Surveillance Report

Disease	**Dourine in Equids**
Coverage Area	**Western Cape – AHS Surveillance Zone**
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Introduction

Dourine is a sexually transmitted trypanosomal (*Trypanosoma equiperdum*) disease of equids. Importantly in the context of potential introduction and surveillance it is not transmitted by invertebrate vectors and there is no know reservoir of the parasite except in the equid host. The parasite is however tissue associated with a humoral immune response so serological testing is the only real option for freedom of disease type surveys. The complement fixation test is universally used for individual animal freedom status, and while the CFT is not always useful in freedom of disease surveys (IFAT and ELISA may be more useful – OIE Terrestrial Manual 2013) it can be used in this setting. Confirmation for surveillance positives can be obtained through the use of clinical signs, microscopy, PCR and the available serological tests mentioned. Clinical signs are relatively pathognomonic; however they may not be present in early or latent cases. Confirmation of the parasite through microscopy, while very specific, has serious sensitivity deficits.

Impact on exports of horses: Dourine is considered in the 2008/698/EC EU Commission Decision regarding the *temporary admission and imports into the EU of registered horses from South Africa*. While the only explicit condition is the testing of horses in quarantine prior to export the preamble in that decision describes a period of freedom from Dourine in the Western Cape during the prior 6 months. Furthermore, in Council Directive 2009/156/EC (*Animal health conditions governing the movement and importation from third countries of equidae*), article 13 (1) c states that equidae from 3rd countries (such as South Africa) must come from countries that have been free from dourine for the prior 6 months. The 2013 FVO (EU Food and Veterinary Office – an auditing arm of the EU) audit in South Africa, pertaining to the controls in place for primarily African horse sickness, had a number of major recommendations, one of these being '*to adopt adequate measures in order to ensure the absence of dourine from the territory of dispatch*.' Since 1999 and the direct exports of horses to the EU, freedom from dourine within the territory of dispatch has relied on primarily clinical passive surveillance by private veterinarians, the active surveillance undertaken within the Thoroughbred breeding system and most importantly the individual testing of horses in quarantine prior to export. The 2013 audit report finding however made it clear that these measures were not considered adequate. The dourine surveillance undertaken looks to address this issue.

Scope

To provide evidence for freedom of Dourine within the same area where active surveillance is undertaken against AHS, i.e. the AHS surveillance zone in the Western Cape Province.

Surveillance parameters

Table 1: Surveillance parameters used in design and evaluation of the surveillance event

Parameter	Value	Comments
Population at risk	14000	All horses in the surveillance and free zones. 14000 is an estimate; there are currently 11658 and 1292 horses registered in the AHS surveillance and free zones respectively.
Design Prevalence	~5%	Minimum expected prevalence in the population should dourine occur; parameter taken from the serological survey requirements of the EU for AHS sentinel surveillance given that the same population was used for the dourine sampling.
Test Sensitivity	90%	Estimate based on best scientific guess. The sensitivity of the complement fixation test (CFT) has not been established, although given the false positive rates (see specificity) the sensitivity is likely to be relatively high. The CFT is seen as the gold standard for individual horse testing prior to export and this also supports a test with relatively good sensitivity.
Test Specificity	Unknown but system specificity of 100% assumed	The CFT test is prone to false positives and probably does not have a particularly good specificity. However, given that any positive CFT result will be investigated in full to establish a final diagnosis a specificity of 100% was used in establishing the outcome of the sensitivity of the surveillance
Type 1 error	5%	Used to provide a final probability of 95% that Dourine was not present if it was not detected using the surveillance parameters.

A goal of 60 serological sentinels per month is the requirement for AHS sentinel surveillance testing for direct exports from South Africa to the EU. Over and above this South Africa sample another 90 horses in the AHS surveillance zone to test approximately 150 horses in the zone using PCR testing. Given that serum samples are taken from all 150 horses, the sampled horses for the dourine surveillance were targeted from the remaining horses sampled that were not tested serologically for AHS. Samples were taken between 1st and 9th February 2018.

Consent

A client consent and information letter was provided to each owner/manager involved in the survey. The survey was voluntary, and owners/managers could withdraw their horses from the survey if they wished. Ethics approval was not obtained nor required for this survey since the samples were used from the existing AHS sentinel surveillance program that runs monthly in the AHS controlled area. Although retrospective samples were used we felt it was ethically responsible to obtain owner/manager consent prior to testing these samples for dourine.

Results

A total of 88 horses were sampled on 34 locations across the AHS surveillance zone. Proportional numbers of horses sampled across the surveillance zone are shown in Figure 1 below. The AHS sentinel surveillance program makes every effort to sample horses in proportion to their relative underlying population at risk using a gridded surveillance system, as depicted in Figure 1. The majority of samples were thus taken from an area of approximately 50 km around the Kenilworth Quarantine Station where horses are exported from.

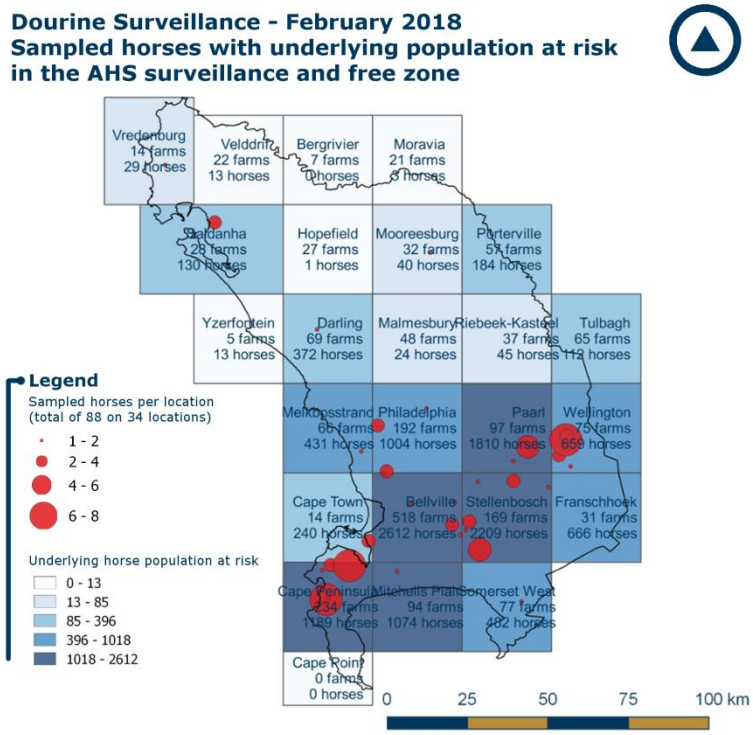

Figure 1: Dourine survey locations showing proportional circles for number of horses tested per location. The underlying population at risk is shown as a light to dark blue gradient, this to show that locations were chosen to reflect the relative underlying population at risk per surveillance grid.

All 88 samples tested negative for Dourine antibody using the CFT test.

The sensitivity of the surveillance program is shown in Table 2 below. While the sentinel surveillance program is based on a single stage sampling strategy (evaluated in Column 2 of Table 2) we have estimates of the underlying number of herds in the surveillance zone as well as estimates of the herd sizes of the sampled herds. This allows an estimate of surveillance sensitivity in a more realistic setting (Column 3 of Table 2). Note that in this latter analysis we reverted to an effective population design prevalence of 2% (within herd design prevalence of 20% and herd level prevalence of 10% throughout the population) – this in an effort to depict a reasonable minimum expected prevalence with so few cases of Dourine reported in the prior 2 decades in the AHS surveillance zone (see Figure 2).

Table 2: Design prevalences with resulting surveillance sensitivity and probability of freedom outcomes for two different scenarios: the sentinel program design prevalence and the generic values used given the history of cases in the AHS controlled area.

Parameter	Descriptions and values based on varying data sources	
	Single Stage population sensitivity	Generic prevalences to result in effective design prevalence of 2% with 2-stage analysis
P^*_U	0.05	0.2
P^*_c	n/a	0.1
Effective population prevalence (P^*_U x P^*_c)	0.05	0.02
MeanSSH - Mean herd level surveillance sensitivity	n/a	0.462
SeP - Population surveillance sensitivity	0.981	0.795
PFreeU - Confidence of population freedom – uninformed prior	0.981	0.829

The probability of freedom in the AHS surveillance zone, given this single surveillance effort, for dourine ranges between 79.5% and 98.1% depending on the effective design prevalence used.

Discussion

Stand-alone surveillance efforts like the one described here supplement the current clinical passive surveillance and Thoroughbred pre-breeding dourine surveillance efforts in South Africa. While the scope is limited to the AHS surveillance zone we believe this will assist in export protocols that require dourine freedom statements where horses are exported from AHS free zone quarantine facilities such as Kenilworth Quarantine Station.

Figure 2 shows all dourine cases reported in South Africa from 1993 through June 2016 (data accessed May 2018 from www.daff.gov.za and collated to South African local municipalities). The disease seems to dominate in the north-eastern parts of the Eastern Cape extending into the south-western Kwa-Zulu Natal (KZN). Other provinces mainly affected have been the North-West and Mpumalanga.

Significant numbers of horses move into the AHS controlled area on an annual basis (over 4400 moved in 2017 – State Vet Boland unpublished data). Given this movement and the lack of cases in the Western Cape (three cases in 2 decades) we can subjectively say that the disease is very much location based. The majority of movements are horses within the commercial sector, and given the nature of dourine and its transmission patterns it is likely that the disease is circulating within specific equine demographics and we suggest given the available data that this would be in working horses in non-commercial settings in South Africa. The last case in the Western Cape in 2012 occurred in a working mule in the Bredasdorp region – details of that case can be found at http://www.elsenburg.com/vetepi/epireport_pdf/February2012.pdf.

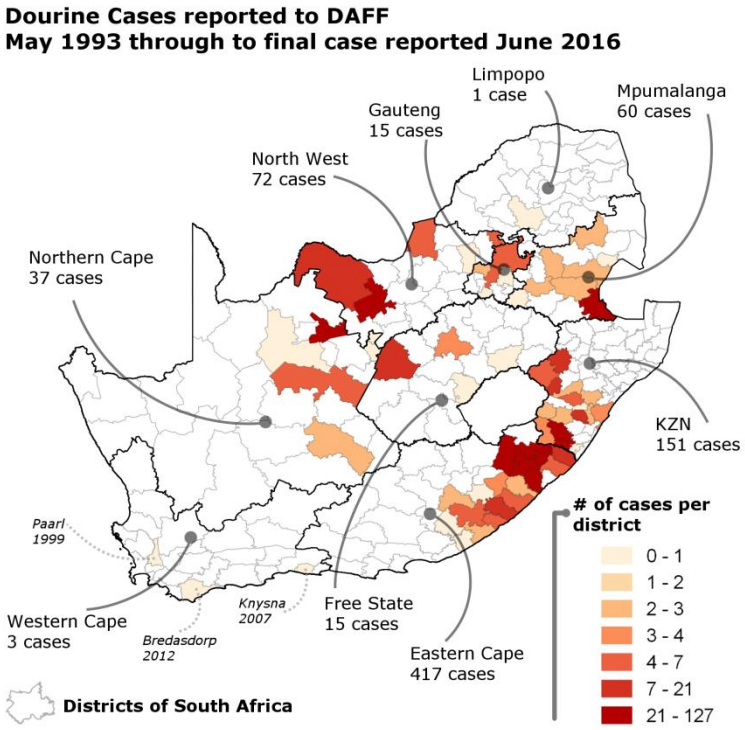

Figure 2: Historical Dourine cases reported to DAFF from 1993 through June 2016 (National Department of Agriculture, Forestry and Fisheries). Cases have been aggregated by district while case totals per province are labelled. The three cases reported in the Western Cape are also labelled specifically.

A recommendation is that this surveillance be repeated every six months in the AHS surveillance zone. This is primarily to comply with the 6 month freedom statement required by 2009/156/EEC and reflects an incubation period of up to 6 months for the disease. More accurate prior probability of freedom can be used and a more realistic confidence of dourine freedom can then be made.

Acknowledgments

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