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HEALTH & PROTOCOLS
EXPORTS SOUTH AFRICA

African horse sickness control

Surveillance report

Sentinel surveillance 2019/2020 season

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2020

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 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. The summary report for last season (2018-2019) can be found in the September 2019 Epidemiology Report while the original detailed report can be found at www.myhorse.org.za.

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 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 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 2019/2020 AHS season from 1 September 2019 to 31 August 2020. 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 600 sero-sentinel samples were analysed from 37 different farms at an average of 50 samples from 24 different farms per month. This was a decrease of 14% from the 2018/2019 surveillance period for the sero-surveillance program. Of the tested serological samples: 589 (average of 49 per month) could be evaluated as they had relevant paired results (Figure 1).

A total of 1746 PCR sentinel samples were analysed from 72 different farms at an average of 146 samples from, on average, 51 different farms per month. This was a decrease of 7% 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 12 samples (2% of the total, a decrease from 3.8% the previous season).

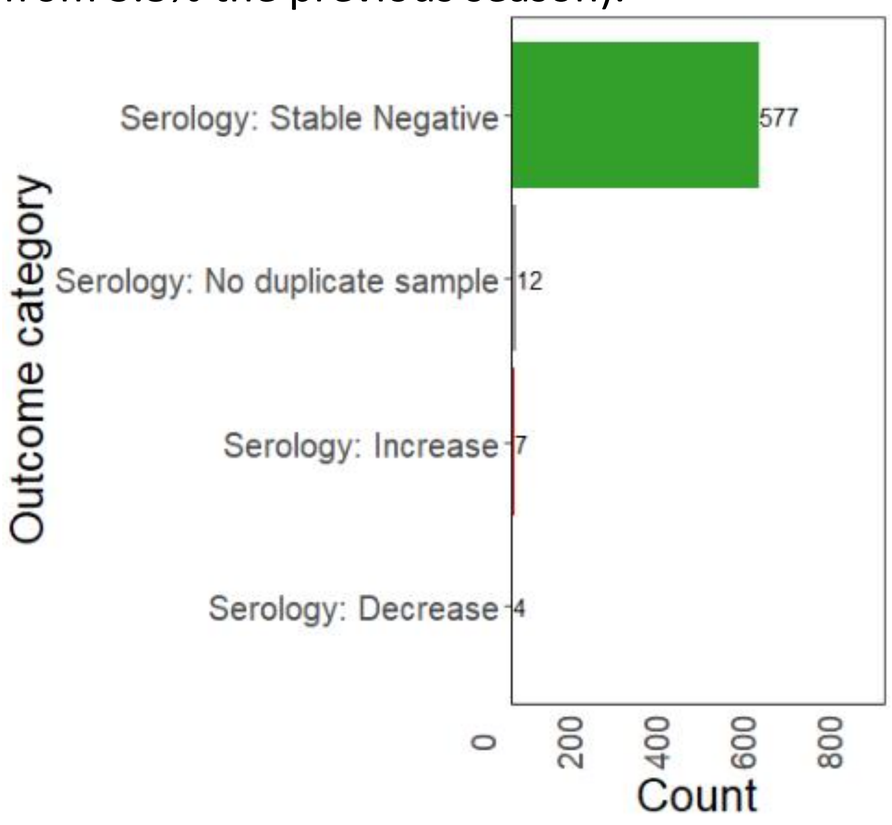

Figure 1: Broad outcomes for serological evaluation for the period under review

PCR

Figure 2 shows the results for the PCR-based surveillance. One sample tested positive (Horse 9797). This horse will be discussed– see individual section below.

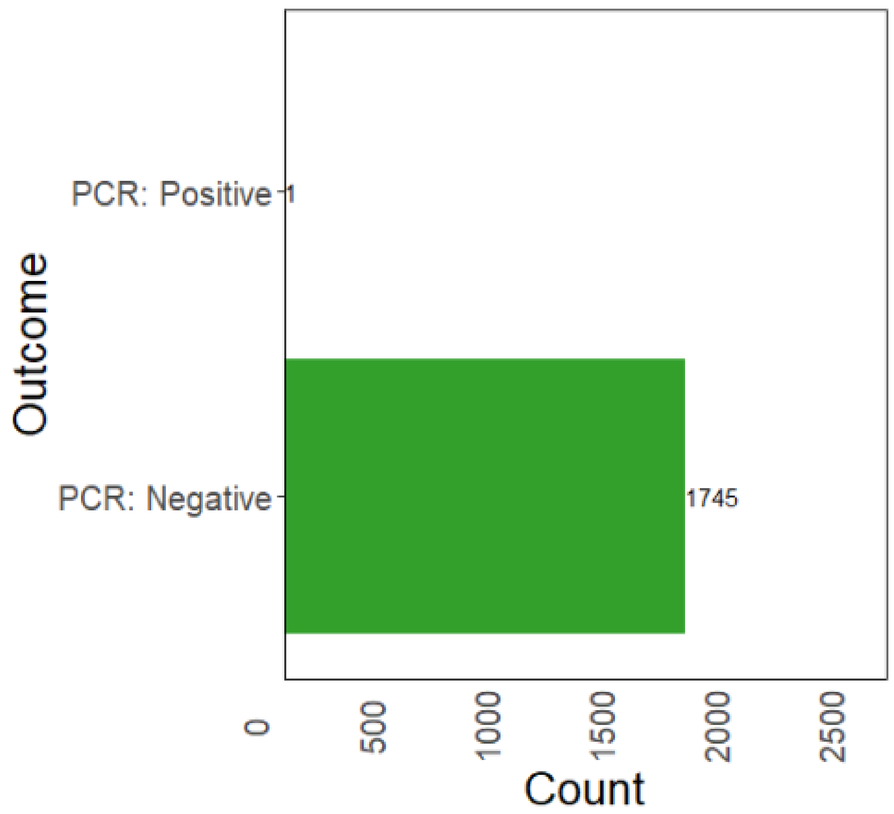

Figure 2: Broad outcomes for PCR evaluation for the period under review.

Results

Follow-up investigations

Like the 2018/2019 season 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 suspect and then weak-positive between February and May 2020.

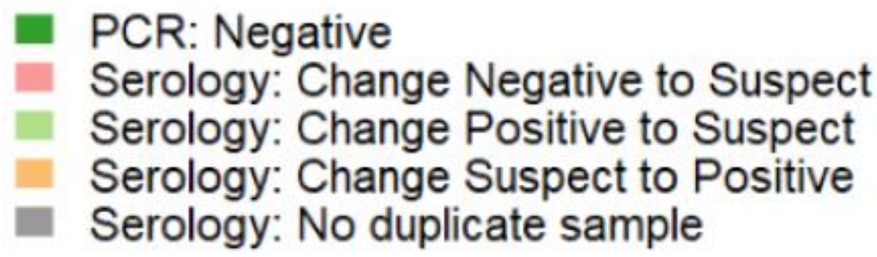

Figure 3: Legend for the individual horse serological and PCR outcomes for the section below

Holding 6020: Horse 28836

Horse 28836 had a changing serological status from negative to suspect, to weak positive and back to suspect between February and June 2020 (Figure 4).

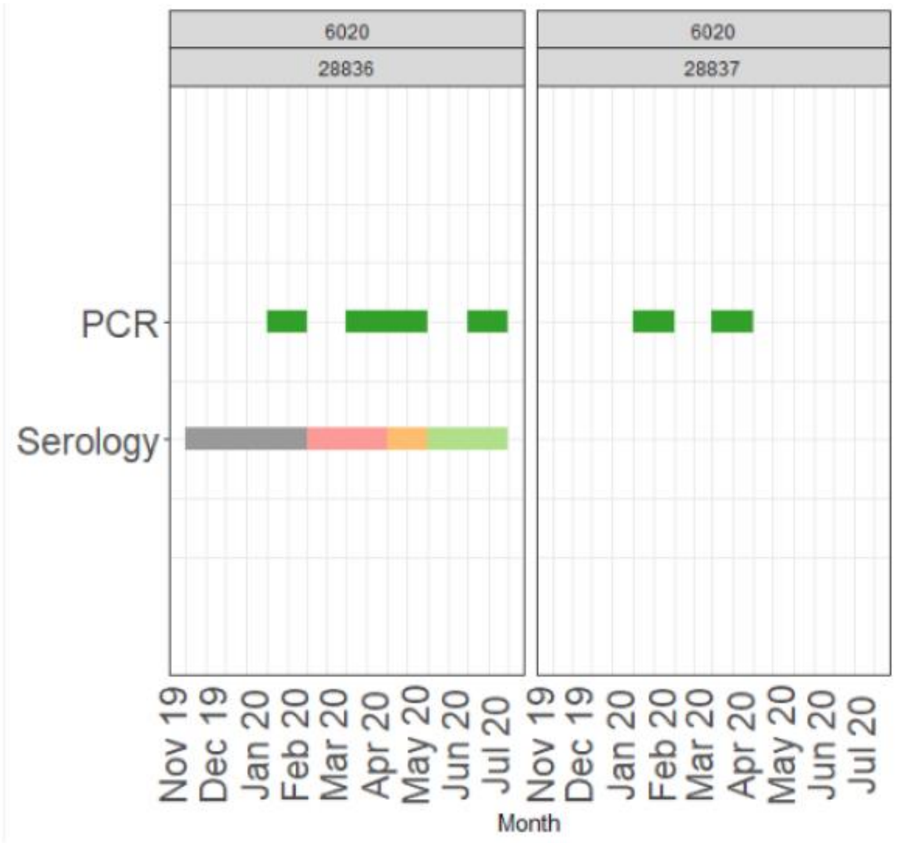

Figure 4: Horse 28836 result series showing the changing ELISA serological status from negative to suspect to positive and back to suspect between Feb and June 2020. Horse 28837 is the other sentinel on the property.

Suspect false positive results on serology do occur from time to time in the program because of the sensitivity of the test. When the horse however tested weak positive on the sample collected in May, a full investigation was undertaken. The affected horse was a 6-year-old Appaloosa mare that had been born in the AHS SZ and to the best of the owner's knowledge it was unvaccinated and had not moved out of the AHS SZ since birth. It and the other horses (n=6 non-sentinels) on the property were tested in mid-July. Other than the suspect result depicted in sentinel 28836 no other samples tested positive.

A trace-back investigation was also undertaken and a radius of 10km around the suspect case was evaluated. Because of COVID-19 movement restrictions there had only been a total of 3 horses moving into the 10 km radius of farm 6020 between 2 weeks prior to the February 2020 sampling event (negative) and the second sample event (suspect) in April 2020. These horses all originated from within the Western Cape Province. Two came from stop over quarantine facilities, and horses moving from these had a negative AHS PCR test prior to movement. The third came from Riversdale, historically, and in 2020, an AHS low risk area, and within ~50 km of the nearest point of entry into the AHS controlled area.

There were an additional 4 sentinel holdings (constituting 12 PCR-sentinels) within 10 km of holding 6020 and results from these holdings were also considered during the investigation. All PCR results during the January – June period were negative – in total 62 sampling events.

The outcome of the investigation in summary: The test results did not indicate that a wild strain AHS virus was responsible for the ELISA suspect and low positive result. The ELISA and SNT levels were low and not what one would expect from an active seroconversion because of AHSV infection. PCR remained negative throughout. Follow-up investigations provided no evidence of suspect or positive AHS infection, and this included active surveillance on both the property affected and surrounding sentinel properties.

In the past in the controlled area AHS vaccine virus has been responsible for several outbreak events. In this case the suspect sero-conversion happened well before the start of the legal vaccination period in the controlled area (1 June 2020), and is unlikely to have been a source of the serological picture seen. Past outbreaks because of vaccine re-assortment or reversion to virulence have also resulted in spread which was not detected in this instance. Finally, the SNT response was not consistent with a either a wildtype or vaccine virus associated response.

Follow-up investigations – Sentinel deaths

No sentinels died during the 2019/2020 season

Follow-up investigations – Other

Horse 8612 tested suspect on ELISA during the months of October and February 2020. This horse has previously had similar results and results indicate either an underlying cross-reaction on serology or residual maternal antibody playing a role in the results noted. The horse has a long history and maintained its negative PCR status throughout the season – it has since been de-recruited as a sero-sentinel and remains on the PCR-only program.

Horse 23470 had a serological profile that reverted between negative, low positive, negative, and finally suspect between May and July 2020. Other sentinels (a further 6) on the property tested consistently negative – 4 of which were sero-sentinels as well as PCR sentinels. Interestingly this horse was EEV positive in April 2020 – whether this plays a role in false-positive AHS ELISA results is not yet known but in future sentinels presenting with similar serological profiles will have prior EDTA tested for EEV.

Horse 9797 had tested both positive on PCR and ELISA in Nov 2019. Investigations revealed that it had been vaccinated illegally in October 2019. While unplanned, this event showed the program detected a 'positive case' albeit because of vaccination. The horse was removed from the sentinel program.

The investigations above account for serological and PCR results that resulted in investigations and are depicted in Figure 1 and Figure 2. There were a further 5 events (4 horses) that required investigation but where results and sample events needed to be removed from the program as a result. **Horse 1530** was incorrectly identified during sampling in Jan 2020. **Horse 5831** was a PCR sentinel whose serum was tested on ELISA in June and July 2020. **Horse 19616** had suspect serological results which were not repeatable on testing by the laboratory and the initial suspect result was retracted. Follow-up samples also tested negative. **Horse 25865** was also a PCR-only sentinel who was tested on serology in Jan 2020.

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.

As discussed below the impact of COVID has resulted in a loss of sampling extent. The areas requiring most improvement remain Paarl and Philadelphia regions for serological sampling. PCR sampling is relatively representative with the Paarl region shy of 7 samples a month on average remaining the worst.

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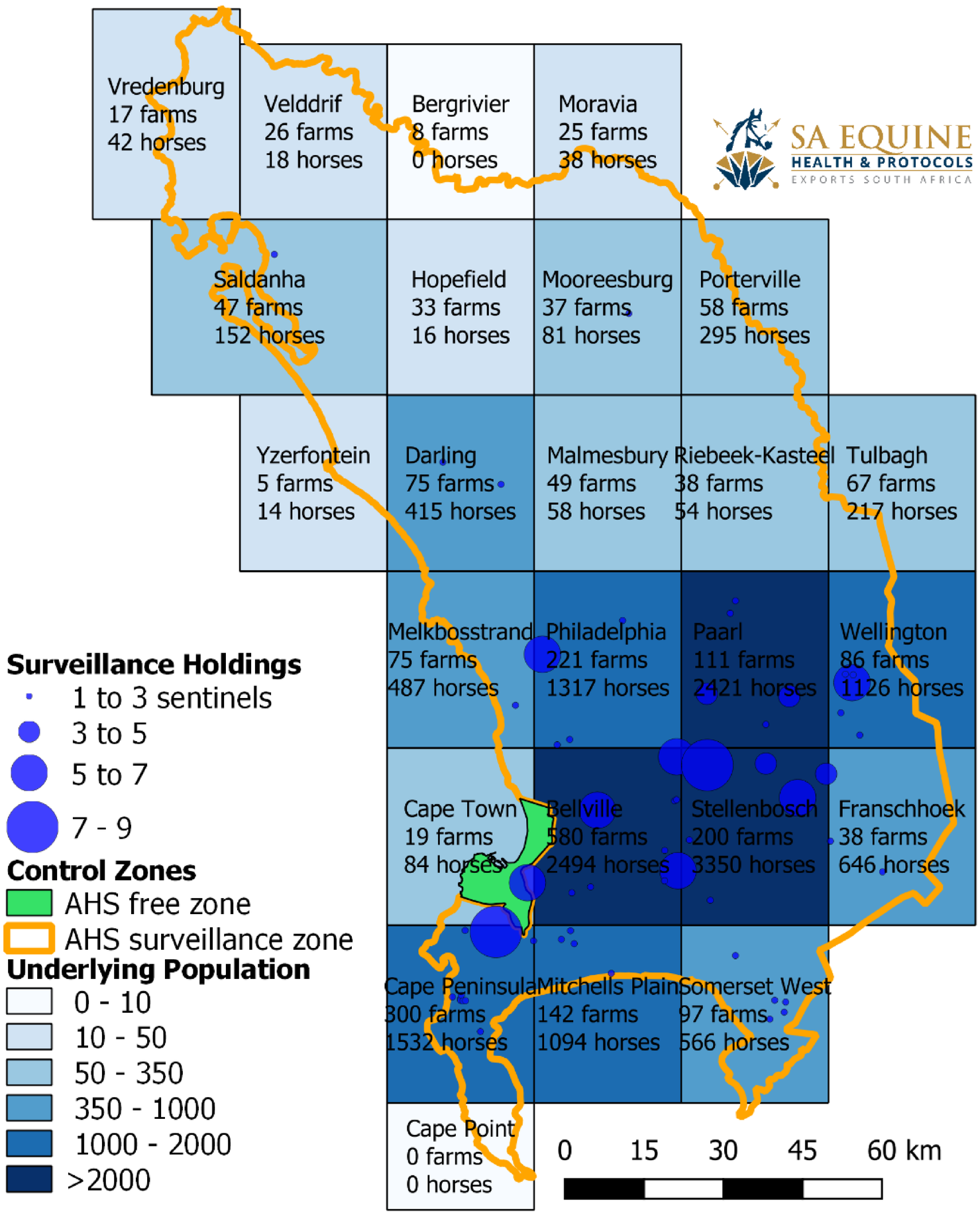

Figure 5: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 September 2020. The proportional circles represent the current sentinel populations.

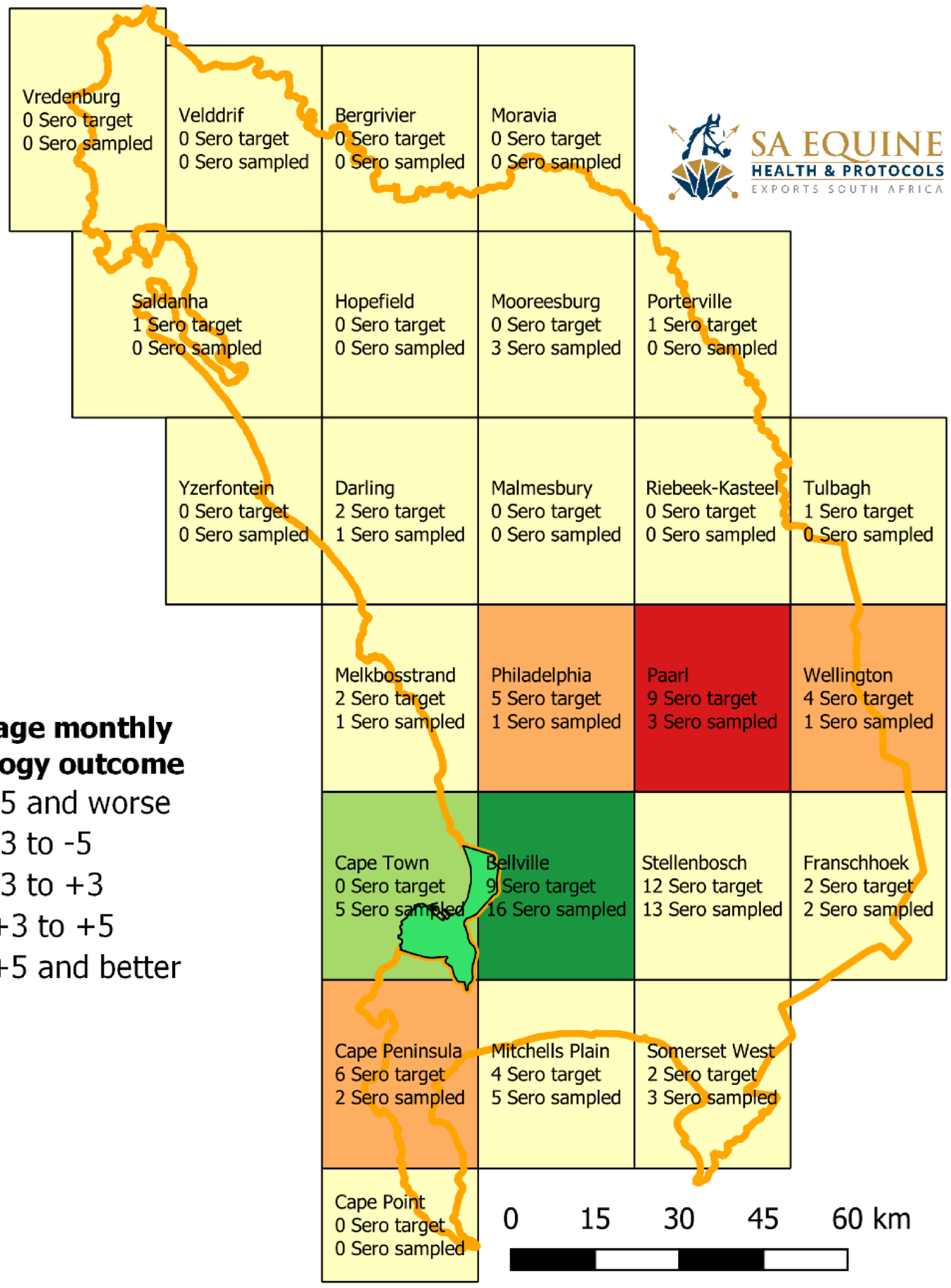

Figure 6: A map showing the AHS surveillance and free zone where sero-sentinel surveillance has taken place for the 2019/2020 season. The map depicts the various areas with their target serology samples to detect a 5% minimum expected prevalence using a proportional sampling frame. The orange areas are 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.

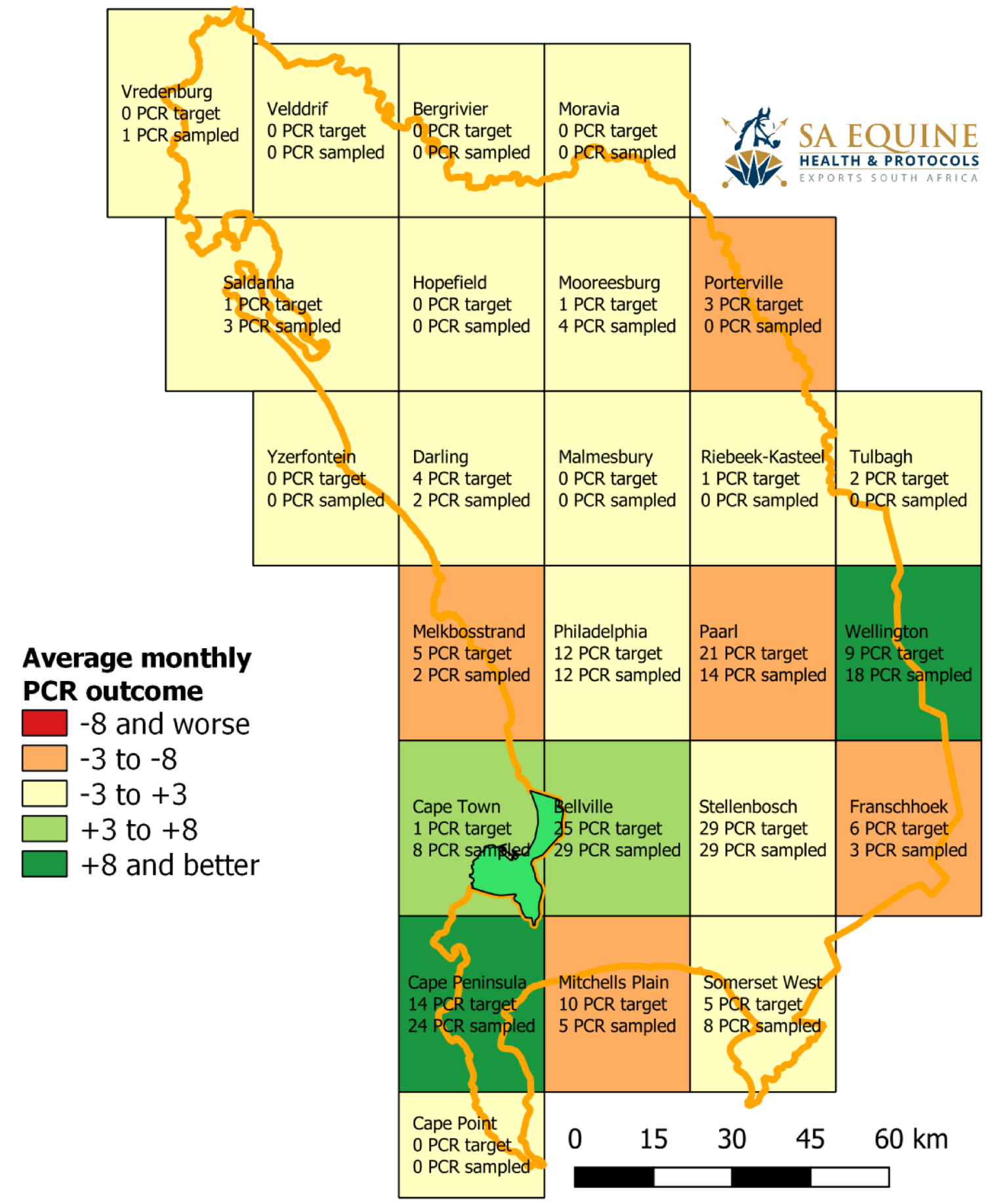

Figure 7: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2019/2020 season. The map depicts the various areas with their target PCR samples to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange areas are areas 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 previous surveillance program is considered as it provides historical information that aids in determining an accurate final probability of freedom as of August 2020. The final probability of freedom at the end of the four-year period (48 months) was 91.3%, a drop of 3% from the previous evaluation (Figure 8).

The sensitivity of the sentinel surveillance alternates around the 30% mark throughout. This is the fourth 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.

Impact of COVID-19 Lockdown

Evaluating the surveillance system gives an insight into the impact that lockdown restrictions had on the ability to detect AHS should it have occurred. While the ability to perform regulatory Veterinary services was considered essential, the ability to move around freely and easily, and the consent for allowing officials onto properties for surveillance was hampered and clearly shows in the number of samples that were taken in April 2020 and after. April 2020 was the month most affected when level 5 lockdown restrictions were in place, and during that month the sensitivity of the program reached a 4 year low of 14.4%. This results in a drop of 56.7% from a pre-COVID April average of 33.18% and a 54.2% drop in sensitivity from the pre-COVID monthly average of 31.42%. The drop in system probability of freedom was affected but because of the ability for prior probability of freedom to inform ongoing probability of freedom the impact was not as dramatic – a drop from 92.6% to 91.15% between March to April 2020 occurred. Relatively though this is high (the visual drop visible in Figure 8 shows it well) – the standard deviation in the plateau phase of probability of freedom between Sept 2018 to December 2019 was 0.45 percentage points.

Overall the 3% drop in probability of freedom relates from Aug 2019 to Aug 2020 relates to the impact of COVID restriction on movement as well as (albeit slightly) on the increase (1259 compared to 1181) in total herds estimated in the surveillance area year on year.

Parameter	Value	Comments
$pIntro$	0.03	Carry over from the previous review and based on historical outbreaks in the region – see the 2018/2019 report for more detail.
Population at risk – total herds	1259	Data captured between 1 April 2016 and 1 Sep 2020 for the AHS surveillance and free zones.
Sentinel farm populations	Various	Based on herd size as of 1 Sep 2020. 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 2020 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

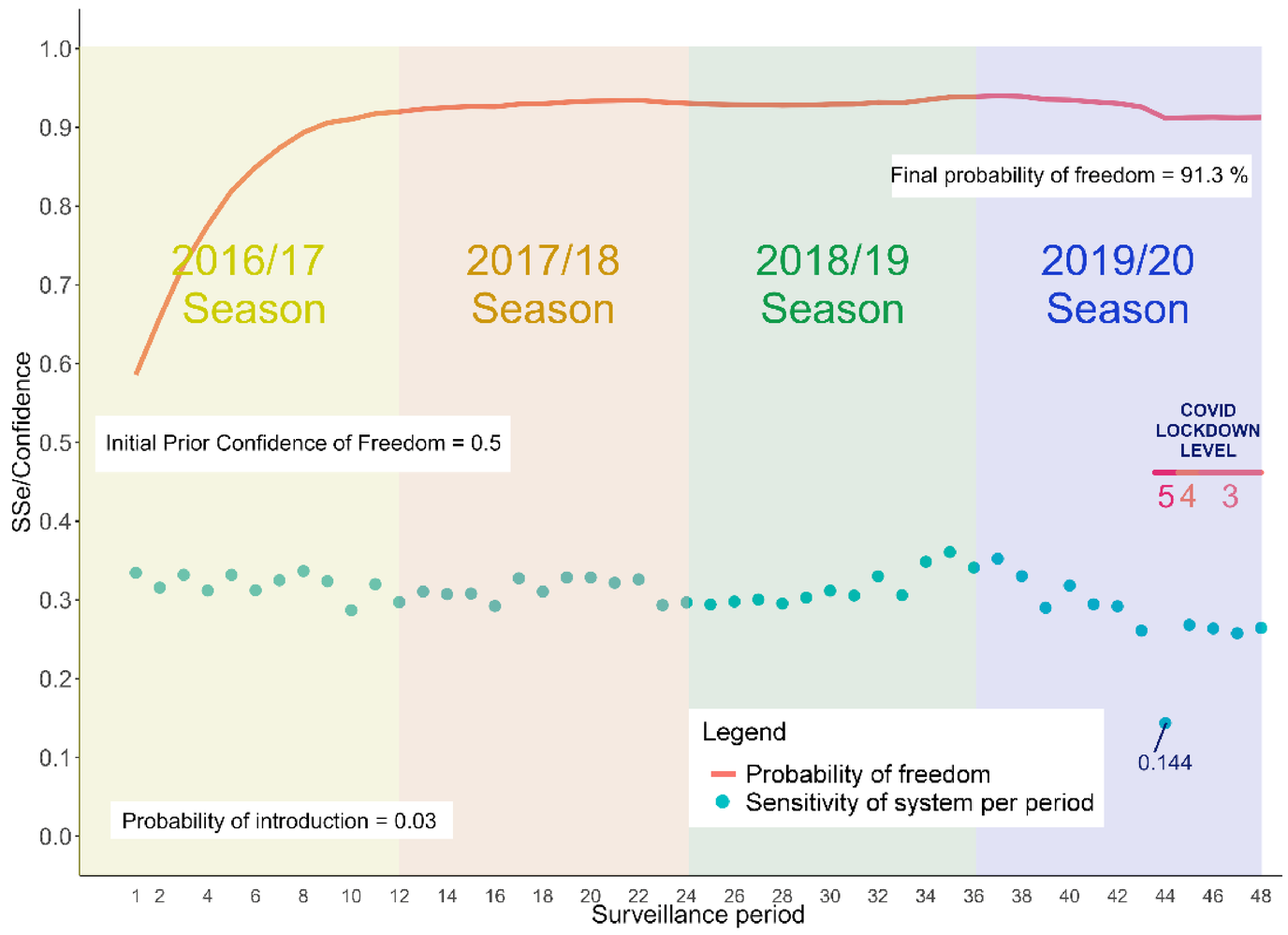

Figure 8: The sentinel surveillance sensitivity of individual surveillance periods (dots) with probability of freedom curve (red line) based on an uninformed 50% prior probability of freedom and a probability of AHS introduction of 3% for the past four surveillance seasons: the season currently reviewed is the right pane – 2019/2020 season running between Sept 2019 and Aug 2020. COVID lockdown periods are also shown starting April 2020 – period 44.

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2019 2020 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 4-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 91.3% probability of freedom from AHS, in the AHS surveillance and free zones, 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, Aliya Davids, Katie Edmonds, Gina Anstey 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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