Guide lyme™

Clear guidance for day-to-day
veterinary management of canine Lyme disease

Introduction

Lyme disease is a controversial topic that attracts a lot of media coverage, especially in human medicine. In veterinary medicine, companion animals are exposed to many of the same vectors and pathogens as their owners. Despite a lower level of awareness about the risk for dogs, the medical importance of this tick-borne disease has increased in parallel with its distribution and frequency.

Veterinary professionals are the most trusted source of information for pet owners. Staying up-to-date on this topic is therefore an important responsibility for veterinary clinics.

Although gray areas in bacteria pathogenicity, clinical manifestations, diagnostic procedures and treatment protocols remain, recent data have shed new light on the transmission speed (S ertour *et al.*, 2018), distribution and epidemiology of Lyme disease (Herrin *et al.*, 2018). This document is designed to help veterinarians put this new science into practice.

Ceva Animal Health is committed to supporting veterinary practitioners facing canine Lyme disease with practical tools for day-to-day practice.

We are glad to share this GuideLyme™ with you.

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The experts

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PROF. BYRON BLAGBURN

Byron L. Blagburn PhD, DACVM (Hon) holds the appointment of Distinguished University Professor at Auburn University. He is Past President of the American Association of Veterinary Parasitologists, the Southern Conference on Animal Parasites, the Southeastern Society of Parasitologists, and the Companion Animal Parasite Council (CAPC). He has served as editor and/or reviewer for numerous publications. He is a recipient of the AAVP Distinguished Veterinary Parasitologist Award, the Zoetis Research Award (1987 and 2010) and several teaching awards.

DR. VALÉRIE CHOUMET

Valérie Choumet (PhD) is a former student of the Faculty of Pharmacy. As Doctor of Science, Valérie Choumet spent most of her career at the Institut Pasteur, where she is currently Group Leader in the Environment and Infectious Risks Unit. Having a background in biochemistry and vector biology, her research activities combine analyses of host-vector interactions mainly using functional genomics and of arthropod-borne pathogen transmission to mammals. Some of her recent publications aimed to improve our knowledge on arthropod-borne pathogen interactions with various tick and mosquito species. Valérie Choumet is also involved in public health studies of vector-borne diseases like Lyme borreliosis.

PROF. FRANS JONGEJAN

Frans Jongejan holds a PhD from Utrecht University, Faculty of Veterinary Medicine in the Netherlands on veterinary parasitology. He is currently director of the Utrecht Centre for Tick-borne Diseases (UCTD) at the Faculty of Veterinary Medicine, Utrecht University. UCTD has been designated FAO Reference Centre for Ticks and Tick-borne Diseases by the Food and Agriculture Organization of the United Nations (FAOUN). He holds an appointment as extraordinary professor in the Department of Veterinary Tropical Diseases of the Faculty of Veterinary Science of the University of Pretoria in South Africa. He provides extensive expert consultations on ticks and tick-borne diseases to the pharmaceutical industry, contract research organizations, and regulatory authorities through TBD International BV, a private company registered in the Netherlands.

PROF. THOMAS MATHER

Thomas Mather is Professor of Entomology of Public Health at the University of Rhode Island and Director of the URI Tick Resource Center (TERC). His work on tick ecology, tick control and tick vaccine development is recognized nationally and internatonally. He is known for the prevention of Lyme disease and is highly sought after for his memorable and entertaining messages on tick prevention. Thomas Mather is involved in the development of a vaccine against Lyme. He developed a tick bite risk index for the U.S., and is the leading voice seeking to educate people about how to avoid getting bitten by ticks. His areas of expertise are bugs, environment, Lyme disease, tick-borne diseases, and ticks.

DR. LINDSAY STARKEY

Lindsay Starkey joined the faculty at Auburn University in May, 2016. She was raised on a rural beef cattle farm in Northeast Kansas, and earned her BS (2007) in Animal Science from the University of Arkansas. She completed both her DVM (2011) and PhD (2015) in Veterinary Biomedical Sciences at Oklahoma State University where her graduate research focused on several *Ehrlichia* spp. of dogs. She is a diplomate of the American College of Veterinary Microbiology, Parasitology sub-specialty, completing her residency training through the National Center for Veterinary Parasitology at Oklahoma State University in 2015. Her work at Auburn involves the teaching of parasitology to first and fourth-year veterinary students, varied research projects, diagnostic parasitology and parasite consultation, and outreach. Lindsay Starkey's research interests include vector-borne infections, primarily those transmitted by ticks and mosquitoes, and food-borne parasitic diseases.

PROF. REINHARD STRAUBINGER

Reinhard Straubinger studied veterinary medicine at the Ludwig-Maximilians-University in Munich. He graduated in 1995 at the LMU in Munich and obtained his Ph.D. at Cornell University in 1997. Three years of research followed at Cornell University and at Leipzig University (2000-2008), where he completed his habilitation in Immunology and Infection Medicine. Since October 2008, Reinhard Straubinger is Head of the the Department of Bacteriology and Mycology at the Institute of Infection Medicine and Zoonoses of the Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München. He is a veterinary specialist in immunology and microbiology. His professional interests are the infectious diseases of animals, the host-pathogen interaction and the application of molecular and genetic engineering methods in research and diagnostics. He has internationally published manuscripts on canine Lyme disease, anaplasmosis, *Helicobacter* infection in dogs and cats, cryptococcosis and the immunomodulators interleukin-1, interleukin-12 and interleukin-23.

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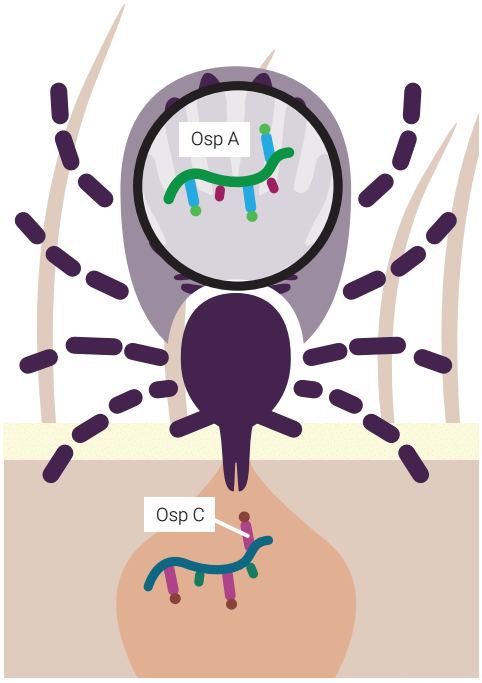
1. THE GOOD, THE BAD AND THE UGLY

A. THE BAD BACTERIUM

Lyme disease, also known as Lyme borreliosis, is a bacterial disease caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex that can be transmitted to humans, dogs and other animals, by ticks belonging to the *Ixodes* genus.

▶ STRUCTURE AND ANTIGENS



Spirochetes reside in the **midgut of unfed ticks**, attached by expressed molecules of Outer surface protein A (Osp A)

During the tick blood meal, *Borrelia* spirochetes **down-regulate the expression** of **Osp A** and **increase the expression** of **Osp C**, which allows migration from the midgut to the **salivary glands**.

Bacteria:

- thin, elongated, spiral-shaped bacteria (0.2 µm x 25 µm)
- practically invisible with normal light microscopy (need dark-field or phase-contrast microscopy)
- cannot survive in the environment outside of the tick or host

▶ TAXONOMY AND GEOGRAPHIC DISTRIBUTION

•Order, family: *Spirochaetales, Spirochaetaceae* - close to *Leptospiraceae*, explaining possible cross-reactions[3].

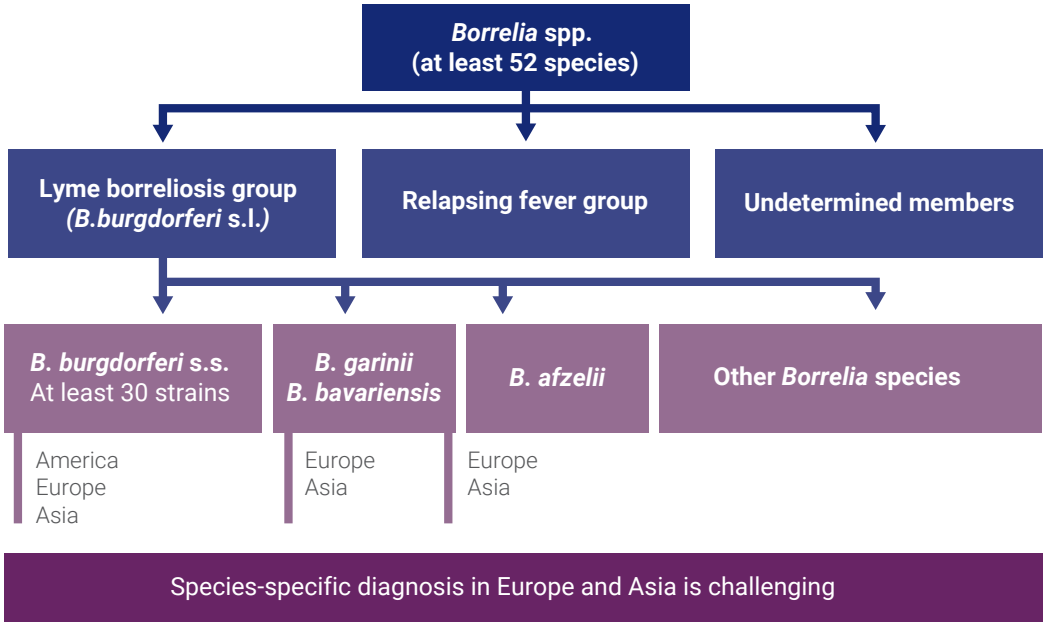


Diagram 1: Taxonomy of *Borrelia* spp.

B.THE UGLY TICK

▶ GENERAL INFORMATION ON *IXODES* TICKS

The main vectors of *Borrelia burgdorferi* sensu lato are species of hard ticks of the genus *Ixodes*. *Ixodes* ticks are particularly prevalent in wooded rural, suburban and even semi-urban areas (ex: inner city parks) in temperate latitudes with higher humidity.

Even at the adult stage, *Ixodes* ticks are small and are easy to miss: depending on the life cycle stage, they range from 0.5 mm (larva) to 3.0 mm (unfed female adults). It is also difficult to differentiate unfed vs. partially-fed nymphs.

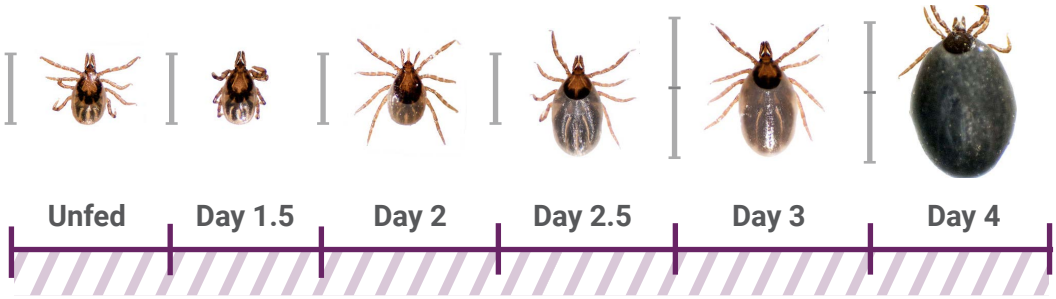


Figure 1: Nymph-stage *I.scapularis* growth comparison (unfed nymph: 1 mm)
From: https://tickencounter.org/tick_identification/tick_growth_comparison

▶ SPECIES AND GEOGRAPHIC DISTRIBUTION

Five species of *Ixodes* have been proven competent vectors of Lyme borreliosis:

- *I. scapularis*
- *I. pacificus*
- *I. angustus*
- *I. affinis*
- *I. ricinus*
- *I. persulcatus*
- *I. hexagonus*

Some other ticks may be competent vectors too, but have not been proven at the moment. Geographic distribution of vectors is continually evolving.

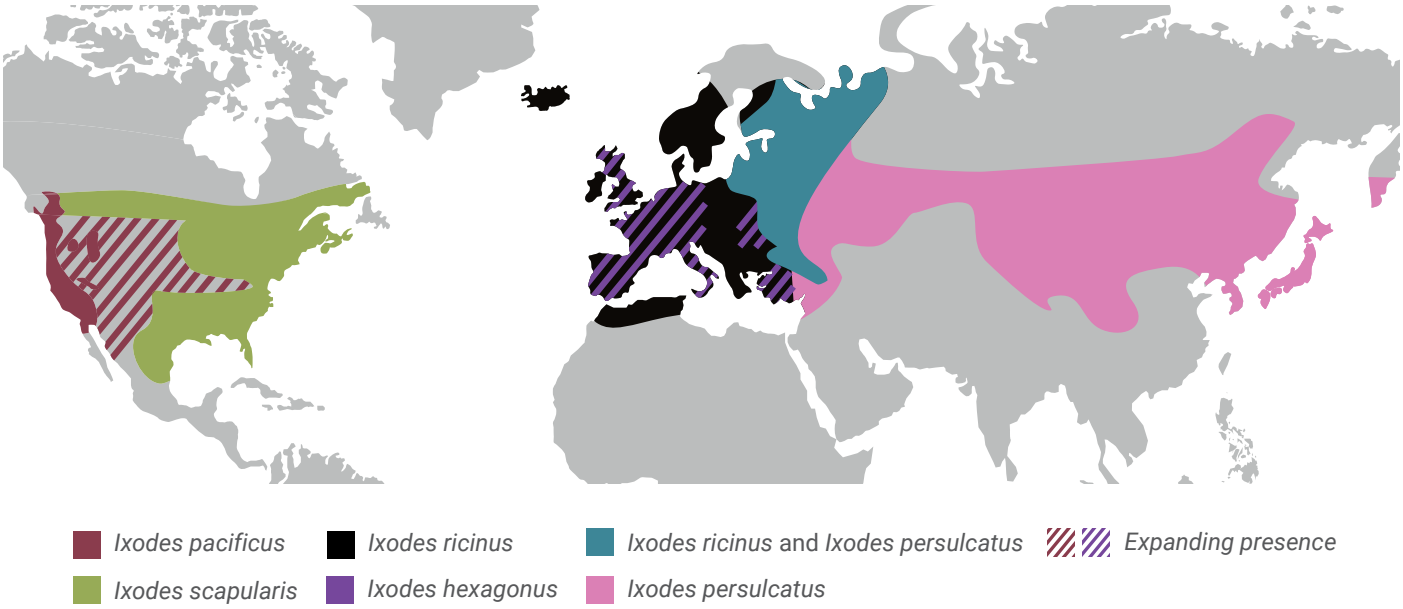
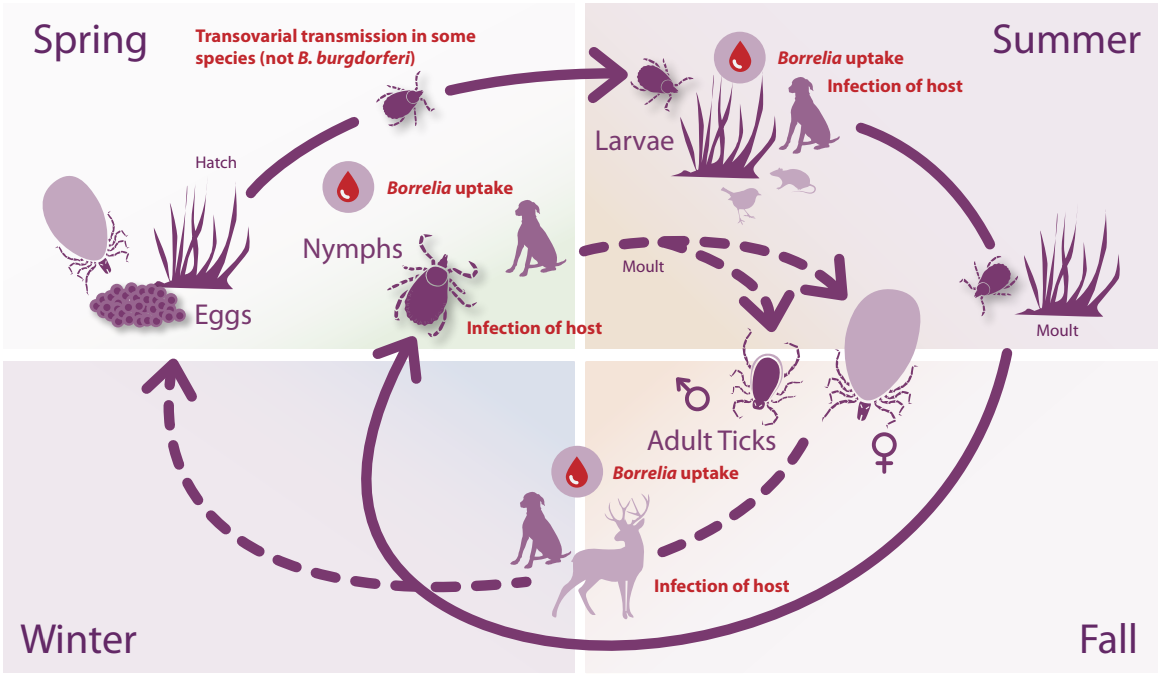


Figure 2: Global distribution of the vectors (*Ixodes* species) of *Borrelia* causing Lyme disease in dogs.[4].

▶ LIFE CYCLE



A single tick can feed on different hosts within one stage[5, 6]

Figure 3: *Ixodes* ticks life cycle

Ixodes ticks have a two to three years life cycle. *Ixodes* can acquire *B. burgdorferi* at every stage of their lives and infect hosts during every, even partial, blood meals. Ticks climb up vegetation and wait for a host. This behavior is called questing.

▶ *BORRELIA* AND CO-INFECTION PREVALENCE IN TICKS:

In some foci, *Borrelia* infection rate can be as high as
63% in USA[7] and
75% in Europe[8]

On average, in highly endemic areas,
1 out of 3 ticks may carry *Borrelia* organisms.

Ticks can carry different pathogens at the same time. Up to 45% of ticks were co-infected in the USA and France[9, 10], the majority with *Rickettsia* spp., but also with other *Borrelia* spp., *Bartonella* spp., Anaplasmatacae, and others.

Co-infections may complicate and worsen the clinical picture.[11]

C. THE GOOD HOST

- Like humans, dogs can be infected with *Borrelia*.
- Dogs are even at greater risk of infection because of their behavior (scouting, hunting, etc.) and their attractiveness to ticks.
- Dogs can be infested with a significant burden of ticks.[12]
- After the tick bite, the dog's immune system tries to eliminate the bacteria, while those bacteria develop escape mechanisms in order to spread and multiply.

A single dog can be infested with **200 ticks** [12], but **one single tick** can infect a dog.[13]

51% of *Ixodes* species ticks removed from dogs were very small nymphs **difficult to see**.[14]

▶ RISK FACTORS

- Living in, travelling to and outdoor activity in an endemic area
- Outdoor lifestyle
- Hunting
- Walking and exploring in wooded areas
- Living next to wildlife area / suburban area [15]
- Other animals in the household

▶ TRANSMISSION TIME TO THE HOST

In general, it has been accepted that the risk of *Borrelia* transmission is negligible during the first 24-48 hours of tick infestation. However, rodent models demonstrated the ability of the pathogen to infect the host within 12 hours of attachment.[1]

Some studies have demonstrated that, under natural conditions, partially-fed ticks [5, 6] can transmit pathogens much faster than previously thought because the **pathogens are already present in the salivary glands**.

This is of vital importance for disease prevention protocols.

Act quickly!

It is difficult to determine the duration of tick attachment and there is a potential of transmission **before 24 hours**.

▶ AFTER ENTERING THE HOST:

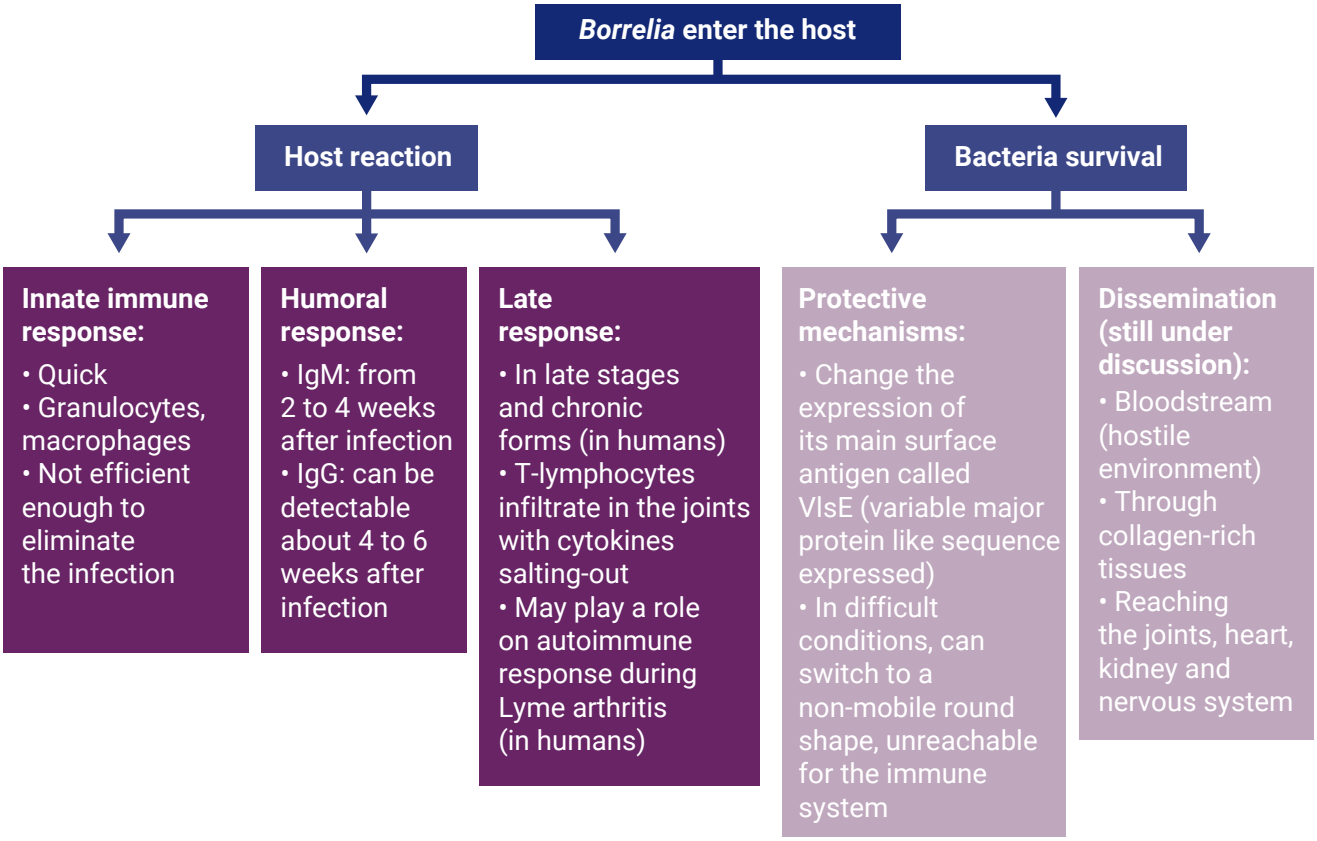


Diagram 2: Host and bacteria reactions after *Borrelia* enter the host

▶ SEROPREVALENCE MAPS

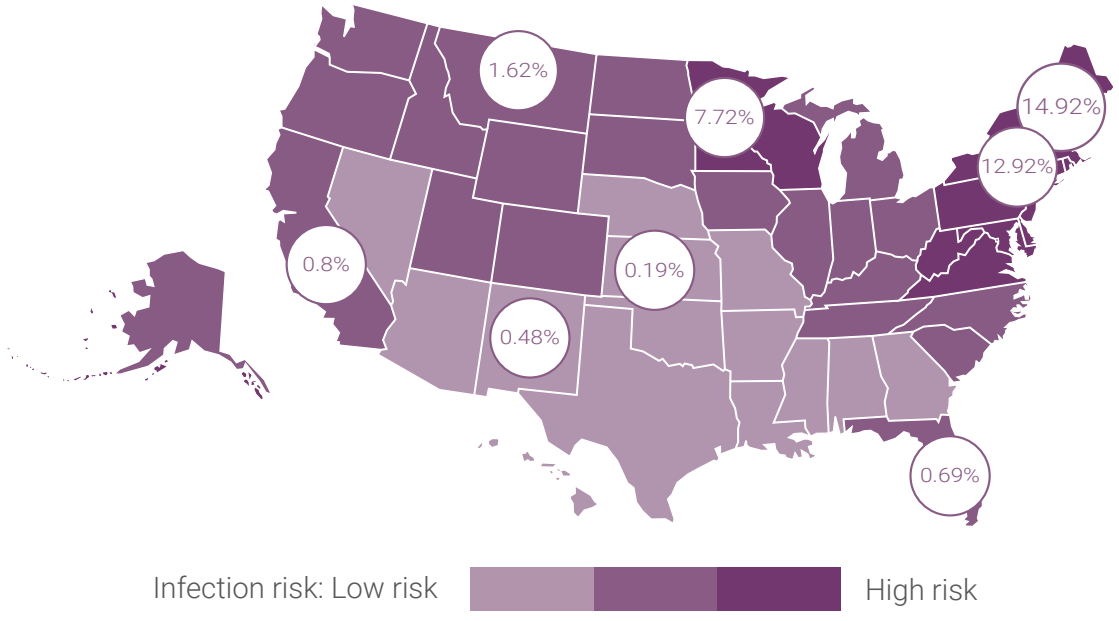


Figure 4: Canine borreliosis serology in North America (2018)
Adapted from: capcvet.org

From 0.13% to 14.92%
of tested dogs were seropositive

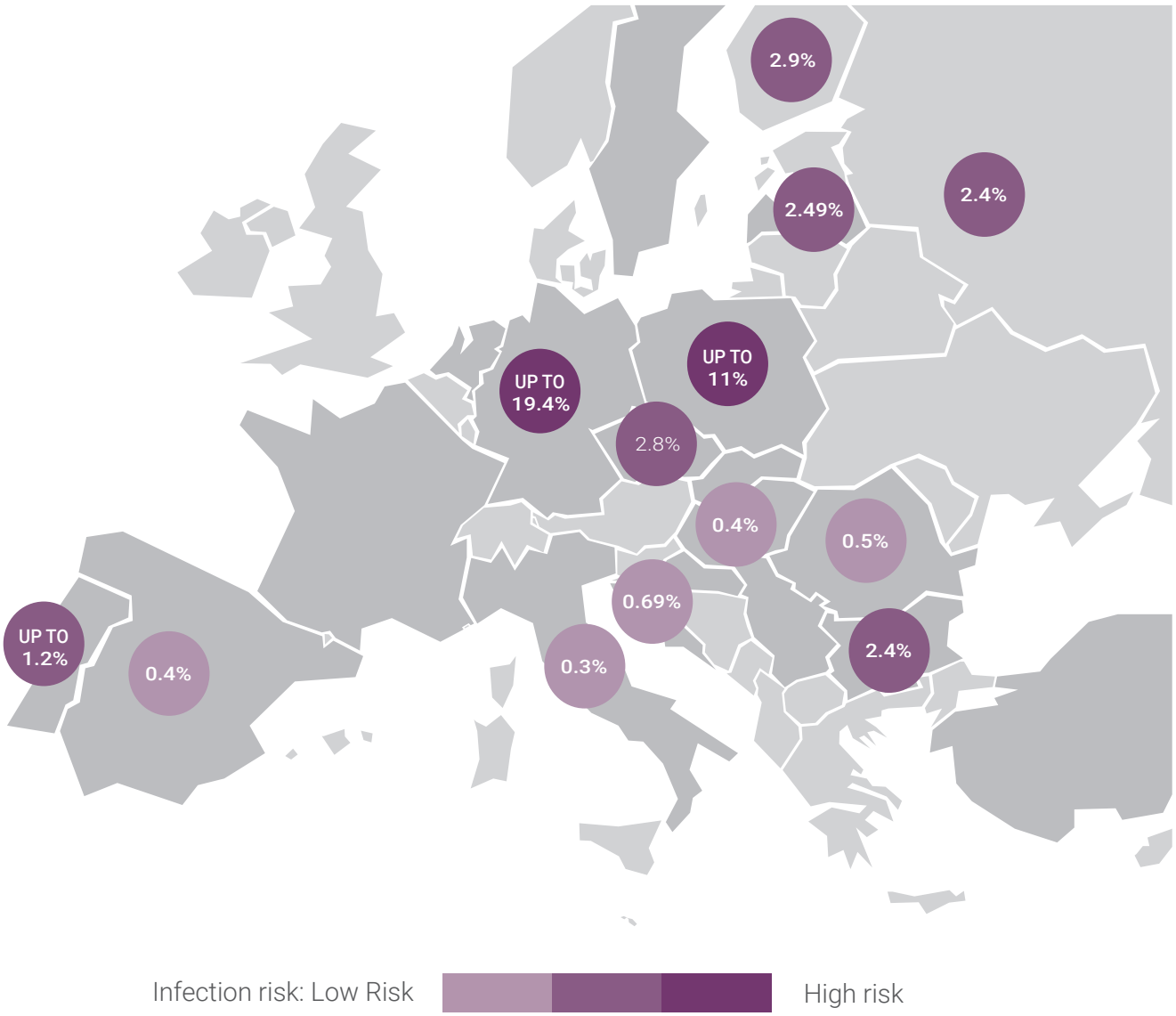


Figure 5: Consolidated seroprevalence (%) of canine borreliosis in Europe[16-29]

2. CLINICAL LYME BORRELIOSIS IN DOGS

In veterinary medicine, we often miss the starting point of the infection with the tick bite. It is therefore complicated to differentiate early from late clinical signs in practice.

Key elements supporting the suspicion of Lyme disease:
- Exposure to ticks and report of past infestation
- Clinical signs consistent with borreliosis
- Exclusion of other causes
- Positive test results

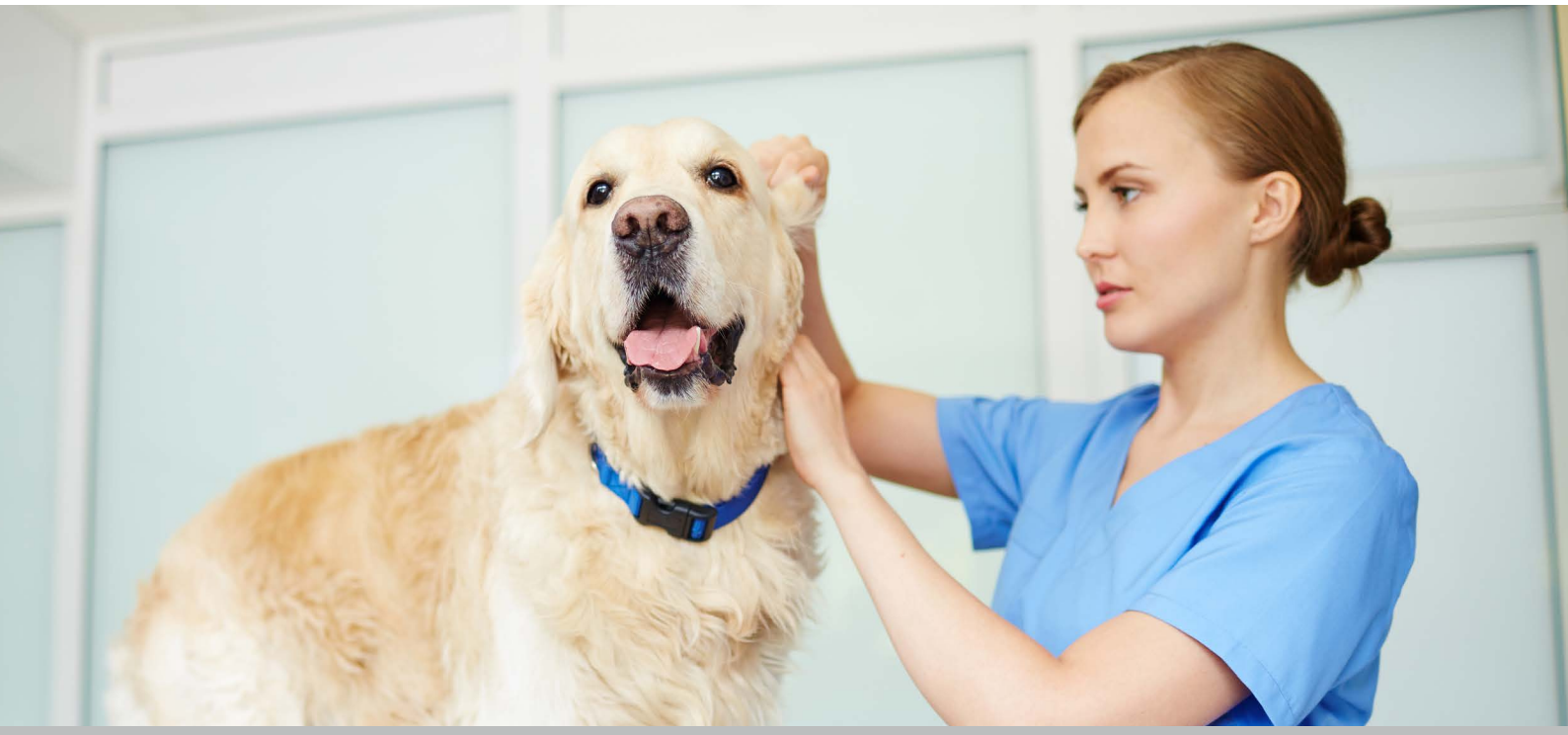
While **clinical signs** were detected in about 5% of seropositive dogs in two 1990s field studies [30, 31], they were observed **in over 57% of experimentally infected dogs** [32-37]. Seropositivity may last for months to years.

Expression of clinical signs might depend on the bacteria genospecies, strains and individual host factors.

A.INFECTION KINETICS

From days to weeks after infection, non-specific signs are often overlooked by the owner because they subsided in a few days without treatment.

Later signs may appear weeks to months after the initial infection.



B.THE CLINICAL PICTURE IN PRACTICE

	Frequent	Uncommon	Rare	Infection kinetics
General signs 	• weakness, fatigue, malaise • fever (mild to moderate) • reluctance to move • anorexia • weight loss	-	-	☑ Early ☐ Intermediate ☑ Late
Orthopedic signs 	• lameness (one or more legs), sometimes during several episodes (usually 2 to 5 in several weeks). • joint swelling (mono or polyarthritis) • lymph node swelling	-	-	☑ Early ☐ Intermediate ☑ Late
Renal signs 	-	• peripheral edema • effusion in body cavities • emesis Blood work results include : • azotemia • proteinuria	-	☐ Early ☐ Intermediate ☑ Late
Cardiac signs 	-	-	• arrhythmia • pale mucous membranes • pulse deficit • slow capillary refill time	☐ Early ☑ Intermediate ☑ Late

Table 1: Clinical picture of Lyme borreliosis

Exclusion of other major diagnoses is often required to complete the clinical picture. Neurologic or muscular manifestations of Lyme borreliosis in dogs are not well documented.

C.CO-INFECTIONS

Ticks are often co-infected, resulting in possible co-transmission to dogs. Most of these pathogens can be managed with antimicrobial therapy, especially doxycycline. The identification of the causative agent is important when using **non-cycline antibiotics**.[38] Co-infections may **worsen the clinical signs** and complicate the diagnosis.[11]

3. LABORATORY DIAGNOSIS

The clinical diagnosis of Lyme disease is difficult to achieve because clinical signs are not specific. However, the identification of the causative agent is important to **warn the owner about their own risk**.

Detection of *Borrelia* can be challenging and diagnosis by culture may take up to 4 weeks. To confirm the diagnosis, testing is required.

A. POINT-OF-CARE ANTIBODY TESTING

There are often no abnormalities in routine blood samples.

- Urinalysis should be run to check proteinuria
- In-house antibody testing is a good first step upon suspicion of Lyme borreliosis

B. LABORATORY TESTING

There are many laboratory tests available: none are considered the gold standard and at this time, indirect detection of *B. burgdorferi* is the best approach.

▶ SEROLOGY TESTING:

	Sensitivity/ specificity	Quantitative	Differentiate natural infection vs vaccination	Differentiate acute vs chronic	Interest in follow-up
ELISA Quantitative C6	Sensitivity and specificity qC6 >= WB or ELISA+WB[39]	✓	Possible (only if vaccination history is known)	Not predictive of clinical illness	Confirmed (decreased more than 58% 6 months after completing treatment)[21]
Full-lysate ELISA	Lack of specificity (often need other test)	✓	-	-	-
Immuno-blot (Western blot, IA)	Very specific	Semi-quantitative	Possible	Possible	-
Serology detecting OspA, OspC and OspF	Lack of specificity	✓	Possible	Possible (Osp C appear earlier than Osp F)	-
IFA	**No longer recommended** (due to its lack of specificity)				

Table 2: Serologic testing in case of suspicion of Lyme borreliosis
Adapted from ACVIM consensus update on Lyme borreliosis in dogs and cats.[40]

Presence of antibodies indicates previous or current *B. burgdorferi* infection, but is not predictive of developing clinical signs.

▶ DIRECT DETECTION OF *BORRELIA*[41]

- Culture: needs up to 4 weeks for a bacterial growth. The technique is difficult to perform because the bacterium is poorly accessible
- Direct detection in blood or synovial fluid: very difficult, spirochetemia is transitory or does not develop at all
- PCR: spirochetemia transitory or absent; difficult to detect in the skin (need for a lesion which is uncommon). Results are inconsistent in the synovial fluid. Moreover, the method cannot distinguish between viable and dead bacteria which can lead to false positive results. False negative results are common.

As a result, blood should not be used as a sample.

C. FIRST STEPS TO FOLLOW IF A DOG TESTS POSITIVE

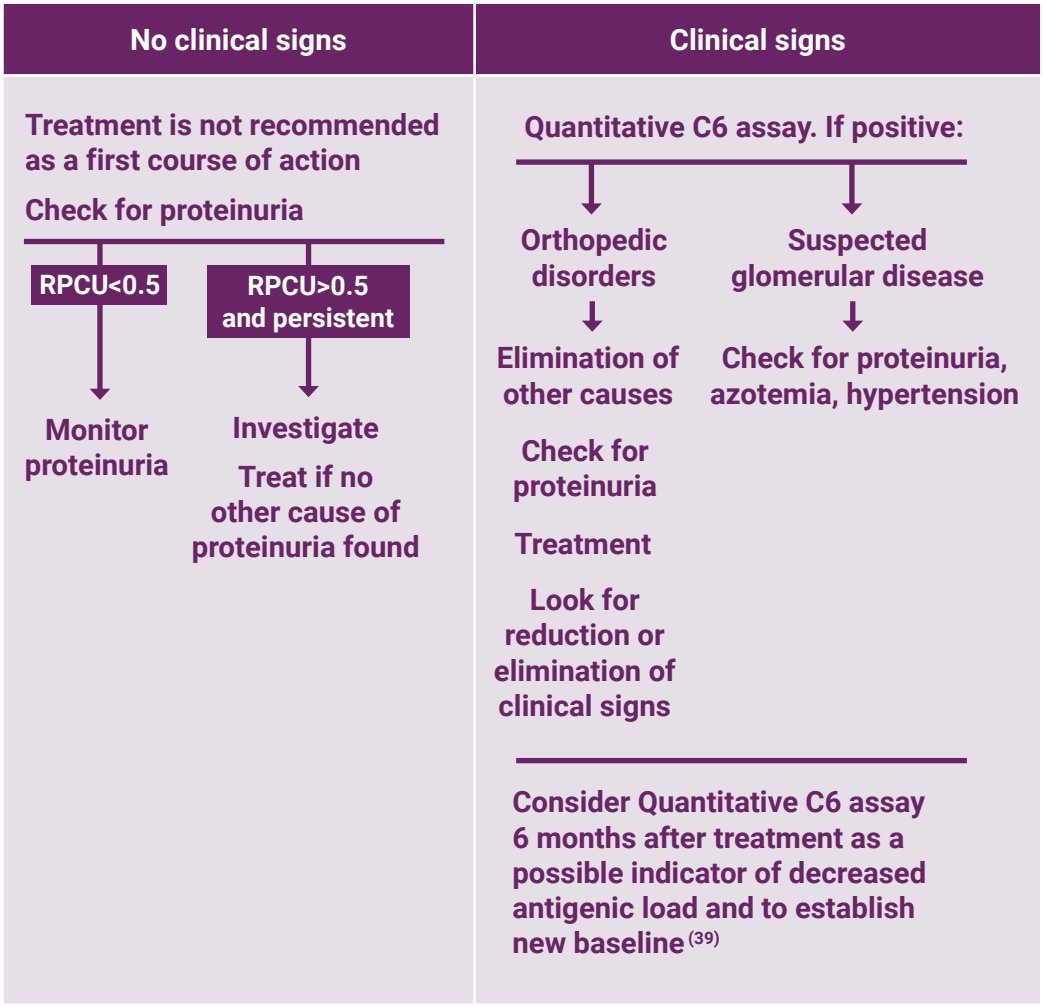


Table 3: What to do in case of positive in-house antibody testing

4. THERAPY

A. ANTIMICROBIAL THERAPY

Doxycycline First choice in case of co-infection suspicion	10 mg/kg SID or 5 mg/kg BID for one month	Clinical improvement in the first week
Amoxicillin	20 mg/kg TID for one month	

Doxycycline may trigger gastrointestinal side effects that are important to mention to the owner.[42]

Other antibiotics are effective, for example in the case of puppies or if doxycycline is not tolerated:

- Minocycline 12 mg/kg BID or 25 mg/kg SID 1 month (minimum)
- Azithromycin 25 mg/kg SID 1 month (minimum) in some countries

Some critical antibiotics should be used in second intention:

- Cefovecin 8 mg/kg SC per 3 weeks (the injection can be repeated once)

B. PAIN MANAGEMENT

Pain management is essential in cases of lameness or nephritis. Even if there is no consensus for the use of nonsteroidal anti-inflammatory drugs (NSAID) rather than corticosteroids you can consider: despite the risk of gastro-intestinal troubles, **NSAIDs are indicated to treat orthopedic pain**. Glucocorticoids should be used only at anti-inflammatory doses.

C. SUPPORTIVE THERAPY

In case of glomerulonephritis, the reaction to treatment may be slower than in dogs with arthritis. See the recommendations of the IRIS Canine Glomerulonephritis Study Group.[43-46]

D. PROGNOSIS

- Clinical signs in most infected dogs are expected to be resolved, but some treated dogs may remain infected without clinical signs.

- Recovered dogs are not protected against re-infection and reactivation of the pathogen is possible.[40]

- Dogs with Lyme nephritis typically have a poor prognosis and often die.

In most cases, canine Lyme borreliosis is not lethal and **most dogs are expected to recover**. Nevertheless diagnosis is complicated, treatment may be prolonged and owners can become distressed when their dog is ill. A strong preventive strategy is justified.

5. MULTI-MODAL PREVENTION

Since no single option provides 100% protection, the prevention of canine Lyme borreliosis should be based on a **multi-modal strategy**:

- general recommendations to reduce the risk of exposure to infected ticks;
- targeting the tick vector with a repellent ectoparasiticide; and
- targeting the *Borrelia* pathogen with vaccination.

A. USE REPELLENT ECTOPARASITICIDES

The selection of an ectoparasiticide to reduce the risk of tick-borne disease is best based on the mode of action. Repellent products with a killing activity provide the appropriate tick control.

Possible effects / Mode of action	**Systemic acting** (some spot-ons + tablets)	**Contact acting topicals** (some spot-ons + collars)
Tick killing	✓	✓
Repellency (preventing tick attachment by contact)		✓

Table 4: Ectoparasiticide effects depend on the mode of action

Recent studies demonstrated there is the potential for *Borrelia* transmission before 24 hours of tick attachment.[1] Since **no minimal transmission time is currently determined for *Borrelia* in dogs**, it is recommended that prevention of infection not be based solely on speed-of-kill claims.

As transmission only occurs after tick attachment, an ideal product should repel and kill the tick **before the bite**. A repellent acaricidal product offers the best protection against tick-borne disease.

Act before the tick bite

For preventing transmission of tick-borne diseases:

- Permethrin-based products are effective [47, 48]
- Permethrin has performed better than fipronil [48]

B. CONSIDER LYME VACCINATION

When tested in field investigations, vaccination was shown to **reduce,** but not eliminate, the risk of infection in dogs. (49)

Recombinant subunit (rOsp A) and bivalent bacterins are available in the USA. They induce anti-Osp A antibodies that, when ingested by a feeding tick, will attack spirochetes in the tick's midgut, halting transmission and reducing the incidence of histological synovial lesions (50).

Multivalent bacterins (Europe) and chimeric recombinant vaccines (USA) are also available. They induce both anti-Osp A and anti-Osp C antibodies. Anti-Osp C antibodies may combat *B. burgdorferi* s.l. inside the host, preventing migration.

Protection seems to be species-specific so highly-effective protection is difficult to achieve in Europe where different species are responsible of borreliosis (51)

There is a lack of expert consensus regarding vaccination. **Be aware of the risk in your local area.** Evaluate your patients' risk factors (living environment, travels, etc.) before considering vaccination in combination with other strategies.

C. REDUCE THE RISK OF INFESTATION

Considering the risk factors:

- Living in, travelling to and outdoor activity in an endemic area
- Outdoor lifestyle
- Hunting
- Walking and exploring in wooded areas
- Living next to wildlife area / suburban area
- Other animals in the household

Recommendations for all dogs:

➜ checking the dog for ticks after each walk

➜ avoidance of highly infested areas

➜ prompt removal of all ticks

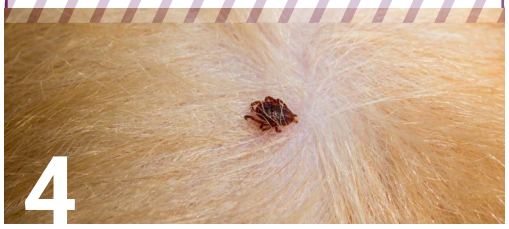
TAKE HOME MESSAGES

Ixodes spp. ticks that carry *Borrelia burgdorferi* can be easily missed in dog's fur. **Stay alert!**

Ixodes spp. ticks are more prevalent in the Northern hemisphere. They are spreading. **Stay up-to-date** on your local risk

One single tick is enough to infect a dog. As dogs are tick magnets and can be heavily infested. **Emphasize prevention**.

There is a potential of transmission **before 24 hours of attachment. Remove ticks as soon as possible**



Lyme borreliosis is a multifaceted disease:

- Exclude other potential causes from the clinical picture
- Clinical diagnosis is impossible, laboratory testing is mandatory

Multi-modal prevention recommended by veterinarians is key to minimize the risk of infection:

- Prevent attachment by using external parasiticides, with a repellent-acaricidal activity
- Reduce the risk of infestation
- Consider vaccination depending on the dog's situation

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