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African horse sickness sentinel surveillance 2018/19

Adapted from The AHS sentinel surveillance program 2018-2019 season report by J.D. Grewar[1] and C.T. Weyer[1]
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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

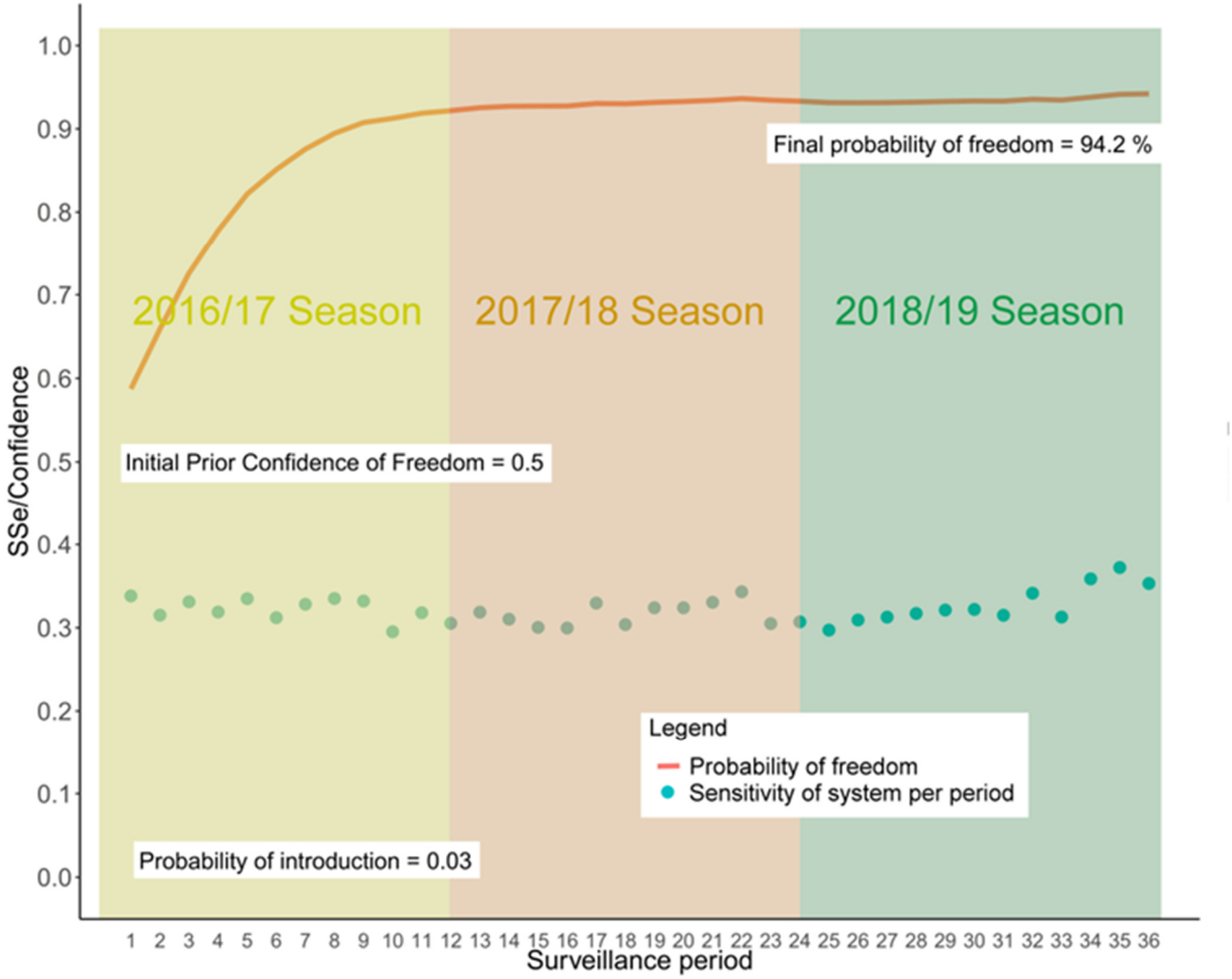

Figure 1: The sentinel surveillance sensitivity of individual surveillance periods (dots) with probability of freedom curve (red line) based on an uninformed 50% prior probability of freedom and a probability of AHS introduction of 3% for the past three surveillance seasons. The right pane represents the period of Sept 2018 to Aug 2019.

recruitment unless vaccination against AHS took place sufficiently recently to result in positive PCR results on their initial testing.

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 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).

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. 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 an increase of 8.3% from the previous season.

Positive results

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). All paired serological samples that could be evaluated showed stable serological results. However, over the course of the season there were three investigations where positive ELISA-results were found to have been a result of sampling and/or labelling errors. Corrective action was taken to ensure that positive identification of sentinels is made by samplers, particularly if they are unfamiliar with a property.

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.

Horse 1791 had a positive screening PCR result in October 2018. 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.

The horse was the only sentinel on the farm (in the Bottlery region of the AHS surveillance zone) on which another eight 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 five 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 included 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 eight 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 or 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.

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

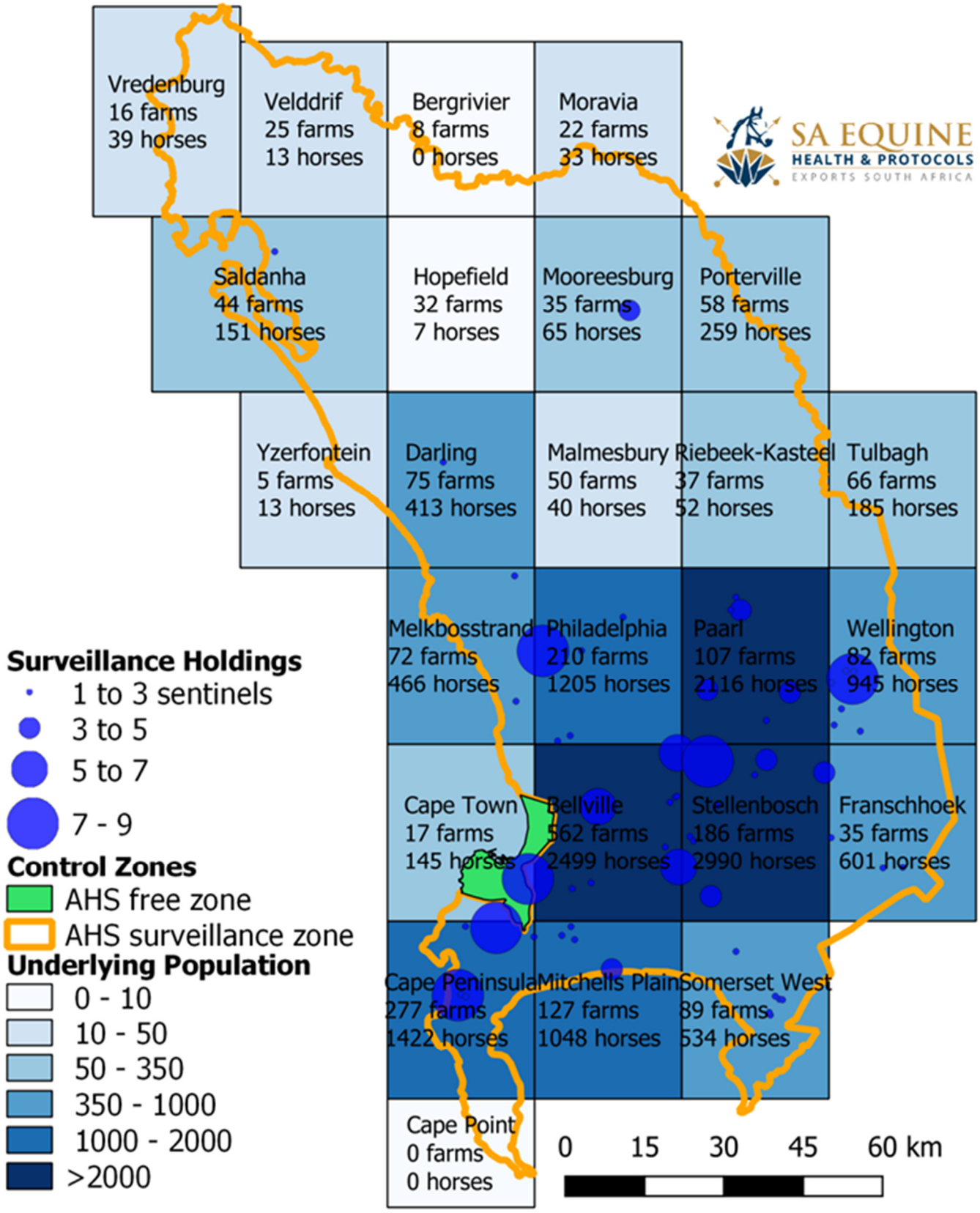

Figure 2: The underlying population of horses in the Surveillance and Free Zones of South Africa. These populations have been revised based on new population data collected between 1 April 2016 and 1 September 2019. The proportional circles represent the current sentinel populations.

of horses. Figure 2 shows the underlying population and current sentinel farms.

There are improvements with spatial representativeness compared to previous years. At worst the sero-surveillance target was, on average, four sentinels per month short in the Philadelphia area and the Paarl area was seven PCR sentinels short per month.

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.

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). The monthly sensitivity of the surveillance program where any sentinel tested negative in the month was established (on paired serology or negative PCR).

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% (fig 1). 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.

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 2017/2018 season was achieved, with a final probability of freedom of ~93%. The PCR testing in conjunction with the serology testing assists greatly in the analysis of the system and for follow up in suspect cases. Furthermore, the use of SNT analysis allows confident categorization of previously seronegative horses into vaccinated or field strain events.

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 three-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.

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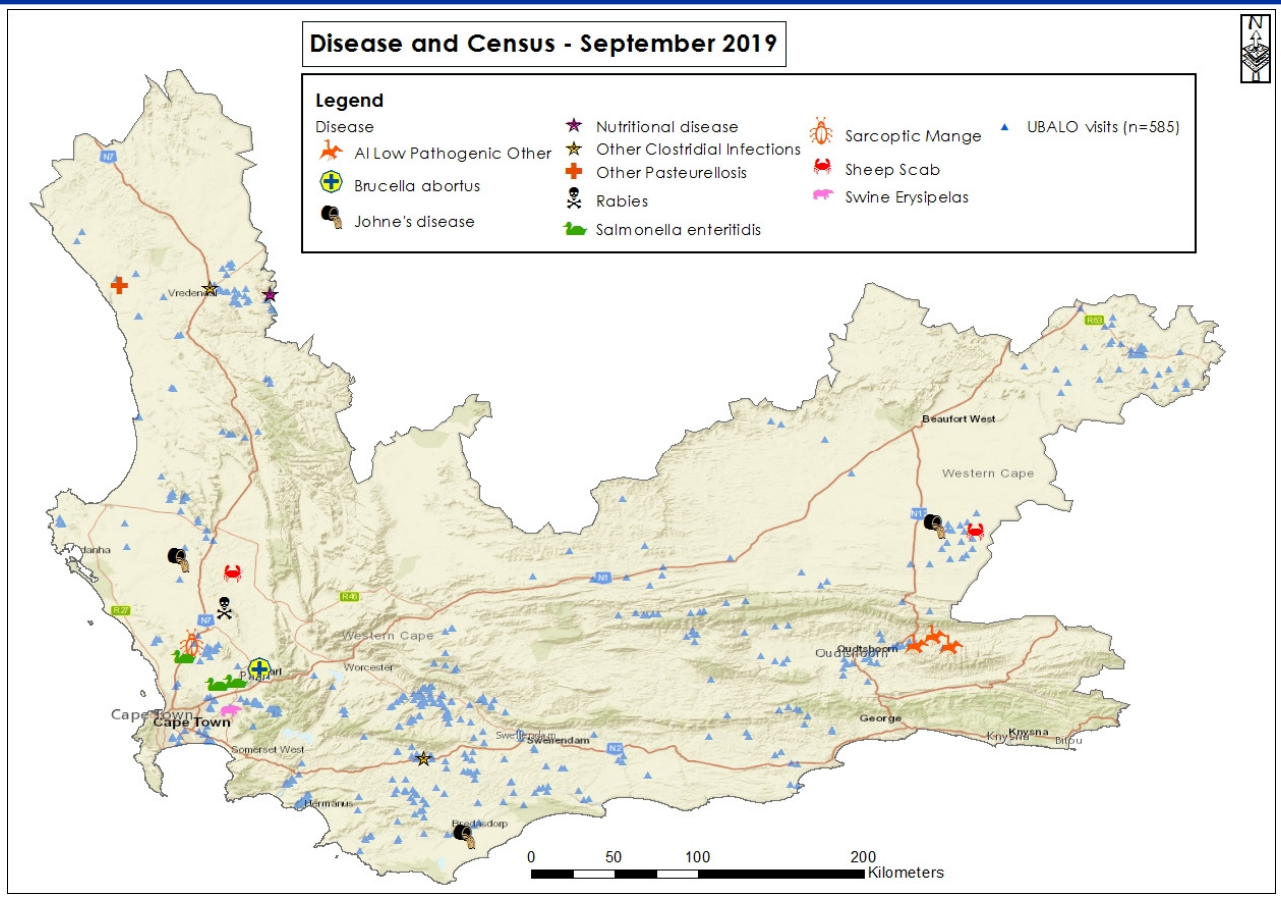
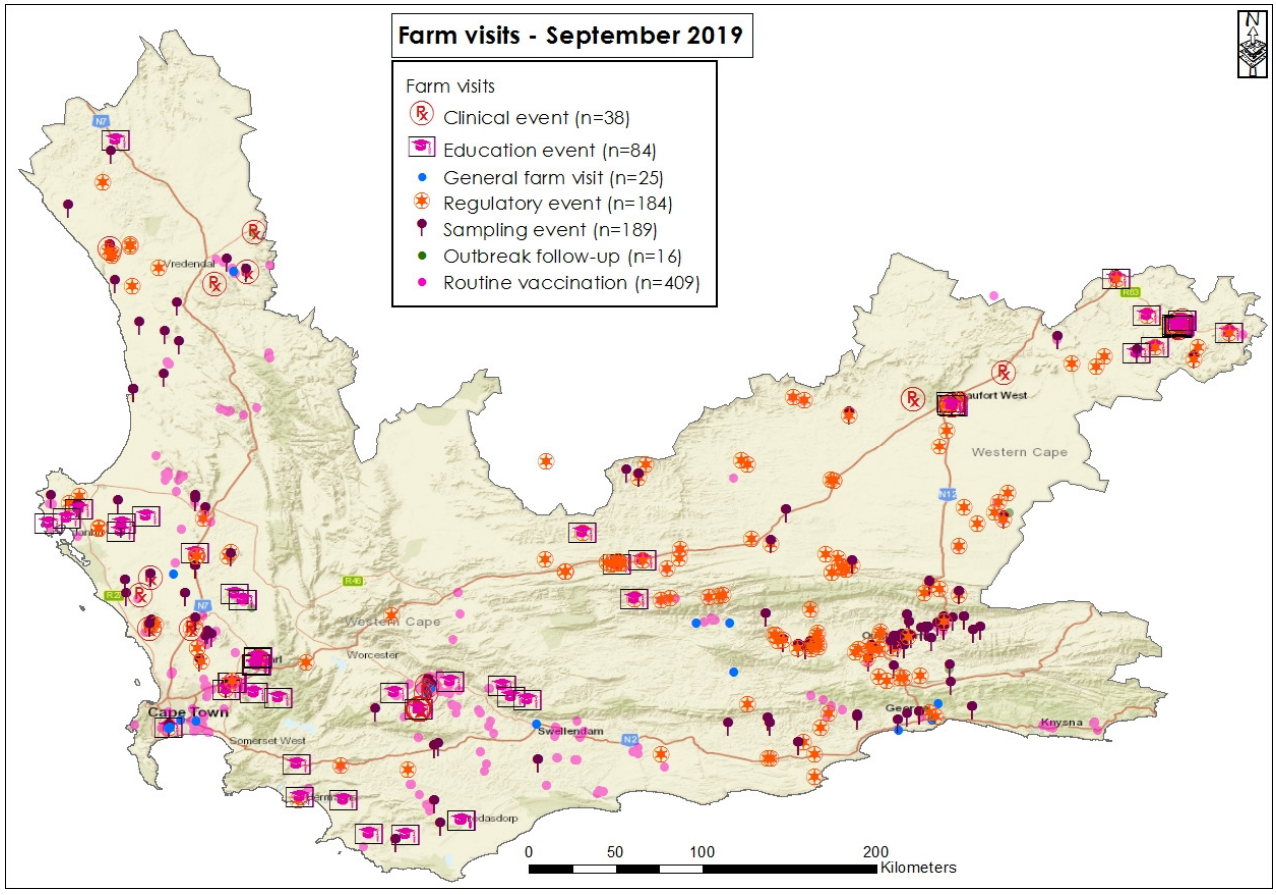
Disease and surveillance





Outbreak events

Cattle belonging to several owners in **Paarl** tested positive for **brucellosis**. The cattle share grazing land that belongs to the municipality and there are frequent movements of livestock in and out of the area. Most heifers in the area were vaccinated by the local animal health technician in 2018 and all test results in that year were negative. Three cows aborted earlier this year, but no investigation took place after this occurrence as it was not reported. All positive reactor cows were slaughtered and all female cattle in the area will be vaccinated with RB51 to control the outbreak.

A farmer near **Riebeek Kasteel** noticed that a car had stopped on the dirt road near his farm. The occupants had seen a **bat-eared fox** rolling and salivating in the road and had wanted to help it. Fortunately the farmer suspected **rabies** and warned them not to touch it. He then drove over the animal to kill it and submitted it for rabies diagnosis. It tested positive. A rabies vaccination day was already organized in the area a few days later for World Rabies Day.

Three **ostrich** farms in the **De Rust** area tested positive for **avian influenza** antibodies in the first two weeks of September. They are within 20km of one another and all showed similar (HxN2) serology patterns when tested at ARC/OVR. One farm tested PCR positive for influenza A but tested negative for H5 and H7 avian influenza. Nine farms within a 10km radius of these positive farms have tested sero-negative on follow-up sampling.

Sheep farmers near **Moorreesburg**, **Bredasdorp** and **Beaufort West** noticed emaciation occurring in their ewes. Necropsies, histopathology and ELISA testing on the affected animals revealed **Johne's disease** (fig 3).

Samples of **chicken** meat taken at an abattoir near **Paarl** tested positive for ***Salmonella enteritidis*** (SE). The products were frozen and investigations done to attempt to trace the source of the infection. All parent flocks that supplied the broilers tested negative. Another broiler farm near **Philadelphia** tested positive for SE on boot cover swabs. *Salmonella* reduction protocols were instituted on the farm in response.

Sheep scab was diagnosed in flocks of pruritic sheep near **Moorreesburg** and **Beaufort West**.

Signs of **erysipelas** were seen in free-range **pigs** from **Stellenbosch** after slaughter at the abattoir.

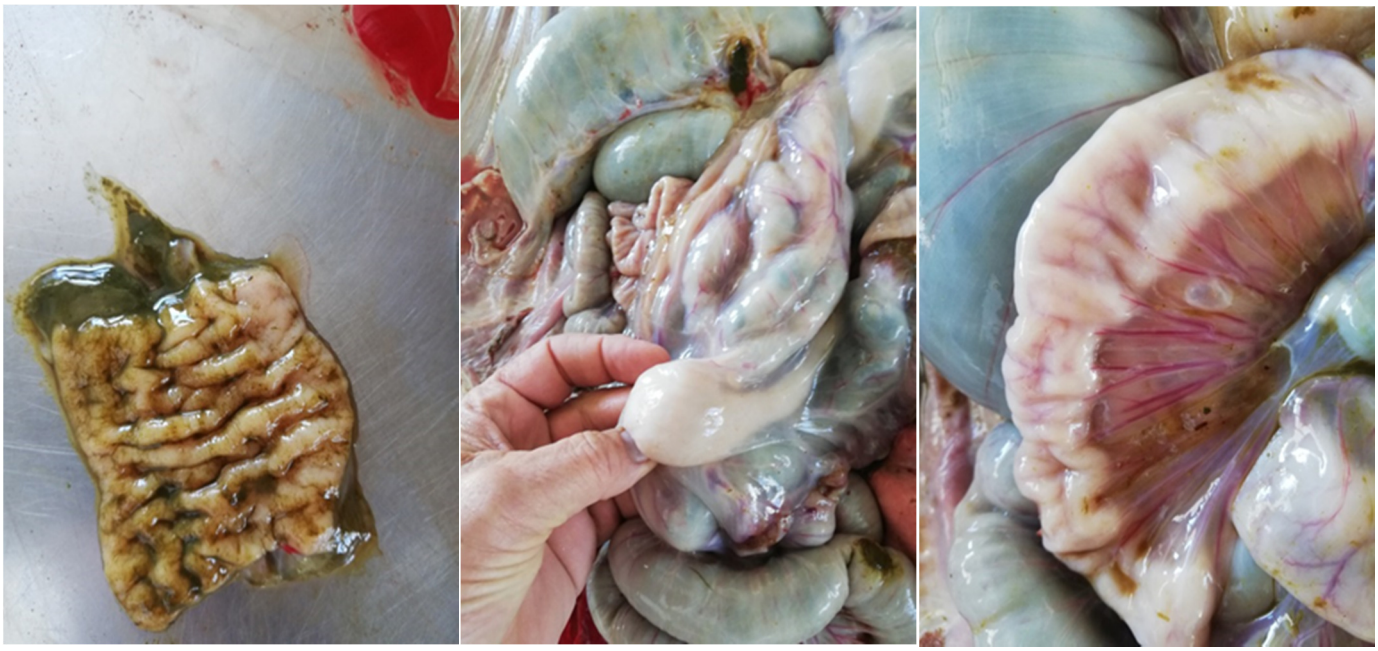


Figure 3: From left to right: Lesions of Johne's disease in the ileum, mesenteric lymph nodes and jejunum of an affected sheep (Photos: J Pienaar)

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