Lyme borreliosis (LB) is the most commonly diagnosed tick-borne disease in Europe, with nearly 85,000 cases reported annually. At least five species of *Borrelia* from the LB complex are known to cause disease in humans, with each species associated with distinct clinical symptoms. Recently, a new species of *Borrelia*, *B. miyamotoi*, has been identified in Europe as the causative agent of tick-borne febrile disease, known as *Borrelia Miyamotoi Disease* (BMD). The primary tick vector for both *B. burgdorferi* and *B. miyamotoi* across Europe is *Ixodes ricinus*, which also transmits a range of other pathogens. Although *B. miyamotoi* is transmitted by the same hard ticks as *B. burgdorferi*, it is genetically closer to the relapsing fever group of *Borrelia* (RF) rather than the LB complex. Despite a growing number of human cases in recent years, our understanding of *B. miyamotoi* distribution, ecology, and epidemiology remains limited.

Due to its shared tick vector with the LB-causing *Borrelia* species, BMD often occurs in the same geographic areas and presents with some overlapping symptoms making differential diagnosis challenging. Current laboratory tests for BMD rely primarily on PCR and serology; however, standardized diagnostic tests are lacking. The short duration of spirochetemia limits PCR detection of *B. miyamotoi* to the acute or relapsing phases of infection. Existing serology-based tests for BMD are still experimental, lacking commercial validation and widespread availability. Recent studies have raised concerns about the sensitivity and specificity of the molecular and serological markers known to date for BM diagnosis, highlighting the need for further research based on new and innovative techniques.

To improve the diagnostic accuracy of BMD serology and facilitate the differentiation of BMD from LB in routine medical practice, it is essential to investigate new, specific antigens with potential as diagnostic markers. This project aims to identify novel diagnostic markers for BMD that could be used in future diagnostic test. By focusing on single epitopes, we aim to enhance diagnostic specificity and minimize cross-reactivity with other *Borrelia* infections prevalent in Europe. Using phage display technology and a 12-mer peptide library, we will select epitopes highly reactive with IgG and IgM antibodies from the sera of *B. miyamotoi* infected mice and humans. Phage display is a high-throughput molecular screening technique with applications in epitope mapping, identifying new ligands for target proteins, and selecting antimicrobial or antiviral peptides for developing diagnostic markers, vaccine candidates, or drugs. Peptide phage display methods have been used to select phages capable of inducing neutralizing antibodies against pathogens such as HIV, hepatitis C, and hepatitis A. To reduce the risk of cross-reactivity with antibodies against other *Borrelia* species, we will focus on short, 12-mer peptides that react with *B. miyamotoi*-specific IgG and IgM antibodies in an experimental model. Using a well-established mouse model of *Borrelia* infection, we will obtain IgM and IgG from mice infected with (i) different *Borrelia* species that cause Lyme disease in humans in Europe, (ii) the relapsing fever-causing *B. miyamotoi*, transmitted by hard ticks as well as (iii) other relapsing fever *Borrelia* species transmitted by soft ticks. Furthermore, we are planning to test the diagnostic potential of selected clones with sera from mice immunized with different infectious dose of *B. miyamotoi* at specific time points of infection (acute and chronic phase of infection) as well as with sera from mice immunized with different *Borrelia* species.

We strongly believe that proposed studies enable us to implement successfully phage display technology to select new, highly immunoreactive *B. miyamotoi* diagnostic markers. This kind of findings will provide the basis for future to explore the clinical utility of selected antigens for early detection of BMD in humans and differentiation between BMD and LB or relapsing fever infections in humans in the endemic regions. Since there is no vaccine and current diagnostic methods are insufficient, developing a new, reliable diagnostic tool will improve diagnostic and, in consequence, therapy process what could ultimately reduce morbidity and mortality caused by BMD.