Schweiz. Arch. Tierheilk.
© 2012 Verlag Hans Huber, Hogrefe AG, Bern
L. Walser-Reinhardt et al., Band 154, Heft 4, April 2012, 149–152
DOI 10.1024/0036-7281/a000318



# Direct detection of *Ehrlichia canis* by PCR in the conjunctiva of a dog with bilateral anterior uveitis

**L. Walser-Reinhardt[1], D. Schaarschmidt-Kiener[2] , J.-L. Forster[3], F. Matheis[1], B. Spiess[1]**

[1]Equine Department, Section of Ophthalmology, University of Zurich, [2]Labor am Zugersee, Hünenberg, Switzerland, [3]Small Animal Practice Letzi AG, Zurich, Switzerland

## Abstract

The following report describes the direct detection of Ehrlichia canis by real-time PCR in the conjunctiva of a 1-year-old female Maltese dog. After being imported from Brazil, the dog was presented because of anorexia, dehydration, fever and palpable mandibular lymph nodes. A few days later, the dog developed bilateral blepharospasm, photophobia and anterior uveitis. Monocytic ehrlichiosis was diagnosed by a positive PCR result and the detection of IgM and IgG antibodies. Because of the massive uveitis a conjunctival sample was taken with a cytobrush, which also tested positive for Ehrlichia canis DNA by real-time PCR. Only one week after starting treatment with systemic doxycycline and local anti-inflammatory and cycloplegic therapy the dog recovered from systemic and eye diseases. After therapy the follow-up examination revealed a full remission of clinical and hematological parameters and a negative PCR result.

Keywords: Ehrlichia canis, cytobrush, real-time PCR, conjunctiva, uveitis

## Direktnachweis von *Ehrlichia canis* mittels PCR in der Bindehaut eines Hundes mit bilateraler Uveitis anterior

Der folgende Bericht beschreibt den direkten Nachweis von Ehrlichia canis mittels Real-Time-PCR in der Bindehaut einer 1-jährigen Malteserhündin. Kurz nach dem Import aus Brasilien wurde der Hund wegen Anorexie, Dehydration, Fieber und tastbaren Mandibularlymphknoten vorgestellt. Ein paar Tage später entwickelte der Hund bilateralen Blepharospasmus, Photophobie und Uveitis anterior. Die Diagnose «monozytären Ehrlichiose» wurde durch eine positive PCR und den Nachweis von IgM-und IgG-Antikörpern gestellt. Aufgrund der massiven Uveitis wurde ein Cytobrush aus der Konjunktiva entnommen und ebenfalls mittels Real-Time-PCR positiv auf Ehrlichia canis-DNA getestet. Nur eine Woche nach Therapiebeginn mit Doxycyclin, entzündungshemmenden Präparaten und Zykloplegika verschwanden alle klinischen Symptome. Eine Verlaufskontrolle nach Therapieende ergab eine vollständige Remission der klinischen und hämatologischen Parameter und ein negatives PCR-Ergebnis.

Schlüsselwörter: Ehrlichia canis, Cytobrush, Real-Time-PCR, Bindehaut, Uveitis

## Introduction

Canine monocytic ehrlichiosis (CME) is a common infectious disease in dogs caused by the gram-negative rickettsia *Ehrlichia canis* (Harrus et al., 1998; Dumler et al., 2001; Cohn, 2003; Skotarczak, 2003). The organism is transmitted primarily by the brown dog tick, *Rhipicephalus sanguineus,* and by the American dog tick, *Dermacentor variabilis* (Groves et al., 1975; Lewis et al., 1977). A wide variety of clinical signs, such as depression, lethargy, weight loss, anorexia, pyrexia, lymphadenomegaly, splenomegaly, bleeding tendencies and ocular signs can be diagnosed (Woody and Hoskins, 1991). Hematologic abnormalities include thrombocytopenia, mild anemia and mild leucopenia in the acute stage, and development of pancytopenia in the severe chronic stage (Woody and Hoskins, 1991; Bulla et al., 2004; Schaarschmidt and Müller, 2007). The main biochemical abnormalities reported

are hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia (Woody and Hoskins, 1991; Bullaet al., 2004; Schaarschmidt and Müller, 2007). Ocular manifestations are among the most common findings in CME (Panciera et al., 2001). Uveitis and meningitis occurred in dogs infected with *E. canis* but were not observed in dogs infected with other *Ehrlichia* species. The inflammatory infiltrate was predominantly lymphocytic, monocytic and plasmacytic. In the histopathologic study reported by Panciera et al. (2001), ocular inflammation was most common and most intense in the ciliary body, becoming less intense in the choroid, iris and retina, respectively. Doxycycline (5 mg/kg twice daily for 21 days) has become the standard drug for treating canine ehrlichiosis. This case report details the clinical and laboratory findings of ocular Ehrlichiosis in a dog, confirmed with PCR amplification and DNA sequencing to be *Ehrlichia canis*.

## Case history

### History and clinical findings

A one-year-old Maltese dog imported from Brazil was presented for the first time to the Small Animal Practice Letzi AG in Switzerland with a history of dehydration, fever and enlarged mandibular lymph nodes. At this time, no ocular clinical signs were present. The dog was treated with an intravenous lactated ringer´s infusion and a combination of NSDs (Metacam®, Boehringer Ingelheim GmbH, Basel, Switzerland) and amoxicillin/clavulanic acid (Clavubactin®, Graeub AG, Bern, Switzerland). A few days later, the dog was presented as an emergency with anorexia and bilateral blepharospasm and photophobia.

### Ophthalmic examination

Ophthalmic examination included slit-lamp biomicroscopy, tonometry and indirect ophthalmoscopy. The dog was severely blepharospastic and photophobic. Menace responses and dazzle reflex were normal. The conjunctiva was hyperemic and chemotic, the cornea was clear. Both pupils were miotic, a 3 + aqueous flare and 3 + cells were detected in the anterior chamber. The posterior segment of the eye could not be visualized in detail. Intraocular pressure in both eyes measured using a TonoPen (Reichert, Depew, NY, USA) was low (left eye: 6 mmHg; right eye: 8 mmHg).

### Laboratory diagnosis

Table 1 shows selected parameters of clinical chemistry (Konelab 30i, Thermo Fisher Corporation, Vantaa, Finland) and hematology (Sysmex XT-2000iV, Sysmex, Norderstedt, Germany). Pathologically high values were obtained for *alkaline phosphatase and ALT* concentrations. The blood count showed a marked anemia, leucopenia and thrombocytopenia, which were confirmed by manual counting. No blood parasites were found in May-Grünwald Giemsa-stained blood smears. DNA was extracted first from EDTA blood and then from a conjunctival sample taken with a cytobrush. These samples tested positive by real-time PCR for *Anaplasma phagocytophilum*, *A. platys*, *and Ehrlichia canis* (Schaarschmidt and Müller, 2007). To determine the definitive bacterial species, the PCR product was directly sequenced. Comparing the nucleotide sequence to GenBank, *Ehrlichia canis* was identified as the causative agent. For confirmative diagnosis, serum was examined by indirect immunofluorescence (IFA) as described (Schaarschmidt and Müller, 2007). An IgM titer of 1:640 and an IgG titer of 1:320 were determined. For differential diagnosis other laboratory tests (IFA for *Leishmania infantum* and PCR for *Babesia canis*) were carried out with negative results.

### Course of infection and therapy

The dog was treated locally with Pred Forte 1 %® (Allergan, 8853 Lachen, Switzerland) and atropine sulfate 1 %® (Ursapharm, 66129 Saarbrücken, Deutschland) eye drops 4 times daily, and once daily, respectively. Systemic doxycycline (5 mg/kg) was administered twice daily for 28 days.One day after initiation of treatment no fever

*Table 1*: Laboratory results before and after therapy. Pathological results are highlighted in bold.

| parameter (range) | Acute infection | After therapy |
|---|---|---|
| Clinical chemistry | | |
| albumin (25–37 g/l) | 26 | 23 |
| total protein (56–71 g/l) | 66 | 79 |
| alkaline phosphatase (< 146 U/l) | 598 | 132 |
| ALT (20–93 U/l) | 288 | 78 |
| Hematology | | |
| erythrocytes (6.0–9.0 x $10^{12}$/l) | 3.7 | 7.1 |
| hematocrit (38–55 %) | 29 | 44 |
| hemoglobin (9.3–12.1 g/dl) | 5.2 | 9.9 |
| leukocytes (6.0–12.0 x $10^{9}$/l) | 2.7 | 7.4 |
| thrombocytes (150–500 x $10^{9}$/l) | 39 | 236 |
| reticulocytes (5–10 o/oo) | 2.6 | 7.3 |
| Microbiology | | |
| morulae in the blood smear | negative | negative |
| *Ehrlichia spp.* DNA (blood) | positive | negative |
| *Ehrlichia spp.* DNA (eye) | positive | negative |
| *Babesia spp.* DNA (blood) | negative | negative |
| Anti-*Ehrlichia canis* IgM (≤ 1:20) | 1:640 | 1:80 |
| Anti-*Ehrlichia canis* IgG (≤ 1:40) | 1:320 | 1:2560 |
| Anti-*Leishmania infantum* IgG (≤ 1:40) | negative | negative |

was present. Blepharospasm and l signs of uveitis disappeared. At a recheck one week later the dog seemed systemically healthy and the ophthalmic examination revealed no signs of uveitis. The pupils were bilaterally wide and unresponsive to light due to the previous cycloplegic therapy. The intraocular pressure was 10 mm Hg for the left eye and 12 mmHg for the right eye, respectively. At this point, indirect ophthalmoscopy ruled out any posterior segment involvement. The cycloplegic therapy was discontinued and the topical prednisolone-acetate therapy reduced to 3 times daily for another week and then gradually decreased over the following three weeks. The systemic treatment with doxycycline was continued for 3 more weeks. A final recheck revealed a full remission of clinical, ophthalmological, and hematological parameters, and a negative PCR result in the blood sample and the conjunctival cytobrush probe (Tab. 1). Six month after initial presentation the dog was in good condition and the eyes remained inconspicuous.

## Discussion

Canine monocytic ehrlichiosis (CME) is considered an important infectious disease of dogs worldwide, particularly in tropical and subtropical areas (Cohn, 2003). The dog in this case report had a travel history to Brazil, an endemic region of the vector *Rhipicephalus sanguineus*. Due to the fact that *E. canis* is not indigenous to Switzerland (Pusterla et al., 1998). CME has long been known as a classic travel-associated infection. However, increasing tourism with dogs and imports of dogs from Mediterranean countries increasingly led to the introduction of infected ticks and autochthon infection in our latitude.
Ocular signs caused by CME have been reported to occur in different parts of the eye including the conjunctiva, cornea, uvea and posterior segment of the eye (Baneth et al., 2009). In a study from Barcelona 37 % out of 46 dogs tested positive for *E. canis*-specific antibodies had ocular signs and 11 (64,7 % of the ocular cases) had only ocular lesions without apparent systemic signs (Leiva et al., 2005). In another retrospective study (Komnenou et al., 2007), 30 out of 90 seropositive dogs present only ocular signs. Unilateral (24,5 %) and bilateral (75,5 %) uveitis were the most common ophthalmic diagnosis. Regarding the causes of uveitis, a study from North Carolina included 102 dogs presented with clinical uveitis. The most common infectious disease was *E. canis* (Massa et al., 2002). In all these studies the diagnosis «CME» was made by serologic detection of specific antibodies by immunofluorescence antibody testing (IFAT). For three reasons, it is still very difficult to make a definitive diagnosis of an infection with *Ehrlichia*. First, microscopic evaluation of a blood smear only rarely gives morphologic clues. Second, seroconversion can be much delayed or may fail to occur. Third, several infectious agents hardly show any cross-reactivity in IFAT. Yet serologic testing for *E. canis*-specific antibodies remains the most commonly used diagnostic method.
In contrast to the reports cited above, the presence of *E. canis* DNA in secretions from conjunctiva of naturally and experimentally infected dog has been reported in a recent study (Baneth et al., 2009). Direct detection of the infectious agent with a highly sensitive molecular genetic method described herein offers the advantage that an acute infection can be differentiated from a possible «serologic scar». As we also looked for and found *E.canis*-specific IgM antibodies strongly supporting the hypothesis of an acute infection the direct detection of the agent in the eye is a much more powerful diagnostic tool. Differential diagnosis with systemic disease and bilateral uveitis include canine leishmaniasis and babesiosis, although ocular manifestations have been described in cases of leishmaniasis with or without systemic signs (Pena et al., 2000). Similar blood results as anemia and thrombocytopenia could be present in canine babesiosis (Schaarschmidt et al., 2006). Both laboratory tests yielded negative results. In a recent publication (Naranjo et al., 2010) *Leishmania spp.* was detected on tissue sections routinely stained with hematoxylin/eosin and immunohistochemistry of ocular associated muscles. PCR detection of *Ehrlichia* DNA from EDTA blood is the laboratory method of choice for diagnosis of CME in the early or acute phase or in reactivated infections. Conjunctivitis is a common clinical sign in CME and the presence of *E. canis* DNA in conjunctival secretions is probably due to inflammatory cells such as macrophages harboring *E. canis* or neutrophils that have phagocytosed cell debris containing *E.canis* DNA. Non-invasive sampling of conjunctiva may serve as an additional diagnostic tool when invasive sampling such as spleen or bone narrow aspirates is not possible. Further studies of *E. canis*-infected dogs with uveitis are necessary to evaluate if this method could be a valuable and powerful new diagnostic method.

## References

*Baneth, G., Harrus, S., Ohnona, F. S., Schlesinger, Y.*: Longitudinal quantification of Ehrlichia canis in experimental infection with comparison to natural infection. Vet. Microbiol. 2009, 136: 321–325.

*Bulla, C., Kiomi Takahira, R., Pessoa Araujo, J., Jr., Aparecida-Trinca, L., Souza Lopes, R., Wiedmeyer, C. E.*: The relationship between the degree of thrombocytopenia and infection with Ehrlichia canis in an endemic area. Vet. Res. 2004, 35: 141–146.

*Cohn, L. A.*: Ehrlichiosis and related infections. Vet. Clin. North. Am. Small. Anim. Pract. 2003, 33: 863–884.

*Dumler, J. S., Barbet, A. F., Bekker, C. P., Dasch, G. A., Palmer, G. H., Ray, S. C., et al.*: Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma,

Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of Ehrlichia phagocytophila. Int. J. Syst. Evol. Microbiol. 2001, 51: 2145–2165.

*Ferreira Sde, A., Ituassu, L. T., de Melo, M. N., de Andrade, A. S.*: Evaluation of the conjunctival swab for canine visceral leishmaniasis diagnosis by PCR-hybridization in Minas Gerais State, Brazil. Vet. Parasitol. 2008, 152: 257–263.

*Groves, M. G., Dennis, G. L., Amyx, H. L., Huxsoll, D. L.*:Transmission of Ehrlichia canis to dogs by ticks (Rhipicephalus sanguineus). Am. J. Vet. Res. 1975, 36: 937–940.

*Harrus, S., Waner, T., Aizenberg, I., Foley, J. E., Poland, A. M., Bark, H.*: Amplification of ehrlichial DNA from dogs 34 months after infection with Ehrlichia canis. J. Clin. Microbiol. 1998, 36: 73–76.

*Komnenou, A. A., Mylonakis, M. E., Kouti, V., Tendoma, L., Leontides, L., Skountzou, E., et al.*: Ocular manifestations of natural canine monocytic ehrlichiosis (Ehrlichia canis): a retrospective study of 90 cases. Vet. Ophthalmol. 2007, 10: 137–142.

*Leiva, M., Naranjo, C., Pena, M. T.*:Ocular signs of canine monocytic ehrlichiosis: a retrospective study in dogs from Barcelona, Spain. Vet. Ophthalmol. 2005, 8: 387–393.

*Lewis, G. E., Jr., Ristic, M., Smith, R. D., Lincoln, T., Stephenson, E. H.*: The brown dog tick Rhipicephalus sanguineus and the dog as experimental hosts of Ehrlichia canis. Am. J. Vet. Res. 1977, 38: 1953–1955.

*Massa, K. L., Gilger, B. C., Miller, T. L., Davidson, M. G.*: Causes of uveitis in dogs: 102 cases (1989–2000). Vet. Ophthalmol. 2002, 5: 93–98.

*Naranjo, C., Fondevila, D., Leiva, M., Roura, X., Pena, T.*: Detection of Leishmania spp. and associated inflammation in ocular-associated smooth and striated muscles in dogs with patent leishmaniosis. Vet. Ophthalmol. 2010, 13: 139–143.

*Panciera, R. J., Ewing, S. A., Confer, A. W.*: Ocular histopathology of Ehrlichial infections in the dog. Vet. Pathol. 2001, 38: 43–46.

*Pena, M. T., Roura, X., Davidson, M. G.*: Ocular and periocular manifestations of leishmaniasis in dogs: 105 cases (1993–1998). Vet. Ophthalmol. 2000, 3: 35–41.

*Pusterla, N., Pusterla, J. B., Deplazes, P., Wolfensberger, C., Muller, W., Horauf, A., et al.*: Seroprevalence of Ehrlichia canis and of canine granulocytic Ehrlichia infection in dogs in Switzerland. J. Clin. Microbiol. 1998, 36: 3460–3462.

*Schaarschmidt, D., Müller, W.*: Diagnostic and Clinical Aspects of Canine Anaplasmosis and Ehrlichiosis. Tierärztl. Prax. 2007, 35 (K): 129–136.

*Schaarschmidt, D., Trachsel, M., Achermann, R., Hartelt, K., Oehme, R., Muller, W.*: [Importance of PCR for the diagnostics of canine babesiosis]. Schweiz. Arch. Tierheilk. 2006, 148: 633–640.

*Skotarczak, B.*: Canine ehrlichiosis. Ann. Agric. Environ. Med. 2003, 10: 137–141.

*Strauss-Ayali, D., Jaffe, C. L., Burshtain, O., Gonen, L., Baneth, G.*: Polymerase chain reaction using noninvasively obtained samples, for the detection of Leishmania infantum DNA in dogs. J. Infect. Dis. 2004, 189: 1729–1733.

*Woody, B. J., Hoskins, J. D.*: Ehrlichial diseases of dogs. Vet. Clin. North. Am. Small. Anim. Pract. 1991, 21: 75–98.

## Corresponding author

Daniel Schaarschmidt-Kiener
Labor am Zugersee
Tiermedizinische Analytik und Diagnostik
Rothusstrasse 2
CH-6331 Hünenberg/Zug
Switzerland
Tel.: +41 (0)41 790 04 40
Fax: +41 (0)41 790 04 42
schaarschmidt@laboramzugersee.ch

*Received: 27 June 2011*
*Accepted: 24 August 2011*