Western Cape Government

EPIDEMIOLOGY REPORT

Agriculture
VETERINARY SERVICES
July 2023
Volume 15 Issue 7

African horse sickness sentinel surveillance 2022

Adapted from the African horse sickness control: Sentinel surveillance report 2022 by John Grewar, Camilla Weyer and Lesley van Helden

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 does not influence their recruitment unless vaccination against AHS took place sufficiently recently to result in positive PCR results on initial testing.

This report covers 1 September 2021 to 31 December 2022. The results confirm that it is unlikely that AHS was circulating in the AHS free and surveillance zone during this period.

The serological test performed is the indirect ELISA (Maree & Paweska 2005). It is a non-quantitative assay and changes 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 Stellenbosch Provincial Veterinary Laboratory (SPVL) with the final month of evaluation's samples tested at the University of Pretoria/Equine Research Center's Molecular Diagnostics Laboratory. The test method used is a University of Pretoria (Equine Research Center) developed and OIE validated real-time RT-PCR (Guthrie et al. 2013).

General overview of sampling

A total of 782 sero-sentinel samples were analysed from 39 different farms, at an average of 49 samples from 25 different farms per month. Of the tested serological samples: 729 (average of 45 per month) could be evaluated as they had relevant paired results (Figure 1).

A total of 2267 PCR sentinel samples were analysed from 73 different farms, at an average of 142 samples from, on average, 50 different farms per month. Average numbers per month were improvements on both serology and PCR compared to the previous season's evaluation.

Results

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 25 samples (3.1% of the total, an increase from 2.6% the previous season).

All samples for the PCR-based surveillance tested negative.

Follow-up investigations

Non-specific ELISA positives

All cases in which positive serological results were obtained were thoroughly investigated and no evidence of infection with African horse sickness virus was found. Details of these investigations are available in the full report, accessible here.

Sentinel fevers

Horse 26561, a sentinel since March 2020, had a fever of over 40 ºC in October 2021. It and a stablemate that also had fever were sampled and tested negative for AHSV and EEV on 15 October. The horse maintained this

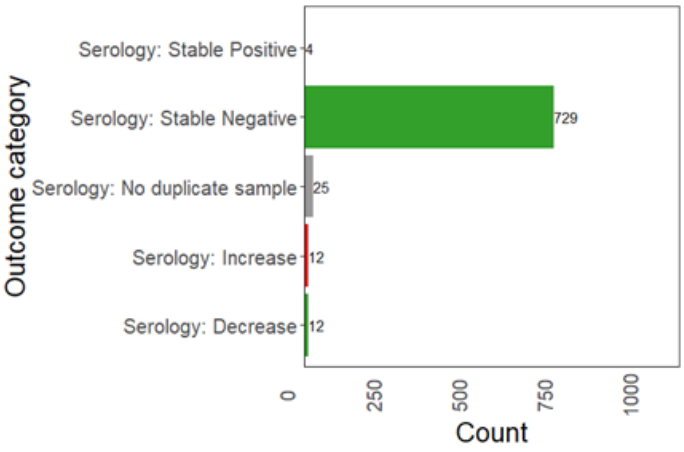

Figure 1: Broad outcomes for serological evaluation for the period under review

negative status throughout the rest of 2021 and 2022.

Horses 34072 and 24273 both had fever and were lethargic in April 2022 and were tested for both AHSV and EEV. The AHSV results were negative, but both were positive for EEV with Ct values of 28.9 and 26.9 respectively. They continued to test AHSV negative for the remainder of the surveillance season.

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 2 shows the monthly average distribution of sentinels in the PCR sentinel programs.

The areas requiring most improvement remain Paarl, Mitchells Plain and Philadelphia (which lacked serum samples only).

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 the full report and analysis is based on evaluating sensitivity of surveillance programs (Martin et al. 2007). The historical surveillance outcome is considered as it provides information that aids in determining an accurate final probability of freedom as of December 2022. The final probability of freedom from Sept 2016 through December 2022 (76 months) was 91.5%, an increase of 17% from the 74.5% of the previous evaluation (Figure 3).

The sensitivity of the sentinel surveillance alternates around the 30% mark throughout. This is the sixth AHS season running where cases of the disease have not been detected in the AHS surveillance and free area, although an outbreak of AHS occurred in the AHS protection zone in 2021.

Discussion and Conclusion

The primary goal of demonstrating AHS freedom for the 2021/2022 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 6-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 91.5% probability of freedom from AHS in the AHS surveillance and free zones. This level was achieved in the face of the AHS outbreak that occurred ~ 88km from the border of the AHS surveillance zone in 2021.

Spatial representativeness remains challenging. The target minimum prevalence of 5% has however been achieved using EDTA sampling and PCR testing; the goal remains however to get as close to the 2% MEP level as often as possible.

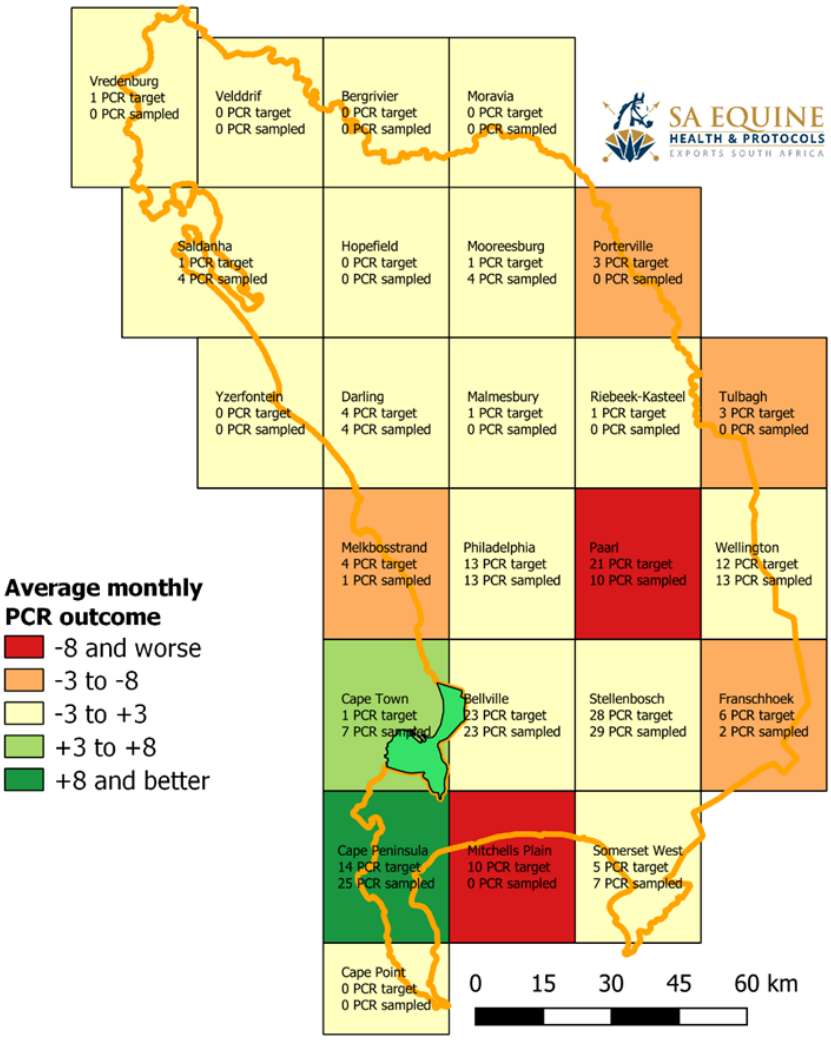

Figure 2: A map showing the AHS surveillance and free zone where PCR-sentinel surveillance has taken place for the 2021/2022 season. The map depicts the various areas with their target PCR samples to detect a 2% minimum expected prevalence using a proportional sampling frame. The orange areas are areas 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.

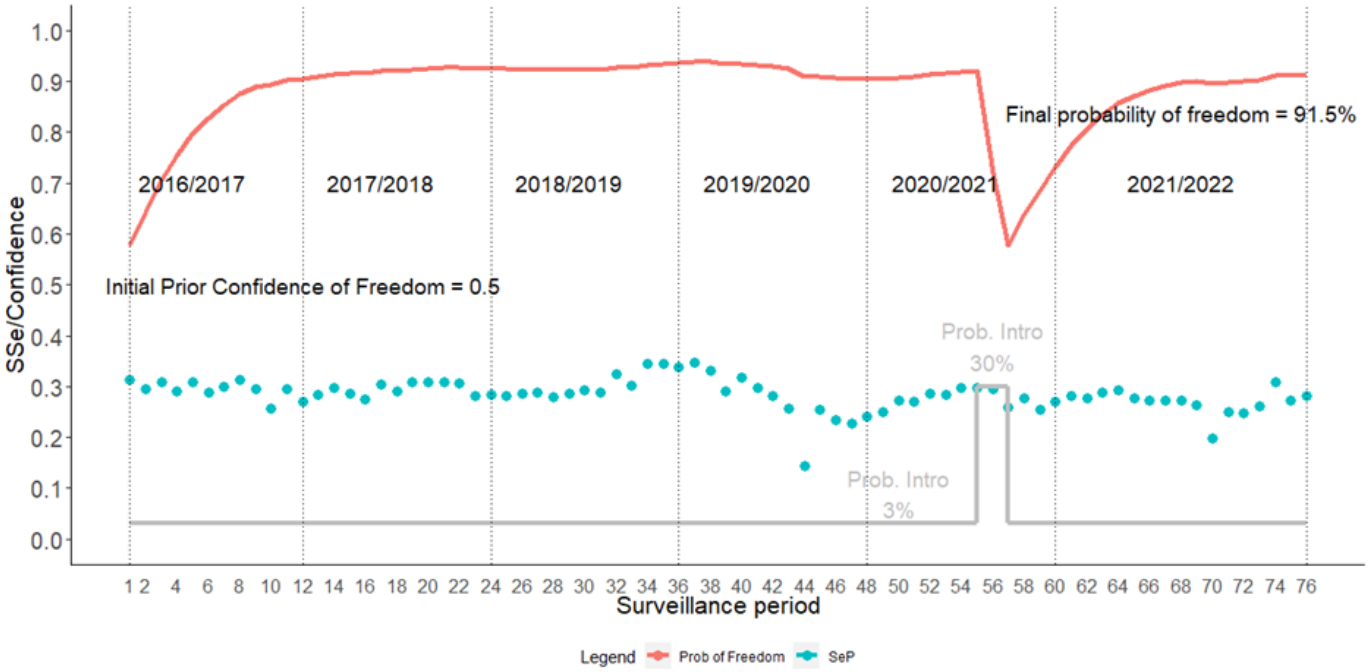

Figure 3: The sentinel surveillance sensitivity of individual surveillance periods (dots) with probability of freedom curve (red line) based on a non-informative 50% prior probability of freedom for the past six surveillance seasons: the season currently reviewed is the right pane – i.e. the 2021/2022 season running between September 2021 and December 2022. Probability of AHS introduction of 3% is set for periods where no AHS outbreaks are present in the AHS controlled area (grey line at 0.03 on y-axis) but at 10X that rate for where outbreaks are present as in April and May 2021 in the Cederberg AHS Protection zone.

Outbreak events

The carcass of a **Hewitt's red rock hare** was found next to the road between Matjiesfontein and **Touwsrivier**. Samples from the carcass subsequently tested positive for **rabbit haemorrhagic disease** virus. On a farm near **Oudtshoorn**, eight out of 20 **domestic rabbits** died suddenly. No samples were taken, but the cause of death is also suspected to be rabbit haemorrhagic disease.

Five **ostrich** farms in the **Oudtshoorn, Merweville** and **Touws River** areas were investigated for **avian influenza** (AI). Two were detected as part of the disease investigation on another farm close by. Avian influenza virus was detected on four of the farms but PCR tests for high pathogenicity subtypes were negative and haemagglutination inhibition serology tests did not indicate HPAI either. Low pathogenicity AI is therefore suspected.

***Salmonella* Enteritidis** was cultured from routine samples taken from five commercial broiler **chicken** properties in the **Malmesbury** state vet area.

A second case of **bovine malignant catarrhal fever** occurred on a property near **Stellenbosch** that had experienced its first case in May. Cases of wildebeest-associated BMCF occurred on a nearby property in February and May this year, but laboratory testing showed that this property's virus was sheep-associated. Sheep are kept on the property with the cattle.

One **pig** carcass from a farm in the **Malmesbury** state vet area was condemned after skin lesions of **erysipelas** were seen at the abattoir after slaughter.

Reproductive syndromes, including a still birth, a weak foal that died within hours of birth and an abortion were reported from a farm keeping **horses** in the **Ceres** area. All affected horses tested positive for **equine herpes virus** (EHV-1).

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