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# OBSERVATIONS ON THE TRANSMISSION, IMMUNOLOGY, CLINICAL SIGNS AND CHEMOTHERAPY OF DOURINE (*TRYPANOSOMA EQUIPERDUM* INFECTION) IN HORSES, WITH SPECIAL REFERENCE TO CEREBRO-SPINAL FLUID

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## Abstract

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This paper is a record of observations on the transmission and clinical signs of dourine in naturally infected cases of known duration, and of temporal and quantitative aspects of the immune response in blood and cerebro-spinal fluid. Included in the record are observations on the presence of *Trypanosoma equiperdum* parasites in these body fluids and methods for their detection. There is evidence that the occurrence of nervous symptoms and lesions in infected horses is associated with the presence of *Trypanosoma equiperdum* parasites in cerebro-spinal fluid. The suitability of cerebro-spinal fluid as an environment for the parasite and its relationship with nervous manifestations of the disease are discussed. Observations support the previously reported lesions of peripheral polyneuritis and suggest a possible correlation between the consistent position of the nervous lesions and the drainage of cerebro-spinal fluid containing the parasite. Chemotherapy with an experimental drug MSbE was used with varying results in 4 horses at different stages of infection.

## *Résumé*

*QUELQUES OBSERVATIONS SUR LA TRANSMISSION, L'IMMUNITÉ, LES SYMPTÔMES ET LA CHIMIOTHÉRAPIE DE LA DOURINE (INFECTION À* TRYPANOSOMA ÉQUIPERDUM*) CHEZ LE CHEVAL AVEC RÉFÉRENCE SPÉCIALE AU LIQUIDE CÉPHALO-RACHIDIEN*

*Cet article est un enregistrement des observations sur la transmission et sur les symptômes de la dourine, portantes sur des chevaux infectés dans la nature et dont la durée est connue et sur des aspects temporels et quantitatifs de la réponse immunitaire du sang et du liquide céphalo-rachidien. Y comprises sont des observations de la présence de* Trypanosoma équiperdum *dans ces liquides ainsi que des méthodes à déceler le parasite. Il existe de la preuve que les manifestations de symptômes et de lésions nerveux peuvent être associées à la présence de* T. équiperdum *dans le liquide céphalo-rachidien. L'auteur discute la convenance du liquide céphalo-rachidien à soutenir le parasite et son rapport avec les manifestations d'ordre nerveuses. Certaines observations viennent appuyer une constatation antérieure de la présence d'une polynévrite périphérique et suggérent la possibilité d'un rapport entre la localité régulière des lésions nerveuses et l'affluence du liquide céphalo-rachidien renfermant le parasite. On constate des résultats variables à la suite de la chimiothérapie avec un médicament expérimental, le MSbE, appliqué à 4 chevaux à différents stades de l'infection.*


## Introduction

Dourine, caused by *Trypanosoma equiperdum* Doflein, 1901, has been recognised as a disease of breeding solipeds for many centuries (Hoare, 1972) and is unique among the trypanosomiases in not involving an arthropod vector. Though it still occurs in many parts of the world, since its eradication from North America and northern Europe, published research on the pathogenesis, immunology and chemotherapy of the disease has been neglected.

The clinical and pathological changes produced by strains of *T. equiperdum* maintained by serial passage or in the laboratory are different from those of the natural disease. Dourine in South Africa occurs as a sub-acute or chronic disease which frequently shows no clinical manifestations in affected horses. Passaged or laboratory-maintained strains give rise, however, to an acute or peracute condition in horses or laboratory animals, the macroscopic lesions differing from those observed in naturally-infected horses (Parkin, 1948; Lavergne, Labert & Raynaud, 1969; Moulton, 1974).

This study was initiated to evaluate the chemotherapeutic sterilizing effect of an experimental compound, registered as MSbE*, in naturally-infected horses, following a favourable report of its acitivity (W. O. Neitz, Veterinary Research Institute, Onderstepoort, unpublished report, 1972). The criteria to be used for infectivity were the ability to infect clean horses by coitus and by sub-inoculation of blood before and after treatment, the detection of trypanosomes in blood and the response of the serum complement fixation reaction. However, the absence of published information on many aspects of the host's response to infection led to observations being made on some of these, especially where the parasite was found to have penetrated the blood-brain-barrier into the cerebro-spinal fluid.

Observations on the transmission, clinical signs, location of the parasite, immune response, pathogenesis and MSbE therapy of naturally-infected horses are recorded in this paper.

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* Manufactured and supplied by Dr. E. F. Friedheim, 5 Avenue Marc-Monnier, 1200 Geneva, Switzerland

## Materials and Methods

### *Experimental animals*

All horses used in this study were sexually mature adults of varying ages. Prior to the experiment, the infected state of the animals was established by a series of complement fixation tests (see below) during a period of isolation. They were vaccinated against horse-sickness and anthrax, treated with Equigard* for intestinal parasites and accommodated in open pens with food and water supplied *ad lib.* All services took place under observation and contact between infected and clean horses was prevented at all other times. The source of *T. equiperdum* parasites was naturally-infected horses originating from the field.

* Shell pharmaceuticals

*Natural transmission*

A naturally-infected stallion (7090) and mare (100), originating from the northern Orange Free State and both positive on complement fixation testing, were used for service with susceptible stallions and mares negative on complement fixation test prior to service. Ovarian activity of the mares was determined by rectal examination, and oestrus was established by the use of a teaser stallion under manual control which was not permitted direct contact with the mares. Mares which conceived but remained uninfected were aborted between 8 and 16 weeks of gestation by saline irrigation of the uterus via the cervix. To stimulate breeding activity in the winter months, the mares were housed overnight in time-controlled lighting which allowed 7 hours darkness.

*Transmission by sub-inoculation of blood into horses*

Blood taken from the jugular vein of infected horses was collected in 10% sodium citrate and transfused into the jugular vein of a serologically-negative stallion or mare within 30 min of collection. Blood samples were subsequently taken from the recipient for complement fixation tests and examination for parasites.

*Transmission by sub-inoculation into other animals*

Blood containing living trypanosomes was collected from infected horses in 10% sodium citrate and injected intra-peritoneally into intact and splenectomized rats, and subcutaneously into 1 dog, within 30 min of collection. Living trypanosomes separated from whole blood on an anion exchange column (see below) were concentrated by centrifugation and injected intraperitoneally into rats. Cerebro-spinal fluid (CSF), collected at post mortem and containing live trypanosomes, was injected into the peritoneal cavity of rabbits within 30 min of collection.

The recipients were kept under observation and thin blood smears were examined daily for a period of 8–10 weeks after sub-inoculation.

*Complement fixation test*

The complement fixation test (CFT) employed was a 6 volume method, using 1 volume of test serum, 1 volume of antigen, 2 volumes of complement containing 2 units and 2 volumes of sensitized sheep erythrocytes, constituting a total volume of 0,6 ml. Sera were inactivated at 58 °C for 30 min prior to the test, and primary and secondary incubations were carried out for 30 min at 37 °C, the results being read immediately afterwards. All positive sera were titrated and read at 50% haemolysis end point. The antigen used was prepared in rats from a laboratory-maintained strain of *T. equiperdum*.

Complement fixation tests were carried out on serum, unconcentrated CSF and CSF concentrated 10 or 100 times, using a Minicon B15* reverse osmosis cell.

*Demonstration of trypanosomes*

Three different methods were used in the attempts made to demonstrate the parasites in blood, viz:

(i) The centrifugation method of Parkin (1948), in which 10 ml of plasma separated from citrated whole blood was centrifuged at 1 500 g (av.) for 15 min. The upper layer being discarded, the bottom 0,1 ml, including cellular deposit, was examined as a wet preparation or prepared as a thick smear, air-dried, fixed and stained with 10% Giemsa for 30 min.

(ii) The column-separation and membrane-filtration technique of Lanham, Williams & Godfrey (1972), in which heparinized whole blood was passed through an anion exchange column of DEAE cellulose. The eluate was centrifuged for 15 min at 1 500 g (av.), and the bottom layer fixed with 2,5% gluteraldehyde and passed through a Swinnex** filter with a 0,45 $\mu$m pore size membrane. The membrane was then stained with 30% Giemsa for 15 min and examined microscopically for trypanosomes.

(iii) A sedimentation/wet preparation method. This involved the collection of 50 ml of blood into 5 ml of 10% sodium citrate in a 60 ml stoppered bottle. The erythrocytes were allowed to sediment at room temperature or in an incubator at 37 °C, for 2 h or more. A sample was withdrawn carefully with a 1 ml pipette from just above the white cell layer and 0,1 ml of the sample spread beneath a cover glass for immediate examination at 400× magnification with a reduced light input.

Samples of CSF were examined directly as wet preparations at 400× magnification and, if no trypanosomes were detected, then centrifuged at 1 000 g (av.) for 15 min. Where trypanosomes were detected in the bottom layer of the sample, thick smears were prepared for staining and morphological examination.

*Electrophoresis*

Electrophoretic separations of serum and CSF proteins were carried out with a Beckman Microzone+ eletrophoresis cell using cellulose acetate membranes. Ponceau-stained protein separations were read and recorded on a Beckman Analytrol+ scanner.

The low concentrations of protein present in CSF necessitated 10× concentration and 5 or 6 applications to the same point on the membrane to obtain measurable readings.

Total serum protein levels were determined by the method of Oellermann (1974), using a Beckman+ spectrophotometer, measuring absorbance at 233 nm and 226 nm and correlation of the difference to a standard curve. Total CSF protein levels were measured by the Farb Test colour method supplied by Boehringer.++

*Cerebro-spinal fluid collection*

The technique used to sample CSF of live horses was a modification of that described by Tufvesson (1963) for anterior epidural anaesthesia.

Sampling was carried out using a 16 gauge needle 15 cm long with a stylet. The lumbo-sacral foramen of the horse lies 6–8 cm posterior to a line connecting the anterior parts of the *tubera sacralae*. An incision 1 cm long is made at this point using local analgesia. When capillary haemorrhage has stopped, the needle with stylet is inserted in the mid-line at an angle 30° posterior to the vertical. A distinct sensation is felt when the needle penetrates the interarcual ligament at a depth of 10–12 cm, and the stylet is then withdrawn. The needle is then carefully inserted a few millimetres to penetrate the dura mater and enter the subarachnoid space. At this stage drops of

* Amicon Corporation, Lexington, Mass., U.S.A.

** Millipore Corporation, Bedford, Mass., U.S.A.

+ Beckman Instruments Inc., Palo Alto, California, U.S.A.

++ Boehringer, Mannheim, B.M., G.m.b.H., West Germany

CSF will appear through the needle and the sample can be collected with a 10 ml hypodermic syringe. On occasion the CSF pressure is insufficient to force the fluid up the needle and negative pressure applied with a syringe may be required. Samples showing obvious contamination with erythrocytes were not used in this study.

Strict asepsis was observed throughout the procedure and no adverse effects were observed in any of the horses sampled in this way. Prior sedation was not required and the animals were restrained in a conventional enclosed crush with a 2,5 metre ceiling.

Post-mortem CSF samples were taken by needle puncture from the atlanto-occipital junction after dissection of the overlying tissues and skin.

*Immobilization tests*

One ml of 100× concentrated CSF from infected Mare 6271 was added to 1 ml of rat blood with a high parasitaemia of a laboratory-maintained *T. equiperdum* strain. The mixture was kept at 37 °C and samples were withdrawn and examined as wet preparations at 10 min intervals. Controls of 1 ml untreated infected rat blood and 1 ml infected rat blood, to which 1 ml 100× concentrated CSF from uninfected Mare 6921 had been added, were treated similarly.

*MSbE*

The experimental drug MSbE was kindly supplied as a powder in 2,0 g vials by Dr. E. Friedheim of Geneva. Prior to administration of the drug, the horses were mass measured on a weighbridge and a dosage of 10 mg/kg body mass was prepared. The drug was dissolved in sterile, pyrogen-free water, 20 ml per 2,0 G MSbE, immediately prior to use and injected into the brachiocephalic muscle halfway down the neck.

## Results

A resumé of the horses used in these experiments and the results obtained with attempted transmission of the disease are given in Table 1.

*Natural transmission*

Infected Stallion 7090, showing pronounced ventral oedema, oedema of the sheath and scrotum (Fig. 5) and a serum CFT titre of 1: 8 192, served 4 serologically-negative mares on 33 occasions over a period of 7 months before a mare was infected. This mare, 6271 (see Fig. 2) was served by CFT-negative Stallion 6568 on Days 43 and 44 following her infectious coitus with Stallion 7090. At this time her CFT titre was 1:48 and trypanosomes were demonstrable in her blood. Stallion 6568 was subsequently shown to be infected by this coitus (see Fig. 3). However, a second CFT-negative stallion, 6569, served Mare 6271 on Days 44, 45 and 46 respectively post infection without contracting dourine. Stallion 6569 was used again to serve Mare 6271, without contracting dourine on Days 76, 77 and 78 respectively post infection when her CFT titre was 1: 96 and trypanosomes were irregularly detectable in her blood.

Infected Stallion 6568 was used to serve clean mares without transmitting the disease on the following days post infection: Days 46, 47, 48, 49, 114, 116, 117, 118, 200, 201, 202, 215, 216 using 3 CFT-negative mares (Table 1) and a total of 16 services. The CFT titre and detection of the trypanosomes in the stallion at these times are recorded in Fig. 3.

TABLE 1 Transmission experiment with dourine-infected horses

(a) With field cases

| Infected animal | Negative horses used for | | Result |
|---|---|---|---|
| | Service | Blood inoculation | |
| Stallion 7090*.. | Mare 6912 | | Negative |
| | Mare 6921 | | Negative |
| | Mare 6961 | | Negative |
| | Mare 6271 | Mare 6645* | Positive |
| | | | Positive |
| Mare 6645..... | Stallion 6568 | | Negative |
| | Stallion 6569 | | Negative |
| | Stallion 7037 | | Negative |
| Mare 100..... | Stallion 7037* | | Positive |
| Stallion 39..... | Mare 6921 | | Negative |
| Stallion 99..... | | | |
| Mare 20..... | | | |

(b) With horses infected from field cases

| | | | |
|---|---|---|---|
| Mare 6271* | Stallion 6569 | Mare 6773 | Negative |
| | Stallion 6568 | | Positive |
| | | | Positive |
| Stallion 6568... | Mare 6961 | Mare 6961 | Negative |
| | | | Positive |
| | Mare 6921 | | Negative |
| | Mare 6912 | | Negative |

* Used for MSbE therapy

Attempts to transmit the infection with other cases of unknown duration from the field gave equally erratic results. Mare 6654, showing no clinical signs of infection but with a serum CFT titre of 1: 64 and no trypanosomes detectable in her blood, failed to infect 3 clean stallions (Table 1) after 19 services over a period of 5 months. Stallion 39, showing a slight ventral oedema and moderate emaciation, with a CFT titre of 1: 192 and no detectable blood trypanosomes, failed to infect clean Mare 6921 through 10 services over a period of 5 days. Mare 100, showing extreme emaciation, posterior paresis, flaccidity of the vulva, a muco-purulent vaginal discharge and a CFT titre of 1: 768 was successful in infecting Stallion 7037 (see Fig. 1, 4 and 8) after 3 services on 3 successive days.

The presence of dourine infection in the stallions used in the above experiments did not appear to interfere with libido or the ability to achieve erection even where there was pronounced oedema of the scrotum and sheath, as in the case of Stallion 7090. This oedema was not observed to involve the penis. Similarly the presence of infection did not appear to affect adversely the fertility of either stallions or mares. On 5 occasions clean mares conceived to services by infected stallions and on 3 occasions infected mares conceived to services by clean stallions. Two foals born to infected mares were normal and were reared to maturity. Stallion 7090 was affected temporarily by an abscess, possibly secondary to the local oedematous swelling, lying above the scrotum and involving the left vas deferens.

*Transmission by sub-inoculation of blood into horses*

The transmission of *T. equiperdum* by sub-inoculation of a large volume of blood from an infected to a serologically-negative horse was easily achieved even during periods when the horse was not infectious by coitus. Sub-inoculation of 1 litre of blood from Stallion 7090 during the 7-month period of coital non-infectivity readily infected Mare 6645 This was demonstrable by the presence of trypanosomes in her blood and positive CFT reaction. Similarly, sub-inoculation of 250 ml of blood from infected Stallion 6568, 10 months after infection, when no trypanosomes were detectable in blood by the methods employed, infected serologically-negative Mare 6961.

*Transmission by sub-inoculation into other animals*

All attempts to transmit the parasite to animals other than horses were unsuccessful, despite the fact that on all occasions the inoculum was known to contain living trypanosomes.

Citrated whole blood, with demonstrable trypanosomes taken from Mare 6271 twenty-one days after infection, was injected into 4 intact and 4 splenectomised rats, 3 ml being given intraperitoneally to each. Thin blood smears, examined daily for a period of 2 months, revealed no trypanosomes, and the rats remained healthy.

Live trypanosomes, obtained by passage through an anion exchange column of 30 ml heparinized blood from Mare 6773 taken 21 days after infection by sub-inoculation of blood, were concentrated by centrifugation and 1 ml of the eluate was injected into the peritoneal cavity of each of 3 rats. Blood smears examined daily over a period of 8 weeks were negative and the rats remained healthy.

Twenty ml citrated blood, with demonstrable trypanosomes, taken 56 days after infection from Stallion 6568, was injected subcutaneously into a dog. Daily examination of blood smears from the recipient over the ensuing 10 weeks revealed no trypanosomes and the animal remained clinically healthy.

CSF, collected at post-mortem from Mare 6271 and containing an estimated 10–40 live trypanosomes per ml, was injected intraperitoneally into 3 rabbits, 2 ml being given to each. Thin blood smears taken from the rabbits during the ensuing 8 weeks were negative and the animals remained healthy.

*Demonstration of the parasite*

*In blood.* In this study the examination of blood taken from long-standing cases of unknown duration and tested by all 3 methods cited gave negative results. The parasite was demonstrated with ease, however, in blood taken from horses in the early stages of the disease.

Nucleopore* filter membranes were found to be more satisfactory for the column separation technique since they did not absorb the stain to any degree and presented a smooth surface for microscopic examination.

The results of blood examinations of Stallion 7037, Mare 6271 and Stallion 6568 are recorded on Fig. 1, 2 and 3. The presence of trypanosomes in Stallion 7037 (Fig. 1) and Mare 6271 (Fig. 2), both of which died as a result of infection, was easily demonstrated until between Days 70 and 90 after infection, when the parasitaemia fell. After this period, the numbers of trypanosomes became irregular and their presence or absence did not coincide directly with fluctuations of the CFT titre of the animal. On the other hand the parasitaemia of Stallion 6568 (Fig. 3), which had no clinical manifestations, was easily detectable until Day 111 post infection when it fell rapidly with only one further positive observation on Day 151. In this case the fall in parasitaemia coincided with the beginning of a marked and consistent rise in CFT titre.

*In cerebro-spinal fluid.* The presence of live trypanosomes in wet preparations of CSF sampled immediately after death was shown in Mare 6271 and Mare 100, both of which died from the nervous form of the disease. It was found that the parasite had only a limited period of viability in the CSF once it was withdrawn from the body or following the death of the host. Whereas large numbers of actively motile organisms were seen on immediate examination, after 30 min exposure at room temperature, only occasional sluggish parasites were found in the same specimen, although, with reduced lighting, the refractile remains of the other parasites could be seen floating in the medium. In Mare 6271 the number of trypanosomes was estimated at 10–40 per ml of CSF.

* Shandon Southern Instruments Ltd., Surrey, England

TABLE 2 Results of CSF examinations of dourine infected and non-infected horses

| Horse number | Sex | Form of disease | Trypanosomes in CSF | | Serum** CFT titre |
|---|---|---|---|---|---|
| | | | Live host | Post mortem | |
| 7037 | Stallion | Nervous | + | | 1:512 |
| 6271 | Mare | Nervous | | + | 1:512 |
| 100 | Mare | Nervous | | + | 1:768 |
| 6961 | Mare | Unexposed | — | | Negative |
| 6961* | Mare | Nervous | + | | 1:768 |
| 99 | Stallion | Interstitial | — | | 1:1536 |
| 99* | Stallion | Interstitial/nervous | — | | 1:1024 |
| 20 | Mare | Interstitial | — | | 1:96 |
| 39 | Stallion | Interstitial | — | | 1:768 |
| 7090 | Stallion | Interstitial | — | | 1:2048 |
| 6568 | Stallion | Asymptomatic | — | | 1:854 |
| 6921 | Mare | Unexposed | — | | Negative |
| 6569 | Stallion | Unexposed | — | | Negative |

* Sampled subsequent to other result recorded
** At the time CSF sample was taken

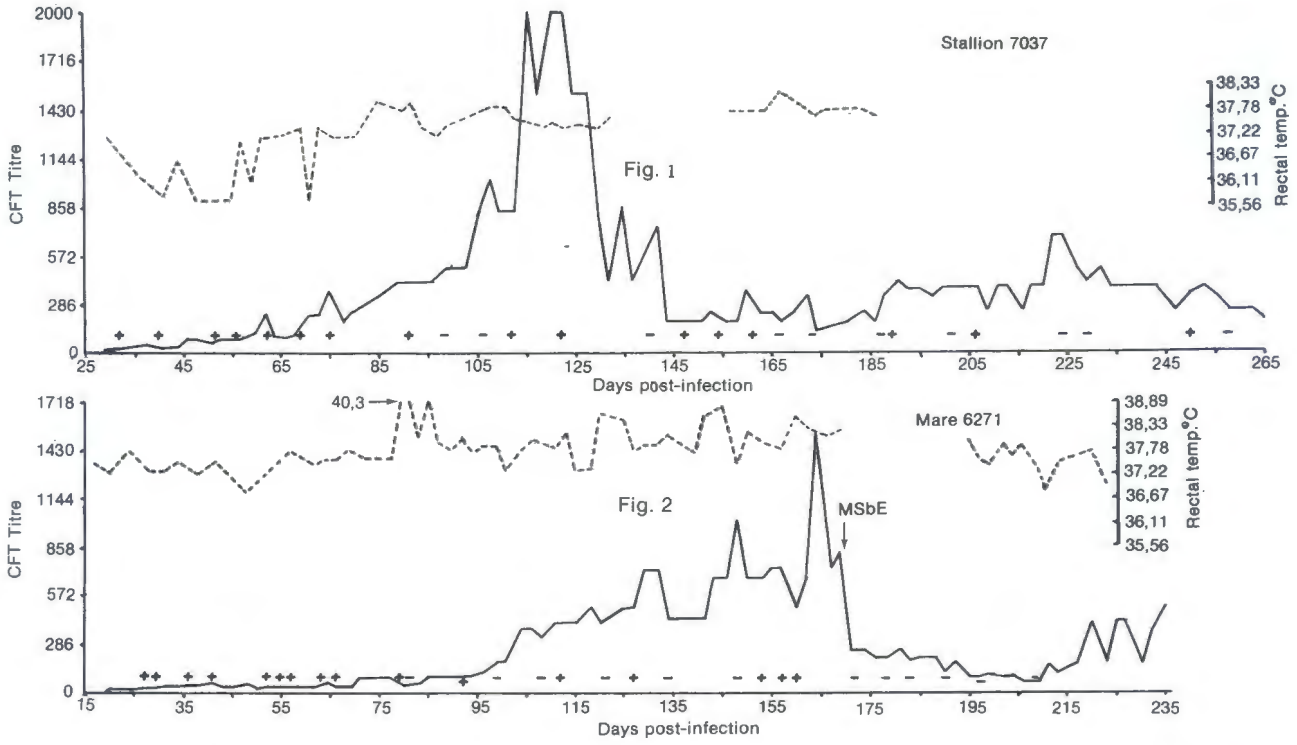


FIG. 1 Stallion 7037, complement fixation titre (——), presence (+) or absence (−) of trypansomes in blood and rectal temperature (.....)

FIG. 2 Mare 6271, complement fixation titre (——), presence (+) or absence (−) of trypanosomes in blood and rectal temperature (.....)

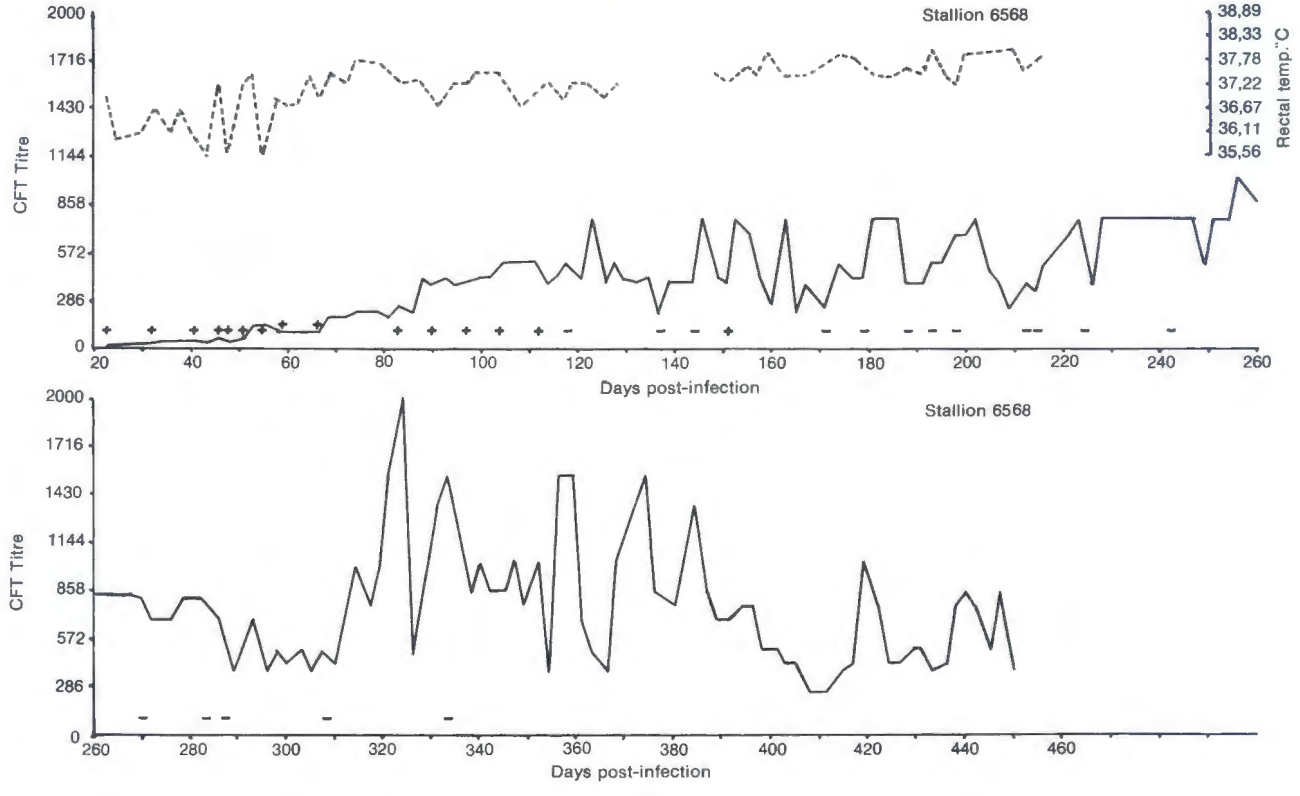


Fig. 3

FIG 3 Stallion 6568, complement fixation titre (——) presence (+) or absence (−) of trypanosomes in blood and rectal temperature (.....)

Finding trypanosomes in animals dying with nervous lesions prompted the sampling of CSF from live horses manifesting the disease in different forms, and from horses with negative CFT reactions. The results of these examinations are recorded in Table 2.

Samples of CSF from 3 CFT negative horses and 5 CFT positive horses, showing clinical signs but no nervous involvement, were negative when examined for trypanosomes. Samples of CSF from Stallion 7037, which subsequently collapsed and died from the nervous form of the disease, contained an estimated 3470 trypanosomes per ml.

Though the CSF of Mare 6961 contained no trypanosomes before infection, trypanosomes were present at the time nervous symptoms developed. Trypanosomes were not found in CSF taken from Stallion 99 twenty-four hours before death, although there were signs of nervous involvement in the last 3 days of its life.

*Complement fixation tests*

The serum CFT titres of 3 animals with dourine infection of known duration are illustrated in Fig. 1, 2 and 3. Stallion 7037, infected by coitus from Mare 100, became CFT positive after 29 days and died on Day 265 after infection. Mare 6271, infected by coitus from Stallion 7090, became CFT positive after 20 days and died on Day 231 after infection. Stallion 6568 became CFT positive after 23 days without showing any clinical lesions, and remained apparently healthy 20 months later. The titres of Stallion 7037 and Mare 6271, both of which died from the nervous form of the disease, rose to a peak which was not maintained. In the case of Mare 6271, the administration of MSbE (see below) may have been responsible for the fall. In contrast Stallion 6568 showed a consistent and sustained rise in CF antibody. Unconcentrated CSF from Stallion 7037 gave a negative CFT reaction, 10× concentration produced a titre of 1: 12 and 100× concentration a titre of 1: 192 at a time when the serum CFT titre was 1: 512. Unconcentrated CSF from Stallion 7090 gave a negative CFT result, a 10× concentration produced a titre of 1: 48 and a 100× concentration a titre of 1: 246. The serum CFT titre at this time was 1: 1536.

*Electrophoresis*

The results of electrophoretic separations of serum and CSF proteins from infected and non-infected horses are listed in Table 3.

The figures show that the only obvious recorded difference between infected and clean animals is a moderate hypergammaglobulinaemia which is reflected in the CSF of most of the infected animals. The gammaglobulin levels recorded for infected horses show an increase, relative to the total protein content, of 1–18% in serum and 0–15% in CSF, compared with the values for clean horses. Stallion 6568, which exhibited no clinical signs of the disease, was the notable exception. The relative percentages of the other serum proteins in infected horses showed no significant change from those of clean horses. Those of the CSF correspond to the serum equivalent, although the total protein level of the CSF is approximately 0,0125–0,0111 ($^1$/80–$^1$/90th) of that of serum.

*Immobilization tests*

The mortality of the trypanosomes, observed as a loss of activity, was seen to be the same under all 3 conditions. The numbers of active trypanosomes began to decline after 30 min; after 90 min they had fallen significantly; 4 h later few motile parasites were seen. No increased trypanocidal activity was observed as a result of the addition of 100× concentrated CSF from infected Mare 6271.

*Symptoms and lesions*

The infected animals under observation exhibited symptoms which can be grouped into 3 categories:

1. Asymptomatic (Stallion 6568, Mare 6645, Stallion 130)

2. Interstitial or oedematous (Stallion 7090, Stallion 39, Mare 131, Mare 20)

TABLE 3 Results of eletrophoresis of serum and CSF from dourine-infected and non-infected horses

| Animal number | Dourine status | Sample | Albumin %* | Globulins %* | | | | | Albumin/ globulin ratio | Total protein mg per ml |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | α1 | α2 | B1 | B2 | γ | | |
| Stallion 7037 | Infected... | Serum..... | 40,5 | 1,4 | 9,4 | 13,5 | 8,1 | 27,0 | 0,68: 1 | 76,0 |
| | | CSF..... | 39,4 | 3,0 | 9,1 | 15,1 | 9,1 | 24,2 | 0,65: 1 | 1,066 |
| Stallion 39.. | Infected... | Serum..... | 42,0 | 5,3 | 7,0 | 10,5 | 8,7 | 26,3 | 0,73: 1 | 89,0 |
| | | CSF..... | 41,5 | 4,9 | 7,3 | 12,2 | 7,3 | 26,8 | 0,71: 1 | 1,066 |
| Stallion 6568 | Infected... | Serum..... | 46,2 | 1,4 | 7,0 | 14,0 | 9,8 | 21,0 | 0,85: 1 | 84,5 |
| | | CSF..... | 54,6 | 0 | 4,2 | 12,6 | 8,4 | 21,0 | 1,2: 1 | 0,98 |
| Stallion 99... | Infected... | Serum..... | 33,8 | 1,5 | 10,3 | 7,4 | 8,8 | 38,2 | 0,51: 1 | 86,0 |
| | | CSF..... | 29,4 | 5,9 | 5,9 | 11,8 | 11,8 | 35,3 | 0,42: 1 | 0,88 |
| Stallion 7090 | Infected... | Serum..... | 52,6 | 2,1 | 5,2 | 10,4 | 6,3 | 23,2 | 1,1: 1 | 86,5 |
| | | CSF..... | 51,0 | 4,1 | 4,1 | 12,2 | 8,1 | 20,4 | 1,04: 1 | 1,147 |
| Mare 6921.. | Non-infected | Serum..... | 47,3 | 1,7 | 10,1 | 10,1 | 10,1 | 20,3 | 0,89: 1 | 91,0 |
| | | CSF..... | 42,0 | 3,0 | 9,0 | 15,0 | 9,0 | 21,0 | 0,87: 1 | 1,25 |
| Mare 6961.. | Non-infected | Serum.... | 44,1 | 3,5 | 10,7 | 12,8 | 9,9 | 19,2 | 0,79: 1 | 83,5 |
| | | CSF | 42,9 | 4,7 | 9,5 | 14,3 | 9,5 | 19,1 | 0,75: 1 | 0,84 |

* Expressed as a relative percentage of total protein in the sample

3. Interstitial or oedematous, accompanied by nervous manifestations in the later stages (Mare 6271, Stallion 7037, Mare 100, Stallion 99)

Asymptomatic cases were detectable by positive CF reactions and, in the case of Stallion 6568, by the demonstration of the parasite during the first 111 days of infection. The CF antibody response in such cases was not greater or less than that in horses exhibiting symptoms of dourine.

The condition in all horses was notably afebrile. A febrile reaction was observed on only 1 occasion, during the initial parasitaemia of Mare 6271, when a rise to 40,3 °C was recorded for 48 h. Skin plaques of transient duration (Fig. 5), containing little fluid and no detectable live trypanosomes, and erupting and subsiding in 72–96 h, were seen on rare occasions on Stallion 7090 and Mare 6271.

The stallions affected with the oedematous form of the disease exhibited persistent oedema of the scrotum and sheath, these organs being 2–3 times their normal size. The presence of ventral abdominal oedema extending to the ventral thorax was observed to be variable and reversible, even though extensive fibrosis was evident from the firmness of this tissue and its failure to pit on pressure (Fig. 5 and 7). Marked ventral oedema (Fig. 5) was present in Stallion 7090 for 18 months; it then subsided to an apparently normal condition over a period of 6 months, and throughout the animal remained in good condition. Stallion 39 exhibited a fluctuating ventral oedema which persisted for several weeks before subsiding and then recurred after several weeks, the animal becoming moderately emaciated.

The mares with the oedematous or interstitial form of the disease showed only slight oedematous lesions of the vulva, perineum and occasionally the ventral abdomen or thorax, these lesions being usually of transient duration and subsiding in a few days. Emaciation was more pronounced in the mares and they remained in poor condition for many months.

The most pronounced and most consistent gross post-mortem change in the animals which succumbed was a chronic lymphadenitis involving most of the internal lymph nodes. These were especially marked in those draining the areas of chronic oedematous infiltration in the inguinal and pelvic regions (Kriek & Barrowman, unpublished observations, 1975).

The following descriptions outline the course of the disease from the date of infection in 2 naturally infected horses, a stallion and a mare. Both cases terminated with nervous manifestations of the disease.

*Mare* 6271. This mare became CFT positive on Day 20 following service and trypanosomes were detectable in her blood at this time. On Day 35 a slight oedematous swelling of the vulval labia was present but it subsided within 48 h. No other lesions were observed until Day 69 when a skin plaque, 5 cm in diameter, developed on the lower thoracic wall and a swelling of the parotid lymph nodes was observed. The skin plaque subsided in 72 h and by this time the mare exhibited a more nervous temperament. The packed red-bloodcell volume (PCV) on Day 79 was 40% and the temperature 40,3 °C. At this time the incidence of trypanosomes in the blood became irregular. On Day 82 the animal exhibited a general malaise, and on Day 83 was lame in the left fore-leg, with oedema of the ventral thorax. On Day 84 the thoracic oedema extended down the left fore-leg. By Day 91 the ventral thoracic oedema persisted but the oedema of the fore-limb had subsided and the mare's demeanour had improved. By Day 107 the ventral oedema had subsided and a unilateral conjunctivitis with lachrymation was observed. This persisted and, from Day 112, was accompanied by photophobia until Day 120 when the conjunctivitis improved. A slight subcutaneous oedema of the ventral thorax recurred at this time and there was diffuse enlargement of cutaneous lymph vessels over the thorax. From this time the mare became progressively more emaciated and dehydrated despite a good appetite, until, by Day 152, she was markedly emaciated and exhibited slight posterior ataxia. The PCV at this stage was 35%. This condition persisted until Day 166 when sciatic nerve paresis of the right hind leg was apparent. By Day 168 the sciatic involvement had worsened, there was a partial bilateral adductor paralysis, the vulva was flaccid, and the animal partly incontinent. The mare showed marked pain on moving and her head and neck were lowered. Her appetite was diminished but this loss of appetite may have been occasioned by pain on moving. On Days 168, 169, 170 and 171 the mare was injected intramuscularly with 10 mg/kg MSbE.

The pain on movement diminished after treatment and the appetite showed a marked improvement. There was a gradual improvement also in the animal's condition and demeanour until, by Day 180, the mare's body mass had increased noticeably, her coat was good and only occasional knuckling of the right hind fetlock was seen. By Day 189 posterior lameness was hardly detectable and a very slight ventral oedema was evident. This improvement continued until Day 218 when signs of posterior weakness recurred. On Day 225 the mare's PCV was 44%. By Day 226 clinical signs of slight sciatic paresis were again seen. By Day 229 the sciatic nerve paralysis had worsened considerably, there was visible obturator nerve involvement, the animal exhibited pain and difficulty on moving, her vulva was flaccid and she was incontinent. On Day 230 she was unable to rise, apparently because of lack of muscular control of her limbs. She remained sitting and her appetite was good. On Day 231 she was laterally recumbent and could raise her head and neck only with difficulty, although she appeared to be fully aware of environmental stimuli. Euthanasia was then carried out, using pentobarbitone sodium (Euthatal*) intravenously. From Day 160 until death no trypanosomes were detected in her blood; at post-mortem, however, they were present in CSF samples at an estimated 10–40 trypanosomes per ml CSF.

In addition to the post-mortem lesions observed with the interstitial form of the disease, there was serous infiltration around the posterior section of the lumbar spinal cord. Serous infiltration was also pronounced around the proximal 3rd of the sciatic nerve, extending intramuscularly from the nerve trunk (Kriek & Barrowman, unpublished observations, 1975).

*Stallion* 7037. This animal became serologically positive on Day 29 following infectious coitus and trypanosomes were demonstrable in the blood at that time. On Day 32 there was oedema of the scrotum, accompanied from Day 33–Day 39 by a slight ventral oedema extending along the abdomen, after which only the scrotal oedema was apparent until Day 54 when this too subsided. The animal showed an irregular appetite for several days thereafter

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but no further clinical signs were seen until Day 206. The PCV was 40% on Day 40, 34% on Day 101 and 32% on Day 188. Trypanosomes were demonstrable in the blood until Day 91, but thereafter they became irregularly demonstrable, although they were seen on occasions up to Day 250 (Fig. 1). From Day 206 progressive emaciation with no oedema was apparent, though the animal's appetite remained healthy. By Day 263 the stallion was grossly emaciated and showed posterior weakness (Fig. 4 and 8). Live trypanosomes were found in CSF drawn by lumbar puncture on Day 259 (estimated at 3,740 ml of CSF) and again on Day 263 when the animal was injected with 10 mg/kg MSbE intramuscularly. The same dose was repeated on Day 264 (see below). On Day 265 the animal had collapsed and, although apparently fully conscious, could raise its head and neck only with difficulty. Euthanasia was carried out as in the case of Mare 6271.

Trypanosomes were not detected in CSF withdrawn from this animal at post-mortem. The lesions corresponded closely to those observed in Mare 6271, including those affecting the nervous system (Kriek & Barrowman, unpublished observations, 1975) (Fig. 6).

In addition to the moderate anaemia observed from PCV measurements of the above animals, a similar lowering of PCV was recorded in infected animals showing no apparent clinical signs and those with chronic oedematous lesions of long-standing. In the latter cases the measurements fluctuated between normal and slightly anaemic (PCV 40% to PCV 34–37%); in one instance a PCV of 28% was recorded from Stallion 7090.

*MSbE therapy*

The results of treatment of 4 infected horses with this drug are outlined.

*Stallion* 7090. The CFT titre of this 4-year old animal was 1: 8 102 on arrival and it showed pronounced oedema of the sheath, scrotum and ventral abdomen. No trypanosomes could be found by examination of sedimented blood or by the anion exchange procedure. At the time of treatment the PCV was 29% and CFT titre 1: 12 288. Prior to treatment, this horse was shown to be infectious by coitus and by sub-inoculation of blood (Table 1). The stallion was injected intramuscularly with 10 mg/kg MSbE and this dose was repeated on 3 subsequent days. The injection was accompanied by a diffuse, non-painful, non-oedematous local swelling which persisted for several days and then subsided. No adverse systemic effects were observed.

After treatment, the CFT titre continued to fluctuate between 1: 6 000 and 1: 12 000. It then fell gradually over the following year but remained at between 1: 1 024 and 1: 4 096. The ventral oedema subsided 1 week after treatment and the swelling of the scrotum and sheath was reduced. These clinical manifestations did not completely disappear, however, and after 5 weeks they gradually returned to their initial state. Sixty-seven days after treatment the PCV had returned to normal (43%). One year after treatment the oedema had subsided until only a mild oedema and induration of the scrotum, together with a serum CFT titre of 1: 1 024–1: 2 048, were observed.

*Mare* 6645. Sedimented blood from this mare was examined daily for the presence of trypanosomes which were demonstrable at a level of 300–500/ml of blood on Day 33 following the transfusion of blood from Stallion 7090. On the same day the mare was injected with 10 mg kg MSbE intramuscularly and the dosage was repeated on 2 subsequent days. A diffuse, non-oedematous swelling developed at the site of injection but no systemic effects were observed.

After treatment no parasites could be detected on Day 34 or on any subsequent day by examination of sedimented blood or by anion exchange separation. The CFT titre was 1: 192 on the day after transfusion as a result of passive transfer of antibody. This titre fell progressively until, by the 48th day after treatment, CFT results remained consistently negative. No rise in CF antibody titre was observed as a result of active trypanosomal antigenic stimulation and the infection appeared to be sterilized.

*Mare* 6271. In this case treatment with MSbE was instituted when there was marked nervous system involvement with sciatic paralysis and partial obturator paralysis, flaccidity of the vulva and incontinence. The CFT titre before treatment was 1: 854. Four successive daily injections of 10 mg/kg MSbE gave rise to a localized, non-oedematous swelling, but no systemic reactions were observed.

There was a decided improvement in the animal's general condition after treatment and a progressive abatement of the nervous condition. The latter condition reappeared, however, on the 55th day after treatment and progressed until the animal was killed *in extremis* on the 60th day after treatment.

The CFT of this animal (Fig. 2) fell progressively to 1: 64 fifty-six days after treatment. It then rose again to 1: 384 and maintained this level until the animal died (1: 512). Live trypanosomes, estimated at between 10–40 trypanosomes per ml, were found in wet preparations of CSF taken at post-mortem. No trypanosomes were detectable in the blood, aqueous humour, abdominal ascitic fluid or synovial fluid from this animal.

*Stallion* 7037. This animal was treated with 10 mg/kg MSbE 263 days after infection by coitus with Mare 100 and 34 days following the detection of live trypanosomes in CSF taken by lumbar puncture. At the time of treatment this horse was extremely emaciated and live trypanosomes were demonstrated in the CSF on the 1st day of treatment before the drug was given. The MSbE injection was repeated the following day and on the 3rd day the animal had collapsed. The animal was then killed and examination of wet and stained CSF smears revealed no trypanosomes.

## Discussion

Reports from the last century on the symptomatology and infectivity of dourine for other species do not correspond entirely with subsequent observations. As these reports originated at a time when taxonomic classification of the trypanosomes was incomplete and from areas where one or more other forms of trypanosomiasis occurred, it is likely that the interpretation of material was complicated to some degree by these factors. The possible complicating presence of *T. brucei* and *T. congolense* is excluded from this study as only small foci of the tsetse fly occur in South Africa, these being found in Natal. Since Theiler (1905) found *T. evansi* in a group of imported camels which were subsequently destroyed, there have been no published records of *T. evansi* in the Republic of South Africa.

FIG. 4 Stallion 7037, showing gross emaciation and weakness, 263 days following infectious coitus

FIG. 5 Stallion 7090, showing ventral abdominal and thoracic oedema and a skin plaque over the scapular region

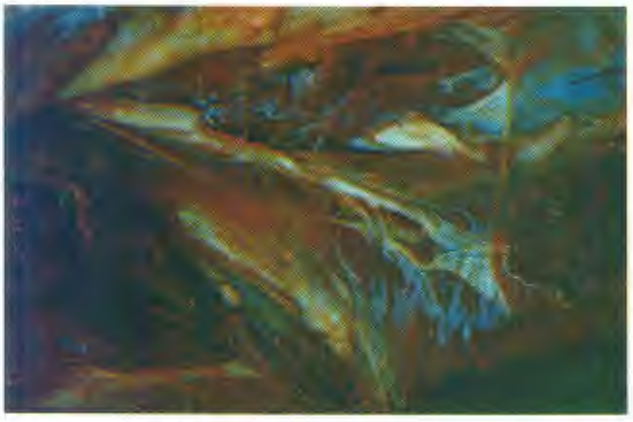

FIG. 6 Dissection of the sciatic nerve trunk in the upper hind limb of Stallion 7037, showing perineural and intermuscular oedematous infiltration. *Note*.—The central section of the sciatic nerve has been resected in this illustration

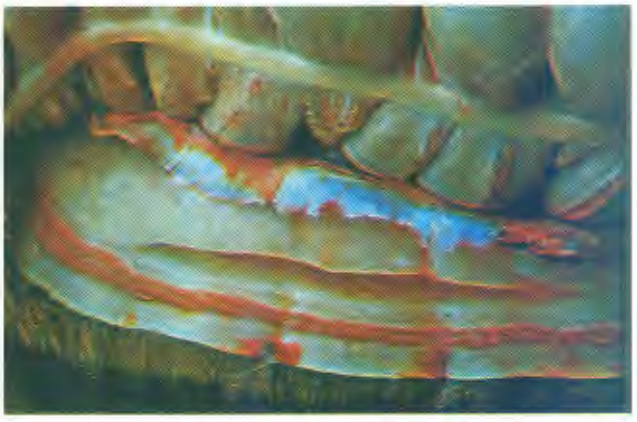

FIG. 7 Longitudinal section through the ventral abdominal wall of a dourine infected stallion, showing gross thickening due to oedematous infiltration and fibrosis

FIG. 8 Stallion 7037, showing extreme emaciation and muscle atrophy 263 days following infectious coitus. Note absence of oedema of genitalia and ventral abdomen

The natural transmission of dourine by coitus was demonstrated before the causative organism was first seen by Rouget (cited by Laveran & Mesnil, 1907). Watson (1920) was the first to study intensively the erratic nature of the transmission of the disease and concluded that "the animal is most likely to transmit the infection during the early stages of the disease, becoming later a less active propagator of this disease". The results obtained from this study seem to indicate that the possibility of transmission is erratic at all stages of infection but may be less so in the early stages and where severe manifestations of the disease are present. This may be dependent on the variable presence of active trypanosomes on the genital mucous membrane at the time of coitus. Parkin (1948) found that trypanosomes were demonstrable in the vaginal washings of recently infected mares but not in those with longstanding infections. This raises the question of whether *T. equiperdum* should be regarded as an obligatory parasite of the genital tract or as an interstitial tissue parasite whose transmission is dependent on its presence on the genital mucosa.

In view of the difficulty experienced by other workers (Watson, 1920; Haig & Lund, 1948) in the direct transmission of *T. equiperdum* from naturally-infected horses to other species, few attempts were made with the use of blood. In all cases where cross transmission was attempted, the inoculum used was known to contain living trypanosomes but no transmission of the organism was achieved.

The CFT titre of infected horses does not appear to be related directly to the severity of the disease, the presence or absence of symptoms, or the infectivity of any given animal. It is not known whether the fluctuations in CFT titre are the result of antigenic variation of the organism or fluctuating antigenic stimulus circulating in the host. There is also no evidence of the extent to which the antibody measured by complement fixation is protective or not.

The clinical picture of the disease corresponds closely to that described in earlier reports (Laveran & Mesnil, 1907; Walker, 1918; Watson, 1920). The presence of skin plaques was also observed by Walker (1918) and Watson (1920) to be a rare symptom, and in this case no trypanosomes could be found in fluid from these plaques. The lesion may, however, have an indirect nervous origin as suggested by Mott (cited by Laveran & Mesnil, 1907). Thus, although the plaque lesion may be pathognomonic, its rare occurrence, transient nature and possible confusion with other skin eruptions limit its diagnostic value.

*T. equiperdum* infection rarely appears to be the immediate cause of mortality in horses where the nervous system is not involved. The debilitating nature of the disease, however, appears to render affected animals more susceptible to other pathogens and external and internal parasites to which they succumb. Horsesickness and bronchopneumonia are prominent among these secondary infections as recorded by Walker (1918) and Robinson (1948). In contrast it appears that when the nervous system is involved the course of the disease is progressive and fatal. The susceptibility of infected horses to secondary infections and other parasites may be the result of reduced resistance in a debilitated animal or the direct immunosuppressive activity of trypanosome infection recently reported by Goodwin, Guy, Green & Voller (cited by Goodwin, 1970). A possible manifestation of this was the observation of a gross *Ascaris equorum* infestation in 2 of the horses necropsied.

The term "oedematous" has been used to describe the clinical signs of the disease. Many horses, and especially mares, however, exhibit only transient oedemas or none at all. In these cases the infection causes a progressive emaciation and, as the parasites cannot be demonstrated in the blood, the term "interstitial" seems more descriptive of this form.

The occurrence of the nervous form of the disease, superimposed on the oedematous or interstitial form, appears to coincide with the presence of the parasite in the CSF. The nervous symptoms were characterised by a short period of hyperaesthesia followed by a prolonged period of hypoaesthesia lasting until death. The motor affectation was one of incomplete ascending motor paralysis of variable duration. The symptons originated in the perineum and hind-limbs and eventually involved the fore-limbs.

Since Rouget (1896) first found a trypanosome in the blood of a stallion suffering from dourine, most workers have experienced difficulty in demonstrating the parasite in naturally-infected horses (Schneider & Buffard, 1900, cited by Laveran & Mesnil, 1907; Watson, 1920; Khalilik, 1973) and have had only occasional success at the onset of the disease. Parkin (1948) recorded the more consistent finding of the parasite during the early stages of the disease in horses infected by sub-inoculation of blood. By using horses whose actual infectious service date was known, it has been possible in this study to demonstrate the more prolonged presence of the trypanosomes in circulating plasma than previously reported. Attempts to demonstrate the parasite in the blood of long-standing cases of unknown duration were unsuccessful.

Contrary to the experience of Fankhauser (1962), the sampling of CSF by lumbo-sacral puncture in the living horse was found to be a relatively uncomplicated procedure. Trypanosomes were shown to be present in the CSF of horses dying from the nervous form of the disease and in live, naturally-infected horses with nervous manifestations, whereas they were not found in CSF from horses without these signs. It may be deduced that the development of the nervous form of the disease is associated with the presence of the parasite in the CSF. Though the portal of entry into the CSF is not known, it is possible that the blood-brain-barrier presents an obstacle which is only penetrated in some cases. Alternatively, the organism may enter and leave the CSF without difficulty. In view of the penetrative capacity of the organism in other tissues, the alternative seems more likely. The chemical composition of CSF compared with that of plasma would suggest that the CSF is a nutritionally inferior environment for the trypanosomes. Evidence of the suitability of the CSF as an "immunologically safe" environment for the parasite is presented by the apparent lack of local humoral immune response by the host. Electrophoretic separations show a direct relationship between the levels of immunoglobulins present in CSF and the serum levels; total protein determinations show the immunoglobulin levels to be approximately 1/80th that of serum. This lack of local humoral immune response is supported by the fact that positive CFT reactions could be obtained only after considerable concentration of the CSF. Thus the presence of trypanosomes in the CSF may

be correlated to their absence from an immunologically hostile circulating plasma. Under these circumstances it is possible that the immunological stimulus to antigenic variation of the trypanosomes in CSF will be considerably reduced, and consequently the antigenic types present in the CSF may differ from those exposed to the total immunoglobulin effect of the systemic circulation. Thus, the lowered levels of immunoglobulins penetrating into the CSF may be non-specific for the antigenic type of organism present. This situation could provide a pool of multiplying trypanosomes or their products for re-entry into the systemic circulation.

The microscopic lesions of the nervous system were found to involve primarily the lumbar and sacral regions of the spinal cord and the sciatic and obturator nerves. The histopathological changes in these tissues have been recorded by earlier workers (Marek, 1904, cited by Laveran & Mesnil, 1907; Mott, 1906, 1907, cited by Laveran & Mesnil, 1907; Formad, 1919; Schoening & Formad, 1923; Watson, 1920), findings which are largely supported by material from the present study (Kriek & Barrowman, unpublished observations, 1975). The primary lesion is one of radiculitis and polyneuritis, involving cellular infiltration and degenerative changes of the spinal nerves and spinal ganglia, extending along the larger peripheral nerves, notably the sciatic nerves. In contrast to the involvement of the central nervous system in *T. gambiense* infections in man (Van Boegart & Janssen, 1957) and experimentally prolonged *T. brucei* infections in horses (McCully & Neitz, 1971), histopathological changes in the central nervous system have not been recorded in natural *T. equiperdum* infections, except that Mott (1906, 1907, cited by Laveran & Mesnil, 1907) found the lesion of the spinal nerves extending into the posterior columns of the spinal cord.

A possible explanation for the site of these nervous lesions in horses so affected is to be found in the mechanism of drainage of CSF which is known to contain trypanosomes and their extracellular products. Work by Weed and by Dandy (cited by Innes & Saunders, 1962) on the drainage of CSF led to the conclusion that: "The CSF leaves the subarachnoid spaces through the arachnoid villi, reaching the dural sinuses and thence the venous blood stream. Dandy concluded that absorption occurred mainly at the sub-arachnoid vessels. Finally, the CSF also flows along the sheaths of the spinal nerves trickling away into the lymphatic stream." If the trypanosomes, their extracellular products or their antigenic components in the CSF drain along the spinal nerves in this way, they could produce a direct effect or elicit a host response at the sites where the nerve tissue is no longer protected by the mechanism of the blood-brain-barrier. At this point the toxic or immunological stimulus would be at its greatest. Within the central nervous system the host tissues are probably protected by the ependymal cells of the blood-brain-barrier. The alternative to this was given by Mott (1906, 1907, cited by Laveran & Mesnil, 1907) in his suggestion that: "the change starts in one seat of primary infection, extends to the inguinal glands, thence presumably by the pelvic lymphatics to the lumbo-sacral plexus and the posterior lumbo-sacral roots to the central nervous system; consequently the lower part of the spinal cord—and especially the posterior column—is first and most affected." Although this route would be contrary to the flow of lymph, the motility of the parasites makes it feasible.

In this study an attempt has been made to esbalish whether the experimental drug MSbE would sterilize *T. equiperdum* infections. The results show that, at the dosage levels used, the drug was able to sterilize the infection at the beginning of the parasitaemia in Mare 6645 infected by subinoculation of blood. This occurred before an active complement-fixing antibody reaction was detectable in the host. The use of the drug in naturally-infected horses, where the parasite had exerted its tissue and CSF penetrative abilities (Stallion 7090, Mare 6271, Stallion 7037), was shown to be ineffective at the dosage used. It is recorded (Rodhain & Van Goidsenhoven, 1944) that complement-fixing activity in *T. gambiense* infections in man may persist for 2 years after drug treatment, and consequently the trial of the drug on Stallion 7090 cannot be assumed to be complete at the time of writing. The persistent oedematous swellings and high CFT titre would indicate, however, that the parasite is still active. It was not established whether the collapse of Stallion 7037 at the time of treatment was the result of the disease or a toxic effect of the drug. The absence of live trypanosomes in the CSF at post-mortem would indicate some degree of penetration of the blood-brain-barrier by the drug.

An interesting aspect of the treatment was the resolution of clinical nervous signs of Mare 6271 following treatment. This would indicate that the nervous lesions are reversible if a trypanostatic or trypanocidal influence is exerted within the host. The nervous lesions recurred, however, and trypanosomes were subsequently demonstrated in CSF from this animal at post-mortem. The reversibility of lesions stimulated by trypanosomes was also observed in Stallion 7090 where a chronic ventral oedema was seen to resolve over a period of weeks.

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## References

FANKHAUSER, R., 1962. Cerebro-spinal fluid. 21–54. *In:* Innes, J. R. M. & Saunders, L. Z. Comparative neuropathology. New York & London: Academic Press.

FORMAD, R. J., 1919. Pathology of dourine with special reference to microscopic changes in nerve tissue and other structures. *Journal of Agricultural Research*, 18, 145–154.

GOODWIN, L. G., 1970. The pathology of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 64, 797–812.

HAIG, D. A. & LUND, A. S., 1948. Transmission of the South African strain of dourine to laboratory animals. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 23, 59–61.

HOARE, C. A., 1972. The trypanosomes of mammals. A zoological monograph. Oxford: Blackwell Scientific Publications.

INNES, J. R. M. & SAUNDERS, L. Z., 1962. Comparative neuropathology. New York & London: Academic Press.

KHALILIK, K., 1973. An investigation of dourine and isolation of *Trypanosoma equiperdum* in Iran. *Archives de l'Institut Razi*, 25, 69–72.

LANHAM, SHIELA M., WILLIAMS, J. E. & GODFREY, D. G., 1972. Detection of low concentrations of trypanosomes in blood by column separation and membrane filtration. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 66, 624–627.

LAVERAN, A. & MESNIL, F., 1907. Trypanosomes and trypanosomiases. London: Ballière, Tindal & Cox.

LAVERGNE, M., LABERT, G. & RAYNAUD, M., 1969. Macroblobulinémie chez le cheval au cours de l'infection experimental a *Trypanosoma equiperdum*. *Annales de l'Institut Pasteur, Paris*, 116, 781–798.

McCULLY, R. M. & NEITZ, W. O., 1971. Clinicopathological study on experimental *Trypanosoma brucei* infections in horses. Part 2. Histopathological findings in the nervous system and other organs of treated and untreated horses reacting to Nagana. *Onderstepoort Journal of Veterinary Research*, 38 (3), 141–176.

MOULTON, J. E., 1974. Pathogenesis of *Trypanosoma equiperdum* in deermice (*Peromyscus maniculatus*). *American Journal of Veterinary Research*, 35, 961–976.

MULLIGAN, H. W., Ed., 1970. The African trypanosomiases. London: George Allen & Unwin.

OELLERMANN, R. A., 1974. The elimination of ribonucleic acid interference in the spectrophotometric determination of protein concentration. *Onderstepoort Journal of Veterinary Research*, 41, 221–224.

PARKIN, B. S., 1948. The demonstration and transmission of the South African strain of *Trypanosoma equiperdum* of horses. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 23, 41–57.

ROBINSON, E. M., 1948. Notes on serological tests carried out on equine species infected with dourine. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 23, 33–36.

RODHAIN, J. & VAN GOIDSENHOVEN, C., 1944. Persistence prolongée de sensibilisatrice fixant l'alexine après guérison de la trypanosomiase. *Annales de la Société belge de Médecine tropicale*, 24, 235–246.

ROUGET, J., 1896. Contribution à l'etude du trypanosome de mannifères. *Annales de l'Institut Pasteur*, 10, 716–728.

SCHOENING, H. W. & FORMAD, R. J., 1923. A study of the serology, the cerebro-spinal fluid and the pathological changes in the spinal cord in dourine. *Journal of Agricultural Research*, 26, 497–505.

THEILER, A., 1905. Trypanosomiasis in camels. *Transvaal Agricultural Journal*, 3, 717–721.

TUFVESSON, G., 1963. Local anaesthesia in veterinary medicine. Astra International. Sweden.

VAN BOGAERT, L. & JANSSEN, P., 1957. Contribution á l'étude de la neurologie de la trypanosomiase humaine. *Annales de la Société belge de Médecine tropicale*, 27, 379–426.

WALKER, J., 1918. The occurrence of Dourine (Slapziekte) in South Africa. *Report of the Director of Veterinary Research, Union of South Africa*, 5/6, 189–206.

WATSON, E. A., 1920. Dourine in Canada 1904–20. History, research and suppression. Dominion of Canada, Department of Agriculture, Health of Animals Branch.