their goal. It would therefore be possible to disturb an insect's flight towards its food goal (a horse) by introducing artificial wind speeds over and above the insects' capable flight speed as well as introducing turbulence in order to remove the possibility of downwind movement of midges.

1.6.3.5 Alternate Hosts

The introduction of alternate hosts into the area where horses are kept may be one of the unexplored ways in which the vector "load" on horses can be reduced. The *Culicoides* spp. is made up of 1400 sub-species (Mellor *et al*., 2000) and many of these species of *Culicoides* have been implicated in virus transmission. They have been found to transmit a selection of Bunyaviridae viruses in rodents, birds, marsupials, bats, sloths, cattle and even humans, Nairobi sheep disease in sheep, goats and humans, Bovine ephemeral fever in cattle, Bluetongue virus in cattle, sheep and deer as well as many other Orbiviruses in cattle. They have even been implicated in transmitting some unclassified viruses whose affected hosts are yet to be discovered (Yuill, 1986). It is therefore plausible that there might be some overlapping of *Culicoides* spp. host preference and

disease transmission. Nevill & Anderson (1972) showed that though many species of *Culicoides* will feed on different animals most have a preference for either avian or mammalian blood. In the same experiment it was evident that one of the species that had a truly mammalian preference, *C. pallidipennis,* did not seem to have a preference for one species over the other between oxen, horses and sheep. As *C. pallidipennis* is more correctly named *C.imicola* (Carter, Ingram & Macfie, 1920; cited by Nevill *et al*., 2007) and is therefore one and the same, and that *C.imicola* is a known South African AHS vector (Mellor *et al*., 2000) then it may be probable that our common AHS vector *C.imicola* will preferably feed on cattle and sheep in the vicinity and thereby reduce the numbers of midge encounters a horse will have to face.

1.6.3.6 Other

There are a few other less documented and untested means that have been used to manage and control *Culicoides* biting. These include using smoke in and around the stable yard to discourage midges from coming near the area and masking the odour and CO_2 release from the animals that would usually attract the midges.

Blanketing horses has been said to prevent midges from being able to bite horses. Though nothing has been published on preferred landing sites of those midges involved in AHS in South Africa, studies have been carried out on other *Culicoides* spp. In Ireland a study on the preferential landing and engorging sites concluded that most species of *Culicoides* preferred the upper and lower legs as well as the mane (dorsal neck area under the mane) (Townley *et al*., 1984). Blanketing may slightly reduce the number of midge bites, but is not an effective preventative method by itself.

Though not one of the preventative or control methods provides 100% protection on its own, the greater the number of these measures that one can put in place simultaneously, the smaller the risk of AHS infection.

CHAPTER 2 SURVEY AND ANALYSIS OF AFRICAN HORSE SICKNESS DATA

2.1 Introduction

To be able to identify and prescribe the best methods that we have to prevent and control AHS, it is pertinent that we evaluate the majority of the currently applicable prophylactic strategies utilised by horse owners in KZN. Some standard equine management practices may help or hinder the occurrence of the disease, and a study was conducted to identify and compare the most prevalent practices. Recommendations need to be made in the field, in addition to improvement in vaccine related practices, in order to reduce the incidence of the disease.

Meiswinkel *et al*. (2000) showed that some species of the AHS vector, namely *C. imicola*, are less likely to enter stables to feed on horses. As *C.imicola* is the most abundant species of *Culicoides* midge (Barnard, 1997; Mellor & Hamblin, 2004) it is possible that stabling may reduce the incidence of AHS. Light is detected by insects (Babrekar, 2004) and acts as an attractant for them, so stable lights may have to be switched off while horses are in them. The use of insect repellents and insect traps as control methods for AHS has always been seen as favorable. Research on the use of repellents has been diverse and each repellent and even each species seems to effect the duration of protection (World Health Organization, 2006). It has been shown that various repellents are effective against the *Culicoides* spp. midge (Schrek *et al*., 1979; Braverman & Chizov-Ginzburg, 1997), acting by repulsing the midges or by killing them as they ingest the repellent. Little is documented on the use of insect traps as a control method for *Culicoides* midges. Traps and especially UV light traps are a popular tool for capturing *Culiciodes* midges for research purposes (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*,

2003, Cêtre-Sossah *et al*., 2004), and could be used just as effectively to reduce midge numbers and control AHS.

Zebra carry the virus without showing any clinical signs (Mellor & Hamblin, 2004), and may act as winter reservoirs for the virus (Mullins, 2007; *pers. comm.*). It is therefore possible that an actively breeding zebra population in an area may increase the probability that horses in that same area will contract AHS. *Culicoides* midges are proven transmitters of the Bluetongue virus in sheep (Nevill and Anderson, 1972; Conte *et al*., 2004). They also transmit various viruses in cattle (Yuill, 1986). *Culicoides* spp. will blood feed on other domestic livestock species. By providing alternate hosts for the midges to select, the bite load on the horses in an area may be reduced.

Annual AHS vaccinations that include the serotypes of the virus present in the environment are supposed to be effective in preventing the disease. It should also be significant at what time of year the vaccination is given, due to the fact that complete immunization may take up to four weeks post-inoculation (Onderstepoort Biological Products, 2006) and that giving the vaccination too late into the AHS season would not have a significant effect on providing adequate control (Lord *et al*., 1997) . Vaccine related deaths from AHS have been observed (Mullins, 2007; *pers. comm.*) and the timing of the vaccination (Crow-Smith, 2005) and the vaccination itself needs to be evaluated in terms of its efficacy in preventing the disease.

The above considerations in terms of the prevention of AHS were collated from survey data in KwaZulu-Natal, as well as data collected by the AHS Research Trust from confirmed AHS cases during the 2005/2006 and the 2006/2007 midge seasons. The data were expected to reveal the predominant prophylactic strategies used in the control of AHS, and those perceived to be effective, and were also used to rank the probabilities that the different approaches would be effective.

PART A AHS SURVEY IN KWAZULU-NATAL

2.2A Materials and Methods

The objectives of the analysis of the survey data were to relate the prevalence of AHS cases to zones and prophylactic interventions, and to create a risk assessment using the different interventions.

Survey Area

KwaZulu-Natal has been shown to be a fast growing area for the incidence of AHS, and has recorded the second most AHS cases in the country over the last ten years (AHS, 2006; OIE, 2006). Limited research into the status of AHS and its control is available in Kwazulu-Natal.

Survey Information

The survey required information on the area in KZN where horses are kept (zone), the number of animals owned by each person, and management practices currently employed with respect to AHS. This included grazing type, vaccinations, timing of vaccination (Lord *et al*., 1997) stabling and time management and repellent usage. Owners were asked to note the year, form and fatality of each incidence of AHS that occurred in the last five years.

Data Analysis

Data obtained was qualitative in nature and did not follow normal distribution. A chi-squared analysis was done on the binomial data (Genstat® version 9, 2002) to determine the significance of the intervention across the survey data using t-probabilities, and were then analysed by a binomial logistic regression model using Genstat® version 9 (2002) (Mc Conway *et al*., 1999). The model was used in order to link each variable (X) with the possibility of getting AHS. Disease status is issued with either a 1 or a 0 depending on

whether animals on a certain farm contracted AHS or not (0 for “non-sick” or 1 for “sick”) (Kleinbaum & Klein, 2002).

The model for the logistic regression is given by the equation:

$$\ln(P_1/1-P_1) = \alpha + ß_1X_1 \ldots ß_iX_i \qquad \ldots(1)$$

Where P_1 = the probability of being “sick”,
$1-P_1$ = the probability of not getting AHS, “non-sick”
$P1/(1-P_1)$ = odds ratio: the ratio of the probability of success (= 1) over the probability of failure (= 0).
X = the value of the predictor variable and
α and ß = parameters of the model.

$LN(P_1/1-P_1)$ is known as the logit or logistic link function (Mc Conway *et al*., 1999) and is used to calculate whether an intervention will increase or decrease the probability of getting AHS, given that the intervention is placebo in nature (=0) or that adding the intervention (=1) to the model will reduce the probability of getting a successful outcome (sickness).

In this manner, one could assess whether the intervention increased the prevalence of AHS in the area.

2.3A Results and Discussion

Survey yield was equal to 41 usable surveys from around the province of KwaZulu-Natal. Survey responses were evenly distributed throughout the province (Figure 6). Though it was thought that one or more areas might show a higher proclivity for AHS cases due to horse density, the analysis of area in relation to AHS cases in the last five years yielded no significant difference.

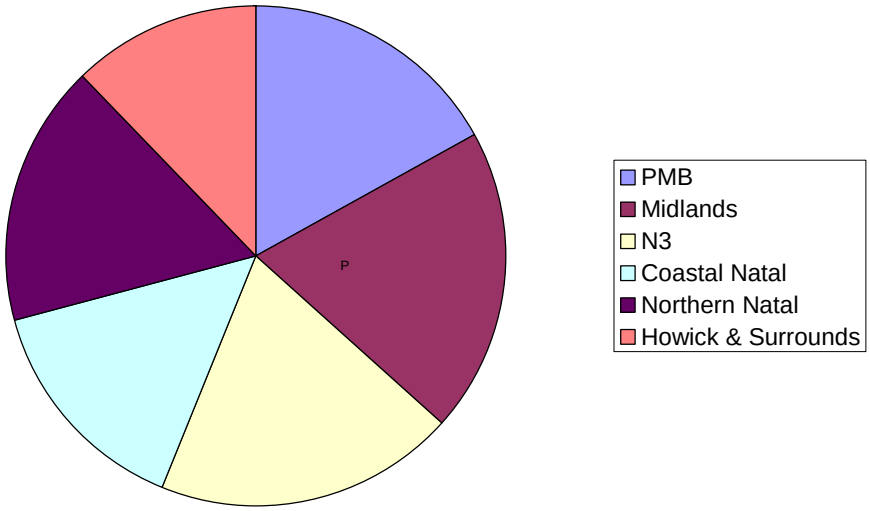

Figure 6 Proportion of respondents to the AHS survey represent six zones in KwaZulu-Natal, from the coast to the midlands.

Results were also obtained from surveys as to the predominant grazing types (Figure7) at each area as well as to the timing of AHS vaccination (Figure 8). Both factors were non-significant in relation to the possibility of contracting AHS.

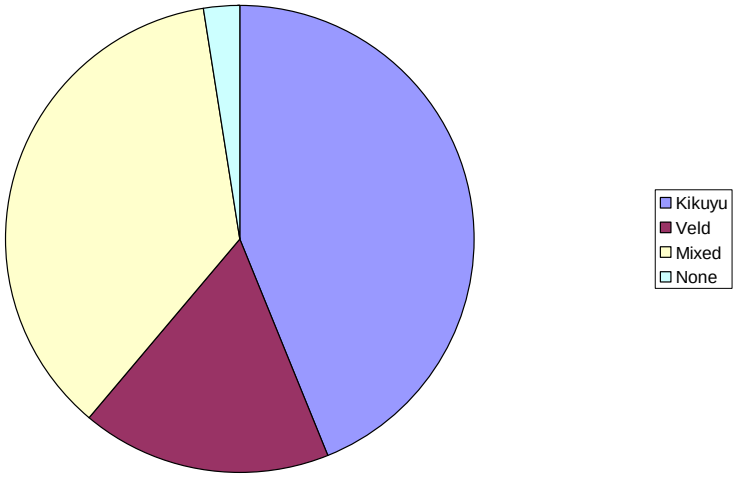

Figure 7 Most horses of AHS Survey respondents offer kikuyu and mixed grazing to horses.

It had been suggested that certain grazing types (due to characteristics) may be more or less likely to offer a breeding habitat to the *Culicoides* spp. (Jenkins, 2007; *pers. comm,*) and there may be a greater predisposition for horses kept on certain pastures to

contract AHS. This may have been less applicable in this survey as most horses were brought into stables during hours of dusk and dawn when the midges are most active thus no matter what the proportion of midges were in different grazing type pastures horses were not exposed to these. Ground cover and vegetation length did not significantly influence the midge catches in these zones, although the permanence of water and incident radiation in these zones may affect midge catches ((Jenkins, 2008).

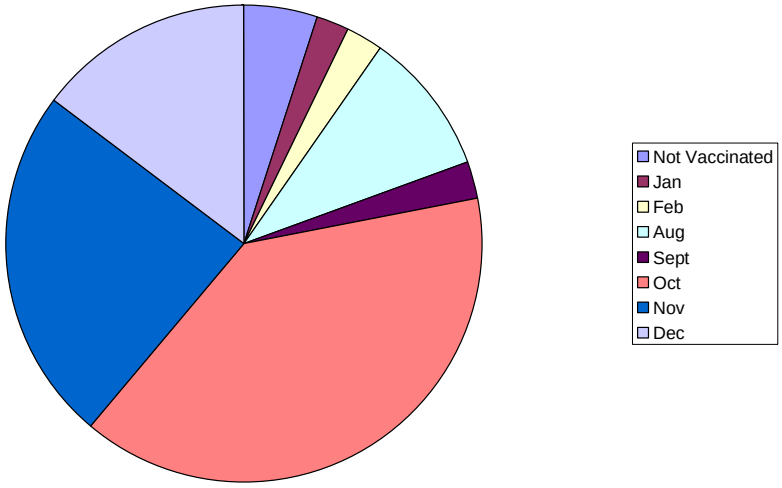

Figure 8 The highest proportion of respondents vaccinate between October and December every year.

There was no significant effect of date of vaccination on the incidence of AHS. It is believed that vaccinating in very late winter or early spring (months August to October) would give the horse a better immunological response against the AHS virus (Lord *et al.*, 1997). Immunity gained from the current vaccination usually starts appearing at 2-3 weeks post vaccination and for some serotypes up to four weeks. The AHS vaccination requires two separate injections given at least three weeks apart (Onderstepoort Biological Products, 2006). This means a total of 7 – 11 weeks before total immunity is reached; it was thought that timing of vaccination might therefore have an impact on the likelihood of AHS infection. This raises the concern about the timing of the competitive seasons in KZN as many competition horses are in fact vaccinated after November

which means that many horses do not have full immunity by the onset of the AHS season (Figure 8).

The main aspect of the survey was to evaluate the use of management/prophylactic strategies for AHS. Figure 9 indicates the proportion of respondents that utilized each of the interventions queried.

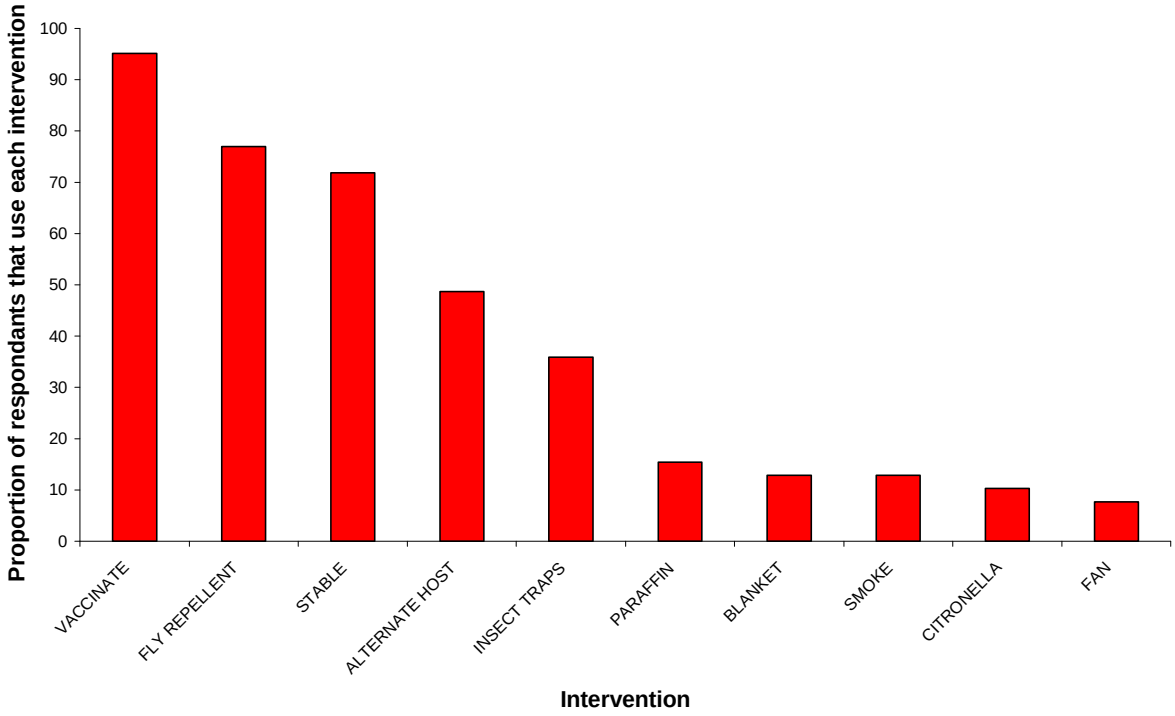

Figure 9 Proportion of respondents surveyed in KwaZulu-Natal that use a given intervention in the control of AHS.

It can be seen from the graph that significantly more people use vaccinations, fly repellents and stabling to control AHS ($p<0.05$). Very few respondents use interventions such as smoke around stables, citronella and fans in stables. This is most likely due to the fact that very little or no hard evidence exists for the use of these interventions against AHS. Respondents are unlikely to use blankets against AHS as the height of the AHS season is during the summer months and blanketing would cause horses to be uncomfortable.

Though there are many and varied interventions used by respondents not many of them were shown to be statistically significant in their effect on decreasing possibility of contracting AHS (Table 3).

Table 3 Chi Squared probabilities of anecdotal prophylactic strategies actually leading to the contraction of AHS.

Intervention	Chi prob.	t-prob
Alternate hosts	0.026	0.036*
Blanketing	0.563	0.581
Citronella	0.788	0.792
Fans	0.788	0.792
Fly repellent	0.286	
Weekly		0.110
Once/day		0.720
Twice/day		0.268
Stabled	0.024	0.027*
Stocking rate	0.159	0.301
Vaccination	0.218	0.717
Insect traps	0.010	0.037*
Lights	0.136	0.135
Paraffin	0.047	0.055**
Smoke	0.046	0.686
Date of Vaccination	0.068	
Jan		0.849
Feb		0.849
Aug		0.900
Sept		0.849
Oct		0.908
Nov		0.912
Dec		1.000

* Significant at 5% level, ** Significant at 10% level

Table 3 gives the chi probability and t-probabilities from logistic regressions employed to discern the effectiveness of a particular intervention in increasing the probability that AHS will be contracted. No interactions were significant in this respect, although the use of alternate hosts, stabling and using insect traps were seen to reduce the incidence of AHS ($p<0.05$), with the use of paraffin significant at the 10% level.

The outcomes of the effect of each of the significant factors is calculated in Table 4 where $LN(P_1/1-P_1) = \alpha + ß_1X_1$ is transformed

to the form p(x). The probability of having contracted AHS in the last five years is equal to 1/[1+exp -α+ ß(x)]] where (x) is 1.

P(x) when x = 1 is therefore the probability of a horse having contracted AHS in the last five years when the intervention was used and p(x) when x = 0 is the probability that a horse **won't** have got horse sickness in the last five years when the intervention was used (Table 4).

Table 4 Transformed Logit probabilities of a horse having contracted AHS in the last five years when the intervention was used, where p(x) when x = 1 is therefore the probability of a horse having contracted AHS in the last five years when the intervention was used and p(x) when x = 0 is the probability that a horse won't have got horse sickness in the last five years when the intervention was used.

Intervention	**A**	**B**		**p(x)**
Alternate hosts	-1.735	1.629	p(sick) 1 p(non-sick) 0	0.033 0.150
Insect traps	-0.241	-2.32	p(sick) 1 p(non-sick) 0	0.889 0.440
Paraffin	-1.139	1.832	p(sick) 1 p(non-sick) 0	0.049 0.243
Stables	0.405	-1.749	p(sick) 1 p(non-sick) 0	0.896 0.600

Stabling has the highest effect on the incidence of AHS, with the probability of contracting AHS in the last five years being equal to 89.6% when horses are stabled. This is contrary to earlier popular belief that stabling horses during the hours of dusk and dawn would protect them against midges. This was thought to be true because initially the *Culicoides* vector *C. imicola* was thought to be exophilic and would stay out of stables or only very reduced numbers would enter stables and Barnard (1997) showed significantly fewer *C. imicola* caught in light trap catches inside stables than outside. However the second proven vector *C. bolitinos,* has been found in large numbers in standard stables (Meiswinkel *et al*., 2000). Though

there are no other proven *Culicoides* vectors it may be that other species of *Culicoides* are vectors and may enter stables to blood feed. More midges were caught in traps placed in stable eves than outside stables (Jenkins, 2008) across KwaZulu-Natal. South African stables are generally not totally sealed and this allows for gases and other attractants to move outside of the stable and attract midges into the stables. The higher concentration of horses in stables per unit area as well as the fact that wind velocities drop at night (Pedgley, 1983) would therefore mean that stabled horses do indeed attract more midges.

The use of insect traps gives the second highest probability of incidence of AHS in the last five years, with an 88.9% probability of a horse getting AHS when insect traps are used. This was not an expected outcome as it was thought that insect traps would draw insects towards the traps and away from horses. The survey though did not indicate what sort of insect traps were being used. Insect traps used primarily to catch flies would not have the same affect on midges as they utilize incorrect attractants for *Culicoides*. The *Culicoides* midge is attracted to its host by CO_2, O_3 and kariomones (Mordue-Luntz, 2003) and therefore only insect traps using the above attractants would be affective against the *Culicoides*. UV light has also proved to be significantly effective in trapping midges (Van Ark & Meiswinkel, 1992) and UV light traps are used widely in research. It is possible that the traps used by respondents were those specified for use against flies and therefore would have had no impact on *Culicoides* numbers. If light traps or other attractant traps are used close to horses, then these might attract midges into areas where horses are kept and therefore would have the negative impact on AHS seen by the statistics in Table 4.

The proximity of alternate hosts for the midges in the form of sheep and cattle has proved to have a significant effect on the incidence of AHS in the preceding five years. A 15% probability that a horse

would not contract AHS is calculated if there was an alternate host in the immediate area. It is widely known that the *Culicoides* midge does not only feed on horses but also on cattle and sheep (Du Toit, 1944; Yuill, 1986) as they also transmit various diseases in these species. It is therefore possible that by having an alternate host to offer as a secondary blood meal, the “bite load’ on horses in the area would be reduced. It is unknown whether the implicated *Culicoides* vectors have a penchant for equines over other livestock species. Due to the fact that *C. bolitinos* is known to breed in the dung of cattle (Meiswinkel & Paweska, 2003) it may be preferable that sheep be used as an alternate host near horses.

The use of paraffin in reducing the likelihood of contracting AHS in the last five years was only significant at the 10% level but showed a 24.3% probability that a horse would not get AHS if paraffin was being used as a preventative method.

None of the given methods of AHS protection was shown to be a 100% effective in reducing the likelihood of AHS and neither did any appear to have a better or more significant effect against AHS when used simultaneously with another.

PART B DATA ANALYSIS OF AHS CASES FOR TWO SEASONS

2.2B Materials and Methods

Data from the African Horse Sickness Research Trust (AHSRT) were taken from confirmed AHS cases and were analysed to link the possibility of mortality from AHS to specific variables.

Data Collection

Data was obtained from the AHSRT (AHS, 2006). Data was collected in KZN over the 2005/2006 and the 2006/2007 AHS seasons (between December and March when AHS cases are most prevalent).

Collection Forms

For each reported case of AHS a collection form was completed by the Trust (Appendix 2 and Appendix 3). Though the forms for each season were laid out differently, each held very similar questions.

Information was requested on the area where each horse was kept, to identify “hotspots” of AHS, and the survey included questions relating to prevention and control strategies such as the use of repellents or pesticides, stabling of horses and vaccination, previous AHS cases in the area, other horses being vaccinated in the area and (in the 2005/2006 collection form) the occurrence of zebra in the area. The survey also included information on the form of AHS that the horse had suffered from. This was reported simply as “Dunkop” for the acute form of AHS and “Dikkop” for the subacute form of AHS.

Data Analysis

Data was analysed by using a binomial logistic regression model on the statistical program Genstat® version 9 (2002). See Equation 1, section 2.2A. Mortality from AHS was used as the explanatory

variable with mortality from AHS (= 1) and survival from AHS (= 0). Percentage outcome of each explanatory variable was graphed. The African Horse Sickness Trust data was collected from horses that were infected with the AHS virus, so that it was possible to link the use of control strategies with the possibility of mortality from AHS or survival.

2.3B Results and Discussion

The majority of cases reported in the 2005/2006 and 2006/2007 seasons came from the Midlands and N3 (extending from Pietermaritzburg to Durban) areas (Figure 10). This is probably related to the fact that a greater number of horses are kept in these areas outside of big towns and therefore there is a greater risk of spread. These areas are also greatly comprised of farming and pasture land and provide more habitats for midge breeding (Jenkins, 2008).

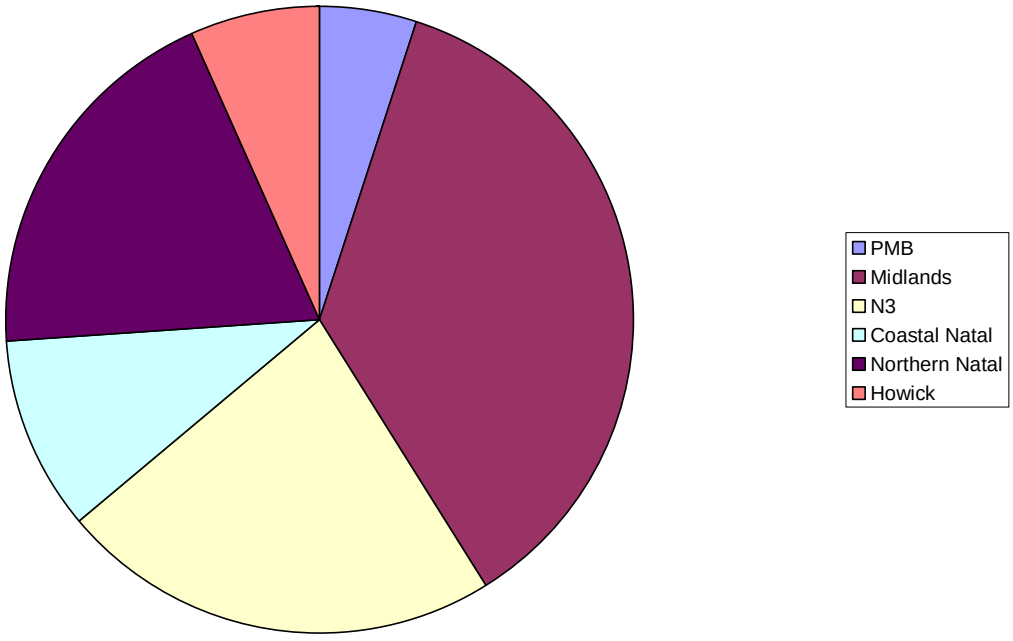

Figure 10 The proportion of horses contracting AHS is shown to be greater in the N3 and Midlands areas of KZN from AHSRT data from AHS positive cases during the 2006 and 2007 seasons.

Although in 60-90% of reported AHS cases, at least one or more preventative interventions were used, horses still contracted AHS. It is evident that in regards to this data no one intervention strategy leads to total protection of horses from the infected *Culicoides* midge.

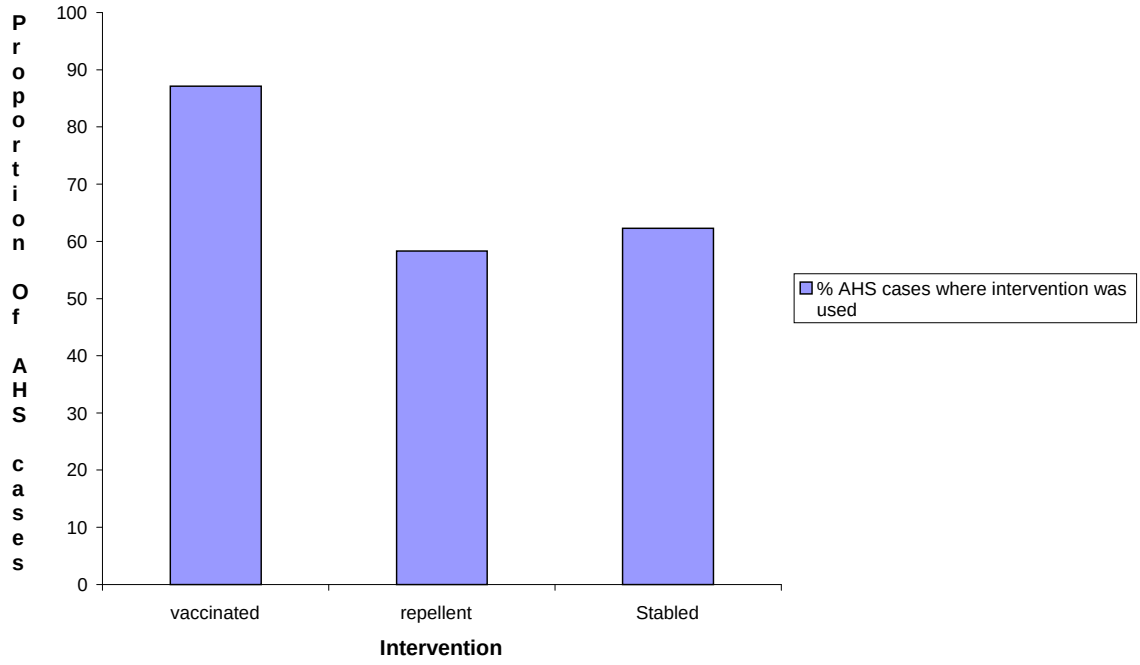

Figure 11 Even where interventions of repellents, stables and vaccinations are used, the incidence of AHS is shown to be high in AHSRT data from AHS positive cases during the 2006 and 2007 seasons.

Figure 12 illustrates the proportions of horses that are AHS positive that fall into the different forms of the disease. In 57% of cases horses were shown to exhibit the cardiac form of the disease. This is a slightly less fatal form of the disease than the pulmonary form (Coetzer & Erasmus, 1994). It is to be discovered whether or not specific serotypes are linked more prominently to certain forms of the disease or whether it is based on the individual horse's immune response. Acute cases made up 24% of the total collected and of these cases, a statistically significant proportion was fatal (see Table 5 below).

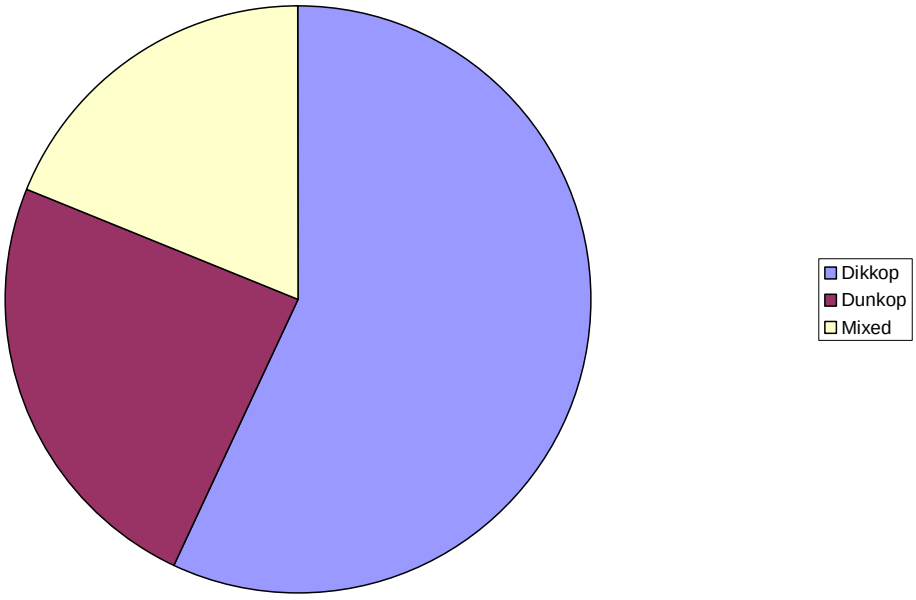

Figure 12 Most horses succumbing to the AHS virus develop the subacute form of the disease in KZN according to AHST data from AHS positive cases during the 2006 and 2007 seasons

Chi-squared probabilities indicated that repellents, vaccination, "dunkop" form of AHS and hot weather influenced mortality from AHS (Table 5). Horses with the acute form of AHS had a highly significant probability of dying of AHS. Mortality figures with Acute AHS often exceed 95% compared to the 'dikkop' or cardiac form of AHS where mortality is around the 50%, as the horse develops hydropericardium and "drowns" (Coetzer & Erasmus, 1994).

Table 5 Chi-squared probabilities that the use of repellents, vaccinations, hot weather and form of AHS increase the incidence of AHS in AHSRT data in KZN across the 2006 and 2007 seasons.

Factor	Chi Prob	t-prob
Age	0.490	0.504
Repellents	0.040	0.043*
Stabling	0.651	0.651
Vaccination	0.007	0.026*
Zebra in area	0.616	0.615
Form Dunkop Mixed	<.001	 0.005* 0.428
Previous AHS	0.457	0.458
Vaccination in area	0.952	0.952
Weather Cold Hot Humid Warm Wet	0.040	 0.077 0.046* 0.832 0.841 0.379

*Significant at 5% level

Interestingly, in a survey of information received on horses succumbing to the AHSV, vaccination does not reduce the incidence of death (Table 5) but increases the probability of survival from AHS of 98.8% (Table 6). This is because the horses that died were vaccinated, but vaccination still remains the most prevalent means of protecting against the virus. Horses vaccinated against AHS are better equipped to fight the virus and this aids in recovery. No correlation was found between vaccination and disease form, with vaccine-related deaths unlikely to be correlated to a type of AHS infection. Horses treated with repellents showed an 80% probability of surviving AHS. No correlation was found to exist between AHS form and repellent used. Horses that contracted the acute/'dunkop' form of AHS rather than the cardiac or AHS fever had a 36.4% higher chance of dying. This can be explained by looking at the differences

in mortality percentages mentioned earlier. The pulmonary form of the disease is far more deadly as it acts far quicker and is often unnoticed until the horse is dying (Coetzer & Erasmus, 1994). The final factor that was shown to contribute significantly to the possibility of mortality from AHS was that of 'hot weather'. Though increased temperatures can increase the possibility of high midge numbers, in the case of mortality from the virus it is more plausible that high temperatures have an adverse affect on the animals due to the heat stress (Hu *et al.,* 2007), and further stressed their compromised systems.

Table 6 Transformed Logit probabilities of a horse contracting AHS in relation to vaccination, repellents, form of AHS and weather, where p(x) when x = 1 is therefore the probability of survival, p(x) when x = 0 is the probability of mortality.

Factor	**A**	**B**		**p(x)**
Vaccination	1.95	-2.43	p(survival) 1 p(mortality) 0	0.988 0.875
Repellency	0.375	-1.068	p(survival) 1 p(mortality) 0	0.809 0.593
Form "Dunkop"	-0.560	2.351	p(survival) 1 p(mortality) 0	0.052 0.364
Hot Weather	0.511	-2.12	p(survival) 1 p(mortality) 0	0.933 0.625

2.4 Conclusion

From the AHS survey it was concluded that stabling may not be as effective at reducing the incidence of AHS cases as was previously believed. AHS cases recorded in this survey were higher in stabled horses. This is possibly linked to the populations of *C. bolitinos* as this specific species has been found in higher numbers inside stables.

Insect traps were also shown to increase the risk of AHS. It was not specified as to what type of insect traps were used and therefore it is possible that insect traps used were not midge specific and therefore had no effect in reducing midge numbers. It is also possible that light traps used in or near stables may serve to increase or lure midges into the stables and are then not effective in trapping or destroying the midges. This would increase the possibility of midges coming into contact with horses to increase bite load. In cases where there were alternate hosts available for the midges the incidence of AHS cases was significantly reduced.

In the data collected by the AHS Trust, it was shown that vaccination contributed significantly to increasing the survival rates of horses that had contracted AHS. Hot weather as well as the contracting of the acute form of the AHS virus increased mortality from the virus.

In order to find more in depth solutions to the AHS crises through survey information and collected data, it is critical that we have initial figures on horse numbers in particular areas as well as in specific stable yards or homesteads. In order to make valuable conclusions from data collected from persons whose horses have contracted AHS we need to know what the ‘base’ number of horses are in that immediate area/ stable yard. Further surveys need to include more specific information related to types of repellents or insect traps used by horse owners so that more accurate conclusions can be drawn.

CHAPTER 3 EFFICACY OF COMMERCIALLY AVAILABLE REPELLENTS

3.1 Introduction

In order to reduce the likelihood of infection with AHS, it is necessary to reduce contact with the viral vector, in this instance the *Culicoides* midge (Venter *et al*., 2000). One way to reduce the bite load is through topically applied insect repellents (Schreck *et al*., 1979). Though repellents are normally effective against most arthropods (Webster *et al*., 1991; in Braverman *et al.*, 1997), it is important to determine the value of repellents in protecting equines against the *Culicoides* spp midge. Population of midges peaks over the months December to March each year (Coetzer & Erasmus, 1994) following a dormancy over the winter months. In summer, horse owners are encouraged to reduce the number of adult midges as well as the hatching larvae (Jenkins, 2008) to augment protection offered by the annually administered vaccination (Mellor & Hamblin, 2004). The peak in the viral challenge of AHS occurs in the summer months when the midge is most prevalent (Coetzer & Erasmus, 1994).

Most studies of repellents consider their efficacy in the protection of humans against biting insects, with very few studies considering horses and other animals. Studies that have been done have shown various repellents to be effective initially but insect catches seem to increase after the fourth or fifth hour post-application and some show little to no significant difference from control catches after nine hours post application (Braverman & Chizov-Ginzburg, 1997).

Commercially manufactured repellents available in South Africa that produce a sustained efficacy against *Culicoides* midges may be useful in reducing the bite load in horses. They can be used in conjunction with other prophylactic strategies for the control of AHS.

Of the many commercially available repellents, there are two main categories of natural or chemical/ synthetically manufactured repellents. In the South African market the majority of the available equine repellents consist of active ingredients from the pyrethrum group. In this group there are the naturally plant-derived ingredients pyrethrins and the semi-synthetic pyrethroids. This group of repellents work by affecting the insect's nervous system and rendering them unable to fly and although referred to as a repellent, are more deterrents or insecticides. Another popular and yet controversial repellent is citronella, which is a typical repellent that deters insects by its odour. The last active ingredient common in insect repellents but not yet specifically in equine repellents is DEET (N,N-diethyl-m-toluamide). DEET is a more typical repellent and though its mode of action is not fully understood it is thought to interfere with the insect's ability to locate a host (McIver, 1981).

While topical applications on the skin of the animal are indicated as most effective (Catton, 2006; *pers comm.*) personal experience showed that it was impossible to conduct field research using applications of repellents on live animals. For this reason, downdraught traps ((Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*, 2003)) with a topical application of the repellent were used (Braverman & Chizov-Ginzburg, 1998) to offer a means of comparison between repellent treatments. Faux fur can be used as a simulation of the animal's skin.

As a result, the following investigation considers each of the most popular, available repellents to discern which strategy in the control of the midges would be best as a support in the control of AHS through vector repellence. While vaccination is still most important, support strategies to control the vector include vector ecology and trapping, but also repellence of the adult midge. It is important to determine the most effective spray treatment against midges, and

how long they are effective for, which is the object of this trial, to assist horse owners in providing effective prophylactic control of adult midges.

3.2 Materials and Methods

During the midge peak in the summer of 2008, downdraught suction traps treated with various repellents were used to catch midges at Ukulinga Research Farm. Trials were carried out from December 2006 to the end of March 2007. Three commercially available equine fly repellents were tested, each of which had a different active ingredient. They contained Cypermethrin with a synergist of piperonal butoxide, Pyrethroid and Citronella respectively. Another human mosquito (*Aedes* spp.) repellent containing the active ingredient DEET (N,N-diethyl-m-toluamide) was tested, as it indicated to have some success against midges.

Trial Facilities

Downdraught light traps were placed in an open field near the stables at Ukulinga Research Farm, Mkondeni, Pietermaritzburg, KwaZulu-Natal. *Culicoides* occur in great numbers in these areas and confirmed AHS cases have been reported in these areas.

Data Collections

Downdraught suction light traps (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*, 2003) (Figure 13) were used to obtain catch numbers of *Culicoides* midges (modified from Braverman & Chizov-Ginzburg, 1998). One trap was used as a control, while repellents were applied to squares of fabric on the netting covering the light trap. Ten randomly placed 8 x 8cm squares of fabric resembling the properties of the coat of a horse were placed on the netting. Enough space existed on the netting for insects to move through the netting towards the light source, which served as the attractant. Repellents were

applied once, but midge catches were taken after five hours and overnight, following the crepuscular activity of the midge (Mellor, *et al*., 2000).

Figure 13 Downdraught suction light traps, modified from Braverman & Chizov-Ginzburg (1998), were used to obtain catch numbers of *Culicoides* midges.

Traps were placed equal distances from each other in a square arrangement (Figure 14).

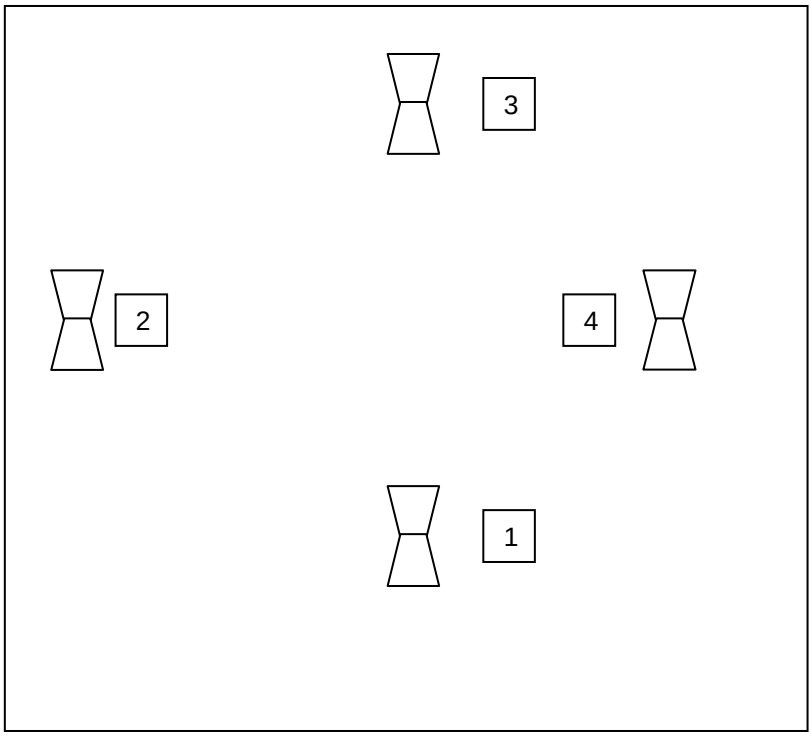

Figure 14 Diagram showing the layout of downdraught light traps to which repellent treatments were applied in a randomised design.

Traps were treated with a repellent at 15:00 and then switched on until 21:00. The collections were then removed and a new vial was placed on the trap. The traps were set to switch on again at 02:00 until 08:00 the following morning. The trial was run for four nights and in each case the traps were moved to a different position. Midges were collected in alcohol filled vials attached to the trap in order to preserve the catches. Repellents were not reapplied in order to assess the longevity of the repellent action of each of the active ingredients.

Data Sorting

Culicoides spp. were separated from other species under a dissecting microscope and then posted to OVI for identification to species of *Culicoides*.

Data Analysis

The number and the species of midge caught were analysed using the generalized linear modelling procedure in Genstat v9 (2006). The data followed a negative binomial distribution, as the data was non-normal count data where the relationship between the variance and the mean was not equal (McConway, 2006). A log link function was used. The significance of the parameter estimates in the accumulated analysis of deviance was used to establish the relevance of the treatments in affecting the total midge catch. Main effects and interactions of treatment and time of application in males and females and *Culicoides* species were analysed. Successful interventions in the control of AHS depend on the identification of the major parameters influencing total midge numbers.

3.3 Results & Discussion

Fewer total midges were caught in the afternoon catch period between 15:00 and 21:00 (mean catch 2.61) than there were in the morning period between 03:00 and 08:00 (mean catch 2.83) ($p<0.01$). This may be due to the fact that the treatments were applied in the afternoon, it can be seen that the effect of the repellents decreased with time, and the repellents did reduce the number of midges entering the trap when they were “fresh” though it is possible (though not documented; Walker, 1977) that the midges are less active at dusk than at dawn. There were however no significant differences ($p>0.05$) in the number of midges caught between the five treatments (Table 7).

Table 7 Log Link Parameter estimates obtained from GLM (Genstat, 2006) of negative binomial data from midge catches in light traps treated with four different repellents at two time periods.

Parameter	Estimate	T pr.	Mean AM catch	Mean PM catch
TRT Citronella	-0.456	0.318	1.87	2.43
TRT Cypermethrin	-0.527	0.251	2.87	2.67
TRT DEET	-0.178	0.692	1.7	5.37
TRT Pyrethroid	-0.342	0.451	5	1.8

- Using the control as the reference level

Only the female midges are implicated in the spread of AHS as they require a blood meal and the effect of repellents on the female midges is an important factor in the control of AHS. In an analysis of the total female catch, a significant difference was found between the trials run over the different months. The different months of the experiment did produce different weather conditions. Midges are extremely weak fliers and increased winds as well as rainfall have an effect on their flight (Kettle, 1977). It has also been shown that *C. imicola* decrease with decreasing temperature (Baylis *et al*., 1999). More female midges may also appear after swarming as they require a blood meal in order to develop a batch of eggs (Wittman & Baylis, 2000). One of the positions of the traps in the hexagonal arrangement favoured less female midges ($p<0.05$) than other trap positions, which may be explained by wind current along that position which was possibly in a more exposed area than the other traps. Care was taken in each replication to circulate the traps evenly over each of the positions during each trial to eliminate confounding in the analysis.

For the female catch alone, no significant difference was found in catch numbers for the different time periods ($p>0.01$). Female midge catches were not different between the periods of dusk and dawn.. Citronella and cypermethrin treatments caught significantly less

midges than the control (Table 8). Both active ingredients have historically been used in insect repellents, although the use of citronella has achieved anecdotal rather than scientific success. As a result, one can suggest that treatments should be refreshed to continue to be effective, and that citronella and cypermethrin-based repellents can be effective in reducing the number of female midges. The mode of this result can be considered to be repellent in action.

Table 8 Log Link Parameter estimates obtained from GLM (Genstat, 2006) of negative binomial data from female midge catches in light traps treated with four different repellents at two time periods

.

Parameter	Estimate	T prob.	Mean AM catch	Mean PM catch
TRT Citronella	-0.902	0.053*	2.53	4.67
TRT Cypermethrin	-0.997	0.036**	5.20	3.67
TRT DEET	0.169	0.672	2.26	10.40
TRT Pyrethroid	-0.111	0.786	9.13	3.33

* The control was used as the reference level

*Significant at the 10% level

**Significant at the 5% level

In order to investigate the interactions between the various treatment factors, the data was analysed initially with species of midge as a factor and then secondly with sex as a factor. No significant differences were seen between the treatments, but the treatment-species interaction analysis showed that significantly more *C.imicola* were caught than any other species while the cypermethrin treatment caught significantly less *C.imicola* (Table 9). These results were also found by Braverman and Chizov-Ginzburg (1998) who tested the repellency of synthetic and plant–derived preparations and found that repellents with permethrin active ingredients significantly (<0.05) repelled *C. imicola*.

Table 9 Log Link Parameter estimates obtained from GLM (Genstat, 2006) of negative binomial data from female midge catches in light traps treated with four different repellents at two time periods using species as a factor.

Parameter	Estimate	T prob.
Constant	0.066	0.848
TRT Citronella	-0.421	0.405
TRT Cypermethrin	0.486	0.284
TRT DEET	0.363	0.428
TRT Pyrethroid	0.000	1.000
Spp. C.imicola	1.121	0.010*
Spp. C. zuluensis	-0.521	0.311
Citronella. imicola	-0.639	0.336
Citronella. zuluensis	-0.125	0.874
Cypermethrin. imicola	-1.671	0.009*
Cypermethrin. zuluensis	-1.032	0.174
DEET. imicola	-0.839	0.170
DEET. zuluensis	-0.668	0.365
Pyrethroid. imicola	-0.703	0.267
Pyrethroid. zuluensis	0.147	0.838

As *C.imicola* is the most prevalent *Culicoides* midge implicated in the spread of AHS it is critical to find that there is a repellent that acts specifically against *C. imicola.*

For the time versus species interaction no significant difference were found between the evening and morning catches in response to the various species.

The final analysis carried out was done with sex as a factor in order to ascertain the interactive affects between treatments and sex. Results showed that there were significantly less males caught ($P<0.001$). The addition of treatments and subsequent interactions did not have any significant effects on the change of the linear model

and therefore there were no significant interactions between sex and treatment factors.

3.4 Conclusion

It was shown that on total midge numbers there was a slight difference between the total catches at dusk and dawn, with fewer midges being caught in the dusk period. There was no significant difference in the number of female midges caught in the two time periods. It is therefore best for horse owners to show equal diligence in the protection of their horses in the mornings and afternoons, as female midge activity does not appear to change.

In terms of the efficacy of commercial repellents it was concluded that both citronella and cypermethrin-based products were effective in repelling the *Culicoides* midges. As none of the other products showed any significant increase in catch numbers to that of the control catch it can be concluded that none of the repellents acts as an attractant to the midges but rather are poor as a repellent and do not deter the midges away from the host.
In the testing of the interactive effects, it was shown that there was a significant interaction between cypermethrin and *C. Imicola,* resulting in less *C. imicola* being caught in the cypermethrin treated traps in comparison to the others. Cypermethrin-containing repellents are therefore the most effective repellent for use on *C. imicola*.

As there were no significant interactions between time and species or species and sex it safe to suggest that the application of a cypermethrin or citronella based product once in the late afternoon just before dusk would provide efficient repellency against the *Culicoides* spp. until dawn, reducing the number of midges in contact with the horse, thus reducing the likelihood of AHS infection. Use of the other repellents would necessitate the repeated application or refreshment of the repellent in order to remain effective. As a

general recommendation then, refreshment of repellents is indicated to increase their efficacy.

CHAPTER 4 ALTERNATE HOSTS FOR *C. BOLITINOS* AND *C. IMICOLA*

4.1 Introduction

As the *Culicoides* spp. is known to transmit Bluetongue in sheep and Three-day Stiff Sickness, *inter alia,* in cattle (Du Toit, 1944; Yuill, 1986) it may be possible that the horse is not the preferred host of either C*. Bolitinos* or *C. Imicola,* which have been implicated in the transmission of AHS (Mellor, 1993; Venter *et al*., 2000). Nevill and Anderson (1972) showed that in the wild, in an area inhabited with mules, fowls, sheep and cattle, the *Culicoides* midges they caught contained blood from each of the mammalian and avian sources. Although some *Culicoides* species demonstrated a preference for either mammalian or avian blood, no species seemed to have a significant predilection for a specific mammalian species. Though none of the tested *Culicoides* spp. were those implicated in AHS transmission in South Africa, *viz* C.*imicola* and C.*bolitinos* (Meiswinkel & Paweska, 2003; Cêtre-Sossah *et al*., 2004), it may be possible that offering an alternate host in the vicinity of horses to the South African AHS vectors may aid in reducing the bite load of horses. This is linked to the fact that out of a population of *Culicoides* females only 10-70% will be positive for AHS infection (Venter *et al*., 2000). Therefore if we reduce the number of midges that are able to blood feed on a horse, in essence reduce the bite load, we reduce the likelihood that the horse will come in contact with an AHS carrying midge. There is also the possibility that AHS infection is linked to the number of midge bites, but this is as yet unproven.

Survey data (refer chapter 2) have revealed that a number of horse owners do have other species on the property, and the presentation of an alternate host to the midge vector may indeed reduce the bite

load on the horses. Without a complex comparison over breeding seasons, considering vector ecology in the mature and immature stages, and strict cognizance of confounding, it may be possible to isolate the host/vector relationship. This study is but a pilot of that, and serves to investigate whether alternate hosts can be attractive to midges.

4.2 Materials and Methods

Trial Facilities

The trial was conducted at Ukulinga research facilities using research horses (Thoroughbreds), feedlot cattle (Santa Getrudis) and feedlot sheep (mixed Dorper and Meat Master). Horses, cattle and sheep were housed in open fronted sheds during the duration of the collection. Each shed was approximately 10m apart and all experienced the same weather/wind conditions, as the collections were run concurrently over a period of three weeks during the peak midge season in February 2007.

Data Collection

Two standard downdraught suction light traps (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*, 2003) were hung inside each of the three housing facilities (as described in Chapter 3, Figure 13). Traps were run from 16:00 until approximately 08:00 the following morning, over the crepuscular period of the midges (Mellor *et al*., 2000). Midges were captured in vials containing alcohol attached to the traps, for preservation before being sorted and sent for identification to species level. Vials were collected at each site every night for five consecutive nights in a series of three replications.

Data sorting and Identification

Catches were sorted under a dissecting microscope removing all non-*Culicoides* spp. *Culicoides* were sent to Dr G. Venter at OVI

(Onderstepoort Veterinary Institute) for further identification, using wing mapping (Venter & Meiwinkel, 1994)

Data Analysis

The catch data was log transformed (log (x+1)) in order to remove skewness brought about by the large variation typical in catch data. The data was then analysed using a standard Analysis of Variance in Genstat v9 (2006). The species of midge as well as the sex were analysed as factors to determine any relationship or interaction between total count, males and females, and host animal.

4.3 Results and Discussion

The analysis of variance showed that there were highly significant differences between the numbers of midges caught of each species, as well as the males and females caught. The numbers of midges caught with the different hosts (Table 10), indicates as well that midge vectors are not only attracted to horses.

Table 10 Analysis of variance of log transformed data (log (x+1)) of total midge catches averaged over three collection periods of five consecutive nights, showing the response in sex and species of *Culicoides* midge to the presence of an equine, bovine or ovine host.

	Species			
	C.imicola	*C. bolitinos*	*C. zuluensis*	Other
	1.011^{a}	0.846^{b}	0.758^{bc}	0.677^{c}
	Host			
	Cattle	Horse	Sheep	
	0.934^{a}	0.749^{b}	0.787^{b}	
	Species			
Sex	*C. bolitinos*	*C. imicola*	*C. zuluensis*	Other
Female	1.568^{a}	1.549^{a}	1.320^{b}	1.143^{c}
Male	0.124^{e}	0.473^{d}	0.196^{e}	0.212^{e}
	Host			
Sex	Cattle	Horse	Sheep	
Female	1.498^{a}	1.408^{ab}	1.278^{b}	
Male	0.369^{c}	0.089^{d}	0.295^{c}	

^{a,b,c} shared superscripts do not differ significantly ($p<0.01$)

No species exhibited a profound interest in a particular host ($p>0.05$), such that the vectors were not host specific. *C. imicola* and *C. bolitinos* are the only two species proven to transmit AHS (Meiswinkel & Paweska, 2003) and neither of these two species display a particularity to horses, so alternate hosts may act as a method to reduce midge load on horses.

More female midges were caught compared to males (mean 0.251) by the animals, but males do not require to take a blood meal and therefore would not be drawn to the host animals (Mellor *et al*., 2000), and seek nectar instead. More *C. imicola* than *C. bolitinos* and *C. zuluensis* were caught at the open-sided sheds. More midges were caught near the cattle than by the horses and the sheep, though there was no difference between the numbers caught at the horses versus the sheep. Cattle therefore seem to be more attractive than either the sheep or the horses. More female *C. imicola* and *C. bolitinos* were caught overall and more females were found at the horses and cattle.

4.4 Conclusion

Offering cattle, sheep or horses to *Culicoides* midges does influence the number of midges caught at each of these locations, particularly in that female midges are found in proximity to the cattle and horses. Nevill and Anderson (1972) when looking at host preferences of *C. pallidipennis* which is the species equivalent of *C. imicola* (Lubenga & Khamala, 1976) found that the species of *Culicoides* was not host specific and was equally attracted to cattle, sheep and horses. Female midges were most numerous in traps by cattle and horses, and may have been dissuaded by the difficulty of penetrating the wool of the sheep. Female midge catches were not significantly higher in horse trap catches, so alternate hosts can be used to reduce the midge load. Introducing alternate hosts into the area where horses are kept, will divide the bite load, which must

necessarily reduce the viral challenge to the horses. This makes alternate hosts a possible avenue of exploration for the control of AHS. The predilection of *C. bolitinos* to breed in cattle dung (Meiwinkel & Paweska, 2003) implies as well that stricter management of vector ecology (Jenkins, 2008) be employed to enhance any alternate host strategies in AHS control. It underscores as well that prophylactic strategies in the control of AHS need to be comprehensive, and no one strategy can be considered to be fool-proof in the eradication of the effects of the disease.

CHAPTER 5 ARTIFICIAL WIND-SPEED AS A METHOD OF VECTOR CONTROL

5.1 Introduction

Effective prophylactic control of African horse sickness has to include a number of strategies, incorporating adult and immature vector control, vaccinations and elements of husbandry practice. As described in the survey data (Chapter 2), horse owners are desperate to implement any method vaguely implicated in the prevention of AHS (AHS, 2006).

The control of AHS rests in the reduction of the bite load. As a result, means of diverting the attention of the midges from the horses have been proposed in this thesis and in the literature (Jenkins, 2008). Repellents (Mellor & Hamblin, 2004) and gauze on the stables (Meiswinkel *et al*., 2000) and alternate hosts (Nevill & Anderson, 1972) and blanketing (Townley *et al*., 1984) are all methods that may prevent the infected *Culicoides* midge biting the horse. All prophylactic strategies rely on characteristics of the midge, which are exploited in their control. Another characteristic is that the *Culicoides* midge is a weak flier. The *Culicoides* midge only achieves forward movement in still air with a magnitude of 0.5m/s (Pedgley, 1983). Furthermore, Kettle (1977), in his review of works on the Diptera: Ceratopogonidea, found that *C.brevitarsis* (the Australian equivalent of *C.bolitinos*) only formed swarms in wind speeds below 1.2m/sec and that swarming ceased altogether at 1.9m/s. If midges are unable to form a swarm in higher wind speeds then it can be assumed that they will be less efficient or totally unable to fly in wind speeds increased over 1.9m/s. Midges take advantage of the decreased wind speeds in the evenings and through the night to fly and find a host.

And this presents a simple, yet effective strategy to prevent the midges biting the horses. Increasing wind speeds artificially can reduce the likelihood of midges entering areas where horses are kept, and so increase the effectiveness of other strategies employed in the control of AHS.

5.2 Materials and Methods

Trial Layout

Five industrial fans, standing at approximately horse shoulder height, were placed in an enclosed corridor each facing into a stable (dimensions 6 x 4m) containing one horse. Each stable contained a standard down-draught light trap with a UV light bulb (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*, 2003) placed in the centre point of the stable (Figure 15)

Data was collected over a period of 12 nights where fans were left on or off on alternate nights. Traps were put on at 16:00 and data was collected until 08:00. Fans produced a wind speed of 4m/s.

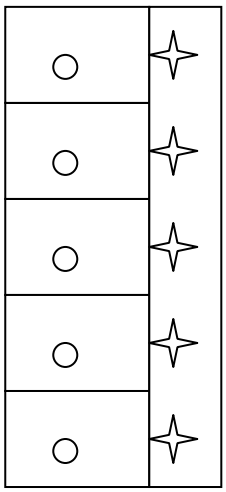

Figure 15 Schematic diagram of the layout of stables and downdraught suction light traps (Nevill, 1967; Van Ark and Meiswinkel, 1992; Rawlings *et al.*, 1998; Paweska *et al.*, 2003; Rawlings *et al.*, 2003) in relation to industrial fans producing 4m/s wind speed.

Data Collection and Sorting

Midges were collected in collecting vials containing 80% alcohol at the bottom of the down draught trap (see Figure 13 of the trap) and at 8:00 each morning, the vials were removed. Fresh vials were placed for each collection. Collections were sorted and all non-C*ulicoides* diptera were removed. All *Culicoides* midges were sent to Onderstepoort Veterinary Institute for identification to species level using a wing mapping technique (Venter & Meiswinkel, 2004)

Data Analysis

The catch data (for total midges, male, female and species catches) was log transformed (log (x+1)) in order to reduce skewness brought about by the large variation in catch sizes (McConway *et al*., 1999). The transformed data was analysed using a standard Analysis of Variance in Genstat v9 (2006), with responses in total catch, males, females and species of midge influenced by the wind speed with stables as a block structure.

5.3 Results and Discussion

The results of the wind speed trial showed that there was a significant difference in the number of midges caught when the fans were switched on in comparison to when they were switched off (Table 11). Midges demonstrate a reduced ability to fly towards an attractant (in this instance a light trap) when the wind speed is increased (Pedgley, 1983).

Table 11 Analysis of variance on Log (x + 1) transformed data to demonstrate the effect of wind speed on the proportion of males and females and species caught

Fan_switch	**On**	**Off**		
	1.00[a]	1.29[b]		
Sex	**Female**	**Male**		
	1.752[a]	0.538[b]		
Species				
C. imicola	Other	*C. bolitinos*	*C. zuluensis*	
1.309[a]	1.184[ab]	1.081[b]	1.006[b]	
Sex				
Fan_switch	Female	Male		
Off	2.014[a]	0.567[c]		
On	1.489[b]	0.510[c]		
Species				
Sex	*C.bolitinos*	*C.imicola*	Other	*C.zuluensis*
Female	1.910[a]	1.774[a]	1.691[a]	1.632[a]
Male	0.253[c]	0.844[b]	0.676[b]	0.380[c]

[a,b,c] shared superscripts do not differ significantly ($p<0.01$)

Table 11 shows that significantly more midges were caught when the fans were switched off than whilst they were on ($p<0.01$). Only the female midges require a blood meal (Wittman & Baylis, 2000) and therefore would be drawn to the hosts (and subsequently the light traps) by CO_2 plumes and other semiochemical cues (Mordue-Luntz, 2003), while the male midges which do not feed on blood (Mellor *et al.,* 2000) would not be drawn to the hosts by these biochemical cues.

C. imicola was caught in the highest number in comparison to *C. bolitinos* and *C. zuluensis* ($p<0.05$). When the fans were off, more females were caught in comparison to the number of females caught

when the fans were on ($P<0.01$), but there was no difference in the number of males caught whether fans were on or off. There were more *C. imicola* and other (combined other species) males caught than there were *C. bolitinos* and *C. zuluensis*. A more rigorous approach should be used in further trials to investigate the critical wind speeds required for the exclusion of midges from stables.

5.4 Conclusion

Culicoides spp. is a weak flier (Kettle, 1984) and this is shown to be an effective means of reducing the midge activity around stables. Furthermore, it is a comparatively cheap and highly successful intervention to assist in the control of the AHS vector.

Increasing the wind speed and air turbulence around the horses reduces the total number of midges but more importantly the total female midges. This supplies a useful strategy in the control of AHS by reducing the contact time or access of the ASH vector to the host.

CHAPTER 6 DISCUSSION AND CONCLUSION

The eradication of the AHS virus through vaccination alone seems to be at the present time non achievable (Mullins, 2006; *pers. comm.*) either through possible ineffiency of the vaccine or through abuse and total non-use of the vaccine by equine owners (Welsh, 2006; *pers. comm.*). This can be seen by the data retrieved through the survey study and AHST data (refer to chapter 2). Between 88 -95% of respondents were shown to have vaccinated their animals, and these were only in a limited survey area not including rural persons that have limited access to vaccinations. It has also been proven through research that in order for a vaccination to be effective, the strategy through which it is rolled out needs to be just as effective (Lord *et al*., 1997). Horse owners need to be made aware of the influence that the timing of vaccination has on the development of an immune response and on the overall control of the disease. Therefore as South African horse owners we are left at a cross roads in regards to the virus where alternative strategies need to be looked into for effective management and possible prevention of infection by this deadly virus.

This leads us to the investigation of a number of different prophylactic methods that are aimed at reducing the likelihood of AHS infections. The first method which can be seen from the survey data as being the most widely used form of prevention strategy is the use of repellents. In the survey analysis it was found that though the application of repellents is the most popular form of prevention it was not significantly effective in reducing the likelihood of AHS infection. It was seen through testing that some repellents significantly reduce the number of midges. The type of repellent was not specified in the survey request and therefore repellents which are not effective may have been included with effective repellents, nullifying the effect of repellents in reducing the incidence of AHS in the survey data.

Survey data showed that Citronella had no significant effect on reducing the likelihood of AHS infection in the survey, while experimentation in Chapter 3 found citronella to be useful as a repellent. Little other experimentation has been carried out on the effectiveness of Citronella as a repellent against the *Culicoides* spp., though it was not found to be an effective repellent against Aedes aegypti (Fradin & Day, 2002) and Braverman *et al.* (1999) demonstrated some attraction of Citronella for *Culicoides*. In the study of survey statistics is was found that the use of paraffin was significant at the 10% level in reducing the likelihood of AHS infection. As mentioned previously the field trial experiment showed that citronella was a significant repellent against the *Culicoides* spp., as was the cypermethrin-based repellent. Cypermethrin in particular caught significantly less *C. imicola* thus making it the most effective repellent against this species. These outcomes shed some positive light on the use of certain repellents in the fight against AHS but more research is necessary to determine this more thoroughly. If populations of midges were easier to grow under laboratory conditions it would be possible to do more intricate tests on the most active ingredient in repellants as well as their duration of effectiveness. More research specific to the species involved in the transmission of AHS would assist horse owners in producing a better strategy for repellent use.

The use of alternate hosts was the third most popular means of control used by horse owners in the survey. Approximately 85% of persons surveyed had either cattle, sheep or both in the immediate area where horse were kept. Because many of the species implicated in the transmission of AHS feed not only on horses but also on cattle and sheep (Nevill & Anderson, 1972), introducing alternate hosts into an area where horses are kept distributes the bite load amongst all these hosts rather than having them all feeding on the horses. This reduces the number of midges biting the horses and therefore the likelihood of an AHS infection. The survey statistics

showed that there was a 15% probability that horses would NOT contract AHS when alternate hosts were present in the area. Further field studies concluded that the male midges were more drawn to the cattle than to the horses or sheep, while no significant difference could be seen between the catches at the horse and sheep. This difference was not seen with the female midges though so one cannot conclude that any of the AHS transmitting *Culicoides* spp. are host preferential which corroborates Nevill and Anderson's (1972) findings. Because midge species are not host specific, the presence of an alternate host may attract some, but not all of the midge population away from the horses and therefore reduce the number of possible encounters that the horse will face with an AHS carrying midge.

The final prophylactic method tested in this research was the use of increased air speed in the confined area where horses are kept. The use of fans in stables is fairly new and an unexperimented method of controlling AHS. Fans increase both the air speed and the turbulence of the air surrounding the horse. Midges are notoriously weak fliers, and are not able to reach the flying speeds to enable them to reach their target in these increased air speeds (Pedgley, 1983). Survey data found that less than 10% of the surveyed population used this method as a control strategy and no significant effect could therefore be found in the outcome of the survey analysis. In the field experimentation though there was found to be a resoundingly significant effect on reducing the number of midges in the stable area when the fans were switched on in comparison to when they were switched off. Though these field trials gave extremely positive results, little evidence of this method as a control system in AHS in the literature was found. More research into the exact wind speed, turbulence or combination thereof required to stop midges from flying would aid owners in installing the correct type and configuration of fans in their stables.

Though all the above results show that managerial methods can significantly reduce the numbers of midges able to gain a blood meal from the horses, it has to be noted that not one of the methods stopped the midges entirely. It is therefore critical in the protection of the horses against the *Culicoides* midge and the deadly AHS virus that we utilise as many of the available resources that we have. Even though the vaccine is not 100% effective either, a greater proportion of vaccinated animals (herd immunity) together with repellents, alternate hosts, traps, larviciding and habitat alteration will increase the probability of protection against African horse sickness.

REFERENCES

AHS, 2006. African Horse Sickness website. 27/07/06 www.africanhorsesickness.co.za

Alexander, R. A., 1935. Studies on the Neurotropic virus of Horsesickness III, The Intracerebral Protection Test and its application to the Study of Immunity. Onderstepoort Journal of Veterinary Science and Animal Industry. 4(2), 349-377

Alexander R. A., W. O Neitz & P. J. Du Toit., 1936. Horse sickness. Immunisation of horses and mules in the field during the season 1934-1935 with a description of the technique of preparation of polyvalent mouse neurotropic vaccine, Onderstepoort Journal of Veterinary Science and Animal Industry. 7,17-30.

Babrekar, A. A., G. R. Kulkarni, B. B. Nath & P. B. Vidyasagar., 2004. Extracellular electrical activity from the photoreceptors of midge. Journal of Bioscience. 29(3), 349-353

Barnard, B. J. H., 1997. Some factors governing the entry of *Culicoides* spp. (Diptera: Ceratopogonidae) into stables. Onderstepoort Journal of Veterinary Research. 64, 227-233

Baylis, M., R. Meiswinkel & G. J. Venter., 1999. A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: ceratopogonidae) in southern Africa. Tydskr. S. Afr. Vet. Ver. 70(2), 80-89

Boyle, D. B., 1994. Viral Vaccines for Animal Disease Control. In: Vaccines in Agriculture. Eds. Wood, P. R., P. Willadsen, J. E. Vercoe, R. M. Hoskinson & D. Demeyer, CSIRO, Australia. pp. 187-191

Braverman, Y., 1989. Control of biting midges Culicoides (Diptera: Ceratopogonidae), vectors of bluetongue and inducers of sweet itch: a review. Israeli Journal of Veterinary Medicine. 45, 124-129

Braverman, Y. & A. Chizov-Ginzburg., 1997. Repellency of synthetic and plant-derived preparations for *Culicoides imicola*. Medical and Veterinary Entomology. 11, 355-360

Braverman, Y. & A. Chizov-Ginzburg, Mullens, B.A., 1999. Mosquit repellent attracts *Culicoides imicola* (Diptera: Ceratopogonidae). Journal of Medical Entomology 36: 113-115.

Capela, R., Purse, B.V., Pena, I., Wittman, E.J.,Margarita, Y., Capela, M., Romao, L., Mellor, P.S., Baylis, M., 2003. Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. Medical and Veterinary Entomology 17, 165-177.

CASA, 2006. Casino Association of South Africa. July 2006. www.casasa.org.za

Cêtre-Sossah, C., T. Baldet, J. Delécolle, A. Perrin, C. Grillet & E. Albina., 2004. Molecular detection of *Culicoides* spp. and *Culicoides imicola*, the principal vector of bluetongue (BT) and African Horse Sickness (AHS) in Africa and Europe. Veterinary Research. 35, 325-337

Conte, A., C. Ippoliti, P. Calistri, S. Pelini, L. Savini, R. Salini, M. Goffredo & R. Meiswinkel., 2004. Towards the identification of potential infectious sites for Bluetongue in Italy: a spatial analysis approach based on the distribution of *Culicoides imicola*. Veterinaria Italiana. 40(3), 311-315

Coetzer, J. A. W. & B. J. Erasmus., 1994. African horsesickness. In: Infectious diseases of livestock with special reference to Southern Africa (vol. 1). Eds. Coetzer, J. A. W., G. R. Thomson & R. C. Tustin, Oxford University Press, Cape Town. pp. 460-479

Crow-Smith, L., 2005. Transfer of passive immunity from the mare to the foal and the timing of the AHS vaccination relative to foaling in Thoroughbred mares. MSc (Agric) Thesis, University of KwaZulu-Natal, South Africa

Du Toit, R. M., 1944. The transmission of blue-tongue and horse sickness by *Culicoides*. Ondersteeport Journal of Veterinary science and Animal Industry. 19, 7-16

Erasmus, B. J., 1972. The pathogenesis of African Horsesickness. Proceedings of the Third International Conference on Equine Infectious Diseases, Paris, 1-11. Basal: Karger.

Fischer-Tenhagen, C., C. Hamblin, S. Quandt & K. Frölich., 2000. Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. Journal of Wildlife diseases. 36(2), 316-323

Fradin, M. S. & J. F. Day., 2002. Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine. 347(1), 13-18

Grubman, M. J. & S. A. Lewis., 1992. Identification and Characterization of the Structural and Nonstructural Proteins of African Horsesickness Virus and Determination of the Genome Coding Assignments. Virology. 186, 444-451

Holbrook, F. R., 1985. Research on the control of bluetongue in livestock by vector suppression. In: Bluetongue and Related Orbiviruses. Eds. Barber, T. L. & M. M. Jochim, A. R. Liss Inc., New York. pp. 617-620

Howell, P. G., 1962. The isolation and identification of further antigenic types of African horsesickness virus. Onderstepoort Journal of Veterinary Research. 29(2), 139-149

Hu, Y., H. Jin, X. Du, C Xiao, D. Luo, B. Wang and R. She., 2007. Effects of chronic heat stress on immune responses of the foot-and-mouth disease DNA vaccination. DNA Cell Biology. 26(8), 619-26

Jenkins, A. B., 2008. A study of the *Culicodes* (Diptera: Ceratopogonidae) vectors of African horse sickness to enhance current practical control measures and research methods. MSc (Agric) Thesis, University of KwaZulu-Natal, South Africa

Kettle, D. S., 1977. Biology and Bionomics of Bloodsucking Ceratopogonids. Annual reviews of Entomology. 22, 33-51

Kettle, D. S., 1984. Arboviruses. In" Medical and Veterinary Entomology. Croom Helm Ltd, Kent, pp. 451-474

Kleinbaum, D. G. & Klein, M., 2002. Introduction to Logistic Regression. In: Logistic Regression; A self-learning text. Eds. Dietz, K., M. Gail, K. Krickeberg, A. Tsiatis & J. Samat, New York. Pp.1-37

Lindley, J.R., 1966. The ovarian cycle in *Culicoides barbosai* Wirth & Blanton and *C.furens* (Poey) (Diptera: Ceratopogonidae). Bulletin of Entomological Research. 57, 1-17.

Lord, C. C., M. E. J. Woolhouse & J. A. P. Heesterbeek., 1996. Vector-borne diseases and the basic reproduction number: a case study of African horse sickness. Medical and Veterinary Entomology. 10, 19-28

Lord, C. C., M. E. J. Woolhouse & P. S. Mellor., 1997. Simulation studies of vaccination strategies in African horse sickness. Vaccine. 15(5), 519-524

Lubenga, B. & C. P. M. Khamala., 1976. Larval habitats of common *Culicoides Latreille* (Diptera, Ceratopogonidae) in Kenya. Bulletin of Entomological Research. 66, 421-425

McConway, K. J., M. C. Jones & P. C. Taylor., 1999. Binary Regression. In: Statistical Modelling using Genstat®. Ed. Rabson, J, Arnold Publishers, Great Britain. pp. 225-241

McIver, S. B., 1981. A model for the mechanism of action of the repellent DEET, N,N-diethyl-*m*-toluamide on *aedes aegypti* (Dipteria: Culicidae). Journal of Medical Entomology. 18, 357-361

Meiswinkel, R., E. M. Nevill & G. J. Venter., 1994. Vectors: Culicoides *spp..* In: Infectious diseases of livestock with special reference to Southern Africa (vol. 1). Eds. Coetzer, J. A. W., G. R. Thomson & R. C. Tustin, Oxford University Press, Cape Town. pp. 69-89

Meiswinkel, R., M. Bayliss & K. Labuschagne., 2000. Stabling and the protection of horses from *Culicoides bolitinos* (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. Bulletin of Entomology. 90, 509-515

Meiswinkel, R. & J. T. Paweska., 2003. Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. Preventive Veterinary Medicine. 60, 243-253

Mellor, P.S., 1993. African horse sickness: transmission and epidemiology. Veterinary Research. 24, 199-212

Mellor, P. S., J. Boorman & M. Baylis., 2000. Culicoides Biting Midges: Their role as Arbovirus vectors. 45, 307-340

Mellor, P. S & C. Hamblin., 2004. African Horse Sickness. Veterinary Research. 35, 445-466

Mordue-Luntz, A. J., 2003. Arthropod semiochemicals: mosquitos, midges and sealice. Biochemical Society Transactions. 31(1):128-133

Moule, L. 1896. Histoire de la Médecine Vétérinaire, Maulde, Paris. 38 (cited by Mellor & Hamblin, 2004)

Mullen, G.R., 2002. Biting midges (Ceratopogonidae). In: Medical and Veterinary Entomology. Eds. Mullen G., & Durden, L. Academic Press. London.

Nevill E. M., 1967. Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. MSc (Agric) Thesis, Onderstepoort University, South Africa

Nevill E. M. & D. Anderson., 1972. Host preferences of *Culicoides* midges (Diptera: Ceratopogonidae) in South Africa as determined by precipitin tests and trap catches. Onderstepoort Journal of Veterinary Research. 39(3), 147-152

Nevill H., G. J Venter, R Meiswinkel & E. M Nevill., 2007. Comparative descriptions of the pupae of five species of *Culiciodes imicola* complex (Diptera, Ceratopogonidae) from South Africa. Onderstepoort Journal of Veterinary Research. 74: 97-114

OIE, World Organisation for Animal Health, 2002. Technical disease data. July 2006. www.oie.int/eng/maladies/finches/A_A110.HTM

OIE, World Organisation for Animal Health, 2006. Handistatus II. July 2006. www.oie.int/hs2/report.asp?lang=en

Onderstepoort Biological Products. 25/07/2006
www.obpvaccines.co.za/prducts.htm

Paweska, J. T., A. S Prinsloo & G. J. Venter., 2003. Oral susceptibility of South African *Culicoides* species to live-attenuated serotype-specific vaccine strains of African horse sickness virus (AHSV). Medical and Veterinary Entomology. 17, 436-447

Pedgley, D. E., 1983. Windborne spread of insect-transmitted of animal and man. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 302, 463-470

Roy, P., P. P. Mertens & I. Casal., 1994. African horse sickness virus structure. Comparative immunology, microbiology and infectious diseases. 17, 243-273

Rawlings, P., Meiswinkel, R.M., Labuschange, K., Welton, N., Baylis, M., Mellor, P.S., 2003. The distribution and species characteristics of the *Culicoides* biting midge fauna of South Africa. Ecological Entomology. 28, 559-566.

Rawlings, P., Snow, W.F., Boorman, J., Denison, E., Hamblin, C., Mellor, P.S., 1998. *Culicoides* in relation to the transmission of African horse sickness virus in The Gambia. Medical and Veterinary Entomology. 12, 155-159.

Roy, P., P. C. Mertens & I. Casal., 1994. African horse sickness virus structure. Comparative Immunology, microbiology and infectious diseases. 17(3-4), 243-273

Schreck, C. E., N. Smith & T. P. McGovern., 1979. Repellency of selected compounds against two species of biting midges (Diptera: Ceratopogonidae: *Culicoides*). Journal of Medical Entomology. 16(6), 524-527

Service, M. W., 1971. Conservation and the Control of Biting Flies in Temperate Regions. Biological Conservation. 3(2), 113-122

Theiler, A., 1921. African Horse-Sickness (Pestis Equorum). South African Department of Agriculture. Science Bulletin 19

Tizard. I., 1992. General Principles of vaccination and vaccines. In: Veterinary Immunology, An Introduction (4th Ed.). W. B Saunders Company, Pennsylvania. pp. 261-275

Townely, P., K. P. Baker & P. J. Quinn., 1984. Preferential landing and engorging sites of *Culicoides* species landing on a horse in Ireland. Equine Veterinary Journal. 16(2), 117-120

Van Ark, H. & R Meiswinkel., 1992. Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). Onderstepoort Journal of Veterinary Research. 59: 183-189

Venter, G. J., S. D. Graham & C. Hamblin., 2000. African horse sickness epidemiology: vector competence of South African *Culicoides* species for virus serotypes 3, 5 and 8. Medical and Veterinary Entomology. 14, 245-250

Venter, G.J., Meiswinkel, R., 1994. The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light trap survey of the colder high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. Onderstepoort Journal of Veterinary Research. 61, 327-340.

Wood, H. A., 1973. Viruses with double-stranded RNA genomes. Journal of General Virology. 20, 61-85

Yuill, T. M., 1986. The ecology of tropical arthropod-borne viruses. Annual Review of Ecological Systems. 17, 189-219

Walker, A.R., 1977. Seasonal fluctuations of the *Culicoides* species (Diptera: Ceratopogonidae) in Kenya. Bull. Ent. Res. 67, 217–233

Welby, M.P., Baylis, M., Rawlings, P., Mellor, P.S., 1996. Effect of temperature survival and rate of virogenesis of African horse sickness virus in *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. Bulletin of Entomological Research. 86 (6), 715-720. (abstract).

Whitmann, E. J & M. Baylis., 2000. Climate change: Effects on *Culicoides*-transmitted viruses and implications for the UK. The Veterinary Journal. 160, 107-117

Whitman, E.J., Mellor, P.S., Baylis, M., 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis.* Medical and Veterinary Entomology. 16 (2),147-156.

Personal Communications

Groenink, S., University of KwaZulu-Natal,

Catton, G., Stride Veterinary Products,

Howell, P., African Horse Sickness Trust

Welsh, D., African Horse Sickness Trust

Mullins, D., Equine Veterinary Services