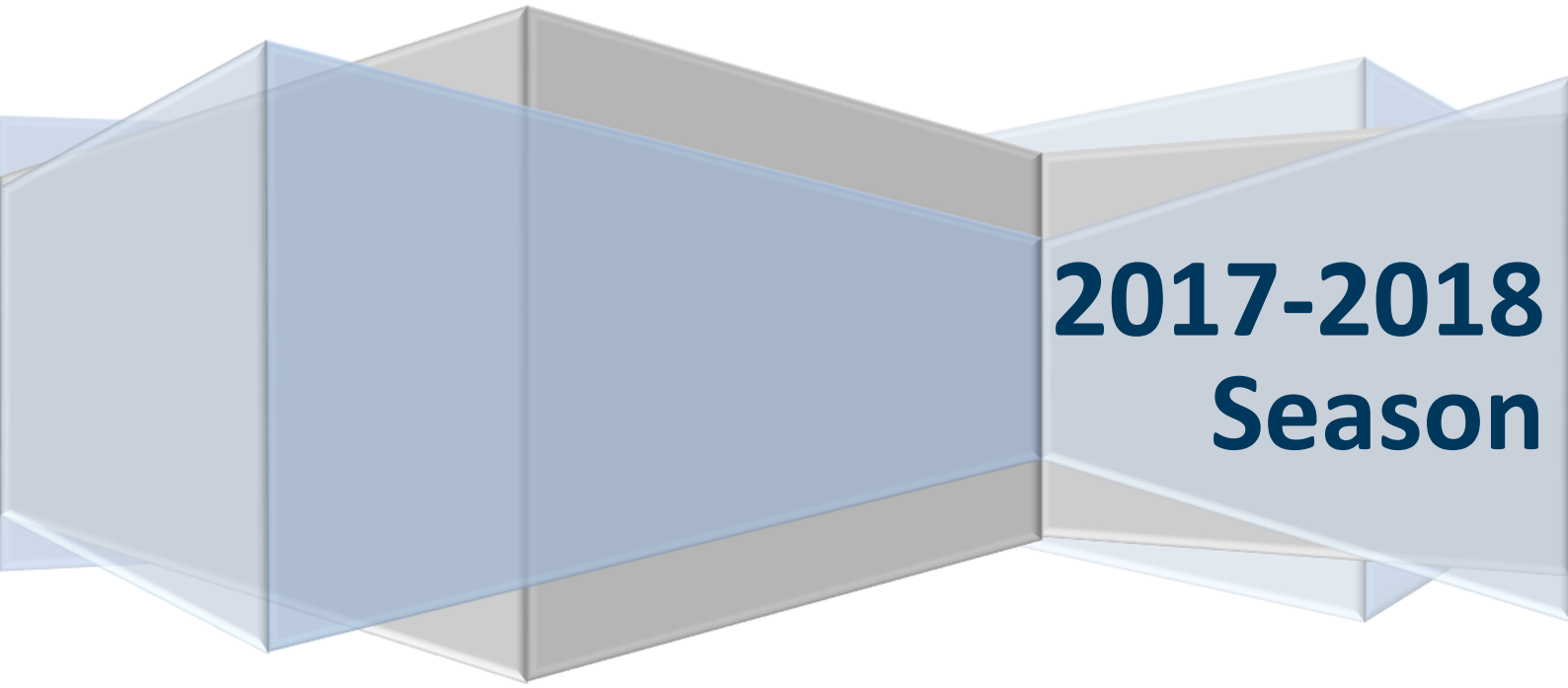
SAEHP

African horse sickness control

Sentinel Surveillance Report



2017-2018 Season

JD Grewar & CT Weyer

Contents

Overview

The African horse sickness (AHS) sentinel surveillance program is aimed at providing additional confidence of AHS freedom in the AHS free and surveillance zones 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 streams of focus – 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 circulating AHS viral genetic material (RNA) within recruits. The sero-sentinel sampling frame 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 unvaccinated for at least the previous two years and are screened using serology prior to recruitment. The vaccination status of PCR sentinels is captured but does not influence their recruitment unless vaccination against AHS took place sufficiently recently to result in positive PCR results on their initial testing.

A detailed description of the program is available in the January 2016 Western Cape Epidemiology Report, obtainable at http://www.elsenburg.com/vetepi/epireport_pdf/January2016.pdf. The summary report for last season (2016-2017) can be found in the November 2017 Epidemiology Report while the original detailed report can be found at http://jdata.co.za/myhorse/#infographics.

The serological tests performed rely on the indirect ELISA (i-ELISA) as the base serological test (Maree & Paweska 2005). It is a non-quantitative assay and changes between positive, suspect and negative results across paired sample events are used for evaluation. Follow-up serological tests include the serum neutralisation assay (SNT), which is AHS serotype specific. All serology is performed at the Agricultural Research Council - Onderstepoort Veterinary Institute (ARC-OVI). Viral RNA testing has historically been performed at the University of Pretoria's Veterinary Genetics Laboratory under coordination of the Equine Research Centre (ERC); however from January 2018 this year the PCR testing, albeit the same test method, was performed at the regional Stellenbosch Provincial Veterinary Laboratory. The test method used is an ERC developed and OIE validated real-time RT-PCR (Guthrie et al. 2013).

This report covers the 2017/2018 AHS season from 1 September 2017 to 31 August 2018. The results confirm that it is unlikely that AHS was circulating in the AHS free and surveillance zone during that period.

General overview of results

A total of 701 sero-sentinel samples were analysed from 37 different farms at an average of 58 samples from 28 different farms per month. This was an increase of 1.7% from the 2016/2017 surveillance period. Of the tested serological samples 678 could be evaluated as they had relevant paired results (Figure 1). This averages to 57 sampling events per month - a 3% increase compared to the 2016/2017 season.

A total of 1757 PCR sentinel samples were analysed from 66 different farms at an average of 146 samples from, on average, 51 different farms per month. This was a decrease of 0.5% from the previous season.

Serology

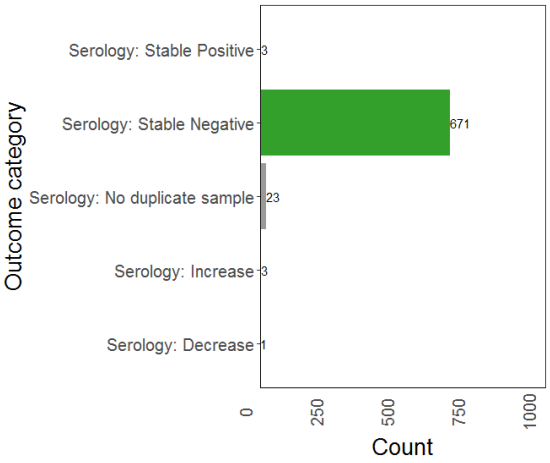


Figure 1 shows the broad serological outcomes for the period. The total serology samples that could not be evaluated for lack of a paired sample amounted to 23 samples (3.3% of the total). This compared to 2016/2017 where 29 samples could not be evaluated (4% of the total). A total of 3 serology evaluations indicated an increase in status warranting investigation – these will be dealt with individually below.

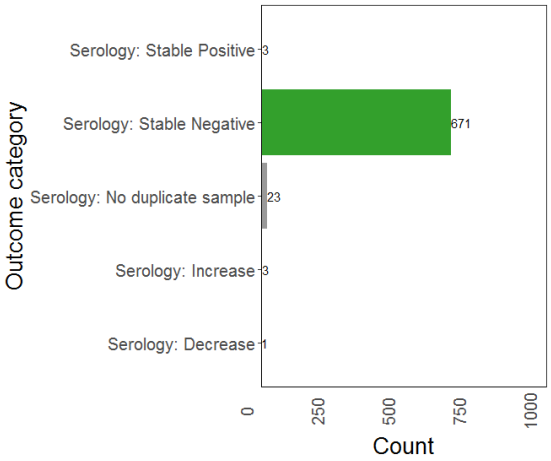


Figure 1: Broad outcomes for serological evaluation for the period under review. Increasing serology incorporates both the negative to suspect/positive and the suspect to positive permutations for serological change across paired samples.

PCR

Figure 2 shows the results for the PCR-based surveillance. All PCR results were negative barring 4 samples from a single horse between November and February of the season. This horse will be discussed– see individual section below.

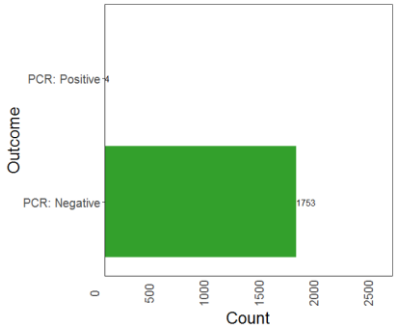


Figure 2: Broad outcomes for PCR evaluation for the period under review.

Results: Increasing sero-status and/or PCR Positive

A total of 3 serological evaluations from 3 different horses returned an increasing serological status for the sentinel in question. The PCR positive results (n=4) were detected in a single horse that also had an increasing serological titers.

The section below provides detail of the evaluation of the 3 horses that had results that triggered specific evaluation. Figure 3

shows the legend for the graphs for Figure 5 through Figure 8.

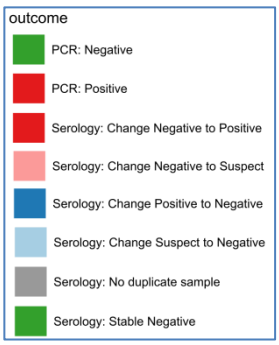


Figure 3: Legend for the individual horse serological and PCR outcomes for the section below

Holding 77: Horse 9685

Horse 9865 had an increase from negative to positive on serology in December 2017 with a return to negative the following month - Figure 4.

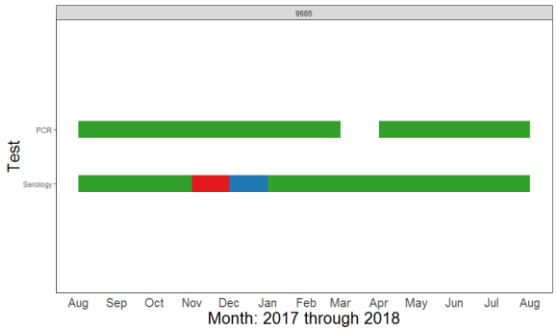


Figure 4: Horse 9685 result series showing a single negative to positive transition on ELISA with an immediate return to negative the following month.

The sample was tested for AHS serotypes on SNT's. All nine serotypes returned positive results which is highly indicative of a prior vaccination and suggested the sample was collected from another horse that had been more recently vaccinated. This almost identical situation arose with the same horse in the prior surveillance season, and the conclusion was the same, i.e. that a sample switch and labelling error was the most likely explanation. The PCR testing for this horse and the remaining 6 sentinels on the property tested negative for both December and January 2018 (Figure 5).

The clinical evidence and testing confirmed that the most likely reason for the December 2018 positive ELISA and multiple serotypes on SNT was an incorrect sample being allocated to this sentinel.

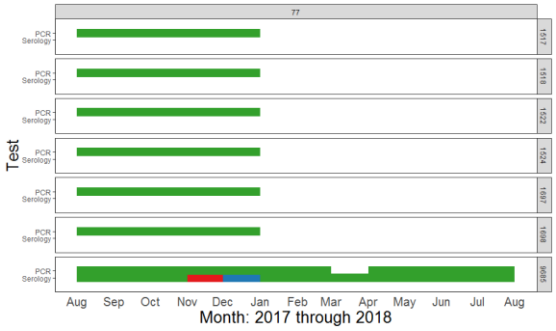


Figure 5: Holding 77 containing Horse 9685 and a further 6 PCR sentinels showing negative PCR throughout the December and January period and the cessation of the program on the farm bar horse 9865 which is discontinued at the end of the 2017/2018 season in Aug.

Given the repeat sample errors and difficulty in management of the horses on the farm it was removed as a sentinel property. Horse 9865 however remained a PCR sentinel for the remainder of the 2017/2018 season – with negative results, both on PCR and serology – and was removed in September 2018 from the program.

Holding 5742: Horse 10804, 15554

Holding 5742 contained horses 10804 and 15554 which were responsible for the remaining investigations in the season.

Horse 10804 triggered the most important investigation of the season. PCR results from the November 2018 sampling were positive on AHS with a Cq value of 34.76 showing a low concentration of RNA in the sample – the cut-off for the test is a Cq of 37.

The November serology result (i-ELISA) was also positive and the sample was sent for SNT's for all AHS serotypes. Eight of nine serotypes (serotype 7 the exception) were positive for AHS.

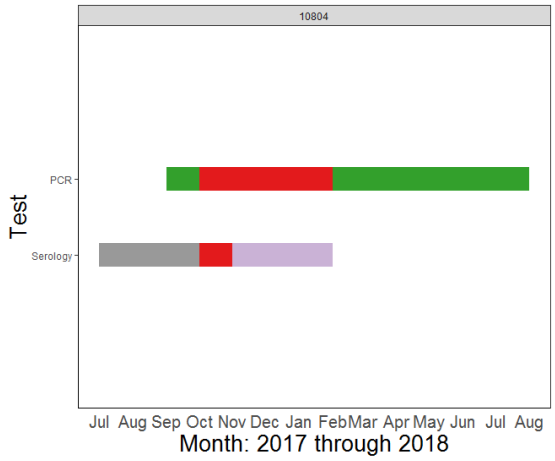


Figure 6: Sentinel results for Horse 10804 showing positive PCR and serology from November 2017.

The December test samples returned similar results: the serum was positive on i-ELISA and the SNT results remained positive, including Serotype 7. The PCR remained positive, with a Cq value of 35.2.

All other sentinels on the property tested negative on RT-qPCR – (Figure 7).

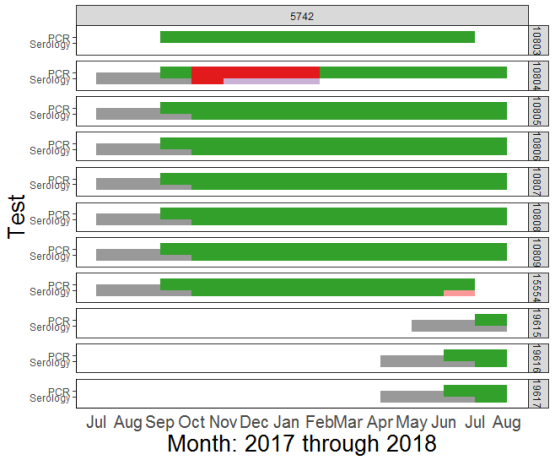


Figure 7: Holding 5742 with its associated sentinel surveillance results showing the events leading to investigations in November 2017 and then in July 2018.

January and February results were as expected – the serum remained positive on i-ELISA (hence the three stable-positive Serology outcomes in Figure 1 and Figure 6). The SNT results from the January sample remained positive for all serotypes. PCR remained positive in the January and February with Cq values of 33.08 and 31.76 respectively. It's important to note that the January and February PCR tests were done in the Stellenbosch laboratory and not the Equine Research Center so the Cq values are not directly comparable, but Stellenbosch still returned relatively high Cq values.

Further epidemiologic investigation was undertaken while the case was being defined. Three unvaccinated foals were included in the farm follow up testing using PCR with negative results (E180021). Farms were visited within 5 kilometers and census, recent movement and vaccination details were updated and/or obtained.

The defining point of the investigation was when it was found that Horse 10804 had been treated on multiple occasions with antibiotics and anti-inflammatories starting on October 17 2018 for a severe laceration of the shoulder. At the same time other horses on the farm were being vaccinated against AHS - the sentinel horses excluded. On further discussion with the owner and investigation it appeared that this horse was mistakenly vaccinated for AHS.

This is supported given the polyvalent nature of the SNT results, which are highly unlikely for a field strain AHS infection, the negative cohort results (7 horses) on PCR (Figure 7), the high Cq values and the lack of clinical signs detected in the horse or its cohorts.

This horse was removed from the serological surveillance program from March 2018 but retained on the RT-PCR surveillance program. The PCR results returned to negative from March 2018.

Horse 15554 returned a suspect i-ELISA in July 2018 (Figure 8). On investigation it was found that this horse was vaccinated against AHS on 25 June 2018 and the owner had forgotten to inform the sampling team. Thus the suspect serological result was consistent with vaccination. The horse has been removed from the sentinel program.

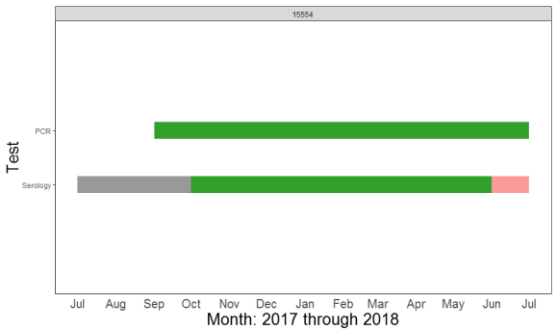


Figure 8: Horse 15554 showing a suspect i-ELISA result in July 2018. The surrounding cohort data is shown in Figure 7.

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 9, Figure 10 and Figure 11 show the underlying population and current sentinel farms and the monthly average distribution of sentinels in the serology and PCR sentinel programs respectively.

In general spatial representation was obtained with only the Paarl grid requiring an improvement in both serology and PCR sentinels. This remains an issue given the post 2016 outbreak vaccination requested from that area as well as a population of horses that travel often to the AHS protection zone and require AHS vaccinations to do so. The latter requirement needs review since the zones of destination and return are in the low risk AHS controlled area.

.

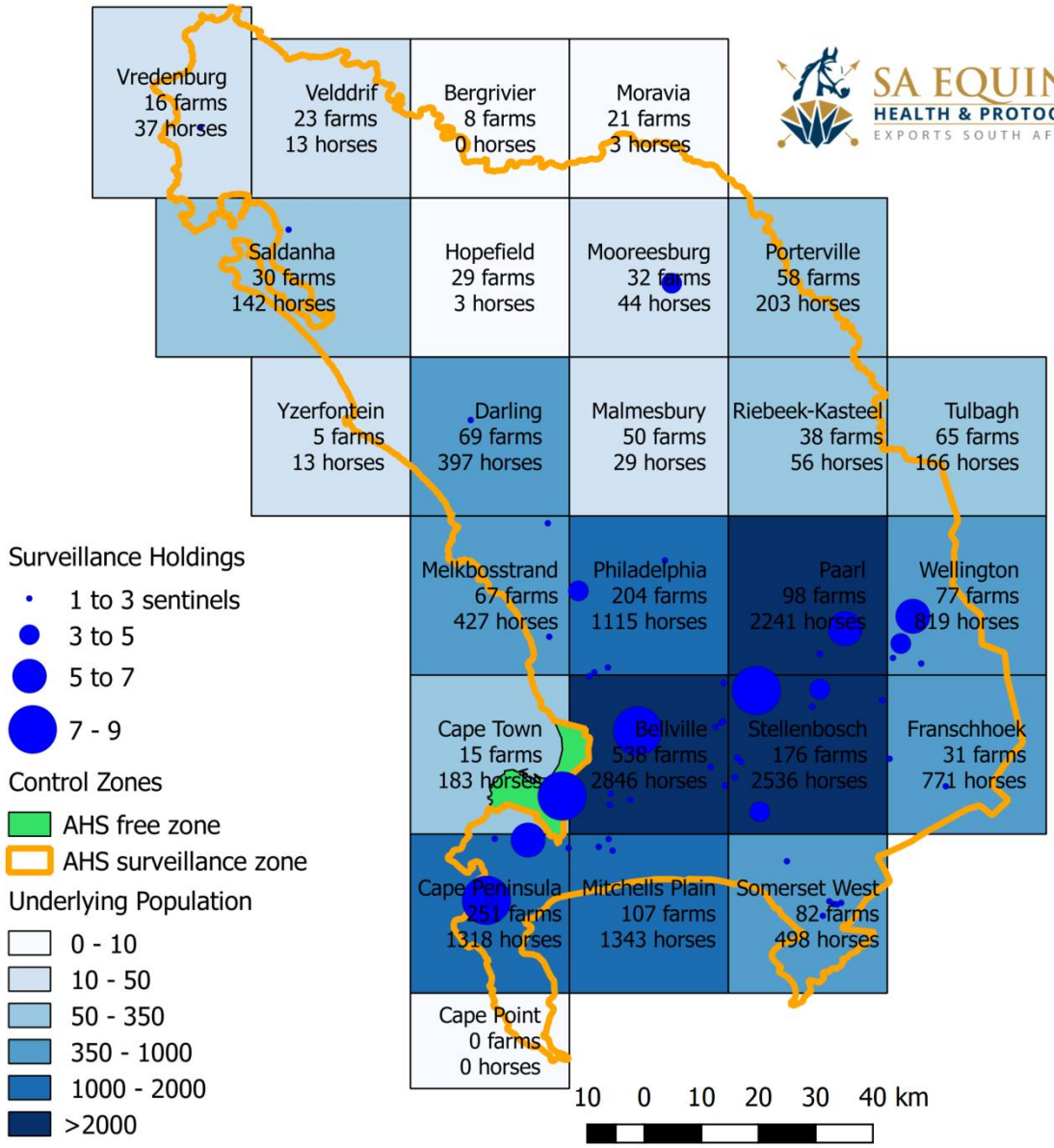


Figure 9: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 October 2018. Proportionally these populations have a similar distribution compared to the original sentinel surveillance plan. The proportional circles represent the current sentinel populations.

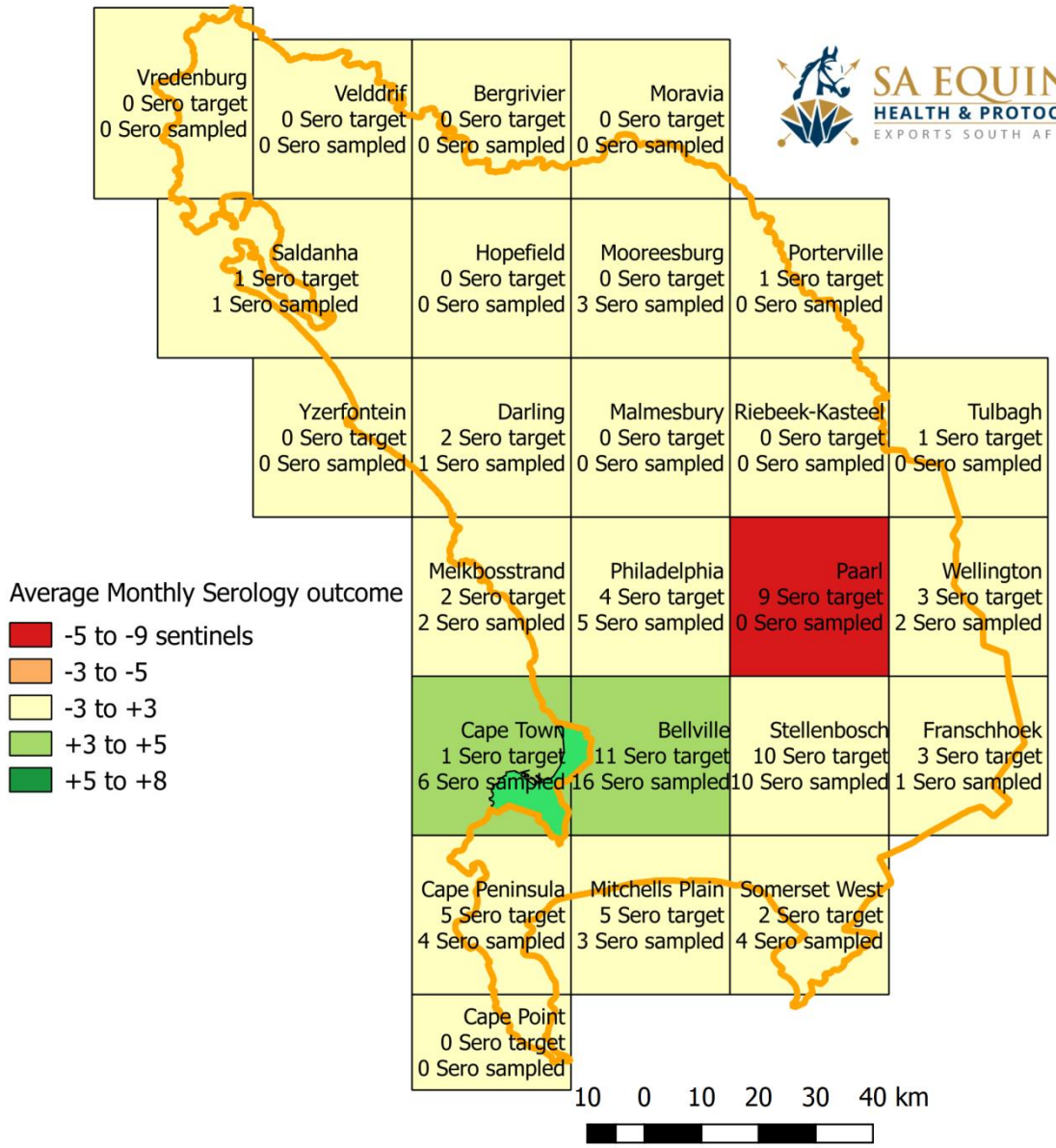


Figure 10: A map showing the AHS surveillance and free zone where SERO-sentinel surveillance has taken place for the 2017/2018 season. The map depicts the various areas with their target serology samples in order to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange to red 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.

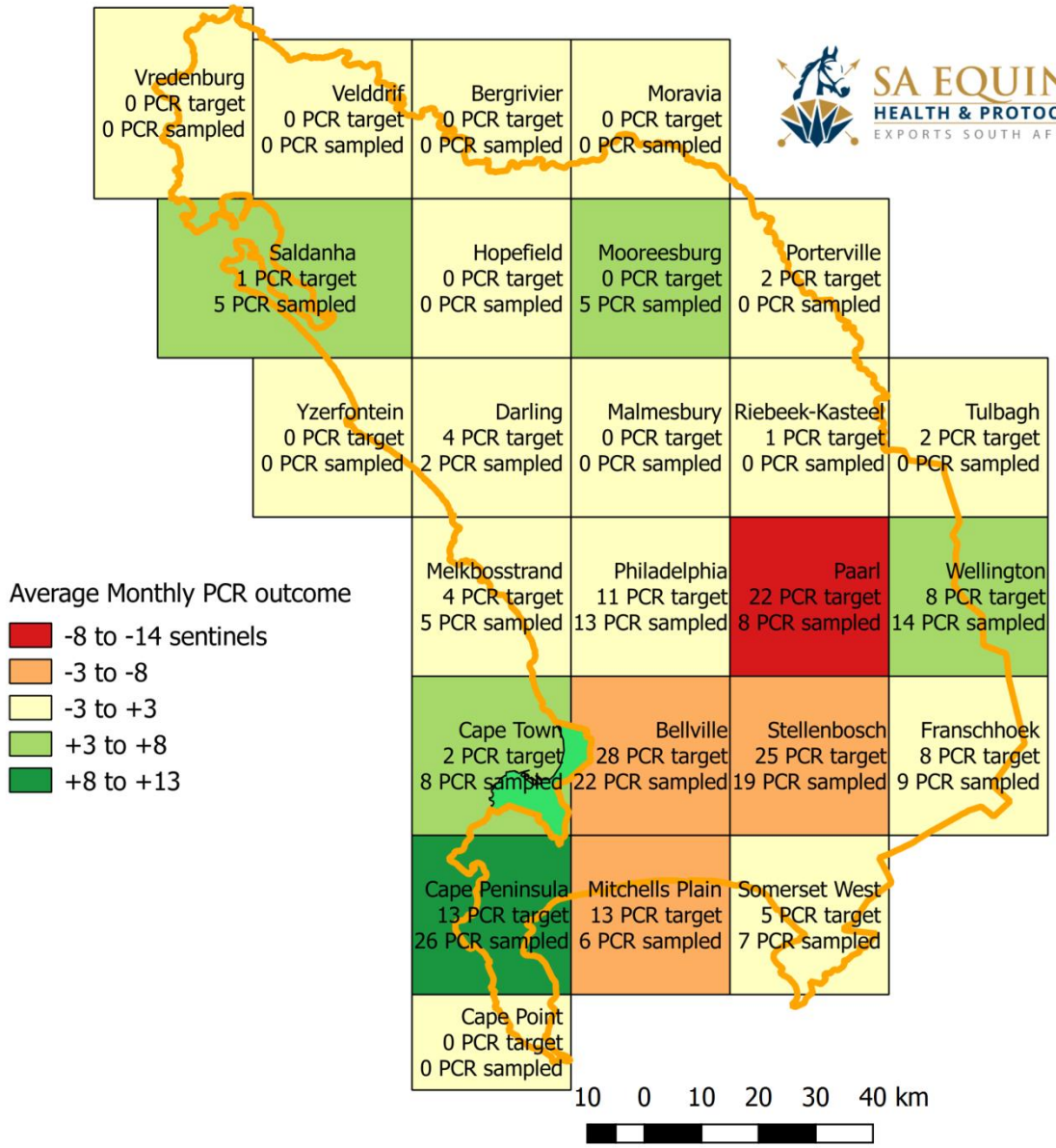


Figure 11: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2017/2018 season. The map depicts the various areas with their target PCR samples in order to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange to red 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.

Sensitivity of Surveillance System

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 previous surveillance program is taken into account as it provides historical information that aids in determining an accurate final probability of freedom as of August 2018. A single season analysis was performed with almost exactly the same outcome (96.5% Probability of freedom). The final probability of freedom at the end of the two year period was 96.6% (Figure 12).

Parameter	Value	Comments
***p*Intro**	0.022	Based on introductions of AHS into the surveillance zone since 1999 (n=5) and the number of months at risk between 1 Jan 1999 and the start of the period under review (n=223)
Population at risk – total herds	1070	Data captured between 1 April 2016 and 1 Oct 2018
Sentinel farm populations	Various	Based on herd size as of 1 Oct 2018. 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^* unit)	0.05	Design prevalence at animal level
Cluster design prevalence (p^* cluster)	0.021	Average number of herds affected across the 5 outbreaks in the surveillance zone (22.8) divided by the number of herds in the surveillance and free zones currently (n= 1070)
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	A conservative prior of 0.5 was used given the AHS outbreak in the Surveillance zone in April and May 2016. When evaluating the single season as discussed in the introduction to this section the final posterior probability of freedom (0.959) for the 2016 2017 season was used as an initial prior for the 2017 2018 season.

Table 1: Parameters used to establish sentinel system confidence of Freedom for African horse sickness

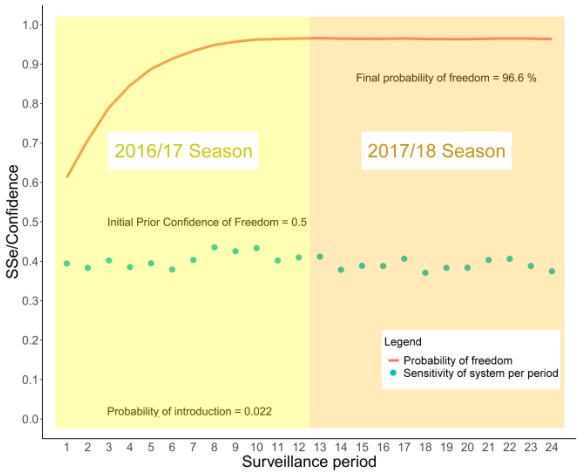


Figure 12: The surveillance sensitivity of individual surveillance periods (blue dots) with probability of freedom curve (red line) based on an uninformed 0.5 prior and a probability of introduction of 0.022 for the past two surveillance seasons: the season now under review shown in the right panel of the graph.

Economic cost of Surveillance

Very similar numbers of horses and farms were tested in 2017/2018 compared to 2016/2017 – and thus the estimated cost of the program for the year is R1.5 Million. This cost is made up of testing, personnel, travel/logistics and equipment costs. Funding comes from the South African Health and Protocols NPC, Wits Health Consortium, the Equine Research Centre (University of Pretoria) and the Western Cape Department of Agriculture (both Animal Health and Provincial Laboratory).

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2017 2018 season was achieved, with a final probability of freedom of 96.5%. 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. Furthermore the use of SNT analysis allows confident categorization of previously seronegative horses into vaccinated or field strain events.

The major investigation of the season was a horse that was inadvertently vaccinated contrary to the owner's intention.

Recruitment still remains a challenge but spatially there is good representativeness, with the Paarl grid remaining a challenge for the recruitment of sentinels. Unfortunately the plans to include the use of sero-negative foals was not realized in that area as a result of logistic constraints, however this remains an option to the surveillance team.

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 Equine Research Center, the Onderstepoort Veterinary Research Institute and now the Stellenbosch Provincial Veterinary Laboratory who performed the testing of samples this season.

In this season we again made use of compulsory community service vets who assisted in sampling. In this regard we specifically acknowledge Tasneem Anthony (who later joined the Provincial laboratory), Marina Lubbinge, Samantha Aaronson, Annemieke Vermaas, Anouska Rixon, Nellma Le Roux, Louie Genis. We are very grateful to our SAEHP team who are directly involved with the program – Phillippa Burger, Esthea Russouw and Lizel Germishuys.

Software and systems references

Evan Sergeant (2016). RSurveillance: Design and Analysis of Disease Surveillance Activities. R package version 0.2.0

H. Wickham (2009). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Hadley Wickham (2011). The Split-Apply-Combine Strategy for Data Analysis. Journal of Statistical Software, 40(1), 1-29.

Hadley Wickham (2016). scales: Scale Functions for Visualization. R package version 0.4.0.

Achim Zeileis and Gabor Grothendieck (2005). zoo: S3 Infrastructure for Regular and Irregular Time Series. Journal of Statistical Software, 14(6), 1-27

Joe Conway, Dirk Eddelbuettel, Tomoaki Nishiyama, Sameer Kumar Prayaga and Neil Tiffin (2016). RPostgreSQL: R interface to the PostgreSQL database system. R package version 0.4-1.

Literature references

Guthrie, A.J. et al., 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(1), pp.30–35.

Maree, S. & Paweska, J.T., 2005. Preparation of recombinant African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. Journal of Virological Methods, 125(1), pp.55–65.

Martin, P.A.J., Cameron, A.R. & Greiner, M., 2007. Demonstrating freedom from disease using multiple complex data sources. 1: A new methodology based on scenario trees. Preventive Veterinary Medicine, 79(2–4), pp.71–97.