Postbus 1
3720 BA Bilthoven

A. van Leeuwenhoeklaan 9
3721 MA Bilthoven

Tel (030) 274 91 11
Fax (030) 274 29 71

info@rivm.nl
www.rivm.nl

rivm

Voedsel en Waren autoriteit
Prof. Dr. E. Schouten Bureau Risicobeoordeling
Prinses Beatrixlaan 2
2595 AL Den Haag

Onderwerp
Framewerk beschrijving tekenonderzoek

Geachte prof. Schouten, beste Evert

Datum
16 oktober 2007
Ons kenmerk
181/07 LZO JvdG
Blad
1/3
Tel (030) 274
Fax (030) 274

De afgelopen tien jaar is het aantal mensen met Erythema Migrans in Nederland verdrievoudigd. Gezien de omvang van de stijging en de ernst van de aandoeningen is de ziekte van Lyme hiermee een toenemend probleem voor de volksgezondheid. Ook lijkt het dat het aantal teken toeneemt. Mogelijke oorzaken hiervan kunnen gerelateerd zijn met een toename van reservoirdieren, bijvoorbeeld door habitatwijzingen (ecologische hoofdstructuur) en klimaatveranderingen, waarbij de periode dat teken actief zijn toeneemt. Daarnaast zijn door mogelijke klimaatsveranderingen ook verschuivingen van tekensoorten uit zuidelijk Europa richting Nederland mogelijk.Hiermee is ook een introductie van nieuwe ziekten een feit. Zo blijkt uit onderzoek van de Faculteit Diergeneeskunde (FD) dat de Dermocentor teek recentelijk tot uitbraken van babesiose bij honden heeft geleid. Een ziekte, die in Nederland niet bekend was. Recent onderzoek van het RIVM (Centrum voor Infectieziekten (CIb),Animal Science Group en Alterra, vakgroep Entomologie en Plant research International van de Wageningen Universiteit en Research centrum (WUR) wijst uit dat in Nederlandse teken niet alleen Borrelia bacteriën voorkomen, die de ziekte van Lyme veroorzaken, maar in teken ook andere voor de mens relevante ziekteverwekkers worden gevonden.
Binnen het Natuurkalenderonderzoek van de Environmental Systems Analysis, Entomologie en Plant Research International van de Wageningen Universiteit en Research centrum (WUR) zijn zeer recentelijk in teken hoge besmettingspercentages van Borrelia bacteriën gevonden.

Het schatten van de risico's en het nemen van effectieve bestrijdingsmaatregelen om de ziektelast te verminderen vereist expertise op vele terreinen, omdat zowel de mens, het dierreservoir, de teek, ecologische omstandigheden en het klimaat hierbij zijn betrokken. De bestudering hiervan vergt daarom een multidisciplinaire aanpak. Dit was in 2004 ook één van de conclusies in het rapport van de Gezondheidsraad over opduikende infectieziekten en het RIVM rapport ' Zoonoses in Europe: a risk to public health'.

Onderzoekers van het RIVM, FD en de WUR hebben vorig jaar het initiatief genomen om een landelijke werkgroep tekenonderzoek te vormen met als doel om expertise van diverse disciplines te bundelen en onderzoeksresultaten uit te

wisselen. Als uitkomst heeft de werkgroep de expertise op dit terrein geïnventariseerd. Hieruit mag blijken dat de intentie voor samenwerking in de toekomst kan leiden tot een geïntegreerde benadering. De werkgroep heeft de huidige lacunes in kennis geïdentificeerd en aanbevelingen gedaan voor nieuw onderzoek. Dit alles staat beschreven in de bijgeleverde notitie: National programme on monitoring ticks and tick-borne diseases in the Netherlands, a framework for collaboration on the study of tick-borne diseases.

Deze notitie is tot stand gekomen op initiatief van de VWA, dat het RIVM hiervoor een opdracht (kennisvraag 9.2.10 beschrijving framewerk onderzoek,contactpersoon drs. R. van Oosterom) heeft gegeven. Gezien de vereiste multidisciplinaire aanpak, willen wij de notitie echter ook bij andere verantwoordelijke ministeries onder de aandacht brengen. Juist opduikende zoönosen vereisen niet alleen een bundeling van krachten van de diverse onderzoeksdisciplines, maar ook van opdrachtverleners. Dit zal mogelijk tot betere geïntegreerde financieringsmogelijkheden leiden. Geïntegreerd onderzoek is de enige mogelijkheid om het toenemende risico van deze multifacteriële gezondheids-problemen aan te pakken.

Graag horen wij of een geïntegreerde programmafinanciering kan worden verwezenlijkt, zodat het voor ons als werkgroep mogelijk wordt om gezamenlijke onderzoeksvoorstellen in te dienen. Hiervoor zouden we graag op termijn in een gezamenlijk overleg met de verantwoordelijke contactpersonen van VWS, LNV en VWA de mogelijkheden nader bespreken.

In afwachting van uw antwoord, teken ik namens de werkgroep tekenonderzoek,

Dr. J.W.B. van der Giessen
Projectleider emerging- en parasitaire zoönosen en vectorgebonden aandoeningen
Laboratorium voor Zoonosen en Omgevingsmicrobiologie (LZO)
RIVM-Centrum voor Infectieziektenbestrijding

c.c:
R. van Oosterom
W. Ooms, VWA buro risicobeoordeling
M. Hoenders, LNV Directie VD
S. Beukers, VWS Directie PG
Leden nationale tekenwerkgroep:
Prof. Dr. F. Jongejans (Faculteit Diergeneeskunde)
Dr. F. Borgsteede (ASG-WUR)
Dr. K. Maassen (ASG-WUR)
Prof. Dr. W. Takken (Entomologie, WUR)
Dr. L. Overbeek (PRI-WUR)
Ir. A. van Vliet (Environmental System Analysis-WUR)
Dr. G. Jagersop Akkerhuis (Alterra-WUR)
Dr. W. van Pelt (EPI-RIVM/CIB)
Dr. P. Wielinga (LZO-RIVM/CIb)

National programme on monitoring ticks and tick-borne diseases in the Netherlands

A framework for collaboration on the study of tick-borne diseases.

Contents

Introduction and background

The last decade studies have shown that tick-borne diseases are forming an increasing problem in the Netherlands and elsewhere in Europe. These include Lyme borreliosis, babesiosis, anaplasmosis, ehrlichiosis, Bartonella infections, rickettsial diseases and tick-borne encephalitis (TBE). The latter disease has not been reported from the Netherlands. In the Netherlands at least seven expert groups are active in the field of tick research at three different institutes and working on different and sometimes overlapping subjects. These groups are 1) the Animal Science Group (ASG), 2) Alterra 3) Plant Research International (PRI), 4) the Laboratory of Entomology and 5) Environmental Systems Analysis Group, all five at Wageningen University and Research Centre (WUR), 6) Utrecht Centre for Tick-borne Diseases (UCTD), Faculty of Veterinary Medicine at Utrecht University (UU) and 7) the Centre for Infectious Disease Control (Cib) of the National Institute for Public Health (RIVM).

These research groups have taken the initiative to coordinate their individual lines of research and exchange of information through the establishment of a National Programme on Tick-borne Diseases. At a kick-off meeting at the RIVM in 2006 the partners from these institutes discussed their current and previous tick research, their different fields of expertise and the possibility to collaborate on future projects. Though tick research in the Netherlands is of world class and many state of the art techniques have been developed by current partners, all have the believe that Dutch research on ticks can benefit from exchange of information, protocols and materials.

A previous collaboration between three of the groups (ASG, Alterra and RIVM) already showed this may lead to synergy. In that latter project (2000-2004) ASG and Alterra studied tick densities in four different habitats (pine forest, heather, city park and dunes). Focusing on the life cycle of ticks in relation to the life cycle of the *Borrelia* bacterium, the research also included population densities of mice and the analysis of ticks on mice and ungulates. Cib studied the infection prevalence of over 1500 of these ticks for several different pathogenic micro-organisms. This collaboration led to better use of the collected ticks and made it possible to study the prevalence of additional pathogenic micro-organisms than the Lyme disease agent *Borrelia burgdorferi* s.l.. These results were only possible because the partners agreed to collaborate and exchange materials and knowledge. This successful project also made clear that collaborative research conducted by different expertise groups is desirable for a better understanding of the tick-borne disease problem in the Netherlands,

In the kick-off meeting the partners already mentioned the need for monitoring more regions in the Netherlands for the presence of *Ixodes* ticks. This could lead to clear improvements in understanding the magnitude of the tick-borne disease problem in the Netherlands and would reveal high risk areas in Netherlands. The finding of our UU partner (Prof. Frans Jongejan) that *Dermacentor reticulatus* is a new tick species in the Netherlands that has become established in certain regions, also shows that it will be necessary to increase the number of surveys to monitor the spread of this tick in the Netherlands. A study of the Cib under all Dutch general practitioners concerning the number of patients with tick bites and erythema migrans (EM) (the first visual manifestation of Lyme disease) in their practice showed a three fold increase in the number of EM patients from 1994 to 2005 and identified certain hot spots of Lyme disease in the Netherlands. The Laboratory of Entomology (Dr Willem Takken) has recently started several projects on population dynamics of ticks and Lyme disease. The weekly dynamics of ticks in 12 different locations across the Netherlands is being studied and the infection rate of ticks with *Borrelia* spp. is established. Through the Natuurkalender programme (www.natuurkalnder.nl) 26 different sites distributed across all provinces are being

monitored monthly for tick presence and density. These ticks will be examined for infection with *Borrelia* spp. and other micro-organisms. The Laboratory of Entomology, jointly with the Plant Research International of WUR will conduct a study to investigate the relationship between pathogen infection in ticks in relation to habitat structure. So far a clear relationship between location and microbial community structure in ticks was found in three areas within the Netherlands. This indicates that local environments may have a strong effect on the establishment of microbial populations, including human pathogenic ones in ticks. This work should answer the question to what extent ecological variation in the Netherlands is associated with tick populations and pathogen infections. As a direct input for the current collaboration Dr. Takken proposed an exchange of the tick material and rodent blood and tissue between the partners who than can study the tick-borne micro-organisms and other relevant questions that are not addressed by WUR at that moment. To make the translation from field data to patient data, the RIVM has proposed to do a prospective study collecting ticks from patients in regions that were identified as hot spots and for which field data show high levels of new types or highly infected ticks.

In their discussions the partners came to several questions that are still unanswered and which may be answered by a national integrated approach:

1. Is there an increasing number of ticks and pathogen-infected ticks?
2. What are ecological risk factors related with tick densities?
3. Can we identify "hot" spots in the Netherlands and what determines them?
4. What is the relevance of the *Rickettsia*, *Ehrlichia/Anaplasma* findings in ticks for public health?
5. How can we design a national monitoring programme for ticks?
6. How can we come to a relevant risk assessment of tick-borne diseases?
7. Is there a relationship between favourable tick habitats and incidence of Lyme disease?
8. Do large herbivores affect the *Ixodes ricinus* populations and/or *Borrelia* infections in ticks?
9. Is there an interaction between bacterial species in the tick gut system?
10. How are *Borrelia* species and other pathogens transferred from reservoirs hosts (e.g. small rodents) to ticks?

Aim of the framework

The framework aims to bundle the different research projects carried out by the different tick expert groups in the Netherlands and to give an overview on which aspect further collaboration might be possible. The proposed collaboration between the five partners and bundling the tick collection data, tick infection data and patient data will provide a good idea of the risks of tick-borne diseases in the Netherlands and it promises to give a better insight in how tick-borne diseases may develop in the future.

Summarizing the direct benefits of the current framework approach:

- Collaborative selection of the different study areas and bundling geographic information on tick densities and infected ticks;
- Exchange of tick material between partners leading to more investigated ticks and a broader coverage of pathogens;
- Exchange of technical information and control materials to standardize common methods used by the partners;
- Production of information on tick distribution, tick infection rates and relevant ecological information from different areas across the Netherlands;

- Collective acquisition for project proposals covering the entire area from pathogen ecology in nature to human health.

In the next sections the different partners present their individual research lines and they will indicate where to collaborate how the other partners can help with this and profit from the obtained new insights.

List of participants and their expertise

Utrecht Centre for Tick-borne Diseases

Prof. Frans Jongejan, f.jongejan@vet.uu.nl
PhD students:
Ard Nijhof
Milagros Postigo
Zoric Zivkovic
Bonto Faburay
Student assistants:
Drs. Christa Bodaan
Linda Franssen
Caroline Spruyt
Lobke Buhrman

Expertise

Tick taxonomy and ecology with particular reference to ticks infesting companion animals Tick rearing facilities and artificial infection of ticks; molecular detection of bacterial and protozoan pathogen in ticks (including Theileria, Babesia, Anaplasma, Ehrlichia, Rickettsia and Borrelia species); tick-host-pathogen interactions with particular reference to Anaplasma marginale in Boophilus ticks; development of attenuated vaccine for heartwater (tropical rickettsial disease transmitted by Amblyomma ticks; modelling tick and tick-borne pathogen distribution using GIS based techniques; genetic manipulation of ticks using RNA interference and development of recombinant anti-tick vaccines

ASG/WUR

Dr. Fred Borgsteede, fred.borgsteede@wur.nl
Cor Gaasenbeek, cor.gaasenbeek@wur.nl
Dr. Kitty Maasen, kitty.maassen@wur.nl

Expertise

Parasitology
Fieldwork: tick collection.

Alterra/WUR

Dr Gerard Jagers op Akkerhuis, gerard.jagers@wur.nl
Wim Dimmers, wim.dimmers@wur.nl

Expertise

Systems ecology of Lyme
Fieldwork: tick infected animal collection.

Entomology /WUR

Dr. Willem Takken, willem.takken@wur.nl
Ir. Fedor Gassner, fedor.gassner@wur.nl
Ing. Frans Jacobs, frans.jacobs@wur.nl
Patrick Verbaarschot, Patrick.verbaarschot@wur.nl
Dr. Ron van Lammeren, ron.vanlammeren@wur.nl
Dr. Elmar Veenendaal, elmar.veenendaal@wur.nl

Expertise
Biology of insect and insect-borne diseases
Ecology of vector-borne diseases
Microbiology (detection of vector-borne microorganisms)
Ecology of plant communities
Geographical Information Systems
Field work: insect collection

Resource Ecology/WUR

Prof. Herbert Prins, herbert.prins@wur.nl
Dr. Sip van Wieren, sip.vanwieren@wur.nl
Ir. Jasper van der Linden

Expertise
Wildlife ecology (birds and mammals)
Ecological modeling
Infectious disease ecology

Environmental Systems Analysis/WUR

Ir. Arnold van Vliet, arnold.vanvliet@wur.nl
Ir. Sara Mulder, sara.mulder@wur.nl

Expertise
Phenology of plants and animals
Interaction between climate change and environment
Public information

Plant Research International

Dr. Leo van Overbeek, leo.vanoverbeek@wur.nl
Piet Kastelein, piet.kastelein@wur.nl
Carin Lombaers, carin.lombaers@wur.nl

Expertise
Molecular Microbial Ecology
Molecular detection techniques
Functional analysis of molecular and microbial isolates (genomics, metagenomics and metabolomics)
Multivariate analyses
Field work (tick collections, description of biotic and abiotic factors)

CIb/RIVM

Dr. Joke van der Giessen joke.van.der.giessen@rivm.nl
Dr. Wilfrid van Pelt, wilfrid.van.pelt@rivm.nl
Ing. Manoj Fonville, manoj.fonville@rivm.nl

Ir. Agnetha Hofhuis, Agnetha.Hofhuis@rivm.nl
Dr. Daan Nootermans, Daan.Notermans@rivm.nl
Dr. Katsuhisa Takumi, katsuhisa.takumi@rivm.nl
Dr. Peter Wielinga, peter.wielinga@rivm.nl
Dr. H. Sprong, hein.sprong@rivm.nl

Expertise
Infectious disease Epidemiology
Medical and veterinary microbiology and parasitology
Molecular detection of tick transmitted pathogens
Detection of tick and insect transmitted pathogens in human patients and animals
GIS applications and risk mapping
Risk assessment
Coordination of national infectious diseases studies

SHORT DISCRIPTIONS OF PROJECTS THAT CAN BE CONSIDERED UNDER THE FRAMEWORK

CIb/RIVM

RIVM has a long history in tick research. The research involved wet and dry research and collaborations with several groups in the Netherlands and elsewhere in the world. Three surveys under all Dutch general practitioners (GP's) showed that from 1994 to 2005 the number of GP consults for tick bites and erythema migrans (the first sign of Lyme disease) has been steadily increasing. The RIVM (amongst others Rijpkema and Schouls) together with UU (Jongejan) have been essential in the now world-wide used reverse line blotting (RLB) technique for genotyping different pathogens in ticks. Using this technique RIVM has been involved in different scientific studies identifying different pathogens in ticks found both in the vegetation and on humans and ticks. These studies have been essential in relating specific pathogens to certain disease phenotypes and determining factors involved in the spread of ticks and their pathogens. The most recent studies involved large longitudinal studies to monitor the tick densities and tick-disease spread in the Netherlands and to compare these factors for different types of vegetation found in the country. The next step RIVM likes to make is to determine the relation between ticks found in the field and the ticks found on humans and the diseases they transmit to these persons. For this RIVM has the appropriate people working in the field of epidemiology, molecular genetics and statistics and RIVM collaborates closely with the other expert groups in the country: UU, WUR, ASG and ALTERA. For the coming period RIVM will conduct the following studies.

1. Together ASG and CIb have been monitoring tick densities and *Borrelia*, *Ehrlichia*, *Anaplasma*, *Rickettsia* and *Babesia* infections in the Duin en Kruidberg sentinel region since 2000 and will do this also the coming years.
Information has been and will again be exchanged to the partners so they are on top of anything new found. ASG may collaborate with UU and Entomology to help cover more areas or specific areas of interest, for instance a region with an outbreak of a tick transmitted disease. RIVM can offer in helping to analyze the micro organisms in the ticks.

2. Prospective national study determining the prevalence of Lyme disease in ticks collected from GP consulting patients in selected hotspot regions in the Netherlands. Regions will

preferably be selected in collaboration with the different partners to include all necessary regions in the Netherlands.
The study will generate a large amount of ticks collected from patients, and information on contamination of ticks with Borrelia. This will be analysed in relation to the patient data (age, sex, region, time of the year, risk behaviour, Borrelia serology, clinical signs etc.). These materials (excluding patient materials) will become available to the partners and can be part of their study.

3. RIVM has molecular detection methods for detection of several of relevant and *Borrelia*, *Ehrlichia*, *Anaplasma*, *Rickettsia* and *Babesia* species. After agreement with the partners RIVM can do these additional genotyping on tick from partners collect these for other purposes or look at selected/different micro organisms.

Vet Med / UU

1. The group of Jongejan runs several projects all focussed on tick transmitted disease found with dogs. In 2005 the UU started a study financed by the Koninklijke Nederlandse Maatschappij voor Diergeneeskunde (the "tick busters" project) asking vets and pet holders to voluntarily send in ticks removed from animals. Ticks were determined to their species level and several tick pathogens were screened. The study was very successful and ticks were sent in amass. As expected mainly *Ixodes ricinus* ticks were send in, however, unexpectedly more than 10 other species were also found. Detection of pathogens is focussed on *Babesia canis*, which has been reported before in the Netherlands to cause fatal diseases in dogs. In particular the tick collection has been very successful and large numbers of tick have been collected. Determination of these ticks and detection of pathogens in them has started. Th preliminary analyzis showed that next to *Ixododus ricinus*, the main species also some less common tick species (e.g. *Rhipicephalus sanguineus, Dermacentor reticulatus, Ixodes hexagonus)* may be found in the Netherlands, which may indicate these may be emerging here, which as a consequence may be indicated that new pathogens may be encountered here in the future. For instance, *Rhipicephalus sanguineus* has been found tot transmit the first *Ehrlichia canis* in the Netherlands. A large number of ticks hase been analyzed already the success of the tick collection was that good that there are still large numbers of ticks that need to be analyzed, which will need more time and funding. The study provides a good idea of the incidence of animals infected ticks and the geographic origins on the encountered tick species and will continue next year.

2. In 2005 the UU discovered the establishment of the tick species *Dermacentor reticulatus* in the Netherlands. Before only *Ixodes ricinus* ticks were found in the Netherlands. The *Dermacentor* ticks are found at several regions in the Netherlands and they may carry for the Netherlands new pathogens from *Rickettsia* family and in contrast to *Ixodes ricinus* these ticks are also active during cold periods. This finding asks for more study of the *Dermacentor* tick and its pathogens and the risk for human and animals health.

3. Vaccination of dogs against Babesia canis may prevent of getting the disease but not the pathogen and dogs taken on holiday may introduce new pathogens coming back to the Netherlands from an endemic region. A risk assesement will be done for dogs taken to endemic regions by serologic testing and by analyzing blood sample for the presence of pathogens by molecular typing methods

4. UU is grant holder of a Wellcome Trust project aimed to develop improved recombinant anti-tick vaccines for livestock in Afria. The project successfully established gene silencing

through RNA interference as a genetic tool at UCTD. Several colonies of European and african tick are being maintained in the tick rearing unit.

ASG/WUR

1. Continuation of the monitoring of tick densities and tick with four week intervals in the period April/May - October/November infestations in the sentinel area Duin en Kruidberg in cooperation with Clb/RIVM. This is a vegetation rich dune area and a favourite site for ticks. Monitoring this area was started in 2000 and since than in the period April - November each month ticks have been systematically collected from the vegetation. Ticks densities, seasonality and distribution of tick development stages have been established for each year. Together with Clb/RIVM these ticks have analyzed for the presence of *Borrelia, Anaplasma, Rickettsia* and *Babesia* species. Three other areas (a city park, a heather area and a forest) could only be monitored for 2000 – 2002, however, comparison showed clear differences between the different habitats and showed that in city parks almost as many ticks and tick transmitted pathogens could be found as in a forestall area.
For this sentinel region ticks from 2000 – 2004 have been examined and those collected last year (2005) and also this year (2006) will be examined in cooperation with Clb/RIVM

2. Collection of mice from Duin en Kruidberg in cooperation with ALTERRA. To compare the result obtain previously for the questing ticks with ticks collected from invested animals, ASG together with Alterra will start to collect ticks from infested animals from in the Duin en Kruidberg sentinel area. Catching the animals and collecting blood samples that may be used for further research will be done by Alterra who have lots of experiences with this type of work. Next tick densities and ticks will be examined together with RIVM for the presence of different pathogens. Also, blood samples of the collected mice which will be used to determined the presence of infective agents or antibodies against different infective agents in these infested mice as well as in control mice.

3. Of several foregoing years (2000 – 2002) there are still several different materials stored that have to be analyzed. Blood samples of tick infested mice and of big mammals will be examined for the presence of different pathogens in cooperation with Clb/RIVM. Also, blood samples of tick infested big mammals (e.g. deer) from two forest areas in the Netherlands has been collected before and will be examined in cooperation with Clb/RIVM.

Alterra/WUR

The expertise of Alterra covers the ecological monitoring of small rodents that are the hosts of ticks. This involves both the trapping of the animals and the taking of physiological measures related to *Borrelia* infection, e.g. blood samples. Although this has not been effectuated in current project, Alterra has the knowledge and equipment for the analysis of *Borrelia* in infected ticks. Alterra will proceed to catch small warm-blooded hosts (mainly mice) in the Duin en Kruidberg which has since 2000 functioned as a sentinel area. They will collect ticks and blood samples from these animals. Ticks will be collected by Alterra, and together with ASG and RIVM the ticks and the blood will be analyzed for tick transmitted pathogens. This study will give insight in differences between the infestation of questing ticks (form the vegetation) and of ticks collected from invested animals. It will show the relation between animal densities and tick densities and may reveal specific animal reservoirs for different tick transmitted pathogens.

Entomology /WUR

1. The Laboratory of Entomology of Wageningen University collects ticks in 25 different locations in the Netherlands. This work is in part financed by the Ruimte voor Geoinformatie (RGI) programme of the Dutch government, and is embedded in the Natuurkalender programme which is coordinated by the Environmental Systems Analysis Group of Wageningen University. Tick collections depend on the work of many volunteers helping with sampling ticks from the vegetation. Several locations have been chosen to overlap with the tick-bites study of the RIVM. Part of the locations overlap with the "high risk" areas and others are in the "low risk" areas. Some of the locations have also been chosen because of the vegetation type and they cover an almost complete set of vegetation types present in the Netherlands, including forest, dunes and heather. The start of the project was 1 July 2006 and will go on for 18 months. The study focuses on *Borrelia* infections in ticks.. There is the possibility that Entomology together with RIVM will further genotype these ticks samples for Borrelia and other tick-borne pathogens. The Natuurkalender collects information via a website (www.natuurkalender.nl) on which humans bitten by a tick can report time and location of their tick bite. Although this is all on voluntary basis and reporting will depend on many different factors, the study is expected to provide information on the sites where people contract a tick most often, the physical activity during the tick bite, as well as the national distribution of ticks. The data can be compared with the RIVM national tick-bite incidence study. Also, data may be used in designing the prospective study of the Cib/RIVM.
Funding: rGO-the Netherlands.

2. Twelve location in the Netherlands are monitored for different insects, as part of an assignment by LNV and VWA to investigate the presence of vectors of human and animal diseases. This study was conducted between July 2005 and November 2006. The project monitors many different insects including ticks in three habitats that are considered high risk because of vector densities and biodiversity: natural peat land, river flood plains and around biological farms. All ticks are being examined on Borrelia infections. They could then be used for further analysis of other microorganisms.
Funding: LNV

3. In collaboration with Plant Research International (PRI) of WUR, the Laboratory of Entomology conducts a study to investigate the relationship between habitat structure and micro-organism infections of *Ixodes ricinus*, using ticks collected under project #1. The specific aim of the project is to establish associations of micro-organisms in Ixodes ricinus with specific habitat and wildlife characteristics. This project began in September 2006.
Funding: Wageningen UR.

3. Entomology works on the relation between ecology and the risk of contracting Lyme disease (submitted project proposal), and together with Resource Ecology (WUR) on the effects of grazing on the survival of *Ixodes ricinus* and *Borrelia*.
Funding: under review.

Plant Research International/WUR

The effect of habitat structure on microbial communities in ticks is studied at Plant Research International (PRI) in close collaboration with the laboratory of Entomology of WUR. This research is financed by the management team of the Plant Science Group of

WUR and is meant to stimulate new research on control of Lyme disease in natural habitats. In 2006 a field study was started where ticks were collected from three different natural habitats in the Netherlands. From individual ticks (nymphs) the presence of *Borrelia* species and the microbial community structure, by PCR-DGGE, were investigated. In ticks from one location the microbial community differed from that of the two other regions. This result was the motivation to further investigate the role of different micro-organisms on *Borrelia* species interactions in the tick gut system. We aim to recover species from ticks via cultivation-based techniques. Also, microbial communities in ticks will be assessed via culture-independent techniques, e.g. by screening of tick DNA clone libraries. We will further improve molecular detection techniques to investigate pathogenic and opportunistic microbial populations in ticks. First, bacterial-group specific PCR-DGGE will allow investigating shifts in different bacterial groups in tick guts. Second, multiplex quantitative detection will be applied to determine quantitative differences between different microbial species in tick guts. With these techniques, the effect of different environmental factors on the tick bacterial community structure can be unravelled. The role of bacterial populations, naturally present in the tick gut system, on suppression of *Borrelia* species and other pathogens may become an important outcome of this approach. The facilities and knowledge are already available at PRI for screening of thousands of target gene sequences via a multiplex quantitative PCR open array (BioTrove) set up.

Possibilities for collaboration.

Next to the exchange of information on the current projects the different partners might potentially benefit from each other in the following ways:

- Exchange of control materials and information to standardize common methods
- Conformational research to reproduce specific results (tick catching, pathogen detection/tick identification).
- Exchange tick, tick biting incidences, host and pathogen data of the different studied area to come up with a better cover of the Netherlands, possibly through common database.
- Exchange of surplus research materials and/or help with analysis of ticks and/or animals materials
- Collaborative action for national and internationals grants

Recommendations for future research on ticks and tick transmitted pathogens in the Netherlands

Surveillance and risk assessement

i. Development and implementation of new molecular tools, real time PCR and micro-array, that can be used for the fast detection of tick transmitted diseases.
ii. Design of a minimal monitoring program for ticks transmitted diseases that monitors tick numbers and the most essential tick transmitted diseases.
iii. Outbreak investigations in case of acute disease problems in e.g. dogs, cattle to trace hot spot areas
iv. A risk assessment for tick transmitted diseases.

Risk mapping and scenario analysis

v. To study the influence of climate and vegetation dynamics on the tick and its pathogens

vi. GIS modeling and scenario modeling of tick transmitted disease to estimate the risk and spread of newly introduced tick transmitted diseases.

Ecology and population dynamics

vii. Estimate the importance of animal reservoirs (rodents, large herbivores) for the maintenance of ticks and the pathogens transmitted by ticks.

viii. Role of different eco-systems on tick populations and the rate of infection of ticks.

ix. Interaction between different micro-organism species in the tick. To study the role of co-infection and pathogenicity.

Control

x. Study different methods for the control of ticks and tick transmitted diseases.