The AHS sentinel surveillance program 2015-2016 season report

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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 of horses. The program has two programs 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 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 in their recent history resulting 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 analysis of the 2015/2016 sentinel program only incorporates recruited sero-sentinels and as far as possible results used for recruitment screening have been omitted. The serological tests performed rely on the indirect ELISA (i-ELISA) as the base serological test. In this circumstance, it is a non-quantitative assay and changes between the permutations of 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 Equine Research Centre (ERC) in collaboration with their Veterinary Genetics Laboratory. The test used is an ERC developed real-time RT-PCR.

This report covers the 2015/2016 AHS season from 1 September 2015 and 31 August 2016. Very importantly, there was an AHS outbreak in the AHS surveillance zone in April and May (2016) of the season under review. The sentinel program, therefore, is largely academic for establishing a timeline of freedom for this season. The results indicate the progress made through the season, highlight the sensitivity of the surveillance on a monthly basis and confirm the detection of the 2016 Paarl outbreak through the program.

General overview of results

A total of 678 sero-sentinel samples were analysed at an average of 57 samples per month. This was an increase of 5% from the 2014/2015 surveillance period. Of these (Figure 1) 622 could be evaluated as they had relevant paired results – this averages out to 52 sampling events per month. Compared to the 509 analysable serological events of the 2014/2015 season there is an increase in this season of 22%.

A total of 1945 PCR sentinel samples were analysed at an average of 162 per month (where the target is 150), an increase of 27% from the previous season. A total of 79 farms were visited during the season, compared to 65 in 2014/2015. The median number of horses per farm was three, with a range of 1-10.

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 56 samples (8% of the total). This compared to 2014/2015 where 137 samples could not be evaluated (21% of the total) although the 2014/2015 evaluation included a higher proportion of recruitment serology tests, inflating the "*No duplicate sample*" classification. A total of 8 serology evaluations indicated an increase in status warranting investigation – these will be dealt with individually below.

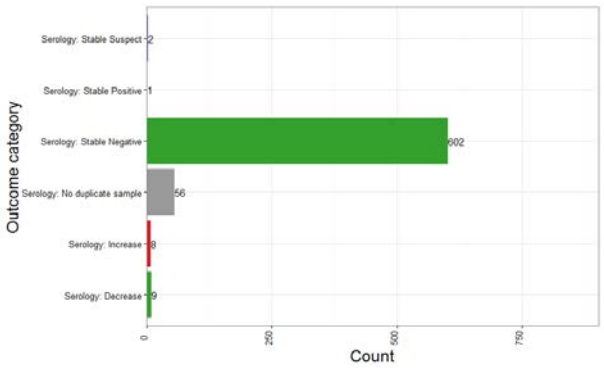

Figure 1: Broad outcomes for serological evaluation for the period under review. Increasing serology incorporates both the negative to suspect/positive and the suspect to positive permutations for serological change across paired samples.

PCR

Figure 2 shows the results for the PCR-based surveillance. By far the majority of results were negative on PCR with 3 positives originating from 2 horses that were infected during the Paarl 2016 AHS outbreak.

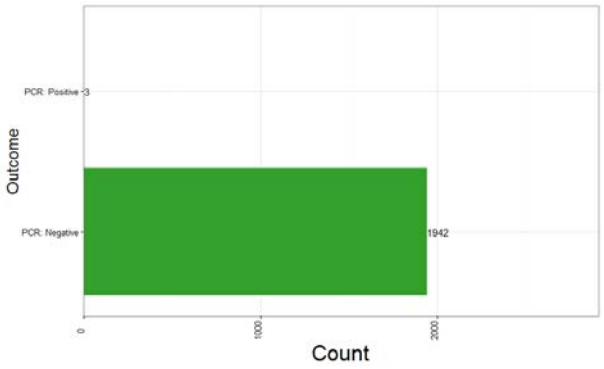

Figure 2: Broad outcomes for PCR evaluation for the period under review. All three positive results (from 2 horses) were part of the 2016 Paarl outbreak which will be discussed below

Results: Increasing sero-status /PCR Positive

A total of 8 serological evaluations returned an increasing serological status for the sentinel in question. These 8 events encompassed 6 horses with one horse (see below) accounting for 3 increasing events.

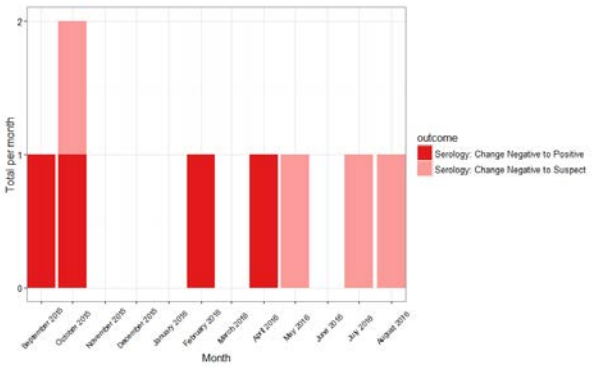

Figure 3: Increasing serological status across the surveillance period.

The April and May 2016 status changes could not be linked to the Paarl 2016 outbreak. This graph differs quite significantly to that of the 2014/2015 report (Figure 8 of that report) because it only encompasses non-recruitment sampling.

The PCR positive results (n=3) were detected in 2 horses in May and June and they are graphically represented in Figure 20 below as white stars.

The section below highlights the evaluation of the 8 horses that had results that triggered specific, detailed evaluation.

Individual horse evaluations

Note: In this section, the date series on the x-axis of the presented graphs are specific to the data available for the individual horse, and date series' do not encompass the entire surveillance period in each case

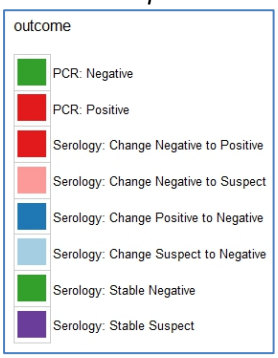

Figure 4: Legend for the individual horse serological and PCR outcomes for the section below

Owner 14: Horse 220

This horse was recruited in April 2016 with a negative serological result (this result would not have had a paired sample hence the greyed "*no duplicate sample*" outcome for April 2016. It, however, had a very low suspect result in May 2016 (i-ELISA percentage positive of 6 with suspect results between 5 and 10) which reverted to negative in June 2016 (Figure 5). It remained negative on PCR throughout and was unfortunately vaccinated in July 2016 and was therefore lost to the program. There were no other sentinels on the farm in question and no other sentinel properties in close proximity. The low suspect result, lack of history of testing and negative PCR throughout resulted in this sentinel not been considered as a positive case.

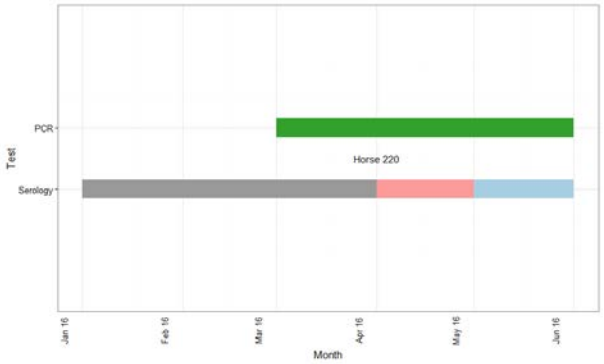

Figure 5: Horse 220 result series

Owner 66: Horse 722 and 723

Both sentinels 722 and 723 were PCR sentinels only and both were positive cases in the Paarl 2016 African horse sickness outbreak. 722 tested positive in May 2016 and 723 tested positive in both May and June 2016 (Figure 6 and Figure 7). These two sentinels showed that the sentinel program would have detected the 2016 AHS outbreak.

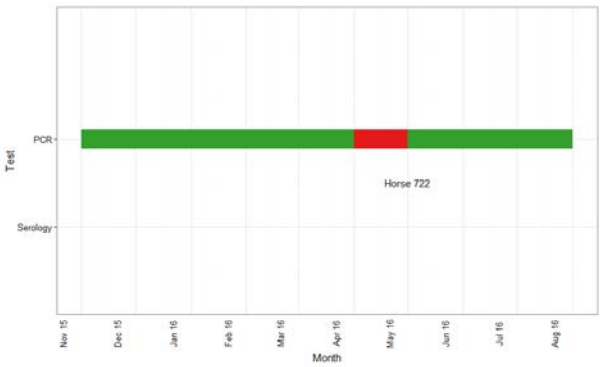

Figure 6: Horse 722 result series

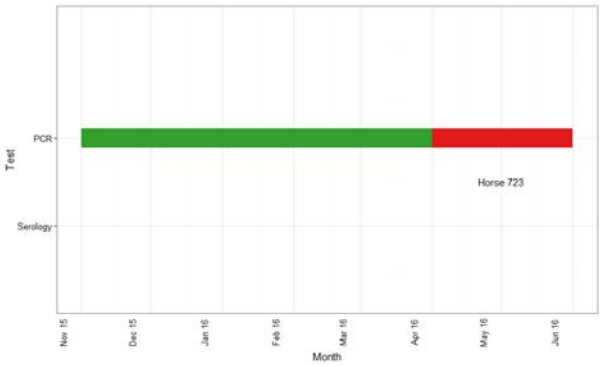

Figure 7: Horse 723 result series

Out of interest, there were another 8 sentinels on the property; all bar one were PCR only sentinels (Figure 8). None tested positive during the surveillance season, including the Paarl 2016 outbreak period. The sero-sentinel (horse 1693) unfortunately relocated during that period so no follow-up data could be analysed in that case.

Figure 8: The farm sentinel cohort containing both sentinels #722 and 723.

Owner 73: Horse 1509

This horse had consistently tested negative to AHS (both on serology and PCR). In April 2016 it had a serological status increase from negative to positive, with a drop immediately to negative in May (Figure 9). SNT was performed on the April sample and types 1,2,5,7,8 were all positive. While no conclusion could be made as to the origin of the positive result, the outcome is not indicative of active infection where one would expect a single serotype to dominate with ongoing positive serology results and a positive PCR result early on in the process. The farm is located approximately 40 km from the Paarl 2016 outbreak events, towards the Atlantic coast. PCR remained negative throughout. All other sentinels on the property (n=4 see Figure 10) tested negative throughout – they were all PCR sentinels only. The horse is reportedly previously vaccinated although a date cannot be provided (it is a 12 year old mare). It has been testing consistently negative as a sentinel, starting in Sept 2014, on both serology and PCR.

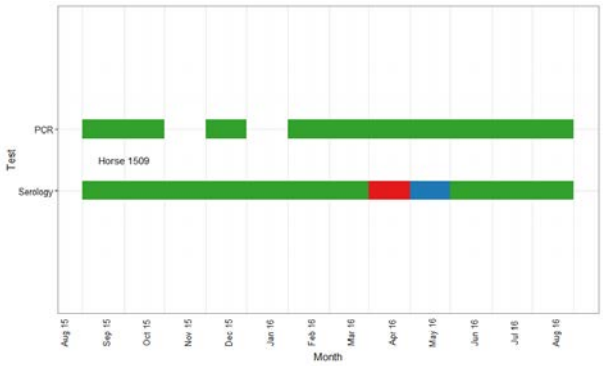

Figure 9: Horse 1509 result series

Figure 10: The farm sentinel cohort associated with horse 1509.

Owner 79: Horse 1536

A single serological change from negative to positive occurred in September 2015 (Figure 11). The i-ELISA was repeated with similar, relatively low positive percentage (21 and 14) results. SNT was performed: it was positive on AHS serotypes 7,8,9. The PCR remained negative throughout and the horse did not test serologically positive again. It relocated in March 2016 and was lost to the program. Within the farm cohort, there were another 7 sentinels, 4 of which were also sero-sentinels. At no time through the year were positive results obtained from any of these horses (Figure 12).

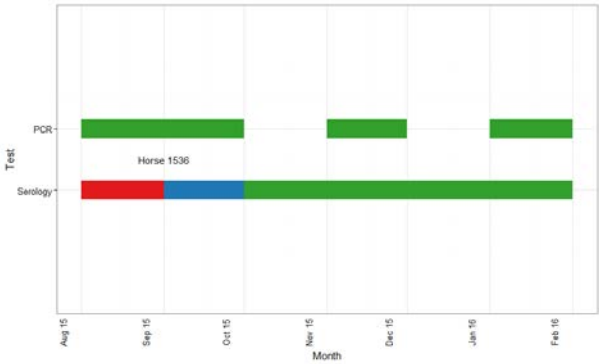

Figure 11: Horse 1536 result series

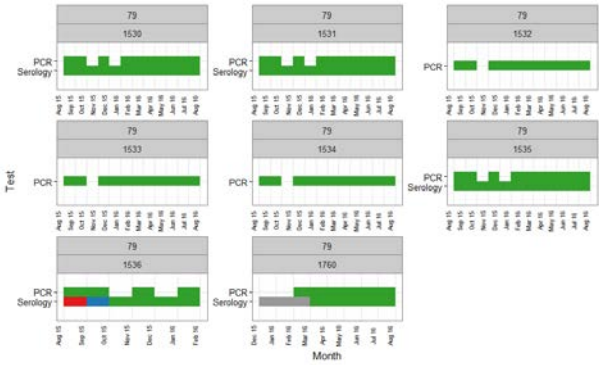

Figure 12: The farm sentinel cohort associated with horse 1536.

Owner 80: Horse 1541

Horse 1541 had fluctuating serological levels between negative, suspect and positive throughout the 2015/2016 season. This had occurred in the previous season as well and in retrospect, this horse was not appropriate as a sero-sentinel. At no point during the season did it have positive PCR results and it has subsequently been removed as a sero-sentinel for the 2016/2017 season. There were another 8 sentinels (all PCR only) on the farm and none of these returned positive results.

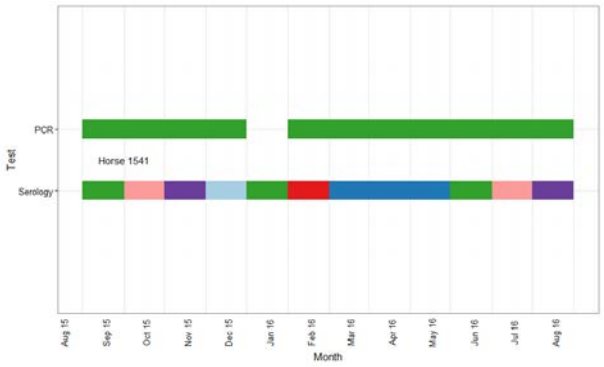

Figure 13: Horse 1541 result series

Owner 109: Horse 1633

Horse 1633 is both a sero- and PCR sentinel with a long history of negative testing in the sentinel program. In August 2016 (the final month of the period under review) it changed serological status from negative to suspect (Figure 14). The i-ELISA result in question had a positive percentage value of 5 where values between 5-10 are considered suspect. The horse tested negative on PCR on the same date and has a history of negative PCR testing.

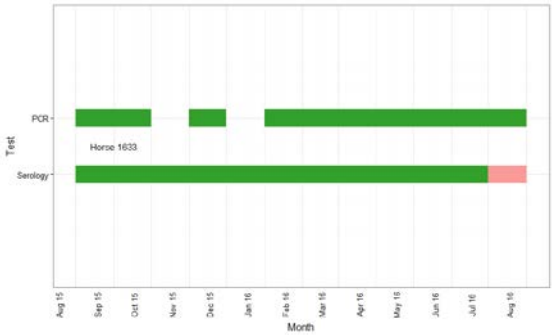

Figure 14: Horse 1633 result series

There are a further 7 sentinels on the property (Figure 15), three of which were also active sentinels during the month in question. None tested positive on PCR during July or August 2016. Furthermore, there are another two PCR sentinels on two farms within 1 km of the property, both of which tested negative on PCR during August 2016.

Given the low suspect i-ELISA result and negative PCR throughout for the suspect horse and the negative PCR results from the cohort and sentinels in immediate vicinity, this horse is not considered a potential AHS case for the 2015/2016 season Follow-up testing of the horses duplicate serum sample for August is being undertaken and the horse has already been sampled for the September 2016 sentinel period. (Duplicate samples are stored should case follow up be indicated).

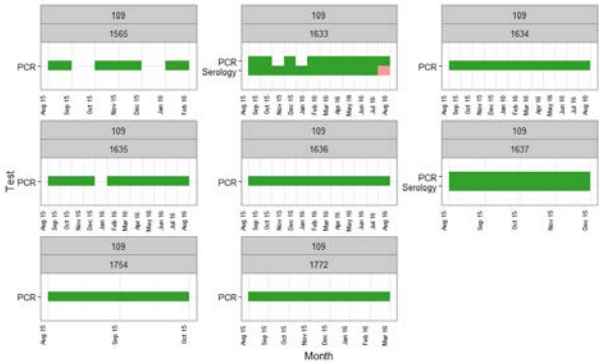

Figure 15: The farm sentinel cohort associated with horse 1633

Owner 1121: Horse 1645

Horse 1645 is equivalent to horse 275 from the 2014/2015 season reported in the January 2016 Western Cape Epidemiology report (the numbering system for horses has changed since then hence the change in ID). In that report, the horse had a negative to positive serological status change in the July-August 2015 period. At the start of the current season under review showed the horse reverted back to negative, again changed to positive in October 2015 and then reverted back to negative and stayed negative from November 2015 onwards (Figure 16). The PCR results for this horse remained negative throughout, as did those of the two other PCR sentinels on the property (Figure 17). Given the erratic fluctuations in late 2015, this horse was changed to a PCR-only sentinel in February 2016. While SNT evaluations were unfortunately not performed on this horse it is not considered an AHS case for the 2015/2016 period of evaluation based on the fluctuations in serology and negative PCR results in the cohort sentinels.

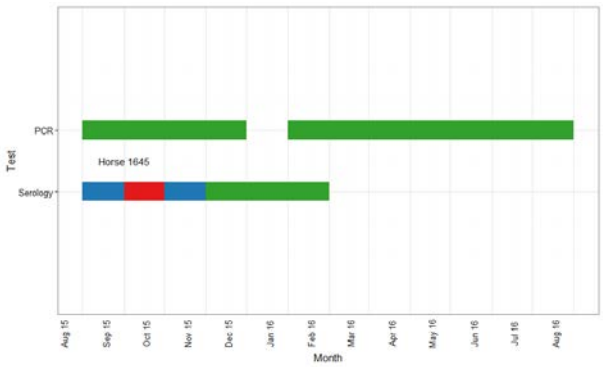

Figure 16: Horse 1645 result series

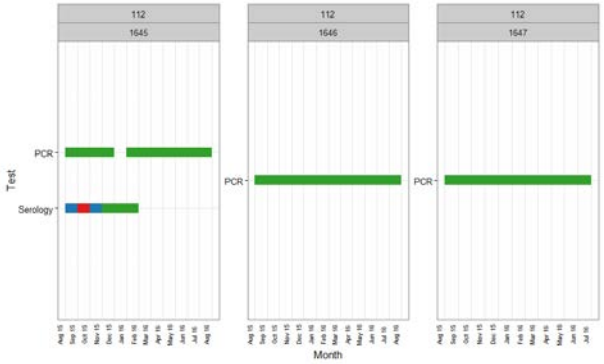

Figure 17: The farm sentinel cohort associated with horse 1645

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.

Figure 18 and Figure 19 shows the monthly average distribution of sentinels used for the sero and PCR sentinel programs respectively. The sero-sentinel areas where improvement can be made are in Paarl and Darling. The deficit of sero-sentinels in these areas was between -3 and -9 sentinels per month. In general, the overall deficit per area averaged out at -0.2 and this does highlight the difficulty in recruiting sero-sentinels, hence the use of PCR testing in the surveillance program. At worst, the PCR sentinel areas have a deficit of -2 PCR sentinels per month.

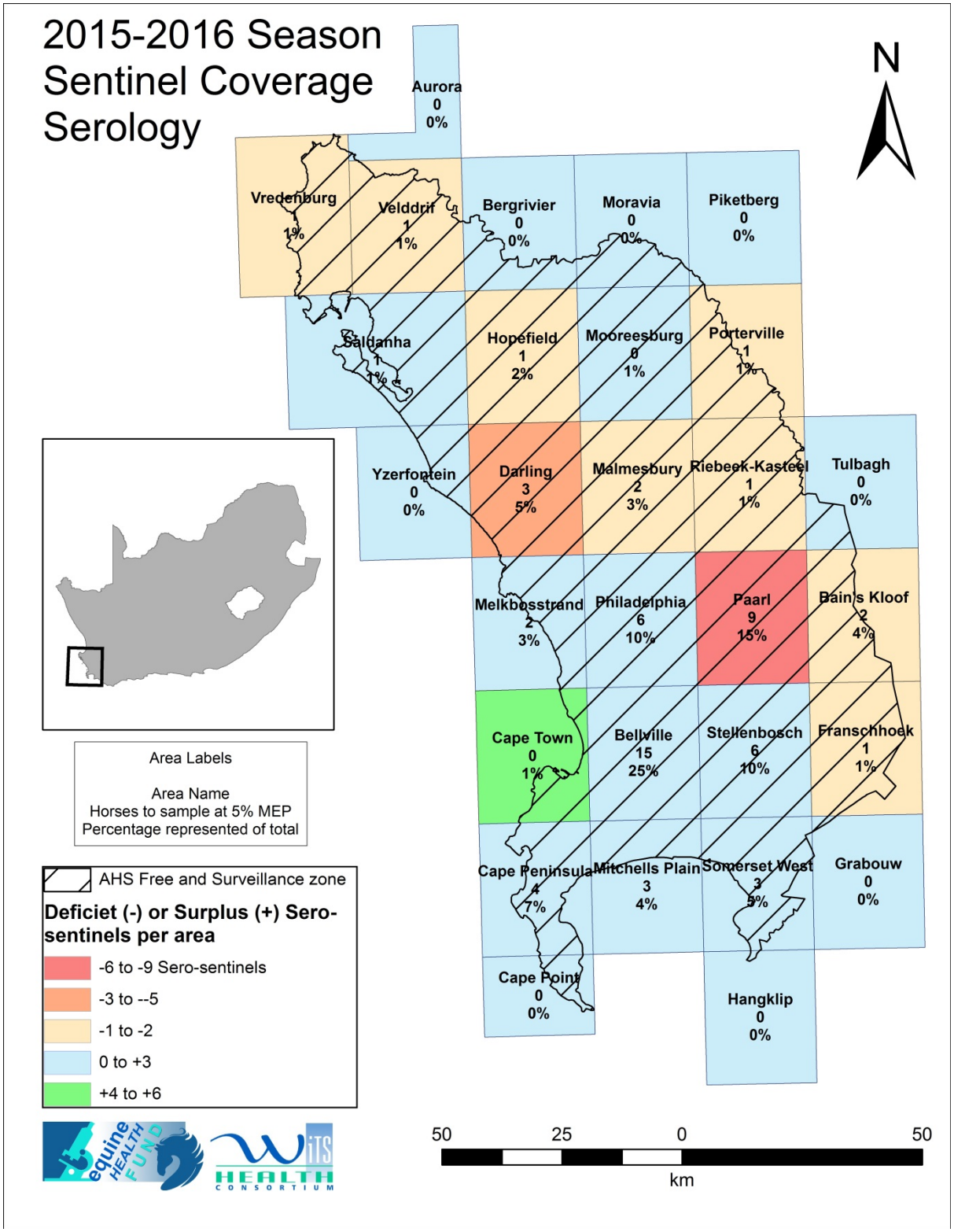

Figure 18: A map showing the AHS surveillance and free zone where SERO-sentinel surveillance has taken place for the 2015/2016 season. The map depicts the various areas with their estimated number of horses labelled that are required to be sampled to detect a 5% minimum expected prevalence using a proportional sampling frame. The yellow to red areas are areas where SERO-sentinels were lacking while the blue to green areas show where surplus SERO-sentinels were sampled.

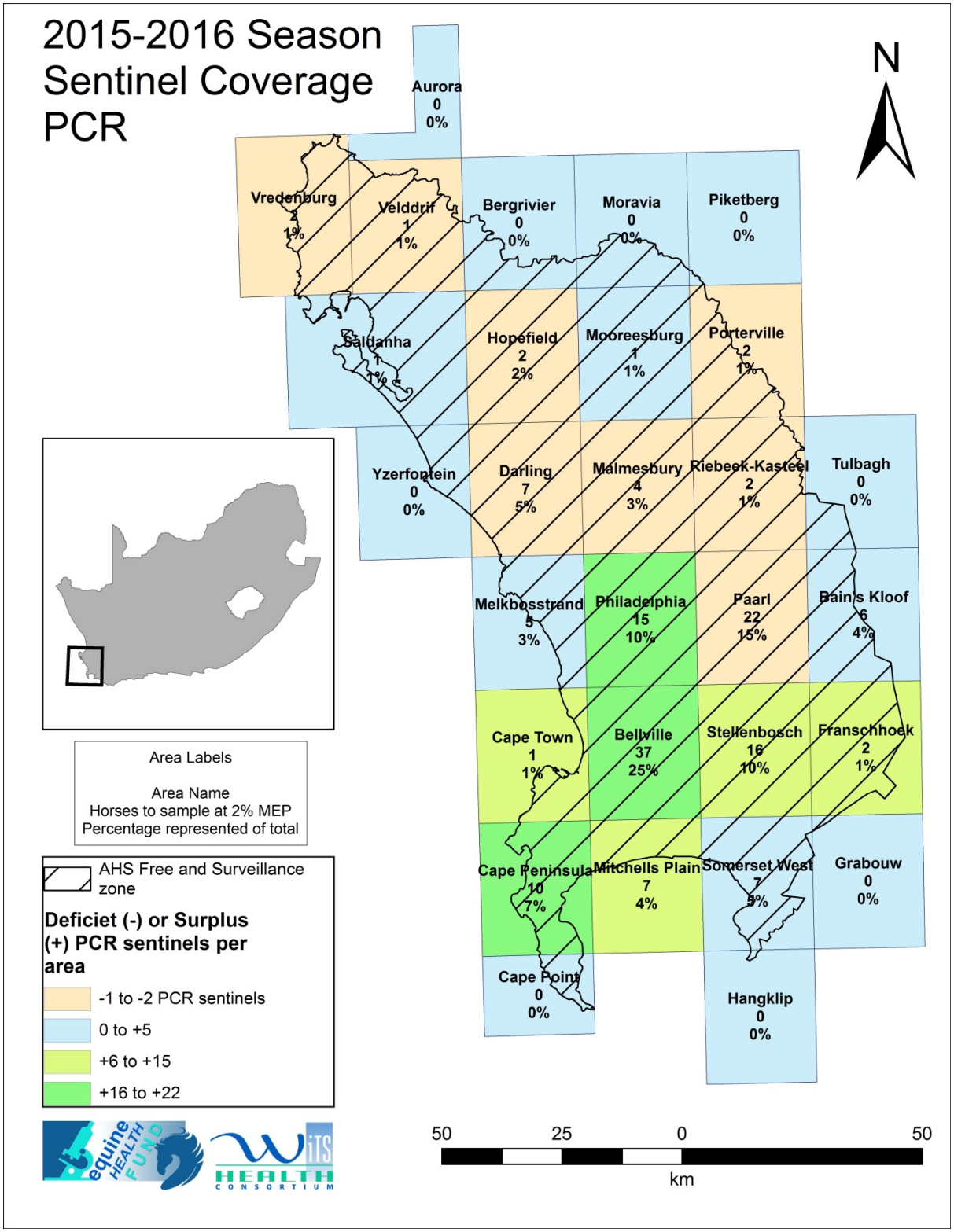

Figure 19: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2015/2016 season. The map depicts the various areas with their estimated number of horses labelled that are required to be sampled to detect a 2% minimum expected prevalence using a proportional sampling frame. The light orange areas are areas where PCR-sentinels were lacking (max of -2 per month) while the blue to green areas show where surplus PCR-sentinels were sampled.

Detection targets of surveillance

The detection target of the 2015/2016 surveillance program is a relatively academic discussion since the Paarl 2016 AHS outbreak occurred in the AHS surveillance zone. What is important is that the PCR-based aspect of the surveillance program would have detected the Paarl 2016 outbreak if it had been missed on passive surveillance. The target levels for both the sero- and PCR aspects of the sentinel program were generally attained (Figure 20). The exception in the sero-sentinel program (section A Figure 20) was in October, November (2015) and May (2016). The reduced sampling in May 2016 was as a result of the 2016 Paarl AHS outbreak. The exceptions in the PCR surveillance were in November 2015 and January 2016. The white stars in Figure 20, Section B, indicate the positive AHS cases detected in two PCR sentinels.

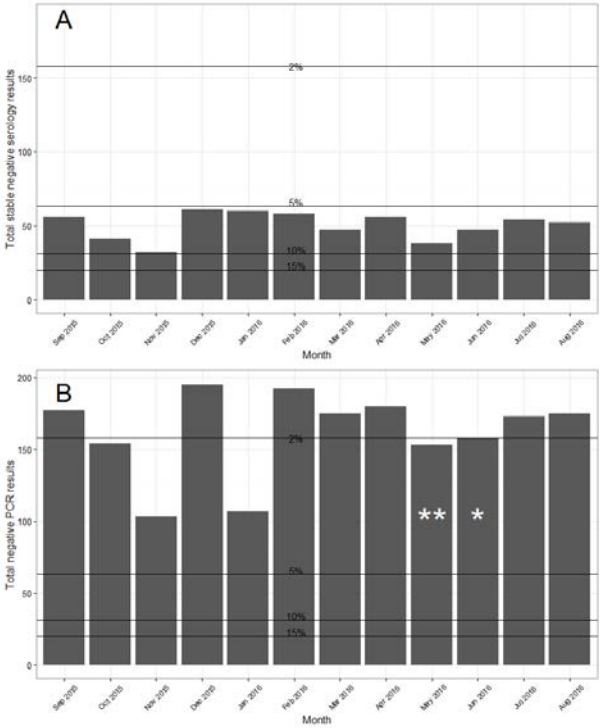

Figure 20: A: Stable negative serological outcomes and B: negative PCR outcomes per month for the period under review. The white stars indicate the positive PCR results (n=3 from 2 horses) that occurred as a result of sentinels testing positive on PCR during the Paarl 2016 outbreak. The horisontal lines indicate the number of samples that would need to be taken to have a 95% confidence that we would detect AHS at the prevalence indicated – i.e. 2%, 5%, 10% and 15% respectively

Figure 21 shows unique sentinels tested in either the serology stream or the PCR stream (no duplicated horses per month) indicating the overall minimum expected prevalence attained (should AHS have not occurred) of the program across the board. The only month where there was a lack of sampling across both programs was in November 2015.

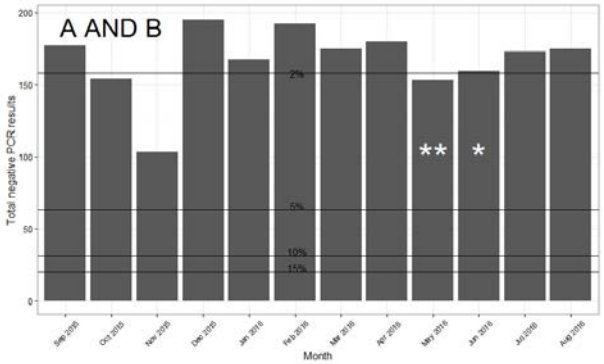

Figure 21: A merger of Figure 20: A and B showing the overall sensitivity of the program per month. Only negative PCR and stable serology considered and sentinels only represented once per month (no duplicated totals for individuals that were both sero- and PCR sentinels concurrently. White stars indicate positive PCR results obtained in May and June 2016.

Discussion and Conclusion

The primary goal of the sentinel surveillance program for 2015/2016 of showing freedom of AHS could not be achieved since there was an outbreak of AHS in Paarl in April/May 2016. What is relevant is the fact that the sentinel surveillance system would have detected this outbreak.

The recruitment of sero-sentinels remains a challenge (See Figure 20 A and Map Figure 18) and this program currently relies on the parallel PCR surveillance system, not only to improve sensitivity but to assist in evaluating positive serological results, given the history of fluctuating results sometimes seen in the serological surveillance.

There have been significant improvements over the past year with regards to the sentinel program. The analysable serological results have increased by 22%. The PCR program

involved the testing of 27% more samples compared to 2014/2015.

8 serological events from 6 different horses required follow up evaluations. None of these indicated a positive case of African horse sickness. The PCR program detected 2 cases of African horse sickness in May and June 2016.

A review of the laboratory processes has been made with regards to the sentinel program. Figure 22 shows the standardised process that will be followed in the coming season for any positive results that are obtained from the two participating laboratories. The sentinel program is managed by a surveillance team that will request follow-up if deemed necessary on a case by case basis. The serological follow-up will be focused on using SNT to type the antibodies, while the PCR follow-up will be focused on typing through type-specific PCR and post isolation plaque inhibition testing as well as sequencing which will assist in differentiating infected from vaccine based positive results. Sequencing is resource intensive and will be undertaken on a case by case basis.

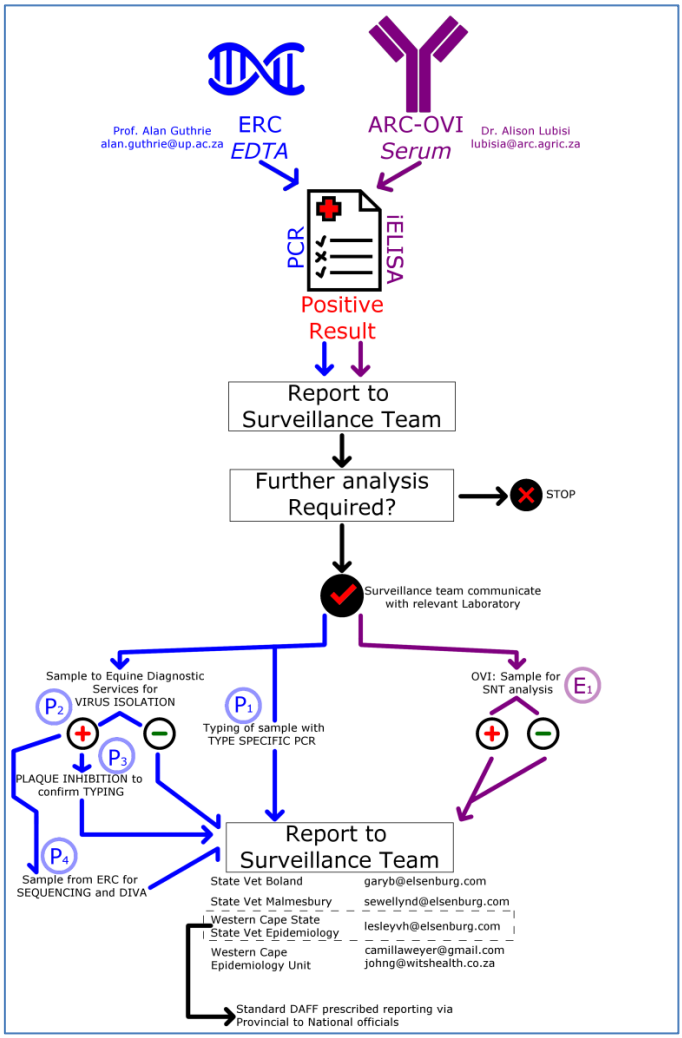

Figure 22: Laboratory processes to follow if positive results are obtained within the sentinel program.

References and Acknowledgements

This work would not be possible without funding from the Equine Health Fund (Wits Health Consortium) and the Government of South Africa. The primary laboratories involved are the Equine Research Centre (Veterinary Genetics Laboratory) and the ARC – OVI, and this surveillance would not be possible without timeous results from them.

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