Department of General Practice	**2nd Protocol**, 1st edition, 15 August 2013 concerning **'Tick-borne diseases in Norwegian general practice'**, Part 2 **A randomised, controlled clinical trial of phenoxymethylpenicillin, doxycycline and amoxicillin in the treatment of erythema migrans with one-year follow-up.** EudraCT no. 2010-023747

2a. Sponsor
University of Oslo (UiO), Institute of Health and Society (HELSAM), Department of General Practice, Antibiotic Centre for Primary Care (ASP), Postboks 1130, Blindern, NO-0318 Oslo, NORWAY

2b. Monitor
Hege Øvergaard at GCP support. The study is monitored during the period 30 May 2013 to 1 March 2015. uxheve@gmail.com

2c. Protocol manager
PhD candidate *Knut Eirik Eliassen*, ASP, Department of General Practice, HELSAM, UiO, Postboks 1130, Blindern, NO-0318 Oslo, NORWAY. k.e.eliassen@medisin.uio.no

3. Principal investigator
Professor *Morten Lindbæk*, ASP, Department of General Practice, HELSAM, UiO, Postboks 1130, Blindern, NO-0318 Oslo, NORWAY. morten.lindbak@medisin.uio.no

The co-investigators are made up of approximately 60 GPs, each of whom will provide three, six or nine patients for the study.

4. Background
Global warming, increased numbers of deer and natural overgrowth of former pastureland are just some of the factors that are leading to a growing population of ticks and the diseases they bring with them (1). The most common disease transmitted by tick bites is caused by the *Borrelia* bacterium (2–6) and is called Lyme borreliosis, but cases of tick-borne encephalitis (TBE) and anaplasmosis have also been registered in Norway (7–13).

Lyme borreliosis
Lyme borreliosis is caused by the spirochete *Borrelia burgdorferi sensu lato*. This is divided into at least 13 species, four of which can cause disease in humans: *B afzelii, B garinii, B burgdorferi sensu stricto* and *B spielmanii* (14). There is no vaccine against *Borrelia* infection. Data from the Norwegian Surveillance System for Communicable Diseases (MSIS) show that the four counties of Telemark, Vestfold, Aust-Agder and Vest-Agder are the most exposed, closely followed by Sogn og Fjordane and Møre og Romsdal. In 2009, there were 5.8 cases/100,000 inhabitants nationwide. This was an increase of 2.1 from 2000. The incidence in these counties was 15–35/100,000 inhabitants/year. Overall, this indicates an increase from 150

to 350 cases per year in Norway, although only disseminated diseases were registered. Numbers peaked in 2008 at 346 registered cases/year compared to 289 in 2010. However, it is unlikely that this indicates a real decline in the incidence of borreliosis in Norway. Borreliosis has many clinical manifestations, primarily from the skin (erythema migrans (EM)), the nervous system (Lyme neuroborreliosis with meningitis or paralysis) and the joints (Lyme arthritis) (15). Apart from cases of EM, the borreliosis diagnosis is based on a combination of tick bites in the patient history, symptoms, clinical examination and microbiological testing. The patient history and symptoms are not always very clear, and borreliosis is often just one of several possible diagnoses.

Erythema migrans

The incidence of the most common form of borreliosis, EM, is not registered in MSIS and is therefore unknown. Data from southern Sweden indicate approximately 500 EM cases/100,000 inhabitants/year (16). EM is a clinical diagnosis that is diagnosed and treated without further laboratory tests. In Norway, it is mostly general practitioners who identify EM, either at a GP surgery or at an out-of-hours clinic. EM does not exist as a separate diagnosis in the diagnosis system used by general practitioners – the International Classification of Primary Care (ICPC-2). This means that EM can be obscured in several different skin and infection diagnoses. Due to public fear of tick-borne diseases, many people tend to consult their GP after a tick bite, even in cases where no infection is present.

Treatment

There is consensus in the Nordic countries that phenoxymethylpenicillin (PcV) is the first-line treatment for both children and adults, as reflected in guidelines issued in Denmark in 2006 (15), Sweden in 2009 (17) and Norway in 2008 (18). Nevertheless, several critics believe that EM should be treated with more broad-spectrum antibiotics in order to prevent neuroborreliosis. The theoretical postulation relates to PcV's inability to penetrate the blood-brain barrier and to permeate between cells (19, 20). Both doxycycline and amoxicillin are presented as alternatives. Early in the history of Lyme disease, spirochetemia was detectable in EM (21). Recent Norwegian research on neuroborreliosis (22) shows that around half of the patients have not had a recognised EM prior to the neurological symptoms. However, there is no evidence that narrow-spectrum antibiotics carry a higher risk of systemic disease. A retrospective analysis of EM patients treated with penicillin and doxycycline respectively, showed no difference in treatment failure (23), and a systematic review of several clinical trials has shown that PcV performs just as well as broad-spectrum medications (24). However, these have greater ecological side effects in the form of increased antibiotic resistance. Recent research suggests that shorter cures and fewer broad-spectrum antibiotics are just as effective as long-term treatments and combinations of several types of antibiotics at the same time (25).

Microbiological diagnosis

The most widely used and most suitable diagnostic laboratory test for borreliosis is demonstrating antibodies to *Borrelia burgdorferi sensu lato* in serum. The current recommended method in Norway is a two-step procedure in which samples that test positive in a primary enzyme immunoassay (EIA) are further tested in a more specific EIA (26). Whichever test is used, the general practitioner is often faced with a problem: a patient with diffuse symptoms and laboratory results in which false positives and false negatives are common. A rational approach to the diagnosis and treatment of borreliosis must be based on a combination of the patient history (focusing on tick exposure and tick bites), symptoms, clinical findings and the ability to interpret the serological results in various stages of the disease. Increased knowledge about the incidence of the various disease manifestations, the

epidemiological situation locally and nationally, and the incidence of IgG and IgM in the background population is therefore important. In samples from patients with suspected borreliosis, 20% in Sogn og Fjordane county tested positive for IgG and 14% for IgM. In blood donors in the Agder counties, 18% tested positive for IgG (27). Although there is no established analysis, as EM is a clinical diagnosis, a Polymerase Chain Reaction (PCR) in a punch biopsy of skin from EM can identify both the presence of the *Borrelia* spirochete and the species of the *Borrelia* bacterium. Since the different species of the *Borrelia* bacterium have different affinity for joints, the nervous system and skin, national guidelines cannot solely be based on international studies. The Norwegian proliferation of subgroups is unknown. In Sweden, 76% *B afzelii* and 24% *B garinii* were found in EM, while the USA has almost 100% *B burgdorferi sensu stricto* (28).

Tick-borne encephalitis (TBE) and anaplasmosis

The TBE virus can be found in the salivary glands of ticks, which means that anyone bitten by an infected tick can immediately be infected. The infection is normally subclinical, but severe neuropsychiatric symptoms and sequelae occur in approximately 10% of those infected. There is no treatment, and TBE can be fatal. In the period 1998–2008, a total of 38 cases were registered in Norway. Twenty-nine of these were infected in Norway. By 2010, this had increased to 44 confirmed domestic cases, and the Norwegian Institute of Public Health (NIPH) now recommends the TBE vaccine in some municipalities on the southern coast of Norway, and to individuals who are historically prone to tick bites (7). Human granulocytic anaplasmosis is a tick-borne disease caused by the *Anaplasma phagocytophilum* bacterium. It is known as a disease of dogs, cats, cattle and sheep, and is called 'sjodogg' in Norwegian. In recent years, it has been found in humans, but is still rare (11–13). The course of the disease is normally subclinical in humans. Sero-epidemiological studies conducted in Telemark have shown 10% seropositivity for anaplasmosis antibodies in patients with *Borrelia* antibodies (11). The significance of this for humans is unclear, but an exacerbated course of *Borrelia* infection through co-infection with anaplasmosis is postulated.

Subjective health complaints and post Lyme syndrome

Borreliosis has received considerable media coverage in recent years. The Norwegian Directorate of Health appointed a working group to give a recommendation for diagnosis and treatment in the autumn of 2009. The group's report serves as a temporary guideline for doctors working with the diseases. A recommendation was given for a laboratory diagnostics strategy, but no consensus was reached for the treatment of EM (26). Public interest is in no small part due to the widespread perception that *Borrelia* infection can play an etiological role in several chronic conditions, from non-specific health complaints, fatigue, muscle pain and headaches to severe neurological and cognitive failure, including fibromyalgia and symptoms of chronic fatigue syndrome (CFS) or myalgic encephalitis (ME). This concept, collectively known as post Lyme syndrome, is controversial among doctors and is difficult to diagnose. A scoring system, known as the *Subjective Health Complaint (SHC) score* has been developed to measure people's perceptions of their own health complaints. This validated scoring system is widely used in terms of the Norwegian population, and data from a *Borrelia*-exposed group can therefore be compared with the background population (29).

5. Purpose, research questions, material and methods

In order to identify the optimum method for conducting a clinical trial to compare the effect of three different antibiotic regimes for the treatment of EM, a summary report on the subject (35) was prepared in collaboration with the Knowledge Centre for the Health Services (the Knowledge Centre). Systematic searches were carried out to find knowledge-based guidelines,

systematic overviews and randomised controlled studies for the treatment of EM in adults. The essence of the discussion about the correct treatment of EM is, in addition to the improvement of symptoms, whether the risk of disseminated diseases, e.g. neuroborreliosis, increases when using a narrow-spectrum antibiotic such as penicillin. This is mainly a theoretical postulation and is not demonstrated in earlier studies. The conclusion of the summary report is that for EM, with the main endpoints of duration of rash and recording of side effects and sequelae, and – for some studies – therapy failure/disseminated disease, the three to four medications that are relevant to Norway have a relatively similar effect (*35*). These are *amoxicillin, doxycycline* and *phenoxymethylpenicillin* (PcV). Macrolides fair slightly worse, but with *azithromycin* being better than *erythromycin*. *Cefuroxime axetil* and the addition of *probenecid* are not relevant for use in Norway.

6. Outcome measures and endpoints

The following *primary outcome measures* are used to improve epidemiological knowledge, diagnosis and treatment of tick-borne diseases in general practice:

- Compare three antibiotic regimes for the treatment of EM in general practice.
- Compare subjective health complaints in EM patients at time of diagnosis and after one year.
- Compare subjective health complaints in EM patients and blood donors.
- Map the incidence of TBE antibodies in EM patients.

Secondary outcome measures:

- Correlations between seropositivity, demographic data, risk factors and tick bites.
- Register the subgroups of *Borrelia* bacterium in EM patients through PCR analysis of a punch biopsy.
- To what extent are national guidelines on the treatment of EM followed?
- Compare the antibody level for *Borrelia* in EM patients and blood donors.
- Improve clinical and microbiological diagnostics of patients with possible borreliosis.

(The blood donors referred to are studied in a parallel project by Reidar Hjetland, head senior consultant, Department of Microbiology, Førde Central Hospital. The same questionnaire was used for EM patients and blood donors in order to enable comparisons. (Tick-borne diseases in Sogn og Fjordane, REC Central Norway 2009/950 and 2009/2248)).

The main endpoint is the *duration of EM*, but subjective health perceptions and parameters for serology, side effects and sequelae are also mapped in order to establish as clear a picture as possible of the patient's state of health after infection. The variables recorded in our study correspond to those used in other clinical studies of EM, but these three medications have never previously been compared in the same study and such a study has never been conducted in general practice.

Sequelae and side effects are recorded in the same way as in several international RCTs for various antibiotic treatments of EM (24,32). Factors such as tiredness, unwellness, headaches, joint pain, neck stiffness, fever, palpitations, muscle pain, sore throats, dysesthesia, dizziness, nausea, chest pain, diarrhoea, chills, flushing and coughing are recorded. In cases of multiple EMs, neurological symptoms or arthritis, therapy failure is considered. The GP performs additional investigations and makes referrals/admits patients to hospital as needed regardless of the study, and this is recorded. Patients receive a one-year follow-up to check for delayed symptoms. The cohort may also receive a five or ten-year follow-up, but this is not part of the study.

7. Design

The study is randomised, controlled and single-blinded. Double-blinding is not possible as the possibilities for handling and repackaging penicillin are extremely limited. The dose of 1.3g of phenoxymethylpenicillin means that any over-capsulation of study medicine for blinding would entail the patient having to take two capsules three times a day, with a dummy capsule being used for the other treatments. This method would have been very costly and not feasible within the budget framework of the project.

8. Calculation of sample size

Figures from southern Sweden indicate an incidence of EM in 5 cases/1000 inhabitants/year (28) in endemic areas, and our preliminary results indicate that figures for Norway are of the same magnitude. With an average of 1200 patients on each GP's list, they will thus see five or more EM patients each season in southern Norway. The study will be conducted in the same four counties as in part 1. The calculation of strength is based on the main endpoint: duration of EM. Size and colour are also recorded. In the analysis, we will compare the duration of EM, sequelae, side effects, subjective health complaints and antibody production for the various antibiotic regimes, and compare the cohort at the time of diagnosis and one year later. The distribution of subgroups of the *Borrelia* bacterium is unknown in Norway, but is assumed to be similar to the distribution in Sweden. Our figures are therefore based on the Swedish data. Median duration of EM from start of treatment is eight days, but with a spread of 1–35 days. In order to aim at a normal distribution, the power calculation is based on log-transformed values. A difference in duration of EM of two days is considered clinically significant. On a logarithmic scale, with a standard deviation of 0.69 (the standard deviation in a log scale is assumed to be 1), and based on the given diffusion, with a significance level of 0.05 and 90% strength, we need 46 patients in each group (30). Since we are going to compare three groups, we should consider a Bonferroni correction and use a significance level of 0.017, and therefore include 58 patients in each group. In addition, the intra-group correlation coefficient (ICC) for treatment measurements for GPs is normally lower than 0.05 (31). With four patients per doctor, this will at worst give a design effect of 1.15 (31), thus increasing the sample size by 15% to 67 patients in each group. In order to allow for dropout, we will recruit 75 patients to each group. This gives three groups where n = 75; 225 patients in total. We will ask 60 GPs to recruit a minimum of three patients each in the period 1 June to 1 November 2011 and, if necessary, to continue in the 2012 season. The patients are included when the doctor gives the clinical diagnosis of EM. If the diagnosis is uncertain and the doctor chooses, for example, to see the patient again after two days, inclusion should be delayed.

(By the end of the 2012 season, we had included 136 patients and decided to continue for another season. Thirty-one of the original doctors are also participating in the new season. This was submitted to and approved by the Regional Committees for Medical and Health Research Ethics (REC)).

9. Inclusion and exclusion criteria and limitations in concomitant medication

Inclusion criteria:

1. The doctor diagnoses *erythema migrans* based on the anamnesis and clinical examination.
2. The patient is over the age of 18 and competent to give consent.
3. The patient is informed about the study orally and in writing, and consents in writing.

Exclusion criteria:

1. Minors and patients not competent to give consent.
2. Known substance abuse or dementia.
3. Known allergy to one or more of the study medications.
4. Pregnancy.
5. Antibiotic treatment in the 14 days preceding inclusion.
6. Ongoing immunomodulatory treatment, for example against autoimmune/rheumatic conditions or cancer treatment.
7. Concomitant use of medications as described in the following list:
    a. The patient uses *warfarin (Marevan),* alternatively *coumarin,* anticoagulant. (Antibiotics can reduce the production of vitamin K from intestinal bacteria and increase the INR, thus leading to a greater risk of bleeding.)
    b. The patient uses *antacids*, either proton pump inhibitors, H- receptor antagonists or other acid-neutralising agents. (Reduces tetracycline absorption.)
    c. The patient uses medication for *epilepsy*, particularly phenytoin. (Enzyme-inducing anti-epileptic drugs increase the elimination of doxycycline.)
    d. The patient uses *Isotretinoin* (*Roaccutan*, for acne) or *Acitretin* (*Neotigason*, for psoriasis). (Concomitant use with tetracyclines may lead to increased intracranial pressure.)
    e. The patient uses *allopurinol* or *probenecid* (for gout – podagra). (Penicillin and allopurinol increase the risk of a skin rash. Probenecid increases the concentration of penicillin.)

Relative exclusion criteria and advice:

1. Patients taking *birth control pills* may participate in the study, but during the 14-day treatment period must use a condom or diaphragm during intercourse as extra protection against pregnancy. (In rare cases, the efficacy of contraceptives is reduced with concomitant use of antibiotics and combination birth control pills.)

2. Patients taking an *iron supplement cannot* participate unless they agree with the study doctor to stop taking the supplement during the study period. (Iron reduces S-doxycycline by 30-90%.)

3. Patients must not ingest liquid dairy products, such as milk or yogurt, 30 minutes before or two hours after taking the medication. Otherwise, the medicine can be taken either during or outside mealtimes. (Milk reduces the absorption of doxycycline.)

4. Patients are advised against having vaccinations, particularly typhoid vaccines, during the treatment period. (Antibiotics can reduce the immunological response to vaccines against typhoid fever.)

10a. Follow-up of study patients

Each GP is medically responsible for his/her own patients. In the study, the treatment administered to the patient for their erythema migrans is randomised, and follow-up is scheduled for day 14, which would not normally happen in an uncomplicated case. Blood tests and questionnaires including follow-up after 3 and 12 months are not usually indicated for EM. It is known that the *Borrelia* infection can cause systemic diseases, even when adequate treatment is received for EM.

Ina case a patient is showing symptoms of a disseminated disease during or after the follow-up period of the study, they should contact their own doctor, or the duty doctor, in the same way they would have done if they had not participated in the study. The doctor must then assess whether further investigation or a referral is needed. Any such events must be reported to the researchers and will be recorded in the study. If necessary, the patient's doctor will request new blood samples to be taken, irrespective of the samples taken in the study.

10b. Registering and reporting unwanted medical events and side effects
All doctors taking part in the study are advised that unwanted medical events are divided into three levels of severity: adverse event (AE), serious adverse event (SAE) and suspected unexpected serious adverse reaction (SUSAR). The forms for reporting the various events are sent to the individual doctors together with the medications and the other forms in the study. Should such an event arise, the individual doctors must report to the sponsor. (Template forms for AE, SAE and SUSAR are available from Oslo University Hospital, section for GCP.) The sponsor stores the detailed information, which, upon request, is sent to the Norwegian Medicines Agency (NOMA). The information is also included in an annual report.

In the case of SAE or SUSAR, the sponsor will report directly to NOMA within one and two weeks respectively so that the report can be sent to the relevant authorities in the EEA countries.

Since this study involves well-tested medications with known dosages for a specific diagnosis, the proportion of reported side effects is expected to be low. Nevertheless, the study will adhere to the regulation for clinical trials of medicines for humans (*Forskrift til klinisk utprøving av legemidler til mennesker*), chapter 7, which covers the duty to report medical events and side effects (*Meldeplikt om medisinske hendelser og bivirkninger*).

10c. Cancellation criteria
Cancellation criteria for individual patients in the study
- Signs of disseminated disease and a need for other treatment.
- Serious side effects or allergic reaction to the medication in the study.
- If the patient wants to drop out of the study.

Cancellation criteria for the actual study
- Where a sufficient number of patients have been recruited.
- (The treatment period is only 14 days, and data on any significantly different outcomes of the treatments will not be available until after the patient's participation is at an end.)

11. Randomisation procedure
In collaboration with Ingvild Dalen, a statistician at the Department of Biostatistics and Department of General Practice, UiO, a procedure has been devised as described below. The randomisation lists are not available to the researchers before the inclusion of patients has been completed, but are delivered directly from the statistician to the pharmacy responsible for dispensing the medications. As this study is open for the patients, there is no need for a blinded procedure in the case of any events arising, as the medication will be known.

Since this is a cluster randomised study (though with a low ICC), we want the design balance to be optimal with regard to the three treatments, and also want there to be a reasonable balance as regards the doctors. The plan is to send the kits to the doctors based on the estimated number of patients, rounded up to the nearest multiple of three. In order to make the study blind for the doctors, the treatment follows a randomised block design. Blocks of six are used, with each

block containing two of each of the three treatments in a randomised order. However, one kit in three will not necessarily contain one of each. Thus, the individual doctor cannot predict what the other patients have received even if one patient reveals their treatment to the doctor. In principle, the treatment is thereby blind for the researcher and doctor, but open for the patient.

Thus, the randomisation occurs in blocks of six, and the patients/kits are numbered from 1001–1300. We have set the total number of medicine boxes at 300 in order to afford a degree of flexibility in connection with dropout/addition of doctors. The kits are labelled with the participant number, and all content (questionnaires, patient diary, requisition form for the microbiology laboratory, patient information and consent form) marked with the same number is placed in numerical order in boxes of three (to increase the likelihood of the doctor distributing them in order) and then dispatched. A doctor who normally has a large number of patients with EM, or who has an agreement with their practice colleagues to 'take' their EM patients during the inclusion period will receive two or three sets for every three patients. Most doctors, however, will be sent kits for the first three patients with the option to request another set when these are used.

Using block randomisation with small blocks means there is a strong possibility that the distribution will be even between the treatments, even if the doctors are unable to recruit all of the patients as planned. It is envisaged that the number of doctors included in the study will be flexible. As we predict a low ICC for treatment effect, and still relatively small clusters, this will have little impact on the requirement for the total number of patients.

(The original medicine used in the study expired on 31 March 2013. New medicine was therefore produced/packaged for the 2013 season by the pharmacy at Oslo University Hospital, as before. The statistician Hiroko Solvang took over this work from Ingvild Dalen, but conferred with her predecessor and used the same model as before to draw up randomisation and package lists).

12. Course of the study

All patients included in the study are asked if they consent to a 4 mm punch biopsy from the skin in the border area of their EM. Those who decline will still be included in the study. The patients are informed about the three blood tests and the fact that the treatment they will receive is randomised. As neither blood tests or punch biopsies are normally indicated for an EM diagnosis, part of the patient consent declaration will entail them agreeing to the results of these tests only being shared with the research group.

As part of the recruitment of GPs for the study, we are arranging a two-day course in tick-borne diseases before each season. Credits for participation in this course will be awarded as part of their specialist education in general medicine. On the course, specialists from various areas, primarily recruited through the research network, NorTick, will teach the doctors in the four counties about diagnostics and treatment of tick-borne diseases, focusing on EM and Lyme disease.

The punch biopsy is taken on day 0. Blood samples are taken on days 0 and 14, and after three months, and are submitted for analysis for *Borrelia* antibodies, IgG and IgM, at the reference laboratory for borreliosis at Sørlandet Hospital Trust, Kristiansand. Part of the blood sample from day 14 is sent to the reference laboratory for TBE at NIPH. The result of the punch biopsy can both confirm the clinical diagnosis of EM in the study and provide information on the

distribution of subgroups of the *Borrelia* bacterium in Norway. However, the RCT is based on the clinical diagnosis of the GP and does not depend on the result of the PCR analysis.

Day 0, consultation:
The patient is asked to participate as soon as the EM diagnosis is made, and will receive randomised treatment; a, b or c:

a. Phenoxymethylpenicillin	1.3g+ 1.3g + 1.3 g	14 days
b. Amoxicillin	500 mg+ 500 mg + 500 mg	14 days
c. Doxycycline	100 mg+ 0 mg + 100 mg	14 days

A voluntary punch biopsy, blood tests of all patients in the study, questionnaires for the doctors and for the patients. Focus on the anamnesis, background information and exposure, the clinical presentation of the rash and how certain the doctor is of the diagnosis. The questionnaire corresponds to the one completed by the blood donors in the microbiologist's subproject, and the answers can therefore easily be compared.

Day 0-14, patient diary:
Accompanying symptoms, better or worse than yesterday? Side effects of the medicine, EM: size and colour. It is also recorded whether there is further contact with the doctor before the control on day 14.

Day 14, consultation:
Questions to the doctor: Has there been further contact with the doctor since last time? Side effects of the medicine? Has the EM cleared up? Otherwise, follow-up to record the duration. Signs of differential diagnosis? Signs of disseminated disease? Referral to hospital or other specialist? (If the doctor decides to take additional samples from the patient at this consultation, this should be done independently of the tests in the study.) No new questionnaire for the patient, but the doctor's secretary collects the diaries when taking blood samples.

Day 90, single contact with the doctor's surgery without consultation:
The patients are called to the doctor's surgery by the research group, based on the date of inclusion, for blood tests. They are given a new questionnaire with the same health complaint questions they received before. First: Has the EM from 90 days earlier disappeared? How long did it last?

Day 360, single contact with the doctor's surgery without consultation:
The patients are called to the doctor's surgery by the research group, based on the date of inclusion, for blood tests. They are given a new questionnaire with the same health complaint questions they received before. A telephone interview was originally considered here, but the one-year control was changed before the first patient was controlled following REC approval.

Questionnaire
Data are collected about participants on gender, age, postcode, marital status, education, occupation, household income, pets and farm animals. Previous tick bites, both in the last year and earlier, are recorded together with health information on relevant symptoms, doctor consultations and antibiotic treatment following a tick bite. They are asked to indicate the presence of ticks in the area, and whether they avoid certain areas due to ticks. In addition, they must state their confidence in the Norwegian healthcare system in connection with tick-borne diseases. Vaccines that can cross-react with the TBE samples are recorded together with trips

to endemic areas for TBE. They are further asked to indicate their daily functioning level and subjective health complaints (SHC). Questions about functioning and health complaints are taken from validated questionnaires to enable data to be compared with the background population (33, 34). In addition, some borreliosis-specific questions have been added.

13. Statistical analysis

The incidence of EM in the study population is calculated together with descriptive statistics on risk factors and demographic data. Descriptive and multivariate analyses will be conducted in the RCT. We will also identify predictors for long-term illness. Subjective health complaints upon inclusion and one year after diagnosis are compared. The relationship between reported subjective health complaints and seropositivity for *Borrelia* antibodies will be analysed.

14. Source data

- Patient consent form
- Doctor questionnaire day 0, including list of inclusion and exclusion criteria
- Doctor questionnaire day 14
- Patient questionnaire day 0, including background information and SHC score
- Patient questionnaire day 90, including SHC score
- Patient diary days 1–14
- Questionnaire, telephone interview, day 360, including SHC score
- Serology result *Borrelia* day 0
- Serology result *Borrelia* day 14
- Serology result *Borrelia* day 90
- Serology result TBE day 14
- Biopsy result, PCR, *Borrelia* day 0
- Forms for AE, SAE and SUSAR

Data from the sources are entered on an ongoing basis in the case report form (CRF) for each patient.

15. Plan for monitoring

This study is to be conducted in accordance with recognised standards for Good Clinical Practice, and in line with the Declaration of Helsinki and the national regulatory framework.

After monitoring was attempted via UiO channels, an agreement was reached with Hege Øvergaard, clinical monitor at GCP support. In the spring of 2013, monitoring visits were carried out with the principal investigator, the hospital pharmacy at Oslo University Hospital and the microbiology laboratory in Kristiansand. The microbiology laboratory at NIPH will be visited when the samples for TBE are ready for analysis there.

16. The project in perspective

Ethical aspects

The punch biopsy is voluntary and is performed under a local anaesthetic. It is considered by the research group to be a minor procedure with few side effects. We believe that giving the participants the results of the blood tests and the punch biopsy will only lead to unnecessary uncertainty about the diagnosis. This is because the samples are taken at a point when they would not normally be taken, a large proportion will be positive and it is difficult to tell the clinical significance of positive tests. With regard to the various antibiotic regimes in the RCT, PcV is the one recommended in applicable national guidelines. Doxycycline and amoxicillin

are not expected to result in a poorer course of infection and are not known to have serious side effects. The clinical trial, including two research biobanks, has been approved by REC.

Social relevance
Most patients with tick bites or tick-borne infections are seen by a GP. Expectations of antibiotic treatment are high. Particularly in the case of disseminated diseases, long courses of broad-spectrum antibiotics and combination treatment with multiple medications are traditionally administered. This can lead to increased antibiotic resistance in addition to side effects in the individual patient. In order to give the patients the best possible diagnosis and treatment, more knowledge is needed on the incidence, sero-prevalence and effect of treatment in Norwegian general practice. This study is intended to contribute to this. It will also enable follow-up of EM patients and blood donors with a view to exploring the long-term prospects of these diseases in terms of daily functioning levels and subjective health complaints, which are controversial and debated aspects of tick-borne diseases.

17. Project management, organisation and network

Project management and cooperation
The head of project and main supervisor is a professor of general medicine at The University of Oslo and general manager of ASP. The doctoral candidate is employed at ASP, which is part of the Department of General Practice at the Institute of Health and Society, University of Oslo. The research environment has extensive expertise in, for example, studies from electronic patient records. The co-supervisors are specialists in infectious medicine and general medicine respectively. For the analyses, cooperation was established with the two national reference laboratories for *Borrelia* and TBE, as well as with the Norwegian University of Life Sciences (NMBU) for competence in subjective health complaints.

Network
Tick-borne diseases are closely linked to the biology and ecology of the tick and its hosts in nature. In addition to the cooperation with various medical specialists, it is important to work with other natural scientists in order to understand the epidemiology of the diseases. In order to meet this need, an interdisciplinary research network, known as NorTick, was established. This is partly funded by ASP, and has held five meetings since April 2008. Project participants are members of NorTick. This national network collaborates with Swedish and Danish researchers. There is contact with the Knowledge Centre, and a knowledge report on the treatment of EM has been produced.

18. Plan for publication

1. *Penicillin vs. doxycycline vs. amoxicillin in patients with erythema migrans – a randomised, controlled clinical trial with one-year follow-up*

2. *Subjective health complaints in patients with erythema migrans compared to blood donors* (In collaboration with Reidar Hjetland, microbiologist at Førde Central Hospital, with a parallel project, and Professor Camilla Ihlebæk, NMBU.)

3. *Borrelia antibody production and Borrelia subgroups in Norwegian erythema migrans patients* (In collaboration with the reference laboratory for *Borrelia*, Sørlandet Hospital Trust, Kristiansand).

4. *Sero-prevalence of TBE antibodies in erythema migrans patients and blood donors in Norway. A foundation for vaccine recommendation?* (In collaboration with the reference laboratory for TBE at NIPH and Reidar Hjetland.)

19. Funding and insurance plan

The study is funded through a doctoral fellowship from the Norwegian Research Fund for General Practice and the Norwegian Medical Association, and part of the operating capital was provided by the Norwegian Centre of Rural Medicine, the Norwegian Surveillance System for Antimicrobial Drug Resistance and the Antibiotic Centre for Primary Care. The biobank for TBE virus is partly funded by NIPH.

All participants are covered by group insurance through the Norwegian Drug Insurance arrangement.

20. Confirmation

We confirm that the study will be conducted in accordance with the protocol, Good Clinical Practice, statutory directives and the regulatory framework.

Oslo, 15 August 2013

Morten Lindbæk
Professor in general practice, head of project

Knut Eirik Eliassen
General practitioner and doctoral candidate

Signature, study doctor:

21. References

1. Randolph SE. Evidence that climate change has caused 'emergence' of tick-borne diseases in Europe? Int J Med Microbiol 2004;293 Suppl 37:5-15.
2. Ljøstad U, Mygland A. Lyme-borreliose hos voksne. Tidsskr Nor Lægeforen 2008;128(10):1175-8.
3. Reiso H, Brage S. Borreliose som årsak til uførepensjon i Norge. Tidsskr Nor Lægeforen 2007;127(23):3061-3.
4. Nygard K, Brantsaeter AB, Mehl R. Disseminated and chronic Lyme borreliosis in Norway, 1995 - 2004. Euro Surveill 2005;10(10):235-8.
5. Larsson C, Comstedt P, Olsen B, Bergstrom S. First record of Lyme disease Borrelia in the Arctic. Vector Borne Zoonotic Dis 2007;7(3):453-6.
6. Hytonen J, Hartiala P, Oksi J, Viljanen MK. Borreliosis: recent research, diagnosis, and management. Scand J Rheumatol 2008;37(3):161-72.
7. Folkehelseinstuttet: Skogflåttencefalitt. http://www.fhi.no/eway/default.aspx?pid=233&trg=MainLeft_6039&MainArea_5661=6039:0:15,5078:1:0:0:::0:0&MainLeft_6039=6041:82867::1:6043:111:::0:0 (14.03.2011)
8. Csango PA, Blakstad E, Kirtz GC, Pedersen JE, Czettel B. Tick-borne encephalitis in southern Norway. Emerg Infect Dis 2004;10(3):533-4.
9. Skarpaas T. Skogflåttencefalitt (TBE) i Norge. MSIS-rapport 2007;35(21).
10. Gunther G, Haglund M. Tick-borne encephalopathies : epidemiology, diagnosis, treatment and prevention. CNS Drugs 2005;19(12):1009-32.
11. Kristiansen BE, Jenkins A, Tveten Y, Karsten B, Line O, Bjoersdorff A. Human granulocytær ehrlichiose i Norge. Tidsskr Nor Lægeforen 2001;121(7):805-6.
12. Bakken JS, Dumler JS. Clinical diagnosis and treatment of human granulocytotropic anaplasmosis. Ann N Y Acad Sci 2006;1078:236-47.
13. Folkehelseinstituttet: Anaplasmose (ehrlichiose). http://www.fhi.no/eway/default.aspx?pid=233&trg=MainLeft_6039&MainArea_5661=6039:0:15,5078:1:0:0:::0:0&MainLeft_6039=6041:82651::1:6043:5:::0:0 (14.03.2011)
14. Brorson Ø. Borrelia – en unik bakterie. Tidsskr Nor Legeforen 2009; 129:2114-7
15. Dessau Ram B et al. Lyme Borreliose. Klinik, diagnostik og behandling. Dansk Selskab for Klinisk Mikrobiologi, Dansk Selskab for Infektions medicin og Dansk Neurologisk Selskab, April 2006.
16. Bennet L et al. Increased incidence of Lyme borreliosis in southern Sweden following mild winters and during warm, humid summers. Eur J Clin Microbiol Infect Dis. 2006 Jul;25(7):426
17. Läkemedelsbehandling av borreliainfektion – ny rekommendation. Information från lakemedelverket 4:2009
18. Nasjonale retningslinjer for antibiotikabruk i primærhelsetjenesten, oktober 2008, IS-1593
19. Bjark P et al. Behandling av erythema migrans. Tidsskr Nor Legeforen 2008; 128:2844
20. Wahlberg et al. Penicillin V- behandling vid erythema migrans kan ge falsk säkerhet. Lakartidningen. 2006 May 3-9;103(18):1454; author reply 1455.
21. Wormser et al. Hematogenous dissemination in early Lyme disease. Ann Intern Med 2005; 142: 751-5
22. Ljøstad U, Mygland Å, Skarpaas T. Nevroborreliose i Vest-Agder. Tidsskr Nor Lægeforen 2003; 123: 610–3.
23. Bennet L et al. Clinical outcome of erythema migrans after treatment with phenoxymethylpenicillin. Scand J Infect Dis. 2003;35(2):129-31.
24. Loewen et al. Systematic review of the treatment of early Lyme disease. Drugs 1999 Feb;57(2):157-73
25. Wormser GP et al. Duration of antibiotic therapy for early Lyme disease. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 2003 May 6;138(9):697-704.
26. Report on diagnostics and treatment of Lyme disease in Norway. The Norwegian Directorate of health. www.helsedirektoratet.no/vp/multimedia/archive/00269/Rapporten__Diagnost_269839a.pdf (14.03.2011)
27. Mygland A, Skarpaas T, Ljostad U. Chronic polyneuropathy and Lyme disease. Eur J Neurol 2006;13(11):1213-5.
28. Bennet L et al. Clinical appearance of erythema migrans caused by Borrelia afzelii and Borrelia garinii-effect of the patient's sex.Wien Klin Wochenschr. 2006 Sep;118/17-18:531-7.
29. Eriksen HR, Ihlebaek C et al. A scoring system for subjective health complaints (SHC) Scand J Public Health. 1999 Mar;27(1):63-72.
30. http://www.danielsoper.com/statcalc/calc47.aspx (14.03.2011)
31. Campbell M K. Analysis of cluster randomized trials in primary care: a practical approach. Fam. Pract. 2000 17: 192-196;
32. Weber K et al. *Azithromycin versus penicillin V for the treatment of early Lyme borreliosis*. Infection. 1993 Nov-Dec;21(6):367-72.
33. Ihlebæk, C. (2002). The epidemiology of subjective health complaints. Doktorgradsavhandling. Institutt for biologisk og medisinsk psykologi, Universitetet i Bergen.
34. Brage S, Fleten N, Knudsrød OG, Reiso H, Ryen A. Norsk Funksjonsskjema – et nytt instrument ved sykmelding og uførhetsvurdering. Tidsskr Nor Lægeforen 2004;124:2472-4.
35. Sæterdal I et al. Antibiotikabehandling av erytema migrans. Notat 2012. ISBN 978-82-8121-461-3. Kunnskapssenteret.no