A CASE OF CANINE SURRA IMPORTED INTO GERMANY

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Case presentation "Hector"

A 9-year old male Jack Russel Terrier (8kg body weight) was presented with polyuria, polydipsia, lethargy, anorexia and unilateral ocular opacity of 3 months duration. The symptoms were first noted during a journey through Thailand and were associated with intermittent fever. The patient also had a previous travel history to Spain and Brazil. In Thailand trypanosomiasis was suspected and therapy was initiated with diminazene aceturate (Berenil®), iron supplementation and doxycycline. The clinical signs improved but lethargy persisted. Upon return to Germany the patient showed lethargy, weight loss, cough and clinical examination by the referring veterinarian revealed pale mucous membranes. Routine hematological and biochemistry profiles revealed moderate non-regenerative normocytic normochromic anemia (Hct: 29%, reticulocytes: 14000/µl), moderate thrombocytopenia (platelets: $89x10^9$/l), marked leukopenia (leukocytes: $1.1x10^9$/l) and mild hyperglobulinemia (globulin: 61g/l). Therapy with allopurinol, itraconazol and amoxicillin/clavulanic acid was initiated. Serological testing for infectious diseases was performed. Serological results were negative for *Babesia sp.* and *Ehrlichia canis*, and doubtful for *Leishmania infantum*. Direct Coomb's test was negative.
Due to the presence of pancytopenia a bone marrow sample was sent to our laboratory for cytological evaluation. No actual blood sample was submitted with the bone marrow sample.

Questions:

What is the Identify of the parasite found during bone marrow examination?
Considering the morphology of the parasite and the previous travel history to Thailand, what is the most likely diagnosis?

Diagnosis, Description and Discussion

Bone marrow cytology

- markedly increased bone marrow cellularity

- mildly decreased M:E ratio

- marked plasmacellular hyperplasia

- moderate numbers of macrophages with cytophagia of erythroid precursor cells and erythrophagia

- numerous hemoparasites compatible with *Trypanosoma sp*.

The cytological picture was highly suspicious for immune-mediated cytopenia secondary to *Trypanosoma* infection.

The patient was then referred to the Small Animal Clinic of the Justus-Liebig University, Giessen. On physical examination the patient showed marked lethargy, pale mucous membranes, mild submandibular lymphadenomegaly and moderate popliteal and prescapular lymphadenomegaly. Marked unilateral left anterior uveitis with secondary glaucoma was found on ophthalmological evaluation.

Hematology

- moderate normocytic normochromic regenerative anemia (Hct: 28%, reticulocytes: $157.6x10^9$/l)

- mild thrombocytopenia ($122x10^9$/l)

- The total and differential white blood cell counts were within normal limits (WBC: $6.4x10^9$/l) with a mild left shift indicative of acute inflammation, along with occasional activated monocytes, occasional medium-sized lymphocytes with fine azurophilic cytoplasmic granulation and a moderate amounts of basophilic cytoplasm.

- MGG stained blood smear: numerous extracellular hemoparasites were present (Figure 2). These were 20-30µm in length, had an elongated thin body with a pointed posterior end, a central nucleus, a large undulating membrane and a flagellum arising near the parabasal body, running through the length of the parasite and ending in a long free flagellum. Some small azurophilic granules were present in the cytoplasm. A subterminal oval kinetoplast was seen.

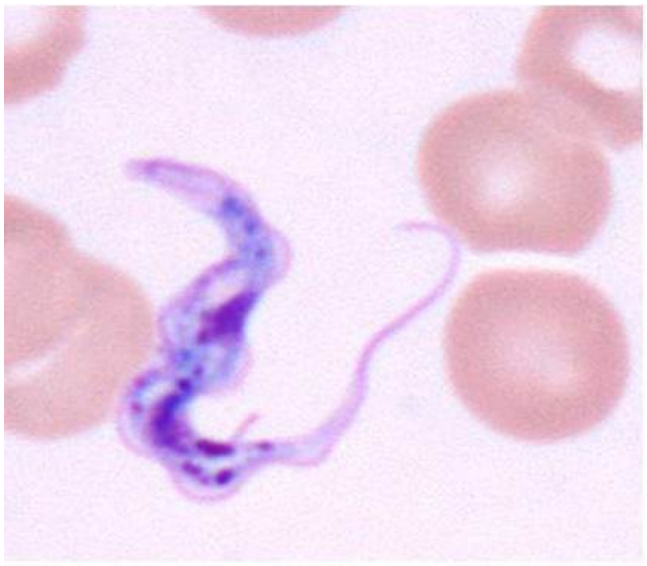


Figure 1 Blood smear. MGG stain, x1000

- Fresh thick blood film preparation: a relatively slow movement of the parasites between the erythrocytes without evident forward progress was observed.

Considering the previous travel history to Thailand, the clinical signs and the parasite morphology, *Trypanosoma evansi* infection was suspected.

Biochemistry

- mild elevation in urea

- mild hypomagnesemia

- mild hyperchloremia

- marked hyperproteinemia

- moderate hypoalbuminemia (18.8g/l)

- marked hyperglobulinemia (69.3g/l)

- mild elevation in the serum activity of ALT

- C-reactive protein was moderately elevated (26.6mg/l, ref. range: 0-11mg/l).

- urinalysis: marked proteinuria (UPC=2.3), mild hematuria, SG=1.016. In the unstained urinary sediment some erythrocytes and rare *Trypanosoma* specimens were seen.

Cardiological evaluation

- Chest X-ray: within normal limits

- ECG: sinus rhythm

Hemostasis

- TEG (Figure 3): moderate increase in the maximal amplitude (MA): 73.9 (ref: 51-69) and G: 14.2K (ref.: 4.6-10.9K) indicative of a hypercoagulable state

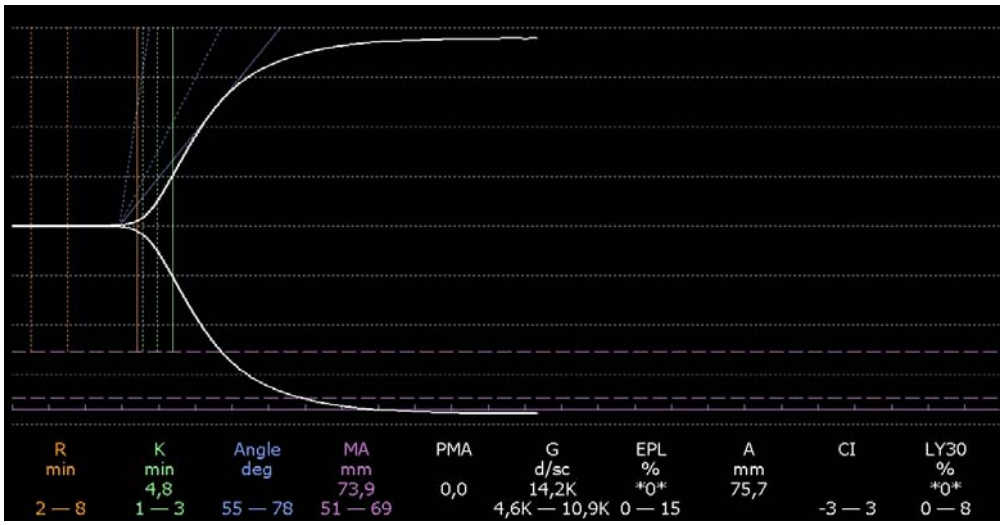


Figure 3 : TEG tracing

- mild prolongation of the aPTT

- no elevation of D-dimers was present

Serum protein capillary zone electrophoresis (Figure 4)

- polyclonal gammopathy

- mild elevation of β2-globulin

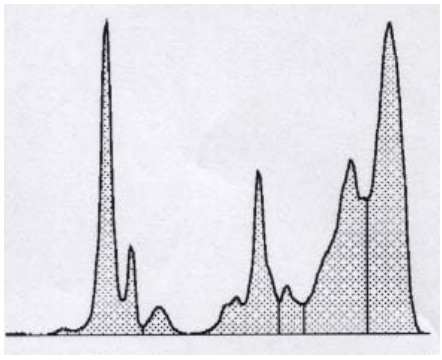


Figure 4 : Serum capillary zone electrophoresis

Peripheral lymph node cytology

- reactive hyperplasia with some well differentiated plasma cells, rare Mott cells and occasional macrophages.

- No parasites were found.

Tests for infectious diseases

Serological testing for *T. evansi* using the card agglutination test (CATT/*T. evansi*) was positive. Molecular identification using a polymerase chain (PCR-18S for *Trypanozoon sp.* and RoTat 1.2 for *T. evansi*) confirmed infection with *T. evansi*. A bone marrow sample was submitted for polymerase chain reaction identification of *Leishmania infantum* and was negative.

Therapy

Monotherapy for canine leishmaniasis (allopurinol) was continued until results of PCR analysis were known, as well as anti-inflammatory (carprofen) and antimicrobial (amoxicillin/clavulanic acid) therapy. Rapid deterioration of vital parameters and aggravation of the pancytopenia (WBC: $1.9x10^9$/l, Hct: 18%, PLT: 42000/µl) were observed and whole blood transfusion was given. At the same time steroid therapy (prednisolone: 1 mg/kg, IV BID) was initiated and coupled to proton-pump inhibitor therapy for prevention of gastric ulceration. Trypanocide therapy was initiated with suramin (70mg IV in 100ml NaCl 0.9% given 3 times every 3rd day). Three days later, after the second suramin injection, no parasites were detected (thick blood film and micro-hematocrit centrifugation technique), and normalization of hematological parameters was achieved (WBC: 7.1 $x10^9$/l, Hct: 56%, PLT: $325x10^3$/µl). Urine specific gravity was hypersthenuric with marked proteinuria (UPC=1.3) and absence of concurrent azotemia or pyuria. After the third suramin injection parasites were not found and clinical improvement allowed the patient to be discharged from the Small Animal Clinic of the Justus-Liebig University, Giessen with the plan to gradually decrease the prednisolone dose.

Follow-up

Monthly follow-up hematology and PCR were unremarkable. The dog was presented again 88 days after initial admission with anorexia, marked lethargy, ataxia and marked dorsal pain localized at the thoraco-lombar junction. Serology was still positive for *T. evansi* (CATT/*T. evansi*).

Magnetic resonance imaging

Magnetic resonance imaging of the central nervous system (CNS) was consistent with meningoencephalitis and cystic lesions were identified in the left frontal lobe. Multiple intervertebral degenerative disc lesions were seen.

Additional tests

On routine hematology, moderate leukopenia (WBC: $2.5x10^9$/l) with mild neutropenia and moderate lymphopenia suggestive of acute inflammation and mild anemia (Hct: 34%) were detected. No parasites were identified on thick blood film preparation or hematocrit centrifugation technique. Cerebrospinal fluid (CSF) evaluation revealed marked pleocytosis (TCC: 596 cells/µl), markedly elevated protein content (TP: 1147mg/l) and a positive Pandy reaction. On cytological evaluation no parasite could be identified. The cytological picture was consistent with marked mononuclear pleocytosis with predominantly reactive lymphocytes and Mott cells. Shortly after, the dog died of sudden cardio-respiratory arrest.

Post-mortem findings

At necropsy mild non-suppurative nephritis was found. Evaluation of the CNS revealed a marked non-suppurative meningoencephalitis with marked mononuclear perivascular infiltration and multifocal hemorrhages. Evaluation of the eyes revealed a mononuclear infiltration of the iris, retina and a perivascular infiltration of lacrymal glands and perineuronal tissue. Parasites were not demonstrated microscopically in any tissue.

PCR and serology were performed on CSF and serodiagnosis performed on serum sample. All results were positive and suggested the presence of parasitic DNA in CSF (Figures 5 and 6).

Discussion

Trypanosoma evansi is the causative agent of Surra and is endemic in large parts of Asia, Africa (also known as "*el debab"),* Latin America (also known as "*mal de caderas"*) and the Canary Islands of Spain. This parasite is an important pathogenic agent for numerous wild and domestic animals including camels, horses, buffaloes, bovines, dogs and cats causing an acute or chronic disease and major economic losses.[1,2,3,4,5,6]

T. evansi is closely related to the African kinetoplastid flagellate *T. brucei* and can be distinguished by a partial or complete loss of kinetoplast DNA.[7] The loss of kinetoplast DNA locks the parasite in the bloodstream stage in the vertebrate host and prevents the formation of the procyclic stage in the insect vector tsetse *Glossina spp.*. In the absence of cyclical transmission by the tsetse fly, *T. evansi* is mechanically transmitted and not bound to the distribution area of tsetse flies. Carnivores being highly susceptible to *T. evansi* infection can be infected mechanically (hematophagous flies) or iatrogenically (injection with contaminated material) or by ingestion of contaminated meat.[4,8,9] The infection is generally fatal in the dog.[9] Parasite stages can be found in blood, hemo-lymphatic tissues and have the capacity to localize extravascularly, especially in the central nervous system.[9,10] Surface protein antigenic variation allows the parasite to evade the host's immune system, thus causing chronic infection, and complicates the development of a possible vaccine.[11]

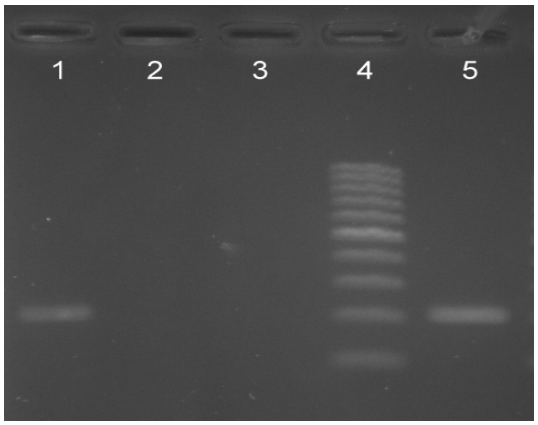
Experimental Surra infection in dogs reveals a prepatent period of 11.2 days with parasitaemia occuring in peaks with aparasitemic periods lasting from 1 to 3 days.[2] This could explain why no parasites could be found at the first examination of our patient. Infected dogs typically display fever, lymphadenomegaly and anemia.[2] Corneal edema was also reported during natural infection.[12]

As clinical signs are not pathognomonic for the disease, identification of the parasite through microscopic and serological testing or molecular testing are required to achieve definitive diagnosis.[4,5,6]

Microscopic evaluation of blood smears is the easiest way to identify the parasite but lacks sensitivity, especially in cases of chronic infection where parasitemia is low. Concentration methods are described to detect cases of low parasitemia and for our patient we used the hematocrit centrifugation technique (also called Woo's technique) after initiating treatment, as no parasites were found on routine stained blood smear. [4,5] Briefly, a microhematocrit

capillary tube is filled with patient's blood and centrifuged at 3000g for 5 minutes then fixed on a microscope slide and examined at high power field at the junction between buffy coat and plasma. Based on the distribution of the different parasite groups and the apparition of clinical symptoms in Thailand, infection with *T. evansi* was suspected, but needed confirmation.[13]

Molecular identification of the parasite can be done using polymerase chain reaction.[13] Several markers have been developed using kinetoplast DNA sequences but fail to identify dyskinetoplastic strains of *T. evansi*. The PCR-RoTat1.2 VG uses primers derived from the RoTat 1.2 variable surface glycoprotein and allows diagnosis of Surra (Figure 5).[13]



1. CSF sediment
2. Negative extraction control
3. Negative PCR control
4. 100 bp DNA marker
5. Positive PCR control

Figure 5 : T. evansi RoTat 1.2VSG PCR on extraacted DNA from CSF sediment.

To detect chronic infection paired serological testing is preferred to PCR as sensitivity of the PCR is dependent on the amount of DNA present and false negative results are possible when low parasitemia is present. In this case we used the CATT/ *T. evansi* to detect agglutinating antibodies in the patient's serum and CSF supernatant. The test uses fixed trypomastigotes of *T. evansi* expressing the variable antigen type RoTat1.2 and can be performed on any host species.[4] The CATT/ *T. evansi* performed here was not repeated, negative and positive controls were used as internal controls and the test results performed on CSF and serum are displayed on Figure 6.

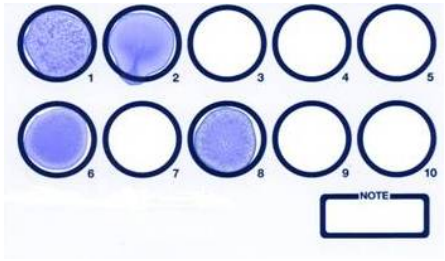


Figure 6: CATT/*T. evansi*

The blue color originates from the blue agglutinating particles, *i.e.* purified, fixed and coomassie stained bloodstream from trypanosomes. When these particles are mixed with antibodies, macroscopically visible blue agglutinates appear which indicate a positive reaction. When there are no antibodies, the particles remain as a homogenous blue suspension. The reagent is mixed on a plastified card with a drop of test serum. After 5

minutes rocking at 60rpm, the reaction is read. Zones 3, 4, 5, 7, 9 and 10 on Figure 3 do not contain anything whether zone 6 contains the reagent and CSF (weak agglutination) and zone 8 contains reagent with the serum (clear agglutination). Similar positive antigen-antibody reactions on the patient's serum were noted before and after treatment.

Therapy of Surra is challenging as immunosuppression following infection is severe.[4,5] Efficacy of treatment may also be diminished in face of chronic infection and possible host´s immunotolerance (also called trypanotolerance).[10] No trypanocides have been registered for use in the dog and no toxicological studies are available for this species. Suramin was historically used in camels for treatment of infection with *T. evansi*. The drug was commercialized as a powder and must be administered carefully and strictly intravenously as the drug is highly irritant at injection site. The drug does not cross the hemato-encephalic barrier.[4,5] Suramin was chosen for therapy in this case as neurological signs were absent at admission and suramin's toxicity in the dog is inferior as arsenical drugs which can cross the hemato-encephalic barrier. Diminazene aceturate (Berenil®) is another trypanocide drug which is administered intramuscularly and commonly used in cattle and small ruminants. The drug is less effective against *T. evansi* and *T. brucei* than against *T. congolense* which limits its use in cases of Surra.[4,5] As our patient was initially treated with Berenil® in Thailand (dose not known) either parasite persistence or selection of drug resistant parasites cannot be excluded and may have led to chronic infection. Melarsenoxide cysteamine is registered for treatment of Surra in camels (Cymelarsan®). It is a trivalent arsenical drug which has been developed for use against trypanosomes of the *brucei* group. It is effective against *T. evansi* strains resistant to suramin and crosses the hemato-encephalic barrier.[4,14]

Anemia is a frequent hematological finding in Surra but its cause remains unknown. At admission the patient presented with a regenerative normocytic normochromic anemia. Cytological evaluation of bone marrow sample was highly suspicious for immune-mediated anemia secondary to infection. Even if direct Coomb's test was negative, a false negative result cannot be excluded. Another possible cause for the anemia has been investigated during experimental infection of Savannah Brown bucks and revealed an association between sialidase production by trypanosomes (*T. evansi*) resulting in altered erythrocytes' sialic acid content and subsequent phagocytosis in the reticuloendothelial system.[15]

Evaluation of hemostasis revealed a prothrombotic state at admission. As anemia was present and an inverse correlation between hematocrit and MA is described, a falsely increase in MA value cannot be excluded. Hypercoagulability may have been related to urinary loss of antithrombin III as glomerular proteinuria was found, to infection, hemolysis or disseminated intravascular coagulation (DIC). DIC seemed unlikely as only two inclusion criteria were present (thrombocytopenia and elevation of aPTT) and no elevation of D-dimer, fragmentocytes or clinical signs of bleeding were present. To date hemostatic disorders secondary to Surra haven't been investigated in the dog. Marked proteinuria (UPC>2) was highly suggestive of glomerular proteinuria. Trypanosomiasis is a recognized cause of glomerular disease (glomerulonephritis) due to formation of auto-antibodies directed against laminin and collagen IV.[16] Serological testing for leishmaniasis performed at initial presentation led to doubtful results. [17] As parasites were not found on cytological evaluation of bone marrow and peripheral lymph nodes and bone marrow PCR results were negative, cross-reactivity following massive infection with *T. evansi* cannot be excluded.

Introduction of *T.evansi* in Europe was previously described in Alicante province, France (2006) and Spain (2008) following the importation of dromedary camels from the Canary Islands.[14,18] This led to sanitary measures as chronic infection is possible and could lead to the parasite becoming endemic in these European countries. As the dog presented parasitemia in the winter (January 2011) and hematophagous flies were absent at this time, probability of transmission to other susceptible vertebrates appeared to be low. In order to avoid importation of new Surra cases into Germany it would be recommended to test any susceptible animal returning from endemic areas using the card agglutination test (CATT/ *T. evansi*). If infection is suspected, therapy with cymelarsan could be performed to achieve diminution of parasite load and to prevent chronic infection.

References

1. Da Silva A, Pierezan F, Wolkmer P, et al. Pathological findings associated with experimental infection by *Trypanosoma evansi* in cats. *J. Comp. Path*. 2010;142:170-176.

2. De Aquino L, Machado R, Alessi A, et al. Clinical, parasitological and immunological aspects of experimental infection with *Trypanosoma evansi* in dogs. *Mem. Inst. Oswaldo Cruz.* 1999;94:255-260.

3. Deplazes P, Staebler S, Gottsein B. Reisemedizin parasitärer Erkrankungen des Hundes [Travel medicine of parasitic diseases in the dog]. *Schweiz. Arch. Tierheilk.* 2006;148:447-461.

4. OIE Terrestrial Manual 2010, chapter 2.1.17.*Trypanosoma evansi* infection (Surra). Page 1-14.

5. Uilenberg G. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. *Food and Agriculture Organization of the United Nations*. 1998;m-27.

6. Vergne T. Epidémiologie de *Trypanosoma evansi* en Thaïlande [Epidemiology of *Trypanosoma evansi* in Thailand]. Thèse ENVT, 2009.

7. Lai DH, Hashimi H, Lun ZR, Ayala FJ, Lukes J. Adaptation of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *PNAS.* 2008;105:1999-2004.

8. Raina A, Rakesh K, Rajora V, Singh R. Oral transmission of *Trypanosoma evansi* infection in dogs and mice. *Vet. Parasitol.* 1985;18:67-69.

9. Antoine Moussiaux M, Desmecht D. Epidémiologie de l'infection par *Trypanosoma evansi* [Epidemiology of *Trypanosoma evansi* infection]. *Ann. Med. Vét*. 2008;152:191-201.

10. Authié E. Trypanosomiasis and trypanotolerance in cattle: a role for Congopain? *Parasitol. Today.* 1994;10:360-364.

11. Pays E. The variant surface glycoprotein as a tool for adaptation in African trypanosomes. *Microbes Infect.* 2006;8:930-937.

12. Singh B, Kalra I, Gupta M, Nauriyal D. *Trypanosoma evansi* infection in dogs: seasonal prevalence and chemotherapy. *Vet. Parasitol.* 1993;50:137-141.

13. Claes F, Büscher P. Molecular markers for the different (sub)-species of the *Trypanozoon* subgenus. In: Developing methodologies for the Use of Polymerase Chain Reaction in the Diagnosis and monitoring of Trypanosomosis. International Atomic Energy Agency. June 2007.

14. Desquesnes M, Bossard G, Patrel D, et al. First outbreak of *Trypanosoma evansi* in camels in metropolitan France. *Vet. Rec.* 2008;162:750-752.

15. Shelu S, Ibrahim N, Esievo K, Mohammed G. Neuraminidase (sialidase) activity and its role in development of anaemia in *Trypanosoma evansi* infection. *Journal of Applied Science.* 2006;6:2779-1783.

16. Bruijn J, Oemar B, Ehrich J, Foidart J, Fleuren G. Anti-basement membrane glomerolupathy in experimental trypanosomiasis. *J. Immunol.* 1987;139:2482-2488.

17. Solano-Gallego L, Koutinas A, Miro G, et al. Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. *Vet. Parasitol.* 2009;165:1-18.

18. Tamarit A, Gutierrez C, Arroyo R, et al. *Trypanosoma evansi* infection in mainland Spain. *Vet. Parasitol.* 2010;167:74-76.