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Canine Ehrlichiosis – from Acute Infection to Chronic Disease

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Quick Digest

Wide Distribution

- Canine monocytic ehrlichiosis (CME) is caused by *Ehrlichia canis.*
- It has been reported in all continents from tropical and subtropical regions and is probably the most widely distributed CVBD (canine vector-borne disease).
- Distribution is driven by the global abundance of its main vector, the Brown Dog tick, *Rhipicephalus sanguineus.*

And … Increasing

- With global warming and expanding tick habitats the spread of disease to former non-endemic areas is of great concern.
- *Ehrlichia* vectors and infections should also be considered in non-endemic areas due to increasing international pet travel and dog importation.

Zoonotic Potential

- *E. chaffeensis* (monocytic ehrlichiosis) and *E. ewingii* (granulocytic ehrlichiosis) also cause canine ehrlichiosis and both can affect humans.
- To date, canine infections with *E. chaffeensis* and *E. ewingii* have only been diagnosed in the United States.

Diagnostic Challenge

- Multiple clinical and subclinical presentations make diagnosis challenging.
- Acute and chronic phases as well as co-infection with other tick-borne pathogens may further complicate therapy.

Silent Infections

- Often, the pathogen cannot be completely eliminated, despite antibiotic treatment and resolution of clinical signs.

Prevention

- A vaccine for ehrlichiosis is not currently available.
- Treatment with an ectoparasiticide product with repelling and killing activity against ticks presents the best option for prevention.

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Canine Ehrlichiosis – from Acute Infection to Chronic Disease

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Ehrlichiosis is a globally distributed canine vector-borne disease (CVBD) transmitted by ticks. Caused by the rickettsial bacteria *Ehrlichia* spp., ehrlichiosis affects dogs and humans as well as other domestic and wild animal species. With global warming, expanding tick habitats and increasing international travel the spread of disease to former non-endemic areas is of great concern.

Ehrlichiosis can have multiple clinical and subclinical presentations making diagnosis challenging. Acute and chronic phases as well as co-infection with other tick-borne pathogens may further complicate therapy. Often, the pathogen cannot be completely eliminated, despite antibiotic treatment and resolution of clinical signs. A vaccine for ehrlichiosis is not currently available, so treatment with an ectoparasiticide product with repelling and killing activity against ticks presents the best option for prevention.

Pathogen / Taxonomy

Ehrlichia spp. are gram-negative obligate intracellular bacteriae with tropism for hematopoietic cells.

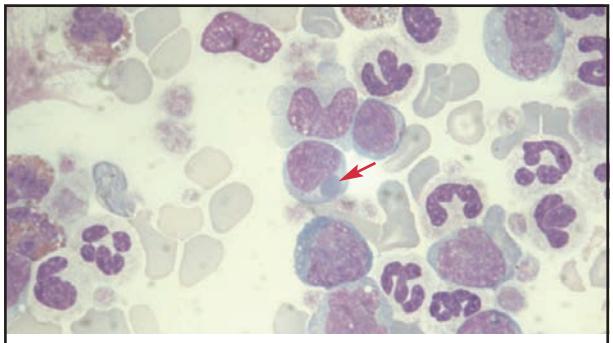

Fig. 1 Intracytoplasmic gram-negative *E. canis* in monocytes forming morulae.
(With kind permission of D. Otranto, Bari, Italy)

Three different *Ehrlichia* species can cause canine ehrlichiosis: *E. canis*, *E. chaffeensis* and *E. ewingii* (see Tab. 1). The term "ehrlichiosis" may still sometimes be used to describe infections by organisms belonging to the former Ehrlichiae tribe. However, with reclassification into the genera *Anaplasma*, *Ehrlichia* and *Neorickettsia* the term now refers specifically to infections by species within the newly reorganized genera (see Fig. 2 and Info Box 1).

E. canis causes canine monocytic ehrlichiosis (CME). This disease, also known as tropical canine pancytopenia, canine rickettsiosis or canine hemorrhagic fever, was first described in Algeria in 1935 by Donatien and Lestoquard.[1] CME has since been reported in many parts of the world, mainly in the tropical and subtropical regions. However, the geographical distribution of *E. canis* is expanding alongside that of its main tick vector, the Brown Dog tick, *Rhipicephalus sanguineus*.

E. canis form microcolonies within a membrane-lined intracellular vacuole (so-called morula), primarily in monocytes and macrophages of mammalian hosts. The pathogen replicates only in the cytoplasm of monocytic cells, and the formation of morulae is a defining characteristic that can be used for diagnosis (see Fig. 1).

Canine ehrlichiosis is also caused by the species *E. chaffeensis* (monocytic ehrlichiosis) and *E. ewingii* (granulocytic ehrlichiosis). Both species can also affect humans. Clinical signs of both related diseases in dogs are indistinguishable from those seen with CME. Discriminating the pathogens by serological testing may be difficult due to a substantial cross-reactivity, mainly between *E. canis* and *E. chaffeensis*, but also to a lesser degree to *E. ewingii*. To date, infections with *E. chaffeensis* and *E. ewingii* have only been diagnosed in dogs in the United States.

Species	Common name of disease(s)	Common natural host(s)	Cells most commonly infected	Primary vector(s)	Distribution
E. canis	Canine monocytic ehrlichiosis (CME)	Dogs and other members of the family Canidae, cats, humans	Primarily mononuclear cells (monocytes and lymphocytes)	*Rhipicephalus sanguineus*, *Dermacentor variabilis*	Worldwide, primarily tropical, subtropical, and temperate climates
E. chaffeensis	Human monocytic ehrlichiosis (HME)	Humans, deer, horses, rodents	Monocytes, macrophages	*Amblyomma americanum*, *Dermacentor variabilis*	USA, Europe, Africa, South and Central America, Korea
E. ewingii	Canine granulocytic ehrlichiosis (CGE), human granulocytic ehrlichiosis (HGE)	Dogs, humans	Primarily neutrophils and eosinophils	*Amblyomma americanum*, *Otobius megnini*	USA, Africa, Korea
E. muris	Not currently associated with disease	Rodents, humans	Mononuclear cells	*Haemaphysalis* spp.	Japan
E. ruminantium	Heartwater disease	Ruminants	Endothelial cells	*Amblyomma* spp.	Africa, Caribbean

Tab. 1 Summary of ehrlichial diseases and their related *Ehrlichia* pathogens.

Co-infections of *Ehrlichia* with *Anaplasma*, *Rickettsia*, *Babesia* or *Bartonella* spp. occur frequently as dogs are naturally exposed to multiple tick-borne pathogens. Little is known about the clinical outcome of concurrent infections with different pathogens. A recently reported study looked at dogs that were simultaneously and sequentially co-infected with *E. canis* and *A. platys*. Lower platelet counts and hematocrit were seen in co-infected animals, along with an enhanced humoral immune response to *A. platys* and a slower clearance of that pathogen.[2] The awareness of co-infections is important in clinical practice, as diagnosis may be complicated by the presence of multiple pathogens.

Transmission / Vector

Ehrlichiae have a complex life cycle involving a tick vector and a mammalian host. Typically, tick nymphs or larvae are infected with *E. canis* after feeding on a persistently infected dog. Transstadial transmission occurs to subsequent stages of the tick vector. A new host is infected via salivary gland secretions during blood feeding. Transmission of the disease has also been reported via blood transfusion.[7]

Note: The failure of canids to completely clear *E. canis* is one important mechanism of this ongoing persistence.

A natural reservoir of infection is maintained in both wild and domestic canids, including but not limited to, dogs, wolves, coyotes, and foxes. The failure of canids to completely clear *E. canis* is one important mechanism of this ongoing persistence and should be considered when selecting canine blood donors from endemic regions.

Zoonotic Potential

A few decades ago, ehrlichioses were considered to only have veterinary relevance. The first human infection with *E. chaffeensis* was diagnosed in 1986 raising the awareness of *Ehrlichia* spp. as zoonotic pathogens.[8]

Note: Nowadays *E. canis*, *E. chaffeensis*, and *E. ewingii* are all known to cause ehrlichiosis in humans.

INFO BOX 1

ANAPLASMA SPECIES

The genus *Ehrlichia* is closely related to that of *Anaplasma*, both of which reside intracellularly. Clinical manifestation of the two resulting diseases is similar; however, there are notable zoonotic and epidemiological differences between them.

The best-known *Anaplasma* species is *A. phagocytophilum*, formerly referred to as human granulocytic ehrlichiosis (HGE) factor, *E. phagocytophila* or *E. equi*. It causes granulocytic anaplasmosis in dogs and humans. Vectors of this pathogen are ticks of the species *Ixodes*. Their reservoir consists of small wild mammals, deer and possibly birds.

A second example is *Anaplasma platys* (formerly *Ehrlichia platys*), which infects platelets. The probable vector for *A. platys* is also the Brown Dog tick *R. sanguineus*, meaning the distribution is similar to that of *E. canis*, and co-infection with the two organisms has been reported.[3,4,5,6]

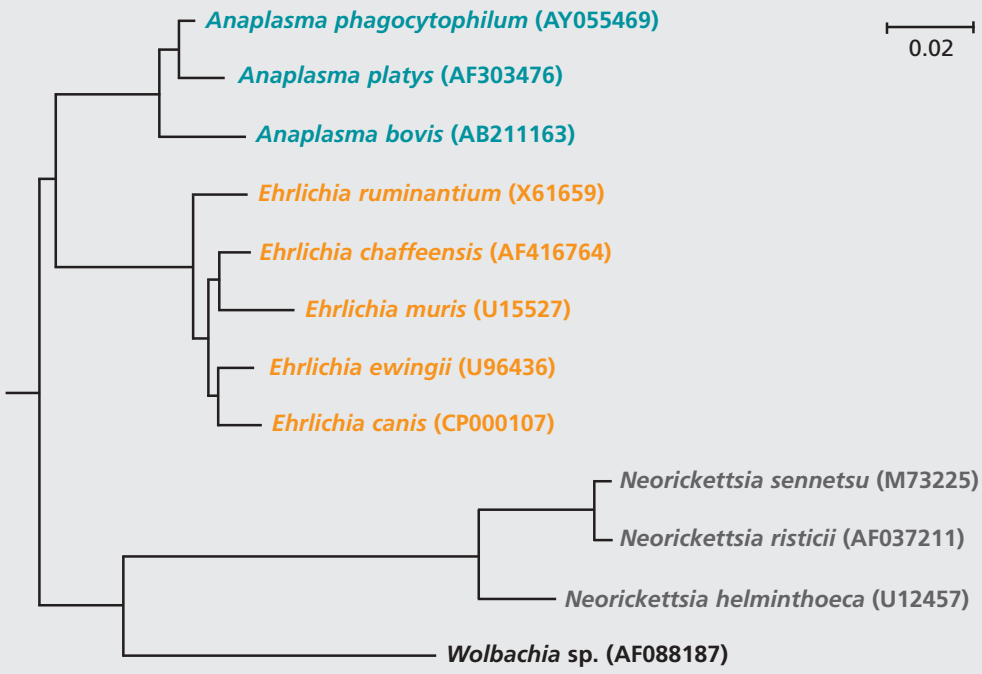

Fig. 2 A detailed molecular analysis of the 16S rRNA and other genes (e.g., gro ESL, encoding heat shock protein) has resulted in a systematic re-arrangement of the genus *Ehrlichia* and *Anaplasma*. *Ehrlichia risticii* has therefore been transferred to the genus *Neorickettsia*. The scale bar indicates the number of substitutions per site.

Today, *E. canis*, *E. chaffeensis*, and *E. ewingii* are all known to cause ehrlichiosis in humans. Most recently, *E. ewingii* – previously regarded as canine-specific – has been confirmed to cause human granulocytic ehrlichiosis (HGE).[9] *E. chaffeensis* targets monocytes, and the disease in people is therefore referred to as human monocytic ehrlichiosis (HME). *E. canis* has been isolated in culture and detected in several human patients with overt clinical signs in Venezuela,[10,11] however, its significance as a human pathogen is not clearly defined at this point.

Fig. 3 Geographical distribution of canine ehrlichiosis in different parts of the world. Countries where the endemic occurence has been reported are highlighted in red. The data were gathered by Bayer HealthCare Animal Health from recent scientific publications to provide a comprehensive picture of the endemic situation of several CVBDs including ehrlichiosis by *E. canis* in Asia-Pacific (Fig. 3a), Europe (Fig. 3b) and Latin America (Fig. 3c). More specific regional information can be obtained from www.cvbd.org.

To date, there is no evidence of direct transmission of *Ehrlichia* spp. from dogs to humans,[12,13] and dogs have not been established as a reservoir for human infection. Additionally, the Brown Dog tick would not appear to be the main vector or reservoir involved in zoonotic transmission because it rarely bites humans.[14]

Distribution

E. canis organisms are found on all continents throughout the world but are more prevalent in tropical and subtropical climates (see Fig. 3). Infections with *E. chaffeensis* and *E. ewingii* in dogs are probably restricted to the United States. With increasing global mobility of dogs, a diagnosis of *Ehrlichia* infection should not be ruled out in non-endemic areas particularly given the chronic stage of the disease.

Note: Due to international pet travel and import of dogs from endemic areas, *Ehrlichia* infections have to be considered also in non-endemic areas.

Clinical Presentation

Clinical signs and the severity of illness seen with ehrlichiosis depend on the species of *Ehrlichia* involved and the immune response of the dog. In general, all breeds of dogs are susceptible to *E. canis* infection, but German shepherds seem to develop severe forms of the disease more frequently than other breeds.[15]

CME is characterized by three stages, acute, subclinical and chronic. These can be difficult to definitively distinguish in practice.

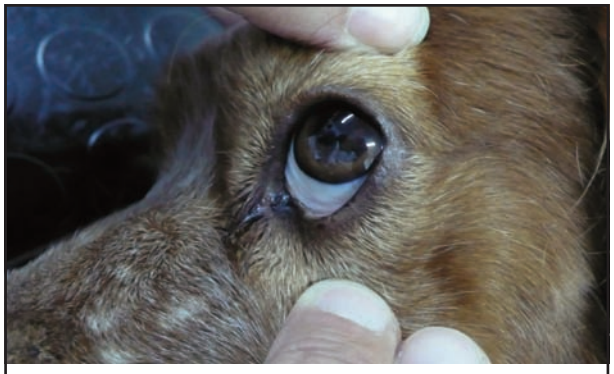

Fig. 4 Pale conjunctival mucosa due to anemia caused by *E. canis* infection. (With permission of D. Otranto, Bari, Italy)

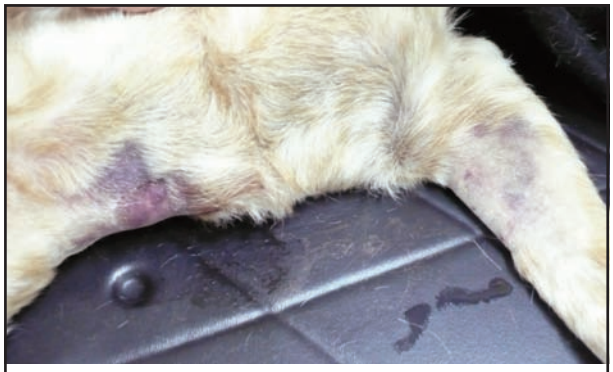

Fig. 5 Ecchymoses as clinical signs of canine *E. canis* infection. (With permission of D. Otranto, Bari, Italy)

INFO BOX 2

LABORATORY FINDINGS (HEMATOLOGY / BIOCHEMISTRY)

- A complete blood count is an important tool for the diagnosis of CME. Moderate to severe thrombocytopenia is a characteristic finding of acute ehrlichiosis.
- Thrombocytopenia appears around day 10 and peaks in the third week post-infection, with platelet counts ranging from 20,000 to 52,000/µl (normal range: 200–450,000/µl). There can also be mild anemia and leukopenia.
- In endemic regions, platelet counts on a blood smear are used as a screening test for CME.[20] True thrombocytopenia can also be distinguished from *in vitro* pseudo-thrombocytopenia by evaluation of platelet numbers on a blood smear.[19] Granular lymphocytosis can occur occasionally during the acute phase and lead to a misdiagnosis of lymphocytic leukemia.
- Hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia (mostly polyclonal, rarely monoclonal) are common in CME. Also moderate increases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) can occur due to hepatocyte damage during the acute phase.
- Dogs in the subclinical phase are clinically healthy, but variable degrees of thrombocytopenia and leukopenia may be present. Thrombocytopenia usually becomes severe in the chronic phase accompanied by marked anemia and leukopenia. Pancytopenia due to bone marrow hypoplasia is characteristic of the chronic severe form.[21]
- A hypocellular bone marrow with varying supression of the erythroid, myeloid, and megakaryocytic cells is seen on aspiration.
- *E. canis* can occasionally induce a protein-losing nephropathy as a result of immune-complex glomerulonephritis with consequent proteinuria and azotemia.

Acute Phase

Acute disease lasts between 3 to 5 weeks with clinical findings of fever, anorexia, depression, lymphadenopathy, and splenomegaly. More variably, ocular discharge, pale mucous membranes, hemorrhagic tendencies (dermal petechiae, ecchymoses, or epistaxis), or neurological signs are seen (see Figs. 4 and 5). The most commonly observed hematological abnormalities are thrombocytopenia and anemia.[16]

Note: The most commonly observed hematological abnormalities are thrombocytopenia and anemia.[16]

Subclinical Phase

A long-term subclinical phase usually follows the subsidence of clinical signs and can last for several years.[17] Dogs that are unable to eliminate the infectious agent develop subclinical persistent infections and become asymptomatic carriers.

Note: Dogs unable to eliminate the infectious agent develop subclinical persistent infections and become asymptomatic carriers.

Chronic Phase

Some infected dogs progress to a chronic phase, which can be mild or severe. This is characterized by recurrent clinical and hematological signs including thrombocytopenia, anemia, and pancytopenia.

Dogs may have weight loss, depression, petechiae, pale mucous membranes, edema, and lymphadenopathy among other signs. In severe cases, the response to antibiotic therapy is poor and dogs often die from massive hemorrhage, severe debili-

tation, or secondary infections. It is very likely that *E. canis* causes immunosuppression but currently little is known about the immunobiology of this infection. A recent study in dogs was unable to demonstrate a marked immunosuppression.[18]

Diagnosis

Light microscopy and blood culture tend to be less sensitive than serology and PCR. Co-infections with other tick-borne pathogens may complicate diagnosis (see Info Box 2).

Note: PCR techniques are suggested to be the most reliable method to diagnose ehrlichial infection.

Blood Smear Microscopy

Detection of typical intracellular *E. canis*-morulae on blood smear examination is highly specific for ehrlichiosis. However, this method is time-consuming and not very reliable because morulae are only found in low numbers in blood smears during the acute phase of infection. Microscopy has an estimated sensitivity of 4 %.[22] Detection of morulae can be improved by evaluation of numerous buffy coat smears.[23]

Cell Culture

It is possible to culture *Ehrlichia* species in specific macrophage cell lines (canine macrophage cell line [DH82] or mouse macrophage cell line [J774.A1]). However, this technique is used more in research laboratories than for diagnosis in practice.

Serology

The indirect fluorescent antibody test (IFAT) is recommended to confirm a diagnosis of ehrlichiosis.[24] Detection of specific IgG antibodies indicates previous exposure to the ehrlichial pathogen, and during the acute disease two tests one to two weeks apart will show rising antibody titers. However, there is extensive serologic cross-reactivity between *E. canis* and *E. chaffeensis* and *E. ewingii*.[25] Thus, results obtained by IFAT need to be interpreted carefully. Low IFAT titers are of low specifity.

Enzyme-linked immunosorbent assays (ELISA) can also be used to confirm a diagnosis of ehrlichiosis and different Dot-ELISA kits for the detection of *E. canis*-IgG antibodies are commercially available. Western immunoblot is a more specific test, which can distinguish between infections with the different organisms causing ehrlichiosis, anaplasmosis, or neorickettsiosis as well as between *Ehrlichia* spp., for example *E. canis* and *E. ewingii*. Dogs will generally become seronegative following antibiotic treatment, but some dogs will show stable antibody titers for years.[26]

Molecular Detection by PCR

PCR techniques are now considered to be the most reliable method to diagnose ehrlichial infection.[19] PCR methods are highly sensitive and enable the detection of *Ehrlichia* DNA as early as 4–10 days post-infection prior to sero-conversion.[27] Numerous conventional and real-time PCRs are available based on different gene sequences.

PCR can be performed on whole blood, serum, splenic aspirates, lymph nodes, or bone marrow. The spleen is the organ most likely to harbor *E. canis* parasites during the subclinical phase[21] and is considered to reveal higher sensitivity than testing of bone marrow or blood samples.[28,29] To evaluate elimination of *Ehrlichia* bacteria following treatment, testing of spleen samples is recommended.

Differential Diagnosis

In general, ehrlichiosis should be suspected in dogs with pancytopenia, thrombocytopenia, and aplastic anemia in areas endemic for the tick vector, *R. sanguineus*. But depending on the geographic region, similar clinical signs can occur with other relevant CVBD pathogens. Anaplasmosis, canine Rocky Mountain spotted fever (another rickettsiosis), babesiosis, bartonellosis, hepatozoonosis, and canine distemper should all be considered as possible differential diagnoses for ehrlichiosis. Molecular characterization by PCR and sequencing may be required to finally determine the specific pathogen involved.

Note: Clinical findings with ehrlichiosis can be similar to other CVBD.

Autoimmune-mediated thromboytopenia, systemic lupus erythematosus or neoplasia (lymphoma or multiple myeloma) should also be considered.

Treatment

Tetracyclines are the treatment of choice for rickettsial diseases. For canine ehrlichiosis, tetracycline (22 mg/kg given every eight hours) or doxycycline (5 mg/kg every twelve hours) administered for four weeks is the recognized treatment. Most dogs recover from the acute and subclinical phases when treated with doxycycline or other tetracyclines at appropriate dosages for an adequate period of time.[28,29]

Note: Due to the fact that no long-lasting protective immunity is developed, dogs can be reinfected with ehrlichiosis.

After initiation of treatment, a rapid improvement in clinical signs is usually seen, but several weeks of therapy are usually required to ensure a full recovery. Persistent infections with *E. canis* often remain as complete bacterial clearance is not guaranteed but has been reported in some cases following antibiotic therapy.[29–33] It has been suggested that the phase of CME could affect the efficacy of doxycycline treatment in clearing *E. canis* infections.[33] The extent to which antibiotic treatment can prevent transmission of the pathogen from an infected dog to feeding ticks remains unclear. Experimentally infected dogs treated with doxycycline for 14 days were still infectious to ticks and thus reservoirs of *E. canis* infection.[33]

Supportive therapy such as blood or fluid transfusions and anabolic steroids may be required in severe cases. The prognosis becomes poor once dogs enter the chronic phase of disease.[34] Co-infections with other pathogens like *Babesia* or *Bartonella* may contribute to the fatal outcome of chronic infections.

As long-term protective immunity does not develop to ehrlichiosis, dogs can be reinfected. Also recrudescence can occur months to years after primary infection.

INFO BOX 3
CANINE EHRLICHIOSIS IN THE WEB

- Background information: www.cvbd.org/4001.0.html
- Menn B *et al.* Parasites & Vectors 2010, 3:34 www.parasitesandvectors.com/content/3/1/34
- Gaunt SD *et al.* Parasites & Vectors 2010, 3:33 www.parasitesandvectors.com/content/3/1/33
- U.S. Centers for Disease Control and Prevention: www.cdc.gov/ticks/diseases/ehrlichiosis/
- ACVIM Consensus Statement: 3 www3.interscience.wiley.com/cgi-bin/fulltext/119824370/PDFSTART

Prevention

There are no vaccines currently available to protect dogs from *Ehrlichia* spp. infections, and further research is needed to define the virulence factors and immunoprotective antigens required to develop one.

The best means of preventing canine ehrlichiosis is by avoiding exposure to the tick vector. Treatments with ectoparasiticides that repel and kill ticks reduce the risk of disease transmission. Spot-on products are applied topically to the dog's skin. Recent studies have evaluated the efficacy of a spot-on formulation containing imidacloprid 10 % and permethrin 50 % (Advantix®) to prevent tick exposure and thus *E. canis* infection in dogs. Preventive efficacies of 95–100 % were demonstrated in treated dogs living under natural conditions in endemic areas.[35,36]

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