



SA EQUINE
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EXPORTS SOUTH AFRICA

African horse sickness control

Surveillance report

**Sentinel surveillance
2022**

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2022**



**Western Cape
Government**
FOR YOU

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Overview

The African horse sickness (AHS) sentinel surveillance program provides additional confidence of AHS freedom in the AHS free (FZ) and surveillance zones (SZ) of South Africa. The program incorporates the monthly sampling of recruited horses proportionately selected within the zones based on the estimated underlying population. The program has two components – a sero-sentinel program that evaluates the changing serological status of horses on a month-to-month basis; and a PCR-based program that is used to detect the

presence of AHS viral RNA within recruits. The sero-sentinel sampling target is drawn up to detect AHS at approximately a 5% minimum expected prevalence (with a 95% confidence level) whilst the PCR surveillance aims for a 2% minimum expected prevalence. Monthly sampling targets are therefore approximately 60 and 150 recruits, respectively. Individual recruits can be part of both programs. Sero-sentinels are required to be completely unvaccinated and are screened using serology prior to recruitment. Recruits used in the PCR-based program are required to be unvaccinated for at least the previous two years. The vaccination status of PCR sentinels does not influence their recruitment unless vaccination against AHS took place sufficiently recently to result in positive PCR results on initial testing.

A detailed description of the program is available in the [January 2016 Western Cape Epidemiology Report](#). The summary report for last season can be found in the [October 2021 Epidemiology Report](#). All other reports can be found at www.myhorse.org.za. This report is different as the reporting period changed in 2022 to the calendar year (from AHS seasons running from September to August).

The serological test performed is the indirect ELISA (Maree & Paweska 2005). It is a non-quantitative assay and changes across paired sample events are used for evaluation. Follow-up serological tests include the serum neutralisation test (SNT), which is AHS serotype specific. All serology was performed at the Agricultural Research Council - Onderstepoort Veterinary Research (ARC-OVR). Viral RNA testing was performed at the Stellenbosch Provincial Veterinary Laboratory (SPVL) with the final month of evaluation's samples tested at the University of Pretoria/Equine Research Center's Molecular Diagnostics Laboratory. The test method used is a University of Pretoria (Equine Research Center) developed and OIE validated real-time RT-PCR (Guthrie et al. 2013).

This report covers the remaining months of 2021 not evaluated and the 2022 calendar year: 1 September 2021 to 31 December 2022. The results confirm that it is unlikely that AHS was circulating in the AHS free and surveillance zone during this period.

General overview of sampling and results

Note: Due to the evaluated period being 16 months, total value comparisons with previous reports of 12-month evaluation periods was not performed.

782 sero-sentinel samples were analysed from 39 different farms at an average of 49 samples from 25 different farms per month. Of the tested serological samples: 729 (average of 45 per month) could be evaluated as they had relevant paired results (Figure 1).

2267 PCR sentinel samples were analysed from 73 different farms at an average of 142 samples from, on average, 50 different farms per month. Average numbers per month were improvements on both serology and PCR compared to the previous season's evaluation.

Serology

Figure 1 shows the broad serological outcomes for the period. The serology samples that could not be evaluated for lack of a paired sample totaled 25 samples (3.1% of the total, an increase from 2.6% the previous season).

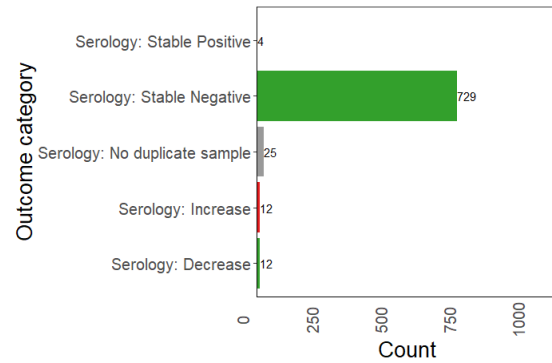


Figure 1: Broad outcomes for serological evaluation for the period under review

PCR

Figure 2 shows the results for the PCR-based surveillance. All samples tested negative.

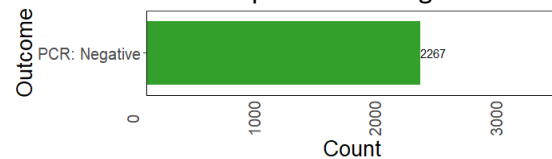


Figure 2: Broad outcomes for PCR evaluation for the period under review.

Follow-up investigations

The follow-up investigations performed in the period are listed below.

Holding 114: Horse 1649

Horse 1649 again had fluctuating serological results as described in the [2020/2021 sentinel surveillance report](#). iELISA results were low positive in September, October and November 2021, and January 2022. PCR results remained negative for the farm throughout. Serum neutralisation tests were flatline negative for October, November 2021, and January 2022 samples, and no clinical abnormalities have been observed or reported from the sentinel holding. EEV serology was performed, and this horse tested strongly positive. Given the history of this horse, it was finally decided to remove it from the sero-sentinel program.

Non-specific ELISA positives- Multiple horses –November 2021

The November 2021 African horse sickness (AHS) ELISA serology results (Lab reference 2021-D-16316) included six (6) samples that tested suspect or low positive with iELISA S/P% values of between 5 and 12 (where > 10 is considered positive). The samples were taken from horses **8564, 10808, 6072, 1531, 34072** and **33794**. These six horses come from six different farms in the AHS surveillance zone – see Figure 3.

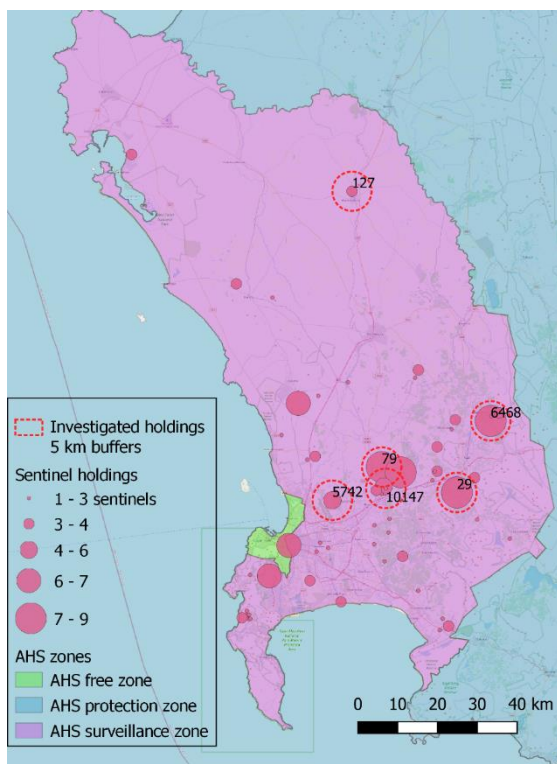


Figure 3: Overview of locations of the six associated sentinel properties in relation to other sentinel properties in the AHS surveillance and Free zone. The six properties are buffered by a 5 km zone and labelled by their farm ID. All sentinel properties are depicted by the number of sentinels on them – ranging from 1-9.

Horses 8564, 10808 and **1531** had consistent negative serological results prior and had been sero-sentinels for some time. **Horses 6072, 34072** and **33794** were relatively new sentinels having started in the program in August and September 2021. PCR results from the

November and December sentinel cohort were all negative and included all six horses investigated. No clinical abnormalities were reported from any of the six farms associated with the results.

SNT results against all serotypes from the original serum samples taken in November 2021 were all negative. Furthermore, all December 2021 serological results were negative, including all six investigated horses.

Having six suspect/low positive iELISA results was unusual for a single month of sampling. All six horses reverted to negative in the month following. PCR results were negative throughout. There was visually no spatial clustering between suspect cases beyond the underlying density of existing sentinel farms, with only 2 of the 6 overlapping at their 5 km buffers. The sentinel farms from which the suspect cases had come are well represented by other sentinels – all of which tested negative. While the iELISA is a highly sensitive test, six false positives from this sample size based on the intrinsic test procedure alone is unlikely. There were three other horses that were positive in the month as well (see Horse 1649 and Other) – these horses either had tested positive before or were incorrectly submitted having been previously vaccinated. Access to the plating layout for the ELISA was not possible. While the exact cause of the false-positive results was not established it is likely to be false positive due to cross contamination with positive samples. Confirmatory PCR and SNT results indicated active AHS as a cause for the suspect and positive results being highly unlikely.

Non-specific ELISA positive – January 2022

Two samples (horses **1535** and **33794**) from two different farms tested low positive on iELISA with S/P% values of 13 and 24 (where > 10 is considered positive). The farms were in the Southern Paarl area – see Figure 4.

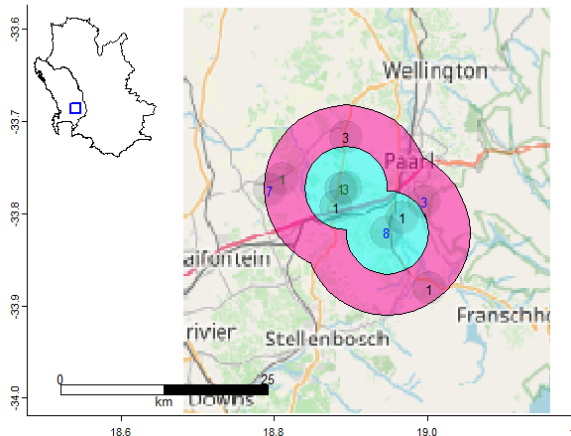


Figure 4: Five and 10 km buffer areas focussed on the holdings affected: Labels by number of sentinels on each holding

There are 10 farms within 10 km of the farm under evaluation (inclusive) containing 29 sentinels in total, representing ~ 20% of the total sentinel population at the time. Of the 29 sentinels, 27 had AHS PCR results that included the potential infection period if the cases were in fact true cases. 29 had PCR results (all negative) in the sentinel season at that point to date. Other sero-sentinels (n=13) in the vicinity were negative. SNT results for the two positive samples returned multiple serotype positives, but at very low titres (maximum of 1:40). EEV testing for both sentinels under evaluation was performed using serum from January. Both tested strongly positive with iELISA levels of 151 and 157 respectively.

Based on underlying EEV seropositivity and across the board negative AHS PCR, the two suspect cases were considered not to be true

AHS cases, as they did not fall within the case definition used for AHS as recommended by the WOA and in use in the province. This was confirmed through further testing where both horses tested negative on both PCR and iELISA for the remainder of 2022 (from February).

Non-specific ELISA positive – November 2022

Two samples (horses **35690** and **38347**) from two different holdings returned low positive iELISA results in November 2022, with S/P% values of 16 and 14. An overview of the location of the two horses is shown in the figure appendix below in Figure 9 and Figure 10 – the holdings were in the Malmesbury (6012) and the Cape Flats (6247) areas respectively.

There were 5 farms within 10 km of the farms under evaluation (inclusive) containing 26 sentinels in total, representing ~ 17% of the sentinel population in total at the time.

Duplicate samples were tested from both animals and tested negative for AHS antibodies. Both samples tested strongly positive for EEV antibodies using iELISA with S/P% values of 86 and 96.

All PCR test results for the two sentinels were negative in the months leading up to and following November 2022 – although **38347** was recruited in October and its first EDTA sample taken in November 2022. December 2022 iELISAs were also negative for both animals.

With the lack of clinical signs, negative PCR, negative iELISA and positive EEV iELISA results we concluded that this was not a case of AHS as defined by the case definition in use.

Sentinel fevers/deaths

No sentinels died during the period evaluated.

Horse 26561, a sentinel since March 2020, had a fever of over 40 °C in October 2021. It, and a stablemate that also had fever, was sampled, and tested negative for AHSV and EEV on 15 October. The horse maintained this negative status throughout the rest of 2021 and 2022.

Horses 34072 and **24273** both had fever and were lethargic in April 2022 and were tested for both AHSV and EEV. The AHSV results were negative, but both were positive for EEV with Ct values of 28.9 and 26.9 respectively. They continued to test AHSV negative for the remainder of the surveillance season. **34072** was interestingly one of the animals with the false positive result from Nov 2021.

Other investigations

Horses 12633 and **33600**, both PCR-only sentinels, were inadvertently tested using

serology in September through December 2022 with positive results (Lab ref's 2021-D12912, 2021-D-14139, 2021-D-16316 and 2021-D-16723). The sample results in these cases were removed from the horses' record, including where SNT was performed as required in follow-ups.

Spatial considerations

The sentinel surveillance program is based on a proportional sampling system with most sentinels in areas of the surveillance area that have the highest population of horses. Figure 5, Figure 6 and Figure 7 show the underlying population and current sentinel farms and the monthly average distribution of sentinels in the serology and PCR sentinel programs.

The areas requiring most improvement remain Paarl, Philadelphia and Mitchells Plain.

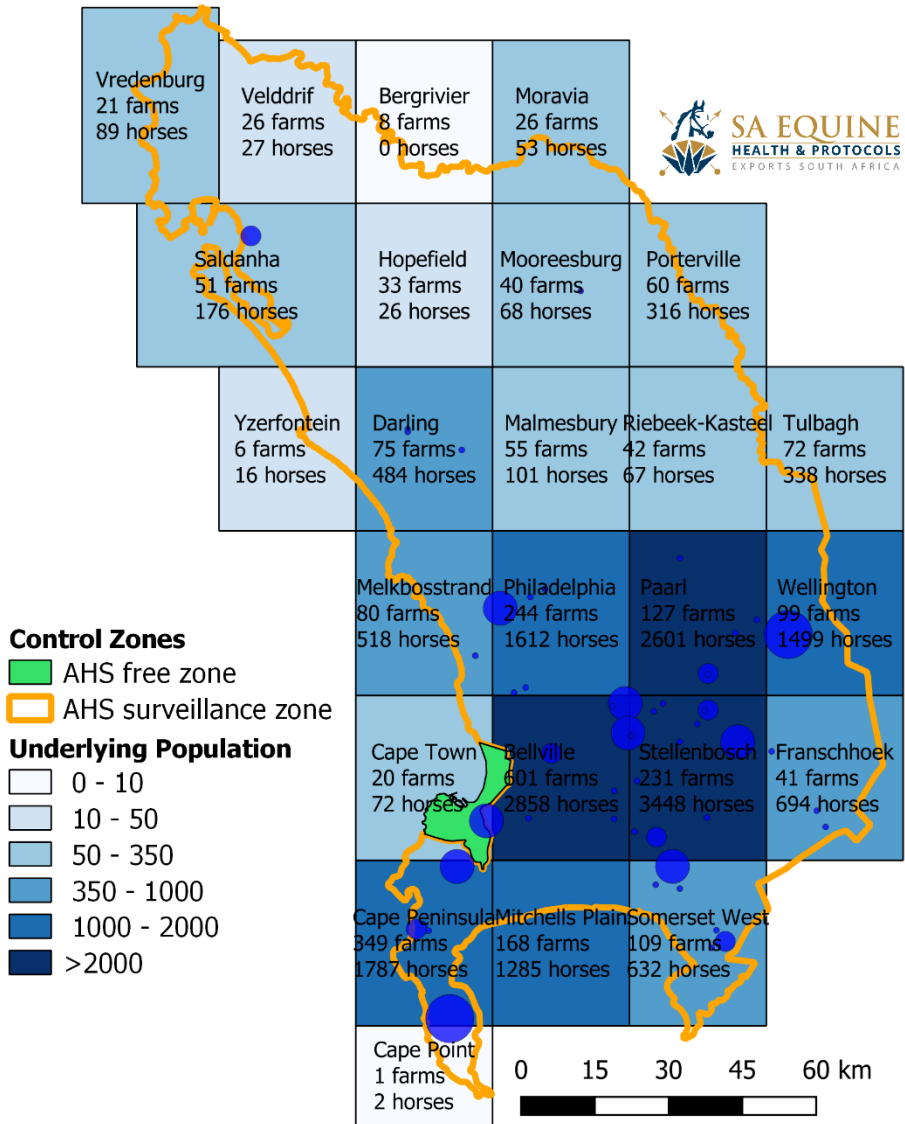


Figure 5: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and April 2023.

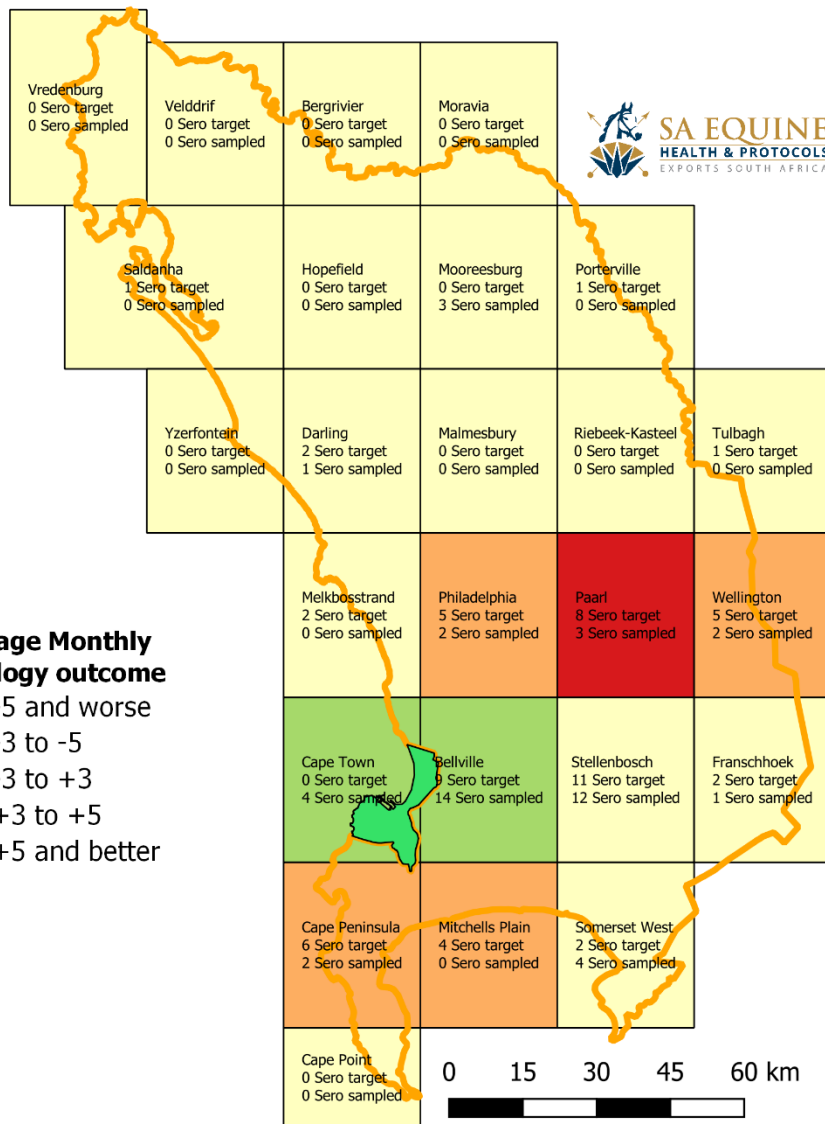


Figure 6: A map showing the AHS surveillance and free zone where sero-sentinel surveillance has taken place for the 2021/2022 season. The map depicts the various areas with their target serology samples to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange areas are areas where sero-sentinels were, on average, lacking while the light green to green areas show where surplus sero-sentinels were sampled. Cream areas depict where the target was generally attained.

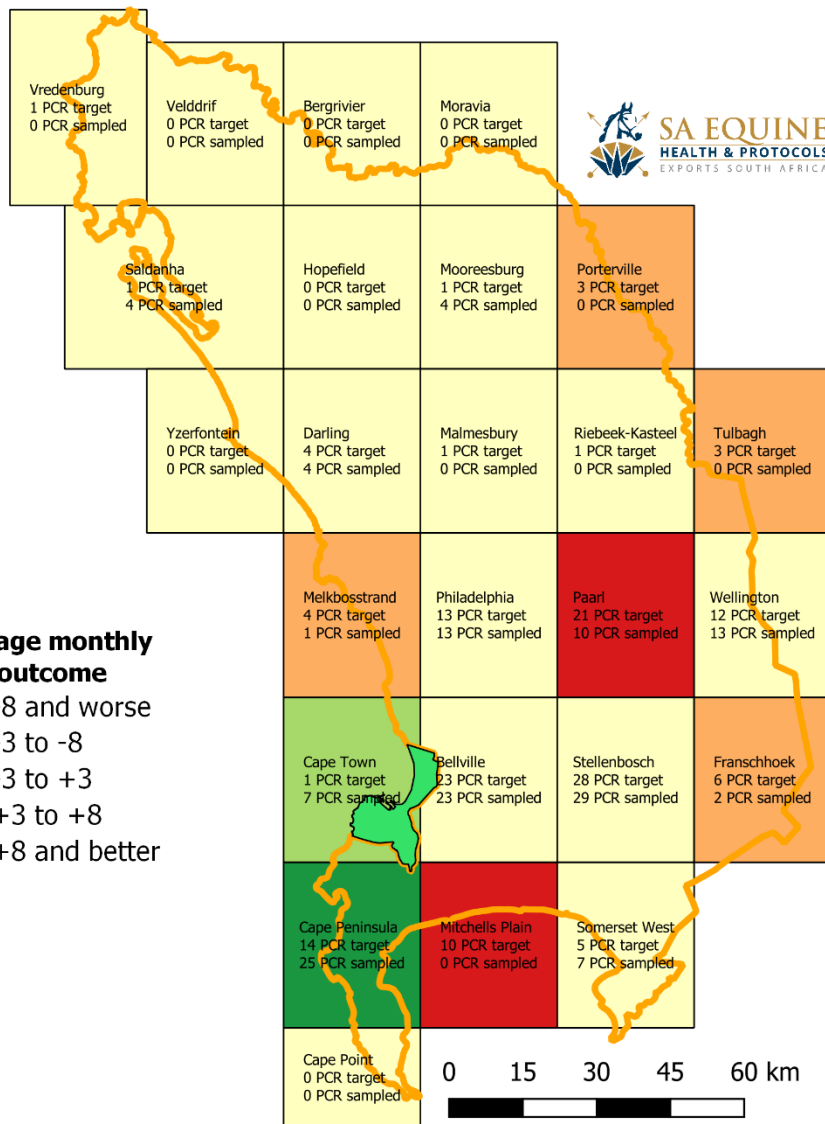


Figure 7: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2021/2022 season. The map depicts the various areas with their target PCR samples to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange areas are areas where PCR-sentinels were lacking on average while the light green to green areas show where surplus PCR-sentinels were sampled. Cream areas depict where the target was generally attained.

Surveillance system evaluation

The surveillance program is designed to detect AHS in the AHS surveillance zone at a minimum expected prevalence of 5% (serology) or 2% (PCR). In this section of the report, we establish the monthly sensitivity of the surveillance program where any sentinel tested negative in the month (on paired serology or negative PCR).

Parameters used in this evaluation are shown in Table 1 and analysis is based on evaluating sensitivity of surveillance programs (Martin et al. 2007). The historical surveillance outcome is

considered as it provides information that aids in determining an accurate final probability of freedom as of December 2022. The final probability of freedom from Sept 2016 through December 2022 (76 months) was 91.5%, an increase of 17% from the 74.5% of the previous evaluation (Figure 8).

The sensitivity of the sentinel surveillance alternates around the 30% mark throughout. This is the sixth AHS season running where cases of the disease have not been detected in the AHS surveillance and free area, although an outbreak of AHS occurred in the AHS protection zone in 2021.

Parameter	Value	Comments
<i>p</i> Intro	0.03	During periods where not outbreaks in the AHS controlled area are present. Based on historical outbreaks in the region.
	0.3	During periods where outbreaks are present in the AHS controlled area – estimate made increasing probability of introduction 10X the normal rate
Population at risk – total herds	1432	Data captured between 1 April 2016 and April 2023 for the AHS surveillance and free zones.
Sentinel farm populations	Various	Based on herd size as of April 2023. The assumption is made that herd size would not change substantially on the sentinel properties over the period reviewed.
Sentinels tested per herd per surveillance period	Various	Actual tested data
Unit design prevalence (P_A^*)	0.05	Design prevalence at animal level as defined by the EU 2008/698 recommendations
Herd design prevalence (P_H^*)	0.02	Design prevalence at herd level based on prior outbreaks (median value taken) in the controlled area assuming a herd PAR of the zones affected by each outbreak.
Test sensitivity	0.978	As published (Guthrie et al. 2013). Note that while serology was taken into consideration, for this analysis all horses that were tested on serology were tested on PCR – hence the use of a single test sensitivity across the analysis
Initial Prior confidence of Freedom	0.5	Note that when evaluating the season independently the prior of 0.5 is used in the first surveillance period (September 2021). When evaluating the past 6 years between Sept 2016 and Dec 2022 the initial prior is 0.5 but relates to September 2016.

Table 1: Parameters used to establish sentinel system probability and sensitivity of freedom for African horse sickness

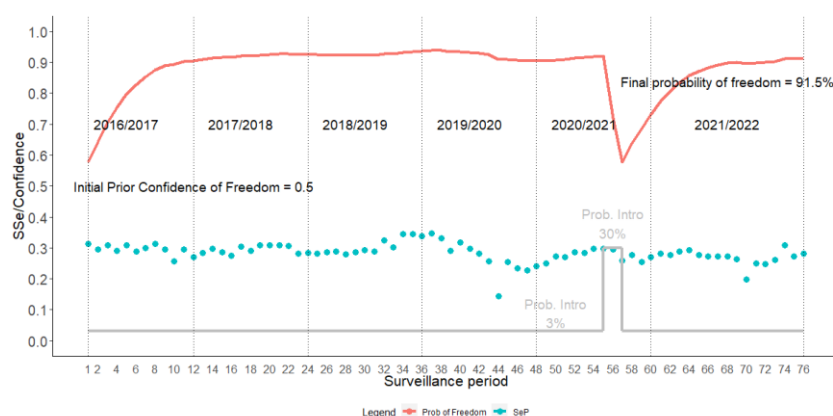


Figure 8: The sentinel surveillance sensitivity of individual surveillance periods (SeP - dots) with probability of freedom curve (red line) for the past six surveillance seasons: the season currently reviewed is the 2021/2022 season running between September 2021 and December 2022. Probability of AHS introduction of 3% is set for periods where no AHS outbreaks are present in the AHS controlled area (grey line at 0.03 on y-axis) but at 10X that rate for where outbreaks are present as in April and May 2021 in the Cederberg AHS Protection zone.

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2021\2022 AHS season was achieved. The PCR testing in conjunction with the serology testing does assist greatly in the analysis of the system and for follow-up in suspect cases. All investigation reports are shared with Provincial and National Veterinary Services.

A 6-year review of sentinel results show that the probability of freedom attained for this program, at an animal design prevalence of 5% and herd-level design prevalence of 2%, shows a 91.5% probability of freedom from AHS in the AHS surveillance and free zones. This level was achieved in the face of the AHS outbreak that occurred ~ 88km from the border of the AHS surveillance zone in 2021.

Spatial representativeness remains challenging. The target minimum prevalence of 5% has however been achieved using EDTA sampling and PCR testing; the goal remains however to get as close to the 2% MEP level as often as possible.

References and acknowledgements

This program would not be possible without the support of the horse owners in the AHS surveillance zone who freely give of their time and resources to allow and facilitate the monthly sampling of horses. We are grateful to the Onderstepoort Veterinary Research Institute and the Stellenbosch Provincial Veterinary Laboratory who performed the testing of samples this season.

In this season we again made use of compulsory community service and Western Cape state vets and AHTs who assisted in sampling. In this regard we specifically acknowledge Drs Tasneem Anthony and Andrea Lau, as well as Malmesbury AHT Janica Chapman. We are grateful to our SAEHP team who are directly involved with the program: Esthea Russouw and Lizel Germishuys.

The cost of surveillance is made up of testing, personnel, travel/logistics and equipment costs. Funding primarily comes from the South African Health and Protocols NPC and the Western Cape Department of Agriculture (both Animal Health and Provincial Laboratory). The sentinel surveillance program is performed in partnership with the Western Cape Department of Agriculture, and we thank Dr Gary Buhrmann (State Vet Boland) who was the primary liaison and supervisor of the program and to whom we reported.

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Figure appendix

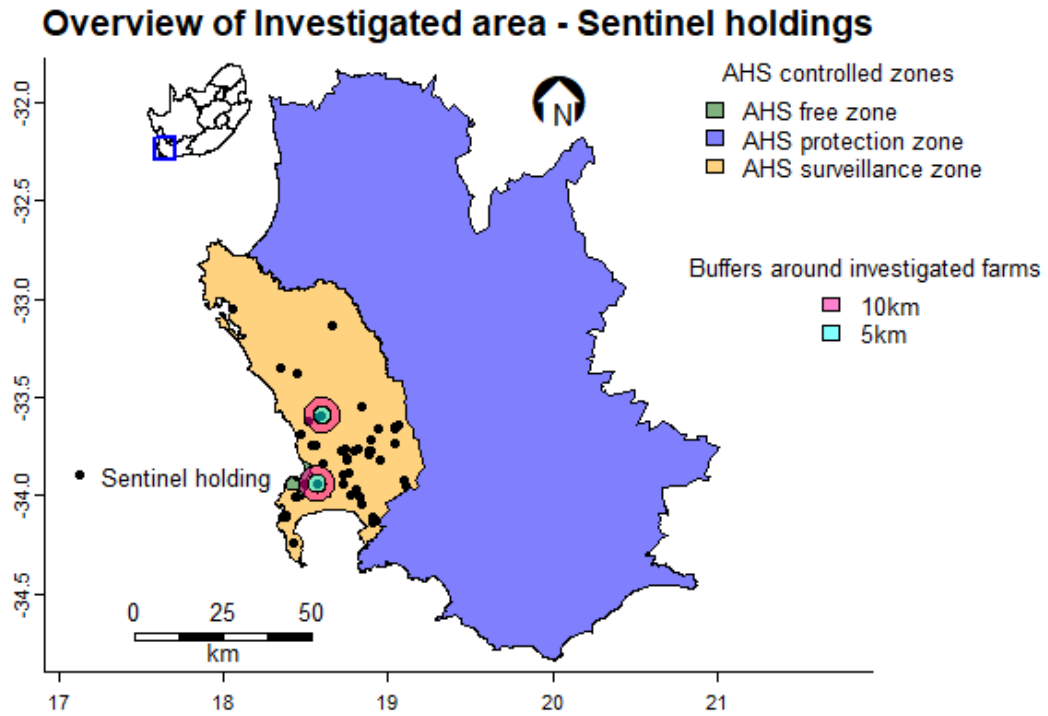


Figure 9: Sentinel holdings currently in the AHS controlled area with the 5 and 10 km buffers around the holdings evaluated for the Non-specific ELISA positive – November 2022 investigation

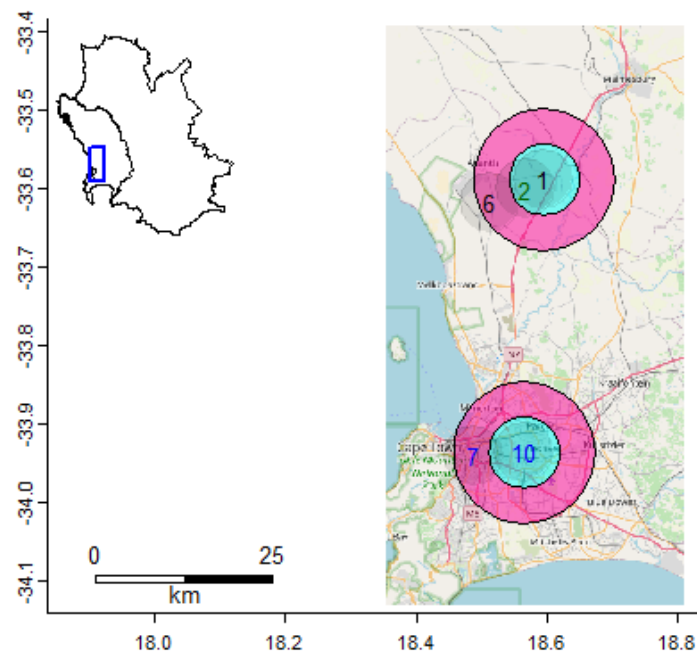


Figure 10: Five and 10 km buffer areas focussed as in Figure 9.: Labels by number of sentinels on each holding