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Reviewer paper

Lyme disease in Bernese Mountain Dogs. Is it a real problem?

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Abstract

Borreliosis is the most frequently diagnosed tick-borne disease caused by spirochete bacteria belonging to the genus *Borreliae* - *Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia afzelii* and *Borrelia garinii*. Clinical manifestations in dogs include fever, lameness, polyarthritis and glomerulonephritis. Diagnosis is mainly serological and is based on an immunoenzymatic test followed by a Western blot confirmatory test. Early treatment with antibiotics such as doxycycline or amoxicillin, for four weeks, usually reduces the risk of chronic disease. Tick control, including tick repellents, is highly reliable in preventing transmission. Vaccines are available to reduce transmission and the clinical manifestations of infection in dogs. Bernese Mountain Dogs are a breed that often test positive for antibodies against *B. burgdorferi* without showing any clinical symptoms of the disease. Quantitative determination of the immunoglobulin level for spirochetes has indicated that Bernese Mountain Dogs may have an increased susceptibility to *Borrelia* spp. infections of a hereditary nature.

Key words: Lyme disease, vector-borne diseases, dogs

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Introduction

Lyme disease is a multi-organ disease caused by an increased immune response of the organism to *Borrelia burgdorferi* sensu lato (Tsao 2009, Stanek and Reiter 2011). These bacteria are transmitted by ticks (Zygner et al. 2008, 2009, Dzięgiel et al. 2014). Despite the development of numerous monitoring and prevention programmes, the disease is still the most commonly diagnosed tick-borne infection in humans and animals in the Northern Hemisphere (Rizzoli et al. 2011). Over the past decades, there has been an increasing number of patients with this disease and the occurrence of cases in areas previously considered free of Lyme borreliosis. The probable reason is global warming, which results in a short, mild winter and early spring. Even slight increases in the ambient temperature allow ticks to colonise areas located higher above sea level, into which Lyme disease is introduced along with arachnids (Rizzoli et al. 2011).

Aetiology and epidemiology

There are at least 51 species of *Borreliae,* of which 21 belong to the group *B. burgdorferi* sensu lato (LB) – whose representatives are responsible for the development of Lyme borreliosis – and 29 are classified as *Borrelia spp.*, which cause recurrent fever. The pathogenicity of the other two spirochete species has yet to be definitively established. The group of *B. burgdorferi* sensu lato currently includes 18 spirochete subspecies (Tsao 2009, Stanek and Reiter 2011). The subspecies that are pathogenic for humans and animals mainly include *Borrelia afzelii, Borrelia garinii, Borrelia burgdorferi* sensu stricto (s.s.), *Borrelia bavariensis* (previously referred to as *B. garinii* OspA serotype 4) and *Borrelia spielmanii.* The pathogenicity of the other subspecies, such as *Borrelia lusitaniae, Borrelia valaisiana* and *Borrelia bissettii* has still not been determined (Margos et al. 2009). The genospecies most often detected in European ticks are *B. afzelii* and *B. garinii*, and – less frequently – *B. burgdorferi* s.s. and *B. valaisiana* (Rauter and Hartung 2005). *B. lusitaniae* occurs locally, mainly in the Mediterranean Basin (Vollmer et al. 2011). Although all of the above-mentioned pathogenic species of bacteria may cause the occurrence of erythema migrans, in many cases – apart from this symptom – they induce varied disorders. The reservoir of spirochetes is very wide and includes animal species, with ticks serving as vectors. Therefore, *B. afzelii* and *B. bavariensis* are most often found in rodents, *B. valaisiana* and most strains of *B. garinii* in birds (Margos et al. 2009), while *B. lusitaniae* in lizards. It has also been demonstrated that the genetic structure of the bacteria in question is related to the species of animals constituting their reservoir hosts (Wilske et al. 2007).

The vectors responsible for the dissemination of the microorganisms, which cause the disease between various species of animals and humans are *Ixodes ricinus* and, less frequently, *Ixodes persulcatus*. The latest epidemiological data from Europe demonstrates that, on average, 13.7% (0-49.1%) of ticks are infected with *B. burgdorferi*. Spirochetes were found more often in adult organisms (18.6%) than in nymphs (10.1%). In Europe, the countries where the percentage of infected ticks was highest were Austria, Czech Republic, Germany, Switzerland, Slovenia and Slovakia (nymphs > 11%, adult ticks > 20%, respectively) (Vitorino et al. 2008, Margos et al. 2009, Tsao 2009, Rizzoli et al. 2011, Stanek and Reiter 2011).

The transmission of the infection from ticks to the host is affected by many factors. It is affected by factors related to the behaviour of ticks, the duration of the individual development stages of arachnids, preferences regarding the host species that they feed on as well as external factors such as weather conditions, the nature of the area where ticks occur, the spirochete reservoir and the actual host species on which they feed and will transmit the infection (Estrada-Peña et al. 2006, Brunner et al. 2008, Brunner and Ostfeld 2008, Faulde and Robbins 2008, Estrada-Peña 2009). In general, during the first 24 hours of ticks feeding on animal or human skin, the spirochetes are not transferred from the organism of the infected tick to the host organism; therefore, early removal of arachnids from the skin is a factor that significantly reduces the risk of the disease development (Rizzoli et al. 2011).

The main reservoir of *Borreliae* spirochetes in Europe constituting a source of bacteria for ticks are small rodents (mice, rats, squirrels) and rabbits, as well as some species of reptiles and birds. Certain species of wild ruminants (deer) and sheep are considered by some researchers as an incompetent reservoir of *Borrelia* spp., which means that the arachnids feeding on them do not get infected (Rizzoli et al. 2011).

The available epidemiological data on Lyme disease in Europe is based on non-standardised criteria and uses a non-unified data collection and processing (Hubálek 2009, Semenza and Menne 2009). Nevertheless, their analysis allows us to assume that in Europe, the cases of Lyme disease are recorded between latitudes of 35°N and 60°N in areas located up to 1,300 m above sea level. The disease is most often diagnosed in Central and Northern Europe, and – less often – in its southern part. The infections occur mainly

in the period from spring to autumn (i.e., in the season of tick activity). This does not mean, however, that the clinical symptoms of the disease must develop during this time. The time between the tick bite causing infection and the development of symptoms may be long; therefore, it is important for the diagnostic procedure to take a thorough medical history from the animal owner, including questions about the presence of arachnids on the animal's skin during the previous year (Rizzoli et al. 2011).

Pathogenesis and clinical symptoms of Lyme disease in dogs

Borrelia spp. initially multiply locally in the skin at the site of inoculation and then disseminate to other tissues, including joints. The research by Levy and Magnarelli (1992) performed on Connecticut dogs demonstrated that the clinical symptoms of Lyme disease, such as lameness, joint swelling and soreness, as well as fever, occur in only 4.8% of dogs that tested positive for *B. burgdorferi* antibodies in serum using ELISA. So far, the factors determining the development of the disease in some dogs and not in others following contact with spirochetes have not been discovered (Skotarczak 2002).

An important element in the pathogenesis of the disease is that the spirochetes persist extracellularly – they multiply in the intercellular spaces in the skin. The clinical symptoms of the disease are related to an increased inflammatory reaction that takes place in the body in the course of the disease. In this way, flagellin, one of the most immunogenic proteins, stimulates the production of antibodies that bind to axons of nerve cells, which may result in the development of neurological symptoms in humans (Fikrig and Barthold 1997, Bockenstedt et al. 2021).

Increased expression of CXCL8 in the synovial membranes is the main cause of polyarthritis, while strong general inflammatory reactions (fever, increased heart and respiration rate) may be associated with the release of increased quantities of cytokines, TNF-α, IL-6 and CXCL8 (Bockenstedt et al. 2021).

The course of Lyme disease varies considerably. Two forms of the disease are distinguished in dogs – articular and renal. The articular form is characterised by fever and shifting lameness. The pathological changes in the joints are generally progressive, and chronic polyarthritis may persist despite treatment (Littman et al. 2018).

The renal form results from glomerulonephritis, leading to renal failure with proteinuria, uraemia, and peripheral oedema (Dambach et al. 1997).

Clinical observations made by many authors indicate that the definition of Lyme disease as a multi-organ disease, or even a multi-system disease, is correct. Still, the most common disorders occurring in its course are related to the locomotor system; however, there are symptoms from the circulatory system and the skin (Hovius et al. 2000, Chang et al. 2001, Goossens et al. 2001, Adaszek et al. 2020).

It should be noted, however, that not every contact with the pathogen leads to the development of the disease. This is evidenced by positive results of serological tests for Lyme disease in animals and humans that do not give rise to symptoms of the disease (Lissman et al. 1984, Magnarelli et al. 1987, Levy and Magnarelli 1992, Adaszek et al. 2009a). Serological screening is extremely helpful in diagnosing asymptomatic infections and determining the epizootic status of Lyme disease in a given area. Most often, immunofluorescence and ELISA tests are used for this purpose. Rapid serological tests can also be helpful (Greene et al. 1991, Joppert et al. 2001, Adaszek et al. 2009a).

Diagnosis

It is difficult to diagnose Lyme disease. The coexistence of at least four elements is required to confirm the condition, including clinical symptoms typical of Lyme disease, positive titres of antibodies against *Borrelia* spp. in the serum of patients suspected of being infected, confirmed patient contact with ticks and a positive patient response to the applied treatment (Adaszek et al. 2009b).

In the serological diagnostics of Lyme disease, ELISA and indirect immunofluorescence are the most commonly used tests (Adaszek et al. 2009a). The antigen for the tests mentioned above is prepared in various ways and, so far, there is no standardised preparation procedure; therefore, the results of the tests differ depending on the laboratory (Greene et al. 1991).

Due to the possibility of cross-reactions (e.g., between antibodies to other spirochetes and *Borrelia* spp. antigen), it is recommended to repeat the ELISA test of positive serum samples using the immunoblotting technique. This allows an increase in the specificity and sensitivity of serological tests for diagnosing Lyme disease (Adaszek et al. 2009b, Rizzoli et al. 2011). Antibodies produced in the body of infected individuals naturally react with *Borrelia* spp. proteins other than the immunoglobulins produced following vaccination. The immunoblotting technique, based on the ability of antibodies to bind to the strictly defined spirochete antigens, also allows the distinguishing of *Borrelia* spp. infections from infections caused by other members

of the *Spirochetaceae* family. In dogs, antibodies produced by natural infection with *B. burgdorferi* react with bacterial proteins of 22 kDa (OspC), 39 kDa (p39) and 41 kDa (flagellin protein) (Chang et al. 1995). In turn, vaccinated animals showed intensive reactions of immunoglobulins with bacteria proteins of 31 kDa (OspA) and 34 kDa (OspB), which are not seen in the case of testing serum from naturally infected dogs. On this basis, it can be assumed that the development of ELISA tests using the purified OspA and OspB proteins as an antigen would help to distinguish vaccinated animals from those with a natural infection (Chang et al. 1995, LaFleur et al. 2009).

The OspC protein is only expressed in warm-blooded organisms. It is not found in bacteria isolated from ticks or obtained from *in vitro* culture. Using this antigen in the ELISA test makes the disease diagnosis more reliable (Mathiesen et al. 1998).

Rapid serological tests for dogs are available on the veterinary product market and allow the demonstration of antibodies against *Borrelia* spp. protein (SNAP 4Dx Idexx, CaniV4 VetExpert) in the body of a suspected animal. In addition to detect immunoglobulins specific for spirochetes, these kits simultaneously facilitate the detection of antibodies against *Ehrlichia canis, Anaplasma phagocytophilum* and the *Dirofilaria immitis* antigens. Their unquestionable advantage is the possibility of obtaining results under clinical conditions within just a few minutes using a small amount of material for their execution (three drops of serum, plasma or complete blood) (Stillman et al. 2014, Liu et al. 2018).

The PCR technique allows quick diagnosis of the disease and identification of the *Borreliae* species responsible for its occurrence. However, this test is not without its drawbacks, and false-negative results are relatively common. The test is based on amplification of the bacterial DNA fragments isolated from the tissues of an infected patient to obtain several million copies. The multiplication does not concern the total *Borrelia* spp. DNA, but its section that is limited by short nucleotide sequences (primers). Primers can be designed for a conserved gene of the *Borreliae* genus, so the PCR will also be positive if there is an infection with non-pathogenic spirochetes. To overcome this draw back, the polymerase chain reaction can be carried out in two steps. In the first step, DNA amplification is performed with primers complementary to the conserved gene found in the entire *Borreliae* genus. If the results are positive, the second step is the amplification with species-specific primers. To determine the spirochete species, it is also possible to analyse the nucleotide sequence of the amplification products (Straubinger 2000, Dzięgiel et al. 2016).

As mentioned above, the disadvantage of PCR is the possibility of obtaining false negative results. They may be caused by the contamination of the tested sample or inappropriately selected test material. When it is blood, it should be kept in mind that the spirochetes are present only in the period of bacteremia. The PCR blood test may be negative if the microorganisms are located in the joints. False-positive results may be a consequence of the persistence of bacterial DNA fragments that PCR can detect in the tissues of infected individuals subjected to antibiotic therapy. Therefore, a big disadvantage of the polymerase chain reaction is that it does not distinguish between living and dead microorganisms (Adaszek et al. 2009a, Dziegiel et al. 2016).

Treatment

Many antibiotics, administered either parenterally or orally, are effective against *Borreliae* spirochetes (Table 1). Doxycycline and beta-lactam antibiotics are the most effective in the treatment of Lyme disease in animals. It should be kept in mind that to be effective, antibiotic therapy should last at least four weeks (Wormser and Schwartz 2009). Doxycycline is considered the drug of choice in the treatment of Lyme disease, mainly because, in addition to its antibacterial activity, it also has anti-inflammatory properties and is also effective in combating other tick-borne pathogens such as *Anaplasma* and *Ehrlichia,* as well as *Leptospira* spp. (Littman et al. 2006). In some countries, this antibiotic is approved for use in puppies and kittens from four weeks of age – although many veterinarians believe that for growing animals, it is better to use amoxicillin or cefovecin (two injections at a 14-day interval) (Littman et al. 2018).

In dogs with Lyme arthritis, the response to antibiotic therapy is generally rapid. The improvement in patients' condition can already be observed after one to three days of treatment. Additionally, analgesics and anti-inflammatory drugs can be used (glucocorticosteroids are preferable to NSAIDs (Non-steroidal anti-inflammatory drugs), especially in the case of suspected immune-related arthritis) (Chang et al. 2001).

The most common form of Lyme nephritis is glomerulonephritis caused by immune complexes (Dambach et al. 1997).

In *Borrelia* spp. seropositive patients with renal failure and proteinuria, hypoalbuminemia, and progressive azotaemia – apart from antibiotic therapy – immunosuppressive therapy may also be indicated (Table 2).

Table 1. Antibiotics used in the therapy of borreliosis in dogs.

Drug	Species	Dose	Route of administration	Frequency (hours)	Duration of treatment (days)	Indications
Doxycycline	Dog	10 mg/kg	p.o.	12	30	Arthritis, dermatitis
Amoxicillin	Dog	20 mg/kg	p.o.	8	30	As above (can be used in puppies)
Azithromycin	Dog	5 mg/kg	i.v.	12	10-20	In early stages of disease
Ceftriaxone	Dog	20 mg/kg	i.v., s.c.	12	15-30	Neurological or cardiac events, chronic arthritis
Cefotaxime	Dog	20 mg/kg	i.v.	8	15-30	Neurological symptoms
Penicillin G	Dog	22.000 U/kg	i.v.	8	15-30	Chronic arthritis, neurological symptoms, cardiac events

Table 2. Immunosuppressants used to treat glomerulonephritis during Lyme disease in dogs.

Drug	Dose	Route of administration	Frequency (hr)
Prednisolone	1 mg/kg	p.o.	12 (4-5 days)
Azathioprine	2 mg/kg	p.o.	24 (14 days)
Cyclosporine	5-20 mg/kg	p.o.	12
Chlorambucil	0.2 mg/kg	p.o.	24-48
Cyclophosphamide	50 mg/m^2	p.o.	7 (than 200-250 mg/m^2 every 3 weeks)

Prevention

Lyme borreliosis is a disease transmitted by ticks and, therefore, any measures limiting the possibility of people and animal contact with arachnids will minimise the risk of its occurrence. The sites of tick foraging should be avoided, especially in their period of activity (Rizzoli et al. 2011). After walks, the fur of animals should be carefully examined for ticks and, if they are found, they should be immediately removed. It should be kept in mind that for infection to occur, the tick must be attached to the skin for at least 12-24 hours; therefore, early removal of arachnids effectively protects against the development of the disease. In animals, it is recommended to use prophylactic preparations against ectoparasites available in the form of tablets, sprays, spot-on preparations and collars.

Several vaccines are available on the veterinary market for the active immunisation of dogs against Lyme disease. They are inactivated preparations containing whole bacterial cells and recombinant ones. Recombinant vaccines based on OspA protein stimulate the production of antibodies that penetrate ticks' intestines while feeding on a dog and inactivate bacteria in its intestines (Chang et al. 1995). Vaccines based on OspC protein stimulate the production of antibodies that inactivate bacteria that have entered the animal's body (Littman et al. 2018).

Lyme disease in Bernese Mountain Dogs

Another aspect is the problem of Lyme disease in Bernese Mountain Dogs. Our own observations and reports from veterinarians indicate that representatives of this breed often test positive for antibodies against *B. burgdorferii* using rapid diagnostic tests without showing any clinical symptoms of the disease. Also, quantitative determination of the immunoglobulin level for spirochetes has indicated that Bernese Mountain Dogs may have an increased susceptibility to *Borrelia* spp. infections of a hereditary nature (Gerber et al. 2007, Adaszek et al. 2009a, Preyß-Jägeler et al. 2016).

This finding was provisionally confirmed by the results of the studies by Gerber et al. (2007). These authors tested 160 Bernese Mountain Dogs and 62 large-breed control dogs (all animals had similar fur

Fig. 1. Percentage of Bernese Mountain Dogs with elevated antibodies against *B. burgdorferi* in different European countries.

and were kept under similar conditions) for the presence of antibodies against *B. burgdorferi.* Serological examination was performed using ELISA and Western blotting. Elevated antibody titres were found in 58% of Bernese Mountain Dogs and only 15% of control dogs. The authors were unable to determine the cause of such a large discrepancy between the groups, suspecting that they might be a consequence of breed predisposition in Bernese Mountain Dogs. Similar conclusions were also reached by German researchers, who showed the presence of antibodies against *B. burgdorferi* in serum of 43.3% of Bernese Mountain dogs and in only 24.6% of control dogs (Preyß-Jägeler et al. 2016).

The results of the study performed by the authors in Poland (data not published) show that as many as 32% of healthy Bernese Mountain Dogs in Poland reacted positively in a rapid test for *B. burgdorferi* (Fig. 1).

Yet another Bavarian study with a dog population living in the same geographic area found antibodies against spirochetes in 92% of Bernese Mountain Dogs (12 out of 13 dogs were seropositive) and only 7% of other breeds (13 out of 187 dogs were seropositive). Since it was excluded that these differences were due to increased exposure of Bernese Mountain Dogs to ticks (all of the animals used in the study came from the same geographical area), it was hypothesised that Bernese Mountain Dogs may be genetically predisposed to *B. burgdorferi* infections, which should be taken into account in clinical practice (Gerber et al. 2009a, 2009b).

The origin of this phenomenon is unknown. It may be a consequence of the intense breeding of Bernese Mountain Dogs, resulting in reduced resistance against infectious agents. This breed is known for intense breeding, and this narrow range of genes might also be true for the gene pool of Bernese Mountain Dogs. It should be noted that Lyme disease is not the only problem that affects this breed. Compared to other breeds, Bernese Mountain Dogs are relatively more prone to blood clotting disorders, epilepsy and malignant histiocytosis.

The predisposition of the breed to parasitic and infectious diseases has also been identified in other breeds (e.g., *Babesia gibsoni* infections in American Pitbull Terriers and related breeds (Hartelt et al. 2007, Beck et al. 2009)) or parvovirus in the Rottweiler, Doberman Pinscher, Pomeranian and German Shepherd breeds. Therefore, it cannot be ruled out that a certain undetermined genetic predisposition in Bernese Mountain Dogs is the reason for the presence of serum antibodies to *B. burgdorferi* in these dogs.

It has also been claimed that low serum concentrations of the third component of complement (C3) are associated with both the susceptibility to infectious agents (such as *B. burgdorferi*) and the development of glomerular disease. Therefore, this breed may have a reduced C3 concentration, which contributes to their increased susceptibility to Lyme disease. However, the findings of Gerber et al. (2010) contradict this theory. The authors studied 83 healthy Bernese Mountain Dogs and 46 control dogs. Antibody titres for *B. burgdorferi* and serum C3 concentrations were determined in all animals. The median C3 concentration was 128.5% in Bernese Mountain Dogs with antibodies against *B. burgdorferi*, 133.5% in *B. burgdorferi*-negative Bernese Mountain Dogs, 87.8% in positive control dogs and 102.2% in negative control dogs. This shows

that in healthy dogs, the serum concentration of C3 is higher than in dogs with antibodies to spirochetes. Furthermore, the concentration of this parameter was higher in *B. burgdorferi*-negative Bernese Mountain Dogs than in negative control dogs. This clearly indicates that low percentage concentrations of C3 do not explain the high prevalence of *B. burgdorferi* infections and glomerular disease in Bernese Mountain Dogs.

It seems interesting to note that in most cases, Bernese Mountain Dogs with serum antibodies to *B. burgdorferi* did not show signs of Lyme disease.

One study attempted to show a correlation between the presence of antibodies to spirochetes in the serum of dogs and the occurrence of glomerular disease. Urine protein excretion was evaluated in 122 clinically healthy Bernese Mountain Dogs and 55 controls. The seroprevalence of *B. burgdorferi* in Bernese Mountain Dogs was 57%, compared to 16% in controls. There were no significant differences in the occurrence of positive dipstick results, microalbuminuria, urine protein--to-urine creatinine ratio or abnormal urine protein pattern between Bernese Mountain Dogs and controls and Bernese Mountain Dogs with and without antibodies against *B. burgdorferi* (Gerber et al. 2009a). The same authors investigated whether Bernese Mountain Dogs with serological evidence of natural *B. burgdorferi* infection more often develop signs such as lameness, azotemia or proteinuria compared to seronegative Bernese Mountain Dogs and to seropositive and seronegative control dogs of other breeds (Gerber et al. 2009b). The study included 53 Bernese Mountain Dogs and 30 control dogs aged 3-11 years with an average of 2.7 years of serological testing for Lyme disease, which showed the presence of antibodies to *B. burgdorferi* in 42% of Bernese Mountain Dogs and 37% of control dogs. A repeat serology (performed after 2.5-3 years) showed the presence of antibodies to spirochetes in 47% of Bernese Mountain Dogs and 40% of control dogs. There were no significant differences concerning poor general condition or lameness between the first and the second evaluation. In seropositive dogs, there was no increase in lameness or signs of renal disease over time. The results of the study indicate that antibodies against *B. burgdorferi* were neither associated with the development of lameness nor with signs of renal disease like azotemia or proteinuria.

There have also been attempts to account for the higher prevalence of serum antibodies to *B. burgdorferi* in Bernese Mountain Dogs in terms of their greater susceptibility to invasion by ticks, which are the vectors of the disease. The dark hair of this breed makes it more difficult to detect the presence of arachnids, so they can remain on the bodies of these dogs for longer and thus have a greater chance of transmitting the disease (Crippa et al. 2002). Gerber et al. (2007) showed that dark-skinned dogs are more likely to have serum antibodies to spirochetes than white-skinned dogs (28% vs. 7%). However, such differences are not identified when comparing the frequency of these immunoglobulins in Bernese Mountain Dogs and in the dark-skinned control dogs of other breeds. This indicates that hair colour does not explain the higher seroprevalence of antibodies against *B. burgdorferi* in Bernese Mountain Dogs.

Conclusions

In conclusion, Bernese Mountain Dogs more often had antibodies against *B. burgdorferi*. Interestingly, most of the studies showing higher Bb-sl-seroprevalence were performed in close proximity to each other in central Europe (Switzerland and southern Germany) – the region with the highest prevalence of Bb-sl infested ticks was found (Rauter and Hartung 2005). One might speculate about a regional effect, a close genetic relationship among the positive dogs, a genetic predisposition for infection or a unique infectious species of Bb-sl in the area. More investigations are needed to evaluate the biological reasons and consequences of infections with *B. burgdorferi* in Bernese Mountain Dogs.

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