Case Report

Reemergence of Dourine in Italy: Clinical Cases in Some Positive Horses

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ABSTRACT

In 2011, Trypanosoma equiperdum reemerged in Italy, almost 10 years after its last appearance. A total of eight infected horses have been observed to date. Six horses were affected by natural outbreaks of the disease, whereas two were infected experimentally. The aim of this study was to offer a recent perspective on clinical cases of dourine in Europe. Investigation of the clinical aspects confirmed the three stages reported in the literature: stage 1 (genital lesions), stage 2 (cutaneous signs), and stage 3 (nervous signs). The most common signs in the horses under study were notable weight loss, edematous skin eruptions and oedemas of the abdomen, mammary glands and hind legs. Three animals presented neurological signs (lip ptosis of lower lip and ataxia). Infections were paucisymptomatic or asymptomatic in some animals. Hyperthermia was not reported in infected animals and considerable anemia was observed. High antibody titers did not always correspond to clinical signs. Positive polymerase chain reaction test results of blood or tissue (skin, eye swab) often correspond to an advanced stage of the disease. Dourine is a variable disease; owing to its low prevalence and chronic manifestation, it can be difficult to make a quick diagnosis when facing a Dourine-positive horse.

1. Introduction

Dourine is a parasitic venereal disease of equines caused by a flagellate protozoan of the species Trypanosoma equiperdum. It is the only trypanosomiasis that is not transmitted by blood-feeding vectors. Dourine can affect horses, mules and donkeys. The latter are generally more resistant and often remain asymptomatic carriers. The infection is endemic in many areas of Asia, Africa, Russia, Middle East and Eastern Europe (World Organisation for Animal Health [OIE] data). Its course and clinical signs vary considerably depending on the virulence of the strain concerned, as described in the literature on this topic. The course of the disease in horses is chronic, varying from a few months to 1-2 years. A number of authors have broken the course down into three stages: stage 1 (genital lesions), stage 2 (cutaneous signs) and stage 3 (nervous signs) [1,2]. Stage 1 involves genital edema and swelling, manifesting 1-2 weeks after infection. In stage 2, typical cutaneous plaques (“silver dollar” plaques) appear, with thickening of the skin, considered pathognomonic by some authors [1,3,4]. Stage 3 is characterized by progressive anemia, neurological disorders and paresis of the hindquarters, often ending in death. Mortality rate is high, at around 50% [3].

Before the recent epidemic, the last case notified in Italy was in 1998, when an asymptomatic stallion in the province of Padova tested positive with a titer of 1:6 on complement fixation (CF) testing. The last reported cases of Dourine-positive horses with clinical signs date back to the epidemic of the 1970s [5]. On May 19, 2011, a stallion undergoing routine serological testing in the province of Catania for stud purposes tested positive on CF. The epidemiological investigation that followed resulted in the identification of other holdings epidemiologically linked...
with the infected animals in southern Italy. The Italian Ministry of Health emanated an ad hoc surveillance plan with mandatory testing of all equines of reproductive age in Italy. This plan has so far led to the identification of seven outbreaks involving 20 positive animals.

Five positive animals previously destined for slaughter were transferred to the Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale” (Istituto G. Caporale) for inclusion in a study to investigate the pathogenesis and attempt the isolation of *T. equiperdum*, which is very difficult, as demonstrated by the low number of isolates in the past decades [3,6].

The long period of the disease’s absence—from the epidemic in the 1970s to that of 2011—has resulted in a knowledge gap in generations of veterinarians. The literature on this topic is generally old and lacking in graphic evidence, as this disease is not generally present in developed countries. Moreover, Dourine is a chronic disease whose signs are not constantly present and whose pathogenicity can vary, depending on the strain concerned. Therefore, the aim of this study was to describe and provide graphic evidence of the signs observed during the recent outbreaks of Dourine.

### 2. Materials and Methods

#### 2.1. Animals

Eight horses were observed (Tables 1-3). One was studied at the holding concerned, whereas five naturally infected and two experimentally infected horses were observed at the Istituto G. Caporale. Experimental infection was carried out by blood transfusion from infected horses with clear signs of Dourine.

The age range of the horses in the study was 4–16 years. Most of them were half-breeds used for meat production. There were six mares and two stallions. The seven animals studied at the Istituto G. Caporale were all stabled under the same conditions: individually, in large boxes, with a small paddock alongside.

#### 2.2. Clinical Examination and Laboratory Tests

The stabled animals underwent the following:

- Daily clinical examination, involved noting any abnormalities or change on a clinical record form;
- Assessment of nutritional condition through a 5-class body condition score (BCS) [7], carried out constantly from the first examination after the animal’s arrival at the Istituto G. Caporale;
- Daily measurement of rectal temperature;
- Twice-weekly draw of 9 mL of whole blood collected in ethylenediaminetetraacetic acid collection tubes and 9 mL collected in anticoagulant-free collection tubes;
- Sampling of mammary secretions, tear secretions and synovial fluid.

The following direct and indirect diagnostic techniques were carried out twice weekly: CF (OIE method) [3], indirect immunofluorescence (OIE method) [3] and real-time polymerase chain reaction (real-time PCR developed by Becker et al. [8]).

The complete blood count was carried out using the ADVIA 120 automated analytical system (Siemens Diagnostics) with specific veterinary software. The following parameters were evaluated: red blood cell count, hemoglobin (HGB), mean cell HGB concentration, mean corpuscular HGB, hematocrit and mean corpuscular volume.

Cytological samples to detect *T. equiperdum* were obtained by puncture aspiration with an 18-G needle. The smears were dried, stained with May–Grunwald Giemsa according to OIE Terrestrial Manual 2010 edition—Chapter 2.1.17 *Trypanosoma evansi* infection (Surra) B. Diagnostic techniques, and observed under an optical microscope.

Fresh mammary secretion and joint fluid preparations were examined for direct identification of the agent at 200× and 400× magnification under an optical microscope.

Blood transfusions were carried out by collecting 900 mL of blood from the jugular vein of infected stallions in two 450-mL Terumo (Terumo Medical Corporation ©) CPDA-1 transfusion bags and transfusing it immediately through the jugular vein of serologically negative mares.

The observation period varied from a minimum of 15 days for horse H0 to a maximum of 6 months for horses H1 and H2.

### 3. Results

In Table 4, a value (0 or 1) is attributed to a given sign in each horse on the basis of its presence or absence. In this way, it is possible to establish which signs were most common and which horse presented the most signs at the same time.

#### 3.1. Progressive Weight Loss, Weakness, and Cachexia

A common clinical sign was substantial weight loss, leading to severe emaciation, weakness and a generally poor condition, with a BCS of 1. The four mares originating

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Period of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0</td>
<td>Friesian</td>
<td>Mare</td>
<td>12 years</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

### Table 1

Naturally infected horse observed at the holding involved in the outbreak

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Period of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Friesian</td>
<td>Stallion</td>
<td>4 years</td>
<td>March 2011</td>
</tr>
<tr>
<td>H2</td>
<td>Friesian</td>
<td>Stallion</td>
<td>6 years</td>
<td>2010-2011</td>
</tr>
<tr>
<td>H6</td>
<td>Half-breed</td>
<td>Mare</td>
<td>7 years</td>
<td>Not Determined</td>
</tr>
<tr>
<td>H7</td>
<td>Half-breed</td>
<td>Mare</td>
<td>12 years</td>
<td>Not Determined</td>
</tr>
<tr>
<td>H15</td>
<td>Half-breed</td>
<td>Mare</td>
<td>6 years</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

### Table 2

Naturally infected horses observed at the Istituto G. Caporale (official outbreaks)

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Period of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Friesian</td>
<td>Stallion</td>
<td>4 years</td>
<td>March 2011</td>
</tr>
<tr>
<td>H2</td>
<td>Friesian</td>
<td>Stallion</td>
<td>6 years</td>
<td>2010-2011</td>
</tr>
<tr>
<td>H6</td>
<td>Half-breed</td>
<td>Mare</td>
<td>7 years</td>
<td>Not Determined</td>
</tr>
<tr>
<td>H7</td>
<td>Half-breed</td>
<td>Mare</td>
<td>12 years</td>
<td>Not Determined</td>
</tr>
<tr>
<td>H15</td>
<td>Half-breed</td>
<td>Mare</td>
<td>6 years</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

### Table 3

Horses infected instrumentally by blood transfusion

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Period of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>H11</td>
<td>Half-breed</td>
<td>Mare</td>
<td>16 years</td>
<td>July 2011</td>
</tr>
<tr>
<td>H12</td>
<td>Half-breed</td>
<td>Mare</td>
<td>10 years</td>
<td>October 2011</td>
</tr>
</tbody>
</table>
from natural outbreaks, whose date of infection could not be determined but was presumably more than 1-2 months earlier, were already thin. In this stage, the antibody titer can be high (1:2560, CF) and PCR results of the blood sample may be positive. There was no appetite loss in any case. Muscle hypotrophy, inelastic skin and a dull coat were observed. The two stallions in the group also lost weight during the observation period, but this cannot be attributed with certainty to the infection, as after testing Dourine-positive their work was stopped and their feed was reduced. Weight loss was gradual in the subjects infected experimentally (Fig. 1).

4. Stage 1: Genital Lesions

4.1. Genital Edema

Slight oedema of the sheath was seen in one stallion. In one mare, oedema of the vulva was accompanied by ulcers along the rim of the vulva. The severity of these genital oedemas varied considerably. They were not observed in the animals infected experimentally (Fig. 2).

5. Stage 2: Cutaneous Lesions

5.1. Edematous Cutaneous Wheals and Plaques

Wheals or plaques were found on the skin of both naturally and experimentally infected animals. The wheals varied in shape (roundish, oval or irregular) and size (from a few millimeters to a few centimeters) and were found on the trunk, neck, chest and shoulders. Numerous wheals appeared contemporaneously, developing quickly and suddenly. They were seen for a few hours to a few days and then disappeared, often reappearing in a different area. A “silver dollar” plaque was seen in just one animal. Wheals and skin plaques were observed both at the beginning of the infection and in the terminal stages (Fig. 3).

5.2. Oedema of the Mammary Gland

Four mares presented cold nonpainful oedema of the mammary glands, with a cloudy off-white mammary secretion testing positive on PCR and with visible parasites

Table 4

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Loss</td>
<td>Edema of the Sheath</td>
<td>Edema of the Vulva</td>
</tr>
<tr>
<td>Naturally infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H15</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Experimentally infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>50%</td>
</tr>
</tbody>
</table>

1, symptom present; 0, symptom absent.
on microscopic examination of wet-mounted samples. This condition remained present until death (Fig. 4).

5.3. Ventral Oedema

When present, ventral oedema extended from the sternum region to the umbilical area. Its onset was fast and it tended to quickly develop to a considerable severity (Fig. 5).

5.4. Oedema of the Limbs

Oedemas were more evident in the hind legs, but the forelegs were also affected in two animals. Their characteristics were as follows: variable severity, extending to above the hock, cold, nonpainful and doughy. They generally developed a few days after the infection, usually after the onset of wheals and could persist for a considerable time (Fig. 6).

6. Stage 3: Nervous Signs

6.1. Nervous Signs

Three animals (Table 4) presented stiffness, weakness, lameness in one or more hind legs, staggering, lack of coordination, inability to stand upright after prolonged sternal or lateral recumbency and ataxia. When standing, animals often stood with the legs well splayed, especially the hind legs. The left hind leg of one animal often dragged on the ground, causing deformation of the hoof wall. As the disease progressed, the difficulties in movement became ever more evident and the affected animals demonstrated reluctance to move.

In two of three cases, there was an apparent improvement before their condition worsened once more. There was no sensory impairment or skin hyperesthesia in any animal.

More or less evident ptosis of the lower lip was seen in three of the eight animals during the observation period,
with ulcerated areas of variable size along the edge of the lips (Figs. 7-9).

6.2. Anemia

Six animals, all seropositive and constantly positive on PCR on blood sample, presented severe anemia, which was essentially normochromic, macrocytic (four of five animals tested) and tended to worsen during the observation period (Table 5). The anemia was moderate on onset and then became more severe in animals with chronic oedematous lesions or nervous signs, with a hematocrit of 20% or less and fewer than 3 million erythrocytes/mm$^3$ in some cases. The two stallions, which were positive on serological testing and sporadically positive on blood PCR, showed no signs of anemia with respect to any of the main erythrocyte parameters, with the exception of a slight macrocytic tendency in one animal.

7. Other Signs Observed

7.1. Increased Synovial Fluid

Increased synovial fluid and consequent joint ectasia were found in 50% of the symptomatic horses (Table 4). The joints affected were as follows: pastern, hock and carpus, with synovial fluid testing positive on PCR. In one animal the parasite could be seen on microscopic examination of synovial fluid from the hock joint.

7.2. Fever

No animal presented significant fever during the observation period.

Table 5

<table>
<thead>
<tr>
<th>Anemia in affected horses</th>
</tr>
</thead>
<tbody>
<tr>
<td>H6 Very severe$^a$</td>
</tr>
<tr>
<td>H7 Severe$^a$</td>
</tr>
<tr>
<td>H15 Moderate$^a$</td>
</tr>
<tr>
<td>H12 Moderate$^a$</td>
</tr>
<tr>
<td>H11 Severe$^a$</td>
</tr>
<tr>
<td>H0 Moderate$^a$</td>
</tr>
<tr>
<td>H1 Absent or mild$^a$</td>
</tr>
<tr>
<td>H2 Absent$^a$</td>
</tr>
</tbody>
</table>

$^a$Assessment based on Feldman’s criteria (2000) [9].

$^b$Mean corpuscular volume values; although remaining within the normal range, showed a distinct and constant increase over time.

$^c$Based on data reported by M. Scacchia et al. 2011 [10].
7.3. Eye Lesions: Conjunctivitis and Keratitis

Mild corneal opacity was observed in just one experimentally infected animal. PCR of the conjunctival swab was positive in some cases, including this horse.

8. Discussion

Data collected in this study demonstrate that most of the signs observed coincide with those reported in the few available literature studies. The stages of the disease described in the past and related to pathogenesis also occur in the same order today. The horses observed can be divided into three main groups: naturally infected mares (group 1), naturally infected stallions (group 2), and experimentally infected mares (group 3).

No rise in body temperature was observed in any of the infected animals. Fever is often reported in the literature [1,2,4], but was not found in other studies [11,12].

Weight loss was observed in all animals, especially in groups 1 and 3. There was no sensory impairment or appetite loss, in contrast with what would be expected from other debilitating diseases leading to the same degree of cachexia. It should be underlined that weight loss is one of the early signs that could lead the veterinarian or owner to suspect Dourine. Mares in group 1 were probably infected between 2-3 months and a few years earlier and were already extremely thin at the beginning of the observation period (BCS: 1-2). A poor body condition and weakness increase the risk of secondary infections. Mare H6 developed a Streptococcus equi infection with fibrinous pericarditis, whereas mare H15 developed pleghm on the left foreleg. Stallions in group 2 lost much less weight in the same period (from BCS 4 to BCS 3), and the weight loss could have been due to inactivity and change of diet after the diagnosis. Mares in group 3 underwent considerable weight loss, although still less than those in group 1.

Ventral oedemas were seen in all group 3 mares. This sign was not constant in group 1 mares, probably because some of them were in the third stage of the disease, when it is not present. Ventral oedema was never variable or reversible, as reported in the literature [11]. Genital oedemas were found in a relatively low number of animals. This could be explained because six of these horses were from natural outbreaks and already showed signs of active disease on arrival at the Istituto G. Caporale suggesting that the theory that the parasite is present in the genital tissues—finding not present in the literature—given that mammary secretions are considered pathognomonic by some authors [1,4], although their presence seems to be inconsistent. Most authors report only the sporadic presence of this sign [1,11-14], although it has been reported more frequently in outbreaks in Europe and North Africa [12]. Some attribute this difference to the T. equiperdum strain involved and others to the immune response. In any case, presence of wheals should lead to suspicion of Dourine infection.

Anemia was observed in a considerable number of horses, which were all female with clear signs, systematic and constant positive PCR test results and high serological titers. In stallions, anemia was marginal or absent, with mild signs, PCR only sporadically positive and high (H1 similar to H11) or low (H2) serological titers. This difference may be owing to the sex or breed (both stallions were Dutch Friesians). However, a different course of the disease should not be excluded. For example, in sheep infected experimentally with Trypanosoma congolense hematological parameters were found to return to normal by 23 weeks after infection [15]. In any case, given the importance of the pathogenesis of anemia in the study of Dourine, the number of animals examined in this study does not permit any definitive conclusions to be reached.

The presence of neurological signs confirms the tropism of T. equiperdum for the peripheral nervous system and the lack of involvement of the central nervous system, in contrast with other trypanosomes [11]. In fact, various nerves (facial, sciatic, optic) in the infected horses tested positive on PCR, whether signs were absent or present, whereas the spinal cord was always negative. The onset of a variable degree of ataxia occurred in the third stage of the disease, confirming past reports. In contrast, ptosis of the lower lip affected one horse in every group and, unlike ataxia, was constant.

The theory that the disease observed in the Italian outbreaks was due to an epidemic of Surra by Trypanosoma evansi was excluded, for diagnostic and epidemiological reasons. It was not possible to identify the parasite in the bloodstream of infected horses by microscopy in almost 80 blood collections and the strain has not been adapted to laboratory animals yet, despite several attempts. According to literature, these diagnostic difficulties are typically due to disease caused by T. equiperdum [16]. Moreover, serologically negative horses were stable in close contact with infected animals in the presence of insect vectors. After a 6-month exposure period, these animals were always found negative on weekly serological and molecular biology testing. The number of cases per holding, absence of the disease in sexually immature animals and geographic distribution of the holdings with positive cases were incompatible with a vector-borne disease.

An interesting finding in the literature was a positive PCR test result from a prepuce swab taken from a Dourine-free stallion immediately after mounting an infected mare. The horse remained negative at all subsequent tests, supporting the theory that the parasite is present in the genital tissues but that sexual transmission is not constant [3,11]. In the study reported herein, direct observation revealed milder signs in the stallions (both Friesians) than in the infected mares. Six months after infection, they were almost asymptomatic, with the exception of a slight oedema of the sheath and ptosis of the lower lip in one animal. The
differences with respect to breed and sex cannot be statistically examined because of low number of considered cases and further studies with a larger caseload are needed.

The antibody titer, owing to genetic rearrangement of the parasites, is related to successive waves of antibody production and does not seem to be directly related to the signs observed. However, there was some correspondence between the presence of signs and a positive PCR blood test.

9. Conclusion

Given the extreme variability of signs in infected animals, difficulties in clinical diagnosis, and the lack of Europe-wide active surveillance plans, it is important to observe and report new cases of Dourine. Difficulties regarding the isolation of the *T. equiperdum* should stimulate more interest in the experts’ community.

References