Ticks: born innocent?

We talk to Assoc. Prof. Anna Bajer from the Department of Parasitology at the University of Warsaw, about ticks and how they transmit Lyme disease.

ACADEMIA: **Ticks have been high in the public consciousness for many years because of the perceived threat they pose to people's health. Should we really be afraid of them?**

ANNA BAJER: This is because ticks are disease vectors – organisms which spread pathogens. Two of the diseases they transmit are of epidemiological significance. Tick-borne encephalitis (TBE) is a serious illness, although fortunately it is relatively rare. There is also an effective vaccine, administered in three doses, although two provide almost full cover. A booster should be given every few years to ensure full coverage. Very few people in Poland are vaccinated, although in some countries, such as Austria, the uptake is around 80%.

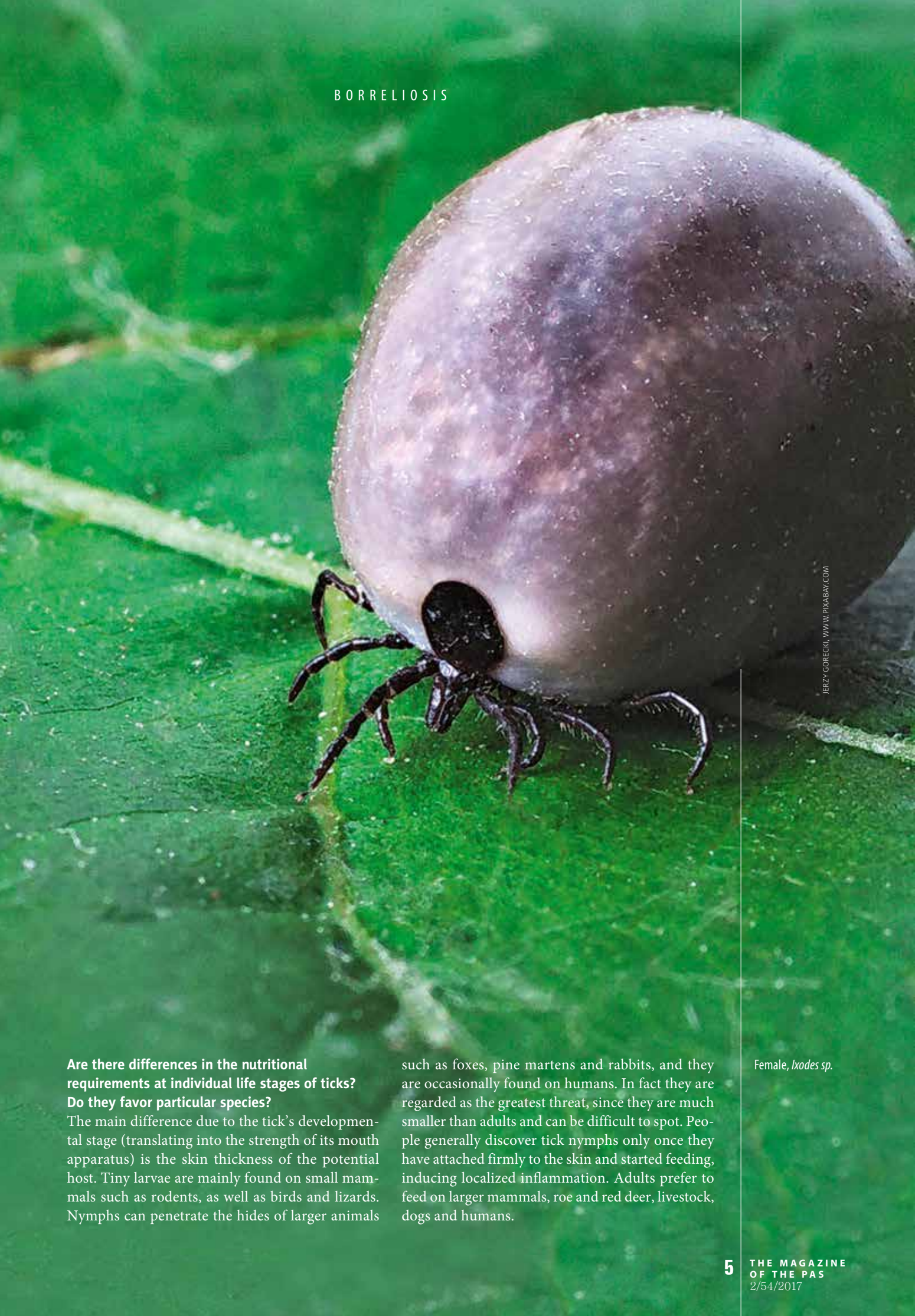
The situation is very different with Lyme disease, isn't it?

Yes, because there is no vaccine and incidence is on the increase in Europe, even in countries with little wild nature, such as the Netherlands.

What's behind this increase?

Tick numbers have skyrocketed in recent years as they expand to new territories. According to experts, the main reason is climate change, although we have yet to collect conclusive data.

Ticks – the vectors or spreaders of Lyme disease – have three developmental stages: larvae, nymphs and adults. Once a tick has found its host and drunk its blood, it detaches itself from the victim and either transforms into the next stage (if it was a larva or nymph) or mates and lays eggs. This means ticks feed just three times in their life cycle and spend the majority of time hungrily awaiting the next host. Their aim isn't to lead long lives but rather to conclude their life cycle as quickly as possible: males die immediately following copulation, while females survive for another two weeks to allow them to lay eggs. According to textbooks, the full cycle takes two to three years, although recent observations suggest that the duration has been reduced by around a year. Contrary to popular belief, ticks aren't resistant to hunger and they aim to complete the cycle as quickly and efficiently as possible, so any conditions which put them in closer proximity to prey are favorable. This certainly explains extended vegetative periods due to increased temperatures, but it's also linked to growing population numbers of wild mammals, in particular roe deer, which in turn is driven by changes to how arable land is being used.



JERZY GORECKI, WWW.PIXABAY.COM

Female, *Ixodes sp.*

Are there differences in the nutritional requirements at individual life stages of ticks? Do they favor particular species?

The main difference due to the tick's developmental stage (translating into the strength of its mouth apparatus) is the skin thickness of the potential host. Tiny larvae are mainly found on small mammals such as rodents, as well as birds and lizards. Nymphs can penetrate the hides of larger animals such as foxes, pine martens and rabbits, and they are occasionally found on humans. In fact they are regarded as the greatest threat, since they are much smaller than adults and can be difficult to spot. People generally discover tick nymphs only once they have attached firmly to the skin and started feeding, inducing localized inflammation. Adults prefer to feed on larger mammals, roe and red deer, livestock, dogs and humans.

Anna Bajer, PhD, DSc
is an Associate Professor at the Department of Parasitology, Faculty of Biology, University of Warsaw and at the AmerLab Diagnostic Laboratory of Parasitic Diseases and Zoonotic Infections. She has spent the last 20 years studying environmental factors influencing parasite infections in humans, focusing on ticks and tick-borne pathogens.

anabena@biol.uw.edu.pl

JAKUB OSTAŁOWSKI

Where are ticks most commonly found?
Contrary to popular belief, they don't lie in wait in trees. They are at risk of drying out and need moisture to survive, so travelling several meters there and back to the ground would be a major problem. In fact they await their hosts on the tips of low-growing green plants or grasses, from where they take regular trips to ground level to rehydrate. This means that hot and dry forests actually harbor few ticks. They are most commonly encountered after rainfall, or in the mornings and evenings when grass is covered with dew.

Which species commonly attacked by ticks are susceptible to Lyme disease?
Ticks feed on a wide range of species, and the only organisms susceptible to the bacteria that cause Lyme are those which don't inhabit forests – including humans.

Where do those bacteria originate from?
The trouble is that forest animals serve as "reservoirs" in that they carry bacteria from the genus *Borrelia*. Different bacterial species favor different hosts; for example *Borrelia lusitaniae* prefer lizards, *Borrelia garinii* live in birds and *Borrelia afzelii* in rodents. It is widely assumed that ticks start off "innocent" – the majority of larvae hatching from eggs aren't carriers of the bacteria even if their mother was infected. Ticks generally become infected with *Borrelia* with their first meal, which is typically the blood of rodents, birds or lizards. Additionally, if several ticks are feeding close to one another on the same host, they can transmit pathogens to one another via pools of blood accumulating under the skin.

Does *Borrelia* cause any disease symptoms in the ticks themselves?
No. The bacteria survive in the digestive system but they can't move to joints or the nervous system because in ticks those organs have a completely different structure than in vertebrates which *Borrelia* are adapted to attack.

There are two theories explaining how the pathogens started coexisting with ticks in the first place. Are ticks the victims of their meals or evil incarnate responsible for generating the disease? The first theory states that the primary source of *Borrelia* is the host, transmitting pathogens to ticks in "contaminated" feed. The theory is supported by the fact that *Borrelia* do not flourish inside ticks; they remain in their digestive tracts and only start multiplying once the tick starts feeding again. The signal to reproduce is probably a sudden increase in temperature and the first portion of blood reaching the tick's guts. It's only then that the bacteria start dividing and move to the host's salivary glands – and thence to the new host.

According to the second theory, it's the ticks themselves that are the source of the pathogens since they have rich microbiotas – complexes of microorganisms, including bacteria, inhabiting their digestive tracts and salivary glands. Of course other animals,

including humans, also have rich floras. While such bacteria are usually harmless to their tick hosts, they can be pathogenic to other organisms. For example, scientists believed for a long time that ticks are vectors for *Francisella tularensis*, responsible for tularemia – a human disease which resembles the plague. However, genetic studies reveal that the strains of the bacteria found in ticks are highly distinct from human pathogenic strains. In fact it turns out that although the bacteria resemble *Francisella*, they are symbiotic microflora (endosymbionts) of ticks. This undermines the hypothesis that such endosymbionts have mutated to become virulent to vertebrates, since no strains of *Francisella* pathogenic to humans have been found in ticks.

You mentioned that *Borrelia* starts multiplying once ticks ingest their next meal. How does this work?

Ticks don't feed in the same way as, say, mosquitos. Rather than gorging themselves, they search for a site with a good supply of blood vessels. They extract blood through a harpoon-like structure known as a hypostome, which is cemented into place. The hypostome damages blood vessels to create a tiny pool of blood under the skin. Ticks also secrete analgesic substances and an anticoagulant to prevent clotting. The entire process takes around two hours, after which the tick starts feeding properly – this is the point at which bacteria and viruses in their salivary glands can be transmitted. Infection with *Borrelia* takes longer since the bacteria need to multiply first. In nymphs, it has been estimated that the migration of bacteria from the digestive tract to salivary glands takes around 36 hours.

Does that mean that removing ticks immediately protects against infection?

It certainly reduces the risk, so ticks should be removed immediately without waiting for medical assistance or specialist equipment. You don't need to worry about the "head" breaking off. The only element which might remain attached is the tick's hypostome and its cement, and there is nothing which can be transmitted from it into the human body, since the salivary glands are located further along the tick's body.

What about putting Vaseline or butter on the tick to suffocate it? Apart from worries about the head breaking off, it's one of the most popular myths about removing ticks.

Absolutely not! A tick vomits as it starts suffocating, which guarantees that any pathogens will enter your bloodstream. People think that dead ticks are easier to remove, but this is simply not the case – their hypotomes are already tightly attached with their cement. Additionally, ticks feed by sucking in blood which thickens in their digestive tracts; they then spit back out any leftover material. It's a cycle of sucking and spitting, so if you try suffocating or burning the tick, it spits more than it sucks, which actually increases the risk of infection.

What's the best way of removing a tick?

Take it off as quickly as possible. Use tweezers if you can – if there is a visible wound on the skin, it's possible for pathogens from the tick to get into the bloodstream. If a tiny fragment of the tick is left behind, simply treat it like you would a splinter.

Because there is a delay before *Borrelia* pass from the tick into the bloodstream, for adults it's enough to check themselves over once a day, for example before showering. Kids should be checked more frequently because their skin is more delicate.

Is it possible to estimate the percentage of ticks carrying pathogens?

Our research points to a figure of around 12%, which is almost identical for urban parks in Warsaw and the wild Białowieża Forest. Around 3% of nymphs and 20% of adult ticks are infected. But because the bacteria only start multiplying after the tick attaches to a host and starts feeding, we have found them in 80% of ticks removed from humans. The difference in numbers is likely due to the fact that the number of bacteria in ticks collected from their environment is below the detection threshold.

Where to get tested

Blood tests and diagnostics on ticks are carried out at the AmerLab Diagnostic Laboratory of Parasitic Diseases and Zoonotic Infections. AmerLab is a spin-off company founded by researchers from the Faculty of Biology at the University of Warsaw and the Medical University of Warsaw.

The lab accepts blood samples collected from associated points or delivered directly by patients. If you have been bitten by a tick, remove it using tweezers and place it in a sealed container of pure alcohol (70–96%). The lab also provides molecular diagnostics to detect *Borrelia* DNA in the patient's blood or in the tick, as well as tests detecting antibodies against pathogens spread by ticks. The cost: between 40 zł and 200 zł, depending on test.

More information on:
www.amerlab.pl
kontakt@amerlab.pl
phone 508 017 683

Are all *Borrelia* strains equally dangerous to humans?

Around a dozen *Borrelia* species have been described, the majority of which are pathogenic. The best known is *Borrelia burgdorferi* responsible for joint inflammation and Lyme disease; the recently described *Borrelia miyamotoi* may cause a relapsing fever syndrome characterized by high temperature, powerful headaches and nausea.

Borrelia are spirochete bacteria similar to those causing syphilis – they can penetrate into almost any kind of cell, with a particular preference for nervous tissue. After they escape from the tick, the bacteria remain in the pool of blood under the skin for a time. If a mark resembling a mosquito bite remains on the skin after the tick has been removed, it may mean that some pathogens have been released.

> One should not put butter on a tick, as it will start to suffocate and may vomit. Then the pathogens it contains will definitely enter the bloodstream.

Is this the infamous rash?

Not yet – it's just a mark left behind the tick's successful feed. The rash starts when the bacteria multiply under the skin and start moving through connective tissue out of the original site. The bacteria remain in connective tissue for around two weeks, after which they find their way into the bloodstream and onwards to organs. If you respond quickly during the first stage of infection and the rash is diagnosed as early-stage Lyme disease, antibiotics eliminate the pathogens quickly and effectively.

Is the rash always present?

Unfortunately not. A type of lump known as a lymphocytoma can be found instead, or there can be no skin symptoms at all. From 50 to 70% of patients present with a rash. According to some researchers, the percentage depends on the exact species; for example, *Borrelia afzelii* causes more changes to the skin while *Borrelia garinii* (found in birds), responsible for a condition known as neuroborreliosis, moves quickly from the skin to the central nervous system where it causes neurological symptoms such as neuropathies, nerve paralysis, headaches, insomnia and even personality changes.

If a rash is not present, it's difficult to say whether the pathogen has made its way into the body, making Lyme disease difficult to diagnose.

Are there ways around this?

Molecular diagnostics provides an answer, although the DNA of *Borrelia* can only be detected in the patient's blood for a short time, between a week and two weeks after the original bite. It can't be detected any earlier because the bacteria are yet to move from the infection site to the bloodstream, and after two weeks they will have moved from the bloodstream to hide from the immune system in tissues and organs. *Borrelia miyamotoi* are easier to detect using molecular diagnostics because they remain in the bloodstream for longer periods.

If you know you were bitten by a tick but you didn't have a rash, it's best to get tested. It is also possible to conduct molecular analysis on the ticks themselves, although detecting pathogens doesn't mean they have been transmitted to the host.

If more than two weeks have passed since the original bite, unfortunately you have to wait and see if you experience symptoms, although it's worth noting that in some cases – especially in young children and the elderly – symptoms can present almost immediately. This depends on the site of the bite. Symptoms can include anything from headaches, fatigue, insomnia, lack of appetite, incontinence, depression, trouble seeing, digestive and heart problems to paralysis.

Researchers believe that each *Borrelia* species has its own preferences: *Borrelia garinii* tends to attack the nervous system, *Borrelia afzelii* causes skin atrophy on the limbs, while *Borrelia borgdorferi* attacks joints. Joints and the brain are highly attractive infection sites, because the immune system works differently there and bacteria can multiply freely. If *Borrelia* end up in any of these locations, the only treatment is high-dose antibiotics.

The current gold standard for detecting infections is serological testing for antibodies against *Borrelia* in the patient's bloodstream. The test is carried out late, around six weeks after the original infection, so in almost all cases symptoms would have already presented. However, it is the only conclusive diagnosis of late-stage Lyme disease. It's perfectly possible that the patient missed the original bite altogether, removed the tick and immediately forgot about it, or that they presented with no rash or other noticeable symptoms. In some cases, patients first start feeling sick in winter and wonder whether they'd contracted Lyme disease in the summer.

Interview by **Agnieszka Kloch**

The art of caution

We talk to **Prof. Joanna Zajkowska, MD**, from the Medical University of Białystok about Lyme disease and how it is treated.

Prof. Joanna Zajkowska, MD is an internist specializing in infectious diseases and epidemiology. She is Deputy Director of the Clinic of Infectious Diseases and Neuroinfections at the Medical University of Białystok.

zajkowsk@umb.edu.pl

ACADEMIA: **A century since the first clinical descriptions of Lyme disease were presented, its diagnosis is no longer a major challenge. So why does the disease have such a bad reputation?**

JOANNA ZAJKOWSKA: Because of the vast amount of information available online and in non-scientific and non-medical media, the truth is frequently mixed in with or even lost in fiction. Although our understanding of Lyme disease keeps improving, outdated myths persist. The once all-encompassing list of symptoms is obsolete and it has been replaced by specific clinical cases. Clinical descriptions of forms of the disease meeting specific criteria define cases such as erythema chronicum migrans (chronic migrating rash). By sticking to definitions, we can compare the incidence of each form of disease in a given area. A rash is a rash, but Lyme arthritis or neuroborreliosis aren't some random, often subjective interpretations but rather they are specific cases which must meet their scientific definition. This is especially important for neuroborreliosis. Many patients would describe it as general fatigue and problems concentrating, yet the specific definition describes it as cranial nerve palsy with inflammation of the cerebrospinal fluid. Progress in medicine reduces the misuse of specific diagnostic terminology.

We also know which tests should be used in routine diagnostics and which ones are largely useless. Tests are continually being improved by competing manufacturers, and the quality and efficacy are progressing rapidly. The most important ones are ELISA tests in preliminary diagnostics and Western blotting (immunoblot) tests confirming the initial diagnosis. The former are highly sensitive: they detect antibodies against the increasingly numerous bacterial antigens responsible for Lyme disease incidence in Europe – home to five *Borrelia* species. The latter are highly specific. In combination with symptoms, all of which are well covered in literature, diagnosing Lyme disease really isn't that difficult.

The number of diagnoses is increasingly steadily in Poland. Are more people really being infected, or are the numbers evidence of the improving quality of tests for Lyme disease?

Environmental and climate changes are causing tick numbers to boom, as confirmed by objective and independent studies. Additionally, changing lifestyles mean more people are exposed to ticks, as outdoor pursuits and sports such as cycling and running become more popular. So, yes, more people are contracting Lyme disease, as indirectly shown by the incidence of tick-borne encephalitis. The neurological disorder, presenting around four weeks after the original tick bite, is routinely registered and can be regarded as an indicator of other tick-borne diseases. The situation is a bit different with Lyme disease. Its incidence is certainly higher and clinical diagnosis is clearer; however, easy access to serological tests means that many people opt for self-diagnosis. However, positive results may in fact indicate a past infection which had been resolved, or simply reveal serological signs of exposure to the bacteria without developing symptoms. Additionally, even the best tests are useless unless we also consider differential diagnosis of symptoms. It's perfectly possible for a patient to test positive even though their symptoms have a different underlying cause. All positive diagnoses need to be reported to the State Sanitary Inspection office, which drives up statistics and – in my view – means the incidence is overplayed by the media.

What treatments are currently recommended in Poland?

European recommendations call for a course of a single antibiotic administered for no longer than a month. The antibiotic and the exact duration of therapy depend on medical signs, age of patient (doxycycline isn't used in children, for example) and the likelihood of coinfection (infection with numerous pathogens during a single tick bite) – in the latter the rash is often accompanied by symptoms such as gener-

al malaise and muscle pains. Any contraindications for individual antibiotics must also be considered. As well as a course of antibiotics, the recommendations also include analgesics and physiotherapy (if required). If or when the patient's condition improves – as shown by a regression of symptoms during the course of therapy – the accuracy of the diagnosis is confirmed. No improvement means a differential diagnosis should be carried out to rule out other potential causes.

The International Lyme and Associated Diseases Society (ILADS) recommends long-term antibiotic therapy even if no symptoms present after a tick bite. Why is this course of action increasingly popular?

It's actually a set of recommendations. Antibiotics should be administered for four weeks after the tick bite, while individual symptoms should be treated until the patient is completely symptom-free, which, unfortunately, may take many months. Doctors have a degree of freedom in their interpretation of the precise course of treatment. The reasons for its increasing popularity aren't clear and I think there are many components, but the phenomenon extends beyond Poland. Many patients experience symptoms without a known cause. There are also cohorts of patients in whom a particular diagnosis – especially neurological – comes with a poor prognosis. People frequently research their symptoms themselves, opting for diagnostics which may not be recommended. Meanwhile experts at ILADS frequently only recommend treatment after they receive complaints from patients or see results of tests which don't meet recommended criteria. If a patient hopes to overturn a diagnosis of a different, serious illness (for example multiple sclerosis), they are likely to agree to prolonged antibiotic treatment. If they still don't experience an improvement, they write it off due to the chronic nature of Lyme disease. At the same time, a regimen of regular antibiotics, improved diet and supplements may bring some relief regardless of the underlying cause. However, in the long term such a strategy can backfire, not just because of the adverse effects of long-term antibiotic use, but more specifically by delaying the correct diagnosis.

> Making a patient doubt their diagnosis, and believe they are not being treated correctly, on the basis of bioresonance is simply unethical.

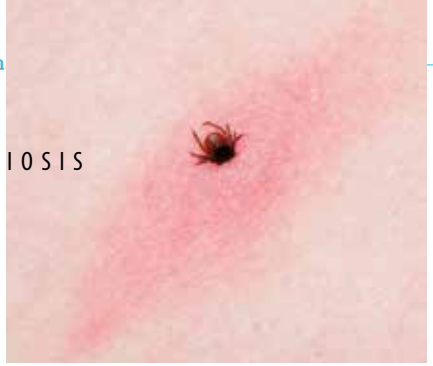
Some doctors believe that bioresonance can be useful in treating Lyme disease. Can you tell us more about it?

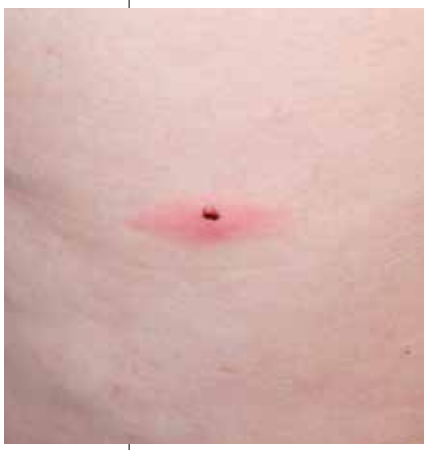
It's a technique based on electromagnetic waves, used for simultaneously diagnosing and treating disorders. There is no evidence that the method is effective, and as a scientist I have never been convinced by results claiming it provides the location and genotype of bacteria. I am not aware of any immunological or microbiological mechanisms which would eliminate selected microorganisms using physical factors inside the body. Perhaps it affects certain metabolic processes; or if it helps the patient and their doctor accept the illness, it may boost the patient's wellbeing. Bioresonance likely has some effect on the body, but it certainly doesn't selective kill bacterial strains. We have around 1.5 kilos of various useful bacteria in our guts and on our skin – more than the total mass of our own somatic cells. If bioresonance really works and destroys bacteria, what happens to them? If this extraordinary method of killing bacteria is effective, why isn't bioresonance used to treat bacterial infections such as diabetic foot, non-healing ulcers or bed sores? Some people who work with Lyme disease make extraordinary statements about it, but none are ever supported by evidence. One of the most dangerous, irresponsible things I've heard is a claimed link between multiple sclerosis and neuroborreliosis. According to someone who works at a bioresonance clinic, "The clinic's experience shows that there is no MS without borreliosis." You may hear that borreliosis resembles MS, and that MS is diagnosed instead of borreliosis. A multiple sclerosis diagnosis is serious and is based on the patient meeting certain criteria such as disease progression over time. A borreliosis diagnosis is based on serological tests, which may be difficult to interpret in autoimmune diseases. But for specialists these difficulties are fairly easy to solve. Making the patient doubt their diagnosis and believe they are not being treated correctly on the basis of the pseudoscience of bioresonance is simply unethical. We see many such patients at our clinic, some with MS, frequently presenting antibodies against *Borrelia*. And you have to take great care to make sure nothing prevents a young MS patient from getting treatment before they develop disabilities. Sometimes borreliosis needs to be treated before administering immunosuppressants for MS.

Patients also often reach for herbal therapies. What are these herbs, and how are they supposed to treat an illness caused by bacteria?

Some of the popular treatments for Lyme disease are individual herbs or their blends, including cistus, teasel and evening primrose.

Herbs can have a positive effect as mild immunomodulators – supporting our own immune system – but it's highly unlikely they have any bactericidal





properties. Additionally, while full mobilization of the immune system is beneficial in most cases, it can be dangerous in autoimmune conditions, so patients should be cautious when taking any supplements which claim to boost immune system function.

Borreliosis is a common diagnosis in the northern hemisphere, mainly in Europe and North America and in a few parts of Asia, with just a few individual cases noted in Africa. Does the treatment vary across continents?

The recommendations are similar. The latest advice in the US recommends a shorter course of treatment than the current standard in Europe. Out of the two main classes of oral antibiotics, penicillin and doxycycline, the latter is recommended because it also treats potential coinfections. In Europe, borreliosis can coexist with anaplasmosis (which can also present on its own); it is a zoonotic disease caused by *Anaplasma phagocytophilum* bacteria, which can present as a rash resembling that found in Lyme disease. Anaplasmosis resolves faster when treated with doxycycline, although it also usually goes away naturally if left without treatment. Borreliosis responds equally well to doxycycline and penicillin, so the former is usually recommended to treat both. In countries where coinfection is rare, it is generally recommended to start treatment with oral penicillin. In neuroborreliosis or infections of the joints, third-generation cephalosporin or ceftriaxone is administered intravenously, and most of the other recommendations are identical.

Tell us about post-Lyme borreliosis syndrome.

Some patients continue experiencing symptoms such as fatigue, low energy and muscle and joint pain after they have completed the course of therapy. Research is being conducted into whether this is actually due to the original infection. The symptoms associated with the syndrome are non-specific, and they are also observed in other infections.

What are the most common complications of untreated Lyme disease?

It's not a complication as such, but late symptoms include recurring joint inflammation (most commonly affecting the knee), probably with an immunological basis, and acrodermatitis chronica atrophicans also known as primary diffuse atrophy. In very rare cases the symptoms can affect the eye, heart or brain. Some of the most common complications which aren't ascribed to Lyme disease are facial nerve paralysis and radiculopathy.

A vaccine was under development in the US in the late 1990s. What happened to it?

The vaccine was based on the OspA antigen. While they are still inside ticks, *Borrelia* present the OspA protein on their cells. As the tick feeds and its digestive tract fills with blood, the bacteria change their antigen coat to one containing the OspC protein. They are both targets for antibodies generated by the vaccine. Unfortunately clinical trials revealed that the OspA antigen is similar to certain structures in the human body, and the vaccine caused symptoms similar to Lyme disease such as recurring joint inflammation. The mechanism could be responsible for recurring knee inflammation in Lyme arthritis. The vaccine failed because it caused an autoimmune response. The OspC antigen found on the bacterial surface at the point when it is ready to move into a vertebrate varies for different *Borrelia* species. There are five genospecies of *Borrelia* occurring in Europe, and they show variability even within a single species. This poses a problem: which protein should we use? Or perhaps a mixture of OspC antigens from different genospecies? In what proportions? Additionally, the occurrence of pathogenic genospecies varies across Europe. All this makes creating a universal vaccine very difficult. Research into a vaccine based on the OspC antigen was conducted in Europe, but the existence of several bacterial species with differences in their OspC protein hindered the studies.

The prevalent opinion in the US is that a vaccine isn't necessary since Lyme disease responds well to antibiotic treatment, so efforts should be diverted into research into vaccines for viral infections for which causative treatment isn't available. The alternative view is that instead of focusing on antigens found in the pathogens, the vaccine should target antigens found in the saliva of ticks to prevent them from attaching to hosts.

INTERVIEW BY **ANNA KILIAN**