THE EPIDEMIOLOGY OF AN AFRICAN HORSE SICKNESS

OUTBREAK IN THE WESTERN CAPE PROVINCE OF SOUTH

AFRICA IN 2004

BY

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List of figures and tables

L i s t   o f   a b b r e v i a t i o n s

%	percent
<	less than
>	more than
°C	degrees Celcius
µg	microgram
µl	microliter
AHS	African Horsesickness
AHSV	African Horsesickness virus
AHT	Animal Health Technician
ARC	Agricultural Research Council
ARGT	annual rye grass toxicity
Cell	cellphone number
CFT	complement fixation test
DEM	digital elevation model
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay

GIS	Geographic Information System
GPS	Global Positioning System
km	kilometre
LST	land surface temperature
m	month
m/s	meter per second
ml	millilitre
mm	millimetre
n	number
Neg	negative
OIE	Office International des Epizooties
OVI	Onderstepoort Veterinary Institute
PBS	phosphate buffered saline
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
s	seconds
SRBCs	sheep red blood cells
TCID$_{50}$	tissue-culture-infective dose

Tel   telephone number

y   year

C h a p t e r   1

SUMMARY

Historically African Horsesickness (AHS) outbreaks are rare occurrences in the Western Cape Province. The 2004 outbreak was particularly troubling since it followed only five years after the previous outbreak and even before any cases were reported further inland, which is traditionally the source of infection for the southern (non-endemic) parts of the country. Following confirmation of the diagnosis, control measures were immediately instituted and an epidemiological investigation was initiated.

The investigation revealed, inter alia, that serological profiles of case horses were inconsistent. A case was subsequently defined as a horse showing typical symptoms of AHS and from which virus could be isolated.

The disease pattern for both the 2004 and 1999 outbreaks can be classified as sporadic epidemics. This type of epidemic pattern is to be expected in a vector borne disease and it is typical in a disease situation where some of the animals are immune.

The temporal pattern revealed that the level of immunity in the equine population of the affected area was higher during the 2004 outbreak than during the 1999 outbreak. In addition, it showed a clustering of cases during the initial stages of both the 2004 and 1999 outbreaks. This illustrated the efficacy of the control measures (including

movement and vector control), which was instituted immediately after the diagnosis of the first case.

The analysis of the spatial pattern during both the 2004 and 1999 outbreaks identified the Eerste-river-valley as a high-risk area for the outbreak of AHS in the surveillance zone.

The population pattern during the 2004 outbreak illustrated that the risk of dying of AHS was higher in horses of 5 years and younger ($p<0.10$).

It was shown that vaccination and stabling offers the best protection against the risk of dying as a result of AHS infection in an exposed population ($p<0.05$).

A questionnaire survey was conducted as part of the epidemiological investigation and it revealed that only 12.4% of equine holdings in the affected area practiced vector control, while a high percentage of horses (69.6%) were protected by means of vaccination, which impacts negatively on the purpose of a surveillance zone.

The number of *Culicoides imicola* midges in the area where the outbreak was detected was extremely high, constituting 94.6% of the *Culicoides* midge population. This is comparable with the 1999 outbreak when 96.0% of the midges collected were identified as *C. imicola*. However, during a similar survey in 1996, *C. imicola* comprised only 11.3% of the population (Neville *et al*. 1988, Venter, G., personal communication 2004).

Furthermore, the outbreak was detected even before significant rainfall was recorded in the region and transmission occurred at average minimum temperatures below 15 °C.

The virus responsible for the 2004 outbreak was typed as AHS serotype 1, while AHS serotype 7 was identified as the cause of the 1999 outbreak.

The source of the infection in the 1999 outbreak was the illegal movement of two horses from the Free State Province in the infected zone into the surveillance zone. Although no absolute proof could be obtained, there is strong evidence that the source of the 2004 outbreak was again the movement of horses, this time from Namibia, accentuating that horse movements constitutes the highest risk to the integrity of the free zone.

Since the ability to control an outbreak successfully is directly dependant on rapid detection and given the large number of vaccinated horses as a result of the outbreaks and the AHS movement control policy, amendments to the export policy and legislation are recommended.

AHS outbreaks in the control area of South Africa cause substantial financial loss to the horse industry and the controlling authorities.

C h a p t e r   2

2. LITERATURE REVIEW

2.1 African Horsesickness

African Horsesickness (AHS) is an infectious, non-contagious disease of equines, which can manifest as a peracute, acute, subacute or mild disease. The disease is endemic to the African continent and is caused by an orbivirus that is transmitted by *Culicoides* midges. Nine serotypes of the virus have been identified.

The incubation period can range from two to ten days and four forms of the disease have been recognized (Coetzer & Guthrie 2004).

- "Dunkop" or 'pulmonary' form:
  This form usually affects fully susceptible horses and foals, and is characterised by severe dyspnoea, paroxysms of coughing and discharge of large quantities of frothy, serofibrinous fluid from the nostrils. Less than 5% of horses suffering from the "dunkop" form recover.

- "Dikkop" or 'cardiac' form:
  The characteristic symptom of this form is subcutaneous swelling of the head and neck, especially the supraorbital fossae. The course of the disease is always more protracted and milder than in the "dunkop" form, with a mortality rate of about 50%.

- 'Mixed' form

  This form is usually only diagnosed during necropsy when lesions of both the "Dunkop" and "Dikkop" forms are observed. Clinically any of the above-mentioned signs can occur in no particular order. The mortality rate in horses suffering from this form of the disease is approximately 70%.

- Horsesickness fever

  This is a very mild form of the disease and is rarely diagnosed clinically. A rise in body temperature lasting up to six days is the characteristic finding. Horses immune to one or more serotypes of AHS usually show this form of the disease.

Mortality rates can be as high as 95% in horses and 70% in mules, while donkeys rarely show clinical signs (Coetzer & Guthrie 2004).

Donkeys can have a persistent viraemia for at least 12 days. They must be considered a potential source of virus for midges and can act as "silent reservoirs", although it is very unlikely that they are long-term reservoirs for the virus (Hamblin *et al.* 1998).

Zebra do not show any clinical signs of disease and can serve as a reservoir of infection. Virus can be isolated up to 40 days after infection from blood and up to 48 days after infection from the spleen (Barnard *et al.* 1994). The capability of zebra to maintain AHS virus is clearly illustrated by the continuing infections during every month of the year, with a peak period in winter. This peak is attributed to the presence of large numbers of susceptible foals in the presence of active *Culicoides* species (Barnard 1993).

Dogs are susceptible to infection with African Horsesickness virus (AHSV) and usually obtain the infection by eating infected meat, but it is unlikely that dogs will act as field reservoir for the virus (Braverman & Chizov-Ginzburg 1995).

The presence of low levels of antibodies against AHS virus in the serum of some free-living elephants has been confirmed. This is judged to be the result of natural hyper-immunization due to frequent exposure to infected biting insects. Elephants should therefore be regarded as poorly susceptible and unlikely to be a source of AHS virus (Barnard *et al.* 1995).

AHS virus is only able to survive through continuous and uninterrupted cycles of transmission between its vertebrate host and invertebrate biological vector, with no 'vector-free' period being longer than the maximum duration of viraemia. When the length of the 'vector-free' period exceeds the duration of viraemia in the local susceptible population, AHS virus will be unable to persist and will only occur in epizootic form (Mellor 1994).

Normally African Horsesickness spreads from the northern (endemic) parts of South Africa into the Western Cape, as was the case with the 1999 outbreak in the Stellenbosch region, and clinical cases are usually only expected from March onwards (Coetzer & Guthrie 2004, Koen, P., personal communication 2004). The southward spread of the disease varies in distance depending on the time of first appearance, the number of susceptible equids and the favourability of climatic conditions for breeding of the insect

vector. The movement of actively infected horses can obviously facilitate the spread. This southward spread is terminated abruptly by the first heavy frost in May (Bosman *et al.* 1995).

2.2 African Horsesickness control measures and exports

African horsesickness is a controlled disease in South Africa. For the purpose of control, the country is divided into two areas, an infected area and a control area. The latter is situated in the Western Cape Province and is further divided into three zones - the protection, surveillance and free zone.

The location of the free zone was based on a historical absence of the disease and not the absence of vectors (Lord *et al.* 2002). This is of great concern, as the introduction of virus in this zone will probably lead to a full-scale outbreak of the disease in the highly susceptible population of this zone.

In order to retain South Africa's export status, strict movement controls is implemented and no clinical cases may have been reported in the surveillance and free zones for at least two years prior to the intended export.

The surveillance zone is meant to act as early warning or sentinel system for the free zone, and horses in this area are only allowed to be vaccinated against AHS with written permission from the State Vet Boland office.

An outbreak in these areas can result in a ban of exports for at least 2 years.

From 2001 until 2003 a total of 487 horses (149 Thoroughbreds, 111 Arabians and 52 other) were exported from the free zone to different countries (including United Arab Emirates, European Union, Singapore, Malaysia, Mauritius, Hong Kong and Bahrain) (Dunn 2003). The exact income from these exports are unknown but is estimated to be at least R65 million annually (Buhrmann 2002).

The benefits of the existence of a free zone include horses being able to return to their point of origin. For example, the system allows shuttle stallions to be imported for a season to improve bloodstock in South Africa and then return to their country of origin (Koen, P., personal communication 2004).

2.3 *Culicoides* as vector

2.3.1 *General:*

Culicoides spp. are tiny biting midges about 1-3 mm in length. These midges are biological vectors of African horsesickness and *C. imicola* appears to be the most important species for virus transmission in southern Africa; although *C. bolitinos* has also been implicated in an outbreak of African horse sickness (Coetzer & Guthrie 2004, Meiswinkel & Paweska 2002). The midges breed in materials high in organic matter, for example damp soil or animal dung. Females lay 100-200 eggs and these develop to larva and pupa within this substrate. The life cycle can be completed in 3-4 weeks (Howell *et al.* 1983).

Adult midges can survive for up to 90 days, but they more often only survive for fewer than 10-20 days (Mellor *et al.* 2000).

Female midges feed on blood to provide the protein necessary for the development and maturation of eggs. Bovines, ovines, equines and poultry can act as hosts for *Culicoides* spp. (Howell *et al.* 1983). One bloodmeal is required for each batch of eggs. The species and ambient temperature determines the frequency of feeding, which in turn influences the rate of egg development. The feeding frequency increases with a rise in ambient temperature, this may increase infection rate as virus transmission may occur at each feed (Wittmann & Baylis 2000).

Most susceptible animals are infected between sunset and sunrise, as this is the period during which *Culicoides* midges are most active (Coetzer & Guthrie 2004).

2.3.2 Climatic influences:
Climate has a remarkable effect on *Culicoides* populations and thus on the epidemiology of AHS.

The rate of virogenesis within a vector is usually faster at higher temperatures and this together with the higher feeding frequency at higher temperatures will enhance the probability of virus transmission. The rate of virogenesis decreases as temperature decreases and will cease altogether at low temperatures, although the lifespan of the vector may be extended in these conditions. Virus replication appears to be minimal at 15°C, causing the titre per infected midge to be generally low and with the

accompanying dramatic fall in infection rate, transmission has not been recorded at this temperature (Mellor *et al.* 1998).

Beside temperature, wind speed also has a profound effect on the activity of *Culicoides.* Activity is almost completely inhibited at wind speeds exceeding 3 m/s (Walker 1977).

Culicoides activity is furthermore stimulated by a high relative humidity (Walker 1977).

Normally the midges can only disperse a few kilometres from their breeding site, but it has been stated that at windspeeds of 10-40 km/h and at temperatures between 12 and 35°C they may be carried at heights up to 1.5 km as aerial plankton for distances up to 700 km (Sellers 1992).

In years with above average rainfall, *Culicoides* numbers have been shown to increase 200-fold compared to the populations during dry years. However it should be kept in mind that irrigation can maintain high numbers of *C. imicola* during the dry season (Meiswinkel 1998).

Two models were developed to predict the abundance and distribution of *C. imicola* in southern Africa. The first model utilises climate data only, and the second uses satellite-derived and climatic variables. In both models the variables most strongly correlated with *C. imicola* abundance were the minimum land surface temperature (LST) and altitude. A positive correlation exists between abundance and the annual mean daily maximum and minimum temperatures, while abundance was negatively correlated with

number of days below 0°C and altitude. *C. imicola* populations decreased as the days of frost or altitude increased (Baylis *et al.* 1999).

C. imicola adults can only survive the winter in areas where the average daily maximum temperature during the coldest month of the year was higher than 12.5°C. It is thus only in these areas that the disease can become endemic (Sellers & Mellor 1993).

2.3.3 Geographical influences

AHS is endemic in the northeastern parts of South Africa (mainly Mpumalanga and Limpopo). Outbreaks in the southwestern parts of the country are apparently the result of introductions of the virus to the local *Culicoides* and equine populations (Lord *et al.* 2002).

Large populations of *Culicoides* midges develop under certain conditions, for example, a frost-free area, good rainfall (average 613 mm/annum), irrigated kikuyu pastures and the presence of vertebrate hosts (Meiswinkel 1997).

During the 1996 outbreak in South Africa, it was found that the largest populations of *C. imicola* occurred in areas with clayey, moisture retentive soils, while the lowest numbers (or none) occurred in areas with sandy and quick-draining soils (Meiswinkel 1998).

The apparent explanation of this phenomenon is that the larvae of *C. imicola* live in the top one centimeter of the soil. Throughout their development (normally a 7-10 day period), they require moisture and micro-organisms. *C. imicola* would therefore probably

be unable to establish itself in sandy soil and/or arid areas where moisture in the upper layer of the soil drains out rapidly or is not regularly replenished (Meiswinkel 1998).

An area close to Port Elizabeth has been found to be *C. imicola* free, probably due to sandy soils and windy circumstances (Meiswinkel 1997). During the 1996 outbreak, two deaths were reported in this area, but the conclusion after an investigation was that the horses acquired the infection outside the area (Meiswinkel 1998). *C. bolitinos* is however present in this area (Meiswinkel 1997).

In contrast with *C. imicola*, *C. bolitinos* achieves dominance in the cooler, wetter, central uplands and in the sandy coastal areas of South Africa. AHSV can thus be circulated in equids almost countrywide (Meiswinkel & Paweska 2002).

From the above it is clear that climate has a great impact on the spread of the disease. It is thus very important to include climatic data in an investigation of an outbreak as this can help determine the origin and explain the pattern of spread.

2.4 Stellenbosch, Western Cape Province

Stellenbosch is situated in a winter rainfall region. Summers are normally warm and dry, while winters are mild and relatively frost free, with an average rainfall of 619 mm/annum. On average, 0.1 days per annum reach a minimum temperature below freezing and for 46.2 days the minimum temperature is below 5°C (Venter *et al.* 1997).

During a study to determine the abundance of *Culicoides* species, the following species dominated in the Stellenbosch region. *C. magnus* (29.0%), *C. gulbenkiani* (27.0%), *C. zuluensis* (25.7%), *C. imicola* (11.3%) and *C. bolitinos* (4.6%) (Venter *et al.* 1997). Relatively large numbers were also collected during winter, suggesting that depending on the availability of circulating virus and susceptible hosts, virus transmission may take place throughout the year (Venter *et al.* 1997).

2.5 Vaccination

According to the Animal Diseases Act (Act 35 of 1984), all horses in the infected area of South Africa have to be vaccinated against AHS by the responsible person annually. In the protection zone, these vaccinations have to be given by a Veterinarian to be valid. In the surveillance and free zones, no vaccination is allowed except by written permission from the State Veterinarian Boland Office. However, in the event of an outbreak in the surveillance or free zones, all horses within a specific radius of the point of infection (usually about 20 km), will be vaccinated to prevent the further spread of the disease and to protect the horses in the danger areas.

As the AHS vaccine is a live-attenuated vaccine, there are some concerns about using this vaccine in non-endemic areas. Potential risks include vaccine virus reassortment with wild-type virus strains in the equine host or insect vector (especially when emergency vaccination is carried out in the presence of field virus during an outbreak), spread of the virus by the vector and reversion to virulence of vaccine strain virus. To

date, no evidence has been published to demonstrate that vaccine strains are capable of infecting *Culicoides* vectors in the field or causing clinical disease as a result of reversion to virulence (Paweska *et al*. 2003).

Theoretically it can be estimated that $10^{5.0}$ 50% tissue-culture-infective doses ($TCID_{50}$) per ml of blood are needed to expose vectors to approximately one $TCID_{50}$. The titre of attenuated AHSV in the blood of vaccinated horses is low and large numbers of *Culicoides* would be needed. A small proportion of these midges will take up virus and only some will become infected and live long enough to feed on and potentially transmit the virus to other uninfected horses. It is thus very difficult to design a laboratory experiment that takes into account the large numbers of vaccinated horses and vectors that would be present in field situations. However, it is postulated that considering the abundance of *C. imicola* and *C. bolitinos* in South Africa and the expected level and duration of viraemia following vaccination, that transmission of at least AHSV-4 and 7 by *Culicoides* from vaccinated to unvaccinated animals may occur. Such an event has not yet been demonstrated (Paweska *et al*. 2003).

2.6 Stabling as preventative measure

The stabling of horses at night provides protection against *C. imicola*. Catches of *C. imicola* inside stables with open doors or ungauzed windows, were less than the numbers captured outside. However, more *C. bolitinos* were caught in open stables than outside, indicating that open structures may protect horses from *C. imicola* (which is exophilic),

but may increase the attack rates from the endophilic *C. bolitinos*. By closing the doors and gauzing the windows, a 14-fold reduction in the numbers of both species were found. Although high wind speeds suppress midge activity outside, ceiling fans had no suppressant effect inside (Meiswinkel *et al.* 2000).

2.7 Previous outbreaks of AHS in the Western Cape Province

Horses and donkeys were introduced into South Africa shortly after the arrival of the first settlers of the Dutch East India Company in the Cape of Good Hope in 1652 and frequent reference was made to AHS in their records (Coetzer & Guthrie 2004). In 1719 nearly 1 700 horses succumbed to AHS in the Cape of Good Hope. The ability of frost to arrest outbreaks was recognized at this time (Coetzer & Guthrie 2004). The worst recorded outbreak since then was in 1855 when nearly 70 000 horses of the Cape of Good Hope died. This constituted more than 40% of the horse population in the region at that time (Coetzer & Guthrie 2004).

In recent history, outbreaks of AHS have only been reported in 1967 (Bosman *et al* 1995) and in 1990 (Du Plessis *et al* 1991) in the Cape Peninsula (now classified as part of the surveillance zone) prior to the 1999 outbreak. Neither of these two outbreaks was completely documented. The 1967 outbreak involved two confirmed cases and during the 1990 outbreak 11 horses succumbed to AHS type 4 in the area between Paarl and Wellington (Guthrie, A.J., personal communication 2005).

In addition, 20 suspect cases were reported in total outside the surveillance zone in the Beaufort-West State Veterinary Area in 1996, 2000 and 2002. This area is part of the infected zone (together with the rest of the country) and outbreaks in this zone do not influence the export of horses.

The first recorded outbreak in the surveillance zone after 1990 was the 1999 outbreak, during which 32 horses died from 21st March to 17th May 1999. The findings of the outbreak investigation by Veterinary Services in 1999 were never published.

2.8 Objectives of the study

The 2004 outbreak was of great concern considering that it occurred only five years after the 1999 outbreak (the previous two outbreaks occurred at intervals of 9 and 23 years respectively) and even before any cases were reported further inland, which is traditionally the source of infection for the southern (non-endemic) parts of the country. No information on previous outbreaks has been published within the scientific literature, hence the 2004 outbreak provided an ideal opportunity to record the epidemiology of AHS and investigate the risk factors pertaining to the Western Cape epidemic.

C h a p t e r   3

3. MATERIALS AND METHODS

3.1 Disease outbreak investigation

3.1.1 Case definition

For the purpose of this study, a case was defined as a horse showing typical symptoms of AHS and tested serologically positive for AHS infection on either enzyme-linked immunosorbent assay (ELISA) or complement fixation test (CFT).

3.1.2 Verifying the diagnosis

For each suspect clinical case, the diagnosis was verified by serological tests including ELISA (Appendix A) or CFT (Appendix B) which was conducted at the ARC-Onderstepoort Veterinary Institute (OVI). This laboratory is the reference laboratory for controlled diseases in South Africa. Blood was collected from 17 horses at Elsenburg and 16 suspect cases in the control area in 7 ml serum (for the above mentioned two tests), heparin and ethylenediamine tetra-acetic acid (EDTA) tubes. The blood collected in the heparin and EDTA tubes were sent to the ARC-Onderstepoort Veterinary Institute (OVI) for antigen detection using reverse transcription polymerase chain reaction (RT-PCR) (Appendix C). The jugular vein was used for the collection of blood. Thirteen dead horses were subjected to a post mortem examination, performed by the personnel of the Provincial Veterinary Laboratory in Stellenbosch, and spleen and

lung tissue, as well as lympnodes was sent to the OVI for virus isolation and typing (Appendix D).

3.1.3 Determining the magnitude of the problem

A questionnaire survey was conducted in the 30 km radius surrounding the index case (see 3.3 for details of this survey). All equine deaths in this 30 km radius were recorded and where possible post mortem examinations were performed at the Provincial Veterinary Laboratory (PVL) in Stellenbosch.

3.1.4 Determining the temporal pattern

The number of cases per day was recorded for the duration of the outbreak from the 31st of January to the 28th of March 2004.

3.1.5 Determining the spatial pattern

ArcView 8.3 was used for GIS manipulation of the data. Coordinates of the affected properties (obtained by the Animal Health Technicians visiting the farms) were recorded in an Excel spreadsheet (Microsoft Office 2000) for easy reference. All the overlays used were obtained from the Elsenburg GIS database and included:

- boundaries of the Western Cape Province
- towns of the Western Cape Province
- Digital elevation model (DEM) of the Western Cape Province
- contour fill layer of the Western Cape Province
- surveillance zone boundary, obtained from Western Cape Veterinary Services
- free zone boundary, obtained from Western Cape Veterinary Services
- major rivers of the Western Cape Province
- major roads of the Western Cape Province

Climatic and geographical data for the relevant areas and time periods during both the 1999 and 2004 outbreaks was obtained from the Agricultural Research Council at Nietvoorbij in Stellenbosch. The average values from four weather stations (Elsenburg, Vergelegen, Môrewag and Nietvoorbij) in the outbreak area were used.

The climatic data was recorded in Microsoft Excel and the average function was used to determine weekly averages for minimum temperature, maximum temperature, wind speed and rainfall. These averages were captured on a separate spreadsheet together with the corresponding number of cases and the chart wizard was used to illustrate these values on combined histogram and line graphs.

In conjunction with Mr. Gert Venter from the OVI Entomology section, midge traps were put up at several sites in the Stellenbosch area throughout the duration of the outbreak to determine the composition of the midge population in the region at that time. These sites included locations where AHS was diagnosed during the 1999 outbreak and a few surrounding properties. The midges were collected in phosphate buffered saline (with an added antiseptic containing Clorhexidane gluconate 0.3 g/100 ml & Cetrimade 3.0 g/100 ml) and sent on a weekly basis to the OVI Entomology section for identification.

3.1.6 Determining the population pattern

Host factors (age, breed, sex), history and relevant clinical data were recorded for 32 horses that presented with clinical signs.

19

Since the equine population at Elsenburg, where the index case was diagnosed, is managed by the Animal Production section of the Department of Agriculture, detailed information could be obtained and recorded on affected as well as unaffected animals. The following information was recorded for each case: identification, breed, age, sex, vaccination status, ELISA (enzyme-linked immunosorbent assay) titre, complement fixation test (CFT) result, health status and information on the environment where the horses were kept.

Three zebra, which were resident on a positive property, were darted by a wildlife specialist and blood in serum, heparin and EDTA tubes was collected from the jugular vein. These samples were analyzed by CFT (complement fixation test) and indirect ELISA (enzyme-linked immunosorbent assay).

3.2 Population at risk

3.2.1 Phase 1

Initially the population at risk was defined as all horses, donkeys, mules and zebra within a 30 km radius surrounding the index case. This radius was decided upon based on the previous AHS outbreak in the surveillance zone in 1999, which spread 17 km from the index case by natural means (i.e. not horse movements) (Koen, P., personal communication 2004).

3.2.2 Phase 2

At the end of March 2004, a single case was diagnosed at Kalbaskraal, 32 km northwest of the epicentre. Subsequently the definition of the population at risk was extended to include a 10 km radius surrounding this focus. The decision of a 10 km radius (instead of the previous 30 km radius) was made to keep the project manageable in the face of manpower constraints.

3.2.3 Phase 3

The questionnaire survey described below (3.3) which was initially only conducted in the 30 km radius, was extended to include the 10 km radius surrounding Kalbaskraal. The results of this survey confirmed that the disease did not spread within a 10 km radius from this focus and since no reports from any suspect cases were received outside the 10 km radius; it was assumed that the new population at risk assumption was correct.

3.3 Questionnaire survey

3.3.1 Objectives

The aim of the questionnaire was to ascertain the following:

- to obtain an accurate, up to date equine census
- to determine the vaccination status (and thus degree of protection) of the horses in the region
- to discover clinical evidence of disease or unexplained deaths of horses
- to obtain information on suspicious recent movements of horses

3.3.2 Information gathered

The following questionnaire was used for the survey:

AHS QUESTIONNAIRE

Owner / Manager* details (*delete the inapplicable)

Initials: [   ] Surname: [   ]   Farm name: [   ]

Physical Address: [   ]

GPS Reading:

	East			South		
	Degrees	Minutes	Seconds	Degrees	Minutes	Seconds

Code [   ]

Postal Address: [   ]   Tel: [   ]

Code [   ]   Cell: [   ]

Census information:			
Horses	Donkeys	Mules	Zebra

Vaccination History: (Implies full vaccination with AHS1 and AHS2)								
Horses vaccinated < 3months ago:			Horses vaccinated >3months, <1year ago			Horses vaccinated >1year ago		
Number vaccinated	Done by Vet?		Number vaccinated	Done by Vet?		Number vaccinated	Done by Vet?	
	Yes	No		Yes	No		Yes	No

Remarks: _____

Movement of horses, donkeys, mules or zebra within the past 3 months:						
From			To			Horses Involved
Departure Date	Property Name	Nearest Town	Arrival Date	Property Name	Nearest Town	Name / Passport nr

Where any of the following symptoms noted among the horses during the past 2 months?: (Mark with ✔)													
Inappetance		Fever		Swelling above eye		Difficulty in breathing		Nasal discharge		Coughing		Sudden death	
Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Date of occurrence:													
Nr of horses affected:													

Diagnosis of Vet:

Are your horses stabled at night?   Yes   No   If yes, how many are stabled?

Do you use insect repellant on the horses?   Yes   No   Name of the repellant?

Remarks: _____   Date:

_____   AHT name: _____   AHT signature

3.3.3 Conducting of questionnaire

Since there was no existing up to date database that could indicate which properties had equines on them, all properties in the 30 km radius surrounding the index case and the 10 km radius surrounding the marginal case were visited by animal health technicians (AHTs) of the Western Cape Veterinary Services. Eleven AHTs were involved in conducting the survey. They started at the epicenter and gradually worked their way out to the edge of the 30 km zone by driving down each road in order to locate all properties. Each morning before starting the survey, a meeting was held during which the previous days work was marked on a map to ensure that every farm would be visited. The questionnaire was completed by the AHTs, while interviewing the owner or responsible manager, on each and every property that had any horses, donkeys, mules or zebra on them. The AHT verified the information given to him by inspecting the horses and their passports (which contain detailed information of movement and vaccination history). The verification process was thus limited to the number of equines on the property, their current health status (as determined by clinical inspection), movement and vaccination history. At the end of each day the completed questionnaire forms were returned to the Boland State Veterinary office where the data was captured on an Excel spreadsheet (Microsoft Office 2000).

After the outbreak an AHT visited all eight affected properties to obtain further information needed for the identification of the risk factors. This missing information included the host factors (age, breed, sex), vaccination history and environmental factors

(housing conditions) of all horses (infected and not infected) that were resident on these properties during the outbreak. This information was obtained by interviewing the owners or managers of the relevant properties and was filed as hard copies that were later used to obtain the necessary input data for further analysis as described below.

3.4 Handling of data and data analysis

As part of the disease outbreak investigation, the host factors, clinical signs, pathology and viral isolations was recorded and written up for each case in a situation report that was updated daily or as new information became available. Hard copies of all laboratory results were filed according to the name of the property. The post mortem findings were summarized by Dr. Jacob Stroebel of the Provincial Veterinary Laboratory in Stellenbosch and presented to the investigator in the form of a report at the end of the outbreak.

Some of the climatic data was received in Microsoft Excel format and others in Microsoft Word format, which was subsequently converted to Excel (Microsoft Office 2000). Weekly averages of minimum temperature, maximum temperature, wind speed and rainfall were illustrated on graphs, together with the corresponding number of deaths.

The data obtained through the questionnaire survey was captured in a Microsoft 2000 Excel database. The sum, count and median functions of Excel was utilized to determine the number of properties visited, the number of properties with equines, the

total number of equines, the number of equines vaccinated within the last three months, more than three months but less than a year ago, and more than a year ago, and the median number of equines per property, respectively.

The two proportions test of the software program NCSS 2004 was used to determine the relative risk (according to the exact method) associated with the exposure of horses to certain possible risk factors. The PASS chi-square estimator function from NCSS 2004 was utilized to calculate the chi-square statistic in order to further evaluate the statistical significance of the association of these risk factors to the occurrence of disease.

The "test agreement" function of the software program Win Episcope 2.0 was used to determine the kappa statistic as measure of agreement between the ELISA and CFT tests.

3.5 Comparison of the 1999 and 2004 AHS outbreaks

Information from the 1999 outbreak was extracted from the filed situation reports and included information on the geographical spread, number of deaths and date of occurrence. The geographical reference points of the deaths during both the 1999 and 2004 outbreaks were mapped and the distribution of the two outbreaks compared. For both outbreaks, the number of deaths were entered into an Excel spreadsheet according to date of occurrence.

The climatic graphs of the 1999 and 2004 outbreaks were compared and evaluated for similarities and differences.

The information gained from a parallel study done by the OVI during both the 1999 and 2004 outbreaks, involving midge trapping for species identification and attempted virus isolation, was correlated with the findings of this outbreak investigation.

C h a p t e r   4

4. RESULTS

4.1 Clinical presentation of cases

Three horses of the sixteen confirmed AHS deaths died peracutely. Three horses died acutely within approximately 12 hours after displaying ataxia and weakness. Ten horses died within one to ten days after showing clinical symptoms. Fever, anorexia, dyspnoea and swelling above the eye was the most obvious clinical signs displayed in these cases.

4.2 Confirmation of the diagnosis

4.2.1 Serological findings

Table 1: *Percheron herd at Elsenburg sampled to determine AHS antibody levels*

Name	Breed [a]	Age(years)	Sex	Vacc status [b]	Elisa titre[c]	CF Titre	Where kept	Health
Lettie	P	2	F	Unvacc	Neg	Neg	Vlei	OK
Lorrainne	P	2	F	Unvacc	Neg	Neg	Vlei	OK
Meaker	P	2	M	Unvacc	Neg	Neg	Vlei	OK
Momsen	P	2	M	Unvacc	Neg	Neg	Vlei	OK
Leroy	P	3	M	Unvacc	Neg	Neg	Vlei	OK
Marcelle	P	7	F	Vaccinated	9,800	1:32	Dam/stable	OK
Merlot	P	11	M	Vaccinated	9,800	1:48	Stable	OK
Mazda	P	10.5	F	Vaccinated	8,500	1:32	Dam/stable	OK
Liezel	P	16	F	Vaccinated	7,700	1:32	Vlei	OK
Vossie	XP	10	M	Vaccinated	6,100	1:16	Stable	OK
Mona	P	6	F	Vaccinated	5,300	1:12	Dam/stable	OK
Lumby	P	7	M	Vaccinated	5,200	1:12	Stable	OK
Mavis	P	6	F	Vaccinated	4,400	1:12	Dam/stable	OK

Lizta	P	6	F	Vaccinated	3,900	1:32	Dam/stable	OK
Sky	XP	10	M	Vaccinated	3,900	Neg	Stable	OK
Mandy	P	4.5	F	Unvacc	1,700	1:96	Dam/stable	Sick
Mariaan	P	1.5	F	Unvacc	Neg	1:192	Vlei	Died
Marinda	P	3	F	Unvacc	-	-	Vlei	Died
Michelle	P	2.5	F	Unvacc	-	-	Vlei	Died
Laura	P	2.5	F	Unvacc	-	-	Vlei	Died
Mara	P	4.5	F	Unvacc	-	-	Vlei	Died

[a] P = Percheron / XP = Percheron Cross

[b] In this table "vaccinated" indicate horses that were last vaccinated in 1999

[c] In this table the cut-off value for an ELISA positive test = 900

Table 2: *Summary of serological findings of unvaccinated horses at Elsenburg during the 2004 AHS outbreak to indicate the agreement between the CFT and ELISA tests*

	CFT +	CFT -	TOTALS
ELISA +	1	0	1
ELISA -	1	5	6
TOTALS	2	5	7

Kappa = 0.6

Table 3: *Summary of serological findings of vaccinated horses at Elsenburg during the 2004 AHS outbreak to indicate the agreement between the CFT and ELISA tests*

	CFT +	CFT -	TOTALS
ELISA +	5	5	10
ELISA -	0	0	0
TOTALS	5	5	10

Kappa = 0

Table 4: *Serological results of horses that showed clinical signs, but survived the 2004 AHS outbreak in the Western Cape Province*

Nr	Name	CFT	ELISA	Vaccination Status	Comment
1	Khamsin	>1:512	Positive	2 years ago	Diagnosed with biliary
2	Pamela	1:32	Positive	2 years ago	Diagnosed with biliary
3	Ash	>1:32	Positive	< 12 months ago	PCR negative

4	Dumble Dan	1:24	Positive	Unknown	Virus isolation negative
5	Déjà vu	Negative	Positive	December 2003	-
6	Melody Fire	>1:32	Positive	< 12 months ago	-
7	Kismet	1:48	Positive	January 2004	-
8	Seattle	1:48	Positive	Vaccinated 3 days earlier	PCR negative
9	Soldaat	-	-	1999	PCR positive
10	Nikita	1:12	Negative	Not vaccinated	PCR positive
11	La Brae Pitts	1:256	Positive	November 2003	-
12	Friesian foal	1:128	Positive	Vaccinated 24 days earlier	Possible vaccination reaction
13	Toffee	1:512	Positive	Unknown	-
14	Welsh Pony	1:12	Positive	Not vaccinated	-
15	Kalbaskraal case	Not available	Not available	Not vaccinated	PCR positive
16	Self Expression	>1:32	Positive	Unknown	Serostable on second bleed.

4.2.2 Post mortem findings of the 2004 outbreak

Thirteen horses were examined by post-mortem at the Provincial Veterinary Laboratory in Stellenbosch, starting on 23 February 2004. AHS virus serotype 1 was isolated from organs (spleen, lung) of them all. They were all adult horses except for two foals: aged 6 months and 8 months respectively. The clinical course of the disease was mostly relatively short: less than 12 hours in the 3 cases (inter alia the foals), 1 – 4 days in 8 cases, 8 - 10 days in 2 cases.

The following lesions were noted in 12 horses (in one case the lesions was not recorded):

- Moderate to severe hydropericardium (n=11).
- Moderate to severe swelling of supraorbital fossae due to oedema of its contained fat (n=9).

- Yellowish gelatinous oedema of intermuscular connective tissue (n=9). The muscles of the shoulders and neck were the most severely affected; those of the chest, back, buttocks and thighs less severely.
- Mild to moderate oedema of the lungs (n=8). In most cases, the trachea contained foam only up to the level of its bifurcation. Only in one case the foam reached the larynx.
- Moderate to severe hydrothorax (n=8).
- Moderate endocardial haemorrhages (mostly in the leftventricle) (n=8).
- Mild to moderate ascites (n=7).
- Serosal haemorrhages ("paintbrush" or ecchymotic) of small intestine, particularly the ileum (n=7). In 2 cases there were also haemorrhages of the proximal part of the ventral colon.
- Oedema (yellowish, gelatinous) of the loose connective around the oesophagus, trachea and larynx (n=7).
- Mild to moderate splenomegaly (n=4). In one case the capsule contained small haemorrhages.
- Ecchymotic haemorrhages of the visceral pleura. (n=4).
- Oedema of the parietal peritoneum (n=4)
- Oedema of the interlobular septa of the lungs (n=4).
- Oedema of the mediastinum (n=4).
- Subcutanious oedema (n=3). The neck and shoulder areas were involved, except in one case where the oedema extended to the ventral chest and abdominal areas. The horse in the latter case was shot after it had been ill for 8 days.
- Epicardial haemorrhages (n=2).
- Mild hepatomegaly with accentuated lobulation (possibly due to congestion) (n=2).
- Haemorrhages in the heart muscle (n=1).

Post-mortem examinations were performed on six other horses that died during the outbreak period. In all these cases, no gross or microscopic lesions suggestive of AHS were detected and virology for AHS was negative. Other causes (e.g. ruptured anterior aorta, ruptured colon, gastric torsion, etc) could be identified.

4.2.3 Virus typing

Virus isolation and typing from the sixteen horses that died identified AHS serotype 1 as the sole cause of the 2004 outbreak.

The cause of the 1999 outbreak was identified as AHS serotype 7.

4.3 Population at risk

4.3.1 Questionnaire survey

The following information was obtained through the questionnaires:

Table 5: *Results of the questionnaire census conducted during the 2004 AHS outbreak in the Western Cape Province*

Total number of properties visited	1 616
Total number of properties with horses	603
Total number of equines	4 484
Total number of horses (included in total number of equines)	4 289
Total number of zebra (included in total number of equines)	44
Total number of donkeys or mules (included in total number of equines).	151
Total number of horses vaccinated within twelve months before the outbreak	2 987
Total number of horses with AHS related clinical symptoms during the preceding 2 months	16
Number of properties on which management practices to repel midges are used	75
Number of horses on these properties	728

The median number of horses on the 595 unaffected properties is three (interquartile range = 298), while the median number of horses on the eight affected properties is 16 (interquartile range = 4.5).

4.4 Temporal pattern

4.4.1 Disease pattern

As illustrated in Figure 1 and 2, the disease pattern for both the 2004 and 1999 outbreaks

can be classified as sporadic epidemic patterns.
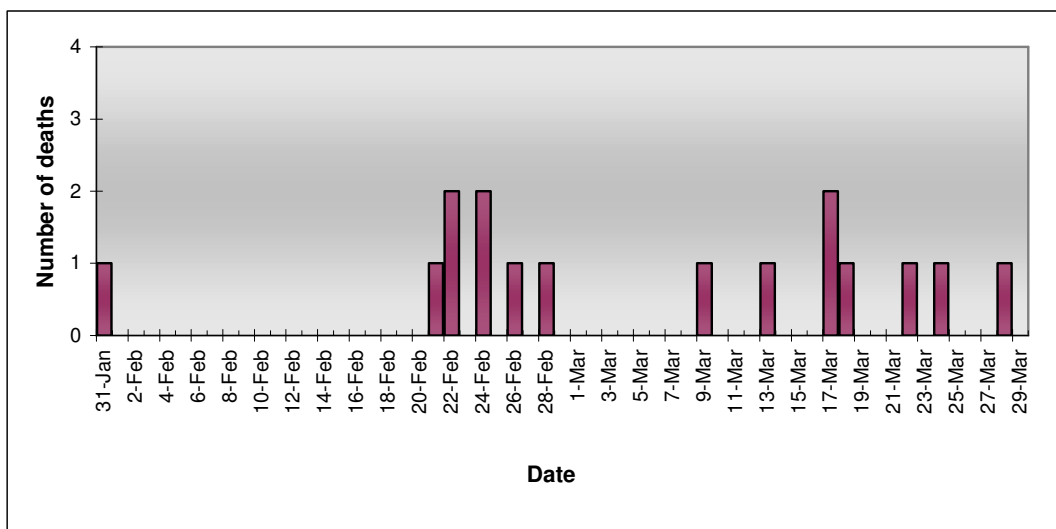
Figure 1: Timeline of deaths during the 2004 AHS outbreak in the
Western Cape Province
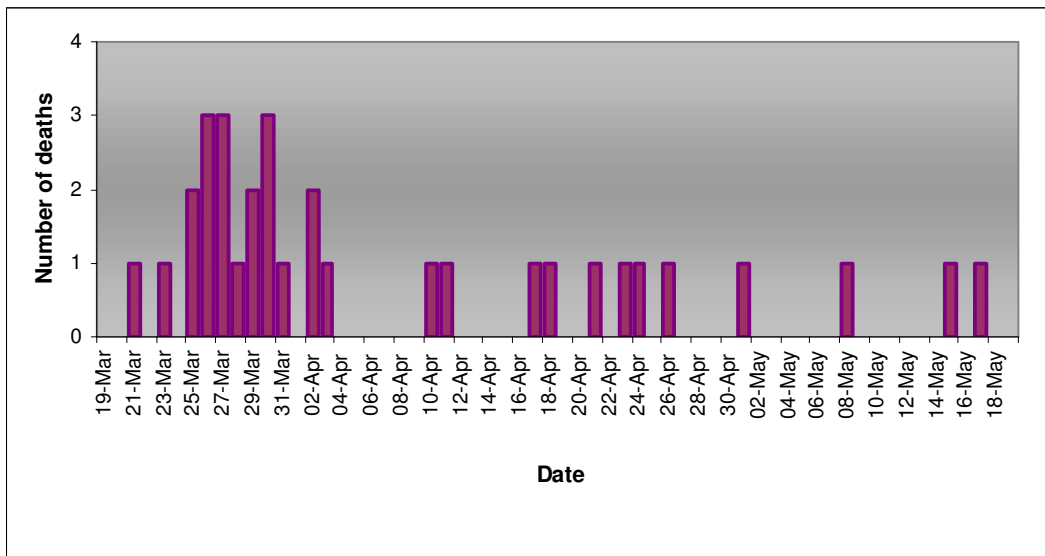
Figure 2: Timeline of deaths during the 1999 AHS outbreak in the
Western Cape Province
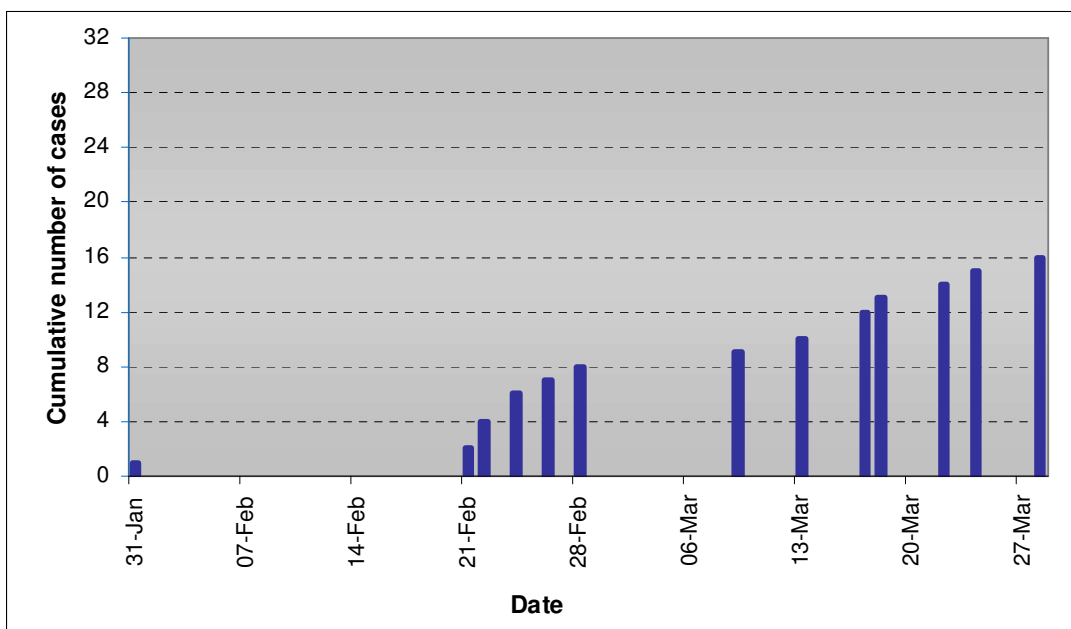
Figure 3: Cumulative case series of AHS during the 2004 outbreak
in the Western Cape Province
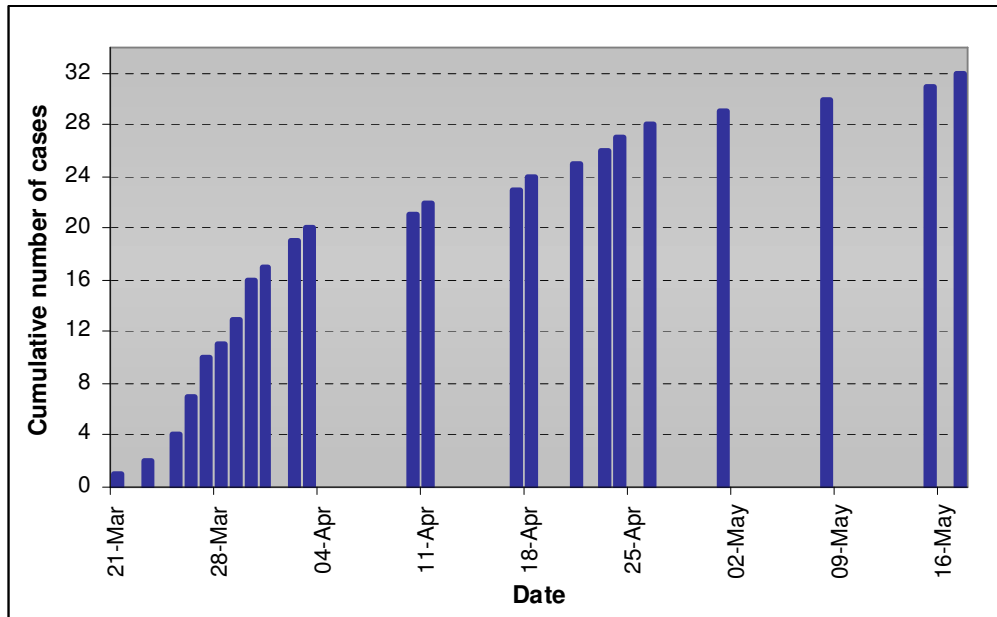
Figure 4: Cumulative case series of AHS during the 1999 outbreak
in the Western Cape Province

4.4.2 Climatic conditions related to the number of cases over time

The windspeed and direction was recorded in relation to the number of cases over time

to determine their possible influence on the vector population and subsequent disease

spread.
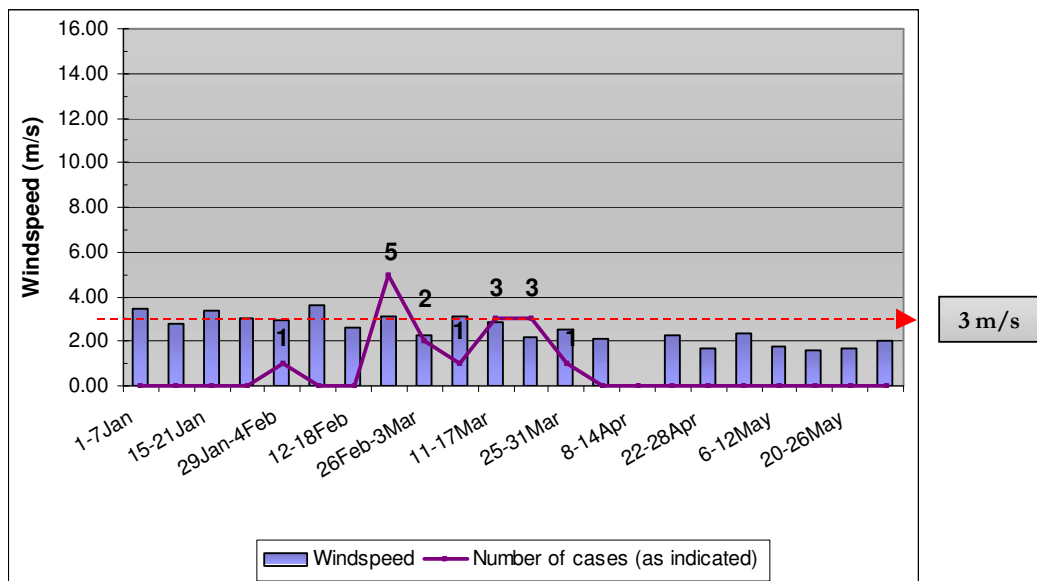
Figure 5: Windspeed and number of cases during the 2004 AHS outbreak in the Western Cape Province
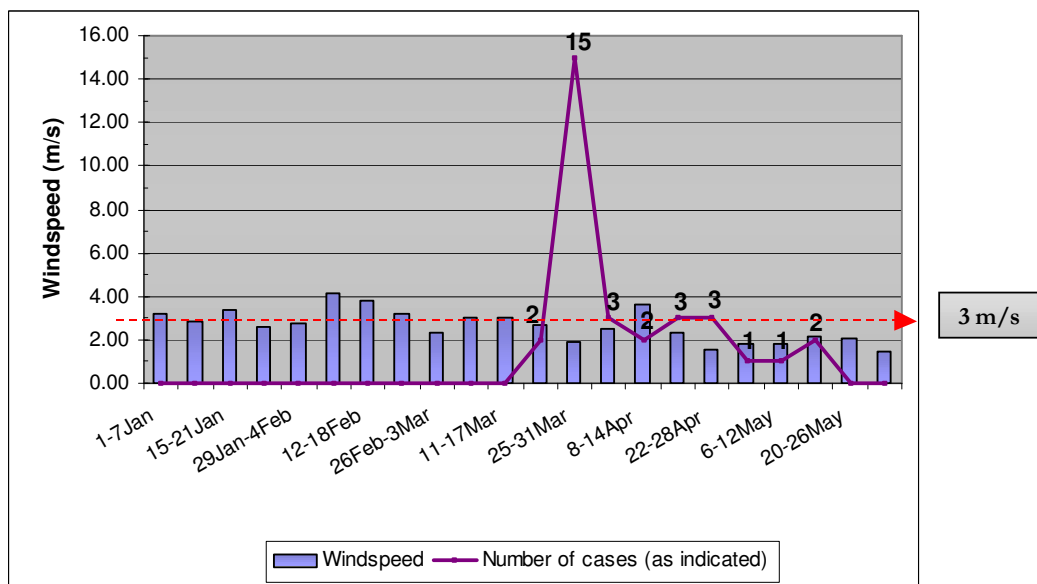
Figure 6: Windspeed and number of cases during the 1999 AHS outbreak in the Western Cape Province

During the 2004 outbreak, the wind maintained a south-south-westerly direction. During the 1999 outbreak the wind altered between a south-south-westerly direction (1-28 January, 5-11 February and 4-24 March) and a south-south-easterly direction (29 January –4 February, 12 February – 3 March and 25 March – 31 May).

The climatic variables (average weekly maximum and minimum temperature and the total weekly rainfall), of this geographical region during the 2004 and 1999 outbreak is summarised in Figures 7 and 8 in conjunction with the number of cases that occurred during that specific time period. Because of a technical malfunction at the weather station, no data was available for the week 25 to 31 March 2004.
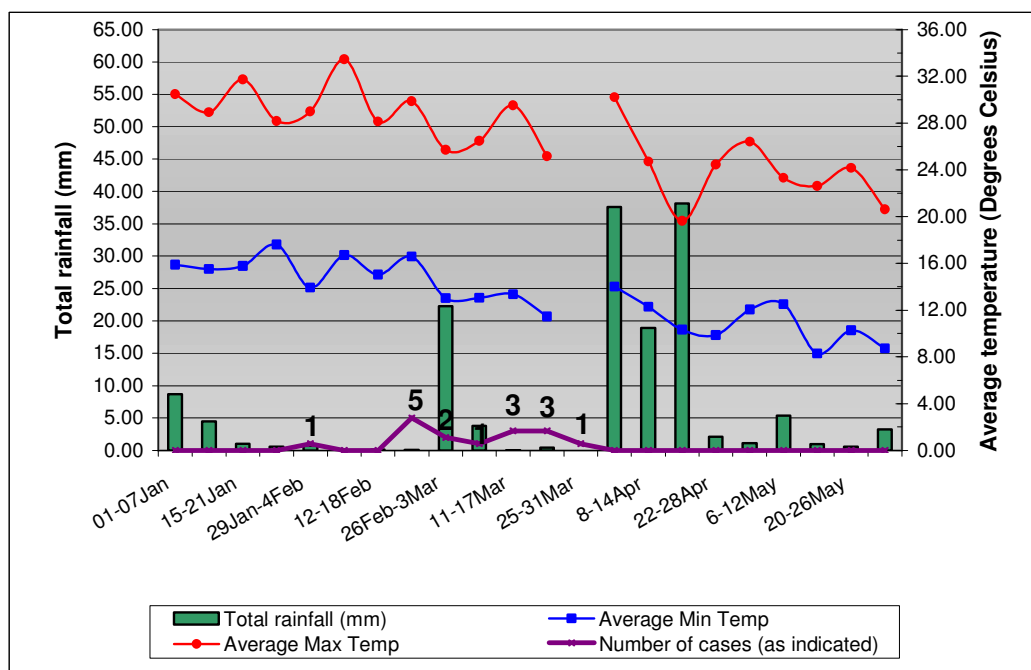
Figure 7: Average temperatures, total rainfall and case distribution during the 2004 AHS outbreak in the Western Cape Province
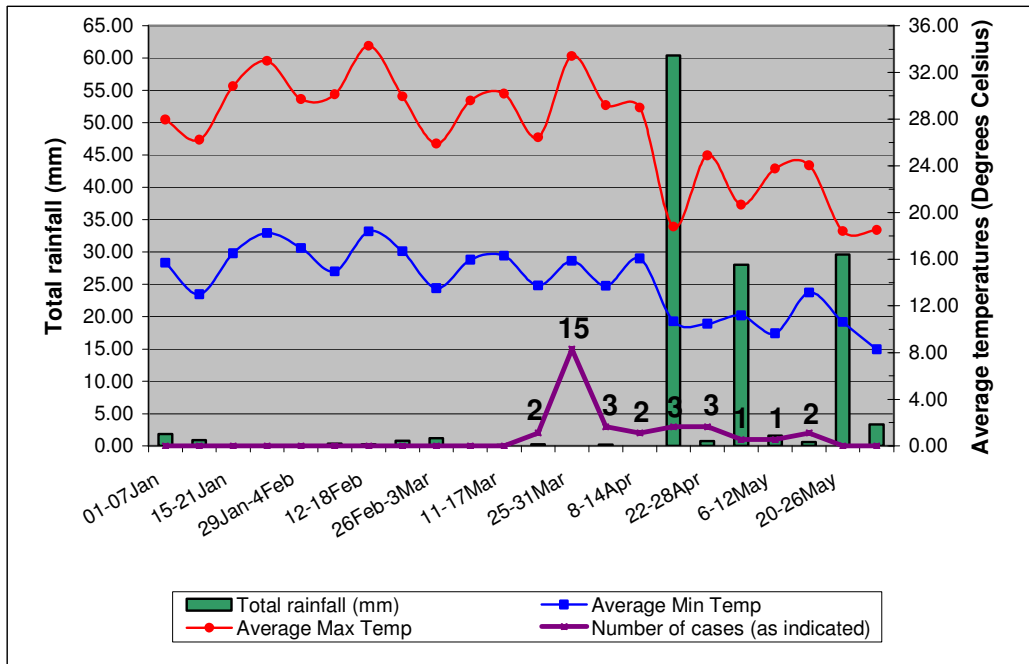
Figure 8: Average temperatures, total rainfall and case distribution during the 1999 AHS outbreak in the Western Cape Province

4.5 Population pattern

4.5.1 Identification of host factors (age, sex, breed) of cases

Table 6: *Epidemic pattern of the 2004 AHS outbreak in the Western Cape*

Case nr	Age	Breed	Sex	Vaccination history	Date of death	Name of property
1	1.5y	Percheron	F	Unvacc	31/01/04	Elsenburg
2	3.5y	Percheron	F	Unvacc	21/02/04	Elsenburg
3	2.5y	Percheron	F	Unvacc	22/02/04	Elsenburg
4	2.5y	Percheron	F	Unvacc	22/02/04	Elsenburg
5	4.5y	Percheron	F	Unvacc	24/02/04	Elsenburg
6	24y	Pony	M	Unvacc	24/02/04	Oakhill Farm
7	8m	SA Saddler	M	Unvacc	26/02/04	Troughend
8	26y	Pony crossbred	M	Unvacc	28/02/04	Daktari

9	14y	TB	F	Vacc	09/03/04	Avontuur
10	5y	Apaloosa-cross	F	Unvacc	13/09/04	Daktari
11	4y	SA Saddler	M	Unvacc	17/03/04	Vredenheim
12	8m	SA Saddler	F	Unvacc	17/03/04	Troughend
13	8y	TB	F	Vacc	18/03/04	Avontuur
14	4y	Boerperd	M	Unvacc	22/03/04	Goedverwacht
15	6m	TB	M	Unvacc	24/03/04	Avontuur
16	12y	TB	M	Unvacc	28/03/04	Kalbaskraal

*TB = ThoroughBred

M = Male / F = Female

Vacc = Vaccinated during the previous 12 months

See Appendix E for a detailed description of the outbreak.

4.5.2 Results of the determination of risk factors

The total population at risk (n = 201) used in the following tables (Tables 7 – 12) represents the total horse population on the eight affected properties at the beginning of the outbreak. Table 7 illustrates the incidence in horses 5 years and younger and more than 5 years old.

Table 7: *2x2 Table for calculating the Relative Risk among younger (5 years and less) and older horses*

	Horses 5 years and less	Horses 6 years and older	Totals
Horses dead	11	5	16
Horses survived	85	100	185
Totals	96	105	201
Incidence	11.5%	4.8%	8.0%

From Table 7 it can be seen that the risk of dying of AHS in horses equal to or less than 5 years old is 2.4 times greater than horses older than 5 years. This is however only

significant at a 90% confidence limit (confidence interval = 1.1, 5.6). The statistical significance of this association is confirmed by the chi-square test statistic of 3.7 (p<0.10).

This procedure was repeated to determine whether there would be a statistically significant association between age and the risk of dying of AHS in exposed horses, when horses at risk were defined as 10 years and younger, this is illustrated in Table 8.

Table 8: *2x2 Table for calculating the Relative Risk among younger (10 years and less) and older horses*

	Horses 10 years and less	**Horses older than 11 years**	**Totals**
Horses dead	12	4	16
Horses survived	129	56	185
Totals	141	60	201
Incidence	8.5%	6.7%	8.0%

From Table 8 the relative risk is calculated as 1.3 (90% confidence interval = 0.5, 3.2), the risk of dying of AHS in horses equal to or less than 10 years old is thus not significantly greater than horses older than 10 years. The chi-square test statistic of 0.2 confirms that the evidence is insufficient to demonstrate the existence of an association using this age risk classification (p<0.10).

Table 9 provides the breed distribution of cases.

Table 9: *Breed distribution of cases during the 2004 AHS outbreak*

Breeds	**Percheron**	**PonyX**	**SA Saddler**	**Apaloosa/ ApaloosaX**	**Thoroughbred**	**Boerperd**
Number of cases	5	2	3	1	4	1
% of Total	31.2%	12.5%	18.8%	6.2%	25.0%	6.3%

Table 10 illustrates the incidence in male and female horses.

Table 10: *2x2 Table for calculating the Relative Risk among male and female horses.*

	Male	Female	Totals
Horses dead	7	9	16
Horses survived	85	100	185
Totals	92	109	201
Incidence	7.6%	8.3%	8.0%

From Table 10 the relative risk is calculated as 0.9 (90% confidence interval = 0.4, 2.1), the risk of dying of AHS was thus not significantly different between male and female horses. The chi-square test statistic of 0.03 confirms that the evidence is insufficient to demonstrate the existence of an association between dying of AHS and the sex of horses ($p<0.10$).

Table 11 illustrates the incidence in vaccinated and unvaccinated horses.

Table 11: *2x2 Table for calculating the Relative Risk among vaccinated and unvaccinated horses*

Vaccination status	Vaccinated	Unvaccinated	Totals
Horses dead	2	14	16
Horses survived	98	87	185
Totals	100	101	201
Incidence	0.02%	13.9%	8.0%

From Table 11 the relative risk is calculated as 0.1 (95% confidence limits = 0.1, 0.6) and vaccination thus provided a protective effect against the risk of dying of AHS. The statistical significance of this association is confirmed by the chi-square test statistic of 9.7 ($p<0.005$).

4.5.3 The zebra population

Only five zebra were resident on an infected property. Blood was collected from three of these animals in serum, heparin and EDTA tubes and sent to the OVI for testing. All three zebra tested negative on CFT and indirect ELISA.

4.6 Spatial pattern

4.6.1 Housing

Table 12 illustrates the incidence in stabled horses and horses that are not stabled.

Table 12: *2x2 Table for calculating the Relative Risk among stabled and not stabled horses*

	Stabled	Not stabled	Totals
Horses dead	2	14	16
Horses survived	75	110	185
Totals	77	124	201
Incidence	2.6%	11.3%	8.0%

From Table 12 the relative risk is calculated as 0.2 (95% confidence limits = 0.1, 0.9) and stabling thus provides a protective effect against the risk of dying of AHS. The statistical significance of this association is confirmed by the chi-square test statistic of 4.9 ($p<0.05$).

4.6.2 Geographical distribution in relation to horse density and topography

See Figure 9 for the geographical distribution of the cases during the 2004 outbreak and Figure 10 for the distribution during the 1999 outbreak. Both maps illustrate where the cases occurred in relation to the boundaries of the free zone and the surveillance zone.
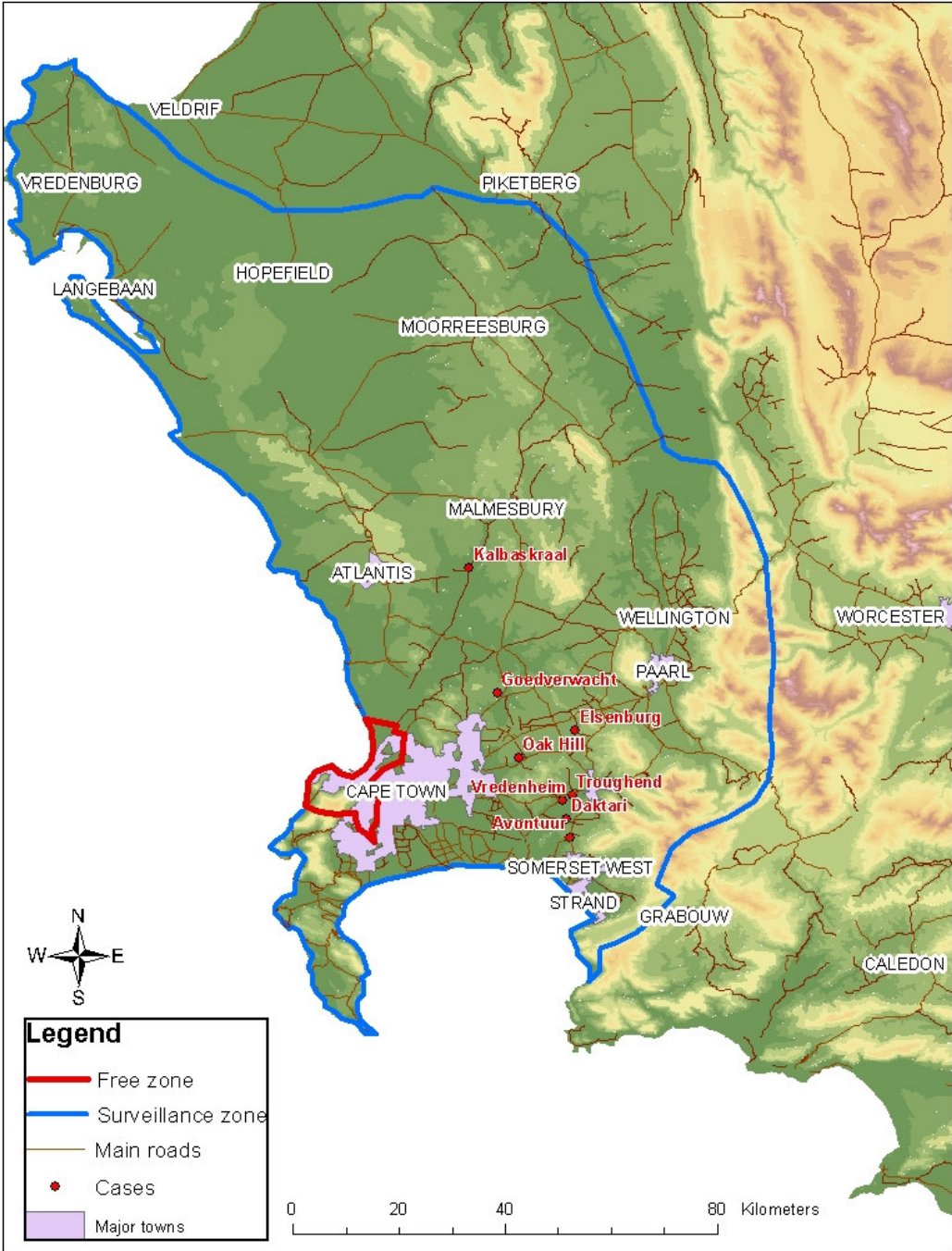
Figure 9: Geographical distribution of AHS cases in relation to the free and surveillance zones during the 2004 outbreak
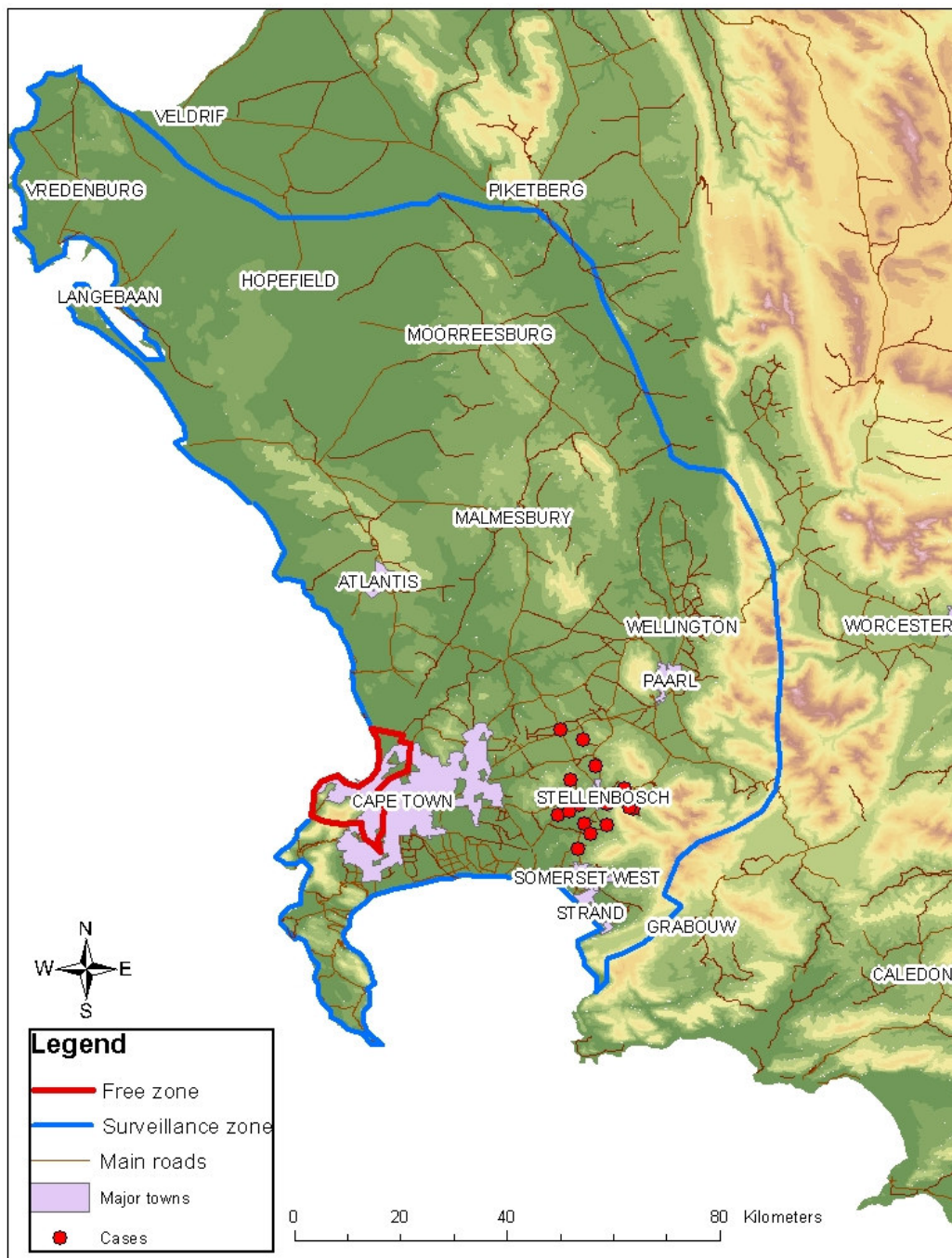
Figure 10: Geographical distribution of AHS cases in relation to
the free and surveillance zones during the 1999 outbreak

Figure 11 shows the distribution of cases during the 2004 outbreak in relation to regional topography, perennial rivers and horse density as determined through the questionnaire survey.

Figure 12 shows the distribution of cases during the 1999 outbreak in relation to the topographical features and perennial rivers of the region. The sequence of occurrence of the cases is indicated by the number 1 – 18. Multiple cases on a single farm are not indicated. No data is available on the horse density in the region during the 1999 outbreak.

Figure 11: Distribution of cases during the 2004 AHS outbreak in the Western Cape Province in relation to horse density and topography
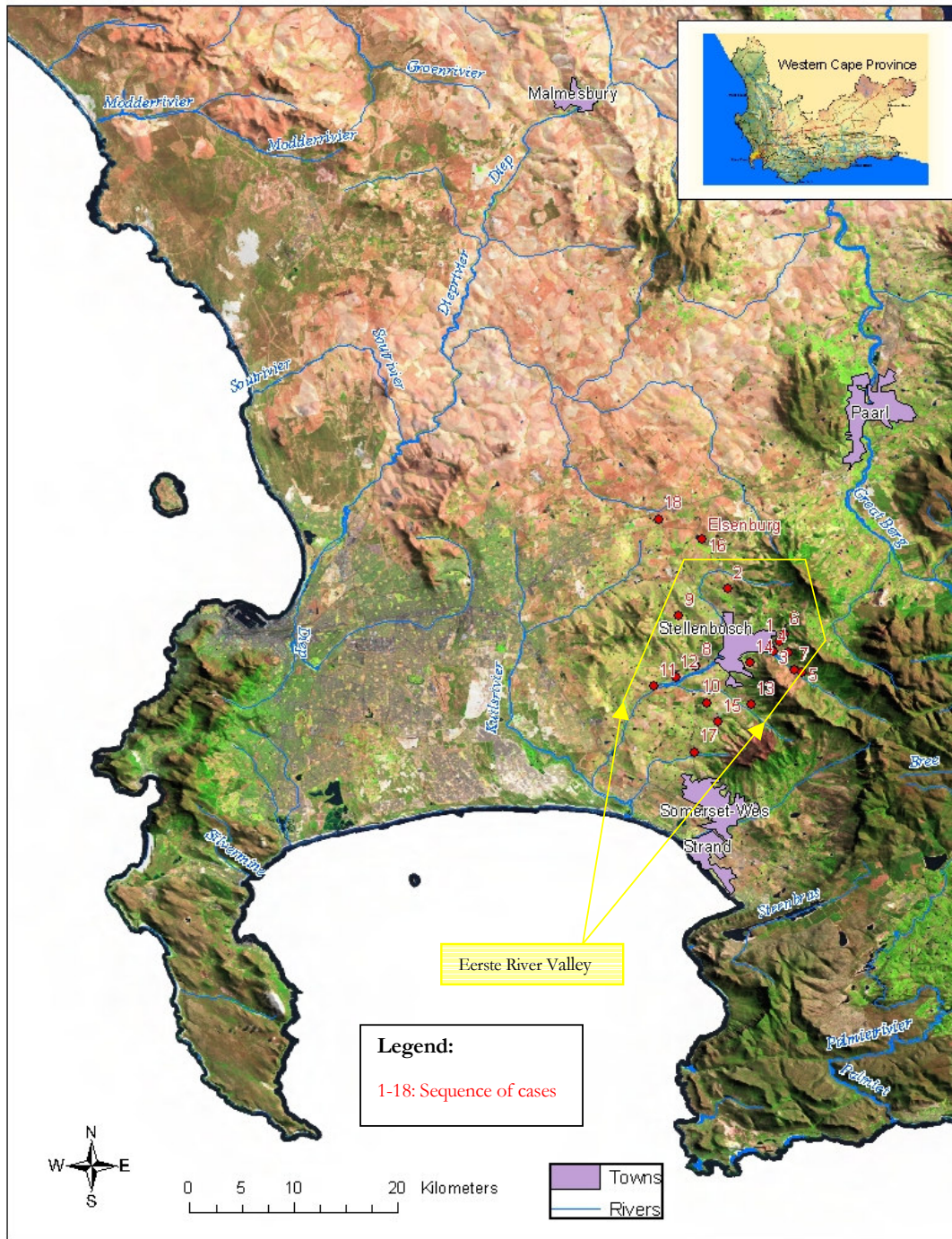
Figure 12: Distribution of cases during the 1999 AHS outbreak in the Western Cape Province in relation to regional topography

Figure 11 illustrates, that most of the cases during the 2004 outbreak occurred in the low laying areas along the major rivers. Elsenburg is not situated directly adjacent to a major river, but this farm has several dams and a vlei area. The five Percheron horses that died at Elsenburg during the 2004 outbreak, all grazed in this vlei area before the occurrence of the outbreak. Since the first case on 31 January was misdiagnosed as annual rye grass toxicity (see Appendix E), all the horses were moved out of the vlei area for 18 days. Three days before the rest of the cases manifested, the horses were moved back into the vlei.

During the 1999 outbreak, the cases were again concentrated along rivers and the infection was initially detected in and subsequently spread along the Eerste River Valley (indicated in Figure 12). Only two farms lay outside this valley; Elsenburg, which has a vlei area as described above and another farm, which is situated alongside a river.

4.6.3 Vector activity and distribution

Table 13 shows the results for the 2004 outbreak and Table 14 shows the results for a similar survey during the 1999 outbreak. This information was received from Gert Venter of the OVI Entomology section. Further entomological analysis of the midges that was caught will be published in the December 2006 issue, volume 25 (3) of the Revue Scientific et Technique de l'Office International des Epizooties.

During the 2004 survey, the midge traps were set up on locations where AHS was previously diagnosed in 1999 and a few surrounding properties. Only a limited number

of traps were available and they were rotated amongst the properties with varied time intervals. For these two reasons it is not possible to present any results regarding the exact spatial distribution of the midges and the difference in population sizes over a specified time period in the area under investigation.

AHS virus was for the first time isolated from midges caught in the Western Cape Province during the 2004 outbreak (Venter, G., personal communication 2004).

Table 13: *Culicoides* **collected during an AHS outbreak in the Stellenbosch area of the Western Cape Province (2004)**

Collection site	Lin quenda	Spier	Rosen dal	Natte Valleij	Delvera	Elsen burg	Vreden heim	Dak tari	Dale wood	Lange berg	Chilanga	Paarl Diamand	Delmerdo (Ashire)	Throug hend	Ivan Stark	Staedfast farm	Total no. of midges	Total %
Collection date	1 March	1-26 March	2 March	3 March	3-30 March	1-26 March	18-19 March	1 March	2 March	3 March	11-15 March	4 March	4 March	2 March	4 March	4 March		
Number of collections made	1	5	1	1	6	10	2	1	1	1	5	1	1	1	1	1		
C. imicola (%)	99.07	99.22	75.49	96.33	98.44	77.61	45.66	79.44	54.99	94.68	79.23	92.31	91.43	94.44	61.54	100	**17 7455**	**94.63**
C. zuluensis (%)	0.48	0.29	17.76	2.01	0.52	13.11	43.11	12.07	41.71	2.88	0.24	3.85	5.71	5.56	0	0	**5 878**	**3.13**
C. bolitinos (%)	0.38	0.27	3.10	0.45	0.78	8.42	6.75	1.16	0.21	0.78	0.35	1.92	0	0	30.77	0	**2 685**	**1.43**
C. magnus (%)	0.02	0.13	3.60	0.80	0.14	0.64	1.44	1.74	2.99	1.11	0	0	2.86	0	0	0	**571**	**0.30**
C. leincostictus(%)	0	0.02	0	0.15	0.01	0.02	1.18	0	0	0.11	16.86	0	0	0	0	0	**547**	**0.29**
C. gulbekiani (%)	0.02	0	0	0	0	0.17	1.04	5.31	0	0	0	0	0	0	0	0	**126**	**0.07**
C. pycnosticus (%)	0	0	0.05	0.19	0	0.03	0	0	0	0.33	2.89	1.92	0	0	7.69	0	**103**	**0.05**
C. glabripennis(%)	0.02	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**97**	**0.05**
C. nivosus(%)	0	0	0	0	0.07	0.01	0.83	0	0.1	0	0.17	0	0	0	0	0	**41**	**0.02**
C. onderstepoortensis (%)	0	0	0	0.08	0	0	0	0.29	0	0	0.03	0	0	0	0	0	**6**	**0.00**
C. milnei (%)	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**5**	**0.00**
C. neavei (%)	0	0	0	0	0	0	0	0	0	0.11	0.10	0	0	0	0	0	**4**	**0.00**
C. angolensis (%)	0	0	0	0	0	0	0	0	0	0	0.07	0	0	0	0	0	**2**	**0.00**
C. similes (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**0.00**
C. engubandei (%)	0	0	0	0	0	0	0	0	0	0	0.03	0	0	0	0	0	**1**	
Total no. of midges	**25 210**	**110 842**	**3 998**	**2 641**	**14 511**	**21 623**	**2 789**	**1 036**	**971**	**902**	**2 870**	**52**	**35**	**18**	**13**	**11**	**187 522**	**100.0**
Average no. of midges / catch	**25 210**	**22168.4**	**3 998**	**2 641**	**2 419.5**	**2162.3**	**1 393**	**1 036**	**971**	**902**	**574**	**52**	**35**	**18**	**13**	**11**		
Max no. / catch		**63 260**			**14 465**	**16 887**	**1 689**			**50**	**2 870**							
Min no. / catch		**48**			**1**	**1**	**1 100**				**166**							

Table 14: *Culicoides* collected during an AHS outbreak in the Stellenbosch area of the Western Cape Province (1999)																	
Collection site	Lin quenda	Thistle down	Stellen kloof	Rem hoogte	Spier	Avon tuur	Vars water	Maties	Through end	Elsen burg	Klein Bosch	Sandring ham	Rosendal	Digteby	Glenn conner	Total no. of midges	Total %
Collection date	27 March - 20 April	22 April	29 March -4 April	26 March	30March - 21April	3-30 April	23 April	28-30 April	2 April	22 April	15 April	24 April	26March – 14April	31 March	29 April		
Number of collections made	3	1	2	1	4	4	1	2	1	1	1	1	14	1	1		
C. imicola (%)	98.52	97.96	97.01	84.88	96.67	89.28	96.93	97.62	99.02	92.29	99.50	84.00	44.17	97.5	43.24	**203 774**	**96.03**
C. zuluensis (%)	0.37	1.84	1.00	7.16	1.23	6.11	1.65	0.02	0.20	2.57	0.45	12.00	17.57	0	5.41	**3 588**	**1.69**
C. bolitinos (%)	1.08	0.07	0.40	0.75	1.76	1.00	0.11	2.10	0.49	3.04	0	1.71	0.25	2.5	0	**2 258**	**1.06**
C. magnus (%)	0.00	0.07	0.10	7.11	0.32	3.37	0	0	0.29	0.76	0	1.71	2.74	0	0	**1 576**	**0.74**
C. leincostictus (%)	0	0	1.1	0	0	0.04	1.31	0.13	0	0	0	0	23.93	0	32.43	**627**	**0.30**
C. gulbekiani (%)	0.02	0.07	0.02	0.05	0.02	0.03	0	0.13	0	1.33	0	0.29	0.12	0	0	**90**	**0.04**
C. pycnosticus (%)	0	0	0.30	0	0	0.03	0	0	0	0	0.06	0.29	8.22	0	18.92	**190**	**0.09**
C. nivosus (%)	0	0	0	0.05	0	0.03	0	0	0	0	0	0	2.31	0	0	**49**	**0.02**
C. angolensis (%)	0	0	0.07	0	0	0.06	0	0	0	0	0	0	0.44	0	0	**33**	**0.02**
C. expectator (%)	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	**10**	**0.00**
C. engubandei (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0	0	**2**	**0.00**
C. coarctatus (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0	0	**1**	**0.00**
C. sp. 107 (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0	0	**1**	**0.00**
Total no. of midges	**107 913**	**10 633**	**13 667**	**8 044**	**25 402**	**24 506**	**4 429**	**8 614**	**3 066**	**2 102**	**1 791**	**350**	**1 605**	**40**	**37**	**212 199**	**100.0**
Average no. of midges / catch	**35 971**	**10 633**	**6 833.5**	**8 044**	**6 350.5**	**6 126.5**	**4 429**	**4 307**	**3 066**	**2 102**	**1 791**	**350**	**114.64**	**40**	**37**		
Max no. / catch	**30 640**		**8 110**		**21 740**	**11 540**		**8 614**					**327**				
Min no. / catch	**2 157**		**5 557**		**294**	**58**		**654**					**5**				

During a survey to determine the *Culicoides* species associated with livestock in the Stellenbosch area during November 1986, *C. imicola* comprised only 19.6 percent of the *Culicoides* trapped (Neville *et al.* 1988).

C h a p t e r   5

5. DISCUSSION

5.1 Case definition

According to Dr. Gerdes from the serology section of the LNR-Onderstepoort Veterinary Institute, the complement fixation test is useful to determine recent exposure and all horses which were vaccinated more than six months previously and have complement fixation titres of 1:32 or higher, should be regarded as positive for infection. However, the Elsenburg outbreak showed that 50% of the horses which were last vaccinated in 1999 showed both positive Elisa titres and CF titres (equal to or higher than 1:32), but no signs of illness, while none of the completely unvaccinated horses, except those that fell ill and died, seroconverted (Table 1). Since all these horses were subjected to similar exposure, some doubt was cast on the use of serology to determine recent exposure. In addition, the kappa statistic of 0.6 (Table 2) indicates only fair agreement between the ELISA and CFT tests in unvaccinated horses and no agreement (kappa = 0) in vaccinated horses (Table 3). The combination of serology and PCR also provided ambiguous results as illustrated by a suspect case named Ash (see Table 4), which showed clinical signs, tested highly positive on CFT and ELISA, but negative on PCR. According to Dr. Gerdes, CFT positive blood should test positive on PCR. For these reasons and for the purpose of this study, a case was defined as a horse showing

typical symptoms of AHS and from which virus could be isolated. Samples for virus isolation was only collected from dead horses and true cases thus only include horses that died.

However, for the sake of completeness, I would like to mention that 16 horses that showed clinical signs and recovered during the outbreak period tested positive on either Elisa or CFT (see Table 4) and could possibly have been true clinical cases. PCR was not performed consistently in all suspect cases, but the available results are shown in Table 4. It is important to remember that as stated above these results could have been influenced by the vaccination status of the animals and the clinical signs may have been caused by biliary fever or equine encephalosis which were also wide spread in the area during the outbreak.

5.2 Pathology

The macroscopic pathology of this outbreak of AHS generally fits the description of the cardiac ('dikkop') form of AHS. The cases of the previous outbreak in 1999 (caused by serotype 7 of the virus) were more typical of the pulmonary ('dunkop') form, in which the severity of the lung oedema is often manifested post-mortem as a frothy, serofibrinous discharge from the nostrils. In addition, the oedema and swelling of the fat in the supraorbital fossae were very mild or absent.

The history of outbreaks of annual rye grass toxicity (ARGT) in the AHS surveillance zone during late summer and early autumn, as well as the diagnosis of ARGT in cattle at

Elsenburg during previous years, contributed to the initial misdiagnosis of the first three Percherons that died at Elsenburg on 31 January 2004 (Appendix E). The first two cases died peracutely and the third was transported to a local animal hospital where it showed nervous symptoms, including convulsions, which are uncharacteristic for AHS but typical of ARGT. In retrospect this convulsions can be attributed to severe brain oedema, which was noted by the private veterinarian during a post mortem examination. Since the horse showed no nervous symptoms at Elsenburg, but only on arrival at the animal hospital, it is thought that the brain oedema might have been initiated or aggravated by the transport. AHS and ARGT share no symptoms or pathological findings other than the possibility of acute deaths. The initial confusion can thus be attributed entirely to the above-mentioned circumstances.

5.3 Questionnaire survey

The first part of the questionnaire detailed the contact information (initials, surname, physical address, postal address, farm name, telephone number and cell phone number) of the owner or manager as well as the geographical weight points of each farm (see p. 22).

The next section of the questionnaire focused on census information for horses, donkeys, mules and zebra, as well as the vaccination history of the horses (see p. 22). On average the affected properties had a higher horse density than the unaffected properties (see p. 32 and Figure 11). The other species were excluded from the

vaccination history since they are not routinely vaccinated against AHS. Vaccination was defined as full vaccination with both the AHS1 and AHS2 portions of the vaccine. The vaccination history was divided into horses vaccinated less than 3 months ago, horses vaccinated more than 3 months, but less than 1 year ago and those vaccinated more than a year ago. This categorization was necessary to distinguish between horses that were adequately protected (those vaccinated more than 3 months, less than a year ago) and those that were not adequately protected (the remaining two categories). Horses vaccinated less than 3 months ago were probably vaccinated as a result of the outbreak and may not have necessarily had adequate time to build up protective immunity. Since AHS should be vaccinated annually according to the manufacturers prescription, horses vaccinated more than a year ago were deemed unprotected. Because the outbreak occurred in the surveillance zone and vaccinations in this zone were only recognized as official if administered by a veterinarian, the vaccination history included a question on whether the vaccine was administered by a veterinarian or not (see p. 22). According to the results of the survey, 69.6% of horses in this area of the surveillance zone were protected by means of vaccination. This high percentage is of concern since the resultant absence of sufficient numbers of sentinels inhibits the objective of the surveillance zone to serve as early warning system.

A question on the recent movement history of horses, donkeys, mules or zebra was included in order to determine the possible source of infection and to be able to do trace back and trace forward procedures should a problem arise on a specific property. The

question was limited to the preceding three months as movement before this time was deemed as most likely unrelated to the outbreak. Details of the question included the departure date, the property of origin and the name of the closest town to this property to establish location. Similarly the details of the destination included arrival date, name of the property and the name of the nearest town. For each movement the names and/or passport numbers of the horses involved in the movement had to be specified (see p. 23). This allowed the state veterinarian to obtain more information on a specific horse in suspect cases (e.g. the vaccination history, disease information and whether the movement was legal or not). This section of the questionnaire was poorly answered and was not included for further analysis for the purpose of this study, although the Boland State Veterinary office followed up all suspicious movements identified from the answers for control purposes.

The questionnaire included a section on clinical symptoms that could possibly be linked to AHS that occurred during the preceding 2 months on the property. The following symptoms were listed: inappetance, fever, swelling above the eye, difficulty in breathing, nasal discharge, coughing and sudden death. The owner had to indicate which (if any) of these symptoms were observed, the date of occurrence, the number of horses affected and the diagnosis of the veterinarian if one was involved in the case (see p. 23). Sixteen horses displaying clinical symptoms (see Table 4) were subjected to further investigation. All of these horses survived and according to the amended case definition, they were not classified as AHS cases, since no virus isolation could be done.

In order to obtain some idea of the management practices in the region, the following questions were added (see p. 23):

- Are your horses stabled at night?
- If yes, how many are stabled?
- Do you use insect repellent on the horses?
- Name of the repellent?

The answers to these questions revealed that only 17.0% of the horses in the area where the survey was conducted were protected by means of vector control (including stabling and/or the use of insect repellents). Considering the previously mentioned high percentage of horses vaccinated, the ratio between the different methods of preventative control, vaccination vs. stabling is 4:1. For the purpose of a surveillance zone, one would rather expect that this ratio would be reversed with significantly more animals being protected by means of vector control rather than vaccination.

5.4 Epidemic pattern

5.4.1 Temporal pattern:

As Figure 1 and 2 illustrated, the disease pattern for both the 2004 and 1999 outbreaks can be classified as sporadic epidemics. This type of epidemic pattern is to be expected in a vector borne disease and it is typical in a disease situation where some of the animals are immune as illustrated in the results of the questionnaire survey during the 2004 outbreak. Although there is no specific information available on the immune status of the horses prior to the 1999 outbreak, one would expect that a significant number of

animals would have also been vaccinated on a regular bases in order to be able to compete in equestrian events, while fulfilling the stipulations of the AHS movement control protocol instituted in 1997. The higher number of cases and the initial steep increase in the cumulative case series (see Figure 4) during the 1999 outbreak does however suggest that fewer animals were immune prior to the 1999 outbreak compared to the 2004 outbreak. This can be explained by the fact that during the 1999 outbreak, all the horses within approximately 30 km radius of the infected cases were vaccinated in order to limited the number of deaths. The severity of the 1999 outbreak left the owners concerned for the welfare of their horses and some continued to vaccinate their horses regularly.

Figures 1 and 2 shows a cluster of cases in the initial stages of the outbreak during both the 1999 and 2004 outbreaks. This can be explained by the institution of strict movement control measures as well as the advocacy of the implementation of vector control soon after the initial cases were diagnosed in both outbreaks. After the implementation of these measures, fewer cases were diagnosed with longer intervals between them as illustrated in Figures 1 and 2, as well as the cumulative case series graphs (Figures 3 and 4).

According to the literature, the incubation period for AHS can range from two to ten days (Coetzer & Guthrie 2004). The most likely period of exposure is thus between 21 and 29 January.

As stated above, the virus involved in the 2004 outbreak was typed as AHS serotype 1, which does not occur commonly in South Africa.   What was even more puzzling was the fact that horse sickness outbreaks had not yet been reported from the infected zone in the rest of the country. It appeared to be too early in the season for AHS and this was partially responsible for the delay in correctly diagnosing the initial death. Once the causal virus had been typed, a clearer picture emerged. In 2002, samples sent from Springbok tested positive for AHS serotype 1. These samples originated from Namibia. It is thus suspected that there may have been an illegal (or legal) movement of a horse incubating the disease from somewhere in Namibia into the surveillance zone in close proximity to Elsenburg. The only recorded movement of horses from Namibia occurred on the 25th of January 2004 to the Stellenbosch area, which ties in with the most likely period of exposure. None of the two horses involved developed clinical disease, although other horses on this property later succumbed to AHS. It is thus quite likely that the primary case was not at Elsenburg, but elsewhere in the Stellenbosch area and that the disease was only first detected at Elsenburg due to the highly susceptible Percheron population at Elsenburg.

The source of the infection of the 1999 outbreak was two horses that moved illegally from the Free State Province to Stellenbosch on the third of March 1999. One of these horses became ill on March the 11th and was diagnosed with conjunctivitis and uveitis by a private veterinarian who treated the horse symptomatically. This horse recovered, but the second horse showed clinical signs of AHS on the 26th of March and was

euthanased. Samples of both these horses tested positive for AHS. The first deaths due to AHS (serotype 7) occurred on the neighbouring farm on 21 March and the disease spread rapidly in the area. In total, thirty-two horses died during this outbreak.

An AHS virus, serotype 7, was isolated from a midge during the 2004 outbreak (Venter *et al.* 2006). Genetic characterization revealed that this virus was not a remnant of the 1999 outbreak but a new introduction from the endemic area of the country where serotype 7 was circulating during the same season in 2004. Therefore, two incursions of AHS must have taken place during 2004, even though only one outbreak was detected (Venter *et al.* 2006). This casts further doubt on the effectiveness of the surveillance zone.

5.4.2 Animal / population pattern of the 2004 outbreak:

The risk of dying of AHS was 2.4 times higher in younger horses than in horses of 6 years and older ($p < 0.10$). (The small sample size accounted for the level of significance that could be obtained.) This association can be attributed to the fact that younger horses had not been vaccinated as much as the older horses and therefore they had a lower level of immunity against AHS.

There was no significant ($p < 0.10$) increase in the risk of horses 10 years and younger dying when compared to older horses. This is to be expected if vaccination history was one of the primary determinants of whether a horse became sick or not.

From the results (Table 11) it can be seen that vaccination has a protective effect against the risk of dying as a result of AHS infection, however this risk mitigation procedure is not desirable on a large scale in a surveillance zone situation where the presence of sentinels are essential.

The breed distribution of cases (Table 9) was not analysed statistically, since the distribution was very specific on each farm, e.g. most affected farms had only one breed present.

Although 44 zebra were identified in the 30 km zone during the questionnaire survey, only five of these were on positive, suspect or dangerous contact properties. All five zebra were resident on the same positive property (Vredenheim). Two of the four horses on this property succumbed to AHS. Unfortunately, as described above, only one case could be confirmed, since the other were buried by the owner and only reported seven days after the incident occurred. However, the clinical signs described were highly suspicious of AHS. This farm is situated in the Eerste-river-valley, where most of the deaths due to AHS occurred, and most of the surrounding farms had positive cases at that time.

A wildlife specialist was contracted to dart the zebra. Of the five zebra, two were pregnant mares and the risk of darting them were deemed too high. Only the remaining three (a stallion of three years and 2 foals between 11 and 13 months) were darted. All

three zebra tested negative on CFT and indirect ELISA, it can therefore be concluded that zebra did not play a role in the dissemination of AHS in the 2004 outbreak.

Unfortunately the animal / population pattern of the 1999 outbreak could not be described, since insufficient information was recorded during that outbreak.

5.4.3 Spatial pattern:

Figures 11 and 12 illustrate the geographical location of cases in the 2004 and 1999 outbreaks respectively. During both outbreaks the affected properties were all situated in low-lying areas alongside a river, and the horses were kept on Kikuyu grazing. One property (Goedverwacht), which was affected during the 2004 outbreak, was situated in a drier area, but the horses had been grazing next to a vlei and a cattle feedlot is situated adjacent to this property. It has been shown that *Culicoides* midges breed in animal dung (Howell *et al.* 1983) and that large midge populations can develop under certain conditions including irrigated kikuyu pastures and the presence of vertebrate hosts which the feedlot provided (Meiswinkel 1997). This explains the infection of the horse on Goedverwacht.

During the 2004 outbreak the infection spread southwards (along the Eerste-river-valley), then towards the north. During the 1999 outbreak the infection initially spread northwards and then towards the south along the Eerste-river-valley. In addition, the midge, from which the aforementioned AHS virus serotype 7 was isolated, was caught on a farm in this valley (Venter *et al* 2006). These findings identify the Eerste-river-valley

as a high-risk area for the incursion and outbreak of AHS in the surveillance zone and it will be beneficial to target this area during routine surveillance.

From the results (Table 12) it can be seen that the stabling of horses has a protective effect against the risk of dying of AHS infection in exposed populations. This supports the findings of Meiswinkel in 2000 and proves that vector control by means of stabling is the most efficient method to prevent AHS infection in a surveillance zone where vaccination is not the prevention method of choice. However, one should bear in mind that this will only be true in the present situation where *C. bolitinos* does not play a significant role since they constitute less than 2% of the local midge population. If the size of the *C. bolitinos* population should increase in future, stabling might not be as effective, since this particular vector is endophilic and will feed on horses in stables where the windows are not sufficiently insect proof (Meiswinkel *et al.* 2000).

As indicated in Table 13 and 14 the main vector of the AHS virus (*C. imicola*) was present in abundance during both the 2004 and 1999 outbreaks. These midges constituted 94.6% and 96.0% of the midge population in 2004 and 1999 respectively, while *C. imicola* constituted only 19.6% of the midge population in 1986 (Neville *et al.* 1988). In 1996, during a study to determine the abundance of *Culicoides* species, *C. imicola* constituted only 11.3% of the population in the Stellenbosch area (Venter *et al.* 1997). The dramatic increase in the *C. imicola* population from 1997 to 1999 and the persistence of the high numbers during 2004, may explain the occurrence of the two AHS outbreaks in the Western Cape Province during the past five years.

Although all the midge traps were placed in the surveillance zone, it is reasonable to assume that a similar situation will prevail in the free zone, and as mentioned previously, the location of the free zone was in fact based on a historical absence of the disease and not the absence of vectors. The introduction of virus in this zone could thus potentially lead to a full-scale outbreak of AHS in the highly susceptible population of this zone.

The climatic variables of this geographical region during the 2004 and 1999 outbreak is illustrated in Figures 7 and 8 in conjunction with the number of cases that occurred during that specific time period.

In both 2004 and 1999, the outbreaks occurred before any significant rainfall was recorded. This is proof that even during the dry season in the Stellenbosch area, there is sufficient numbers of *Culicoides* midges present to transmit disease. This is of great concern since the results of the questionnaire survey indicate that on only 75 out of the 603 properties (12.4%) with equines, some kind of method is used to prevent vector contact with the animals. In other words, only 728 out of 4 289 (17.0%) horses in the investigated area are protected from vector contact.

During the 2004 outbreak 63.0% of the cases occurred during a time-period when the recorded average minimum temperatures were below 15°C. This contradicts the previous findings that transmission has not been recorded at 15°C (Mellor, *et al.* 1998).

As previously illustrated in Figures 5 and 6, the wind speeds seldom exceeded 3 m/s during the apparent transmission periods in both 2004 and 1999. This is in agreement with the previous findings in the literature (Walker 1977).

The wind direction (south-south-westerly) did not seem to influence the dispersal of the midges and the subsequent spread of the disease significantly, since the disease spread more in a southerly direction during 2004 as apposed to the expected northerly direction (with the exception of the Goedverwacht and Kalbaskraal cases where wind direction could well have played a role). The same is true for the 1999 outbreak where the infection spread only in the Eerste River Valley, more in a southerly direction, where as one would expect a northerly spread if wind direction played a role since a south-south-westerly and south-south-easterly wind prevailed during the outbreak period. This might be explained by the fact that the windspeed was not high enough to substantially influence the activity of the midges as explained above.

5.5 Control measures instituted

On The 26th of February 2004, an emergency contingency planning meeting was called at Elsenburg with all the relevant role players. This included local equine specialist veterinarians, personnel from the Disaster management team of the Western Cape and staff of the provincial Directorate of Veterinary Services. The following actions for implementation were agreed upon:

- A 30-kilometre buffer zone was created using the first diagnosed case at Elsenburg as the epicentre.

- Animal Health Technicians (AHT's) were called in from neighbouring State Veterinary areas to assist with surveillance on equine holdings in the area. Within a period of about 3 weeks 1 616 farms had been visited, representing some 4 289 horses.

- The Department of Agriculture of the Western Cape immediately made 1 400 doses of AHS vaccine available free of charge to private veterinarians, who were requested to agree on charging a reasonable fee to all their clients. The AHT's assisted in vaccinating in resource-poor communities and no charge was levied in these instances.

- An immediate total embargo was placed on all horse movements within the whole of the surveillance and free zones and the Minister of Agriculture issued the first of many Press Releases on 27 February 2004. This Press Release stipulated the movement restrictions and highlighted the importance of vector control.

- The Provincial and Municipal Traffic Departments within the Disaster Management team played a vital role in assisting the Directorate by monitoring and restricting movements of horses.

- Apart from the Press releases, several interviews were done with the national television stations and regular updates were broadcasted over the local radio stations throughout the duration of the outbreak. A 15-minute documentary was also produced for Teletrack on Channel 34 for Digital Satellite Television (DSTV). This program highlights important events, which affect the Race Horse community, and has wide coverage in the racing fraternity. Situation reports were also compiled intermittently to keep the relevant authorities and private veterinarians informed of the evolution of the outbreak, progress with disease control measures and the status of the quarantine restrictions.

The complete ban on horse movements within the entire surveillance zone appeared to have had a significant economical impact on the race horse community and associated businesses. The Department of Agriculture called another stakeholder meeting on Sunday 29th February in order to review the situation in the light of the apparent localization of the outbreak in the Stellenbosch area, which is approximately 35 kilometres away from the Racing Stables at Milnerton, the Kenilworth race track and the horse Export Quarantine Station. The Minister of Agriculture chaired the meeting, which was attended by representatives from Gold Circle Racing (GCR), Thoroughbred Breeders Association (TBA), trainers, the Directorate Veterinary Services and the media.

Following intensive discussions it was agreed to allow restricted movement of race horses between the racing stables situated in low risk areas, to Kenilworth, provided they met certain stringent conditions. These included Veterinary health certification, treatment of the horses and horse boxes with insecticides, vaccination status, stabling overnight from 2 hours before sunset until 2 hours after sunrise, restricted movement times only between 2 hours after sunrise until 2 hours before sunset and issuing of Red Cross permits for each movement.

Disaster Management team meetings were held two to three times per week initially at Elsenburg to keep the Provincial and Municipal traffic authorities informed on progress and the disease status. Only horses with Red Cross permits issued by the State Veterinarian Boland were allowed to move. Anyone caught without the correct documentation was instructed to return to point of origin immediately. This system

worked extremely well and was implemented very efficiently by the traffic officers on duty.

Due to the apparent limited spread of the virus and after comparing the risk involved with the negative effects on the industry, the quarantine in most of the surveillance zone was lifted on 11 March 2004. However, the Stellenbosch and Somerset West magisterial districts, which were considered to harbour the primary focus of infection, 11 properties in the Bottelary district and a single farm in the Franschhoek district (where suspect cases were monitored), remained under strict quarantine.

An additional area was placed under quarantine after the last case at Kalbaskraal (15 kilometres south of Malmesbury and approximately 32 kilometres northwest of Elsenburg) died peracutely on Sunday 28th March. As soon as the diagnosis of AHS was confirmed, all the horses in this area were vaccinated.

After the last confirmed death on 28th March the quarantine in the designated areas (with the exception of Malmesbury) was maintained for a further period of 7 weeks before the quarantine was lifted on 17th May 2004. This enabled sufficient time for any further outbreaks (clinical or deaths) to occur and allowed time to process all the results from the horses sampled during the initial survey in the 30-kilometre zone.

With no further cases occurring in the Malmesbury district, the quarantine was lifted on the 21st June 2004.

5.6 General considerations

The immediate disease control actions taken during the 2004 outbreak, especially the rapid vaccination campaign, the advisement to use insecticides and to stable horses where possible, along with the strict movement controls, ensured that only 16 horses died in the Stellenbosch district where the main focus of the disease was situated. The two other isolated deaths that occurred outside this area, were linked to the main core as they were all confirmed as the same serotype of the AHS virus (serotype 1) and they occurred along the prevailing wind direction. The number of deaths is however relatively low, compared to the morbidity and mortality rates in Gauteng, where 75 cases developed between February and March 2004.

The impact of the outbreak in the Western Cape on the racing community and other equestrian events was immense. Several race meetings and numerous large shows were cancelled as all horse movement ceased completely and subsequently five thousand casual workers were out of work. In addition exports were placed under embargo and the export industry came to an immediate standstill, which represented a loss estimated to be at least 30 million Rand in foreign exchange during the 24 months following the outbreak (Gibson, P., personal communication 2006). Fortunately, during this 24 month period (after the outbreak ceased), Mauritius allowed the importation of approximately 200 horses from South Africa, if this was not the case, the loss in foreign exchange would have been up to 60 million Rand (Gibson, P., personal communication 2006).

The similar outbreak during 1999, with a mortality of 32 horses and an enforced 2-year ban on exports by the European Union, resulted in a substantial loss in foreign income for the racing industry in South Africa and breeding animals for local owners.

The AHS free status of the Western Cape was again compromised with the recent outbreak, but the deaths were far less, due to stringent and immediate measures taken to control the disease and the presence of a less susceptible horse population.

During the 1999 outbreak the disease could be detected fairly quickly since the horses in the vicinity of the index case were susceptible to disease. The surveillance zone thus fulfilled its function excellently. However, during the 2004 outbreak the primary case could not be pinpointed and this impeded our ability to determine the source of infection. The disease only became apparent when it reached the fully susceptible horses of Elsenburg. This is a further indication that the surveillance is not as functional as it was five years earlier. The main reason is probably an increase in the number of vaccinated horses in the surveillance zone due to strict movement controls under the current AHS protocol. As part of the control measures implemented by the State Veterinarian Boland Office, all unvaccinated horses in the 30 km radius surrounding the epicenter as well as in the 10 km radius surrounding the Kalbaskraal focus, were vaccinated at the start of the outbreak with the aid of private veterinarians. At the end of this operation, an estimated 94% of horses in the mentioned areas were vaccinated. Unfortunately, this essential procedure to safeguard the remaining population of horses in the area, further impaired the main function of the surveillance zone.

C h a p t e r   6

CONCLUSION

Illegal horse movements remain the biggest threat for AHS introduction into the AHS control area.

The main vector of AHS (*Culicoides* midges) is abundantly present in the surveillance zone (even during the dry season) and should the virus thus be carried into this zone by any means (e.g. horse movement), the disease has a high probability of spread.

This study identified the Eerste-river-valley as a high-risk area for the outbreak of AHS in the surveillance zone.

According to the results of this study, regular vaccination and stabling offers the best protection against the detrimental effects of AHS.

During the 2004 outbreak, 69.6% of horses were protected by means of vaccination, while only 17.0% of horses were protected by means of vector control.

The ability to control an outbreak successfully is directly dependant on rapid detection and response.

Due to the large number of vaccinated horses in the surveillance zone, this zone is currently not fulfilling its main function to allow rapid detection.

The institution of strict control measures (movement and vector control) soon after the initial cases were diagnosed in both outbreaks, successfully limited the spread of the disease.

According to the results of the study, virus transmission can occur at average minimum temperatures below 15 °C.

The current export protocol is not ideal since a large part of the equine industry does not benefit from exports, but is negatively influenced by the limiting effects of the current movement restrictions.

C h a p t e r   7

RECOMMENDATIONS

The movement restrictions on horses within the AHS control zone of the province place an additional burden on all interest groups involved in the horse industry. It is therefore recommended that alternative options should be investigated to facilitate exports while at the same time not inhibiting the needs of other non-export interest groups within the equestrian society. Any alternative option would however, imply a change in the international standards for the trade in horses recommended by the World Organisation for Animal Health (OIE) and will therefore have to be negotiated with both the OIE and the European Commission.

Both compartmentalization and seasonal exports (where exports will only be allowed during the winter months) are alternatives to the current export protocol. From the results of this study I would recommend compartmentalization, since the analysis of the climatic data suggested that midge activity is still sufficient to transmit disease even when the temperatures decreased to 15°C. It will thus be very difficult to determine specific time-intervals when export can be deemed safe. Further studies to determine *Culicoides* abundance throughout the year, might be helpful with a seasonal export protocol in mind. However, the existing vector-proof export quarantine station at Kenilworth

offers excellent bio-security and will be ideal to serve as a compartment for export purposes.

As discussed above, the large number of vaccinated horses in the surveillance zone has a detrimental effect on its main function. In order to improve this undesirable effect, the AHS control policy needs to be reviewed. The best option would be to decrease the size of the surveillance zone in order to enable Veterinary Services to improve disease surveillance and control in this zone. Since the Eerste-river-valley was identified as a high-risk area in the surveillance zone, it is recommended that routine surveillance should be increased in this area. Accompanying the decreased size of the surveillance zone, should be less strict movement controls for movement between the protection and surveillance zone, which are both areas of low risk, allowing movement without vaccination, but under permit issued by the local State Veterinarian for traceability purposes. Presently horse owners have to vaccinate their horses in order to move between protection and surveillance zone. Since most equestrian events in the area are held in these two zones, a high percentage of horses in the surveillance zone are vaccinated in order to be able to compete. By relaxing these measures, fewer horses in the surveillance zone will have to be vaccinated and the resulting presence of more sentinels will increase Veterinary Services' ability for early detection of disease. The relaxing of movement controls in these two zones will not constitute a higher risk, since the measures for movement of horses from the infected zone to the combined control area will remain the same. The suggested measures of decreasing the surveillance zone

and relaxing movement controls in the low risk areas, will thus result in a decreased risk to export since an outbreak will be detected early and will be less likely to threaten the integrity of the free zone or compartment. In addition, the financial burden on local competitors in equestrian events will decrease significantly and these events will once again gain popularity.

To address the issue of animal welfare an alternative method would be to use donkeys, which rarely show any clinical signs, as sentinels in the surveillance zone. If sufficient numbers of these alternative sentinels can be obtained, horses, which experience high morbidity and mortality, do not have to act as sentinels and can be vaccinated on a regular basis.

Furthermore, a single CF titre of greater than 1:32 should not be used to diagnose a case of AHS, as was illustrated by the ambiguous serological results obtained in this study. Paired serum samples, tested either by CFT or ELISA should rather be used in the diagnosis of AHS. Alternatively, new serological diagnostic techniques should be developed to allow for prompt diagnosis of AHS, this would decrease the reaction time and increase the success of control considerably.

Lastly it is recommended that an effort should be made to raise public awareness regarding the importance of vector control in the prevention of AHS infection, since this was only practiced on 12.4% of equine holdings prior to the 2004 outbreak.

C h a p t e r   8

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Appendices

Appendices

Appendix A

Method for ELISA

The ELISA used was a VP7 indirect ELISA, according to the method described by Maree and Paweska (Maree & Paweska 2005). Plaque-purified recombinant AHSV-3 and AHSV-9 VP7 baculoviruses were used to infect Sf-9 cells and VP7 expression and crystals were then analysed by SDS-PAGE and microscopy. AHSV-3 VP7 crystals were purified for use as antigen in the ELISA. Optimal concentrations of ELISA reagents were determined by the standard checkerboard titration procedure. The purified AHSV rVP7 stock antigen (1:50 dilution in 50% buffered glycerol) was further diluted 1:60 in 0.05 M carbonate-bicarbonate buffer (pH 9.6) to yield a final dilution of 1:3000. Routinely, 50 µl/well diluted antigen was then passively adsorbed onto ELISA plates (NUNC C96 Polysorb) overnight in a humidity chamber at 4°C. Plates were then washed three times with 250 µl per well of TST buffer (0.8 M Tris; 0.15 M NaCl; 0.05% Tween-20 pH 8.0). The same washing procedure followed each subsequent stage of the assay. The coated plates were then blocked by the addition of 100 µl blocking buffer comprising 3% fat-free milk powder ("Elite'" Clover SA, Pvt. Ltd.) in TST at 37°C for 30 minutes. After washing, 50 µl of each test and control serum diluted 1:100, were added in duplicate. Following incubation at 37°C for 1 hour, plates were washed and a volume of 50 µl recombinant protein G conjugated with horseradish peroxidase (Zymed Laboratories) diluted 1:20 000, was added. Plates were incubates in the dark for 10 minutes at room temperature. Reactions were terminated by adding 50 µl/well of 1 M H_2SO_4, and optical densities

(OD) were measured at 450 and 690 nm reference filters. The mean OD readings were converted to a percentage of high-positive control serum (PP) value using the equation: (mean OD of test sample/mean OD of high positive control) x 100. The cut-off value was determined to be 11.9 PP of an internal high-positive control serum using misclassification cost term (MCT) option of the two-graph receiver operating characteristics (TG-ROC) analysis, at 95% accuracy level. The test specificity was determined to be 100% and sensitivity 99.4% (Maree & Paweska 2005).

Appendix B

CFT method

The CFT was used according to the procedure described in the fifth edition of the World Animal Health Organisation's (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2004, Gerdes, T., personal communication 2005).

The reagents for this test included:

1) Veronal buffered saline containing 1% gelatin (VBSG).

2) Serum samples, free from erythrocytes, must be heat inactivated: horse serum at 56°C, zebra serum at 60°C and donkey serum at 62°C, for 30 minutes.

3) The antigen is a sucrose/acetone extract of AHSV-infected mouse brain. The control antigen is uninfected mouse brain, extracted in the same way. In the absence of an international standard serum, the antigen should be titrated against a locally prepared positive control serum. In the test, four to eight units are used.

4) The complement is a normal guinea-pig serum.

5) The haemolysin is a hyperimmne rabbit serum against sheep red blood cells (SRBCs).

6) The SRBCs are obtained by aseptic puncture of the jugular vein and preserved in Alsever's solution* or sodium citrate. (*20.5 g dextrose [114

mM], 7.9 g sodium citrate 2H 2 O [27 mM], 4.2 g NaCl [71 mM], H 2 O to 1 litre. Adjust to pH with 1 M citric acid.)

7) The haemolytic system (HS) is prepared by diluting the haemolysin to contain two haemolytic doses and using this to sensitise washed SRBCs. The SRBCs are standardised to a 3% concentration.

8) Control sera: A positive control serum is obtained locally and validated. Serum from a healthy antibody-negative horse is used as the negative control serum.

The sera, complement and antigen are reacted in 96-well round-bottom microtitre plates, or in tubes if the macro-technique is used, at 4°C for 18 hours. Sensitised SRBCs (3%) are added to all wells on the microtitre plate. The test plate is incubated for 30 minutes at 37°C. Plates are then centrifuged at 200 g, and the wells are scored for the presence of haemolysis. The following controls are used: (a) serum and complement; (b) serum and SRBCs; (c) CF antigen and control antigen each with 4 CH50 (50% complement haemolytic units), 2 CH50, and 1 CH50 of complement; (d) CF antigen and SRBCs; (e) control antigen and SRBCs; (f) complement dilutions of 4 CH50, 2 CH50, and 1 CH50, and (g) SRBCs. Results are read using 50% haemolysis as the end point. The inverse of the highest dilution of serum specifically fixing complement with the CF antigen is called the titre. A titre of 1/10 or more is positive, under 1/10 is negative (OIE 2004). No information is available on the

sensitivity and specificity for this test at the ARC-OVI (Gerdes, T., personal

communication 2005).

Appendix C

Method for RT-PCR:

1 ml Volumes of blood in heparinized tubes was transferred to Eppendorf tubes and plasma was separated from the blood cells by low speed centrifugation (500 x g) and removed. RNA was then extracted directly from the packed blood cells. Alternatively, blood cells were stored at –20 °C or –70 °C before RNA was extracted. Samples from organs were stored at either 4 °C or –20 °C before RNA extraction (Bremer & Viljoen 1998).

For RNA extraction a suspension was made of approximately 300 μg of organ material or of the blood cell pellet contained in 1 ml blood, in 500 μl solution D (4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarkosyl and 0.1 M β-mercapto ethanol). Thereafter, 50 μl of 2 M sodium acetate (pH 4), 500 μl water saturated phenol and 100 μl chloroform:isoamyl alchohol (49:1) were added. After centrifugation (18 000 x g for 15 min), the RNA in the supernatant fluid was precipitated with isopropanol. In certain instances 10-20 μg glycogen was added. The pellets were washed in 75% ethanol and resuspended in distilled pyrogen-free water. In some instances a second extaction was carried out on samples to ensure that all inhibitory substances were removed (Bremer & Viljoen 1998).

Total extracted RNA resuspended in distilled pyrogen-free water, was added to 50 ng of each primer and the volume was adjusted to 5 μl with distilled pyrogen-free water. The sample was heated for 3 min at 96 °C and cooled rapidly on ice. A 5 μl volume

of a mixture containing 10 U M-MLV reverse transcriptase (RT) (Promega), 2 x M-MLV buffer and 10 mM of each dNTP and approximately 25 U human placental Rnase inhibitor (Amersham) was added. Samples were incubated at 37 °C for 45 min (Bremer & Viljoen 1998).

PCR was carried out by adding 40 μl of a mixture containing 2 U thermostable DNA polymerase, 1.2 x Dynazyme buffer and 200 μM of each dNTP to each sample. The reaction was carried out in a Perkin Elmer 9600 PCR machine using the following conditions: 96 °C for 3 min, followed by 40 cycles of 96 °C for 20 s, 57 °C for 30 s and 72 °C for 30 s. The RT-PCR amplicons were analyzed by agarose gel electrophoresis (Bremer & Viljoen, 1998). The sensitivity of this procedure was determined to be 94% and the specificity 100% (Romito, M. personal communication 2005).

Appendix D

Method for virus isolation and virus typing

Virus isolation was done by intracerebral inoculation into newborn mice as well as inoculation of cell cultures. These two procedures was set up concurrently and both are performed according to the specifications as described in the fifth edition of the World Animal Health Organisation's (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2004, Gerdes, T., personal communication 2005).

Cell culture

The cell cultures used was either baby hamster kidney (BHK) or African green monkey kidney (Vero) cell lines. Spleen and lung tissue was used and a 10% tissue suspension was prepared in phosphate buffered saline (PBS) or cell culture medium, containing antibiotics. A cytopathic effect (CPE) usually appears between 2 and 8 days post-infection. Three blind passages were performed before the samples were considered to be negative (OIE 2004).

New born mice

This method involves the intracerebral inoculation of two families (7 babies / family) of 1 or 2 days old mice. In positive cases, animals develop nervous signs between 3 and 15 days. The brains from sick animals must be collected, homogenized and re-inoculated intracerebrally into at least six 1 to 3 day old mice. This second passage

should present a shortened incubation period (2 to 5 days) and 100% infectivity (OIE 2004).

Virus typing

Virus neutralization, as described in the fifth edition of the World Animal Health Organisation's (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, is the method used for serotyping (OIE 2004, Gerdes, T., personal communication 2005).

The test is performed as follows:

1) Stock virus is diluted to yield 30 to 100 $TCID_{50}$ (50% tissue culture infective dose) per 25 µl, and 25 µl is added to each of four microtitre wells containing 25 µl serum dilutions. For screening, a final serum dilution of 1/10 is used. Doubling dilutions are used for titrations.

2) Serum/virus mixtures are incubated for 60 minutes at 37°C prior to the addition of 0.1 ml of Vero cell suspension (200,000 cells/ml) to each test well.

3) A back titration of virus stock is prepared for each test using four wells per tenfold dilution, 25 µl per well. Test plates are incubated at 37°C, 5% CO_2, 95% humidity for 4 to 5 days, until the back titration indicates that the stock virus contains 30 to 100 $TCID_{50}$.

4) The plates are then fixed and stained in a solution of 0.15% (w/v) crystal violet in 2% (v/v) glutaraldehyde and rinsed. Alternatively, they may be fixed with 70% ethanol and stained with 1% basic fuschsin.

5) The 50% end-point titre of the serum is calculated by the Spearman-Kärber method and expressed as the negative $\log_{10}$ (OIE 2004).

Appendix E

Detailed description of the outbreak

The first indication that there was an outbreak of African Horse Sickness in the surveillance zone was when four Percheron horses died within a few days of each other from 21st to 23rd of February 2004 at the Elsenburg Agricultural College (S 33:50:30.6 E 18:49:49.2). This research farm is situated 10 kilometres north of Stellenbosch, and about 38 kilometres east of the Horse Export Quarantine Facility at Kenilworth in Cape Town. However, in retrospect it would appear that the first detected case occurred on the 31st January 2004, when a Percheron horse named Mariaan (mare, 1½ years old, unvaccinated) died peracutely at about 18h00 on the same farm in the Stellenbosch District. This horse was seen by the private veterinarian who suspected annual rye grass toxicity, since outbreaks of annual rye grass toxicity are known to occur during late summer and early autumn in the area, a fact that contributed to the initial confusion. A post mortem was done as well as histopathology, but no specific diagnosis was made by the private pathologist. Serum was collected but not sent away to the laboratory until 25 February 2004 when other cases died on the farm at which time this serum sample was sent to the OVI and subsequently tested positive.

The second peracute death, a Percheron named Marinda (mare, 3½ years old, unvaccinated, pregnant), occurred on the 21st of February, on Elsenburg at about 19h00. Again, a private veterinarian attended to the case. He also surmised annual rye grass

toxicity due to the previous death of the other horse on the same property 3 weeks earlier.

The third Percheron, named Michelle (mare, 2½ years old, unvaccinated), died acutely on Elsenburg on the 22nd of February. She showed ataxia and nervous signs and did not respond to treatment (MgSO$_4$ and dopamine). On the same day, the fourth Percheron, named Laura (mare, 2½ years old, unvaccinated), died acutely following initial detection early in the morning.

Two days later (24 February), the fifth Percheron, named Mara (mare, 4½ years old, in utero when dam vaccinated in 1999), died acutely on Elsenburg. On the same day a gelding (Pony) named Murphy, aged 24 years, died on Oakhill Farm (S 33:53:15.0 E 18:44:01.0) off the Bottelary road in the district of Kuilsriver, about 9 kilometres west-south-west of Elsenburg. He became ill on 19th February and was previously unvaccinated. Seven other horses on the farm were unaffected.

Two days later a colt (SA Saddle horse), unnamed foal, 8 months of age and unvaccinated, died in the afternoon on the farm Troughend (S 33:57:05.9 E 18:49:30.8) about 4 kilometres outside Stellenbosch alongside the Eerste River (10 km's south of Elsenburg). He showed symptoms of weakness and ataxia a few hours before death.

Another two days later (28 February) a Pony crossbred named Murphy Brown, gelding, approximately 26 years old and no known vaccination against AHS, died in the early hours of the morning following clinical symptoms for 3 days on the farm Daktari (S

33:59:27.9 E 18:48:55.4) off the Annandale road about 8 kilometres form Stellenbosch and approximately 4 km's south of Troughend. He first showed symptoms on 25th February.

The next case was only detected more than a week later when a Thoroughbred named Bow Street Belle (mare, 14 years old, vaccinated annually – last vaccination during May 2003) were euthanased at Avontuur stud (S 34:01:33.5 E 18:49:24.3) on the Stellenbosch-Somerset West district boundary, about 19 kilometres due south from Elsenburg. This animal was ill for at least a week before being put down.

On 13 March a second horse on the farm Daktari, Apaloosa-cross named Calypso (mare, 5 years old, unvaccinated) died after becoming ill on the 3rd March.

A SA Saddler, 4 years old, male named Bruin Perd, became ill on the evening of 15 March on the farm Vredenheim (S 33:57:37.7 E 18:48:27.9), about 6 kilometres from Stellenbosch along the Eerste River valley. This animal was vaccinated on 2nd March 2004 with the first bottle of AHS vaccine and remained on pastures without use of insect repellent. He died on the 17th of March. Further investigation revealed that another horse died acutely on this property on the 25th February, but was not reported by the owner until about 7 days after its burial. No samples could thus be collected to confirm the diagnosis.

On the same day a SA Saddler foal, mare, 8 months old, died on Troughend after starting to show symptoms on the 10th March. She was vaccinated on 27th February 2004 with the 1st fraction of the AHS vaccine.

The next day a Thoroughbred, named Special Edition (8 year old mare with foal at foot, vaccinated annually, last vaccinated May 2003), died on Avontuur after becoming ill on the 15th March.

On the 22 of March, the infection was detected away from the Eerste River Valley, for the first time since the Percherons died at Elsenburg, when a Boerperd, 4 years old, gelding, died on Goedverwacht farm (S 33:46:37.8 E 18:41:50.3) off the Spes Bona Road (just off the R312) between Durbanville and Klipheuwel (magisterial district of Bellville). This property is approximately 16 km's northwest of Elsenburg. Four horses ran wild on the property and were unvaccinated. Prior to the one horse succumbing, they had been grazing next to a vlei.

Another death occurred on the farm Avontuur on 24 March, when a Thoroughbred foal, colt, approximately 6 months old, died acutely. This animal had not been vaccinated and had been stabled since the previous deaths.

The last case, a Thoroughbred, 12 year old, colt, unvaccinated, died peracutely on a plot just outside Kalbaskraal (S 33:34:00 E 18:39:00) about 15 kilometres south of Malmesbury and about 32 kilometres northwest of Elsenburg. Three other horses on the farm, all unvaccinated were unaffected. This was the furthest point from Elsenburg

where infection was detected. Surveillance by means of the questionnaire survey was extended to include a 10 km radius surrounding this case. Fortunately, it was determined that the infection did not spread from this focus.

In total, sixteen horses died in this 10-week period. (See Figure 9 for geographical distribution.) The infection spread the furthest in a northerly direction, with the closest case to the free zone occurring a distance of 24 kilometres from its eastern boundary.