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African horse sickness control

Surveillance report

Freedom from dourine
Cape Town Metropole
April 2020

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Surveillance Report

Disease	Dourine in horses
Surveillance type	Freedom from disease
Coverage area	Cape Town Metropole, Western Cape Province, South Africa
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Contents

Introduction, purpose and objectives.....	2
Underlying legislation, disease occurrence and historical surveillance.....	2
South Africa.....	2
EU legislation.....	3
OIE legislation.....	5
Introduction summary	5
Scope.....	5
Case definition and surveillance parameters.....	5
Case definition and testing strategy	5
Survey parameter definitions	6
Research ethics, Section 20 and manager consent	7
Results.....	7
Discussion, considerations and conclusion.....	8
Funding and Acknowledgments.....	8

Introduction, purpose and objectives

Dourine is a sexually transmitted trypanosomal (*Trypanosoma equiperdum*) disease of equids. Dourine freedom is required for the Cape Town Metropole for horses exported from South Africa to the European Union (EU). This surveillance requirement is over and above the pre-export testing of horses in Kenilworth Quarantine Station (KQS), which is done approximately 21 days prior to export. This report details a freedom from disease survey undertaken in the Cape Town Metropole in February 2020 to provide further evidence of freedom from this disease to facilitate the trade of live horses from South Africa.

Underlying legislation, disease occurrence and historical surveillance

Dourine is not only a controlled (and therefore notifiable) disease in South Africa (Animal Diseases Act – No. 35 of 1984) but is both an OIE listed disease (OIE Terrestrial Animal Health Code Chapter 12.3) and a disease requiring risk mitigation for importation of equidae into the European Union (EC2009/156; 2008/698/EC; EC 2018/659).

South Africa

The disease is considered present in South Africa with an average of 29 cases reported a year between 1993 and January 2018 – Department of Agriculture, Forestry and Fisheries (DAFF – now the Department of Agriculture, Land reform and Rural Development – i.e. DALRRD) disease database - see **Figure 1**. The Western Cape Province, within which the EU territory of dispatch is located (i.e. the African horse sickness (AHS) free zone), has reported only 3 cases in this 25 year period, in Paarl, Knysna and Bredasdorp in 1999, 2007 and 2012¹ respectively.

Export associated testing for dourine has been historically the primary surveillance focus in the current territory of dispatch. The majority of permanent horses in this area are, however, Thoroughbred racehorses in training. This industry has an active dourine surveillance program within its Stud Health Scheme² where all maiden and barren mares are tested for a variety of diseases, one of which is dourine. Furthermore all registered stallions are tested annually prior to the breeding season.

As a prelude to the surveillance described here, two active surveillance events in February 2018 and 2019 where 88 and 95 horses were tested respectively have been performed in the AHS free and surveillance zone using sera from current AHS sentinel animals. No positive cases were detected and these reports are available online at www.myhorse.org.za.

¹ WCDOA. Dourine. West Cape Dep Agric Epidemiol Rep. 2012;4(2):1. http://www.elsenburg.com/vetepi/epireport_pdf/February2012.pdf.

² <https://www.nhra.co.za/index.php/studbook/stud-health/stud-health-scheme>

Dourine Cases reported to DAFF May 1993 through to final case reported Jan 2018

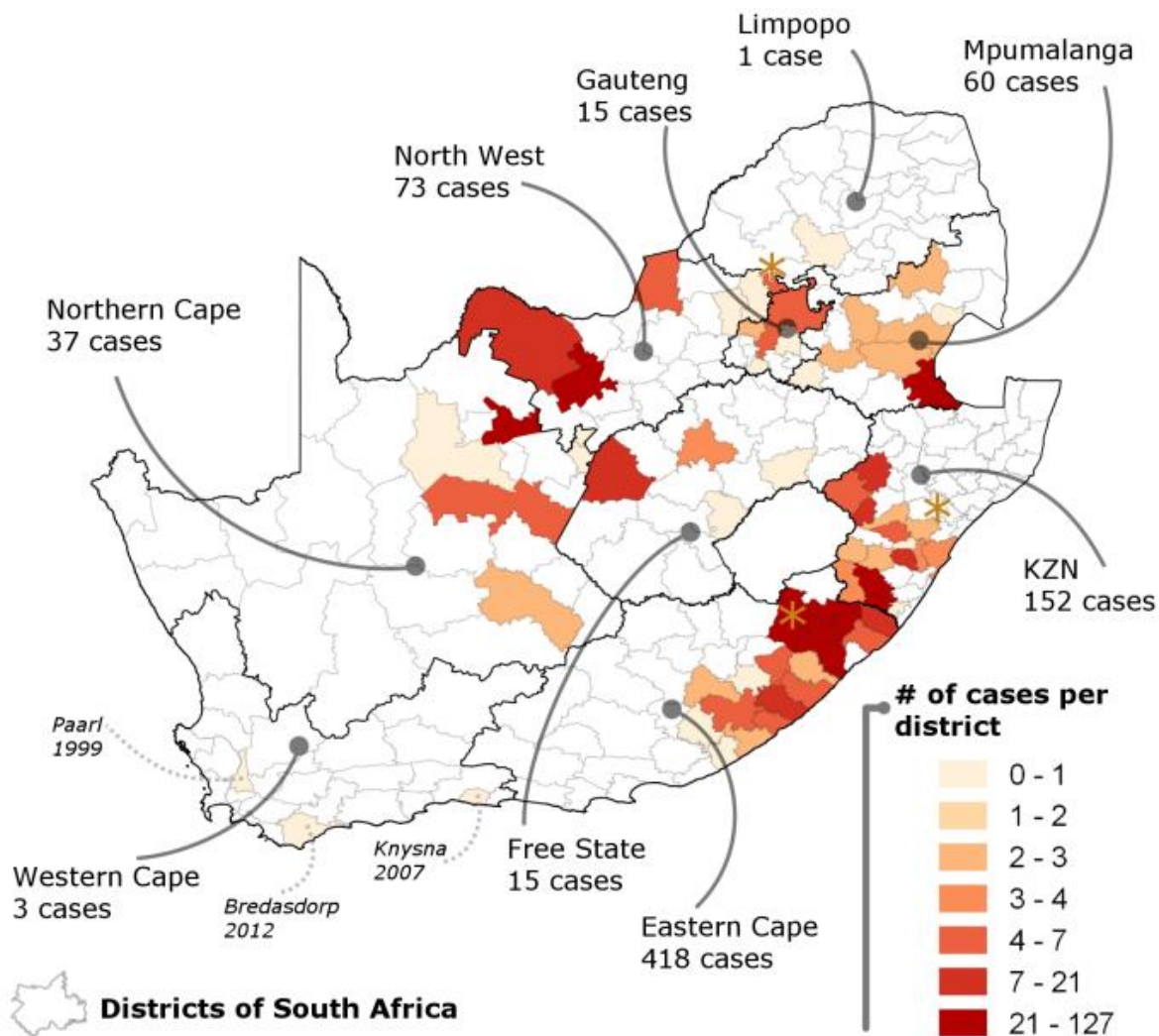


Figure 1: Historical dourine cases reported to DALRRD from 1993 through January 2018 (National Department of Agriculture, Forestry and Fisheries). Cases have been aggregated by district while case totals per province are labelled. The three cases reported in the Western Cape are labelled specifically with a location and year of occurrence.

EU legislation

There are a number of EU legislative documents that guide the surveillance and requirements concerning dourine and the trade in live horses from South Africa.

EU 2018/659

Commission implementing regulation (EU) 2018/659 of 12 April 2018 on the *conditions for the entry into the Union of live equidae and of semen, ova and embryos of equidae*, registers the territory of dispatch for South Africa as ZA-1: Metropolitan area of Cape Town. Specifically this area is equivalent to the AHS free zone of South Africa. A map of this zone showing this delineation is depicted in **Figure 2**.

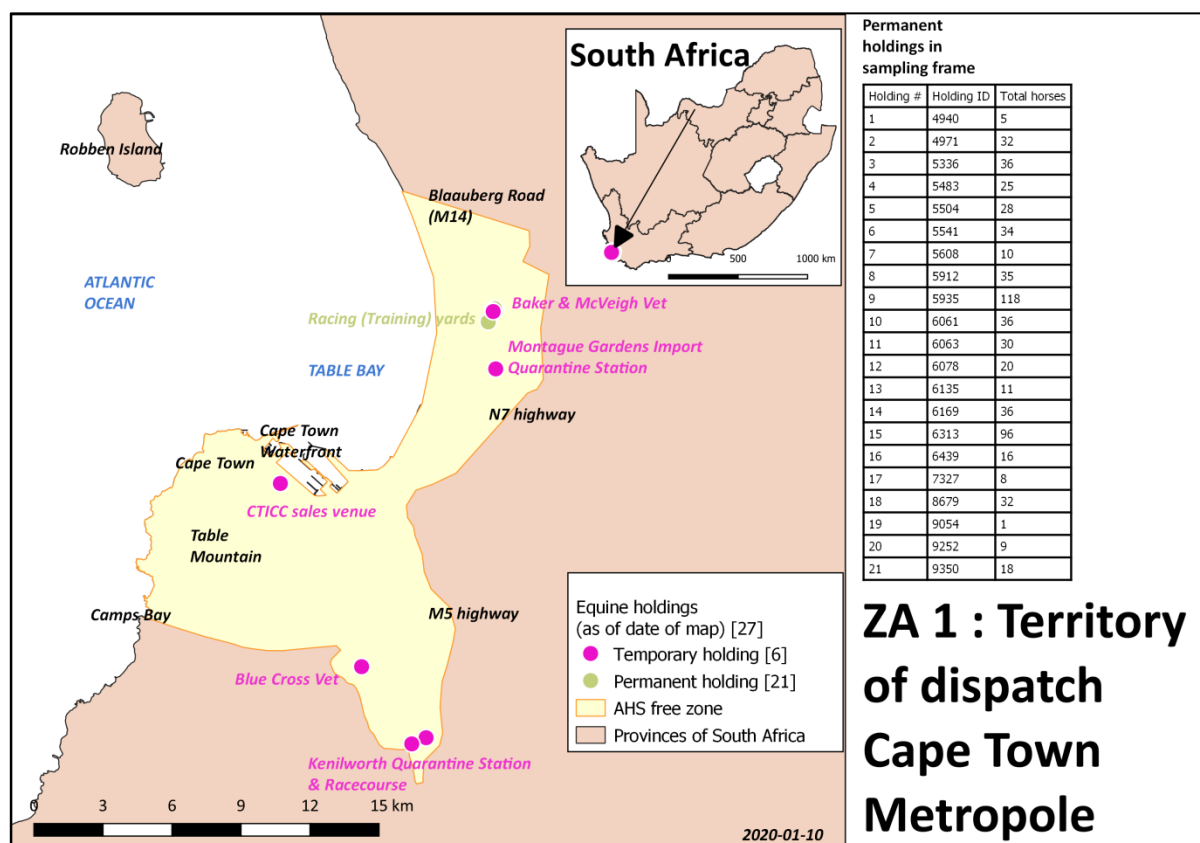


Figure 2: ZA 1 - Cape Town metropole area defined in EU 2018/659 including the initial sampling frame for this surveillance activity

2008/698/EC

The requirements for dourine surveillance are not explicit in 2008/698/EC which is the *Commission Decision of 8 August 2008 on the temporary admission and imports into the Community of registered horses from South Africa*. The text relating to dourine is limited to:

- dourine is endemic in certain parts of South Africa. However, the Western Cape Province has been free of dourine for more than six months.
- dourine must be notifiable
- during the isolation period the animal health tests must include dourine

2009/156/EC

2009/156/EC is the *Council Directive of 30 November 2009 on animal health conditions governing the movement and importation from third countries of equidae* and is also pertinent to South Africa in terms of dourine and export to the EU and in this legislation text relating to dourine reads:

- in addition to the requirements laid down in Article 5, the equidae must not come from a holding which has been the subject of one of the following prohibition orders
 - six months in the case of equidae suspected of having contracted dourine (or been in contact with equidae from a holding suspected of having dourine), beginning on the date of the last actual or possible contact with a sick animal. However, in the case of a stallion, the prohibition shall apply until the animal is castrated
- dourine must be notifiable

EU legislation summary

The EU legislation is not explicit on the surveillance requirements for dourine – rather the notifiable status of disease, a 6 month freedom period prior to export and testing for the disease in horses due for export. 2018/659/EC gives the most explicit area from which freedom must be ensured, and this principle is corroborated by the

comments made by the Food and Veterinary Office (FVO) audit team in 2013 who, when referring to deficiencies found in dourine surveillance, noted that:

“The actions do not ensure absence of dourine from “Metropolitan area of Cape Town (which would also encompass prevention of introduction of the disease), or the proposed area to be listed for export of live horses.”³

Considering this the target area for the surveillance described is the AHS free zone (i.e. Metropole of Cape Town).

OIE legislation

Dourine is discussed in the OIE’s Terrestrial Animal Health Code (Article 12.3.4) with the corresponding chapter in the Terrestrial Manual regarding testing for the disease. There are no exact surveillance targets specified: but rather recommendations on importation from an infected country requiring certification stating that animals exported:

- *showed no clinical sign of dourine on the day of shipment;*
- *were kept for the six months prior to shipment in an establishment where no case of dourine was officially reported*

Introduction summary

Freedom from dourine infection is required to be shown in the territory of dispatch to comply with EU certification requirements for the export of live horses from South Africa to the EU. While historically there have only been a handful of cases in the Western Cape Province, the 2013 EU audit indicated that additional information would be required. Two rounds of active surveillance within the AHS sentinel group have been completed in 2018 and 2019 respectively, and this surveillance report focusses on the actual territory of dispatch and shows freedom from dourine at a point in time in the first quarter of 2020.

Scope

The scope is limited to the population of horses that are resident in the AHS free zone (see **Figure 2**) of South Africa.

Case definition and surveillance parameters

Case definition and testing strategy

The OIE’s Terrestrial Animal Health Code does not explicitly state the case definition to be used for dourine. The Manual however, in the *Identification of the agent* section states that:

Definitive diagnosis depends on the recognition of clinical signs and identification of the parasite. As this is rarely possible, diagnosis is usually based on clinical signs, together with serological evidence from complement fixation (CF) tests.

Importantly, in the context of potential introduction and surveillance, the disease is not transmitted by invertebrate vectors and there is no known reservoir of the parasite except in the equid host. The parasite is however tissue associated with a humoral immune response therefore serological testing is the only practical option for freedom from disease type surveys. The Complement Fixation Test (CFT – CF test) is universally used for individual animal freedom status, and while the CFT is not always useful in freedom from disease surveys (IFAT and ELISA may be more useful – OIE Terrestrial Manual 2013 – see **Table 1**) in South Africa it is the only available test. The CFT is used routinely internationally for pre-export testing.

³ https://ec.europa.eu/food/audits-analysis/act_getPDF.cfm?PDF_ID=10679

Table 1: Test methods available for the diagnosis of dourine and their purpose. Extracted as published from the OIE Terrestrial Manual 2018

Method	Purpose			
	Population freedom from infection	Individual animal freedom from infection	Confirmation of clinical cases	Prevalence of infection – surveillance
Agent Identification¹				
Microscopy	–	+	+++	–
PCR	–	+	++	+
Detection of immune response				
CFT	+	+++	+++	+++
IFAT	++	+	+	++
ELISA	++	+	+	++

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
PCR = polymerase chain reaction; CFT = complement fixation test;
IFAT = indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay.

Confirmation for surveillance positives can be obtained through the use of historical records of animal breeding, clinical signs, microscopy, PCR and other available serological tests. 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.

For the purposes of this surveillance the case definition was based on the CF test which was used to screen all selected horses. While information from each horse regarding its sexually active status, its dam and sire and its sex and date of birth were obtained, no positive results were obtained and further follow up and clinical investigation of sampled horses was not necessary.

Survey parameter definitions

Unfortunately the epidemiologic description and published information regarding dourine occurrence is sparse, and survey parameters could not be chosen on well-defined proportions. A single stage random sampling frame was chosen for this survey since:

- the population all occur in a very small area within the greater survey area at risk (see **Figure 2**)
- they are all horses that are imported into the survey area (no breeding occurs in the survey area to the best of our knowledge) and will come from a variety of different holdings/locations
- planned sexual contact is unlikely between animals and this is true for all establishments in the survey area
- a census level individual horse sampling frame was available

Minimum expected prevalence

In the absence of international, regional and trade partner guidelines, the minimum expected prevalence (design prevalence) of 2% of horses was chosen.

Test sensitivity and specificity

The test sensitivity of the CFT has not been explicitly published, particularly in the South African context. Internationally it has been shown that the CFT may be less sensitive than other serological tests for dourine. The CFT has been the global standard for international movement of horses and the sensitivity should be relatively high – a best scientific guess of 90% CFT sensitivity was used to determine the sample size. A test specificity of 100% was assumed since any suspect or positive result (there were none) would have been followed to a final decision based on the case definition used.

Population-level sensitivity

A type one error rate of 5% was used reflecting a required 95% probability level of detecting dourine should it exist within the survey parameters.

Population size

Population data (herd (N=21) and individual horse level (N=636)) were obtained through census derived from existing systems within the Western Cape Province. Census information (on individual horse level) is available since the permanent holdings are part of the existing AHS Multiple Movement Scheme⁴ which requires ongoing census updates. The sampling frame was extracted on the 10th January 2020. There were 21 holdings with a total of 636 horses on them. Holding's averaged 30 horses; had a median of 28 horses; and ranged between 1 – 118 horses residing on them. Evaluation of the surveillance after it was performed used an updated population census reflecting the changes between January and February when the surveillance was performed.

Random replacement horses were selected from the same holding where pre-selected horses were not available to sample.

Horses to sample calculation

Calculations for the total number of horses to sample were made in EpiTools (Ausvet (Pty) Ltd: <http://epitools.ausvet.com.au/>) using the “Sample size to achieve specified population level sensitivity” option⁵. Using the variables mentioned a sample size of 146 horses was established. The 146 horses in the final sample selection were selected randomly from the outbreak population using a random sample selector, also in EpiTools – “Random sampling from a sampling frame”, with a simple random sampling strategy, sampling without replacement, and, as mentioned earlier, no stratification or subgrouping.

Research ethics, Section 20 and manager consent

Owner/manager consent was attained prior to sampling of selected holdings. Exemption from a Section 20 permit was confirmed by DALRRD. Ethics approval was obtained from the Western Cape Department of Agriculture Research Ethics Committee (DECRA reference DS20/133).

Results

A total of 145 horses were sampled on 18 holdings across the AHS free zone between 17-18 February 2020. A total of 596 horses were present in the free zone in this period. All 145 samples tested negative for dourine antibody using the CFT (tested at the Agricultural Research Council's Onderstepoort Veterinary Institute – Laboratory reference 2020-D-2747). Post-surveillance evaluation was performed and the sensitivity of surveillance was 94.8% with a 95% probability of freedom if a prior probability of freedom of 50% was assumed, and a 99.4% probability of freedom if a prior probability of freedom of 90% was assumed.

A total of 68 of the pre-selected horses were not available for sampling when the survey took place. The majority (n=40; 59%) were not present when sampling took place, with the remainder not sampled for the following reasons: due to race within 7 days (n=11); recently treated by a veterinarian (n=2); trainer not present to give consent (n=9) and unsafe to sample (n=6). Replacement horses were selected randomly.

Over and above the trainer that was not available (Holding 6169) to give consent, there were a further 3 holdings (ID's 7327, 9054 and 5608) that were no longer training (and did not have horses present) in February when the sampling was performed. Holding 9054 only had one horse in the initial sampling frame and it had not been selected for sampling. In the interim however, a new trainer (holding 9546) had started in the Milnerton training yards. A random selection of horses from this yard were used to replace the three holdings (6169, 7327 and 5608) that had been selected to be sampled but were not available to sample.

⁴<http://www.myhorse.org.za/ahsvpn/Documents/AHS%20Control%20SOP%20Multiple%20Movement%20Permit%20System.pdf>

⁵ <https://epitools.ausvet.com.au/freedomss>

Discussion, considerations and conclusion

We conclude that, if dourine was circulating in the AHS free zone in February 2020 at a level of 2% or higher, we would have been 95% sure that our surveillance would have detected it, and there is a 95-99% probability that the area is free from dourine. This surveillance activity was primarily driven by the definitions of freedom required for trade of equines between South Africa and the EU. The geographical scope of the event was very limited and by default limited to a very distinct population of horses. This underlying population at risk would be low risk for dourine infection as they are young Thoroughbred's in training from a sector of the industry that has a Stud health scheme which includes dourine surveillance. This program is just a part of the overall dourine surveillance undertaken in the Western Cape, and adds to the evidence that allows dourine freedom statements to be certified by exporting officials.

The historical case occurrence within the Western Cape Province is very low (**Figure 1**) and cases have not, to our knowledge, occurred within the targeted surveillance area. While cases have occurred in the country, reported cases have occurred sporadically. This situation makes it difficult to establish a minimum expected prevalence to survey for, should dourine exist within the population.

Funding and Acknowledgments

The surveillance event cost approximately R51 000. Funding of this project was obtained from the South African Equine Health and Protocols (80% - sampling, logistic and testing costs) and the Western Cape Department of Agriculture (3% - sample kits). Dr Aliya Davids is a compulsory community service veterinarian and is allocated to the SAEHP for 2020; however her salary costs are covered by the DLRARD (17% of total cost). Administration and client communication was conducted by Marie van der Westhuizen from SAEHP. We are very grateful to the owners and managers of the training horses in the AHS free zone in the Western Cape, for their kind assistance.