The 2011 outbreak of African horse sickness in the African horse sickness controlled area in South Africa

Authors:
John D. Grewar[1]
Camilla T. Weyer[2]
Alan J. Guthrie[2]
Pieter Koen[1]
Sewellyn Davey[1]
Melvyn Quan[3]
Dawid Visser[1]
Esthea Russouw[1]
Gary Bührmann[1]

Affiliations:
[1]Veterinary Services, Western Cape Department of Agriculture, South Africa

[2]Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, South Africa

[3]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa

Correspondence to:
John Grewar

Email:
johng@elsenburg.com

Postal address:
Private Bag X1, Elsenburg 7607, South Africa

African horse sickness (AHS) is a controlled animal disease in South Africa, and as a result of the high mortality rates experienced, outbreaks in the AHS controlled area in the Western Cape Province have a significant impact on affected properties as well as on the exportation of live horses from the AHS free zone in metropolitan Cape Town. An outbreak of AHS serotype 1 occurred in the surveillance zone of the AHS controlled area of the Western Cape during the summer of 2011. The epicentre of the outbreak was the town of Mamre in the magisterial district of Malmesbury and the outbreak was confined to a defined containment zone within this area by movement control of all equids and a blanket vaccination campaign. A total of 73 cases of AHS were confirmed during this outbreak, which included four confirmed subclinical cases. The morbidity rate for the outbreak was 16% with a mortality rate of 14% and a case fatality rate of 88%. Outbreak disease surveillance relied on agent identification using polymerase chain reaction (PCR)-based assays, which is novel for an AHS outbreak in South Africa. The source of this outbreak was never confirmed although it is believed to be associated with the illegal movement of an infected animal into the Mamre area. This detailed description of the outbreak provides a sound scientific basis to assist decision making in future AHS outbreaks in the AHS controlled area of South Africa and in countries where AHS is an exotic or emerging disease.

Introduction

African horse sickness (AHS) is a vector-borne, life threatening disease of equids caused by African horse sickness virus (AHSV), a member of the genus *Orbivirus* in the family *Reoviridae*. This virus causes vascular injury that can result in four forms of disease: the pulmonary, cardiac, mixed, or horse sickness fever forms (Erasmus 1974). The virus is transmitted to horses by midges (*Culicoides* spp.) and the disease is most prevalent in areas where these vectors are most abundant. Outbreaks in South Africa generally occur during late summer in the summer rainfall areas of the country (Coetzer & Guthrie 2004). AHS is a controlled disease in South Africa (South African Government 1984:s.2, ss.53) and is a World Organisation for Animal Health (OIE) listed disease as it causes high mortality rates and has the potential for rapid spread (Mellor & Hamblin 2004). AHS is endemic to sub-Saharan Africa but the disease has occurred sporadically outside of this region (Mellor 1994), for example the outbreaks of AHS in Spain in the 1960s and 1980s, where hundreds of horses succumbed to the disease while hundreds of thousands were vaccinated during its control (Rodriguez, Hooghuis & Castano 1992).

The area around the Cape of Good Hope in South Africa has historically been free from AHS (Coetzer & Guthrie 2004), with sporadic outbreaks as a result of the introduction of AHSV positive horses from other provinces. On this basis a protocol was submitted to the European Community (now the European Union – EU) proposing the establishment of an AHS free zone in the Cape Peninsula, from which the export of horses could resume provided certain conditions were met (Bosman, Bruckner & Faul 1995). This proposal was accepted by the EU in 1997 (Fischler 1997). That same year, an AHS controlled area was established in the Western Cape Province of South Africa (South African Government 1984:s.2, ss.53), consisting of the Metropolitan Cape Town AHS free zone, the AHS surveillance zone and the AHS protection zone (Figure 1b).

Since implementation of this regionalisation, two other AHS outbreaks have occurred within the AHS surveillance zone, in the Stellenbosch district (Sinclair, Buhrmann & Gummow 2006; Venter, Koekemoer & Paweska 2006). These outbreaks were as a result of AHSV serotype 7 in 1999 and AHSV serotype 1 in 2004, and the equine deaths associated with these outbreaks totalled 32 and 16 in 1999 and 2004 respectively. In February 2011, another outbreak of AHS occurred within the AHS surveillance zone in horses resident in the town of Mamre in the Malmesbury magisterial district. This resulted in the suspension of horse exports directly from South Africa to the EU

and other countries. The aim of this study was to provide a descriptive evaluation of the events and control measures employed for this outbreak of AHS.

Materials and methods
The outbreak
On 26 February 2011, State Veterinarian (SV) Malmesbury was requested by a private veterinarian from the Malmesbury district to assist in the necropsy of a horse that had died that day in the town Mamre in the Western Cape Province of South Africa. The private veterinarian had seen the horse earlier in the day and it had exhibited clinical signs suggestive of infection with AHSV. Blood and tissue samples were collected for laboratory analysis. On the same day, the SV sampled a dead horse found next to the road in Mamre. On 02 March 2011, another horse was reported dead by an owner from Mamre and a necropsy with sampling was performed. Results of quantitative real time polymerase chain reaction (RT-qPCR) assays were also received, showing that the samples from the first two mortalities were positive for AHSV. The Department of Agriculture, Forestry and Fisheries (DAFF) was notified immediately and the implementation of control measures and plans to attempt to contain the outbreak were initiated by the Directorate of Veterinary Services of the Western Cape Province.

A movement ban was immediately instituted by means of a press release on 03 March 2011 that limited movement of horses into, within and out of the Malmesbury magisterial district. These movement restrictions remained in place until 09 June 2011, one month after the last confirmed case (03 May 2011) of AHS within the containment zone.

Advice was given to horse owners to stable their horses from two hours before sunset until two hours after dawn to decrease the risk of vector contact with the horses. Owners were also advised to apply insect repellent to their horses daily.

Ring vaccination was initiated on 09 March 2011 in the immediate vicinity of the initial cases in Mamre. The vaccine used was produced by Onderstepoort Biological Products (OBP) and is presented as two separate injections with different AHSV combinations represented in each injection. The outbreak strain was identified as AHS serotype 1 and therefore the relevant vaccine combination was used during the primary outbreak response vaccinations to provide protection against this serotype. Private veterinarians in the area were authorised to vaccinate clients' horses resident in the Malmesbury magisterial district, provided that they kept detailed records of these vaccinations. This authorisation for private veterinarians to perform emergency vaccination in the Malmesbury magisterial district was rescinded after the conclusion of the outbreak. The ring vaccination provided an opportunity for a concurrent equine census to be taken in the outbreak area. The population at risk used in the denominator for the estimations of disease morbidity and mortality was calculated from these data.

Sample collection and laboratory testing
Samples collected from dead horses for laboratory testing included lung and spleen samples. When carcasses were badly decomposed, a sample of body fluid was placed in ethylene-diamine-tetra-acetic acid (EDTA) vacutainer tubes or a piece of tissue was collected. When a veterinarian was present, EDTA blood samples were collected by jugular venipuncture from each horse presented to outbreak response personnel prior to vaccination.

Blood and organ samples were subjected to testing using either a RT-qPCR (Guthrie *et al.* 2013; Quan *et al.* 2010) or a hemi-nested PCR (hnPCR) assay (Bremer, Dungu-Kimbenga & Viljoen 1998). In the case of the RT-qPCR, the cycle threshold (C_T) is the number of cycles taken for fluorescence in the sample to exceed a threshold of 0.1. For the purposes of this outbreak, RT-qPCR results were classified as positive if the fluorescence exceeded the threshold of 0.1 within a maximum of 30 cycles.

In order to rapidly establish the serotype of AHSV involved in the outbreak, partial, direct sequencing of the S10 gene (coding for the non-structural protein NS3) and the L2 gene (coding for the outer capsid protein VP2) was performed using the samples collected from the first confirmed case of AHS of the outbreak. Nucleic acid was extracted using a MagMAX™ Express Magnetic Particle Processor and a MagMAX™-96 Total RNA Isolation Kit (Lifetech), according to the manufacturer's instructions. Sequencing of S10 was performed as described previously (Quan *et al.* 2008). Primers for sequencing L2 were designed from AHSV sequences available on Genbank. L2 was amplified in approximately 600 nucleotides (nt), overlapping fragments and the sequences assembled using the Staden package. MEGA4 was used to construct a bootstrapped (1000 replications) neighbour-joining phylogenetic tree of approximately 1000 nucleotides of the 5′-end of the AHSV L2 gene.

Virus isolation (VI) and serotyping by the plaque inhibition neutralisation test were performed as described previously (Quan *et al.* 2008).

Outbreak case definition
The AHS case definition used by the Directorate of Veterinary Services, Western Cape Province at the beginning of the outbreak was clinical and/or post-mortem signs indicative of AHS with laboratory confirmation by PCR and/or VI assays. Initially, only horses that showed clinical and/or post-mortem signs indicative of AHS were included as suspect cases. This was subsequently modified when it became evident that some horses that did not show clinical signs indicative of infection with AHSV tested positive on PCR, and in some cases AHSV serotype 1 was isolated from samples collected from these horses. A case definition code system was instituted in order to facilitate processing of results (Table 1). Subclinical cases were defined as an animal that was found to be RT-qPCR ($n = 14$) or hnPCR ($n = 1$) positive, with or without positive AHS VI results, but showing no discernible clinical signs of

AHS. AHSV serotype 1 was isolated from four of these cases, which were subsequently classified as confirmed cases. The case definition for an AHS case according to the OIE is:

> AHSV has been isolated and identified from an equid or a product derived from that equid; or viral antigen or viral RNA specific to one or more of the serotypes of AHSV has been identified in samples from one or more equids showing clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed case; or serological evidence of active infection with AHSV by detection of seroconversion with production of antibodies to structural or non-structural proteins of AHSV that are not a consequence of vaccination have been identified in one or more equids that either show clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed case (World Organisation for Animal Health 2010).

According to this definition, all the suspect horses falling into the S2 ($n = 12$) case definition category should also have been considered as confirmed AHS cases owing to the animals being epidemiologically linked to other confirmed cases, and having a positive molecular diagnostic result. This should be taken into consideration in future outbreaks as well as reporting of cases from the infected part of the country.

Carcass disposal

Horse carcasses on which *post-mortem* examinations were performed at the Stellenbosch provincial veterinary laboratory were disposed of by the laboratory. Dead horses that were not transported to the laboratory for *post-mortem* were sampled *in situ* and transported to the Vissershok waste disposal site, which is registered for this purpose. There were exceptional cases where disposal was not possible and this was when carcasses were too decomposed to move or where attempts by local residents had been made to burn or bury them locally.

Ethical considerations

Samples collected during this outbreak were done under State authorisation using standard veterinary procedures and according to disease detection and surveillance protocols.

Observations

Quantification and spread of the outbreak

The epidemiological variables have been separated into the outbreak as a whole and the outbreak epicentre (Table 2). The outbreak epicentre was the area within the Mamre town limits where most cases occurred and it is approximately 3900 ha in size. Outlying cases (Figure 1) extended as far as Darling to the north-west (15 km), Riverlands in the east (12 km), Groenfontein to the south-east (13 km) and just south-east of Atlantis to the south (13 km).

The confirmed AHS cases totalled 73 horses. The incidence of AHS during the outbreak period in the epicentre was 0.18 (95% Conf. 0.14–0.22) compared with an incidence of 0.16 (95% Conf. 0.13–0.20) for the outbreak as a whole. The total number of deaths recorded during the outbreak (both AHS confirmed ($n = 64$) and suspect cases ($n = 6$) was 70. This relates to an AHS-specific mortality rate of 0.16 (95% Conf. 0.12–0.20) in the epicentre and 0.14 (95% Conf. 0.11–0.18) for the outbreak as a whole. The majority of these deaths impacted indigent owners. The case fatality rate was 0.88 both within the outbreak epicentre and for the outbreak as a whole.

There were a number of subclinical cases recorded in this outbreak, both suspect ($n = 11$) and confirmed ($n = 4$), which is also a unique feature for an outbreak in the surveillance zone, but has recently been described in endemic areas (Weyer *et al.*

TABLE 1: The outbreak case definition codes categorised according to the clinical signs and laboratory results of each case, and the number of cases in each category.

Case definition	Total of cases per case definition	Code	Sub definition	Number of cases per sub definition
Negative	172	N1	Death or suspect AHS clinical and/or PM signs with confirmation of another cause of disease and confirmed negative AHS laboratory results	14
		N2	Routine testing for surveillance with negative AHS laboratory results	158
Positive	73	P1	Clinical signs and/or PM signs of AHS with laboratory PCR and/or VI positive result	69
		P2	PCR and VI AHS positive result with no accompanying clinical or PM signs	4
Suspect	16	S1	Clinical signs and/or PM signs of AHS with no laboratory positive confirmation	4
		S2	No clinical or PM signs of AHS with a positive PCR result and VI negative	12

PM, *post-mortem*; AHS, African horse sickness; VI, virus isolation; PCR, polymerase chain reaction.

TABLE 2: Various epidemiological variables measured during the outbreak.

Aspect measured	Outbreak area category			
	Epicentre	95% Confidence intervals	Entire outbreak	95% Confidence intervals
Estimated population at risk based on census data	319	-	447	-
Animals evaluated: clinically, *post-mortem* or laboratory tested	186	-	261	-
Total positive cases	57	-	73	-
Total AHS confirmed deaths	50	-	64	-
Morbidity rate	0.18	0.14–0.22	0.16	0.13–0.20
Mortality rate	0.16	0.12–0.20	0.14	0.11–0.18
Case fatality rate	0.88	0.76–0.94	0.88	0.78–0.94
Total confirmed sub clinical cases	4	-	4	-
Total suspected sub clinical cases	9	-	11	-

Note: Equines evaluated include all equines which were surveyed using quantitative real time polymerase chain reaction (RT-qPCR) testing by the Equine Research Centre (University of Pretoria). The population at risk is based on the census performed in the outbreak area by the veterinary services of the Western Cape.
AHS, African horse sickness.
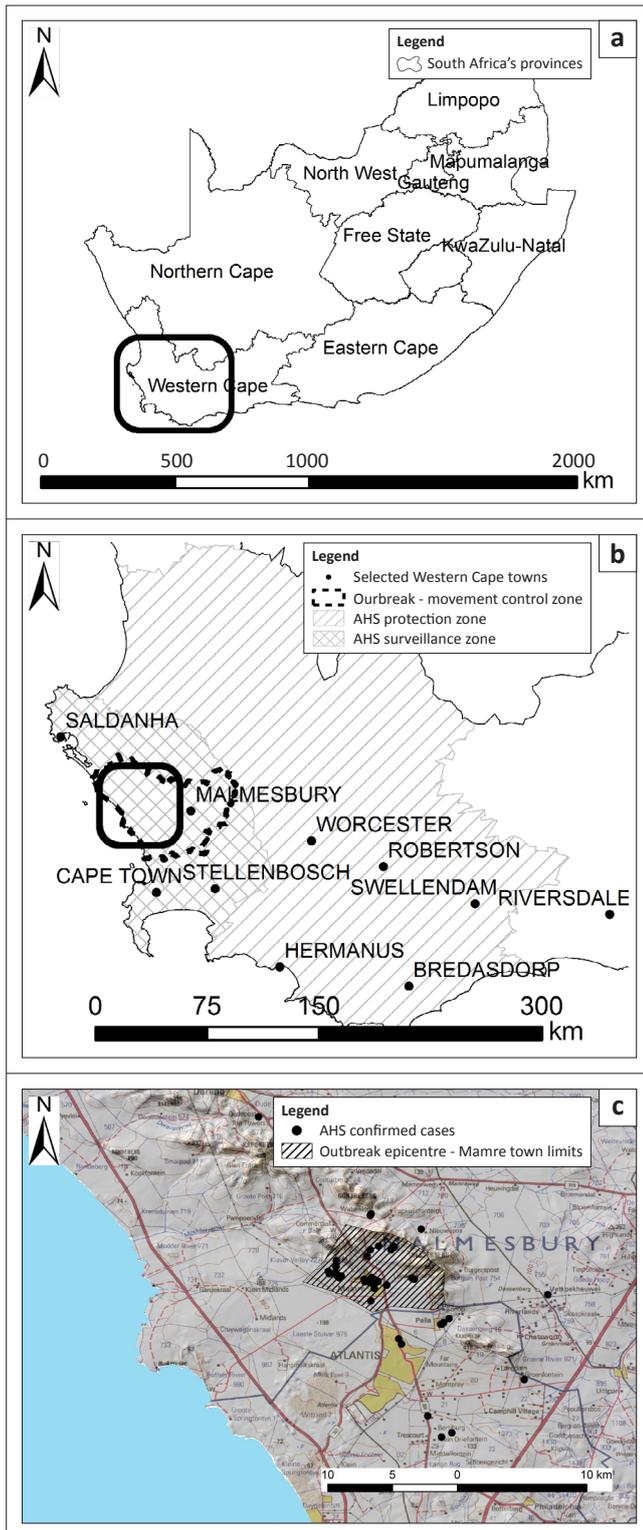
Note: Section A shows the geographical extent of South Africa with the Provincial boundaries as well as the extent frame of section B (labelled as such). Section B shows two of the AHS control zones within the Western Cape Province, namely the protection zone, which is an area of compulsory vaccination for domestic equids against AHS, and the surveillance zone, which is where vaccination is only performed after permission from the Provincial Director of Veterinary Services is obtained. Also in Section B is the extent of the Malmesbury magisterial district, the boundaries of which were used to delineate the movement restriction zone implemented during the outbreak. Section C shows the extent of the outbreak by individual confirmed cases (*n* = 73) within the Malmesbury district as well as the extent of the Mamre town limits which was the epicentre of the outbreak and where the majority (*n* = 57) of cases occurred.
AHS, African horse sickness.

FIGURE 1: A map indicating the extent of the AHS outbreak in the Western Cape: (a) geographical extent regarding the Provincial boundaries (b) shows two of the AHS control zones and (c) shows the extent of the outbreak by individual confirmed cases.

2013). Ten of the 11 suspect subclinical cases were RT-qPCR positive, some for an extended period of time, but isolation of viruses from these blood samples proved unsuccessful. Of the 11 suspect cases, nine were sampled prior to vaccination, so the positive RT-qPCR result was most likely a result of field infection and not vaccination. The other two were sampled between one and two weeks after vaccination, so the positive result may have been due to either vaccination or field infection. There was one remaining suspect case that was not classified as subclinical, as the horse died. However, although the horse was found to be RT-qPCR positive, the post-mortem findings were not typical of AHS, the sample was negative on VI and the horse's vaccination history was unknown. It was therefore decided to classify this horse as suspect rather than confirmed. One other limitation in the subclinical case category is that the number of suspect subclinical cases is dependent on the specificity of the PCR assay used in their definition. The median specificity of the RT-qPCR used in the outbreak (defining 10 of the 11 suspect cases) is described as being greater than 0.992 (Guthrie *et al.* 2013).

A total of 447 equids were vaccinated against AHS by state veterinary authorities in the outbreak area, the majority in early March 2011, approximately one week before the outbreak peaked (Figure 2). Reports from the Malmesbury district showed that a further 189 horses were vaccinated by private veterinarians during the outbreak period in the surrounding district. This brought the total of vaccinated equines during the outbreak period to 636.

Virus identification

A sequence of about 320 nt long at the 5' end of AHSV S10 was obtained. The sequence shared the highest identity with
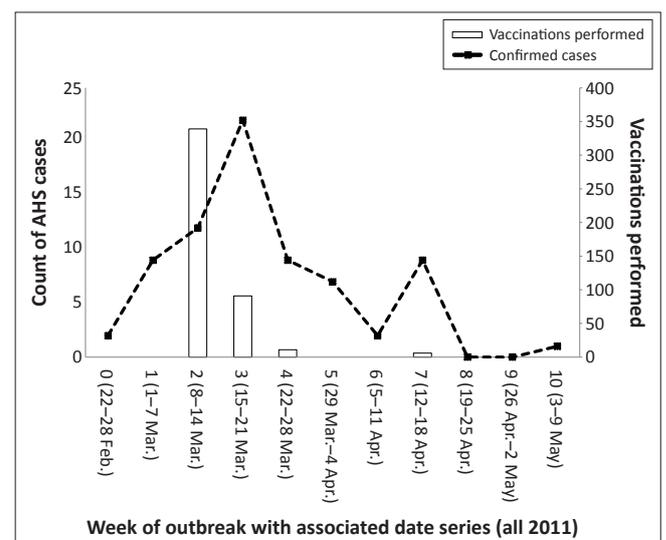
Note: The curve drops off sharply with a small secondary peak during the eighth week of the outbreak. The vaccinations performed as a result of the outbreak (private veterinarian vaccinations not included; totals per week shown as bars with the totals on the right axis) show that the peak vaccination occurred in the week before the peak in confirmed cases.
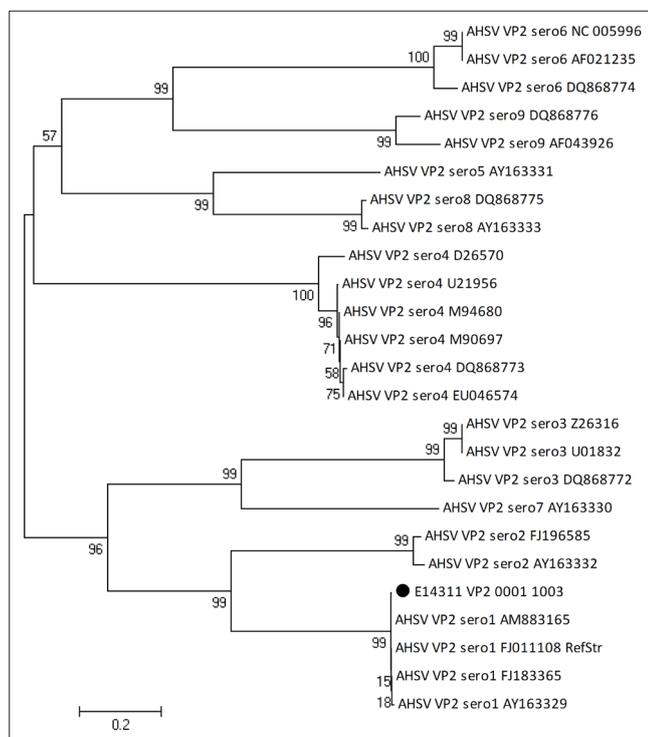AHS, African horse sickness.

FIGURE 2: The epidemic curve (dashed line) of the outbreak depicting only AHS confirmed cases (*n* = 73). The peak of cases occurred during the fourth week (including week zero) of the outbreak with 22 cases occurring.

two serotype 1 AHSV isolates: 99.4% with E03404 (GenBank accession number EU433546.1) and 99.6% with Jane 1 (EU433410). Sequencing of approximately 1000 nucleotides of the 5'-end of the AHSV L2 showed that the isolate was AHSV serotype 1. Alignment of the sequence with other AHSV VP2 sequences available on GenBank showed a 99.8% – 99.9% identity with other AHSV serotype 1 isolates (Figure 3).

Discussion

Spread and source of disease

The demographics in the outbreak environment meant that it was initially difficult to prevent the movement of infected animals within the Mamre area and immediate surroundings, as there were no formal horse societies to advise and pressurise owners and no incentive not to move horses as they normally did. It was found that personal communication with each individual horse owner in Mamre and surrounding areas was necessary in order to explain the reasons for movement restrictions. This was one of the biggest challenges in control of this outbreak compared with past outbreaks of this disease, which occurred in wealthier areas where formal stabling and management existed. The strategies to control movement into, out of and through the movement restriction zone were effective in preventing formal movements by vehicles, which was easier to control as there was cooperation from traffic officials and reporting from other concerned horse owners.

Note: Genbank accession numbers are indicated in the labels.
AHSV, African horse sickness virus.

FIGURE 3: A bootstrapped (1000 replications) neighbour-joining phylogenetic tree of approximately 1 000 nucleotides of the 5'-end of the African horse sickness L2 (VP2) gene showed that the outbreak isolate (E14311 VP2 0001 1003) grouped with other AHSV serotype one sequences.

The source of the outbreak could not be confirmed, although investigations by the SV did show that there were suspected illegal movements of horses into the AHS surveillance zone. The partial sequence of the S10 gene of the outbreak virus was 98% – 99% identical with other AHS serotype 1 viruses including the Jane reference strain and field isolates from Mpumalanga, Gauteng and from the Stellenbosch area of the AHS surveillance zone. Further phylogenetic studies of these AHS serotype 1 viruses and serotype 1 viruses isolated in South Africa prior to and following the 2004 outbreak may provide data from which the most likely source of the outbreak virus could be identified. Such studies would also be necessary to confirm that the outbreak viruses involved in 2004 and 2011 were sufficiently different to be associated with separate introductions and were not due to re-emergence of the 2004 outbreak virus in 2011. As the prevailing wind direction on the Cape Peninsula in the summer months is from the south or south-east and the only AHS cases reported in other parts of South Africa prior to the outbreak in the surveillance zone in 2011 were in excess of 800 km from the outbreak in a north-easterly direction, it is highly unlikely that wind-borne dissemination of infected *Culicoides* was responsible for initiating this outbreak.

Outbreak surveillance strategy

A unique feature of the surveillance approach to this outbreak was the method of surveillance employed in the apparently healthy equine population. This ensured that active AHSV nucleic acid detection surveillance using an almost census-level sampling frame was accomplished and this provided a dataset from which accurate determination of epidemiological variables could be made, as test results improved the accuracy of confirmed negative cases in the population at risk (Table 2). Serological surveillance techniques have been utilised in previous outbreaks but there is always the aspect to consider of baseline vaccine antibody levels present in the horse at the time of sampling and invariably paired serum samples are a necessity. With PCR techniques, and in particular in future with serotype-specific real time PCR, one sample per horse examined or counted in the initial stages of an outbreak will assist greatly in determining the initial extent and magnitude of the outbreak.

Making use of a nucleic acid detecting test, in this case PCR on EDTA blood samples, for surveillance during an outbreak does also pose its own set of challenges. The window of opportunity for detecting nucleic acid is reliant on the period these molecules are present at detectable levels in the tested substrate. Also, as occurred in this outbreak, once the blanket vaccination of equines in the area has taken place, the value of PCR surveillance diminishes dramatically, as would the value of serological surveillance, since in neither case can positive results due to vaccination be distinguished from those due to the outbreak strain. PCR or serological testing making use of a DIVA (differentiating infected from vaccinated individuals) assay will greatly assist in any outbreak situation including AHS.

The subclinical cases found in this outbreak, although substantially fewer than the clinical cases, are still a cause for concern in terms of spread of disease. This is of further concern when considering that these cases, without the aid of molecular surveillance tools like the RT-qPCR used during this outbreak, are unlikely to be diagnosed in field conditions because the relevant samples would generally not be taken from a horse that did not show clinical signs. An evaluation of all positive (P1 and P2 definitions) and suspect (S2 definition) cases where PCR and VI results were available showed that only 66% (n = 54) of the positive PCR results had collaborating positive VI results. Of the total 15 suspect subclinical cases, only four (27%) had positive VI results, again showing the value of the more sensitive PCR in an outbreak situation.

Vaccination effect

A dramatic decrease in the number of cases occurring per week was observed within two weeks after the majority of the equine population in Mamre had been vaccinated (Figure 2). The majority of equids vaccinated (n = 289) were within the Mamre town limits. This accounted for 64.6% of equines vaccinated in the outbreak zone and covered 90.0% of the equines within Mamre.

As AHS is a vector-borne disease, one would normally expect (if no counter measures are taken) a propagating epidemic curve with multiple case peaks during an outbreak. Except for a very small peak at the end of April, the outbreak in Mamre showed only one peak in disease cases. This is most likely due to vaccination in the Mamre area and particularly in the surrounding areas of Pella and Atlantis, which prevented significant seeding of the outbreak.

Impact of the outbreak

The direct costs of this outbreak were borne by many different industries and state departments. The lowest cost estimate for the outbreak totalled R850 000 and the bulk of this cost was due to laboratory testing of samples. The equine industry paid many of the monetary expenses associated with the outbreak and the role-players involved included the Equine Research Centre (University of Pretoria), Racing South Africa, the Cape Breeders' Association and the Acorn Group of Companies. State departments involved included the Western Cape Department of Agriculture - Veterinary Services Animal Health, City of Cape Town Disaster Management and the Stellenbosch Provincial Veterinary Laboratory.

At the time of the outbreak, South Africa was exporting on average 200 horses per annum. The revenue loss to industry stakeholders directly involved in the logistics of exporting horses is estimated at R20 million per annum. There was also loss of foreign investment as a result of a decrease in direct exports which was estimated at R200 million per annum. This was partly (by 33%) offset by importers utilising alternative shipping routes via Mauritius (P. Gibson, Racing South Africa, pers. comm., 05 October 2012).

Seventy horses in total (64 confirmed as AHS) died during the 2011 AHS outbreak in one of the poorer communities in the Western Cape. Apart from the tradable value of these horses, the direct and indirect cost as a result of the death of these animals is difficult to determine. Direct costs include the loss of a working animal used for transport and for herding livestock. Indirect costs include all products and services related to the maintenance of the horses in this community. Emotional costs and the loss of breeding stock are also important factors to consider, as horses are considered as part of the community's heritage.

Conclusion

The response to this outbreak highlighted the importance of partnerships in the control of diseases such as AHS. Private-public partnerships between members of equine industries and the State Veterinary service ensured a rapid control response. The State was able to start vaccinations immediately with the AHS vaccine donated by the Equine Research Centre and the personnel of the different bodies were able to work together in an efficient manner. This also helped in building a relationship with the community of Mamre, as all parties involved were seen to have the same objectives and the best interests of the community at heart. Reporting to the public was also made easier by help from the AHS Trust (http://www.africanhorsesickness.co.za) who allowed the use of their website to disseminate the situation reports for the outbreak to the public.

The vaccination requirements described by the movement control protocol of horses (Western Cape Government: Agriculture 2006) between the various AHS control zones in South Africa are primarily aimed at preventing infected horses entering the AHS surveillance zone, which in turn decreases the risk of AHS infection in the AHS free zone. The various equestrian societies that rely on movements of horses for their events must continue to ensure that participating horses have undergone the correct procedures when moving between the various AHS control zones to minimise the risk of future incursions.

Acknowledgments

those private veterinarians consulted during the outbreak who assisted with information dissemination to their clients and who contributed to the overall vaccination campaign. Publication of this article was sponsored by the South African Equine Veterinary Association of the South African Veterinary Association.

Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

Authors' contributions

J.D.G. (Western Cape Dept. of Agriculture) and C.T.W. (University of Pretoria) made equal contributions to this manuscript with assistance from A.J.G. (University of Pretoria), S.D. (Western Cape Dept. of Agriculture) and M.Q. (University of Pretoria). C.T.W. (University of Pretoria) performed the research on the nucleic acid detection surveillance while J.D.G. (Western Cape Dept. of Agriculture) performed the quantitative, spatial and temporal analysis of the data. S.D. (Western Cape Dept. of Agriculture) and D.V. (Western Cape Dept. of Agriculture) co-ordinated the outbreak response and logistics. J.D.G. (Western Cape Dept. of Agriculture) and C.T.W. (University of Pretoria) maintained the outbreak sampling and results data. A.J.G. (University of Pretoria), P.K. (Western Cape Dept. of Agriculture) and G.B. (Western Cape Dept. of Agriculture) assisted during the outbreak with pertinent scientific and historical information on AHS. E.R. (Western Cape Dept. of Agriculture) was practically involved throughout the outbreak in census, sampling and community outreach procedures. J.D.G. (Western Cape Dept. of Agriculture) submitted the manuscript. M.Q. (University of Pretoria) was responsible for laboratory diagnosis and sequence analysis during the outbreak.

References

Bosman, P., Bruckner, G.K. & Faul, A., 1995, 'African horse sickness surveillance systems and regionalisation/zoning: The case of South Africa', *Scientific and Technical Review, Office International des Épizooties* 14, 645–653.

Bremer, C.W., Dungu-Kimbenga, B. & Viljoen, G.J., 1998, 'Detection of African horse sickness virus in zebra by RT-PCR and the development of different methods for confirming AHSV specificity of RT-PCR products', in U. Wernery, J.F., Wade, J.A. Mumford & O.R. Kaaden (eds.), *Equine Infectious Diseases VIII: 8th International Conference Proceedings,* Dubai, UAE, March 23–26, 1998, p. 529, R & W Publishers (Newmarket) Ltd., Newmarket.

Coetzer, J.A.W. & Guthrie, A.J., 2004, 'African horse sickness', in J.A.W. Coetzer & R.C. Tustin (eds.), *Infectious diseases of livestock*, 2nd edn., pp. 1231–1246, Oxford University Press, Cape Town.

Erasmus, B.J., 1974, 'The pathogenesis of African horse sickness', in J.T. Bryans & H. Gerber (eds.), *Equine Infectious Diseases: 3rd International Conference,* Paris 1972, pp. 1–11, Karger, Basel.

Fischler, F., 1997, '97/10/EC: Commission Decision of 12 December 1996 amending Council Decision 79/542/EEC and Commission Decisions 92/160/EEC, 92/260/EEC and 93/197/EEC in relation to the temporary admission and imports into the Community of registered horses from South Africa', *Official Journal of the European Communities* 40(L3), 9–24.

Guthrie, A.J., Maclachlan, N.J., Joone, C., Lourens, C.W., Weyer, C.T., Quan, M., Monyai, M.S. & Gardner, I.A., 2013, 'Diagnostic accuracy of a duplex real-time reverse transcription quantitative PCR assay for detection of African horse sickness virus', *Journal of Virological Methods* 189, 30–35. http://dx.doi.org/10.1016/j.jviromet.2012.12.014, PMid:23291102

Mellor, P.S., 1994, 'Epizootiology and vectors of African horse sickness virus', *Comparative Immunology, Microbiology and Infectious Diseases* 17, 287–296. http://dx.doi.org/10.1016/0147-9571(94)90048-5

Mellor, P.S. & Hamblin, C., 2004, 'African horse sickness', *Veterinary Research* 35, 445–466. http://dx.doi.org/10.1051/vetres:2004021, PMid:15236676

Quan, M., Lourens, C.W., Maclachlan, N.J., Gardner, I.A. & Guthrie, A.J., 2010, 'Development and optimisation of a duplex real-time reverse transcription quantitative PCR assay targeting the VP7 and NS2 genes of African horse sickness virus', *Journal of Virological Methods* 167, 45–52. http://dx.doi.org/10.1016/j.jviromet.2010.03.009, PMid:20304015

Quan, M., Van Vuuren, M., Howell, P.G., Groenewald, D. & Guthrie, A.J., 2008, 'Molecular epidemiology of the African horse sickness virus S10 gene', *Journal of General Virology* 89, 1159–1168. http://dx.doi.org/10.1099/vir.0.83502-0, PMid:18420793

Rodriguez, M., Hooghuis, H. & Castano, M., 1992, 'African horse sickness in Spain', *Veterinary Microbiology* 33, 129–142. http://dx.doi.org/10.1016/0378-1135(92)90041-Q

Sinclair, M., Buhrmann, G. & Gummow, B., 2006, 'An epidemiological investigation of the African horsesickness outbreak in the Western Cape Province of South Africa in 2004 and its relevance to the current equine export protocol', *Journal of the South African Veterinary Association* 77, 191–196. http://dx.doi.org/10.4102/jsava.v77i4.376, PMid:17458343

South African Government, 1984, *Animal Diseases Act: Act No. 35 of 1984,* Pretoria.

Venter, G.J., Koekemoer, J.J. & Paweska, J.T., 2006, 'Investigations on outbreaks of African horse sickness in the surveillance zone in South Africa', *Scientific and Technical Review, Office International des Épizooties* 25, 1097–1109.

Western Cape Government: Agriculture, 2006, *African Horse Sickness (AHS) Control Policy,* Elsenburg.

Weyer, C.T., Quan, M., Joone, C., Lourens, C.W., Maclachlan, N.J. & Guthrie, A.J., 2013, 'African horse sickness in naturally infected, immunised horses', *Equine Veterinary Journal* 45, 117–119. http://dx.doi.org/10.1111/j.2042-3306.2012.00590.x, PMid:22612775

World Organisation for Animal Health, 2010, 'Infection with African horse sickness virus', in *Terrestrial Animal Health Code*, pp.669–675, OIE - World Organisation for Animal Health, Paris.