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

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Abstract

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

Keywords

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

1. Introduction

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

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

2. Materials and Methods

2.1 Initial events and control

In April 2016 the Equine Research Centre, University of Pretoria, reported a confirmed positive result for AHS using a real-time reverse transcriptase PCR (RT-qPCR) test on a blood sample taken by a private veterinarian from a clinically ill horse on 2 April 2016 in the Paarl region of the Western Cape. A containment zone of 15 to 50 km radius around the index property was established the day after the outbreak was confirmed and was later reduced to a 15 to 25 km radius. All subsequent cases occurred within the revised containment zone which was dissolved on 13 June 2016, 40 days after the last case on 4 May. All unauthorised movement of horses into, within, through and out of the containment zone was prohibited. Where movements were permitted measures were implemented to protect against the dissemination of AHS infection. These measures included: pre-movement AHSV RNA-based testing; vector protected stabling prior to or post-movement; pre-movement veterinary health examinations and same day movement with departure and return to the property of origin within daylight hours.

2.2 Case definitions

The case definition used in this outbreak was based on the World Organisation for Animal Health (OIE) AHS case definition of an infected animal (Infection with African horse sickness virus, 2016). For analysis, a differentiation was made between animals showing clinical signs of AHS or not (Table 1). Negative animals were categorised as laboratory tested for AHS or not. Properties were classified positive if at least one positive AHS case occurred on the property.

Table 1

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

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

RT-qPCR: Real-time reverse transcriptase PCR

2.3 Surveillance

For best estimates of the animal and disease patterns in the outbreak, a 10 km zone around the index case was considered the outbreak epicentre where the most thorough data collection and sampling took place. In addition, sample-based surveillance took place within 5 km of all infected properties. When logistically possible all horses were sampled from properties where sample-based surveillance was performed. Whole blood in EDTA was collected from live horses for AHSV detection by PCR. Samples from dead horses included lung and splenic tissue. Clinical surveillance on properties in the containment zone was through property visits by officials and owner and/or private veterinarian reporting of suspect clinical cases. Results of the surveillance zone's AHS existing sentinel surveillance program were also considered.

Two Onderstepoort 220 V suction light traps were set up on the property neighbouring the index property the day after the outbreak was confirmed. This property had a population of

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

2.4 Laboratory testing

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

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

2.5 Climate

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

2.6 Data capture and analysis

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

3. Results

3.1 Clinical findings

Fourteen (67%) of the 21 cases did not exhibit clinical signs associated with AHS and were classified as subclinical cases (Table 1). In the remaining seven cases the clinical signs consisted primarily of fever, anorexia and swollen supraorbital fossae. There were four cases where death or euthanasia occurred. Individual case clinical presentation, detection, demographic, temporal and concurrent infection information is shown in supplementary table S1.

3.2 Disease, affected proportions, temporal and animal patterns

Overall frequency data by vaccination status, breed, sex and colour are shown in Table 2. Non-tested, clinically negative horses were considered AHS negative during the outbreak and this may have resulted in an underestimation of the case totals given that subclinical AHS can occur (Weyer et al., 2013). To account for this, affected proportions were established for both overall and tested populations at risk.

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

Table 2

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

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

†only breeds/colours with at least 30 per category in total were included as separate categories

‡Fisher's exact p; calculations only performed on known factor classifications; unknown/other categories excluded

CI: confidence interval

An average of 10 horses (median = 3) were sampled per property and in total 784 unique horses from 81 properties were tested. All AHS cases occurred within 5 weeks of the index case (Figure 1). The overall crude AHS affected proportion within the horse population was 0.01 (21/1817; 95% CI: 0.01, 0.02). For laboratory tested horses it was 0.03 (21/784; 95% CI: 0.02, 0.04) overall, 0.05 (18/381; 95% CI: 0.03, 0.07) within the outbreak epicentre and 0.07 (21/296; 95% CI: 0.04, 0.10) on the eight positive properties. Subclinical cases were detected only from late April to early May 2016. The crude property-level AHS affected proportion was 0.10 (8/81; 95% CI 0.04, 0.19) for properties where at least one horse was laboratory tested, while for epicentre properties the crude property-level AHS affected proportion was 0.14 (6/48; 95% CI: 0.05, 0.29).

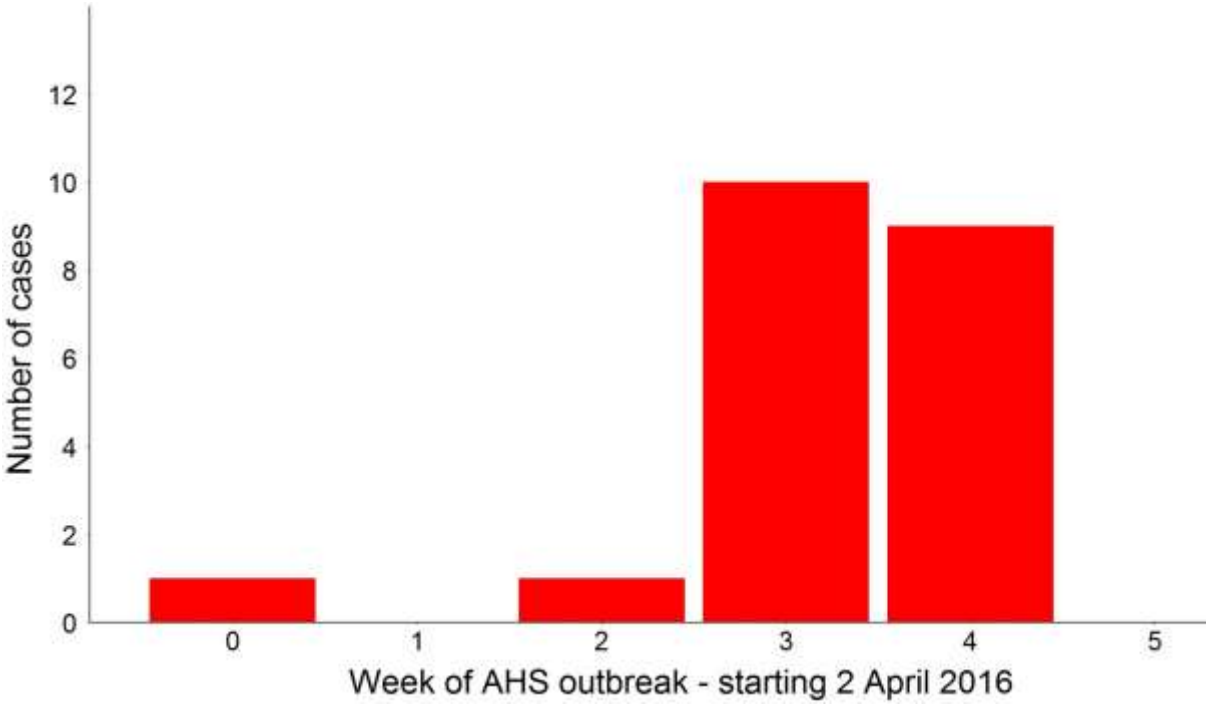

Figure 1. The epidemic curve of the 2016 African horse sickness (AHS) outbreak in the controlled area of South Africa. Week zero indicates the first week of the outbreak starting 2 April 2016.

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

3.3 Spatial considerations

Six of the eight AHS infected properties were within 10 km of the index property, which, along with a neighbouring property that later became infected, was the most south-eastern affected property (Figure 2).

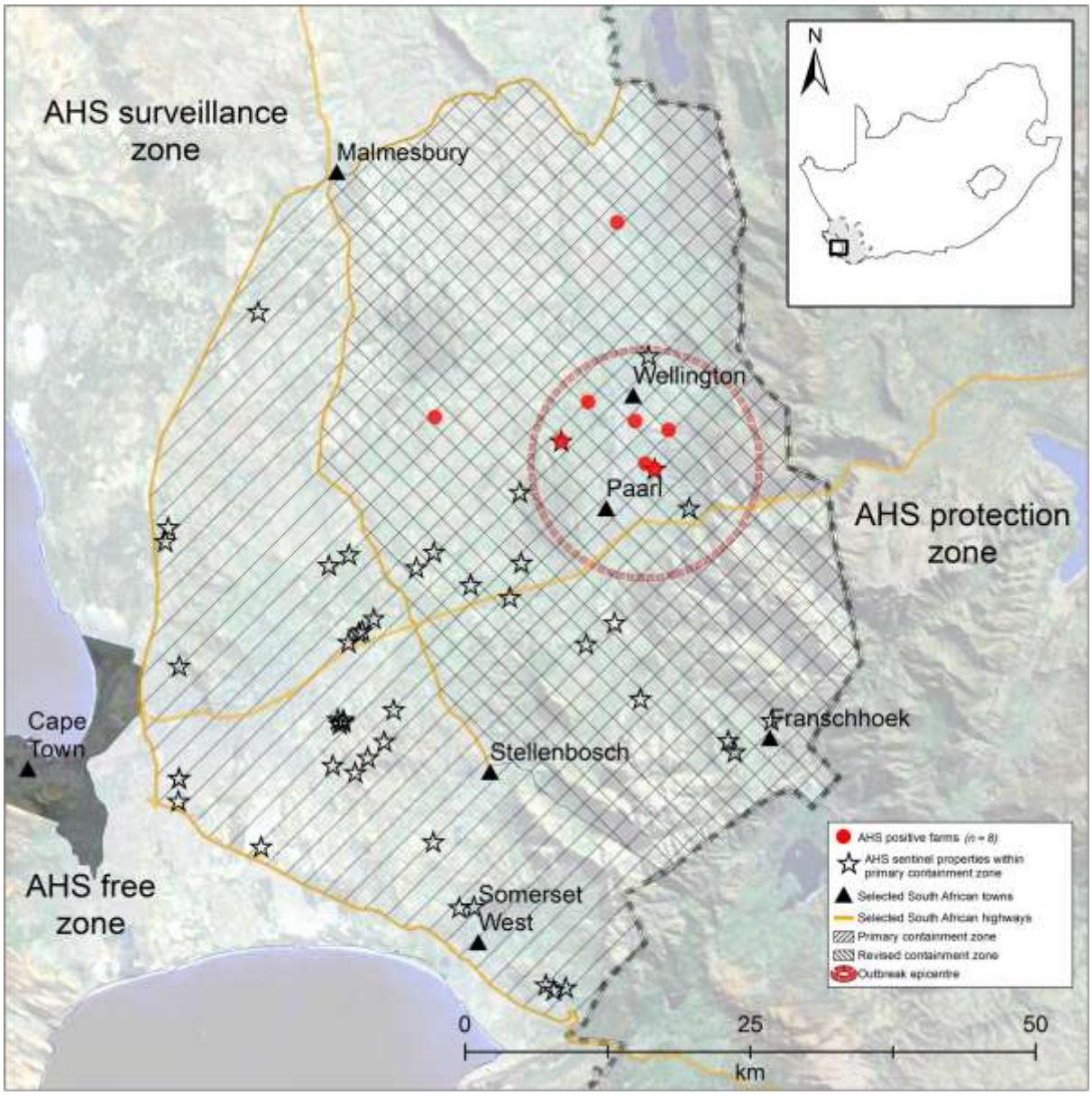

Figure 2. A map of the 2016 African horse sickness (AHS) outbreak that occurred in South Africa's controlled area depicting the positive AHS properties as well as the sentinel properties within the outbreak containment zone. The epicentre was defined as a 10 km radius around the index property. Major roadways associated with the outbreak are depicted and the primary and revised containment zones are also shown.

3.4 Vector and Climate

The outbreak occurred in mid-autumn in a winter rainfall area of South Africa where the April and May mean long term temperature ranges between 11.1^{o}C - 22.8^{o}C (Schulze et al., 2008). The summer (approximately October to March) in the Western Cape, and South Africa in general, during 2015/2016 was hot and dry because of the El Niño influence of 2015 which extended into 2016. There were consistently high daily temperatures with maximums above 30^{o}C until a rainy period towards the latter part of the outbreak in late April (Figure 3). This wet period was immediately followed by a spike in temperature with a parallel increase in vector abundance.

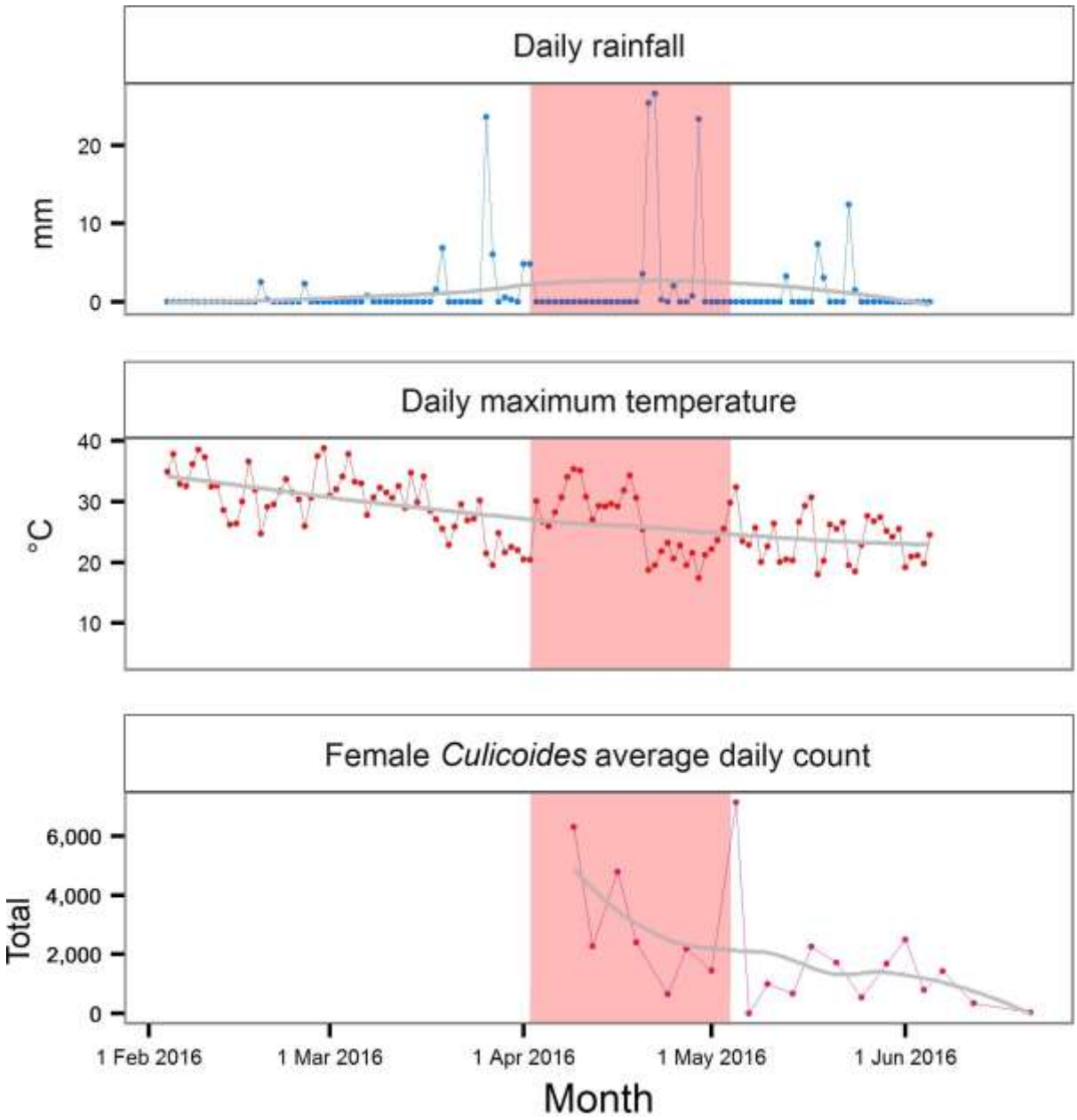

Figure 3. Environmental and vector parameters measured prior to, during (shaded pink band) and after the 2016 African horse sickness outbreak period in the controlled area of South Africa (outbreak period considered between first and last detected case date). Midge data is the average catch allocated to the respective median day of the catch period for each sampling event. Midge trapping took place on one of the outbreak positive properties which neighboured the index property. Environmental data was obtained from the closest weather station (within 2 km) to the index property.

Wind patterns present in the outbreak epicentre immediately prior to and during the outbreak are depicted in Figure 4. A diurnal pattern of wind direction was present (Figure 4A), with the majority of wind occurring from the south and south-east between 19:00 and

09:00 and more of a north/north-westerly origin during the daylight hours. Wind speed was highest generally in mid to late afternoon with wind speed tailing off in the evening and during the night (Figure 4B).

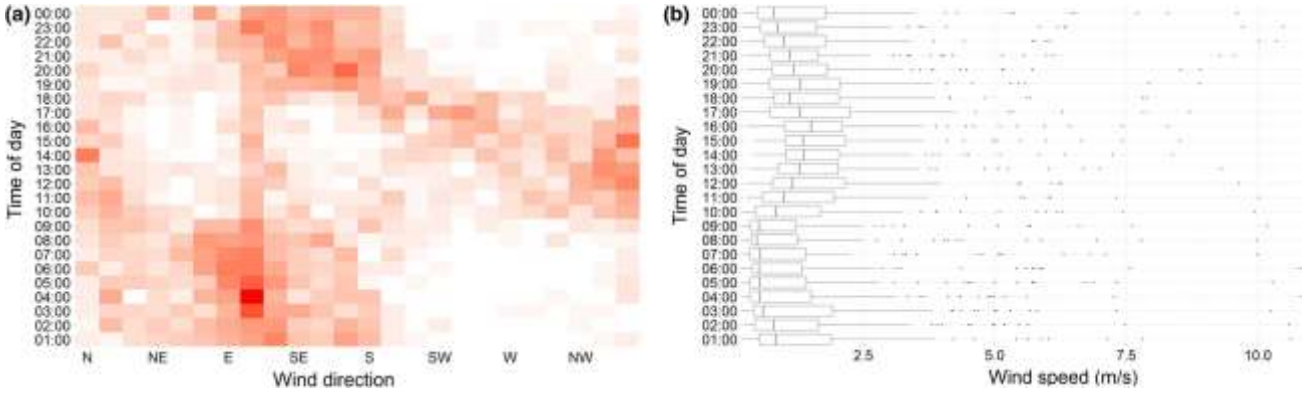

Figure 4. Wind direction (A) and speed (B) as measured between 1 March 2016 and 1 June 2016 by the closest weather station (within 2 km) to the index property of the 2016 African horse sickness outbreak in the controlled area of South Africa. Time of day (hourly classification) is represented on the Y-axis with wind direction (in 15^{o} increments and labelled based on the points of the compass) and wind speed (m/s) represented on the x-axis in A and B respectively. The colour range from white to dark red in (A) indicates the amount of time, for that time of day and wind direction, that wind was present with white being seldom present and red indicating often present, all relative to other time/direction combinations. N: north; E: east; S: south; W: west; NE: north-east; SE: south-east; SW: south-west; NW: north-west

The proportional breakdown of *Culicoides* species detected during the outbreak is shown in Table 3. In total 17 species were identified, although >95% were *Culicoides imicola*. The majority (>90%) of the midges collected consisted of nulliparous and parous females. One midge pool, collected over three nights from 11 to 14 April, tested inconclusive for the presence of AHSV RNA (Cq value of 38.05 where the positive cut-off is 37).

Table 3

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

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

3.5 Movement control

A total of 323 movement events involving 903 horses occurred into, out of, through or within the outbreak containment zone. The movement direction and various control measures are summarised in Table 4. The majority of horses moving out of the AHS containment area (309/513; 60%) were bound for destinations in the AHS infected zone of South Africa while the majority of horses moving into the containment zone (129/232; 56%) originated from within the AHS controlled area.

Table 4

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

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

3.6 Sentinel findings

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

3.7 Source of the outbreak

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

3.8 Discussion

The outbreak occurred in April and May 2016 which is consistent with previous AHS outbreaks in the same region since 1999. The case total, overall affected proportion and case fatality proportion for this outbreak compared to previous outbreaks was low, with a comparatively high subclinical proportion (Grewar et al., 2013; Weyer et al., 2016). One of the limitations of the disease proportions established during this outbreak was that case clustering is likely to have occurred on affected properties. To account for this, affected proportions were established for individual properties, the outbreak epicentre and the outbreak containment zone as a whole. The epidemic curve of the outbreak suggests that the index case could have been the primary case, or closely associated with it, given the

time period between it and the next reported case, which was followed closely by the bulk of the cases (Figure 1). Herd immunity in the outbreak area is likely to have played a role in preventing further propagation of the outbreak, with 81.4% of horses censused in the containment zone having been previously vaccinated against AHSV.

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

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

Local weather conditions will influence *Culicoides* abundance, and while we have considered temperature and rainfall data two months prior to the outbreak (Figure 3), abundance would have been influenced by climatic conditions preceding this. On a local scale the climatic conditions of high temperature with a spike of rainfall, which was evident both prior to and towards the end of the outbreak, would have promoted increasing vector

populations (Meiswinkel, Venter, & Nevill, 2004). The south-easterly wind pattern during this outbreak and general case distribution in a north-westerly direction from the index property makes it plausible that local spread of virus was through the wind dispersal of infected midges. However, the topography of the outbreak area cannot be ignored, since it occurred in a well-defined valley bordered by mountains on its eastern/south-eastern and western edges. This influences horse population distribution, so vector dispersal associated spread of infection could initially only have taken place in a general northerly or southerly direction.

The dominant species of midge collected during the outbreak was *C. imicola*, a species considered a proven vector of AHSV (Meiswinkel et al., 2004). The testing of selected midge pools from the first two months of the outbreak did not result in the detection of AHSV. This is consistent with results found during the 1999 and 2004 AHS outbreaks in Stellenbosch, approximately 30 km south-west of this outbreak, and indicates very low field infection prevalence in the vector (Venter et al., 2006).

This is not the first time that an AHS live attenuated vaccine virus, with evidence of reversion to virulence, has been the source of an outbreak of AHS within the AHS surveillance zone of South Africa (Weyer et al., 2016), although the SNV's were not identical to those previously described (Guthrie, A.J. Unpublished data). Introduction of the virus into the outbreak area could have occurred in several ways. There could have been circulation of vaccine associated virus in the region; however this is unlikely given that such circulation was not detected through any ongoing sentinel surveillance, passive surveillance by private veterinarians or a post-2014 freedom of disease AHS surveillance study, all of which took

place in the 2016 outbreak area (Western Cape Department of Agriculture, Unpublished data). Vaccination in the AHS surveillance and free zone of South Africa is by State authorisation only and may only be done between June and October. The wind dispersal of AHS from the infected area of South Africa is also an unlikely scenario for introduction. The prevailing wind conditions during summer in the south-western Cape region of South Africa: in the 2015/2016 AHS season the closest detected AHS (type unknown) outbreak was 600 km to the east (*Final 2016 African Horse Sickness Season Report (Amended)*, 2016). Future research into wind dispersal of *Culicoides* midges in South Africa is required to explore the patterns and risk of this dispersal method. The most likely route of introduction of the virus was either via an illegal AHS vaccination within the AHS surveillance zone in the vicinity of the index property or via a horse harbouring a reverted or reverting vaccine strain being moved illegally into the area.

4. Conclusion

This is a detailed description of an AHS outbreak in the AHS surveillance zone in South Africa. The outbreak source virus originated from the reversion to virulence of the live attenuated vaccine, presumably introduced through illegal vaccination or movement within the AHS controlled area. This outbreak was detected by a private veterinarian through the existing passive surveillance program in the AHS surveillance zone of South Africa, highlighting the importance of this form of surveillance in AHS. The climatic conditions promoted the initial transmission of the virus but a combination of control measures, vaccination status of the exposed population and cooler climatic conditions decreased the case numbers and the local scale impact of the outbreak. The global scale impact remains significant, preventing the direct export of live horses from South Africa to the EU under

existing EU legislation for at least two years. This outbreak emphasises the importance of the judicious use of the live attenuated vaccine within the AHS controlled area of South Africa.

5. Acknowledgments

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6. Conflict of Interest

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

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