

A summary of the beneficiaries experiences from being part of the NorthTick project

1: Which finding of your part of the project was the most important one?

WP3

During the project ticks as well as blood and tissue samples have been collected systematically from rodents, roe deer, migratory birds and pets to gather new surveillance data on tick-borne infections in animals, and to determine the abundance/activity of ticks and tick-borne pathogens in different areas (both geographic and ecological).

Hein Sprong

During the project several tens of thousands of ticks has been collected in the NSR and analysed for the presence of tick-borne pathogens. This information has among other things been used to create so called risk-maps to high-light high risk areas of contracting tick-bites. The study has also shown that some of the tick-borne pathogens are highly depend on the geographic distribution of their reservoir hosts. This is important knowledge for future research to further characterize the association between environmental-animal-human disease risk.

Erik Matthysen

A citizen science project involving the general public was performed to study tick abundance and tick-species in private gardens across Flanders. The response from the public was very good. The data showed that ticks are present in about 40% of the participants' gardens and on various substrates. Interestingly, tick numbers were lower on lawns (regardless of grass height) than on soil, leaf litter or wild vegetation.

We have also studied recreational forests. Here it was found that tick densities were lower along trails than in the forest interior, but prevalences of *Borrelia burgdorferi s.l.* higher, leading to similar tick infection risks in both types of locations. We explain this by the different types of larval hosts that are active in these different locations.

Christina Strube

One very important finding was the nationwide spread of the meadow tick, *Dermacentor reticulatus*, which may transmit potentially fatal canine babesiosis, but also tick-borne encephalitis virus. This finding resulted in updated recommendations for veterinarians and pet owners with regard to tick protection, which was widely communicated in the media, and thereby raised awareness for ticks in general.

Moreover, we confirmed occurrence of tick-borne encephalitis virus in several locations of Lower Saxony, which are not yet officially recognized as risk areas. This information was also made publicly available, among others by being featured in a TV show broadcasted at "prime-time" (8:15 pm) by a regional TV station, with the goal to raise awareness and increase the TBE vaccination rate.

Karen Krogfeldt

As part of a citizen-science project, websites where people can register human-tick encounters were set up by different beneficiaries. These provide novel data on human-tick encounters, that we would otherwise not be able to collect. Furthermore, the websites provide a platform for communicating to the public, launching new projects and providing information on tick and tick-borne diseases.

Neoehrlichia mikurensis was found to be present also in Danish ticks. Therefore we also established diagnostics of *N. mikurensis* in Danish patients through collaboration within NorthTick.

WP4

High quality laboratory analyses are important parts in the clinical diagnosis of tick-borne infections. Different methods are also more (or less) suitable in different clinical settings, patient groups and during different stages of the inflammatory process following infection by a tick-borne pathogen. New tests need to be evaluated against old ones in different settings, and new innovative diagnostic strategies and approaches are required to combat tick-borne disease and to shorten time to diagnosis.

Christine Wennerås & Anna J Henningsson

During the project we have compared different diagnostic tests, both molecular biology based and those detecting antibodies against different tick-borne pathogens in blood samples as well as in cerebrospinal fluid. The utility of tests detecting the early (IgM) and late antibody response (IgG) to *Borrelia* in different patient groups, mainly in Lyme neuroborreliosis in the young vs adult patients has been described. The limitations and strengths with different tests have been identified and communicated between clinical laboratories in the NSR. These findings are of importance to the clinical laboratory and when diagnosing patients.

Alan Bowman

In Europe mainly three genospecies of the *Borrelia* bacterium exists; *B. garinii*, *B. Afzelii*, *B.s.l.s*. We have developed a simple blood test that can inform the clinician or researcher which of the three main Lyme Borreliosis bacterial species was infecting a patient. This may be of importance as to determine which patients needs extra monitoring for the risk of for example Lyme neuroborreliosis which is mainly related with *B.garinii*, whereas *B.afzelii* mainly affect peripheral tissue, although there is some overlap. This may also have implications for the planning of the patients care.

Joppe Hovius

A diagnostic test for *B. miyamotoi* disease, a rare but emerging tick-borne disease in the NSR, has been validated. The method and information regarding it were shared in a scientific publication. The emergence of this pathogen in the Northern hemisphere was investigated and described in a publication, in collaboration between NorthTick beneficiaries. This will facilitate timely diagnosis and treatment of future patients and will fuel future research.

Anne-Mette Lebech, Anna J Henningsson

To cause neuroborreliosis and effects on the central nervous system the borrelia bacterium has to pass the blood brain barrier (BBB). However, to study the effect of different *B. burgdorferi* s.l. isolates on the BBB in neuroborreliosis is complicated. A novel 3D human in vitro BBB organoid model, which at the same time offers a controlled study environment and avoids the use of animal models has been established at Kopenhagen University. In a collaboration this BBB organoid model was evaluated in a first set of experiments and highlighted the organotropism between *B. burgdorferi* s.l. genospecies and their ability to cross the BBB contributing to CNS infection.

Increasing our knowledge on the BBB will be of paramount interest to not only understand the mechanisms driving the development of neuroborreliosis, but for the development of preventative and adjunctive treatments.

WP5

By reducing the time to diagnosis and to treatment, by reducing the unnecessary use of antibiotics and misdiagnosis, the management of tick-borne infections may improve and the number of patients with long-term complaints may in turn be reduced. The beneficiaries have compared their current guidelines for the management in general and a comprehensive overview and consensus document has been summarised. The adherence to national guidelines regarding antibiotic use has been studied and a specialized centralized and multidisciplinary approach for the management of patients with suspected tick-borne infection has been evaluated and proven beneficial.

Randi Eikeland

The process and result of producing the report: Tick-borne diseases in the North Sea Region - a comprehensive overview and consensus of diagnostic, treatment and management strategies, and other tick-borne diseases in the North Sea region, has strengthened the cooperation between the beneficiaries. There is a high degree of consensus on diagnostics for borreliosis, tick-borne encephalitis and other tick-borne diseases in the North Sea region. Some minor differences in treatment strategies was noted as to the choice and duration of antibiotics, and in mode of deliverance - oral or intravenously. These differences reflects different therapy traditions between the countries, not differences in research-based knowledge.

The process of forming the consensus document has been an important output of the work, bringing clinicians, researchers and stakeholders together in fruitful co-operation.

A prototype-App for recognizing tick-borne disease (TBD) skin infections was developed. The intention was to guide patients as when to contact the health-care based on the evaluation of the tick-bite appearance. The work has involved many different professions such as clinicians, data engineers, lawyers and institutions securing civil rights and data-protection issues, and is now continued by a commercial company.

Sally Mawin

The project has provided information about the burden of Lyme disease and prevalence of tick-borne pathogens in the NSR, and even showed that this burden in certain geographic areas is larger than previously thought. In the NHS highland for example, the annual incidence of Lyme borreliosis may be approximately 5 times higher than expected.

Anne-Mette Lebech

By evaluation of data from 215 patients referred to the specialized Unit of Tick-borne diseases at Rigshospitalet (collected under other funding) it was found that 34% of patients suspected of a tick-borne infection actually suffered from another disease. This underscored the complexity in diagnosing Lyme borreliosis and the importance of ruling out other diseases through careful examination. This study is a good proof of the benefits of a multidisciplinary approach in managing patients referred to clinical care under the suspicion of tick-borne infection and who often present with complex symptoms. The approach has a potential of bringing down the number of patients with suspected long-term complaints from a suspected tick-borne disease with 34% by finding the correct diagnosis, and to give advice for optimal management based on current medical standards.

Also, the presence of *N. mikurensis* DNA in retrospectively collected plasma from a well-characterized cohort of 239 immunocompromised patients was investigated. *N. mikurensis* DNA was detected in 3/239 patients, all of whom primarily had a hematological disease. Thus, testing for *N. mikurensis* in immunocompromised patients should be considered. Future awareness of *N. mikurensis* among physicians is important to diagnose neoehrlichiosis and to avoid diagnostic delay in this group of patients

To delineate current practice in antimicrobial management of adults with neuroborreliosis and to prioritize future trials needed to optimize treatment recommendations, a questionnaire-based survey was performed among specialists in infectious diseases and neurology based in Norway, Sweden, and Denmark was carried out between October 2021 and February 2022. In total, 290 physicians participated in the survey. In Sweden, all respondents preferred treating neuroborreliosis with oral doxycycline for 10-14 days, whereas 5% in Norway and 19% in Denmark still treat neuroborreliosis with IV antibiotics for the entire treatment course. Following this trial a road-trip to spread the results aiming to increase adheres to guidelines and harmonize the administration route of antibiotics between countries were undertaken. This is important to avoid over-use of antibiotics in the NSR and to highlight the importance to regularly evaluate adherence to treatment guidelines.

2: What did your cooperation in the NorthTick project mean for your activities and organisation?

The participation in NorthTick has, and will be, extremely important for tackling tick-borne diseases within the NSR and will unequivocally benefit the general population, especially as tick-borne diseases are spreading to new areas as a result of climate change. The project has been very important for the participating beneficiaries, to expand their network, initiate new collaborations, but also for the students and co-workers of the research groups, as well as

for the role of National Reference Laboratories for the diagnosis of *Borrelia* and other tick-borne bacteria and as Lyme borreliosis expertise centers active within the NSR. With the help of the NorthTick partners we could realize analyses on occurrence and spread of tick-borne infections which would many times have been difficult or not feasible alone.

A good example of this is that during the NorthTick project period, tick borne encephalitis as well as emerging pathogens such as *Neoehrlichia mikurensis* and *B. miyamotoi* and Babesia has been detected in new geographic areas not earlier reported. The importance to consider emerging pathogens, especially *N. mikurensis*, in special immune compromised patient groups has also been evidenced. This knowledge is important to the general practitioners and specialized clinics phasing these patients.

The relationships and network that has been established will allow us to continue to work and collaborate with partners within the NSR who has expertise in the different tick-borne infections, which will aid the further development of surveillance, diagnostic and patient management pathways within our respective countries. They will form the basis for future collaborative research and activities and will be guiding in the much needed concerted action at a European level to combat tick and tick-borne diseases. The knowledge and material exchange across institutions and organizations has made the setup of protocols, validation of different types of diagnostic tests and projects possible that would otherwise have taken much longer to complete.

Taking part in this highly multidisciplinary project helped to broaden our perspective on tick-borne infections and allowed us to extend our fundamental research on ecology of ticks and tick-borne infections into more applied topics. Through NorthTick we have for example also gained insight in the role of animals such as hedgehogs as an important link in tick-borne disease scenarios, illustrating the interdisciplinary focus and a "One Health" perspective of the project. Moreover, involvement in NorthTick strengthened the relationship with the general public, regional and national public health authorities, and also increased the visibility of the research and institutions both nationally, as well as abroad and in the form of many joint scientific publications and reports that are important for clinicians and laboratories in the management of tick-borne pathogens. The resulting network forms the basis for future collaborative research and activities and will be guiding in the much needed concerted action at a European level to combat tick and tick-borne diseases

During meetings, workshops, and conferences data and information on the topic has been shared between the project beneficiaries. One major output of the project is also reports and scientific publications made available to the wider scientific society. We have jointly produced many articles, some of which were published in very high-impact journals such as Lancet Microbiology and the British Medical Journal. Finally, reaching out to the general public about ticks, Lyme borreliosis and other tick-borne diseases is also of key importance. In that regard, we have written a book on ticks and tick-borne diseases and produced two movies (in Dutch and in English) about chronic symptoms attributed to Lyme borreliosis for a laymen audience to raise awareness and to correctly inform and empower (future) patients. Activities at scout camps and the citizen science projects also represent a few examples on how the beneficiaries have worked to increase the interaction with the general public. The project has also gained interest in the more population scientific literature, local radio and TV as it covers a subject many people can relate to and certainly have feelings about.

3. Do you consider that the NorthTick project has contributed to the overall goals set up in the application, e.g. to delimit the suffering of people affected by tick-borne diseases and to decrease the number of affected people? If so, how and in what way?

Absolutely!

NorthTick's extensive findings on the distribution of disease-carrying ticks throughout the North Sea region including in less-well recognized areas of residential gardens and public recreational areas in cities involved the help of thousands of members of the public. These activities have led to better understanding of the risks and the consequent mitigation steps for local, regional and national public health bodies, NGOs, academics and the general public. Such information and its dissemination will lead to a decrease in the number of people affected by tick-borne diseases.

Participation in the NorthTick project has heightened awareness of all of the tick-borne diseases present (and emerging) within the North Sea Region. Our results also highlight the importance of investigating patients referred for tick-borne diseases carefully and to rule out other conditions for their symptoms. If each partner can raise awareness of these diseases and pathogens within the public and medical populations and continue to further develop surveillance, diagnostic and patient management pathways for each one then the number of people suffering from long-term effects as a result of tick-borne diseases will only decrease.

We have made advances towards even better laboratory analyses with higher quality, as well as more targeted use of particular analyses. Thanks to the validation and up-take of commercial as well as the distribution of in-house developed analytical protocols between beneficiaries, it has been possible to diagnose patients for, for example *Borrelia miyamotoi* at the Swedish reference laboratories for tick-borne infections. A new tick-borne pathogen, *Spiroplasma ixodetis*, has been identified thanks to the network of NorthTick. Additionally, more diagnosed neuroborreliosis cases may prevent potential life-threatening thromboembolic event and more diagnosed cases of *B. miyamotoi*-meningoencephalitis may prevent patients from having impaired cognitive functions.

Taken together all the activities within NorthTick have in different ways contributed to delimit the suffering of people affected by tick-borne diseases and to decrease the number of affected people. For example by increased awareness of tick-borne infections, and how to take preventive measures, by improved laboratory diagnostics, by the comprehensive overview and consensus report describing diagnostic, treatment and management strategies, and other tick-borne diseases in the North Sea region, by the evaluation of a multidisciplinary management approach as well as outreach activities and scientific publications that constitute important support for clinicians and laboratories in the management of patients with a suspected tick-borne infection.

Reported by each beneficiary:

C Wennerås

We've diagnosed patients in the Västra Götaland region with *Borrelia miyamotoi* and identified a new tick-borne pathogen, *Spiroplasma ixodetis*, thanks to this network.

A Bowman

NorthTick's extensive findings on the distribution of disease-carrying ticks throughout the North Sea region including in less-well recognized areas of residential gardens and public recreational areas in cities involved the help of thousands of members of the public. These activities have led to better understanding of the risks and the consequent mitigation steps for local, regional and national public health bodies, NGOs, academics and the general public. Such information and its dissemination will lead to a decrease in the number of people affected by tick-borne diseases.

S Mavin

Yes! As mentioned in 2., the distribution of tick-borne diseases differs throughout the NSR. Participation in the NorthTick project has heightened awareness of all of the tick-borne diseases present (and emerging) within the NSR. If each partner can raise awareness of these diseases within the public and medical populations and develop surveillance, diagnostic and patient management pathways for each one then the number of people suffering from long-term effects as a result of tick-borne diseases will only decrease.

H Sprong

The awareness and general knowledge of the presence of TBP, especially the "other TBPs" in all regions has increased among civilians and medical/health professionals. Also, I think that advantages have been made towards the diagnosis and consequently the treatment of TBDs. To this end, this has resulted to a better medical help to civilians in the NSR.

E Matthysen

I am confident that the various publications and outreach activities have helped to raise awareness among the public and increased people's knowledge on prevention. Significant improvements were also made in strategies for surveillance, diagnostics and treatment.

A J Henningsson

This is of course difficult to assess in actual numbers but we do believe that the NorthTick collaboration has led to publications that constitute important support for clinicians and laboratories in the management of patients with suspected TBDs

C Strube

Communicating the above-mentioned results to the public certainly has raised awareness for ticks and TBDs and thereby contributed to decreasing the number of affected people.

R Eikeland

Improved diagnostics and treatment contributes to delimit the suffering of people affected by tick-borne diseases, and thus probably also contributes to decrease the number of persons getting prolonged symptoms after tick-borne infections.

Increased awareness of the less known tick-borne infections and diagnostics among clinicians and clinical microbiologists will reduce long-term tick-borne infections associated with complications. Typically, more diagnosed neoehrlichiosis cases may prevent potential life-threatening thromboembolic event and more diagnosed cases of *B. miyamotoi*-meningoencephalitis may prevent patients from having impaired cognitive functions. Serum *Borrelia* IgM is as common when *Borrelia* is unlikely, as when infection is possible. These data suggest that IgM testing with poor indication, often leads to uncertainty rather than correct diagnosis. IgM in CSF occurs more often in children, and seem to have a stronger correlation to actual infection.

The NorthTick project has contributed to cooperation across national borders and has helped to shed light on the topic from different points of view.

A-M Lebech

Definitely. Our results highlight the importance of investigating patients referred for Tick-borne diseases carefully and rule out other conditions for they symptoms.

Our data have demonstrated the presence of *Neoehrlichia micurentis* in Danish patients. We have now established diagnostics and increased awareness of this condition, that was not known among physicians in Denmark. During the last 9 months > 6 patients have been diagnosed in the clinic and cured after antibiotic therapy.

J Hovius

The knowledge, products, information and collaborations that came from the NorthTick project have certainly contributed to lowering the impact of Lyme borreliosis and other tick-borne diseases in the NSR by raising awareness among the general public and by providing in-depth insights, novel information and new tools to clinicians and scientists assisting in better prevention, diagnosis and treatment of tick-borne diseases

K Krogfelt

We now have an improved diagnostic for *Neoehrlichia mikurensis* which leads to improved treatment.

We also have better knowledge of distribution of tick-borne diseases across the participating countries, e.g. through the different citizen-science based websites.

Finally, through collaborations with the participating beneficiaries we were able to get an overview of the different levels of surveillance for borreliosis and tick-borne encephalitis in the participating countries. Clarifying the difference in surveillance can help future collaborations and make comparisons between countries easier to navigate.