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Diagnosis of dourine in outbreaks in Italy

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ABSTRACT

Dourine is trypanosomosis that affects equids, it's mainly sexually transmitted. The disease was first eradicated in Italy in the 1940s, but there was then a serious epidemic in the mid-70s. After sporadic reports at the end of the 1990s, in May 2011 it was reported once more.

Clinical diagnosis of dourine can be complex, as clinical signs and gross lesions are not always present. Direct laboratory diagnosis is also problematic, given the low number of parasites normally present in infected tissues and the mild, short-lasting parasitaemia. This article describes the epidemiological, clinical and laboratory data enabling confirmation of the suspicion of dourine in Italy in the 2011 epidemic.

1. Introduction

Dourine is a parasitic disease of equids caused by *Trypanosoma equiperdum* of the subgenus *Trypanozoon*. This subgenus also includes the three subspecies of *Trypanosoma brucei (Trypanosoma brucei brucei*, *Trypanosoma brucei gambien*se and *Trypanosoma brucei rhodesiense*).*T. b. brucei* causing nagana in domestic animals and *Trypanosoma brucei rhodesiense* and *T.b. gambiense* causing sleeping sickness in human. Further: *Trypanosoma evansi* also causes African trypanosomiasis, as *T.b. brucei*.

Dourine is the only trypanosomosis not transmitted by insect vectors. It is generally transmitted sexually from infected to healthy animals, although there are literature reports of transmission of *T. equiperdum* to foals during birth and through maternal milk (Brun et al., 1998).The infection is endemic in many areas of Asia and Africa and is also found in the Middle East, South America and Eastern Europe. In Italy, after eradication in the 1940s, there was a serious epidemic in the mid-70s (Caporale et al., 1977a, b; Caporale, 1978; Caporale et al., 1980a; Caporale and Semproni, 1980) It was then eradicated once more, and the last cases before the 2011 outbreaks reported herein, were notified to the World Organisation for Animal Health (OIE) in 1996 (OIE, 1996).

Diagnosis of dourine can be difficult (Zablotskij et al., 2003). The parasite is found only rarely, both due to the low number normally present in infected tissues and the short-lasting, mild parasitaemia (OIE, 2008). Diagnosis is made more problematic by the non-constant presence of clinical signs (urticarial plaques, genital depigmentation and oedema), non-pathognomonic lesions and the existence of forms with few or no symptoms. Clinical signs are in many ways similar to those of surra, caused by *T. evansi*. The two parasites are genetically and antigenically similar (Caporale et al., 1980b, 1981; Brun et al., 1998; Giardina et al., 2003; Claes et al., 2005), making differential diagnosis problematic in areas where the two diseases coexist (OIE, 2008, 2010). In Italy, no reports on *T. evansi* exist so far. This article describes the epidemiological, clinical and laboratory findings enabling diagnosis of dourine in Italy.

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2. Materials and methods

2.1. Epidemiology

A Friesian stallion, the index case for the 2011 outbreak of dourine, was discovered in May 2011 in Scordia in the province of Catania, Sicily, during statutory controls of breeding horses required by Italian legislation to prevent the spread of diseases that can be transmitted sexually or by direct contact during coitus. According the national legislation, all stud horses must be authorised by the veterinary authority and tested annually for glanders, equine arthritis, infectious anaemia, equine encephalitis, contagious metritis, rhinopneumonitis and dourine (Anonymous, 1991). The stallion had been imported from the Netherlands on 6 September 2009. It presented depigmentation of the glans and was positive on complement fixation test (CFT) testing at a titre of 1:640. Since the stallion was previously included in the network of sentinel horses in the national surveillance plan for West Nile virus, aliquots of serum, collected on 21 May 2010, 30 August 2010, 28 September 2010 and 18 April 2011 were stored in serum bank of CESME (Centre for foreign animal diseases of ICT). Aiming to identify the possible time of infection, the sera were tested by CFT for dourine; the examination of sera collected on 21 May 2010, 30 August 2010 and on 28 September 2010, gave negative result, whereas the sample collected from the same animal on 18 April 2011 resulted positive by CFT at titer 1:640. Thus the time of contagion was likely narrowed down to between September 2010 and May 2011. Four adult horses and 2 foals present in the farm gave negative results to the CFT performed during the trace back activity.

In Italy, a confirmed case of dourine was defined as an equid positive on a CFT carried out by the national reference laboratory for exotic diseases (CESME) and confirmed by indirect immunofluorescence (IFAT), which

- presents clinical signs compatible with the disease, *or*
- is epidemiologically linked to a confirmed case of dourine, *or*
- shows increase of serological CFT titer in two consecutive testing.

The epidemiological investigation carried out after confirmation of this first case of dourine enabled the identification of second confirmed dourine case. It was a mare (mare no. 1) coming from another farmer in Catania Province. From the farmer's declarations it was verified that mare 1 was hosted in the same stable as the infected Friesian stallion in the period between February and March 2011, all of 16 horses living in the same stable tested after the discovery of the clinical the mare no. 1 were negative by CFT. Considering that she was recognized the most likely source of the stallion's infection. The mare tested positive on the CFT at a titre of 1:2560 and on the IFAT at a titre of 1:640, and showed severe clinical signs attributable to the third phase of dourine (Caporale, 1946). The animal's worsening signs and suffering caused her to be euthanized on 30 May 2011.

She had been imported from the Netherlands on 29 September 2009 to a stable in the province of Caserta, Campania, which at the time of the investigation was free of animals. From here she was transferred on 24 November 2009 to another stable in the province of Naples, Campania, and then on 7 February 2011 to the stable in Scordia (Scacchia et al., 2011). Two stallions present in the Naples stable through which Pandora has passed tested positive for dourine on CFT at titres of 1:5 and 1:40. Six serologically positive animals were found in two further holdings, also in the province of Naples and linked with this stable. One of these, located in the municipality of Nola, held 17 horses. Out of them, 5, all mares, had clinical symptoms suggestive of dourine, subsequently confirmed by laboratory testing. Out of these five, three were euthanized, while two were transferred to CESME for further diagnostic investigation.

3. Clinical signs and lesions

The clinical signs described in this article refer to the mares euthanized in the outbreaks in Scordia and Nola and in the two mares from the latter outbreak, which were transferred to CESME. The lesions and laboratory tests of organs and of tissues taken prior to death also refer to the animals from these outbreaks. The animals discussed in this study will hereafter be identified as follows:

Euthanized animals:

- Mare 1 Outbreak in: Scordia (Catania)
- Mare 2 Outbreak in: Nola (Naples)
- Mare 3 Outbreak in: Nola (Naples)
- Mare 4 Outbreak in: Nola (Naples)

Living animals transferred to CESME:

- Mare 5 Outbreak in: Nola (Naples)
- Mare 6 Outbreak in: Nola (Naples)

3.1. Real-Time PCR

3.1.1. DNA extraction

All samples were processed using the Maxwell™ 16 automated nucleic acid extraction system (Promega). DNA was extracted from whole blood samples by the Maxwell™ 16 Blood DNA Purification kit (Promega), from tissues by the Maxwell™ 16 Tissue DNA Purification Kit (Promega), and from biological fluids and vaginal and conjunctival swabs by the Maxwell™ 16 Cell DNA Purification kit (Promega), according to the manufacturer's instructions.

3.2. Real-Time PCR

Trypanosoma sp. DNA testing was performed using a Real Time PCR developed by Becker et al. (2004), based on the amplification of a 135 bp portion of the 177 bp sequence of the highly repeated region within the Trypanozoon subgenus. The method was carried out on a 7900HT Fast Real-Time PCR System (Applied Biosystems) and raw data were analysed by the software program SDS 2.4 (Applied Biosystems). The reaction mixture (20 μL total volume) contained the two primers (Tb177F and Tb177R) at a final concentration of 100 nM, the KAPA SYBR FAST ABI Prism 2X Mix (Kapabiosystems) and 5 μL of the extracted