ORIGINAL ARTICLE

Resistance and rational actions as effective countermeasures in the struggle against Lyme disease

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Abstract

Recent years have witnessed a growing interest in Lyme borreliosis, a diseases transmitted by ticks, which occurs in Poland and many other countries across world. Previously, foresters, hunters, and farmers were considered a high-risk group, however, the increasing physical activity in woodland areas means athletes are also at risk of contracting the disease. The aim of this study is a general review on the possibilities of counteracting Lyme disease, and efforts to combat the disease in the light of recent scientific findings.

Fundamental survival strategies of living organisms include mutual interaction, independent of the elements that form it, cell structure complexity, size, or mass, as well as the ability to select the right countermeasures to vector-borne diseases.

This paper specifically presents the issues of dependence and interrelationships between humans, ticks, and Lyme spirochete through a detailed presentation of the problem of Lyme disease in athletes. Furthermore, this article presents strategies used by the Lyme spirochete to infect the host's organism, strategies used by the arachnid to cause infection and its preferences in choosing a host, and principles of modern microbiological diagnostics and prophylaxis as a tool in the fight against a tick infected with *B. burgdorferi*.

Keywords: agonology • borreliosis • combat strategies • interactions • interrelationships • ticks

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Physical activity – *noun* exercise and general movement that a person carries out as part of their day [39].

Physical training – *noun* same as **physical education** [39].

Contact sport – *noun* any sport in which physical contact between players is an integral part of the game, e.g. Boxing, rugby or taekwondo [39].

Chronic – *adjective* **1.** used for describing a disease or condition that lasts for a long time. Compare **acute 2.** used for describing severe pain [39].

Preventive – *adjective* used for describing an action taken to stop something happening, especially to stop a disease or infection from spreading [39].

Combat sport – *noun* a sport in which one person fights another, e.g. wrestling, boxing and the martial arts [39].

Martial arts – *plural noun* any of various systems of combat and self-defence, e.g. judo or karate, developed especially in Japan and Korea and now usually practised as a sport [39].

INTRODUCTION

For several years, there has been a growing interest in tick-borne diseases – especially Lyme borreliosis, which is currently one of the common diseases transmitted by ticks, both in Poland and across the world. Laboratories perform dozens or even hundreds of tests per week to diagnose Lyme disease. Formerly, ticks were feared mainly in the summer and autumn, but nowadays, due to global warming, contact with ticks is possible even during winter. Every year, there is a large increase in the number of Lyme disease cases, as well as a greater number of ticks in the urban environment. Previously, foresters, hunters, and farmers, i.e., people associated with being in the same environment as ticks infected with *B. burgdorferi*, were considered a high-risk group. However, increasing physical activity outdoors, exercising in woods, parks, and meadows, means athletes are also vulnerable to this disease (but also officers of the border guard, because the forested areas of countries between, which run through the closed borders are a convenient place for increased smuggling activity and other criminal acts). As a result, for many people the "tick environment" is not only a place individual's carry out work-related activities but where they pursue their passions or preventive health care, and are at risk of falling ill.

Lyme disease is an infectious bacterial disease that affects all types of tissues in the human body. It has several stages, may be mild, or chronic and very destructive [1]. Females, males, children, adolescents, adults, the elderly, pets, and wild animals can be infected with Lyme borreliosis. This disease may show symptoms for the first time a few days after a tick bite, but sometimes it cannot be diagnosed until years later. Furthermore, the symptoms of Lyme disease are extremely diverse and similar to other illnesses. During the diagnostic stage, there are often false-positive or false-negative results. What is the difficulty of detecting this disease? The answer lies in the microbiology of the etiological factor causing Lyme disease – in bacteria, *B. burgdorferi* spirochete. Most of the spirochete illnesses have recurrent nature. Lyme disease owes its "cunning" to gram-negative bacteria, which have the appropriate morphological features but also apply molecular survival strategies. It can "anesthetize" the host's immune system alertness and attack at a convenient time [2]. In this case, ticks are the vector of infection, but they also have their methods that facilitate reaching the host. Moreover, ticks have individual preferences, which guide their selection of the hosts. In recent year cases of Lyme infections have increased. Therefore it could be considered that a persistent "fight" between *B. burgdorferi* spirochete (constantly searching for improvement in its methods of invading the host's organism), and humans' trying to prevent it, by simple methods as well as using their immune system, and by the latest diagnostic tools and the capabilities of molecular biology.

Jarosław Rudniański, the founder of the theory of an unarmed struggle and the specific theory of compromise, defined the fight in a more extensive way than his predecessor, Tadeusz Kotarbiński, founder of the general theory of struggle, known as agonology. The word "fight" is used in numerous contexts: for example popular nowadays "fight with the pandemic", or "fight with the elements", "fight with fire", "fight with tuberculosis", but also, due to its increasingly common occurrence, "the fight against Lyme disease". Rudniański stated that the actions called a fight, in which the most specific feature is reckoning with the opponent's countermeasures, fall within a very broad class of actions. This class of actions is characterized as "a reckoning throughout the whole action with strong and varied resistance, located in a constant and independent of the performing entity of movement or material, or the environment, or altogether". This mutual interaction, independent of the elements that create them, is one of the basic elements of the survival strategy of living organisms. It must be noted that it is not the complexity of the cell structure, size, or mass that matters, but the ability to select the appropriate countermeasures to the action of this specific "opponent". Rudniański formulates a rule – as he claims – of the highest extent of generality: "(…) in an action in which the material or the environment is in motion independent of the performer during the entire time of the action while creating strong and varied resistance, acts in such a way, that you can change both the action plan and its method at any moment" [3].

The aim of this study is a general review on the possibilities of combatting Lyme disease with wider use of scientific understanding.

Lyme disease in athletes

The occurrence of Lyme disease in athletes is a rarely discussed issue, both by the scientific community in Poland and internationally [4]. However, some studies have researched the epidemiology of Lyme disease, clinical symptoms, diagnosis, treatment, and prevention of Lyme borreliosis [5].

Athletes who train or compete in the woodland areas of the endemic regions are at an increased risk of getting infected by Lyme disease. Variable clinical symptoms and limitations in testing may lead to misdiagnosis. Early diagnosis and treatment can completely cure most of the athletes with Lyme disease; however, symptoms may appear for months or years, particularly when the diagnosis is delayed [6]. European studies show a statistical dependence between doing outdoor sports in the tick-infected forests and catching the tick-borne infection [7]. On the other hand, early case studies in the United States did not show a significant increase in the risk associated with practicing outdoor sports, yet a study by Smith et al. [8] revealed that people who spent more than 30 hours a week training outdoors in the endemic areas, were tested positive for Lyme disease even 2.5 times more often. Based on research conducted by Vetillard [9], it was found that the risk of contracting with Lyme disease is higher by 6.3% in those athletes, who train in the forested environments.

The risk of sporting activity for infected people varies significantly depending on the location of the infection, its degree, and microbial cause, as well as the intensity and a type of sports discipline. Intensive physical training, and even mental stress, may reduce the organism's defensive response against the infection, and aggravate the infection. What is more, an asymptomatic complication of an infection, such as myocarditis, aggravates by vigorous exercises. The risk factor is generally higher for a trained athlete, especially at a highly skilled level than for an ordinary athlete. Physical effort in athletes stimulates the immune system which enhance defensive mechanism against the infection. An athlete who begins to exercise systematically, gradually strengthens his immune system functions, thus reduces its vulnerability to infections. Intensive endurance training, such as jogging for at least an hour, initially provides a strong stimulation of the immune system, which later changes to the opposite. Predominantly, the period of temporary weakening of the immune function occurs after training or competition, which temporarily heightens the susceptibility to infections [4].

Athletes participating in outdoor training in endemic regions are at increased risk of contracting Lyme disease. Diagnosis should be based on results of clinical and serological tests. However, this can be complicated due to non-specific symptoms, which are common in the sports population, and the limitations of laboratory testing. Clinical signs include skin lesions (especially erythema migrans), neurological disorders, musculoskeletal symptoms, and arrhythmias. The incidence rate of infections appears to be increasing in Great Britain and the rest of Europe. In addition to the common symptoms of Lyme disease – fatigue, joint pain, neck stiffness, and brain fog – Christine Green says, that some athlete-specific symptoms may indicate Lyme disease: "You may notice longer recovery time or more prominent symptoms of delayed onset muscle soreness (DOMS)", says Green. "Strained muscles with prolonged healing time are also something that is often mistaken as a normal part of the training, but could imply something else". The occurrence of injuries in athletes, e.g. during contact fights (especially in contact sports), can lead to various types of joint damage or neurological disorders, which the coach or doctor does not consider as a symptom of Lyme disease.

The sooner the treatment for tick-borne disease starts, the better for the patient, and the worse for the microbes. Only 50% to 68% of patients have a pronounced erythema migrans rash, and only 26% notices a tick that causes Lyme disease. Unfortunately, the majority of athletes do not know that the flu in late spring, summer, or early fall can be a sign of Lyme borreliosis. In the treatment of Lyme disease, properly selected physiotherapeutic techniques can cause a favorable effect on the health and well-being of an athlete. They can effectively relax the muscles, relieving the condition. Joseph Burrascano [11] emphasizes that a patient with Lyme disease will not recover without physical activity, because during exercise, T cells are produced, previously destroyed by the borreliosis. Therefore, to maintain the effects of pharmacological treatment and the diet, it is necessary to perform a set of exercises appropriately selected to the needs and capabilities of the patient. The training in Lyme disease cannot be anaerobic, the workout cannot be done every day either. However, it is important to achieve therapeutic goals such as an enhancement of muscle strength, and also improvement and maintenance of functional ranges of motion in the joints. The implementation of an appropriate set of exercises not only improves the general health of the patient but also contributes to the removal of excess lymph. In Lyme disease, the flow of lymph is significantly impeded due to its enormous amounts produced by the immune system to get rid of the excessive amount of toxins and dead bacteria.

Tools used to combat *B. burgdorferi*

B. burgdorferi has a spiral, irregular shape and is one of the largest spirochetes. It belongs to the group of gram-negative bacteria, although it also has the ability to stain, which is characteristic of gram-positive bacteria [12]. *B. burgdorferi*, due to its cilia, has a great motility, which is an advantage in the context of movement in the bloodstream and tissues. Lyme disease spirochetes have a triple cell wall, and it is distinguished among other bacteria of this type by having an additional mucosa made of proteoglycans on its surface. It has a protective function, providing "a camouflage" from the human immune system [2, 13]. The cell wall gives shape to the cell. It turns out that *B. burgdorferi* can change the structure of the cell wall by the exclusion of the genes that form it. Then, the spirochete takes the shape of a sphere – also called the L-form. It causes major difficulties for doctors and microbiologists in diagnosis [2].

Of all known bacteria, *B. burgdorferi* has the most complicated genome. Molecular studies have allowed researchers to distinguish about 20 of its genospecies. The genome size is relatively small, only 1 x 5 Mb. The main element of the genome is the linear chromosome, on which most genes are located and its size is about 1 Mb. Besides, as many as 21 plasmids can be distinguished in the genome, which is the largest number among other bacterial genomes [14]. The genetic material of the Lyme spirochete is also distributed differently – along the cell wall line, so no free-floating chromosomes in the cytoplasm are observed. Another feature that distinguishes *B. burgdorferi* and irritates the human immune system is its formation of structures called vesicles. The spirochete duplicates the genes and then places them in the cell wall. This particular fragment of the cell wall, after exocytosis, is transferred to the human body along with the vesicle. The cell division of pathogens is usually rapid. It lasts about 20 minutes. It is not the case for *B. burgdorferi*, which has the ability of division from 12 up to 24 hours. This is important during antibiotic therapy, which uses bacteria division to fight them and destroy the newly formed cell walls. No antibiotic can be effective when the bacteria are dormant (between divisions) [2, 15].

B. burgdorferi vs. human – strategies used by Lyme spirochete to infect a host

The processes that enable *B. burgdorferi* to survive in the host organisms occurs at the molecular level. The spirochete proteins are encoded by a series of plasmids and chromosomal genes, and the regulation of their expression is initially assisted by the tick environment, and later by the host – vertebrate. Surface lipoproteins (Osp) – mainly OspA – play an important role in the colonization of the tick intestine. In the beginning, *B. burgdorferi* has to move from the tick's intestine to its salivary glands in order to enter the host. Bacterial migration occurs by the reduction of regulation of OspA, and the incensement of regulation of the OspC lipoprotein. The interaction between a humans and *B. burgdorferi* begins with a tick bite. Then, Lyme disease spirochete encounters the first line of defense of the human immune system, which releases antibodies and chemokines, begins the immediate production of reactive oxygen species, and activates the complement system cascades (C3). *B. burgdorferi* suppresses the immune system by acquiring appropriate surface proteins (CRASP) and Erp proteins together with H or FHL factor (factor-H-like). Factor H affects the regulation of the complement system, while factor FHL, as a cofactor of C3 convertase, facilitates its cleavage and binds OspE proteins. *B. burgdorferi* receives help from the infection vector itself, i.e. the tick, which, due to the SALP15 salivary protein, plays an important role in exterminating the host's defense mechanisms. The SALP15 protein inhibits the activity of T lymphocytes and the binding of the OspC surface protein, which makes *B. burgdorferi* impossible to annihilate [1, 16]. Another salivary protein in ticks is Salp25D, involved in the neutralization of reactive oxygen species produced by neutrophils. These processes allow the Lyme spirochete to transmit and initiate the infection. Once *B. burgdorferi* reaches the inside of the host's organism, bacteria's important feature reveals, which permits to deceive and manipulate the human immune system. It is the ability of Lyme spirochete to polymorphism. As a result, during the period of division, *B. burgdorferi* has the ability to change surface antigens, sequences of coding genes, e.g. OspC, and also to change the structure of the cell wall, which ultimately causes genomic changes, making it more difficult to locate the bacteria as an intruder. Therefore, Lyme disease spirochete gains time to hide in the host and wait for the immune system to stop searching for it [17]. When the immune system encounters a foreign body, it is absorbed by the macrophage to transmit and store information about the cell. The immune system also tries to detect an antigen on the surface of an intruder's cell to produce an attacking antibody. In the case of the Lyme spirochete, the situation is complicated because it hides its antigens in the inner membrane, which makes it invisible to the human immune system and prevents the immune response. Due to the ability to change surface antigens, *B. burgdorferi* distracts the immune system, forcing the production of new antibodies when the infection continues and the bacteria hide better and better [2, 14]. Another survival strategy for Lyme spirochete is genetic recombination, which takes place at the major sequence protein gene locus (vls). Expression takes place in ViSE (vls expression site) and causes the formation of new antigenic variants and changes in DNA sequences [18]. The success of *B. burgdorferi* pathogenesis is also influenced by lateral gene transfer with the usage of bacteriophages. It has been proven that a phage transfers DNA from one spirochete to another. Moreover, frequent transfers of OspC genes within species and between *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii* have been reported. The high frequency of gene exchange between spirochetes enables them to respond effectively to the pressure of selection in the environment [19].

Tick vs. humans – bite strategies used by tick arachnids and preferences in host selection

Nowadays, ticks are well-known vectors of human and animal pathogens. Among the arthropods, they are characterized by the greatest variety of transmitted infectious organisms, such as protozoa, viruses, bacteria, and fungi. The pathogens transmitted by ticks cause a series of tick-borne diseases: babesiosis, anaplasmosis, Q fever, tularemia, but the highest percentage of cases in humans is noted due to Lyme disease [20]. The activity, reproduction, abundance as well as the survival of the tick depends on many biotic factors. Temperature, humidity, and photoperiod have a significant effect on arachnids from this group. Current climate changes are the driving force behind the expansion of ticks around the world [21].

When choosing a host, ticks are guided by certain distinguishing features that suggest them host's qualities. Ticks are primarily stimulated by smells, radiant heat, vibrations, and breath. The odor attractants include ammonia in urine, animal waste, as well as butyric acid and lactic acid, which can be found in sweat. The search for a host is additionally triggered by stimuli including vibrations, sounds, and touch. The vibration of the grass immediately triggers the arachnid's reaction [22]. Studies have shown that the ticks are also stimulated by the electromagnetic waves at a frequency of 800-900 Hz, typical for TV antennas or smartphones [23]. The *Ixodes* genus ticks can crawl around the host even for several hours to find a suitable feeding place. This selection process is supported by the heat and smell that are released by a potential hosts. Ongoing research is carried out determine the preferences of ticks for blood group of a potential host; for example in 2018, in Czechia, an experiment investigating this took place. The results showed statistical preference for particular blood groups among ticks was A>0>AB>B. However, these are only initial findings that require further investigation [24]. From the moment of penetration of the skin, the tick begins to fight for survival, and for the possibility of starting a blood meal [25]. Immediately after attachment, most arachnids secrete a substance that helps them to secure in the wound. Next, the tick salivates and sucks blood from the exact place of the hematoma, which starts to develop after its bite. In the saliva, ticks have numerous anticoagulant and antihistamine compounds that cause tissue lysis and a constant flow of fluid. The success of the meal depends on the secretion of anti-hemostatic, anti-inflammatory, and immunemodulating proteins in the tick's saliva, which inhibit the host's responses that may otherwise reject the intruder. Many species of these arachnids secrete proper proteins regulating platelet aggregation and proteins that degrade the mediators of pain, itching, inflammation, especially histamine and serotonin. The properties and action of tick sputum suggest its active participation in the transmission of an infectious organism between infected and uninfected arthropods living on the same host. This process contributes to the further development of the infection [25, 26].

Modern microbiological diagnostics and prevention as a tool in the struggle against ticks infected with *B. burgdorferi*

Many methods are used to diagnose Lyme disease. Among them, microbiological, serological, and molecular biology methods can be distinguished. Serological tests focus on the response of the immune system. Their principle of operation is to recognize the appropriate antibodies in the patient's serum. In microbiological methods, bacteria are grown in vitro on substratum that provides them with appropriate growth conditions. The methods of molecular biology are the techniques mainly based on the PCR reaction (polymerase chain reaction) and its variants, which allow for the amplification of bacterial genetic material [1, 27]. Therefore, if a Lyme infection is suspected, it would seem that the patient has a lot of diagnostic possibilities. Unfortunately, contrary to appearances, this disease is not so unambiguous when it comes to making an accurate diagnosis.

According to the recommendations, the two-stage serological diagnosis procedure is advised. The first step is to perform an enzyme-linked immunosorbent assay (ELISA). Its task is to detect antibodies against *B. burgdorferi* and then assign them to the IgM or IgG class. This examination selects patients who require further diagnosis. The ELISA does not confirm Lyme disease, it only confirms the presence of a specific class of antibodies to spirochetes [1, 28].

The second stage of the serological diagnostics is the Western blotting, which is used to verify the result of the screening test. It classifies antibodies by size and weight. When antibodies bind to the *B. burgdorferi* antigens, characteristic bands are formed, which indicates a positive result. Each band corresponds to a distinct bacterial protein. The most frequently detected antigens are VIsE – cell wall lipoprotein (*B. afzelii*, *B. burgdorferi*, *B. garinii*), p83 – cell wall-related protein, flagellin – p41, *Borreliella* p39/BmpA, p31/OspA, p30, p25/OspC, p21, p19, p17/Dbpa [1, 29].

Both tests are based on the host's ability to produce antibodies against *B. burgodrferi*. However, there are challenges using this diagnosis method. Lyme disease spirochetes, having an additional mucosa and the capacity to change surface antigens, effectively avoid the immune system response. This type of test can only detect free antibodies that are not bound to the antigen. Immune syndromes are beyond the scope of ELISA and Western blot [18].

In the early stages of the disease, the serological test results may be false-negative due to the inability to detect low levels of IgM antibodies. On the other hand, false-positive results may appear when other viral infections and autoimmune diseases coexist, e.g. EBV [1, 30]. In the case of the serological tests, it is impossible to distinguish between the past and current infection. Even if a single bacterial cell survives after the antibiotic treatment, it may cause the recurrence of disease [2].

Advances in molecular biology have provided enormous diagnostic possibilities. They can be used to confirm Lyme disease, typify and identify spirochetes, identify co-infections between different species of *Borreliella*, as well as other tick-borne pathogens. The arachnid itself can also be material for molecular studies. Modern techniques allow the analysis of the infection of a tick by a pathogen. Molecular methods significantly shortened the time of DNA analysis of *Borreliella* spirochetes, and in comparison to other methods, they are characterized by high sensitivity, easy standardization, and require a negligible amount of genetic material for the research [1].

100% protection against a tick bite does not exist. There are, however, a few basic ways to limit contact with these arachnids. The prevention includes avoiding forested and grassy areas, where ticks patiently wait for the arrival of a host. During trips to nature, it is necessary to wear appropriate light-colored clothing with long sleeves covering as much of the body surface as possible. The light color of the clothes will make any intruder immediately visible, so it can be easily removed before it finds a feeding place. For basic prophylaxis, there are also various tick repellants available on the market – preparations with DEET (N,N-Diethyl-meta-toluamide). After returning from the areas where contact with arachnids was possible, it is necessary to carefully examine your body and evaluate whether any of them got beyond the first line of human defense [31]. A tick bite is not synonymous with getting sick, as not every tick is infected with the pathogen. The second line of human defense is triggered in the moment of contact with the arachnid and is associated with the host's immune response. Initially, the infection causes a non-specific, innate reaction. Over time, the immune system widens the host's responses. After 2-3 weeks, as a result of the humoral response, the early IgM antibodies appear. On average, in 2 months from the moment of infection, they achieve their highest value. When their titer starts to drop, the immune system begins to produce late antibodies – IgG, which persist even for several years [1].

There is no "golden mean" in a person's fight against Lyme disease. Simple and basic prevention, including avoiding the habitats of ticks, especially at the peak of their occurrence, appropriate clothing, and the use of repellent preparations, are the only actions that a person can take lest catching tick-borne diseases. When an infection develops, the immune system responds to the pathogen, trying to neutralize it naturally. People began to use new technologies, alongside the development in many fields of biology and medicine, to be able to win against the pathogen by getting to know its structure, genome, and genes. Understanding the properties and functions of the infectious organism, ticks, which are the vector of infection which of *B. burgdorferi*, allows for the constant improvement of diagnostic methods and the development of effective means of protection against arachnid bites and Lyme infection.

Athletes constitute a high-risk group due to their increased exposure to different people and environments, and sometimes to their lifestyle behavior. Primary prophylaxis can be promoted through appropriate vaccination, good hygiene practices, and a modification of behaviors to minimize high-risk activities. Secondary prevention can be achieved through vigilant surveillance of reported diseases, adequate education, and a limitation of containment in case of occurrence of disease.

CONCLUSIONS

A demanding reader may ask the reasonable question about the legitimacy of publishing this work in a journal dedicated to the broadly understood prophylaxis, health therapy, and personal safety with the use of martial arts and combat sports. According to Jarosław Rudniański's theory of unarmed struggle, especially to the general rule of a controlled environment, provides a partial explanation [3]. However, the key argument is associated with the fact that since 2011 [32], the journal Archives of Budo has been popularizing science as a unique knowledge about innovative agonology (detailed science of struggle) in the global space. The underestimated value of this science (starting with the general theory of struggle, agonology by Tadeusz Kotarbiński [33]) are theorems that can be connoted on the verge of various sciences – even as distant as fiction [32, 34], or sports science [35, 36]. In our opinion, the primary criterion of the application of innovative agonology with other scientific disciplines is a real success in counteracting health or life-threatening situations from the micro to the macro scale (from an individual to the human population), regardless of whether the opponent (the enemy) is a man, an animal, a force of nature, lack of psychomotor competencies of an individual operating under certain circumstances, unreliable technology, or bacteria and microbes invisible to the naked eye [37]. In this thesis, we merely indicate the issue of the rule of a controlled environment, which is so universal that – apart from the source of the threat – can be used by any entity that resists the threat rationally [38].

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