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Haemotropic mycoplasmas of cats and dogs: transmission, diagnosis, prevalence and importance in Europe

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Summary

Haemotropic mycoplasmas (or haemoplasmas) are the causative agents of infectious anaemia in many mammalian species. They were previously known as *Haemobartonella* and *Eperythrozoon* species. The development of sensitive, specific PCR assays has expanded our knowledge of these agents and PCR is the method of choice to diagnose and differentiate haemoplasma infections. In felids, *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' have been described. They vary strongly in their pathogenic potential and co-factors may influence the disease severity. In dogs, *Mycoplasma haemocanis* and '*Candidatus* Mycoplasma haematoparvum' are known; clinical signs are mainly found in immunocompromised dogs. Transmission of haemoplasmas may occur via infected blood (aggressive interaction, transfusion) or blood-sucking arthropods. Infections can be treated with Doxycycline, although it is disputable whether the infection is completely eliminated. Feline haemoplasmas must be expected in cats all over Europe, while canine haemoplasmas are mainly encountered in dogs in Mediterranean countries but should also be considered in Swiss dogs with a travel history.

Keywords: haemotropic mycoplasma, transmission, prevalence, diagnostics, companion animals

Haemotrope Mykoplasmen bei Hund und Katze: Übertragung, Diagnose, Prävalenz und Bedeutung in Europa

Haemotrope Mykoplasmen (Haemoplasmen, früher *Haemobartonella* und *Eperythrozoon*) sind die Erreger der infektiösen Anämie bei verschiedenen Säugetierarten. Die Einführung sensitiver, spezifischer PCR Methoden führte jüngst zur Erweiterung des Wissen über diese Erreger und sie stellen den Goldstandard zum Nachweis und zur Differenzierung von Haemoplasmen dar. Bei Katzen sind *Mycoplasma haemofelis*, '*Candidatus* M. haemominutum' und 'Candidatus M. turicensis' beschrieben. Die Erreger haben unterschiedliche Pathogenität und Co-Faktoren können die Erkrankung beeinflussen. Beim Hund wurden *Mycoplasma haemocanis* und 'Candidatus M. haematoparvum' beschrieben; klinische Erkrankungen werden vor allem bei immunsupprimierten Hunden gesehen. Als Übertragungswege werden infektiöses Blut (aggressive Interaktionen, Transfusionen) und blutsaugende Arthropoden diskutiert. Infektionen können mit Doxyzyklin behandelt werden; eine komplette Elimination der Erreger wird jedoch kaum erreicht. Feline Haemoplasmen kommen in ganz Europa, canine Haemoplasmen vor allem im Mittelmeerraum vor. Letztere sollten jedoch auch in der Schweiz bei Hunden mit einer Reiseanamnese in Betracht gezogen werden.

Schlüsselwörter: Haemotrope Mykoplasmen, Übertragung, Prävalenz, Diagnose, Kleintiere

Introduction

In recent years, there has been a growing interest in haemotropic mycoplasmas, also known as haemoplasmas. Haemotropic mycoplasmas are small (< 1 µm), cell wall-less, discoid-shaped bacteria, closely attached to red blood cells (RBC) of infected animals (Fig. 1). They were formerly known as *Haemobartonella* and *Eperythrozoon* species and are the causative agents of infectious anaemia in a variety of mammalian species. Increasing knowledge on the bacterial genomic sequences and phylogenetic relationship led to the reclassification of these organisms within the genus *Mycoplasma* as haemotropic mycoplasmas (Neimark et al., 2001). Infections with haemoplasmas can induce acute haemolysis, associated with anorexia, lethargy, dehydration, weight loss and sudden death of infected animals. The inability to culture these agents *in vitro* has limited the possibilities to investigate

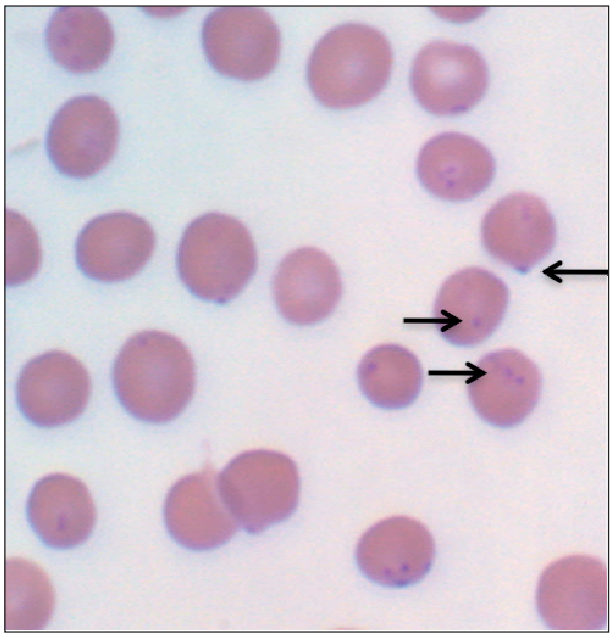

Figure 1: Giemsa-stained blood smear from a cat infected with Mhf. Multiple basophilic coccoid structures (black arrows), around 0.5 µm in diameter, some of them arranged in pairs or chains, are seen attached to RBC.

haemotropic mycoplasmas. However, in recent years species-specific conventional and real-time TaqMan PCR assays have been developed for sensitive detection of feline and canine haemoplasmas. The application of these assays has been allowing investigating the pathogenesis and epidemiology of haemoplasma infections in cats and dogs in more detail.

Haemotropic mycoplasmas in cats

In cats, early studies described two distinct haemoplasma species: the Ohio isolate (large form) and the California isolate (small form) of *Haemobartonella felis* (Berent et al., 1998; Foley et al., 1998; Messick et al., 1998). Along with the suggested reclassification within the genus *Mycoplasma*, these isolates were renamed *Mycoplasma haemofelis* (Mhf) (Neimark et al., 2001) and '*Candidatus* Mycoplasma haemominutum' (CMhm) (Foley und Pedersen, 2001; Fig. 2). In 2002, a third haemotropic *Mycoplasma* species was identified in a privately owned Swiss cat that presented with haemolytic anaemia; this third species was designated '*Candidatus* Mycoplasma turicensis' (CMt) (Willi et al., 2005; Willi et al., 2006a;

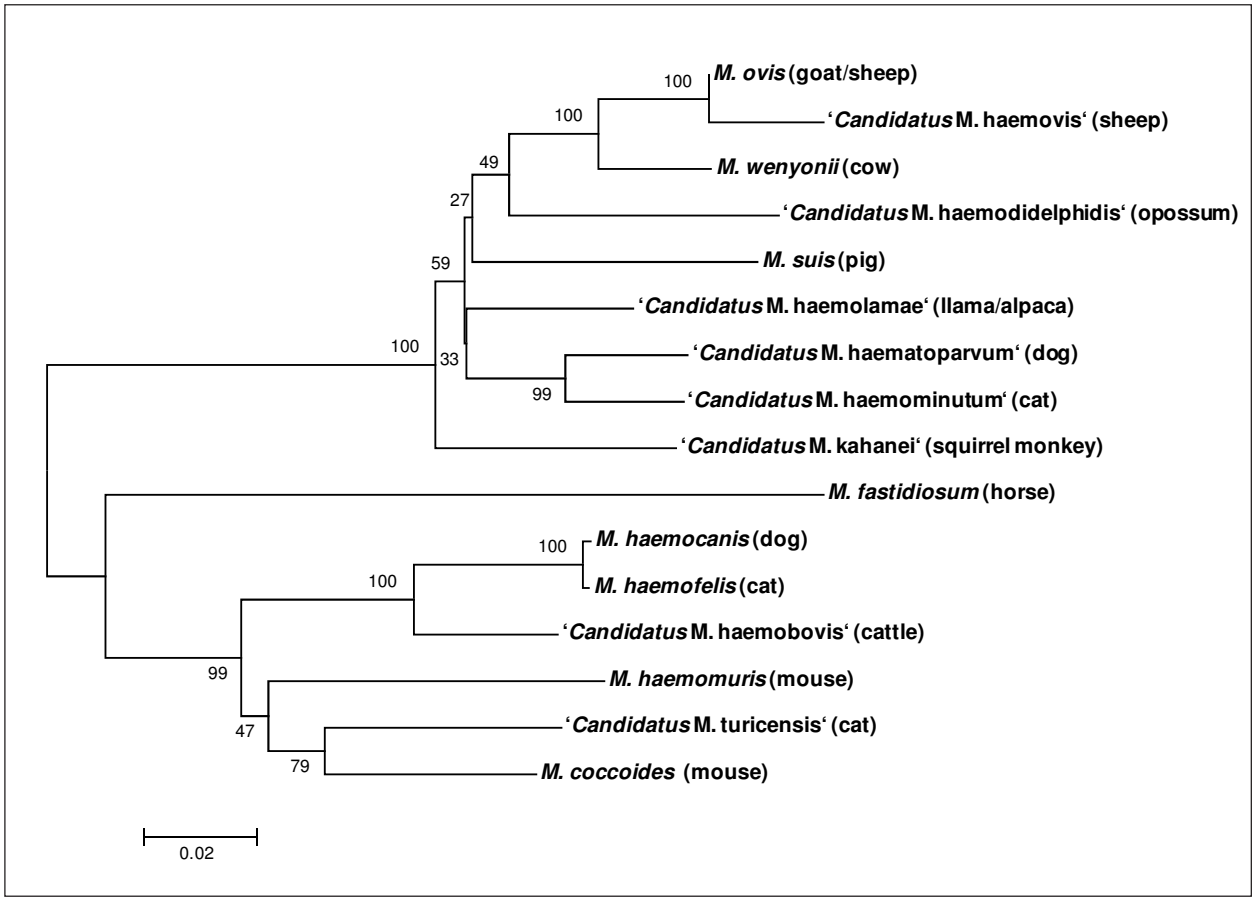

Figure 2: Phylogenetic tree of common haemoplasma species. The main host species are indicated in parenthesis. Bootstrap values are given at the nodes of the tree. Evolutionary distances are shown to the scale. GenBank accession numbers are available upon request from the authors.

Fig. 2). Recently, a fourth species similar to the canine haemoplasma '*Candidatus* M. haematoparvum' (CMhp) has been detected in cats in California (Sykes et al., 2007). By applying PCR-based methods, haemoplasma infections have been diagnosed in domestic cats and wild felids worldwide (Willi et al., 2007b). Co-infections with several feline haemoplasmas were reported. In the West and South of Switzerland, feline haemoplasma infections were more prevalent than in the remainder of the country (Willi et al., 2005).

The pathogenic potential of the different feline haemoplasma species varies and co-factors, such as immunosuppression or pre-existing retroviral infections, may increase the disease severity. In general, Mhf was found more pathogenic than CMhm (Foley et al., 1998; Westfall et al., 2001). CMt can induce mild to moderate anaemia in experimentally infected domestic cats, but anaemia does not always result (Willi et al., 2005; Museux et al., 2009; Tasker et al., 2009). In naturally infected cats, risk factors for feline haemoplasma infections, such as male gender, old age, cat bite abscesses, retroviral infection, non-pedigree lineage and outdoor access have been identified. The clinical presentation may depend on the stage of infection: while acute Mhf infection was found to induce severe anaemia, chronic carrier state may present asymptomatic even with high bacterial loads (Willi et al., 2006b). Overall it is not well understood why some cats show life-threatening anaemia, while others stay asymptomatic, and individual susceptibilities – or possibly the blood type of the infected cat (Museux et al., 2009) – may play a role in the severity of the disease development. Doxycycline, enrofloxacin and marbofloxacin treatment have been shown to reduce feline haemoplasma blood loads and clinical signs in infected cats (Dowers et al., 2002; Tasker et al., 2004; Tasker et al., 2006a, 2006b). Since some cats treated with high or intravenous doses of enrofloxacin may develop acute blindness from retinotoxicity, doxycycline (10 mg/kg/day po) represents the antibiotic of choice to treat feline haemoplasma infection. Esophagitis and esophageal strictures may occur subsequent to oral doxycyline treatment; thus, it is recommended to provide water or food following the tablet administration to encourage complete swallowing. To date, it is assumed unlikely that antibiotic treatments completely eliminate haemoplasma infections and infected cats may become asymptomatic carriers for months or years. However, reactivation of clinical disease seems rare (Foley et al., 1998).

Haemotropic mycoplasmas in dogs

Haemobartonella canis infections associated with anaemia have been sporadically reported in dogs. Subsequently, *H. canis* was reclassified as *Mycoplasma haemocanis* (Mhc), and a second canine haemoplasma, CMhp (Fig. 2) has been described in an anaemic splenectomised dog undergoing chemotherapy (Messick et al., 2002; Sykes et al., 2004). Both agents seem to exhibit worldwide distribution, but only limited prevalence data based on molecular detection methods are yet available. Using specific PCR assays to investigate the prevalence and clinical importance of canine haemoplasma infections in Europe, a higher prevalence was recently reported in countries with Mediterranean and sub-Mediterranean climate when compared to Switzerland (Kenny et al., 2004; Wengi et al., 2008; Novacco et al., 2009). Additionally, in some populations young animals and male dogs seemed more susceptible to canine haemoplasma infections than adult and female dogs, respectively (Novacco et al., 2009; Barker et al., 2010). Severe haemolytic anaemia has only occasionally been described in haemoplasma-infected dogs, mainly in immune-compromised or splenectomized animals. PCR-based investigations of canine haemoplasmosis in Europe also support the low pathogenic potential of these agents (Wengi et al., 2008; Novacco et al., 2009). Early studies suggest that co-infections with parvovirus, *Ehrlichia* and *Babesia* species, or concurrent neoplasia may play an aggravating role for canine haemoplasma infections. More recently, mange infection was associated with canine haemoplasma infections (Novacco et al., 2009). The mites may play a role in the mechanical transmission of haemoplasmas, or mange infection may signal a compromised immune system in affected animals. Most haemoplasma-infected dogs present with chronic, asymptomatic infections. These animals seem unable to clear the infection. In a recent study using quantitative real-time PCR, all samples collected from three infected dogs throughout a follow-up period of up to 13 months tested positive (Wengi et al., 2008). As described for other haemoplasma infections, antibiotic treatment may be unable to eliminate canine haemoplasma infections completely, but was found to reduce clinical signs of infection.

Transmission

The natural mode of transmission of feline and canine haemoplasmas has not been definitely elucidated. Blood transfusions have been reported as a source of Mhf and CMhm infections (Gary et al., 2006; Willi et al., 2006b). Furthermore, blood-sucking arthropods may be involved in the transmission of feline and canine haemoplasmas. Mhf and CMhm DNA was detected in the cat flea, *Ctenocephalides felis*, and in flea faeces, but a recent experimental transmission study for Mhf and CMhm via *C. felis* was not conclusive (Shaw et al., 2004; Woods et al., 2005; Lappin et al., 2006; Woods et al., 2006; Willi et al., 2007a; Kamrani et al., 2008). In Switzerland, positive results for CMhm and CMt were obtained from some *Ixodes* and *Rhipicephalus* spp. ticks collected directly from animals but no haemoplasma DNA was detected in *Ixodes* ticks collected from the vegetation, which indicates that *Ixodes* ticks are not a major reservoir for haemotropic mycoplasmas

in Switzerland (Willi et al., 2007a; Willi et al., 2009). In contrast, the brown dog tick, *Rhipicephalus sanguineus*, is likely to play a role as a vector and reservoir for canine haemoplasmas (Seneviratna et al., 1973). In Europe, the brown dog tick is commonly encountered in areas with Mediterranean and sub-Mediterranean climate and the high prevalence of canine haemoplasma infections found in these countries supports the hypothesis of it being a possible tick vector for the transmission of the infection (Kenny et al., 2004; Novacco et al., 2009; Barker et al., 2010). CMt was detected by PCR in saliva and faeces of infected cats during early infection and CMhm was detected in the saliva and salivary glands of experimentally infected cats (Willi et al., 2007a; Dean et al., 2008; Museux et al., 2009) indicating that direct transmission of haemoplasmas via saliva may be important. However, a recent *in vivo* study that modelled CMt transmission via social contact among cats was unable to infect cats by subcutaneous or oral inoculation of CMt PCR-positive saliva (Museux et al., 2009). In contrast, transmission by subcutaneous inoculation of as little as 10 µl of PCR-positive blood was successful, which may indicate that aggressive interaction is necessary to transmit CMt between cats (Museux et al., 2009). This would also be in agreement with observations for Mhc, for which the prevalence was higher in Japanese fighting dogs compared to other breeds (Sasaki et al., 2008). The latter was attributed to direct transmission of Mhc via infected blood during aggressive contact (dogfights).

Diagnosis

Specific conventional and quantitative real-time TaqMan PCR systems have been introduced and are now considered the gold standard for the detection and differentiation of feline and canine haemoplasma species (Tasker et al., 2003b; Willi et al., 2007b; Peters et al., 2008; Wengi et al., 2008; Willi et al., 2009; Barker et al., 2010). They were also applied to investigate the pathogenesis and haemoplasma tissue loads in experimental studies (Tasker et al., 2009). No *in vitro* culture system has been established to date to propagate feline and canine haemoplasmas outside their hosts. The light microscopic investigation of Giemsa-stained blood smears from infected animals was shown unreliable to diagnose haemoplasma infections. A diagnostic sensitivity of less than 20 % has been reported for this method, and the diagnostic specificity is often hampered by confusing organisms with stain precipitates or Howell-Jolly bodies. In particular, light microscopy is unfit to diagnose CMt infection because of the usually low CMt blood loads. Even at peak CMt bacteriemia

Table 1: Percentage of feline and canine haemoplasma PCR-positive animals in the sampled populations in Europe.

	Switzerland	Germany	UK	Spain	Italy	Portugal	France
Feline haemoplasmas							
	n = 615	n = 397a	n = 2011	n = 30[b]	n = 307		
- Mhf[c]	0.5 %	5.3–7.4 %	1.6–2.8 %	20 %[b]	5.9 %		
- CMhm[c]	8.5 %	8.9–23.3 %	11.2–17.1 %	10 %[b]	17.3 %		
- CMt[c]	1.0 %	2.2 %	1.7–2.3 %	nt	1.3 %		
- Co-infected	1.0 %	0.8–3.0 %	1.6 – 1.9 %	nt	5.5 %		
References	(Willi et al., 2006b)	(Just und Pfister, 2007; Bauer et al., 2008)	(Tasker et al., 2003a; Willi et al., 2006c; Peters et al., 2008)	(Criado-Fornelio et al., 2003)	(Gentilini et al., 2009)		
Canine haemoplasmas							
	n = 889			n = 200	n = 600	n = 50	n = 460
- Mhc[c]	0.9 %			0.5 %	4.5 %	40 %	5.8 %
- CMhp[c]	0.3 %			2.0 %	5.8 %	0 %	12.2 %
- Co-infected	0 %			0 %		0 %	2.6 %
References	(Wengi et al., 2008)			(Novacco et al., 2009)	(Novacco et al., 2009)	(Novacco et al., 2009)	(Kenny et al., 2004)

[a] including some pre-selected samples (anaemic cats).
[b] all samples pre-selected (cats with suspected haemoplasma infection).
[c] including co-infected animals. nt = not tested

only one CMt copy per 10^3 to 10^4 RBC may be expected, a number that is undetectable by routine light microscopy (Museux et al., 2009). Most recently, we were able for the first time to demonstrate CMt using electron microscopy. The discoid-shaped CMt organisms measure about 0.3 µm in diameter and are closely attached to RBC of infected cats. Serological assays are not yet routinely available but are expected to be established in the near future.

Prevalence and importance in Europe

The sample prevalence of haemoplasma infections in dogs and cats in Europe based on real-time PCR investigations are listed in Table 1. Feline haemoplasma infections were found in all investigated populations. In Switzerland, canine haemoplasma infections are less prevalent than in most of the other examined countries, particularly those with a Mediterranean climate (Wengi et al., 2008; Novacco et al., 2009). Moreover, the infected dogs in Switzerland had either been imported from or visited regions where *R. sanguineus* is indigenous (Wengi et al., 2008). This observation supports the hypothesis that canine haemoplasmas may be indirectly transmitted by blood-sucking arthropods, in particular those that rely on a warm climate for their survival. In a recent study on the situation in Europe, we identified several risk factors for canine haemoplasma infections, e.g. living in kennels, young age, and non-pedigree lineage (Novacco et al., 2009). A higher prevalence of canine haemoplasma infections in kennel-kept dogs may be due to the fact that dogs in kennels are group housed, which could increase the risk of direct haemoplasma transmission among dogs and their risk of exposure to fleas and ticks.

The close relationship between CMt and rodent haemoplasmas as well as among certain feline and canine haemoplasmas suggests a potential interspecies transmission of these agents (Sykes et al., 2004; Willi et al., 2005). While CMt has not yet been detected in rodents (Willi et al., 2007a), CMhm infection has been reported in a dog in China (Zhuang et al., 2009). Moreover, Mhf has recently been detected by PCR in the blood of a human AIDS patient (dos Santos et al., 2008), and PCR-positive results for porcine haemoplasma-like organisms have been obtained from blood of Chinese farm workers and swine veterinarians (Yuan et al., 2009). This substantiates the risk of an interspecies transmission of haemoplasmas. Nevertheless, so far the zoonotic potential of haemoplasma infections has been largely neglected.

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Mycoplasma hémotropique chez le chat et le chien: mode de transmission, diagnostic, prevalence et incidence en Europe

Le mycoplasme hémotropique (anc. Hémoplasmose) est l'agent responsable de l'anémie infectieuse chez de nombreux mammifères. Il était préalablement décrit en tant qu'Hémobartonelle et Eperythrozoon. Le développement d'un test PCR sensitif et spécifique a nettement amélioré nos connaissances de cet agent, et la PCR est maintenant la méthode de choix pour le diagnostic et le différentiel des infections à hémoplasmes. Chez le chat, les mycoplasme haemofélis, candidatus mycoplasme haemominutum et candidatus mycoplasme turicencis ont été décrits. Leur potentiel pathogénique est très variable, ainsi que leur importance en tant que co-facteurs pathogènes.

Chez le chien, les mycoplasme haemocanis et candidatus mycoplasme haematoparvum sont connus, les symptômes cliniques étant plus fréquents chez les animaux immunodéprimés. La transmission des hémoplasmes par voie hématogène est possible (transfusion, agression ou par le biais des arthropodes hématopha-

Micoplasmi emotropi nel cane e nel gatto: trasmissione, diagnosi, prevalenza e importanza a livello Europeo

I micoplasmi emotropi (o emoplasmi) rappresentano l'agente patogeno dell'anemia infettiva in molte specie di mammiferi. In passato erano conosciuti come Haemobartonella ed Eperythrozoon. Il recente sviluppo di specifiche e sensibili metodiche basate sulla PCR ha permesso di approfondire le conoscenze riguardanti questi agenti infettivi. Tali metodiche sono attualmente considerate la prima scelta per la diagnosi e la differenziazione delle infezioni da emoplasmi. Nei felini sono stati descritti i seguenti emoplasmi: *Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum' e '*Candidatus* Mycoplasma turicensis'. La loro potenziale patogenicità è molto differente e possibili fattori concomitanti possono influenzare la gravità della patologia. Nel cane sono noti *Mycoplasma haemocanis* e '*Candidatus* Mycoplasma haemominutum'. La manifestazione clinica della patologia è prevalente nei soggetti immunodepressi. La trasmissione degli emoplasmi avviene attraverso sangue infetto (interazioni

ges). L'infection peut être traitée par la doxycycline, mais, il n'est pas certain que l'infection soit totalement éliminée. Les hémoplasmoses félines sont courantes dans toute l'Europe, alors que les hémoplasmoses canines sont rencontrées le plus souvent dans les pays du pourtour méditerranéen. Cette pathologie devrait êtres considérés comme possible en Suisse chez des chiens ayant voyagés dans le sud.

aggressive fra soggetti, trasfusioni) o attraverso artropodi ematofagi. Le infezioni possono essere trattate con doxiciclina, tuttavia, non è ancora stato chiarito se l'infezione venga eliminata completamente. L'emoplasmosi felina deve essere presa in considerazione nei gatti di tutta Europa, mentre nel cane viene osservata prevalentemente nei paesi meditteranei. In Svizzera tale patologia deve essere tenuta in considerazione anche nel cane, specialmente nei soggetti che riportano in anamnesi viaggi o spostamenti in zone a rischio.

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