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African Horse Sickness - Sentinel Surveillance Report 2014/2015

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Contents

1. Overview of the program

The African horse sickness (AHS) sentinel surveillance program is aimed at providing additional confidence of AHS freedom in the AHS free and AHS surveillance zones in the Western Cape Province.

Serological sentinel surveillance candidates are selected based on their history of a lack of AHS vaccination while PCR candidates have not been vaccinated in at least the last 2 years. In the initial phases of the program vaccinated horses/ horses of unknown vaccination status were also selected during recruitment in an attempt to identify true sentinels. It is for this reason that horses with a serological outcome of "Stable positive" were detected (*see 2.2 below - Serology: Total broad outcomes*). This recruitment will continue into the 2015/2016 season. Some horses fell out of the sentinel program during the current period under review and this is due to results showing unsuitability of the horse as a sero-sentinel. However, for the sake of completeness in this report all results have been included and evaluated. In future horses that are recruited but found to be not suitable for either the serological or PCR surveillance will be removed both from the sentinel cohort and from the analysis.

The serological sentinel process is simple. Each horse in the program is tested monthly and on evaluation the previous month's test is selected as the initial sample in a series of two samples (paired samples). If no samples are taken for the previous month then we retrospectively select back to a maximum of 3 months prior to the month under review.

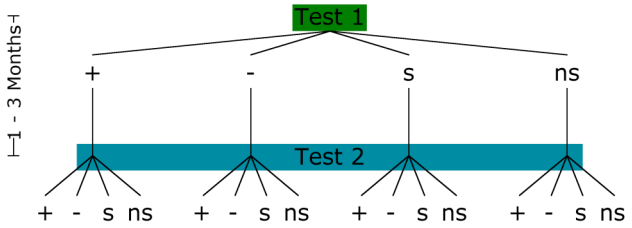

Figure 1: An overview of the permutations of outcomes of monthly sero-sentinel surveillance.

ns - Not sampled, + Positive, - Negative, s - suspect

For the sero-sentinels there are 16 permutations for each horse per month of analysis when tested in this program and these are illustrated in Figure 1. Seven of these consist of horses that were either not tested in the month of the paired serum sample analysis (i.e. figure 2 Test 2), or alternatively not sampled in the 3 months prior to the month under analysis (i.e. figure 1 Test 1). Analysis of each month therefore excludes any occurrences of "not sampled" events - see Figure 2 – "*No duplicate sample*" - in this analysis this totaled 137 events (21%) which could not be analysed.

The PCR sentinels are evaluated on an individual sample basis with either a positive or negative outcome. When analysing PCR results the entire review period result set per horse is taken into consideration.

1.1 Tests performed

PCR tests are performed by the Equine Research Center using the techniques for group specific quantitative RT PCR as described in Guthrie et al in 2013.

Serology tests (i-ELISA) were performed by the Onderstepoort Veterinary Institute as described by Maree and Paweska in 2005.

Period under review

2014-09-01 to 2015-08-31 - this is the standard annual AHS surveillance range adopted in South Africa.

2. General overview of results

2.1 Total number of samples tested in period

Serum: 646 samples tested
PCR: 1528 samples tested
Farms involved in program: 65

2.2 Serology: Total broad outcomes

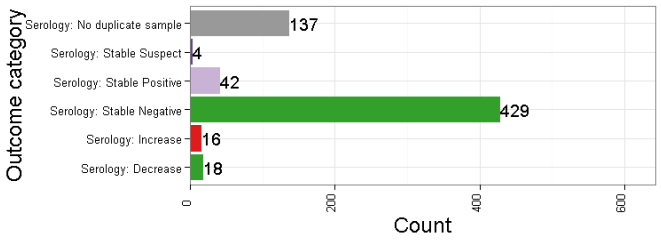

Figure 2: Broad outcomes of the period under review. Note that an increase in serology indicates both when a horse moves from negative to suspect/positive or from suspect to positive. The converse is true for the *"Serology: Decrease"* category

There were a total of 16 increasing serological levels which constitutes 3% of the total serological events (n=509) that could be evaluated.

2.3 Serology: Total detailed outcomes

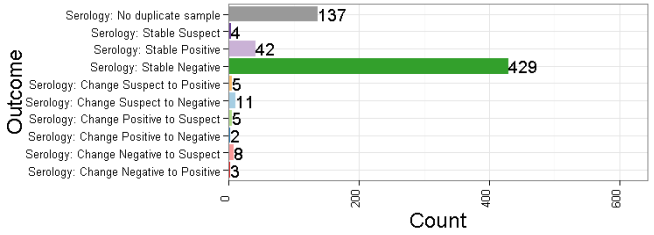

Figure 3: Detailed serological outcomes of the period under review.

2.4 PCR: Total outcomes

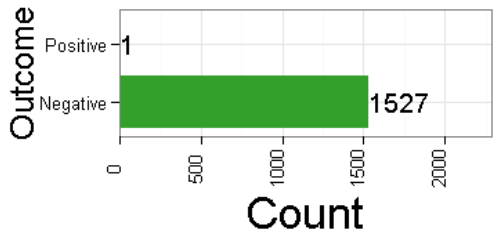

Figure 4: Detailed PCR outcomes of the period under review.

3. Detailed overview

3.1 Stable serology and negative PCR results overview and sensitivity of surveillance

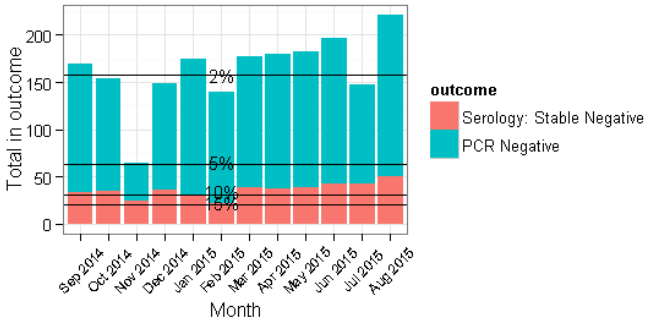

Figure 5: The number of stable negative serology results and negative PCR results per month for the period under review. The horizontal lines indicate the number of samples that would need to be taken to have a 95% confidence that we would detect AHS at the prevalence indicated – i.e. 2%, 5%, 10% and 15% respectively

3.2 Non-stable Results

3.2.1 Serology

3.2.1.1 Serology: Summarised non-stable results

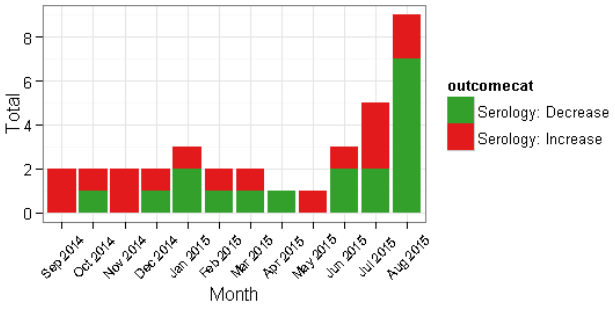

Figure 6: Summarised serological analysis where stable results were not achieved for each month of analysis

3.2.1.2 Serology: Detailed non-stable results

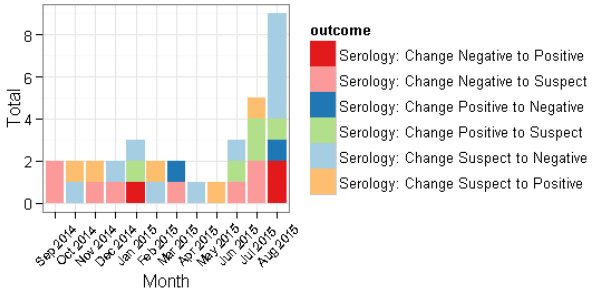

Figure 7: Detailed serological analysis where stable results were not achieved for each month of analysis

3.2.1.2.1 Serology: Increase in positivity: Detailed

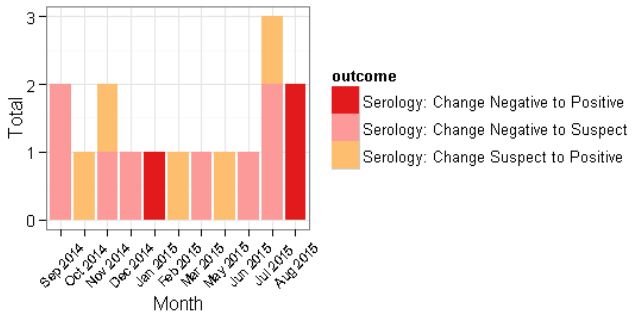

Figure 8: Detailed serological analysis where an increase in positivity was found for each month of analysis

The increases in positivity for a paired serological series are the important results to evaluate in a sero-surveillance program since these have an impact on the outcome of the program. Complete individual horse results for the period under review are necessary to evaluate the individuals that needed follow up. In the section below each horse represented in Figure 8 is evaluated, including, if applicable, results from horses on the same property. The reference numbers for the horses are indicated within each caption and where multiple horses are evaluated their reference numbers, along with that of their resident property, are shown. Both PCR and serology results have been added to each graph to assist in individual analysis. The date series below each graph is unique to that horse, so null data outside the range of testing is not shown.

3.2.1.2.1.1 - Horse 12

This horse had alternating suspect and positive serological results when tested during the year (figure 9). It started the sentinel program in Sept 2013 and was suspect on the first sample that was collected, so the results seen in the period under review, especially in conjunction with the negative PCR results, are certainly due to residual antibody from either a previous vaccination or previous exposure to AHSV. The vaccination history indicates that the horse was not vaccinated since 1999, but prior to this the vaccination history is unknown. The horse is situated in an area that was under movement restriction during the Mamre 2011 outbreak, however cases were not reported in the immediate vicinity. The ELISA percentage positive value (PP) remained very low for positive results in this horse (<20).

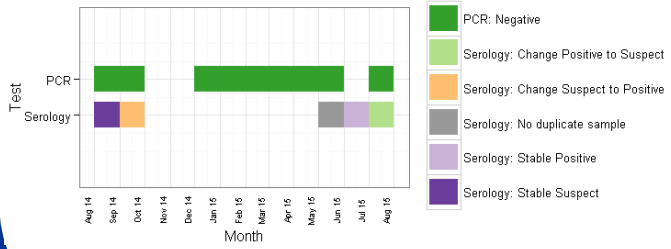

Figure 9: Individual PCR and serology results for the serological increase in horse number 12

3.2.1.2.1.2 - Horse 45

This horse had alternating suspect and positive serological results when tested during the year (figure 10). As with horse 12, this horse started the sentinel program in Sept 2013 and was suspect on the first sample that was collected, so the results seen in this period under review, especially in conjunction with the negative PCR results throughout the year, are certainly due to residual antibody from either a previous vaccination or previous exposure to AHSV. ELISA PP values also were very low (<20). This horse was previously vaccinated for AHS in 2001.

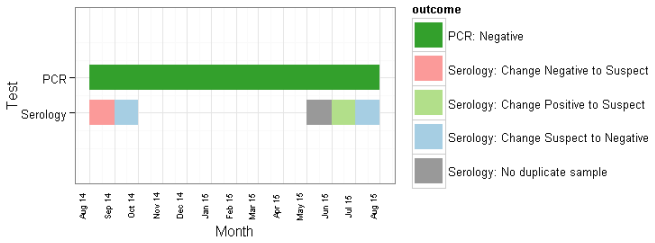

Figure 10: Individual PCR and serology results for the serological increase in horse number 45

3.2.1.2.1.3 - Horse 118

The results set for this horse appear to be a false suspect result in March 2015, with serology returning to negative the following month and consistent negative PCR results in the months leading up to and following the suspect result. This horse was previously vaccinated in 2009.

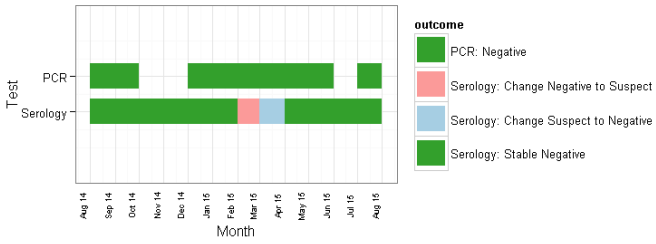

Figure 11: Individual PCR and serology results for the serological increase in horse number 118

3.2.1.2.1.4 - Horse 142

This horse's results are a good example of the potential difficulties of serological surveillance. In the face of negative PCR this horse has had 3 increases and 3 decreases in serology category over the year making analysis difficult (figure 12). Having a look at the rest of the sentinels on the property (figure 13): horse 140 only had PCR testing and was negative for the entire period under review, while horse 141 was serologically negative, stable for the entire period under review with negative PCR from Oct 2014 through to July 2015. Horse 142 therefore does not follow this trend. The negative PCR results do point towards no active circulation, especially seen in light of the other horses' test results on the property.

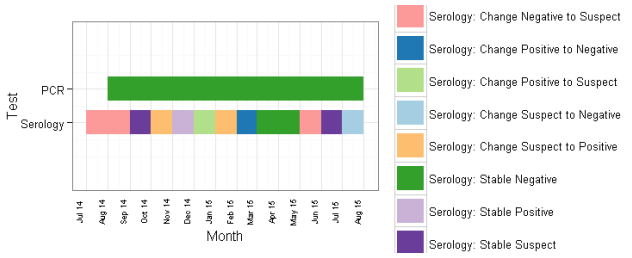

Figure 12: Individual PCR and serology results for the serological increase in horse number 142

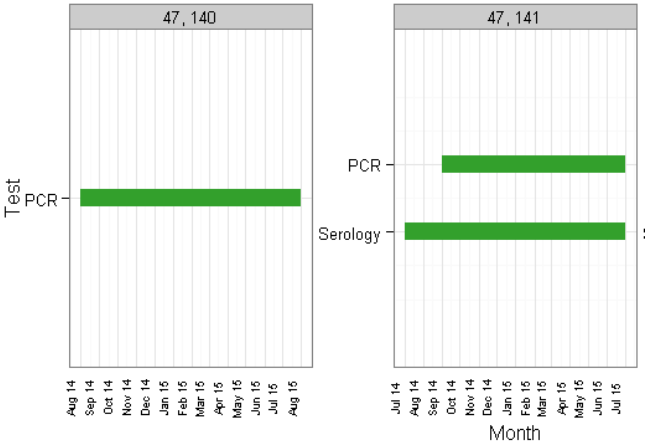

Figure 13: Property cohort results for horse 142 – Owner number 47 - legend as for figure 12 and horse 142 has been excluded

3.2.1.2.1.5 - HORSE 165

This horse had 2 events where negative results went to suspect but back down to negative immediately in the following month. Interpreting these results along with the negative PCR, this horse is not considered a possible positive. It was also the only horse on the property that was included in the program so no comparison between horses in close proximity is possible.

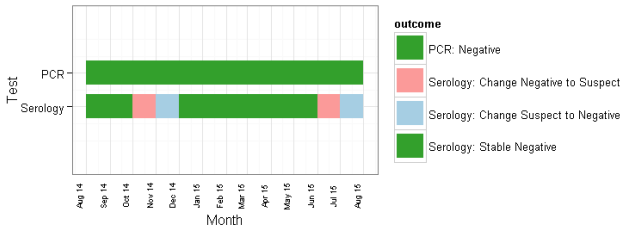

Figure 14: Individual PCR and serology results for the serological increase in horse number 165

3.2.1.2.1.6 - HORSE 214

In similar fashion to horse 165 above, this horse had a suspect result that carried through for one month longer than horse 165 but then reverted to negative. Again, along with the negative PCR (right throughout the period) this horse is not considered a possible positive. It was also the only horse on the property that was included in the program so no comparison between horses in close proximity is possible.

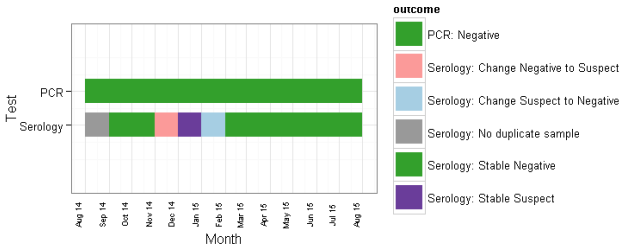

Figure 15: Individual PCR and serology results for the serological increase in horse number 214

3.2.1.2.1.7 - HORSE 223

This horse had a serological jump directly from negative to positive in Jan 2015 (with a few months of negative results prior to the jump) and stable positive results for the following 3 months (figure 16). There were negative PCR results throughout the period but unfortunately no further serology results. Having a look at horse 223's property cohort (figure 17): there were a total of 7 horses (including horse 223) on the farm. The PCR results were negative throughout with a few gaps in testing and one other horse was a sero-sentinel and had negative results throughout the year (horse number 6). Certainly the PCR results don't point towards a positive result but the freedom of disease cannot be ruled in completely with this serological response. Previous vaccination history for this horse is unknown. Fourteen of the non-sentinel horses on the farm were vaccinated in Nov, Dec and Jan (2014/2015) with both AHS bottle 1 and 2. The transmission of vaccine virus is a consideration as a possible source of the seroconversion of horse 223 (although again the negative PCR adds some uncertainty to this possibility) - see concluding remarks regarding vaccination protocols in the AHS control zones.

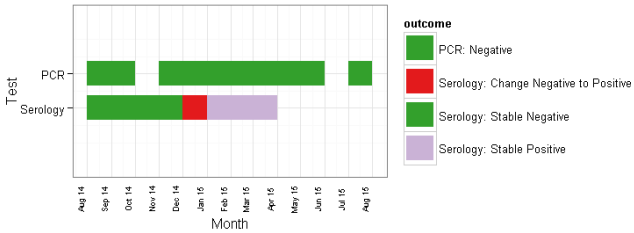

Figure 16: Individual PCR and serology results for the serological increase in horse number 223

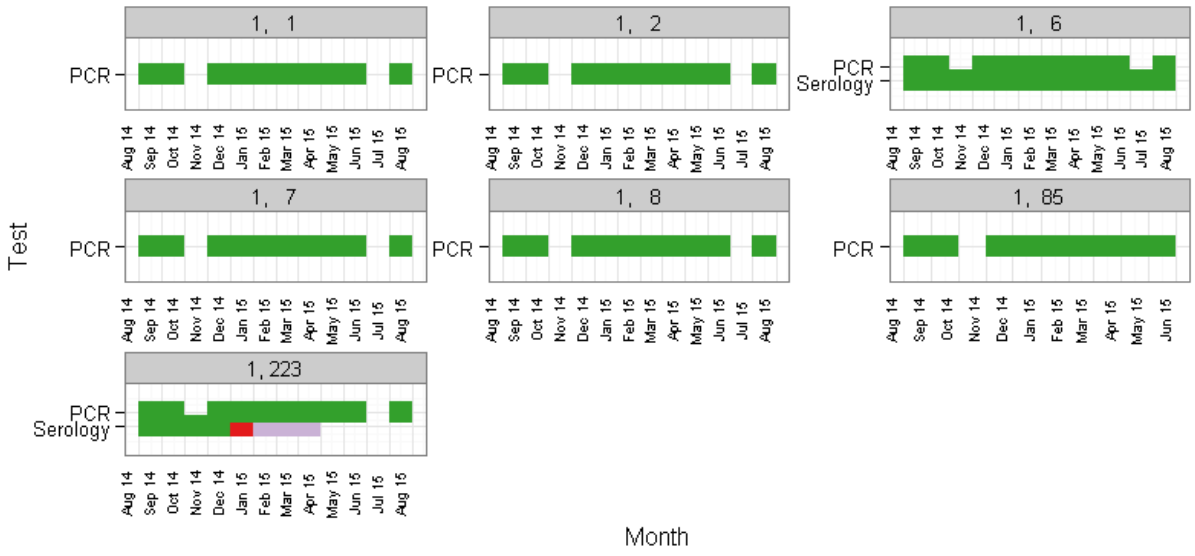

Figure 17: Property cohort results for horse 223 – Owner number 1 - legend as for figure 16

3.2.1.2.1.8 - HORSE 242

Horse 242 started the period with a suspect result but immediately reverted to negative (figure 18). This was repeated in Jul and Aug 2015. Because of the negative PCR this horse is not considered a possible positive. The rest of the horses on the farm included in the sentinel program (figure 19) totaled 6 horses, including horse 242. Two horses (240 and 310) were removed as sero-sentinels for starting with positive results – they had no testing prior to the period under review and previous vaccination history was unknown. Two horses had stable negative serology results for much of the period under review and for every event that they were tested. All PCR results for horses belonging to the same owner were negative.

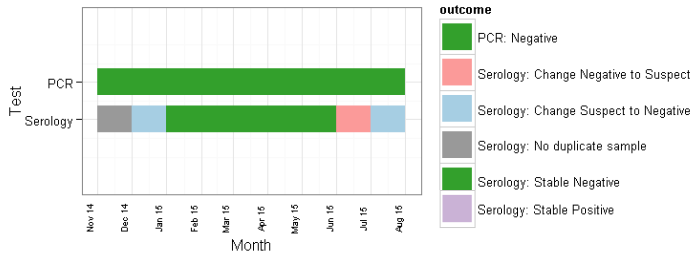

Figure 18: Individual PCR and serology results for the serological increase in horse number 242

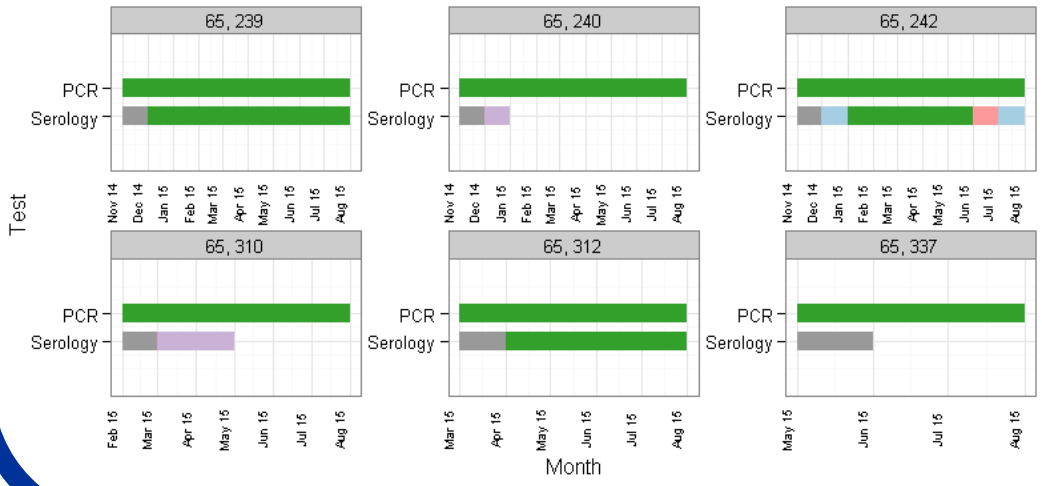

Figure 19: Property cohort results for horse 242 – Owner number 65 - Legend as for figure 18

3.2.1.2.1.9 - HORSE 256

This horse is clearly a true positive with a positive PCR result and a positive change from negative to positive in serology for the same month under observation (figure 20). For the same owner (Owner 66) there were a total of four horses in the program including horse 256 (figure 21). Two of the four had consistent negative PCR and serology results throughout the period under review and the remaining horse was a PCR sentinel only (its initial serology was positive and it was thus

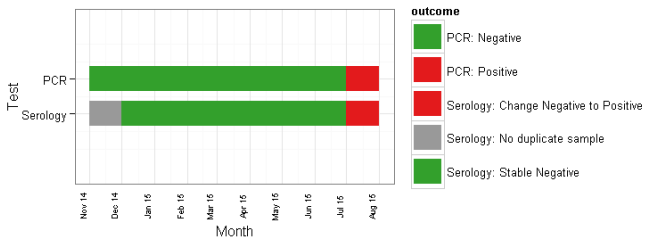

Figure 20: Individual PCR and serology results for the serological increase in horse number 256

not used further in the sero-sentinel program). It had negative results, albeit with a gap in testing during March and April 2015. After horse 256's results were received the owner was contacted and it was established that between the July and August sampling (1st July 2015 and 15 August 2015 respectively) the horse had been vaccinated with AHS bottle 1 (8th July) and bottle 2 (5th August). This horse is therefore considered as a false positive for the AHS surveillance program since vaccine strain AHSV was detected by PCR and the serological response was as a result of the vaccination.

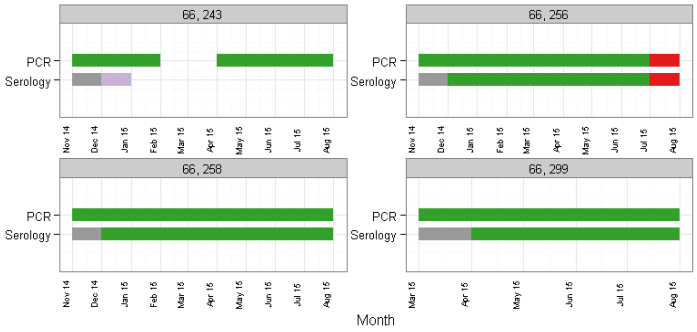

Figure 21: Property cohort results for horse 256 – Owner number 66 - For legend see figure 20

3.2.1.2.1.10 - HORSE 275

Horse 275 had an increase in serology from negative to positive right at the end of the period under review (figure 22). Its PCR results were negative throughout the year making it a different scenario to that of the vaccinated horse 256 (Figure 20). The positive result falls in the middle of winter making it an unlikely true positive and the PP value was very low (PPV 14). Furthermore, the rest of the horses in the property sentinel cohort (Figure 22) were both consistently negative on PCR throughout the review period, although they were not part of the sero-sentinel group. On the first test in the next surveillance period that this horse was involved in (Nov 2015) the iELISA AHS serology result was negative.

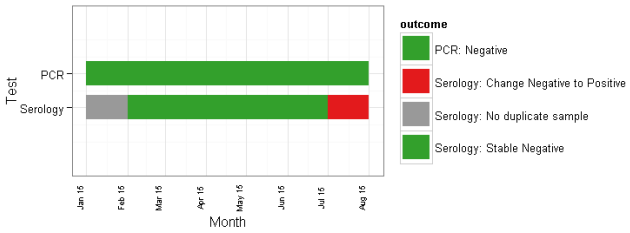

Figure 22: Individual PCR and serology results for the serological increase in horse number 275

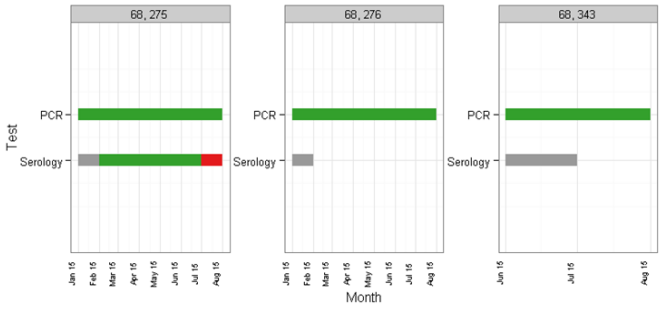

Figure 23: Property cohort results for horse 275 – Owner number 68 - Legend as for figure 22

3.2.1.2.1.11 - HORSE 311

Horse 311 started the sero-sentinel program in April 2015 and tested suspect on serology on the initial test and then alternated between suspect and positive on serology throughout the rest of the period under review (figure 24). This along with the negative PCR results indicates it was likely to have been vaccinated or exposed prior to the period under review and these were residual antibodies that were being detected, making it a false positive result. Also the rest of the surveillance cohort on the property (figure 25) showed consistent negative PCR results and the one other horse that was a sero-sentinel (horse 250) had stable negative results throughout.

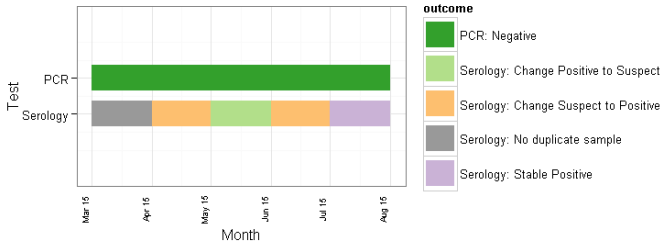

Figure 24: Individual PCR and serology results for the serological increase in horse number 311

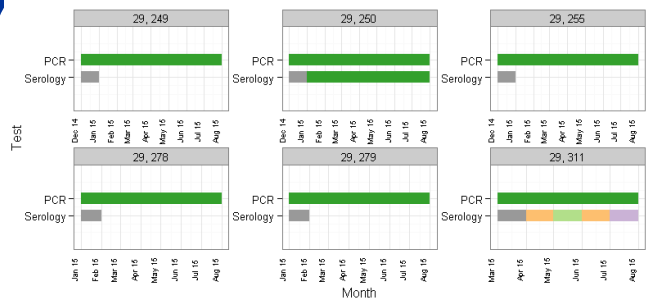

Figure 25: Property cohort results for horse 311 – Owner number 29 - legend as for figure 24

3.2.2 PCR

3.2.2.1 PCR: Positive results

A total of 1 sample tested positive for the period under review. This horse (horse 256) was also positive on serology and has been discussed under that section – see figure 20 and figure 21 – this horse had been vaccinated just prior to the positive result and was thus a false positive.

4. Location of sentinel farms

The ideal spread of sentinel properties and horses is illustrated in Figure 26. Under each area block's name is the ideal required number of horses to include in the program and below that the percentage of the total that should be covered by sampling in that area (for the concept of proportional sampling to be maintained) to detect a 2% minimum expected prevalence (MEP) of AHS. Overlaid on Figure 26 is a color range indicating the attained number of sentinels during the period under review with red, orange and yellow indicating where targets were not attained, green indicating where targets were either attained or very close to attained and then light blue through purple showing areas where more than the required number were attained. Remember that in Figure 5 the target of 2% MEP was reached on most occasions so the attained versus deficit levels will generally balance out for the entire surveillance area.

The highest requirement for sentinels is in the 4 block area of Philadelphia, Paarl, Belville and Stellenbosch (center of the map). In this area the targets of three of the four blocks either were attained or surplus sentinels were sampled, with the Paarl area showing the highest deficit (14 sentinels) for the entire area.

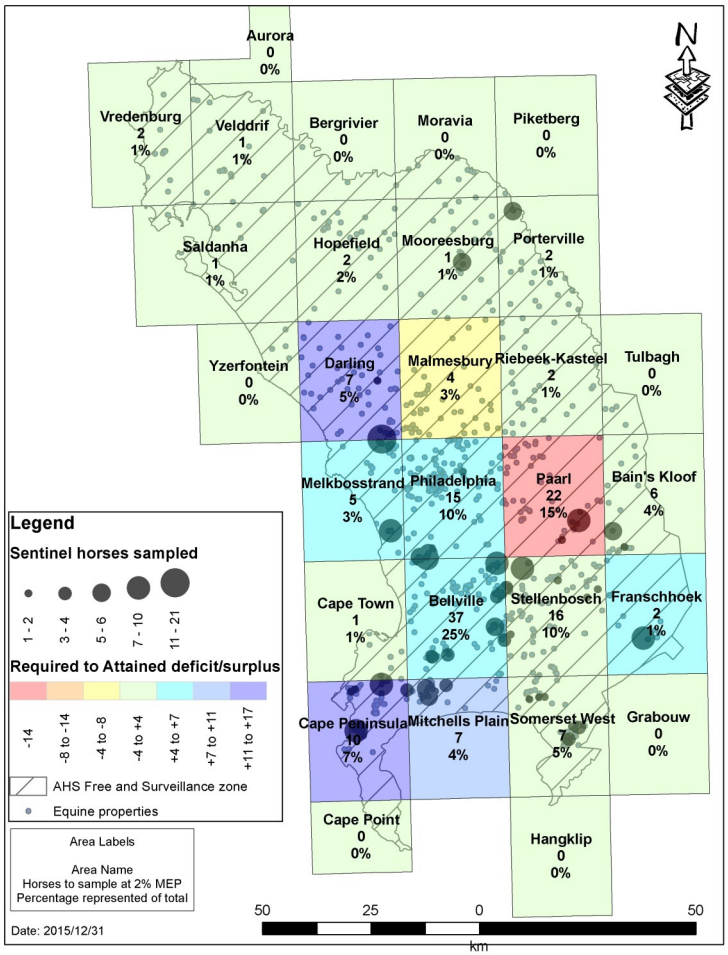

Figure 26: A map showing the AHS surveillance and free zone where sentinel surveillance has taken place. The map depicts the various areas with their estimated number of horses labelled that are required to be sampled to detect a 2% minimum expected prevalence.

The yellow to red areas are areas where sentinels were lacking while the bluer areas show where a surplus of sentinels were sampled.

continued on next page

5. Results and discussion

This is the first attempt at a detailed analysis of the sentinel program in the AHS surveillance zone which includes both PCR and serological testing. The program is not without its challenges, and the recruitment of sero-negative animals for the sero-surveillance aspect has been difficult, which has forced the recruitment of either horses of previously unknown vaccination status or of horses that have been vaccinated some time ago. Also, the surveillance zone has had outbreaks of AHS so the exposure status of some sentinels is unknown, leading to results which are difficult to analyse.

A total of 21% of the samples taken could not be used as part of the analysis because they did not fall within a period of 3 months of another serological result for the same horse. This number will hopefully decrease given that the program has now been established and for the next period sentinels will be selected based on their results this year and should be more representative of the "true" sentinel status.

From a serological point of view there were 16 events in total (from 11 horses) of the 509 events that had an increase in serology from negative to suspect/positive or suspect to positive. Of these there was one definite positive that was recently vaccinated – see Horse 256.

There were a further 2 horses (Horse 223 and Horse 275) that had results showing an increase in serology that could not be definitively confirmed as non-AHS associated. Horse 223, however, had negative PCR throughout the period (figure 16) under review, as did the other 6 horses on the same property with one other horse on the property having stable negative serology throughout (figure 17). The positive result, however, was in January 2015 which is a seasonally possible time for AHS to occur.

Horse 275 had the increase in serology in August 2015 after stable negative results from March of the same year. It also had negative PCR throughout, which was mirrored by the other two horses on the same property, although neither were involved in the sero-sentinel program. Certainly a positive result in August is seasonally very uncharacteristic of AHS and this result should be seen in this light. Also, the next test (iELISA) that was performed on the horse in Nov 2015 was negative for AHS, which would not be expected after a true seroconversion.

Figure 6 shows that in a program like this there are going to be horses with increases in serology pretty much throughout the year, and it is very important to follow these up to try reach some resolution, making a final survey analysis like this one more powerful. This also shows how important adding PCR to the program has been as most of these events can be shown to be false positive increases given serial negative PCR results for each horse. It also illustrates that results must be timeously analysed so that immediate follow up can be performed, for instance possibly the use of SNT (serum neutralization tests) could be incorporated into increases in positivity results.

The AHS vaccination protocol was amended in mid 2015 with either permissions to vaccinate (free and surveillance zone) or compulsory vaccinations (protection zone) now only allowed to occur during the low vector activity period (1 June through 31 October). This will impact positively on the sentinel surveillance program given that potential transmission of vaccine strains will be less of a consideration for potential seroconversions (see horse 223).

6. Conclusion

If negative PCR prior to, during and after an increasing serological result can be considered as categorising that result as false positive then the surveillance results show that it is unlikely that AHS was circulating during the 2014/15 AHS surveillance period in the AHS surveillance zone of the Western Cape at greater than a 2% minimum expected prevalence of detection with a 95% confidence level. Even allowing for false negative PCR (the period of detection for PCR is shorter than that of antibody detection) then there were only 2 horses which showed results that could be considered to be associated with AHS, one of which occurred in a season when AHS circulation is highly unlikely.

The results have been influenced by difficulties in recruitment of true sero-negative sentinels and future analysis will hopefully be easier given that horses not meeting sero-sentinel requirements have been removed from the program throughout the year (note that these horses have still been included in this analysis of the 2014-2015 review period).

The indirect ELISA that is being used in this program is not a truly reliable quantitative test, meaning that it's difficult to analyze a titre difference between stable positive results for instance for a horse that repeat tests positive – like horse 223.

Some positive general outcomes from this program are that cart horse owners in the City of Cape Town area (Mitchells plain and Cape Peninsula in Figure 26) have been recruited during the period reviewed. Also the analysis of monthly data is now automated to prepare a report similar to this one on a monthly basis. This should assist in timelier follow up of increasing serological results.

7. References and Acknowledgements

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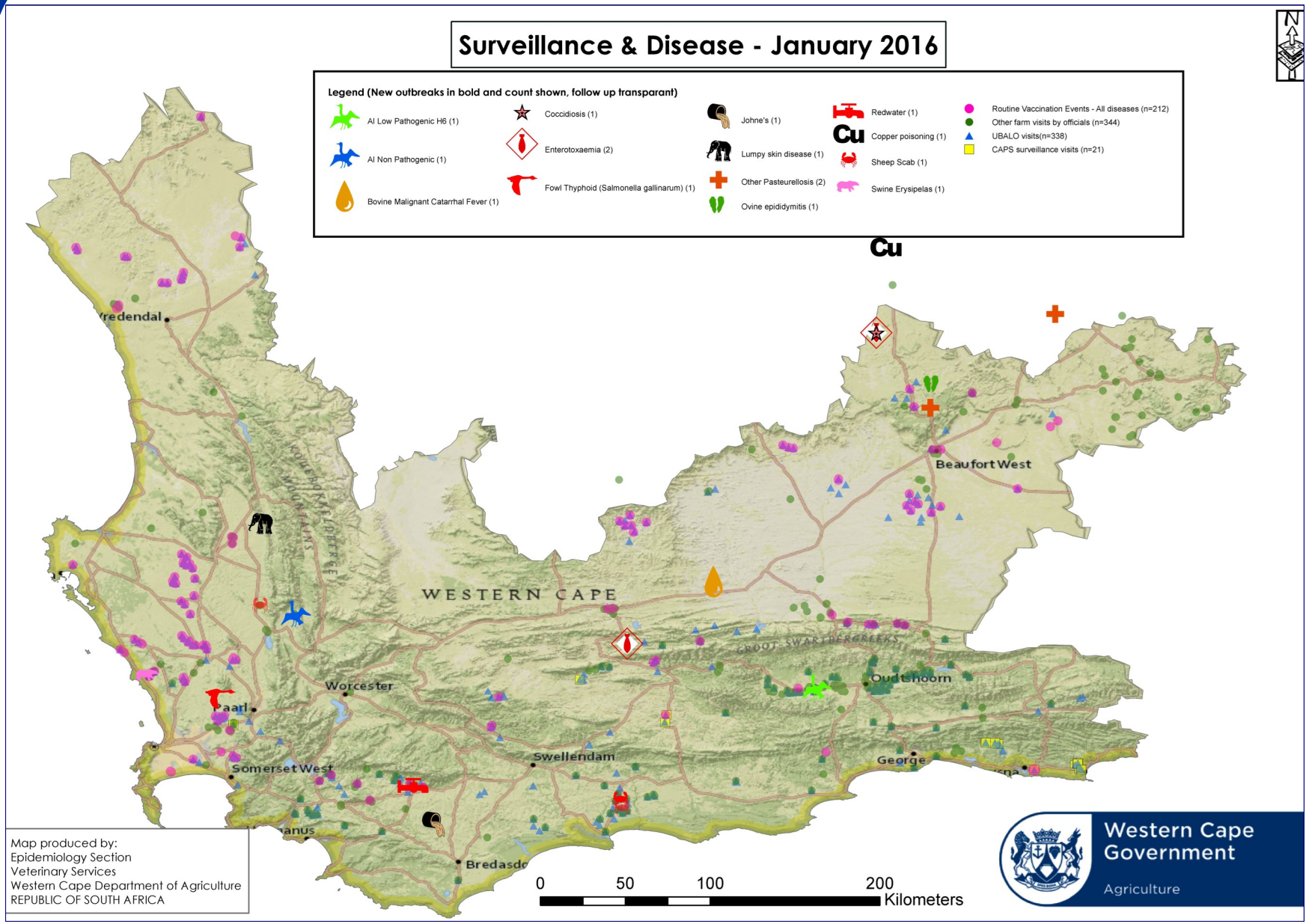

Figure 27: Surveillance and disease map of the Western Cape for January 2016 .

Outbreak events

- A **sheep** farm in the **Bredasdorp** area was confirmed positive for **Johne's disease** in October 2015 after chronic emaciation and diarrhoea was seen in the flock.
- A sheep farm in the **Heidelberg** area was diagnosed with **sheep scab** after a clinical inspection by a private veterinarian. The farmer had received weaner sheep from another farm treated for an outbreak of sheep scab in 2015. Early skin lesions were seen on the sheep in December 2015. This farm and the neighbouring farm have been put under quarantine and the first treatment of all sheep has been done under official supervision.
- A case of **lumpy skin disease** was picked up on ante-mortem examination by one of our newly graduated veterinarians doing his community service year at an abattoir in Hermon. The affected cow had been sold at a slaughter auction, but was returned to her farm of origin near **Piketberg** to recover from the disease before she could return to the abattoir.
- ***Brucella ovis*** was detected on a sheep farm near **Beaufort West**.
- ***Salmonella gallinarum*** (fowl typhoid) was diagnosed using culture on a layer farm near **Klipheuwel** after mortalities on the farm increased suddenly. This is the third farm reported infected with *S. gallinarum* in the province in the last six months, prior to which the province had been free of the disease. Poultry farmers are encouraged to institute strict biosecurity measures on their farms to prevent becoming infected, as well as to remain vigilant for signs of the disease and report it promptly if suspected.
- **Pneumonia** caused by ***Pasteurella*** was diagnosed as the cause of death in 3-week old dorper **lambs** and 4-month old boergoat **kids** near **Beaufort West.**
- **Coccidiosis** was identified as the cause of diarrhoea in **lambs** near **Beaufort West**.
- **Goats** near **Laingsburg** and a **lamb** near **Beaufort West** died of **enterotoxaemia**, identified on post-mortem examination.

Figure 28: Enlarged livers with a green-bronze sheen are often seen in chickens that die acutely of fowl typhoid.

- Serological surveillance (pre-slaughter) on an ostrich farm in the **Oudtshoorn** area detected **H6 N2/N8 avian influenza**. Follow up PCR was negative and since this was the final group to be slaughtered for this season all birds were slaughtered for local consumption and quarantine could be lifted
- Serological testing of a ostrich farm in the **Tulbach** area detected **avian influenza** on **ELISA** with negative HI results and thus far negative PCR results. This is therefore difficult to categorise and has been allocated as an Undefined AI event. The relatively high prevalence level of the ELISA results mean the farm remains under quarantine until absence of circulation of whatever AI is involved is confirmed.
- Not shown in Figure 27 is a potential **H5 avian influenza outbreak** on a **duck breeder farm** in the **Joostenburgvlakte** area. This farm was one of those affected last year by H6 avian influenza (see the June and July epi reports for some of those details) and sampling was being undertaken to establish whether that event could be finalised. Serological results showed however that H5 AI could either be currently circulating or had circulated in the recent past - HI results returned positive values on the H5N2, H5N1 and H6N2 antigens making H5N2 the likely responsible virus. The PCR testing of swabs on the affected farm were negative and follow up testing on serology showed relatively stable prevalences which point towards a detection of a historical outbreak. The farm however remains under quarantine as well as farms within 3 km (which have or soon will be tested).
- An case of **bovine malignant catarrhal fever** was detected in a **heifer** in the **Beaufort West area**. Interestingly the event occurred shortly after Wildebeest were introduced onto a neighbouring farm but the type found was **sheep associated MCFV** (tested twice for confirmation). The young heifer affected was in a herd that had been grazing with sheep on the farm but for the past many years raising interesting questions as to why it was affected only now,.

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Disclaimer: This report is published on a monthly basis for the purpose of providing up-to-date information regarding epidemiology of animal diseases in the Western Cape Province. Much of the information is therefore preliminary and should not be cited/utilised for publication