SA EQUINE
HEALTH & PROTOCOLS
EXPORTS SOUTH AFRICA

African horse sickness control

Surveillance report

Sentinel surveillance
2020/2021 season

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Contents

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 September 2020 Epidemiology Report. All other reports can be found at www.myhorse.org.za.

The serological tests 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). 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 2020/2021 AHS season from 1 September 2020 to 31 August 2021. While an outbreak of AHS occurred in the AHS protection zone during the season, the results confirm that it is unlikely that AHS was circulating in the AHS free and surveillance zone during that period.**

General overview of sampling and results

A total of 527 sero-sentinel samples were analysed from 30 different farms at an average of 44 samples from 23 different farms per month. This was a decrease of 12% from the 2019/2020 surveillance period for the sero-surveillance program. Of the tested serological samples: 513 (average of 43 per month) could be evaluated as they had relevant paired results (Figure 1).

A total of 1661 PCR sentinel samples were analysed from 72 different farms at an average of 138 samples from, on average, 52 different farms per month. This was a decrease of 5% from the previous season.

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 14 samples (2.6% of the total, an increase from 2% the previous season).

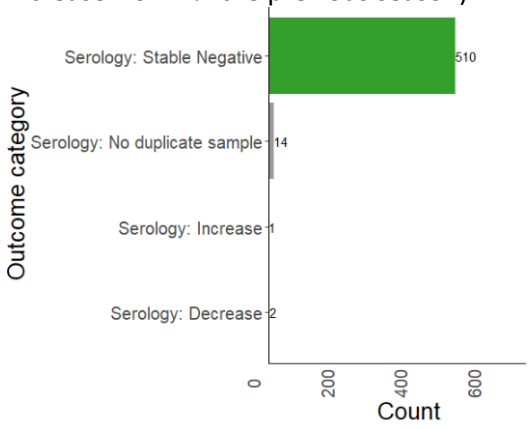

Figure 1: Broad outcomes for serological evaluation for the period under review

PCR

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

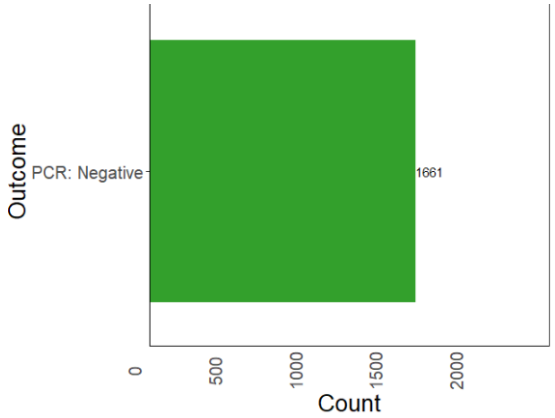

Figure 2: Broad outcomes for PCR evaluation for the period under review.

Results

Follow-up investigations

As with the 2018/2019 and 2019/2020 seasons, there was one investigation of importance for the period reviewed – in this case it was a horse that went from a negative serological status to positive, suspect and negative (remaining so) over four months in early 2021.

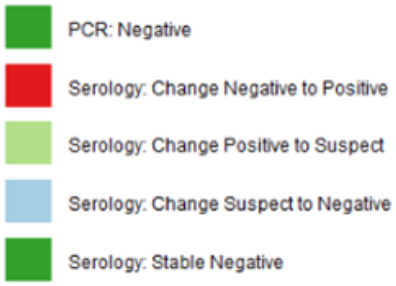

Figure 3: Legend for the individual horse serological and PCR outcomes for the section below

Holding 114: Horse 1649

Horse 1649 had a changing serological status from negative to positive, to suspect and back to negative between January and April 2021 (**Error! Reference source not found.**A)

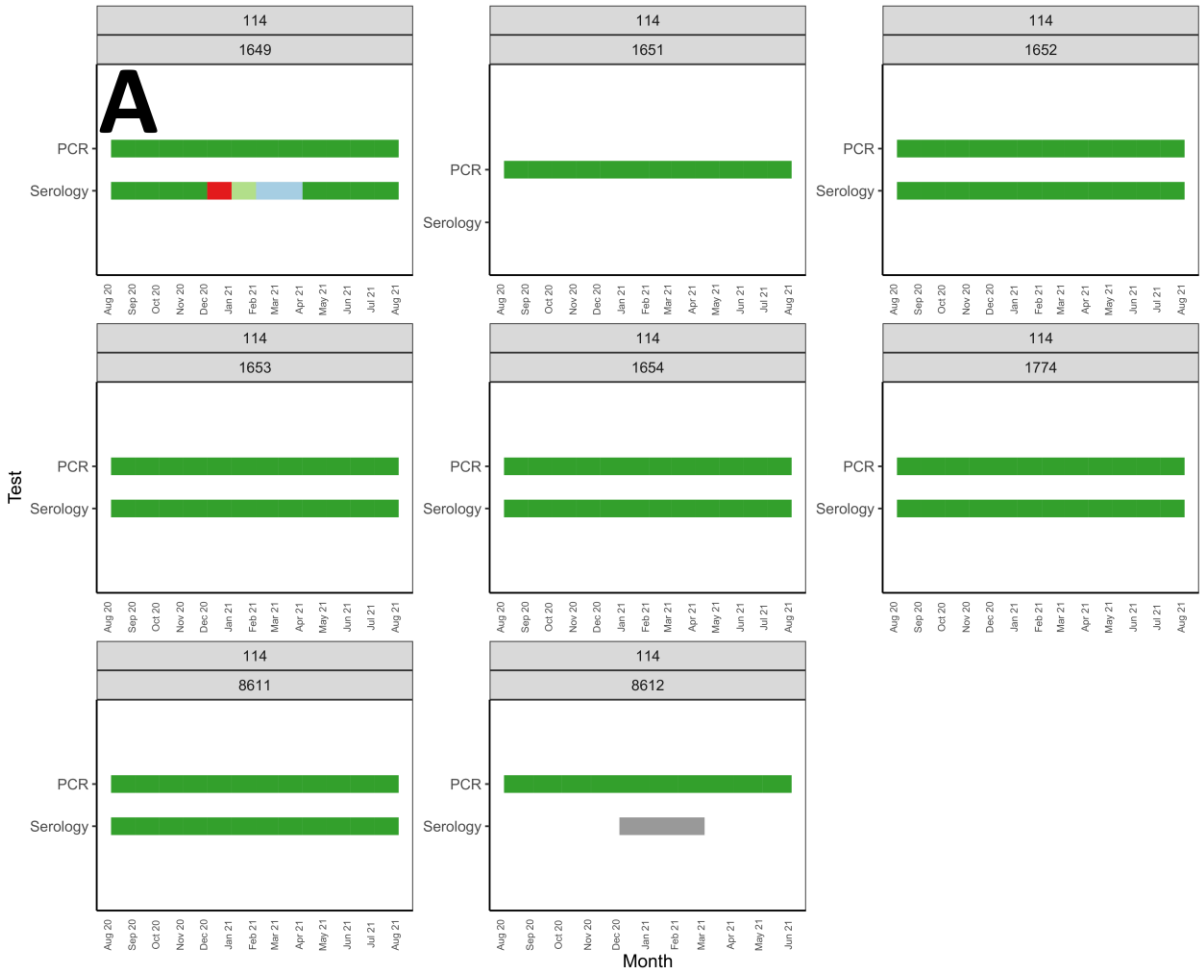

Figure 4: Horse 1649 result series showing the changing ELISA serological status between Jan-Apr 2021 with stable negative results from April for the rest of the season. All other horses depicted (n=7) are sentinels on the same property showing negative results including 5 which were both sero- and PCR sentinels.

A detailed report was submitted to the Provincial and National Veterinary services and a summary is included here.

The initial positive sample was repeat tested at the laboratory with similar results (i-ELISA S/P% of 18 compared to 20 initially - <5 is considered negative; 5-10 is considered suspect). The positive horse and all other sero-sentinels from the same property had their duplicate samples tested with the same outcome (horse 1649 still positive at an i-ELISA S/P% of 23). SNT results from the positive horse returned negative results for all 9 sero-types. Follow up farm testing was performed in February with the previously positive horse now returning a suspect i-ELISA result (S/P% of 7) and all others remaining negative. All horses on the farm had been and remained PCR negative throughout. No clinical abnormalities were detected on the farm. PCR testing for EEV was performed on the Dec and Jan 2020/2021 samples with negative results.

This type of serological fluctuation was more common in past sentinel analyses before more effort was made to establish clean baseline serological levels in sero-sentinels – see annual reports from 2015 and 2016 as examples. More recent investigations have resulted in unspecified serological conversions although

in Horse 1649's case it is purely on iELISA and not SNT. Based on the investigation the iELISA conversion is not likely to have arisen from circulating AHSV and this case was classified as negative and as an unspecified serological conversion. Horse 1649 remained in both the RNA and serological sentinel program to monitor serological status – it remained negative throughout the rest of the season.

Follow-up investigations – Sentinel deaths

No sentinels died during the 2020/2021 season

Follow-up investigations – Other

Horse 31270 tested positive on ELISA during the month of November 2020. An investigation revealed that it was very likely that the incorrect sample was associated with this horse in the November sampling as a result of a processing error, where labels had been separated from tubes during centrifugation of samples. All other results for this horse and all other sentinels on the property remained negative throughout. The sample result in this case was removed from the horses' record which is why it is not included in Figure 1.

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 respectively.

The areas requiring most improvement remain Paarl and Philadelphia regions for serological sampling. Additionally in this season sampling of the horses in the Mitchells plain area was impacted due to security concerns. PCR sampling is relatively representative with the Paarl and Mitchells plains regions requiring attention.

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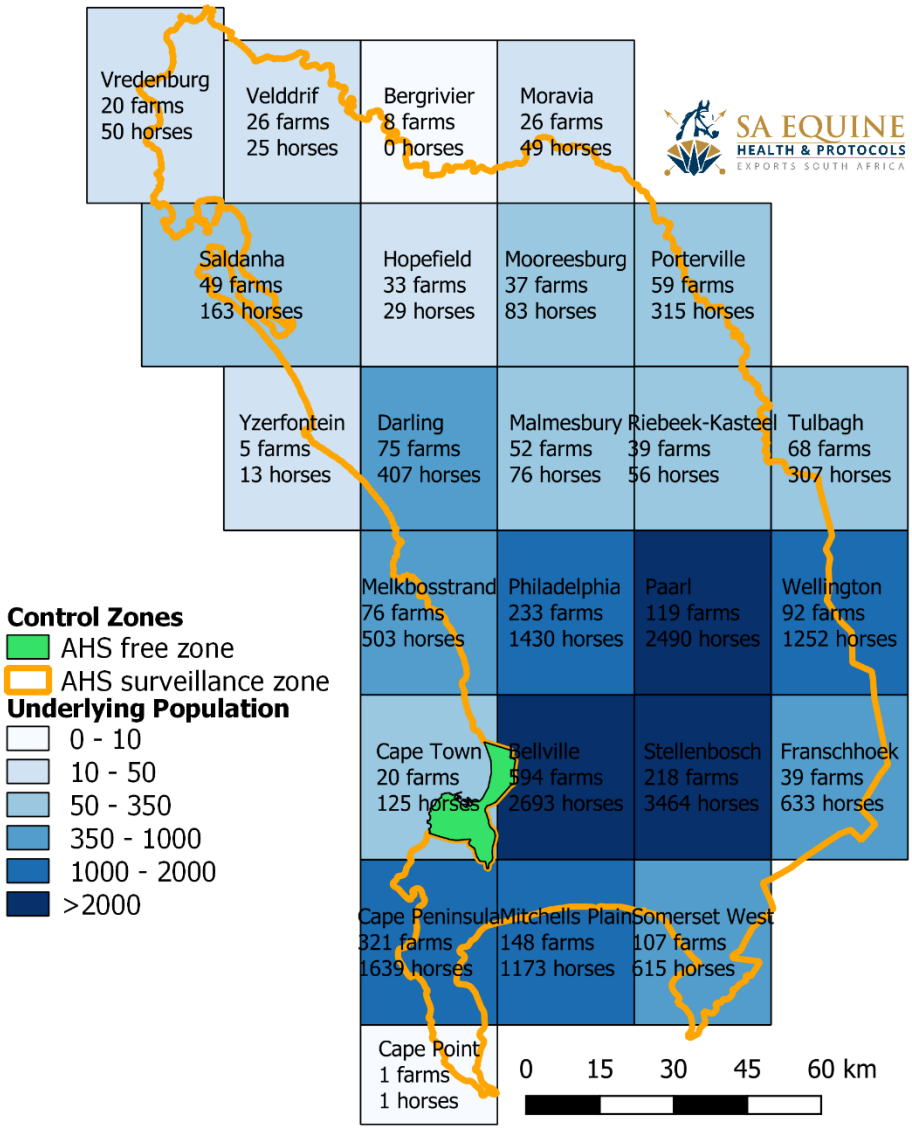

Figure 5: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 September 2021.

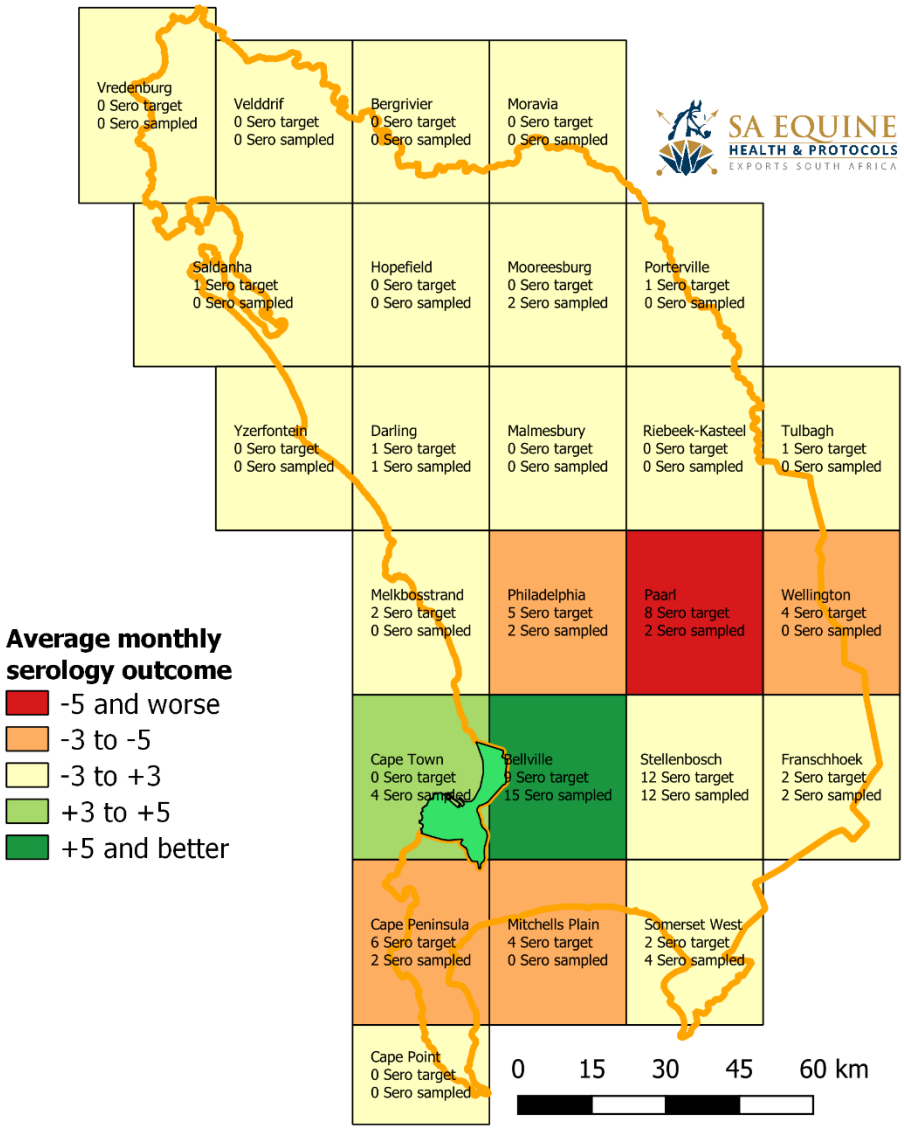

Figure 6: A map showing the AHS surveillance and free zone where sero-sentinel surveillance has taken place for the 2020/2021 season. The map depicts the various areas with their target serology samples to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange areas are 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.

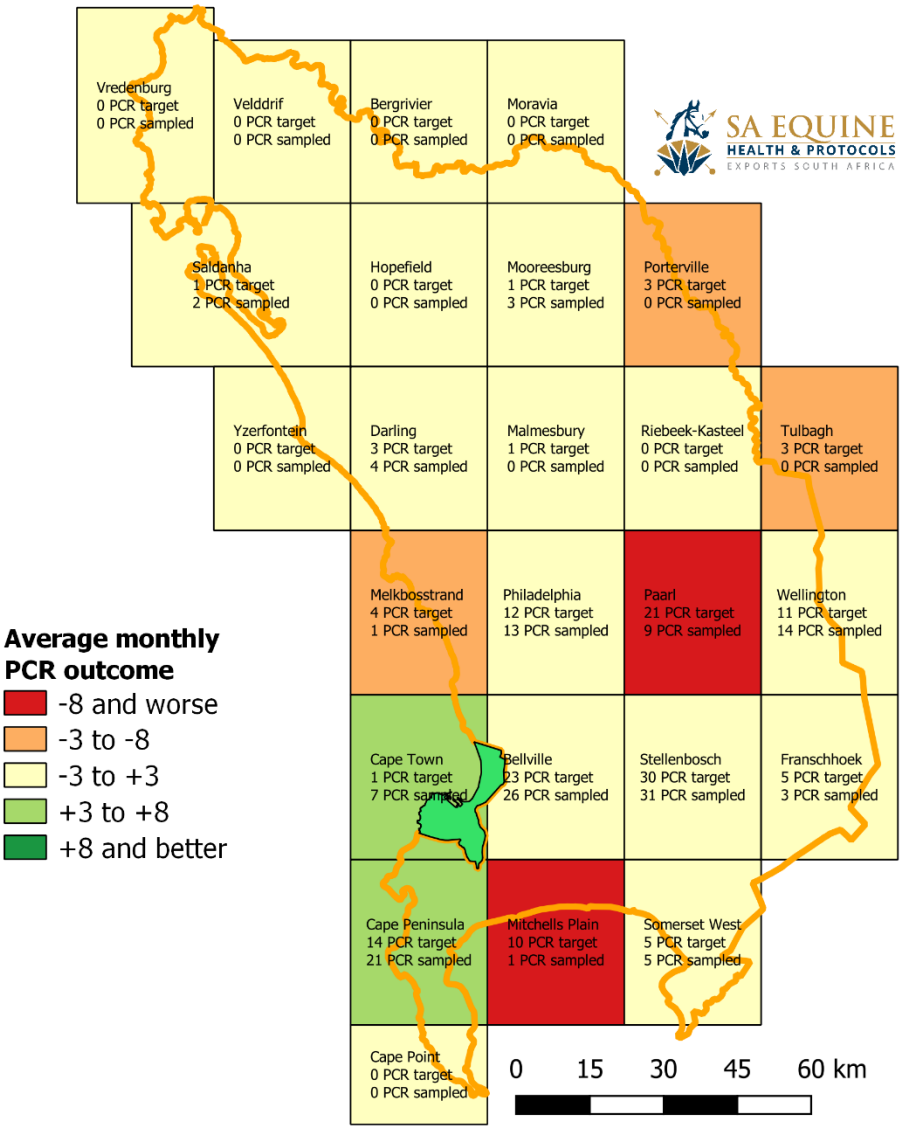

Figure 7: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2020/2021 season. The map depicts the various areas with their target PCR samples to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange areas are areas 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 August 2021. The final probability of freedom at the end of the five-year period (60 months) was 74.5%, a drop of 16.8% from the previous evaluation (Figure 8).

The sensitivity of the sentinel surveillance alternates around the 30% mark throughout. This is the fifth 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 (Figure 9).

Impact of the Cederberg AHS outbreak in the AHS Protection zone

While AHS was not detected in the AHS free and surveillance zone in the period reviewed, the outbreak in the controlled area impacts the final probability of freedom provided by the system since it is realistic to estimate that the probability of introduction of AHS into the AHS surveillance and free zones is increased during the outbreak period. The AHS outbreak occurred ~ 88km to the closest point in the AHS surveillance zone (Figure 9), although the highest density of horses in the surveillance zone is still further South than that point. Be that as it may, for the outbreak period we increased the probability of introduction to 10X the 3% generally used to provide some insight into the impact of AHS cases in relatively close proximity to the Surveillance zone. The impact is clear to see in Figure 8 where the probability of freedom drops substantially in April and May of 2021 with recovery up to the final 74.5% in August 2021.

Parameter	Value	Comments
$pIntro$	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	1348	Data captured between 1 April 2016 and 1 Sep 2021 for the AHS surveillance and free zones.
Sentinel farm populations	Various	Based on herd size as of 1 Sep 2021. The assumption is made that herd size would not change significantly 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 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 2018). When evaluating the past 5 years between Sept 2016 and Aug 2021 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

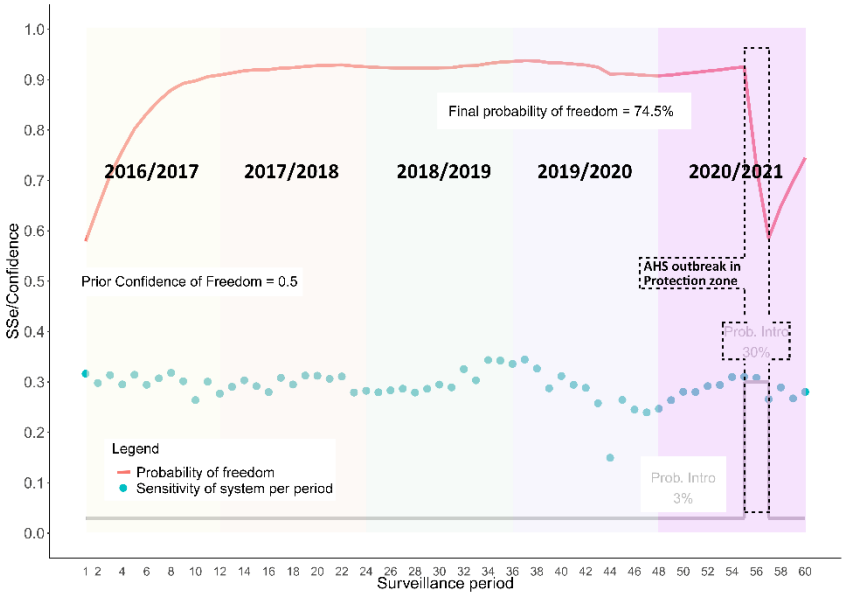

Figure 8: The sentinel surveillance sensitivity of individual surveillance periods (dots) with probability of freedom curve (red line) based on an uninformed 50% prior probability of freedom for the past five surveillance seasons: the season currently reviewed is the right pane – i.e. the 2020/2021 season running between Sept 2020 and Aug 2021. 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 (black dotted period indicated in 2020/2021).

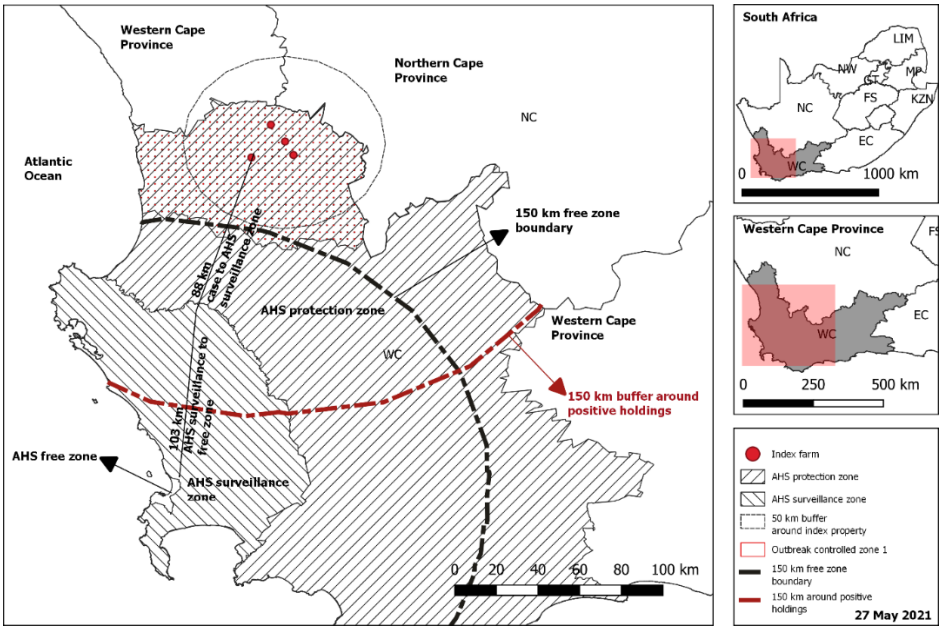

Figure 9: Map of the AHS cases associated with the 2021 Protection zone outbreak in relation to the existing AHS controlled area and the provincial borders of South Africa. The outbreak control zone is shown in the main figure as well as the distances from the cases to the border of the AHS surveillance zone and from the AHS surveillance zone border to the AHS free zone in Cape Town. The red dot-dash line indicates a 150km buffer around all infected properties. WC: Western Cape Province; EC: Eastern Cape Province; NC: Northern Cape Province; FS: Free State Province; KZN: KwaZulu Natal Province; MP: Mpumalanga Province; LIM: Limpopo Province; NW: North West Province; GT: Gauteng Province

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2020 2021 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 5-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 74.5% 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.

Spatial representativeness still remains challenging, particularly in conjunction with continued COVID-19 related restrictions in April-June 2021. The target minimum prevalence of 5% has however been achieved through the use of 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 who assisted in sampling. In this regard we specifically acknowledge Drs. Tasneem Anthony, Aliya Davids and Leandri Klopper. We are grateful to our SAEHP team who are directly involved with the program – Esthea Russouw and Lizel Germishuys.

The sentinel surveillance program costs in the region of R1.5 million a season. This cost 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 is the primary liaison and supervisor of the program and to whom we report.

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