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HEALTH & PROTOCOLS
EXPORTS SOUTH AFRICA

African horse sickness control

Sentinel surveillance report 2018/2019 Season

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In partnership with
Western Cape Government

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 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 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 (2017-2018) can be found in the October 2018 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 Research (ARC-OVR). Viral RNA testing was performed at the regional 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 2018/2019 AHS season from 1 September 2018 to 31 August 2019. 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 40 different farms at an average of 59 samples from 27 different farms per month. This was a sampling increase of 1.4% from the 2017/2018 surveillance period. Of the tested serological samples 684 (average of 57 per month) could be evaluated as they had relevant paired results (Figure 1). This is a 0.7% increase compared to the 2017/2018 season.

A total of 1902 PCR sentinel samples were analysed from 74 different farms at an average of 158 samples from, on average, 56 different farms per month. This was a increase of 8.3% 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 27 samples (3.8% of the total). This compared to 2017/2018 where 23 samples could not be evaluated (3.3% of the total).

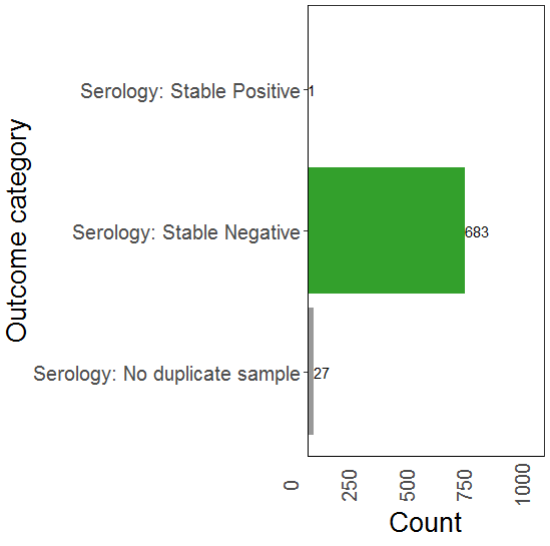

Figure 1: Broad ELISA evaluation outcomes of the sero-sentinel surveillance program for 2018/2019

PCR

Figure 2 shows the results for the PCR-based surveillance. All PCR results were negative barring 1 sample from a single horse in October 2018 (Horse 1791 on farm 5356). This horse will be discussed– see individual section below.

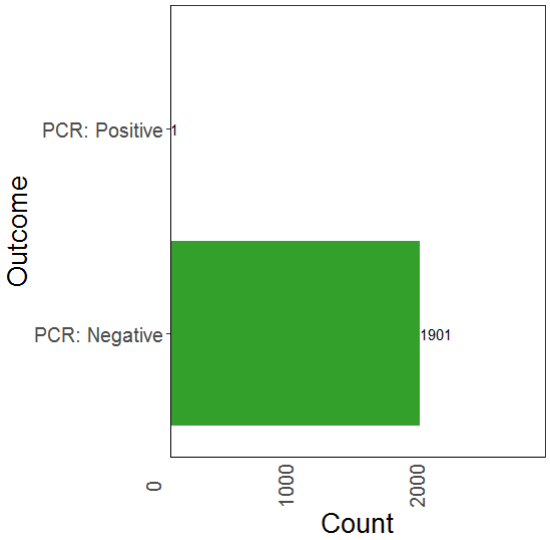

Figure 2: Broad outcomes for PCR evaluation for the period under review.

Results

Follow up investigations

There was one investigation of importance during the 2018/2019 season – this was as a result of a screening positive PCR in horse 1791 on property 5356 in October 2018.

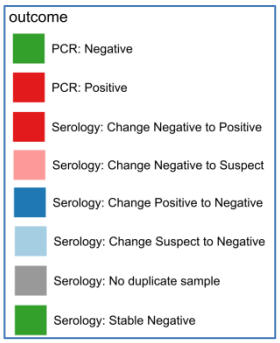

Figure 3: Legend for the individual horse serological and PCR outcomes for the section below

Holding 5356: Horse 1791

Horse 1791 had a positive screening PCR result in October 2018 (Figure 4). It was a PCR-sentinel only; however, serum samples

that had routinely been taken from this animal were also subsequently tested during the investigation. The ELISA results confirmed the prior vaccination history of the animal and returned positive results from both September and October. This *Stable Positive* result correlates with the same category in Figure 2 above.

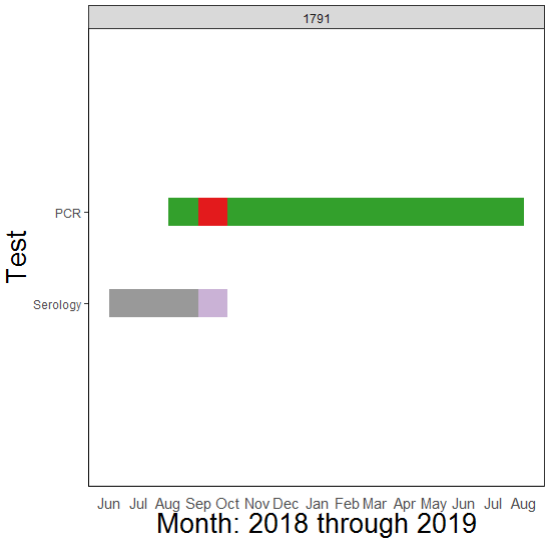

Figure 4: Horse 1791 result series showing a single positive PCR result in October 2019 with subsequent negative results. The retrospective ELISA testing confirmed prior vaccination.

The horse was the only sentinel on the farm (in the Bottlery region of the AHS surveillance zone) on which another 8 horses were resident at that time. The owner confirmed that neither Horse 1791 nor the other horses on the property had been vaccinated against AHS during 2018.

Trace-back analysis of movements into the surrounding 10 km showed that a total of 5 horses moved in two separate movements from the AHS infected zone in September 2018. These horses originated in Midrand, Gauteng (n=1) and Parys, Free State (n=4). At that point there had been no confirmed cases of AHS yet in the AHS infected zone for the 2018/19 season. Two suspect cases were reported in November from Midrand, but the Midrand farm of origin with regard to the movement was outside a 30 km zone around both suspect cases.

Laboratory follow up testing including: re-testing at the SPVL; testing using the same PCR at the Equine Research Center at the University of Pretoria; and both hemi-nested PCR and an attempt to type and sequence at the ARC-OVR. Repeat testing at SPVL returned similar results (Ct-value 32.5). ERC results were negative for both AHS and EEV while the OVR hemi-nested PCR was positive. Sequencing and typing at OVR was unfortunately not possible due to low levels of RNA in the sample. SNT results from the suspect horse returned a polyvalent response as was expected from a previously vaccinated horse.

Follow up sampling on the farm included full farm population sampling in November and December. All samples were negative for AHS.

Results from surrounding sentinel farms were evaluated: there were 8 farms with 24 sentinels present on them within 10 km of farm 5356. All sentinel results from these farms were consistently negative going back from July 2018 and throughout the rest of the 2018/19 season.

The final conclusion reached was that the positive result was a false positive PCR reaction. This conclusion was based primarily on:

- The high Ct value and inability to type of sequence the PCR product
- The lack of clinical signs in the horse and horses on the same property
- The negative follow up testing in both the affected horse and horses on the same property
- The negative status of 24 sentinels surrounding the affected farm

• The negative results from the entire sentinel cohort in November through February 2018
• The negative outcome of the trace back for the month preceding the suspect case.

Follow up investigations – Sentinel deaths

A component of the sentinel program is that deaths are investigated from recruits. During the 2018/19 season sentinel 16423 died from a holding based in the AHS surveillance zone near Morning Star. In the days leading up to her death, the horse had been in the process of being treated for abdominal colic and it died on the 11th December. The last time the horse had been sampled for the sentinel program was on the 10th December which was negative on PCR for AHS.

Follow up investigations – Sampling errors

Over the course of the season there were three investigations where positive ELISA-results were found to have been as a result of sampling and/or labelling errors. This occurred in April 2019 (once) and June 2019 (twice). The April event was for horse 1530 where the wrong horse was presented for sampling by inexperienced grooms to a sampler who had just taken over that property and was not familiar with the sentinels. Corrective action was taken to ensure that positive identification of sentinels is made by samplers, in particularly if they are unfamiliar with a property.

The cases in June were both due to incorrect labelling of samples prior to submission. Generally horse names are written on sample tubes and overlaid by a barcoded sticker which is supposed to be performed at the time of sampling. Some inexperience resulted in a lag between sampling and labelling in both these cases and the incorrect samples were linked to horses not due for serological testing. Corrective action was taken to enforce labeling on farm rather than retrospectively and the remainder of the season's sampling was uneventful. All samples associated with the incorrect labelling and incorrect sentinel presented for sampling were discarded in the sampling event, information and results table of our database. They are not included in the aggregated outcomes presented in the first paragraphs of this report.

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.

There are improvements with spatial representativeness compared to previous years. At worst the sero-surveillance target was, on average, 4 sentinels per month short in the Philedelphia area (Figure 6) and the Paarl area was 7 PCR sentinels short per month (Figure 7).

Over the past few years where formal analysis of this program has taken place this result is the best representativeness that has been present over an AHS season.

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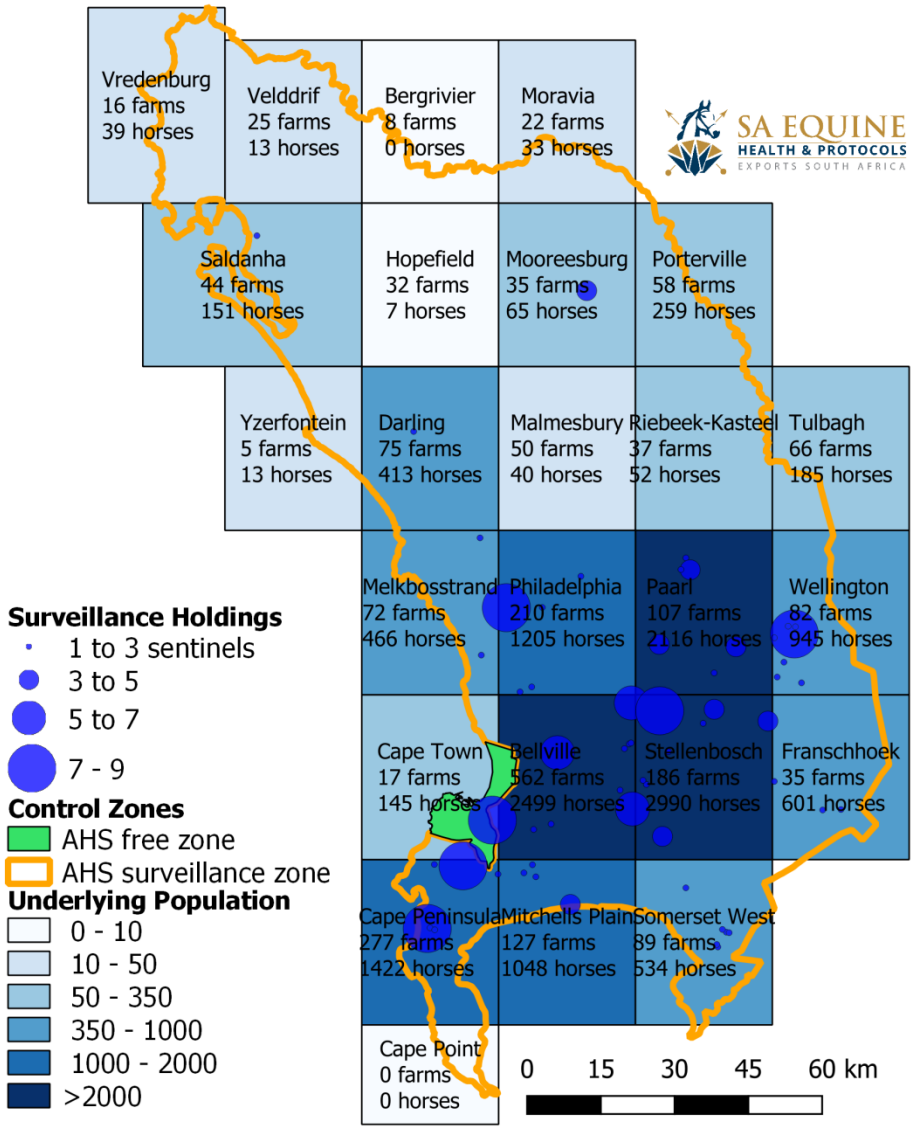

Figure 5: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 September 2019. The proportional circles represent the current sentinel populations.

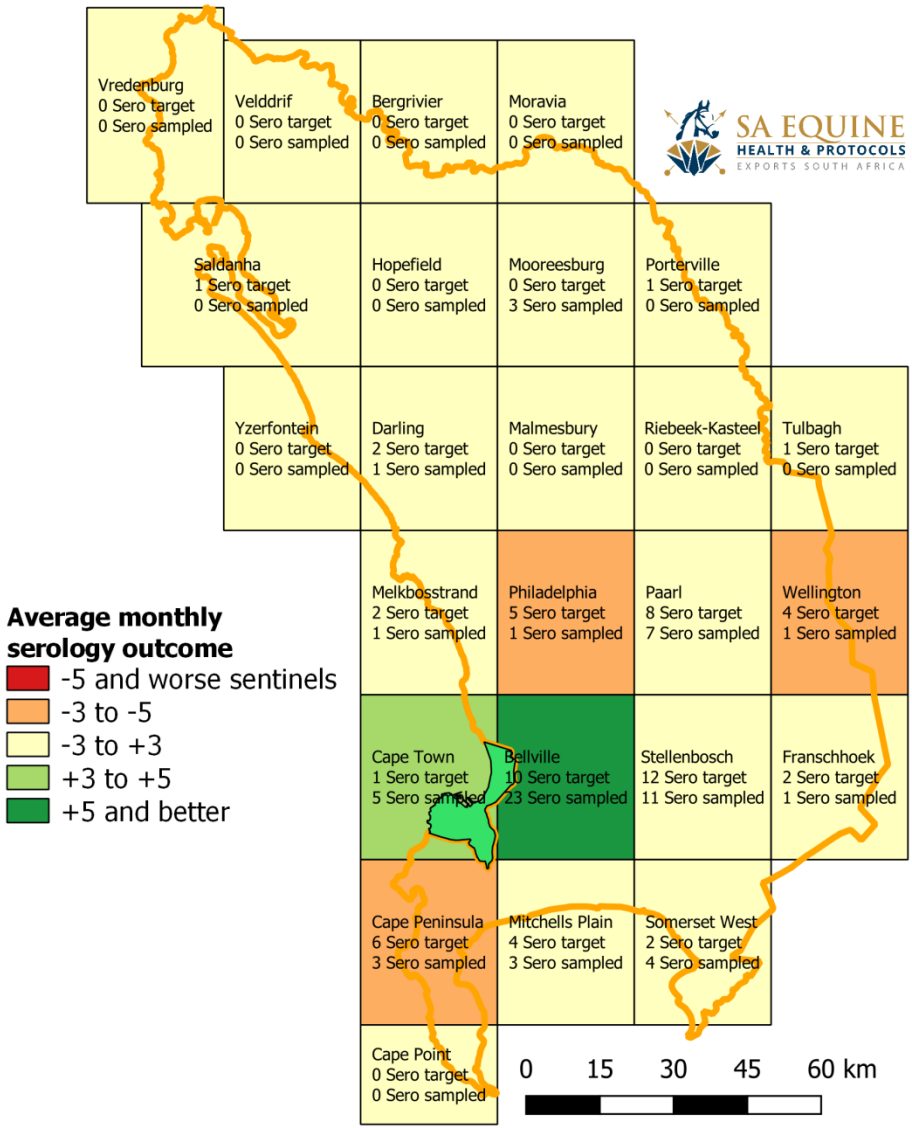

Figure 6: A map showing the AHS surveillance and free zone where sero-sentinel surveillance has taken place for the 2018/2019 season. The map depicts the various areas with their target serology samples in order to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange 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.

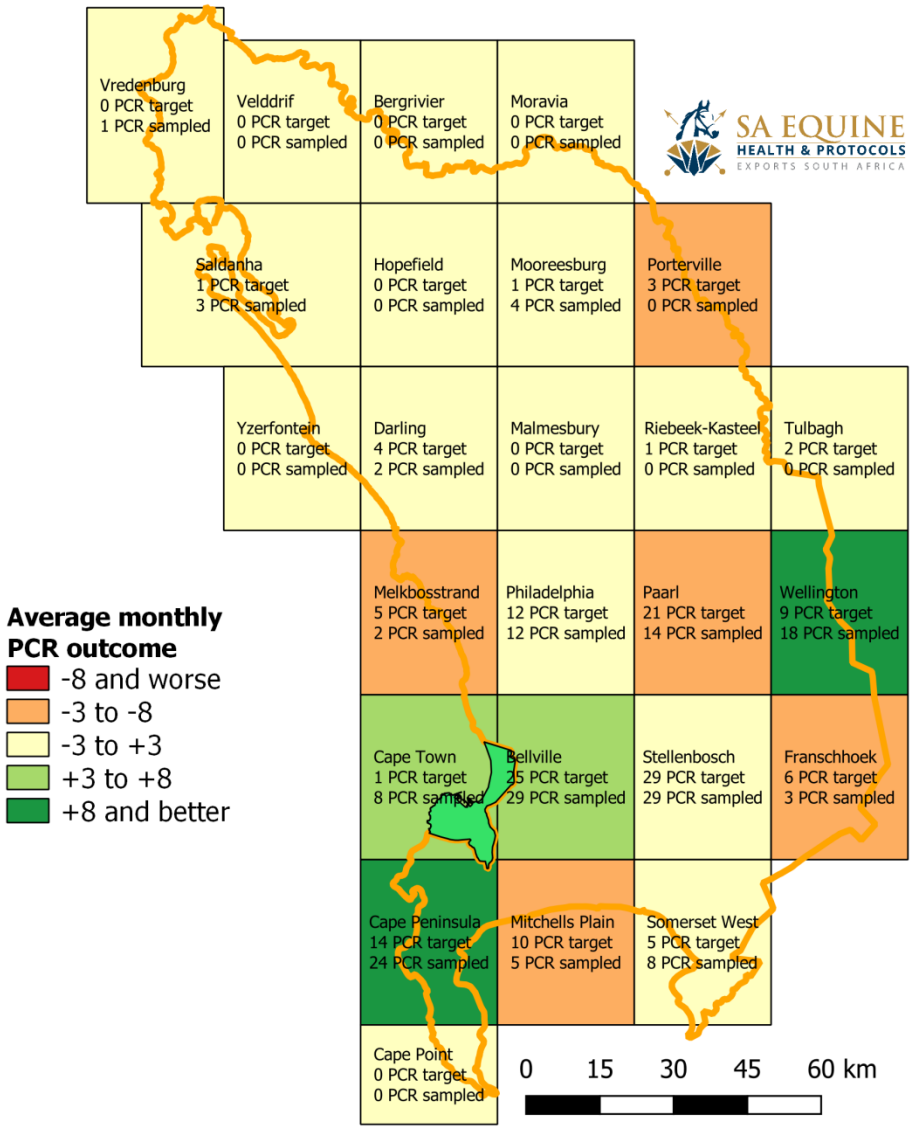

Figure 7: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2018/2019 season. The map depicts the various areas with their target PCR samples in order to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange areas 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 2019. A single season analysis was performed with a final posterior probability of freedom of 93% assuming an uninformed prior probability of freedom in Sept 2018 of 50%. The final probability of freedom at the end of the three year period was 94.2% (Figure 8).

The sensitivity of the sentinel surveillance alternates around the 30% mark throughout. This is the third AHS season running where cases of the disease have not been detected in the AHS controlled area. The last time this occurred was in the period between the 2006 and 2011 outbreaks where, for four full seasons running. the area was AHS free.

Parameter	Value	Comments
***p*Intro**	0.03	Based on introductions of AHS into the surveillance zone since 1999 (n=7) and the number of months at risk between 1 Jan 1999 and the start of the period under review (n=235). This has changed from last year: here we are more conservative and including outbreaks that occurred in the AHS Protection zone (n=2 – 2006 and 2014)
Population at risk – total herds	1181	Data captured between 1 April 2016 and 1 Sep 2019 for the AHS surveillance and free zones.
Sentinel farm populations	Various	Based on herd size as of 1 Sep 2019. 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 3 years between Sept 2016 and Aug 2019 the initial prior is 0.5 but relates to September 2016.

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

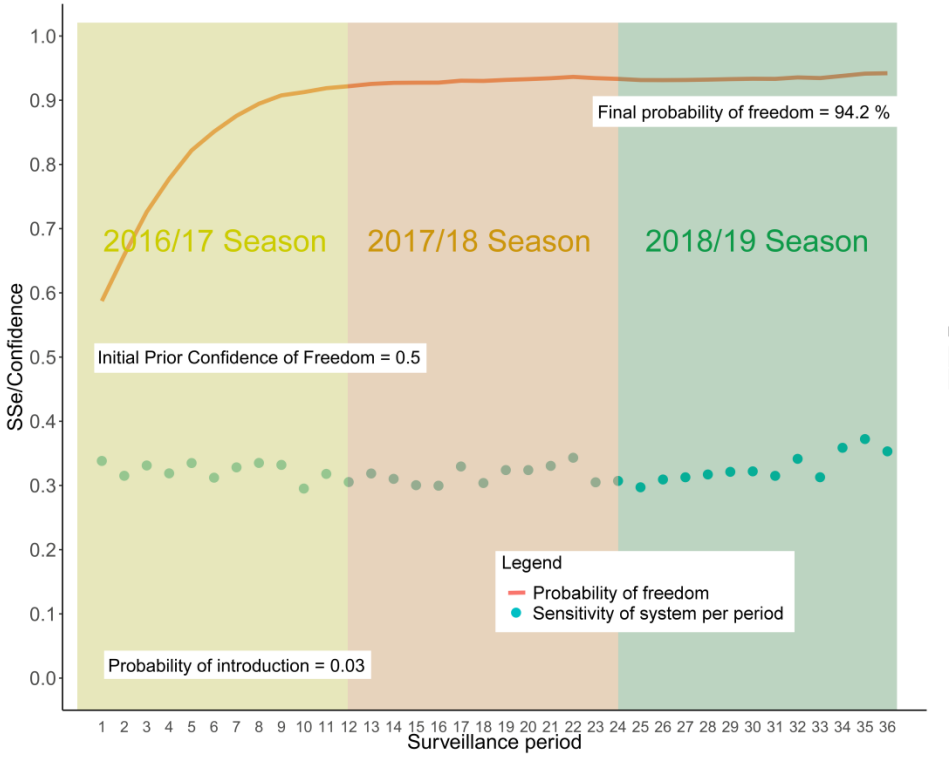

Figure 8: The sentinel surveillance sensitivity of individual surveillance periods (dots) with probability of freedom curve (red line) based on an uninformed 50% prior probability of freedom and a probability of AHS introduction of 3% for the past three surveillance seasons: the season currently reviewed is the right pane – 2018/2019 season running between Sept 2018 and Aug 2019

Economic cost of Surveillance

Very similar numbers of horses and farms were tested in 2018/2019 compared to 2017/2018 – and thus the estimated cost of the program for the year remains R1.5 Million. 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).

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2018 2019 AHS season was achieved, with a final probability of freedom of ~93%. 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 major investigation of the season was a horse that returned false positive PCR results; this after an extensive investigation was undertaken once initial results were available.

While there are still areas that remain a challenge in terms of representativeness this is the first year where no area had a major lack of sentinels, either serological- or PCR categories.

A 3-year review of sentinel results show that the probability of freedom attained for this program, at an animal design prevalence of 5% animals and herd-level design prevalence of 2%, shows a 94% probability of freedom from AHS as a result of sentinel surveillance.

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, Katie Edmonds, Louie Genis, Gina Anstey, Anouska Rixon and Nellma le Roux. We are very grateful to our SAEHP team who are directly involved with the program – Esthea Russouw and Lizel Germishuys.

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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