# The AHS sentinel surveillance program 2016-2017 season report

*JD Grewar[1], CT Weyer[1], P Burger[1], E Russouw[1], B Parker[1] and A. Guthrie[2]*

[1] *Wits Health Consortium: Equine Health Fund*
[2] *Equine Research Centre, University of Pretoria*

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## 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 systems 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 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 last 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 report for last season (2015-2016) can be found in the September 2016 Epidemiology Report at http://elsenburg.com/vetepi/epireport_pdf/September2016.pdf. 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 is performed at the University of Pretoria's Veterinary Genetics Laboratory under coordination of the Equine Research Centre. The test used is an ERC developed real-time RT-PCR (Guthrie et al. 2013).

This report covers the 2016/2017 AHS season from 1 September 2016 to 31 August 2017. According to the results, the sentinel surveillance program did not detect an incursion of AHS during the 2016/2017 season.

## General overview of results

A total of 689 sero-sentinel samples were analysed from 39 different farms at an average of 57 samples from 29 different farms per month. This was an increase of 1.6% from the 2015/2016 surveillance period. Of the tested serology samples 660 could be evaluated as they had relevant paired results (Figure 1) – this averages out to 55 sampling events per month. This is a 6% increase compared to the 2015/2016 season.

A total of 1766 PCR sentinel samples were analysed from 59 different farms at an average of 147 samples from, on average, 50 different farms per month. This was a decrease of 9.2% 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 29 samples (4% of the total). This compared to 2014/2015 where 56 samples could not be evaluated (8% of the total). A total of 3 serology evaluations indicated an increase in status warranting investigation – these will be dealt with individually below.

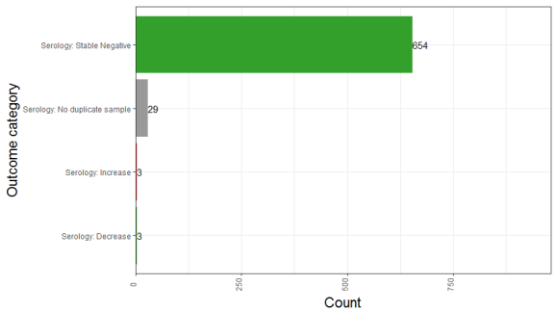


**Figure 1: Broad outcomes for serological evaluation for the period under review. Increasing serology incorporates both the negative to suspect/positive and the suspect to positive permutations for serological change across paired samples.**

## PCR

Figure 2 shows the results for the PCR-based surveillance. Except for one result, all PCR results were negative. One positive result was from a vaccinated horse – see individual section below.

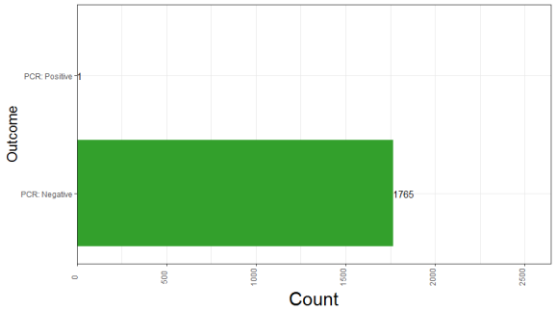


**Figure 2: Broad outcomes for PCR evaluation for the period under review. The single positive was a vaccinated horse that was also responsible for one of the increasing serology titres shown in Fig 1.**

## Results: Increasing sero-status /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 result (n=1) was detected in a horse that also had an increasing serological titer in the same month.

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

# Individual horse evaluations

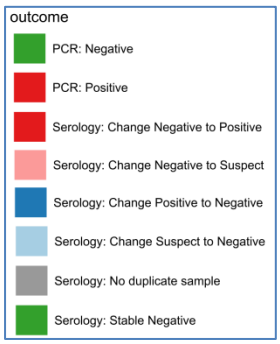


**Figure 3: Legend for the individual horse serological and PCR outcomes for the section below**

## Holding 139: Horse 1783

Horse 1783 was a test case for the sentinel process. We were made aware that the horse was vaccinated by the owner in June 2017. This information was not revealed to laboratory staff. Both the PCR and ELISA returned positive results in July 2017 (Figure 4) and the sentinel was removed from the program in August.

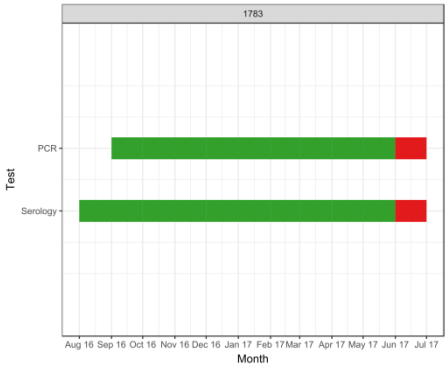


**Figure 4: Horse 1783 result series**

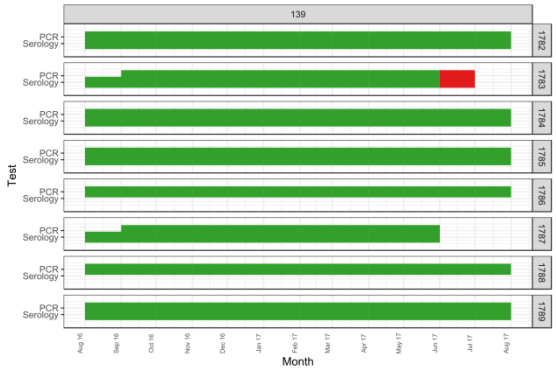


**Figure 5: Holding 139 sentinels - cohort of horse 1783. No further adverse findings were seen other than the vaccinated horse.**

There were no adverse findings from the other sentinels on the same property (Figure 5)

## Holding 77: Horse 9685

Horse 9685 had an increase in serological titre from negative the previous month to positive in June 2017 (Figure 6). SNT's were requested on the sample. There are 8 PCR sentinels on the property, only one of which takes part in the sero-sentinel program. When sentinels were sampled, serum and EDTA samples were collected from all 8 sentinels. The initial concern was that samples had been incorrectly labelled and the incorrect sample submitted for serology. There was no clinical indication of an infectious disease on the property.

SNT results showed diffuse positives (all serotypes bar AHS type 6) indicating likely prior vaccination. PCR results were negative for April, May, June, July and August for this horse indicating unlikely exposure prior to, during or after the sero-positive result. The July serum sample returned a negative result which remained negative in August. All cohort animals were negative on PCR, with 6/9 sentinels tested on PCR in May and June over and above the affected horse (Figure 7).

The clinical evidence and testing confirmed that the most likely reason for the June 2017 positive ELISA and multiple serotypes on SNT was an incorrect sample being allocated to this sentinel.

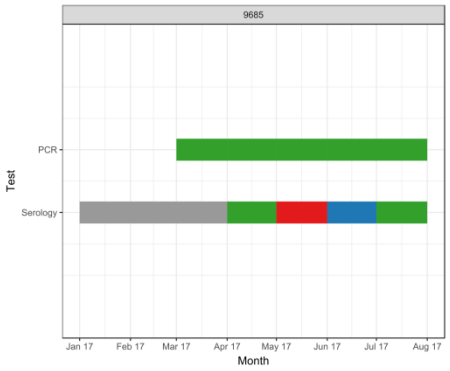


**Figure 6: Horse 9685 result series showing a single negative to positive transition on ELISA with an immediate return to negative the following month.**

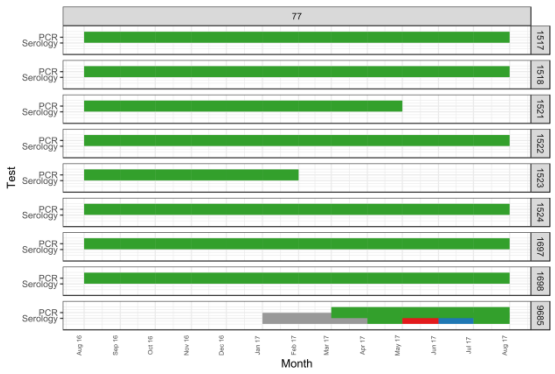


Figure 7: Holding 77 cohort of horse 9685.

## Holding 112: Horse 9797

Horse 9797 had an initial recruitment sample collected in March 2017 when the foal was 6 months old. Serology was positive but as the foal was young it was decided to continue sampling it as a sero-sentinel (Note the recruitment sample is not shown in Figure 8). The April 2017 sample was negative and the May 2017 sample tested suspect on i-ELISA (ELISA value of 6 where 0-10 is suspect). The horse was 8 months old in May 2017.

Horse 9797's July serological result was negative and it remained stable negative in August, which is the end of the period currently under review. The foal was sampled for PCR testing in May, July and August and all results were negative. Two cohort horses that were tested over the same time period were negative on PCR and one was a sero-sentinel that remained negative throughout the period (Figure 9).

It was concluded that maternal antibody is the most likely source of the suspect result in May.

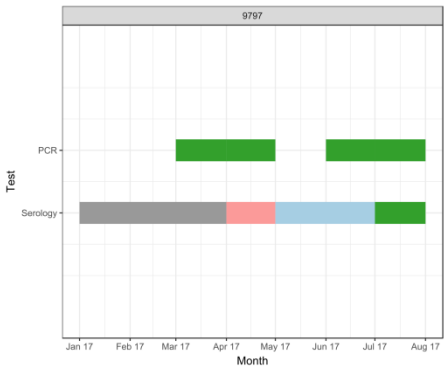


Figure 8: Horse 1509 result series showing a single suspect result with a return to sero-negativity.

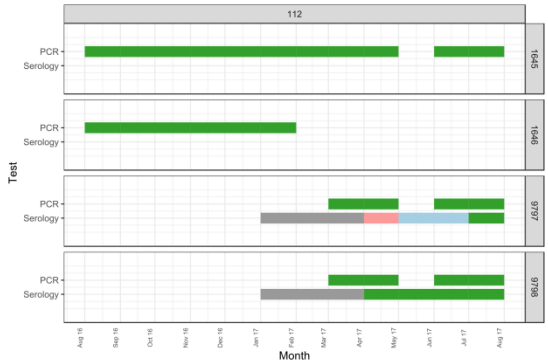


Figure 9: The farm sentinel cohort associated with horse 1509.

# 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. Every year an evaluation of the distribution of the sentinels is undertaken to establish whether there are areas where improvements are required. This is going to be increased to evaluating on a quarterly basis for the next season.

Figure 10, Figure 11 and Figure 12 shows the underlying population and current sentinel farms and the monthly average distribution of sentinels in the sero and PCR sentinel programs respectively.

In general representativeness was obtained spatially with only the Paarl grid requiring an improvement in both sero and PCR sentinels.

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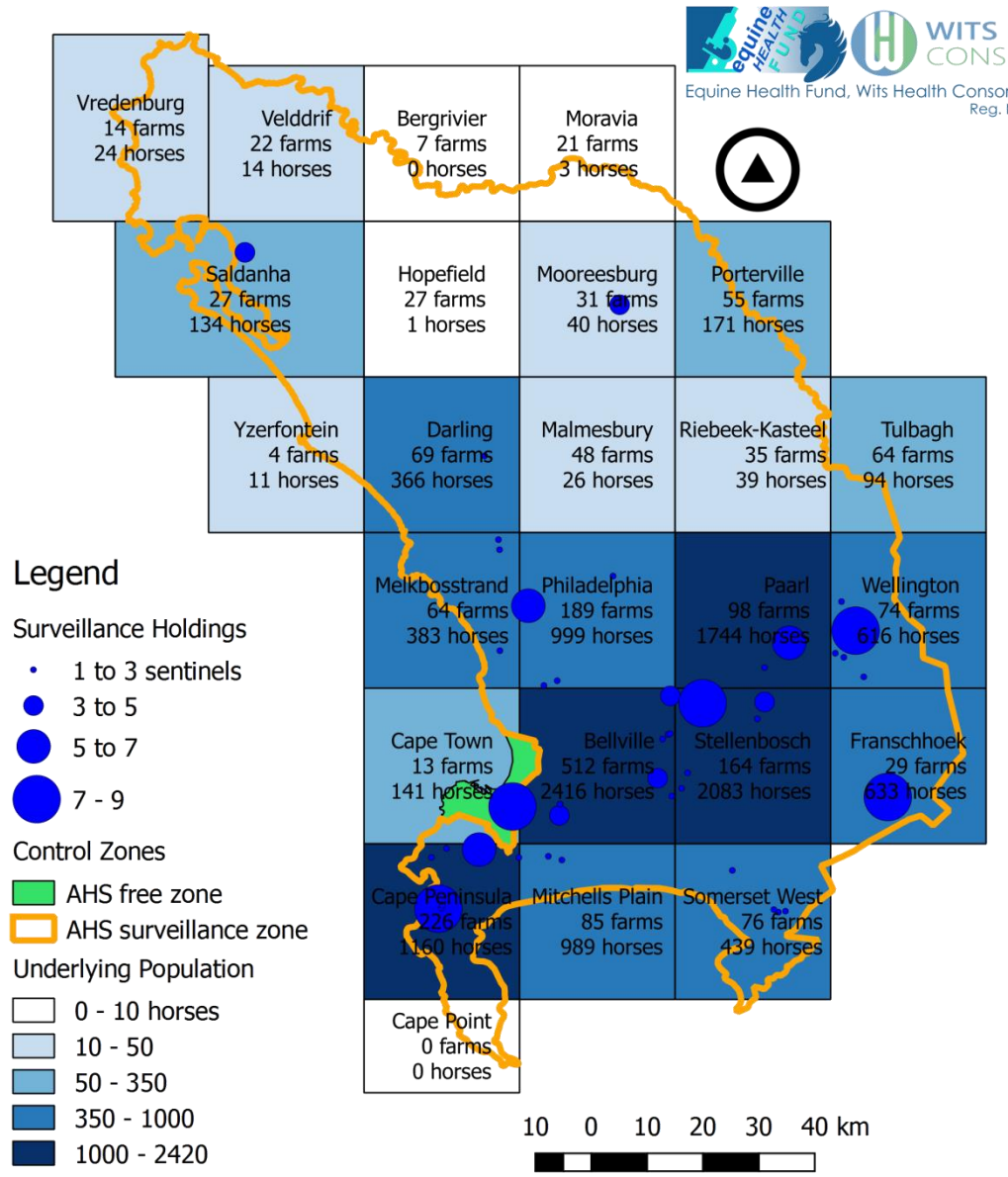


**Figure 10: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 October 2017. Proportionally these populations have a similar distribution compared to the original sentinel surveillance plan. The proportional circles represent the current sentinel populations.**

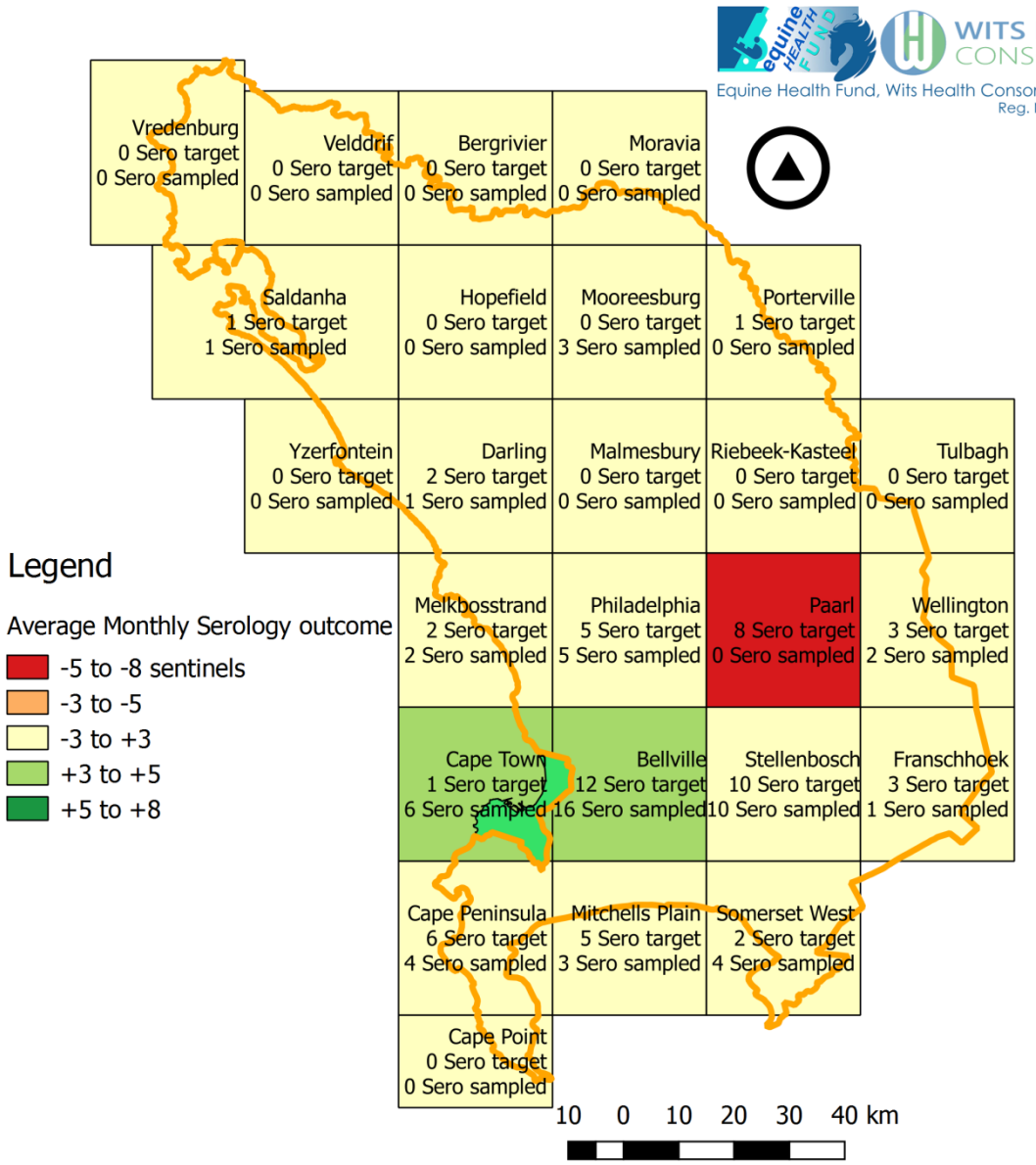


**Figure 11: A map showing the AHS surveillance and free zone where SERO-sentinel surveillance has taken place for the 2016/2017 season. The map depicts the various areas with their target serology samples in order to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange 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.**

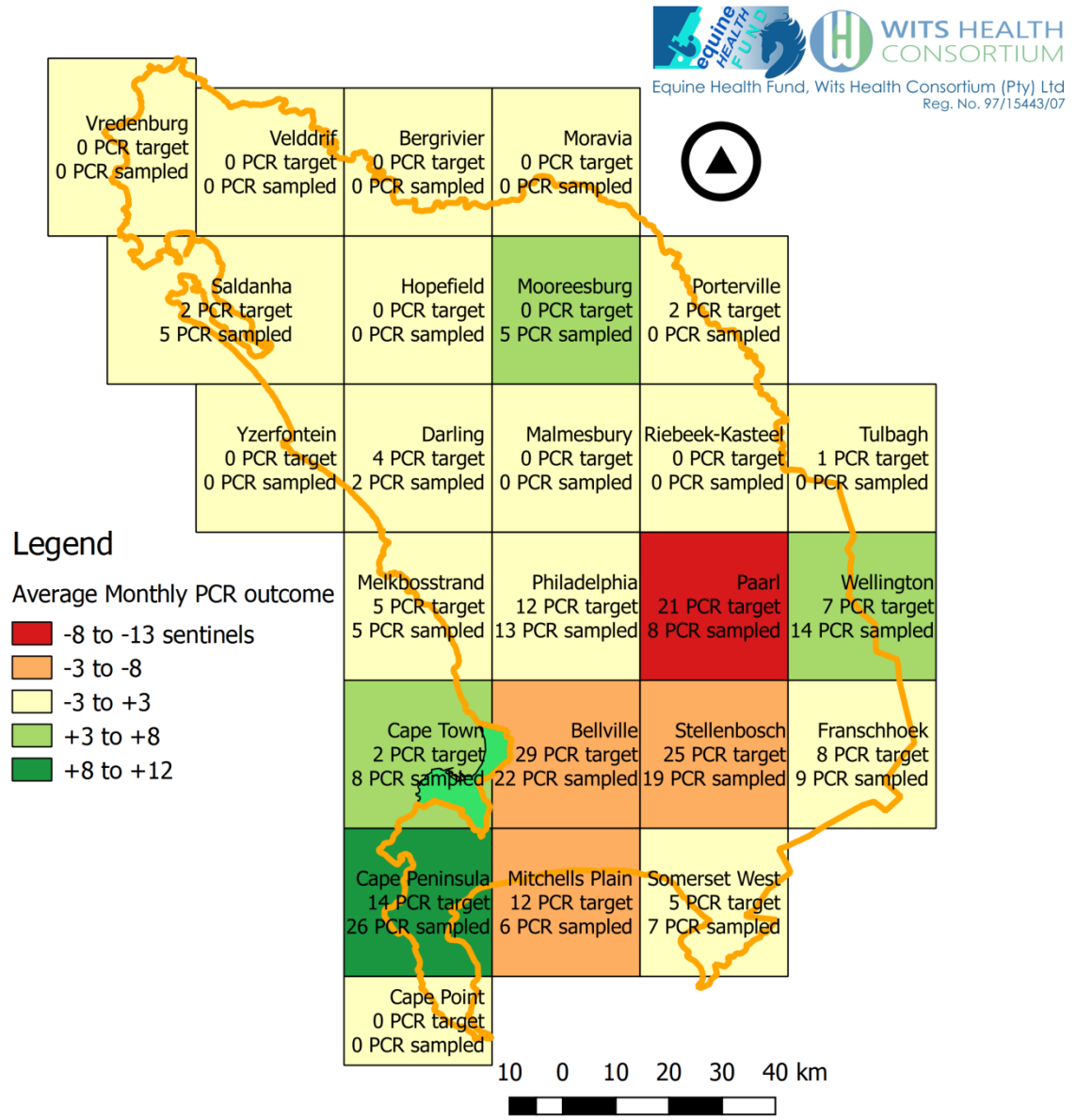


**Figure 12: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2015/2016 season. The map depicts the various areas with their target PCR samples in order to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange to 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 final probability of freedom at the end of the period was 95.9% (Figure 13).

| Parameter | Value | Comments |
|---|---|---|
| ***p*Intro** | 0.024 | 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=211) |
| **Population at risk – total herds** | 977 | Data captured between 1 April 2016 and 1 Oct 2017 |
| **Sentinel farm populations** | Various | Based on herd size as of 1 Oct 2017. Assumption is made that herd size would not change significantly on the sentinel properties over a single year. |
| **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)** | 0.023 | 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= 977) |
| **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 |

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

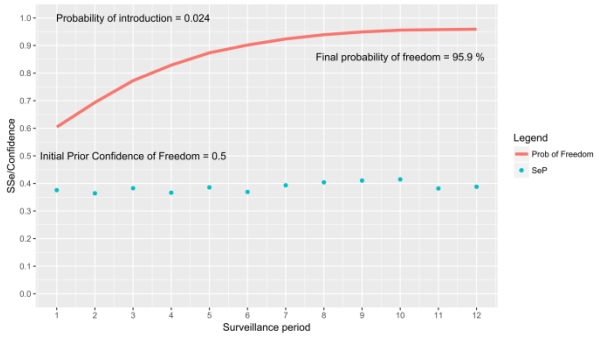


**Figure 13: The surveillance sensitivity of individual surveillance periods (blue dots) with probability of freedom curve (red line) based on an uninformed 0.5 prior and a probability of introduction of 0.024.**

## Economic cost of Surveillance

The total estimated cost of the AHS sentinel surveillance program for the 2016/2017 season amounted to R 1.476 Million. This cost is made up of testing costs (R936 000), personnel costs (R454 000), travel and logistics (R77 500) and equipment costs (R 7 500). Funding comes from the Equine Health Fund of the Wits Health Consortium, the Equine Research Centre (University of Pretoria) and the Western Cape Department of Agriculture.

## Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2016 2017 season was achieved, with a final probability of freedom of 95.9%. 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.

The system detected a vaccinated horse during the winter of 2017, and this was detected in two separate labs doing PCR and serology respectively.

Recruitment still remains a challenge but spatially there is good representativeness, with only the Paarl grid still being a challenge for the recruitment of sentinels. Plans for the 2017 2018 season included the use of sero-negative foals to supplement sentinels during the AHS higher risk period.

# 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 both the Equine Research Center and the Onderstepoort Veterinary Research Institute who perform the testing of samples.

The 2016 2017 season represents the first time that compulsory community services veterinarians did field work in the sentinel surveillance program. We specifically acknowledge Drs Annemieke Vermaas, Tasneem Anthony, Marina Lubbinge, Lizanne Murphy and Friedl le Roux.

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