



## Apparent prevalence of dourine in the Khomas region of Namibia

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### ABSTRACT

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A 15-year record of the results of horse sera from the Khomas region of Namibia tested by the complement fixation test for dourine at the Central Veterinary Laboratory in Windhoek before clearing the respective animals for export and competitive sport were subjected to statistical analysis. The range of percentage positive, taken as the apparent prevalence of dourine for the region, during the period of study, was 0–29.09 %; the average regional level of apparent prevalence was 8.33 %. These figures were thought to be lower than the real situation due to some bias in the sampling criteria. For more accurate results, the more reliable enzyme-linked immunosorbent assay techniques are recommended for use in sero-surveys for dourine in Khomas and other regions of Namibia to provide a basis for development of effective control strategies against the disease.

**Keywords:** Complement fixation test, dourine, horses, prevalence

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### INTRODUCTION

Dourine is an insidious venereal infection of equine species caused by the protozoan parasite *Trypanosoma equiperdum*. The disease is clinically characterized by inflammation of external genitalia, cutaneous lesions and nervous involvement. Natural transmission of the infection is almost entirely by coitus. The source of infection may be an infected male animal or a healthy male acting as a physical carrier after serving an infected mare (Radostitis, Blood & Gay 1994). Some concern has been raised about the possibility of dourine transmission through imported semen (Metcalf 2001). Dourine was first clinically diagnosed in Namibia in 1914 and is believed to have been introduced into the country in horses imported by German settlers.

Documentation exists about the wide distribution of the disease in southern Africa, including Namibia (Barrowman & Van Vuuren 1976; Williamson & Herr 1986). Outbreaks of the disease are notifiable by law in Namibia but compulsory testing of susceptible animals followed by slaughter and destruction of positive cases, as recommended by Derbyshire & Nielsen (1997), is not enforced. No comprehensive sero-survey of equid populations has been undertaken for dourine in Namibia and so the magnitude and pattern of spread of the infection remains unclear. According to the 1997 stock census of the Directorate of Veterinary Services, Ministry of Agriculture Water and Rural Development (MAWRD), the domestic equid population of Namibia consists of 57 000 horses and 166 000 donkeys. These are an important national resource that contributes in various ways to the socio-economic well-being of many people in terms of transport, animal power,

recreation, as well as an important export commodity to the South African market. An estimated 49 000 zebras are an important component of the wildlife resource-base of the country. Dourine constitutes a risk factor for this national wealth.

The central veterinary laboratory (CVL) in Windhoek handles all serological diagnostic tests for dourine in Namibia. The complement fixation (CF) test, also known as the dourine test, is the test of choice, and all racehorses and those destined for export must be certified free of dourine. The animal owner meets the costs of dourine tests performed and undertakes to eliminate the animals that test positive for the presence of anti-trypanosome antibodies. This is why it is mainly the more prosperous commercial farmers, who breed horses for export, who submit sera for testing.

The present paper presents the findings of a retrospective study on results of dourine tests carried out during a 15-year period (1985–1999) at the CVL. The aim of the study was to shed light on the prevalence and the risks posed by dourine to horses in the Khomas region of Namibia and to provide some basis for speculation about the situation among equid populations over the entire country and what should be done to control the disease.

## MATERIAL AND METHODS

### Study area

The Khomas region of Namibia is situated in the central part of the country and consists mainly of the central highlands where the capital city of Windhoek is located. As in most parts of Namibia, livestock farming is the main economic activity in the Khomas region and is strictly commercial. Movement of equid species and all farm animals into and within the region is strictly controlled.

### Statistical analysis

The number of animals whose sera were tested, represented the sample size for the Khomas region. The domestic equid population figures for the region, which has remained stable during the last 15 years, are 4 500 horses and 1 900 donkeys (Directorate of Veterinary Services 1997). The sample size required to estimate dourine prevalence in horses at the 99% level of confidence with an error of estimation of < 10% was calculated for the Khomas region and found to be 51 animals. The apparent prevalence of dourine in the region

was calculated as the percentage of positive cases for all sera tested.

### Details of CF test carried out in the CVL

CF testing at the CVL is done according to the standard procedure. Briefly, test sera are first incubated in water bath at 56 °C for 1 h to inactivate endogenous complement. Twofold serial dilutions of each test serum are made in equal volumes (25 µl) of calcium-magnesium veronal (CMV) buffer (Bio-Merieux) in U-shaped 96-well microtitration plates (Nunc). To each well 25 µl of diluted *Trypanosoma* antigen (Onderstepoort Biological Products Ltd) is added, followed by 25 µl of reconstituted purified guinea pig complement (Dade Behring Marburg GmbH). Plates are then incubated at 37 °C for 1 h. Thereafter 25 µl of a 3% suspension of sheep red blood cells (SRBC) in CMV and amboceptor solution (Dade Behring Marburg GmbH), the haemolytic system, is added to each well and plates are again incubated at 37 °C with constant shaking. Results are read after 1 h of incubation. Controls consist of known positive and known negative horse sera for anti-trypanosome antibodies.

SRBC are obtained from sheep raised at the Bergflug experimental farm just outside Windhoek. The animal is bled by jugular venipuncture. Blood is immediately mixed with an equal volume of an anticoagulant (Alsever's solution) and stored at 4 °C for 7 days before use. Just before use SRBC are washed twice in CMV at 1 500 rpm. The indicator reaction system (3% suspension of RBC) is prepared by adding 6% SRBC suspension in CMV to an equal volume of amboceptor solution.

## RESULTS

The study revealed that only horse sera are sent by farmers to the CVL for testing for dourine. Data on CF test results carried out on these sera from the Khomas region between 1985 and 1999 are found in Table 1.

In none of the 15 years of study was the total number of animals tested lower than 51, the minimum sample size to estimate the prevalence of dourine among horses in the region at 99% level of confidence with an error of estimation < 10%. The highest prevalence of positive cases found in the region was 29.09% in 1986 and the lowest was 0% in 1996. Apparently the incidence of dourine has tended to fluctuate from year to year in the Khomas region. The overall average apparent prevalence of



TABLE 1 Prevalence of dourine in the Khomas region of Namibia (1985–1999)

Year	No. of horses tested	Positive horses	Percentage positive
1985	63	1	1.59
1986	368	96	29.09
1987	380	42	11.05
1988	328	17	5.18
1989	263	10	3.80
1990	271	11	4.06
1991	198	6	3.03
1992	181	19	10.50
1993	193	11	5.70
1994	143	12	8.39
1995	76	7	9.21
1996	148	0	0
1997	208	17	8.33
1998	107	4	3.74
1999	175	5	2.86
Total	3 098	258	8.33

the disease among horses in the region was 8.33%. Limited findings obtained for other regions (not presented here) pointed to the same trend.

## DISCUSSION

Although dourine is widespread in equine populations throughout Namibia, only data available for the Khomas region are statistically analyzable. However, the overall prevalence of 8.33% found for the disease in this region is probably lower than the real situation due to a certain level of bias in the kind of sampling criteria on which depended this study. Not only are suspected sera submitted for testing predominantly from the commercial farms, but, even at this level, it is mainly horses destined for competitive sports or for export that are routinely tested for dourine as demanded by the regulations of Namibia. It is obvious that such animals would normally be physically fit and in apparent good state of health. Older and weak animals, much more likely to harbour infectious agents such as *T. equiperdum*, are not generally subjected to the same sero-examination. African strains of this parasite have been shown to more often give rise to the inapparent and chronic forms of dourine (Radostits, Blood & Gay 1994). In neighbouring Botswana, the overall national level of dourine prevalence was found to be 9.0%, with a range of 0–20% in different districts (Masupu & Majok 1998).

It is difficult to explain why dourine should remain endemic and at an elevated level amongst horses

in the Khomas region since, as in all other commercial farming regions of Namibia, the latest technologies and most advanced management techniques are employed for disease control. On these farms, mating is strictly controlled and effective control measures are in place to prevent contact with domestic and wild equids that might be found on neighbouring farms. It is, however, possible that the infection is maintained on commercial farms in animals of which sera are not routinely subjected to serological tests.

Furthermore, anticomplement activities have been shown in dourine tests on sera of donkeys and mules to result in false negatives. Although, according to Williamson & Herr 1986, the phenomenon does not seem to present a problem when horse sera are tested, enzyme-linked immunosorbent assay (ELISA), not susceptible to anti-complement factors, has been shown to be a more sensitive serological test (Bishop, Rae, Phipps, Boid & Luckins 1995; Wassall, Gregory & Phipps 1991; Williamson, Stoltz, Mattheus & Schiele 1988). Katz, Dewald & Nicholson (2000) reported competitive ELISA to be a technically more reproducible, objective and convenient approach for a number of protozoal infections, including dourine, to screen animals for export or disease eradication programmes. These observations indicate that negative results obtained with the CF test are not always an indication that the animal concerned is free of trypanosomiasis and that false negatives might be responsible for maintenance of dourine amongst horses in the Khomas and other commercial farming regions in Namibia.

Positive results obtained with the CF test for dourine are likely to be accurate for most of Namibia, since the tsetse fly vectors (*Glossina* spp.) of most other *Trypanosoma* spp. are absent because the country lies outside the tsetse-infested zones of tropical Africa. Consequently, no other *Trypanosoma* spp. are expected to occur in Namibian horses and be the cause of antigen/antibody cross-reactivity. However, for a more effective control programme for dourine to be introduced in Namibia, use of the more sensitive ELISA in sero-surveys should be considered.

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