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A clinical case of dourine in an outbreak in Italy

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Summary

In May 2011, dourine was reported in Italy following the declaration of a positive result observed in a stallion undergoing routine testing for stud purposes. Clinical signs, anatomo-histopathological findings and laboratory results that resulted in the confirmation of diagnosis of dourine in a clinically affected mare, which was the likely source of infection in the stallion, are described.

Keywords

Diagnosis, Dourine, Horse, Italy, Sicily, *Trypanosoma equiperdum*.

Introduction

In May 2011, a stallion undergoing routine testing for stud purposes tested positive for dourine in Sicily. Prior to this outbreak, the disease that was eradicated the first time in the 1940s, had caused a major epidemic in the mid-1970s (3) and was consequently eradicated a second time between the 1970s and 1980s. The last official notification to the World Organisation for Animal Health (*Office International des Épizooties*: OIE) was in 1996.

This short communication describes the clinical signs, anatomo-histopathological findings and results of diagnostic tests that confirmed the diagnosis of dourine in a mare in Sicily that was the likely source of the infection of the stallion.

Materials and methods

The animal was imported from the Netherlands on 29 September 2009 and, after two journeys in Campania Region, was transferred to the Scordia stables in the Catania Province of Sicily on 7 February 2011. The mare tested positive with a complement fixation test (CFT) titre of 1:2 560 and was slaughtered on 30 May 2011, after clinical signs deteriorated.

At the time of slaughter, the mare was cachectic, dehydrated and in a poor condition, with general muscle hypotrophy. The animal tested positive for neutrophilia and lymphocytopenia, and had a hematocrit of 23.1%. Her skin was dull and hypoelastic, with numerous grazes around protruding bones, depigmentation in the perineal area and an oedematous plaque on the skin of the right thigh. Her right udder was warm but not sore and there was general lymph node enlargement. Neurological examination revealed walking difficulties. There was also marked ataxia of the hindquarters, with straddle of the limbs.

Upon necropsy, skin lesions on the right thigh revealed mild subcutaneous oedema, while the lymph node draining the injury (popliteal lymph node) was enlarged. The parenchymatous organs were undamaged, with the exception of the spleen which was congested, with hyperplasia of the white pulp. There was also abundant synovial fluid in the tarso-metatarsal right joint. The right udder was hard, with slight reactivity of the tributary

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lymph nodes. High titre serum for *Trypanosoma equiperdum* was collected. Organ and fluid samples were taken for *T. equiperdum* testing using molecular and traditional methods and for histological examination using haematoxylin-eosin staining and immunohistochemistry (IHC).

All matrices were collected for microscopic examination of *Trypanosoma* spp. and bacteriological tests to exclude concurrent bacterial infections.

A real-time polymerase chain reaction (PCR) method amplifying a highly repeated region specific for the *Trypanozoon* subgenus was used (1).

Haematoxylin-eosin staining was performed on samples prior to fixing and embedding in paraffin.

On skin tissue sections, IHC using the mare-positive serum in combination with an anti-equine IgG monoclonal antibody was performed. Skin samples from healthy horses were included as negative controls.

Cerebrospinal fluid and serum collected after slaughter were tested using the CFT for *T. equiperdum* antibodies. The same serum sample underwent Western blot testing with *T. equiperdum* Onderstepoort Veterinary Institute (OVI) antigen (5, 6).

Results

All matrices were negative for *Trypanosoma* spp. on microscopic examination of fresh samples, and bacteriological tests excluded any concurrent bacterial infections

Tissue samples from both mammary glands, one of the left tributary lymph nodes, skin lesions, ipsilateral popliteal lymph nodes, cerebrospinal fluid, a clitoral groove smear, urine and tarso-metatarsal joint fluid all tested positive when examined by real-time PCR.

Histological examination of haematoxylin-eosin-stained tissue sections from the skin lesions revealed an unusual picture of pustular dermatitis, characterised by severe inflammation with cell detritus exudate. These cells were identified as eosinophils, presumably the bodies of free protozoa. This type of lesion was described as 'trypanosomal sand'.

The superficial area of trypanosomal sand of the same skin section gave positive results for trypanosomes using IHC methods.

The CFT for *T. equiperdum* antibodies in cerebrospinal fluid and serum collected after slaughter gave positive results (+++1:20 for cerebrospinal fluid and 1:5 120 for serum). The same serum sample underwent immune-Western blot testing (5, 6). Preliminary results revealed that positive serum from the mare identified bands ranging from 48 kDa to 37 kDa, 25 kDa and 19-14 KDa. These bands were not identified when negative sera were used.

Discussion

The clinical signs and macroscopic appearance of the lesions revealed in this case agree with evidence published in the literature. Specifically, the nervous signs with antibodies in cerebrospinal fluid and the skin lesions that tested positive using real-time PCR and IHC were similar to those described in the literature for the second and third stages of the disease (2). The localised monolateral involvement of the udder and joint lesions were of particular interest and require further investigation. IHC revealed *Trypanosoma* in the oedematous plaque. Although often described as characteristic of clinical cases of disease (2, 9), no microscopic description of such lesions is available in the literature. Pustular dermatitis in the presence of the finding described as 'trypanosomal sand' is new.

The evaluation of Western blot results requires further studies.

The availability of highly sensitive methods, such as molecular tests, enables even low levels of the parasite to be detected. However, despite this sensitivity, some doubts remain over the differentiation of *T. equiperdum* within the *Trypanozoon* subgenus. In fact, the unequivocal genetic differentiation of *T. equiperdum* and *T. evansi* is not possible on the basis of genetic characterisation studies performed (4, 7, 8). Some authors report that most *T. equiperdum* strains isolated possess

similar molecular features to *T. evansi*, supporting the theory that both species indeed originate from successive differentiations of *T. brucei* (7, 8). However, epidemiological evidence from this outbreak (prevalence, age, reproductive activity and the relationship between the affected animals) revealed that the origin of contagion was likely to be sexual.

This infection route specific to *T. equiperdum* is not reported for *T. evansi* which is transmitted by mechanical vectors and causes surra which causes clinical signs in horses that closely resemble those of dourine (9, 10).

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