DOURINE (INFECTION WITH TRYPANOSOMA EQUIPERDUM)

AETIOLOGY

Classification of the causative agent

Dourine is a parasitic venereal disease of equines caused by the flagellate protozoan *Trypanosoma* equiperdum of the order Trypanosomatida, family *Trypanosomatidae* and subgenus *Trypanozoon*. Other species within this subgenus are *T. brucei* and *T. evansi*. Recent genomic studies propose that *T. equiperdum*, along with *Trypanosoma evansi*, are subspecies of *T. brucei*. One hypothesis asserts that the disease condition "dourine" is actually a host-specific immune response to either *T. equiperdum*, *T. brucei* or *T. evansi* infection.

Resistance to physical and chemical action

This agent does not survive very long outside its hosts and is not transmitted by fomites, therefore, parameters associated with resistance to physical and chemical actions (i.e. temperature, chemical/disinfectants, and environmental survival) are not meaningful.

EPIDEMIOLOGY

Dourine is not transmitted by an invertebrate vector. It is transmitted during breeding or infected mares may occasionally pass infection to foals. Average mortality associated with acute disease approaches 50% (especially in stallions)

Hosts

- Horses, mules and donkeys
- No known natural reservoir of the parasite other than infected equids
- Rats, mice, rabbits and dogs can be infected experimentally; rodents are used to prepare antigen for diagnostic tests

Transmission

- Natural transmission occurs directly from animal to animal during coitus:
 - mainly from stallion to mare, but may also be transmitted from mare to stallion
 - infection is not always transmitted by an infected animal at every copulation
- There is currently no evidence that arthropod vectors play any role in transmission
- Rarely, foals may be infected via the mucosa (conjunctiva), during parturition or by drinking milk from an infected dam. Foals may then transmit disease when they are sexually mature

Sources of infection

- *T. equiperdum* may be found in vaginal secretions of infected mares, and seminal fluid, mucous exudate of the penis, and sheath of stallions
- Rarely, infected mares have been reported to pass the infection to their foals, possibly from infected mare to fetus via placenta. Trypanosomes have been detected in the mammary secretions of some infected animals.

Occurrence

Dourine is endemic in some African, Asian and Latin American regions, as well as the Middle East and Eastern Europe. It was once spread more widely, but has been eradicated in many countries. In Italy, an outbreak occurred in 2011.

For more recent, detailed information on the occurrence of this disease worldwide, see the OIE World Animal Health Information Database (WAHID) Interface [http://www.oie.int/wahis/public.php?page=home]

DIAGNOSIS

Incubation period is very variable and could be from one week to a few months or longer. For the purposes of the OIE *Terrestrial Animal Health Code*, the incubation period for dourine is 6 months.

Clinical diagnosis

Severity and duration of disease vary considerably. Though the disease is often fatal, spontaneous recoveries do occur but may result in latent carriers. Diagnosis is most commonly based on clinical evidence supported by serology.

- Clinical manifestations include:
 - fever
 - o local oedema of the genitalia and mammary glands
 - o oedematous coetaneous eruptions
 - o knuckling of the joints, incoordination and unilateral facial and lip paralysis
 - o ocular lesions
 - o anaemia
 - o progressive weight loss and emaciation
 - o nervous form may set in after emaciation and oedema and lead to weakness, lameness mostly of the hind legs resulting in a 'staggering movement', and gait abnormalities
- Clinical signs are marked by periodic exacerbation and relapse, ending in death, sometimes after paraplegia or, possibly, recovery; acute disease lasts only 1–2 months or, exceptionally, 1 week
- A chronic, usually mild, form of the disease may persist for several years
- Subclinical infections occur; donkeys and mules are more resistant than horses
 - may remain inapparent carriers
- In fatal cases, the disease is usually slow and progressive, with increasing anaemia and emaciation, although the appetite remains good almost throughout

Lesions

- Raised, oedematous or cutaneous urticarial skin plaques ("silver dollar plaques"), 5–8 cm in diameter and 1 cm thick
 - plaques usually appear over the ribs, although they may occur anywhere on the body, and usually persist for between 3 and 7 days
 - o not a constant feature and when present not easy to identify
- Oedema disappears and returns at irregular intervals causing a thickening and induration of affected tissue; gelatinous exudates are present under the skin
- In mare,
 - o vulva, vaginal mucosa, uterus, bladder, and mammary glands may be thickened with gelatinous infiltration
 - o vaginal mucosa may show raised and thickened semitransparent patches
 - o folds of swollen membrane may protrude through the vulva
- In the stallion,
 - the scrotum, sheath, and testicular tunica are thickened and infiltrated
 - testes may be embedded in a tough mass of sclerotic tissue and may be unrecognisable
- Depigmentation of the genital area, perineum, and udder may occur
- Lymph nodes, particularly in the abdominal cavity, are hypertrophied, softened and, in some cases, haemorrhagic
- Spinal cord of animals with paraplegia is often soft, pulpy and discoloured, particularly in the lumbar and sacral regions

Differential diagnosis

- Coital exanthema
- Contagious equine metritis
- Surra
- Nagana
- Anthrax
- Equine viral arteritis
- · Equine infectious anaemia
- Purpura haemorrhagica
- Other conditions leading to weight loss and emaciation: malnutrition, verminosis, dental pathology, chronic infections

Laboratory diagnosis

Samples

- Trypanosomes are present, in low numbers only, in lymph and oedematous fluids of the external
 genitalia, in the vaginal mucus, and exudates of plaques and mammary gland exudates; may be
 found in the urethral or vaginal mucus collected from preputial or vaginal washings or scrapings 4
 5 days after infection
- Aspirate from plaque: skin of the area over the plaque should be washed, shaved and dried, and the fluid contents aspirated by syringe; blood vessels should be avoided
- Several thick blood smears
 - thick films are made by placing a small drop (approximately 20 μl) of blood on to a clean glass slide, droplet is air-dried, heat-fixed at 80°C for 5 minutes, and stained in 10% Giemsa for 15–20 minutes
 - unstained blood smears should not be stored with formalin solutions as it may affect staining quality
- Whole blood (in EDTA or heparin), plasma and serum
- Trypanosoma equiperdum strains are best stored in liquid nitrogen

Procedures

Identification of the agent

- A definitive diagnosis depends on the recognition of the clinical signs and the demonstration of the parasite
- Rarely possible due to:
 - although the clinical signs and gross lesions in the developed disease may be pathognomonic, they cannot always be identified with certainty, especially in the early stages or in latent cases
 - they can be confused with other conditions, such as coital exanthema (moreover, in some countries [e.g. in South America], T. evansi infections give rise to similar clinical signs);
 - the trypanosomes are only sparsely present and are extremely difficult to find, even in oedematous areas: and
 - the trypanosomes are only fleetingly present in the blood, and in small numbers that defy detection
- Microscopic examination of fresh aspirate
 - motile trypanosomes are present for a few days only, so that lesions should be examined at intervals
- As the parasite is rarely found in thick blood films, the use of concentration techniques is recommended, such as capillary tube centrifugation, and mini anion exchange centrifugation technique
 - o dourine is the only trypanosome to affect horses in areas free from Nagana or Surra diseases, observation of agent thick blood films is sufficient for a positive diagnosis
 - differentiation on the basis of morphological criteria in countries with other members of the subgenus Trypanozoon, is difficult

Serological tests

Humoral antibodies are present in infected animals, whether they display clinical signs or not, however, diagnosis of dourine must include history, clinical, and pathological findings as well as serology. Methodologies are described in the OIE *Terrestrial Manual*

- Complement fixation test
 - o used to confirm clinical evidence and to detect latent infections
 - uninfected equids, particularly donkeys and mules, often give inconsistent or nonspecific reactions because of the anticomplementary effects of their sera
- Indirect fluorescent antibody (IFA) test
 - o in the case of anticomplementary sera, the IFA test is of advantage
 - o cross-reactions are possible due to the presence in some countries of other trypanosomes
- Enzyme-linked immunosorbent assay
 - competitive ELISA has also been described for detecting antibody against *Trypanosoma* equiperdum
 - o cross-reactions are possible due to the presence in some countries of other trypanosomes
- Other serological tests
 - Immunochromatography and immunoblotting tests have been developed but are not yet validated

Molecular tests

Although no *T. equiperdum*-specific polymerase chain reaction (PCR) method is available, subgenus *Trypanozoon*-specific conventional PCR and real-time PCR can be used to detect low numbers of parasites in samples of horses with dourine. Primers and probes are described in the OIE *Terrestrial Manual*

There is an OIE Reference Laboratory for Dourine (consult the OIE Web site for the most up-to-date list: http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapter 3.5.3 Dourine (infection with *Trypanosoma equiperdum*) in the latest edition of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading "Diagnostic Techniques".

PREVENTION AND CONTROL

Sanitary prophylaxis

- Control of the disease depends on compulsory notification and slaughter of infected animals
- Movement control enforced by legislation in most countries
- · Good hygiene at assisted matings is also essential
 - fences may help control the spread, although stallions have been reported to serve mares over fences

Medical prophylaxis

- No vaccines are available for this disease
- Pharmaceutical therapy is not recommended because animals may improve clinically but remain carriers of the parasite

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the OIE *Terrestrial Animal Health Code*

REFERENCES AND OTHER INFORMATION

- Brown C. & Torres A., Eds. (2008). USAHA Foreign Animal Diseases, Seventh Edition. Committee
 of Foreign and Emerging Diseases of the US Animal Health Association. Boca Publications Group,
 Inc.
- Brun R., Hecker H. & Lun Z.R. (1998). *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Vet. Parasitol.*, **79**, 95–107.
- Büscher P., Gonzatti M.I., Hébert L., Inoue N., Pascucci I., Schnaufer A., Suganuma K., Touratier L.
 & Van Reet N. (2019). Equine trypanosomosis: enigmas and diagnostic challenges. *Parasit. Vectors.* 12, 234
- Carnes J., Anupama A., Balmer O., Jackson A., Lewis M., Brown R., Cestari, I., Desquesnes M., Gendrin C., Hertz-Fowler C., Imamura H., Ivens A., Koreny L., Lai D.H., MacLeod A., McDermot, S.M., Merritt C., Monnerat S., Moon W., Myler P., Phan I., Ramasamy G., Sivam D., Lun Z.R., Lukes J., Stuart K. & Schnaufer A. (2015). Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Negl. Trop. Dis.*, 9(1): e3404.
- Claes F., Agbo E.C., Radwanska M., te Pas M.F.W., Baltz T., De Waal D.T., Goddeeris B.M., Claassen E. & Büscher P. (2003). - How does Trypanosoma equiperdum fit into the Trypanozoon group? A cluster analysis by RAPD and Multiplex-endonuclease genotyping approach. *Parasitology*, 126, 425-431.
- Coetzer J.A.W. & Tustin R.C. Eds. (2004). Infectious Diseases of Livestock, 2nd Edition. Oxford University Press.
- Cuypers B., Van den Broeck F., Van Reet N., Meehan C.J., Cauchard J., Wilkes J.M., Claes F., Goddeeris B., Birhanu H., Dujardin J.-C., Laukens K., Büscher P. & Deborggraeve S. (2017). Genome-wide SNP analysis reveals distinct origins of *Trypanosoma evansi* and *Trypanosoma equiperdum*. *Genome Biol. Evol.*, 9, 1990–1997.
- Fauquet C., Fauquet M., & Mayo M.A. (2005). Virus Taxonomy: VIII Report of the International Committee on Taxonomy of Viruses. Academic Press.
- Kahn C.M., Ed. (2005). Merck Veterinary Manual. Merck & Co. Inc. and Merial Ltd.

- Lai D.H., Hashimi H., Lun Z.R., Ayala F.J. & Lukeš J. (2008). Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *T. equiperdum* and *T. evansi* are *petite* mutants of *T. brucei*. *Proc. Natl Acad. Sci. USA*, **105**, 1999–2004.
- Pascucci I., Di Provvido, A., Cammà C., Di Francesco G., Calistri P., Tittarelli M., Ferri N., Scacchia M. & Caporale V. (2013). Diagnosis of dourine outbreaks in Italy. Vet. Parasitol., 193, 30–38.
- Scacchia M., Cammà C., Di Francesco G., Di Provvido A., Giunta R., Luciani M., Marino A.M.F., Pascucci I. & Caporale V. (2011). - A clinical case of dourine in an outbreak in Italy. Vet. Ital., 47, 473–475.
- Perrone T.M., Gonzatti M.I., Villamizar G., Escalante A, Aso P.M. (2009). Molecular profiles of Venezualan isolates of *Trypanosoma sp.* by random amplified polymorphic DNA method. *Vet. Parasitol.* 161, 194–200.
- Spickler A.R. & Roth J.A. Iowa State University, College of Veterinary Medicine http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.htm
- Suganuma K., Narantsatsral S., Battur B., Yamasaki S., Otgonsuren D., Musinguzi S.P., Davaasuren B., Battsetseg B. & Inoue N. (2016). Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia. *Parasit. Vectors*, 9, 481.
- World Organisation for Animal Health (2020). Terrestrial Animal Health Code. OIE, Paris.
- World Organisation for Animal Health (2020). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. OIE, Paris.

* *

The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Science Department (scientific.dept@oie.int). Last updated December 2020.