# Workshop on Ticks and Tick-borne Diseases

## Berlin, September 30th-October 2nd, 2014

Joint Meeting of the
German Society for Medical Entomology and Acarology (DGMEA)
and the
Workshop on Tick-borne Diseases
of the National Reference Laboratory for Q-Fever,
Jena, Germany

Nationale Forschungsplattform für Zoonosen

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Cover: © Birgit Habedank, *Ixodes ricinus* at a hiking trail at the river Elbe near Rathen, 2013/05/02
Page 1: © Reiner Pospischil

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# Workshop on Ticks and Tick-borne Diseases

## Berlin, September 30th –October 2nd, 2014

**Scientific programme and local organization**

Dr. B. Habedank, Dipl.-Biol. A. Vander Pan
Umweltbundesamt - Federal Environment Agency, Section IV 1.4 – Health Pests and their Control

Dr. K. Henning
Friedrich-Loeffler-Institut - Federal Research Institute for Animal Health,
German National Reference Laboratory for Q-fever, Jena

Prof. Dr. G. von Samson-Himmelstjerna, Dr. A.M. Nijhof, Dr. J. Krücken
Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Berlin

Dr. R. Pospischil, President of the DGMEA

**Workshop venue:**

Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine
*Registration and presentations:*
Institute of Veterinary Pathology, Robert-von-Ostertag-Str. 15 (Building 31)
*Poster sessions and practical workshop:*
Institute for Parasitology and Tropical Veterinary Medicine, Robert-von-Ostertag-Str. 7-13 (Building 35)

**Acknowledgement**

The workshop is funded by the National Research Platform for Zoonoses (http://www.zoonosen.net) and the registration was assisted by Ms I. Schmid.

# Content

# Welcome

Dear Colleagues,

We welcome you to the Workshop on Ticks and Tick-borne Diseases which is a joint meeting of the German Society of Medical Entomology and Acarology (DGMEA) and the "Workshop on Tick-borne Diseases" of the National Reference Laboratory for Q Fever of the Friedrich-Loeffler-Institut.

This joint meeting is organized by:

the **Umweltbundesamt** (UBA) - Federal Environment Agency, Section IV 1.4 – Health pests and their control, in close cooperation with the **German Society for Medical Entomology and Acarology** (DGMEA),

the **Friedrich-Loeffler-Institut** (FLI), National Reference Laboratory for Q Fever and

the **Freie Universität Berlin** – Free University Berlin, Institute for Parasitology and Tropical Veterinary Medicine.

The annual meetings of the German Society for Medical Entomology and Acarology usually are addressed to a special arthropod group of medical and veterinary importance. Traditionally, these meetings include at least one day for scientific presentations and a second day for practical training. Trainings for the identification of species of a specific arthropod group were offered and sometimes biotope excursions were made to provide insight into the ecology of hematophagous parasites. The last DGMEA meeting which focused on ticks and tick-borne pathogens was held in the year 2000. Since that time there were many important developments and new findings. We will focus again on this interesting subject during this year's meeting which was organized mainly by the Federal Environment Agency, Section `Health Pests and their Control`.

The "Workshop on Tick-borne Diseases" was initiated in 2012 by the National Reference Laboratory for Q Fever of the FLI and has been conducted annually. The aim is to offer a platform for the scientific exchange and cooperation between scientists of European countries in the area of tick-borne diseases. So, it was a natural step for both the FLI and the DGMEA to organize the workshop in 2014 as a joint meeting.

The Federal Environment Agency (UBA) provides the expertise in different fields important for the research on ticks and tick-borne diseases: ticks and tick control (Section IV 1.4 - Health Pests and their control), authorisation and environmental assessment of acaricides (Section IV 1.2 – Biocides) and the assessment of climate change impact and adaptation needs (Section I 1.6). Gaps in tick control measures in the prospect of climate change and the responsibility for our environment led to initiation and support of tick research projects as well as to advance the scientific discussion by the organisation of the international workshops "Vector-borne Diseases: Impact of Climate Change on Vectors and Rodent Reservoirs" 2007 in Berlin (1,2) and "Ticks as Vectors and their Control: The Present and Future under the Perspective of Global Climate Change" 2012 in Speyer, Germany (3). Our workshop is a further step in the discussion on development of strategies for tick control.

The Institute for Parasitology and Tropical Veterinary Medicine, as an important German center for veterinary entomology, supports the workshop with their expertise in tick research. The institute´s facilities will be used for the practical tick identification course and its employees will provide practical insight into the *in vitro* feeding of hard ticks.

The close cooperation of Federal and Regional Authorities and Universities brings together the interdisciplinary expertise from all sides so that we are able to present important aspects of tick

research (tick biology and ecology, transmitted diseases and measures to prevent tick bites and control ticks) together with practical training.

Our aim is to present the results of ongoing research and to support the scientific exchange and cooperation but also to identify essential gaps and discuss options for solutions in research, education, public information and control measures.

We wish to express our gratitude to all attendees, plenary speakers, workshop facilitators and the German Research Platform for Zoonoses for their contribution.

On behalf of all colleagues of the Organising Committee we wish you a rewarding and pleasant workshop.

Yours

B. Habedank, G. v. Samson-Himmelstjerna, R. Pospischil and K. Henning

**References**

(1) Umweltbundesamt (2007): International conference „Vector-borne diseases: Impact of Climate change on vectors and rodent reservoirs", Berlin, Germany, 27.-28.09.2007, 104 pp. http://www.umweltbundesamt.de/sites/default/files/medien/419/dokumente/programme-and-abstracts.pdf [assesed 05 September 2014].

(2) Habedank B. and Klasen J. (eds.) (2008): Vector-borne diseases and climate Change. Parasitol Res 103 (Suppl 1): S1-S160.

(3) BMU IG II7 and Habedank B. (2012): Ticks and climate change. BMU-Umwelt 6/2012: 52-53. (in German)

# Scientific Programme

## Tuesday, September 30

Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Building 31 (Auditorium)

10:00 Registration

11:00 – 11:30 **Opening of the Workshop**

A. Gruber (Vice-Dean for Research, Faculty of Veterinary Medicine, Freie Universität Berlin)
and G. von Samson Himmelstjerna (Freie Universität Berlin)

K. Henning (National Reference Laboratory for Q-Fever, FLI, Jena)

R. Pospischil (German Society for Medical Entomology and Acarology)
and B. Habedank (Umweltbundesamt - Federal Environment Agency, Berlin)

### Session: Ecology I

Invited contribution, *Moderation: B. Habedank*

11:30 – 12:30 **The Ecology of Ticks (and Some Caveats to Interpret and Model it)**

A. Estrada-Peña, J.S. Gray, O.Kahl, R.S. Lane, A.M. Nijhof
(Zaragoza, Spain; Dublin, Ireland; Berkeley, USA; Berlin, Germany)

**Lunch**

*Chairs: A. Estrada-Pena, G. v. Samson-Himmelstjerna*

13:30 – 13:50 **Ticks in Romania and Recent Trends of Spread**

L. Chitimia-Dobler (Bucharest, Romania)

13:50 – 14:10 **Dynamics of a TBE focus over a period of 6 years**

G. Dobler, S. Frey, S. Eßbauer (Munich, Germany)

14:10 – 14:30 **Monitoring *Ixodes ricinus* questing activity on field plots over several years – implications on our knowledge of the life cycle and the phenology of the tick**

H. Dautel, D. Kämmer, J. Heger, O. Kahl (Berlin, Giessen, Germany)

14:30 – 14:50 **ZUP – Zecken, Umwelt, Pathogene Baden-Württemberg. An Interdisciplinary Project to Study the Ecology of Ticks as Vectors of Pathogens in Baden-Württemberg Germany**

M. Pfäffle, S. Norra, R. Oehme, O. Kahl, H. Dautel, J. Steidle, P. Sebastian, N. Littwin, D. Böhnke, T. Petney (Karlsruhe, Stuttgart, Berlin, Germany)

**Coffee break with Poster session**

Session: Tick-transmitted pathogens I

*Chairs: V. Fingerle, J. Krücken*

15:30 – 15:50 ***In vitro* Cultivation and Development of Diagnostic Tools for the Emerging Pathogen *Borrelia miyamotoi***

R. Venczel, G. Margos, L.Knoke, M. Pavlova, E. Dzaferovic, K. Binder, G. A. Schaub, A. Sing, V. Fingerle (Oberschleissheim, Bochum, Germany)

15:50 – 16:10 **Abundances of *Ixodes ricinus* and Prevalences of *Borrelia* sp. in the Nature Reserve Siebengebirge, Germany**

G.A. Schaub, , L.R. Knoke, , A.-K. Steinmann, A. Muminovic, C. Balczun, A. Schwarz, A. Venczel, G. Margos, V. Fingerle (Bochum, Oberschleissheim, Germany)

16:10 – 16:30 **Lyme-Borreliosis – Aspects on the Diagnosis and the Obligation to Report**

V. Fingerle, G. Margos, A. Sing (Oberschleissheim, Germany)

**Coffee break**

Session: Ecology II

*Chairs: T. Petney, H. Dautel*

17:00 – 17:20**Vegetation vs. Vertebrates. Activity Patterns of *Ixodes ricinus* on Vegetation and on Small Mammal Hosts in Baden-Wuerttemberg Forest Areas**

N. Littwin, M. Pfäffle, H. Taraschewski, T. Petney (Karlsruhe, Germany)

17:20 – 17:40 ***Dermacentor reticulatus* – Host Preferences of Immatures and Their Role as Vectors in the Hardtwald, Karlsruhe, Germany**

M. Pfäffle, N. Littwin, P. Sebastian, R. Oehme, T. Petney (Karlsruhe, Stuttgart, Germany)

17:40 – 18:00 **Abundance and Species Diversity of Rodent-Attached Ectoparasites Trapped in Differently Structured Habitats in Germany**

A. Obiegala, M. Pfeffer, A. Balling, M. Kiefer, D. Kiefer, C. Silaghi (Leipzig, Munich, Germany; Zurich, Switzerland)

19:30 **Workshop Dinner**

## Wednesday, October 1

Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Building 31 (Auditorium)

09:00-09:10 **Opening Day 2**

P. Greiner (Umweltbundesamt - Federal Environment Agency, Dessau-Roßlau, Germany )

### Session: Tick control and tick bite prevention

*Chairs: A. Nijhof, R. Pospischil*

Invited contribution

09:10 – 10:10 **Vaccinomics: Understanding Tick-Host-Pathogen Interactions for Vaccine Development**

J. de la Fuente (Ciudad Real, Spain; Stillwater, USA)

10:10 – 10:30 **Tick Control - A Challenge in the Fields of Health and Environment Protection**

B. Habedank (Berlin, Germany)

10:30 – 10:50 **Biocidal Product Authorisation – General Procedure and Outlook for Insecticides/Acaricides and Repellents**

N. Ludwig, D. Frein (Dessau-Roßlau, Germany)

**Coffee break with Poster session**

*Chairs: J. De la Fuente, B. Habedank*

11:30 – 11:50 **Efficacy Testing of *Ixodes ricinus* Tick Repellents: Comparison of Two Test Protocols for Human Subject Trials**

H. Dautel, C. Dippel, A. Werkhausen, R. Diller (Berlin, Jena, Germany)

11:50 – 12:10 **Repellent Efficacy of DEET, Icaridin, and EBAAP Against *Ixodes ricinus* and *Ixodes scapularis* Nymphs (Acari, Ixodidae)**

K. Büchel, J. Bendin, A. Gharbi, H. Dautel (Berlin, Germany)

12:10 – 12:30 **The Effects of *Metarhizium anisopliae* and *Steinernema carpocapsae* on Different Developmental Stages of *Ixodes ricinus***

M. Wassermann, E. Wurst, P. Selzer, J. Steidle, U. Mackenstedt (Stuttgart, Germany)

12:30 – 12:50 **The Biology of the Chalcid Wasp *Ixodiphagus hookeri* and its Suitability for the Biological Control of Ticks in Europe**

J. Steidle , P. Selzer , J. Collatz , C. Pfaff, M. Koban , M. Haas, M. Pfäffle , T. Petney , R. Oehme , P. Sebastian , U. Mackenstedt (Hohenheim, Stuttgart, Karlsruhe, Germany; Zurich, Switzerland)

**Lunch**

Session: Tick-transmitted pathogens II

*Chairs: G. Dobler, K. Henning*

13:40 – 14:00 **Phylo-geography of Tick-borne Encephalitis Virus in Central Europe**

G. Dobler, S. Frey, M. Pfeffer, S. Eßbauer (Munich, Leipzig, Germany)

14:00 – 14:20 **Evolution of TBE Virus in a TBE Focus over a Period of 5 Years**

G. Dobler, D. Höper, M. Beer, S. Eßbauer, S. Frey (Munich, Riems, Germany)

14:20 – 14:40 **Case of Human Granulocytic Anaplasmosis ex Scotland**

P. Hagedorn, M. Imhoff, C. Fischer, C. Domingo, M. Niedrig (Berlin , Germany)

14:40 – 15:00 **Prevalence of *Rickettsia* spp. in Tanzanian ticks**

L. Chitimia-Dobler, M. Starke, M. Nurtsch, N. Heinrich, M. Hölscher, G. Dobler (Bucharest, Romania; Munich, Germany)

**Coffee break with Poster session**

Session: Tick-transmitted pathogens III and ticks

*Chairs: N. Mencke, A. Nijhof*

15:30 – 15:50 **Evaluation of DNA Extraction Methods from Blood Samples Applied on FTA Cards for Molecular Diagnosis of Tick-Borne Pathogens**

Z. Hailemariam, P.-H. Clausen, J. Ahmed, A. M. Nijhof (Berlin, Borstel, Germany)

15:50 – 16:10 **Canine Vector Borne Diseases (CVBD): 2006 – 2014. An Initiative to Exchange Interdisciplinary Scientific Information and Increase of Vector Transmitted Diseases in Animals and Humans**

N. Mencke, M. de Lourdes Mottier, B. Schunack (Leverkusen, Germany)

16:10 – 16:30 ***In vitro* Feeding of *Dermacentor reticulatus***

B. Böhme, B. Bauer, P.-H. Clausen, A.M. Nijhof (Berlin, Germany)

16:30 – 16:50 **A Manipulation of Wound Healing Process by Tick Saliva**

P. Bartikova, I. Stibraniova, M. Slovak, V. Holikova, V. Hajnicka (Bratislava, Slovakia)

**Coffee break**

Session: Present and future of tick control

*Moderation: B. Habedank, A.Nijhof, K. Henning*

17:10 - 17:30 **Assessment of Climate Change Impacts and Adaptation Needs in Germany**

I. Schauser (Berlin, Germany)

17:30 – 18:00 **Plenary Discussion: Control of Ticks in the Prospect of Global Climate Change**

**19:30** **Dinner**

## Thursday, October 2

Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Building 35 (Practical course room, ground floor)

## Practical Workshop

| 9:00 – 12:00 | |
|---|---|
| **Tick Identification Workshop**<br>T. Petney, M. Pfäffle, N. Littwin (Karlsruhe, Germany) | **Demonstration: Artificial Feeding of Ixodid Ticks**<br>C. Krull, A. Nijhof (Berlin, Germany) |

12:00- 12:15 **Closing of the Workshop**

12.30 - 13.30 **General Assembly of the German Society of Medical Entomology and Acarology (DGMEA)**

## Poster presentations

**P01** **A New Map of Geo-Referenced Tick Locations in Germany**

F. Rubel, K. Brugger, H. Dautel, O. Kahl, S. Leverenz (Vienna, Austria; Berlin, Germany)

**P02** **Materials on the Biology of Preimaginal Phases of Ticks (Acari: Ixodidae) in the South of the European Part of Russia**

L. Shaposhnikova, E. Lazarenko, N. Ermolova (Stavropol, Russia)

**P03** **Lyme Disease Ecology in British Wildlife**

L. Perrin, R.J. Birtles, J. Seikel, R.J. Delahey, A. Tomlinson (Woodchester, Salford, UK)

**P04** **Molecular Evidence of Tick-Borne Diseases in *Dermacentor reticulatus* Ticks Collected from Dogs from Eastern Austria**

M. Wijnveld, A.-M. Schötta, G. Duscher, M. Leschnik, H. Stockinger, G. Stanek (Vienna, Austria)

**P05** **Tick-Borne Pathogen Xenodiagnosis in Ticks Collected from Ruminants in Maban County, South Sudan**

T. F. Mota, P.-H. Clausen, Z. Hailemariam, A. M. Nijhof (Salvador, Brazil; Berlin, Germany)

**P06** **OAKS: Optimization and Automation of Artificial Tick Feeding**

B. Boehme, C. Krull, P.-H. Clausen, A. M. Nijhof (Berlin, Germany)

**P07** **Molecular Detection and Genetic Characterization of the Crimeancongo Hemorrhagic Fever Virus in Ticks from South of Russia**

A. Volynkina, Y. Levantsova, E. Kotenev (Stavropol, Russia)

**P08** **Genetic Typing of *Coxiella burnetti* Isolates from Separate Areas of the North Caucasus**

E. Kotenev, A. Volynkina, Y. Levantsova (Stavropol, Russia)

**P09** **The Reverse Line Blot for Borrelia Detection and Discrimination of Different *Borrelia* Genospecies**

A.-M. Schötta, M. Wijnveld, M. Reiter, A. Müller, H. Stockinger, G. Stanek (Vienna, Austria)

**P10 iSpot Lyme: A Sensitive and Specific ELISPOT Assay for the Detection of Antigen-Specific T-Cell Response to *Borrelia burgdorferi***

R.E. Kneusel, W.E. Grose, B. Peacock, T.B. Gherezghiher, G. Kellermann (Freiburg, Germany; Osceola, USA)

**P11 Strong Systemic Th2 Responses in Nematode Co-Infection do not Influence Susceptibility to Ticks and Lyme Disease Spirochetes**

D. Maaz, S. Rausch, D. Richter, J. Krücken, A.A. Kühl, J. Demeler, F.R. Matuschka, G. v. Samson-Himmelstjerna, S. Hartmann (Berlin, Potsdam, Braunschweig, Germany)

**P12 Av-PDI Protein, a Candidate for Anti-Tick Vaccine?**

I. Stibraniova, M. Slovak, M. Kazimirova (Bratislava, Slovakia)

**P13 An Immunological Strategy for the Control of Poultry Mites**

G.R. Makert, M.-E. Krautwald-Junghanns, F. Mozafar, M. Voss, S. Ulbert (Leipzig, Cuxhaven, Germany)

# Abstracts of Oral Presentations

# The Ecology of Ticks (and Some Caveats to Interpret and Model it)

A. Estrada-Peña[1], J.S. Gray[2], O.Kahl[3], R.S. Lane[4], A.M. Nijhof[5]

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[2] *UCD School of Biology and Environmental Science, University College Dublin, Dublin, Ireland*

[3] *Tick-radar GmbH, Berlin, Germany*

[4] *Department of Environmental Science, Policy and Management, University of California, Berkeley, USA*

[5] *Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

Increasing interest in tick-borne zoonotic agents, accompanied by the development of accurate and accessible molecular tools, has resulted in a marked upsurge of published papers on the topic over the past three decades. Although this has resulted in distinct progress in knowledge, some of the hard-learned lessons of the past have inevitably been overlooked during this period of intense activity. Typical problems include premature or superficial data on the abundance and seasonal activity of ticks, and inappropriate use of statistical methodology to correlate the observed patterns of distribution with abiotic (climate) variables.

We assume that the procedural and analytical errors in current tick and tick-borne zoonotic disease research are often a consequence of a lack of knowledge or of suitable training. This presentation is not intended as an exhaustive review on tick biology and behavioural ecology. We paid special attention to the variables regulating the activity of the ticks, and how these should be recorded and interpreted. Most ixodid ticks are inactive in the lowest layers of vegetation or in the leaf litter or soil before they begin to quest. The combination of a set of suitable conditions, which normally involves an activation temperature in the spring, triggers the activity causing the ticks to climb to the top of the vegetation to quest for hosts. During questing, ticks may lose water that they normally regain by descending at intervals into the litter zone where the ticks actively reabsorb water vapor from the atmosphere (Rudolph and Knülle, 1974; Kahl and Alidousti, 1997). After the ticks are rehydrated, they are ready to ascend the vegetation. Ticks vary in their ability to retain or to gain water (Kahl and Knülle, 1988) and there is an interspecific variability in the management of their water balance. Extrinsically, tick water balance is affected by the saturation deficit of water in the air (affecting water loss) and by relative humidity (affecting the possibility of water gain by active water vapor uptake). The energy reserves of the tick plus its ability to maintain an acceptable level of body water are the factors mainly regulating the short-term questing behaviour of ticks. Host stimuli may also affect tick activity.

Climate change and climatic variations within seasons are likely to influence the epidemiology of vector-borne diseases (Patz et al., 2003). This has kindled interest in capturing the basic patterns of climate and other environmental features regulating the geographical ranges of ticks and their associated pathogens. We want to outline some misconceptions that may affect the performance and conclusions drawn from modelling exercises. The capture of data to estimate the direct and indirect effects of weather on the distribution, phenology or spread of ticks is commonly based on the idea of “climate” or “environmental” niche (Soberón and Nakamura, 2009). This is defined as the “intersection” of values of the climatic variables at which optimal development and mortality

occur, resulting in the best performance of the population. This concept assumes that the most important factors driving the performance of ticks are related to weather and that niches can be reconstructed by relating data on the occurrence of the tick with datasets summarizing climate, topographic, edaphic, and other "abiotic" or "ecological" variables. It is also assumed that (1) there is a niche conservatism and the organism tracks the sites where adequate conditions exist (not taking into account adaptations of local populations to local weather) and (2) the complete distribution range of the organism has been surveyed. The tick's niche is evaluated to infer its associations with environmental variables. Inference is later projected into a target area to obtain a map that explains "how similar" the conditions in space are, compared with the ones where the tick has been collected. Such a measure of similarity is not an estimate of abundance or of distribution, although it is incorrectly assumed to be a "risk map," in which "climate similarity" is interpreted as a direct estimator of abundance. Many other variables, including the fine scale distribution of hosts, human factors altering the habitat, geographical barriers to host movements and the uncertain effects of the vegetation, influence the abundance of ticks (Estrada-Peña et al., 2012) and therefore the crude map is not a direct projection of the spatial risk.

Other procedural gaps in the evaluation of the environmental niche of a tick involve the inadequate utilization of interpolated climate datasets, which inflate the models because of autocorrelation (Legendre, 1993) and collinearity (Storch et al., 2003). Both factors strongly modify the apparent influence of variables that affect the distribution of an organism, resulting in the false perception of a well-fitted model. Determination of climate niche is also affected by the partial and subjective use of a limited number of collections of the tick to be modelled, by the inclusion of variables that are correlated with others to build the model, such as elevation and temperature or by the selection of variables based on the predictive performance of the best model, but lacking a biological significance. The utilisation of the elevation in attempts to relate tick occurrence patterns to environmental variables is misconceived. At a local scale, elevation greatly affects the patterns of climate, decreasing the temperature over the altitudinal gradients and influencing the rainfall. In every case the performance of the model will be inflated giving the false perception of a robust model, biasing the conclusions and the projections. We propose herein the use of satellite-derived information transformed through a harmonic regression to remove the collinearity correlated variables. We further demonstrate the abilities of "hybrid models" (those including both correlative and process-driven methods) to develop a framework against which test the effects of the trends of climate on the life cycle of ticks. We illustrate such hybrid approach with an example involving the impact of the climate of the last 110 years on the development and mortality processes of the tick *Hyalomma marginatum*.

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Estrada-Peña, A., Farkas, R., Jaenson, T. G. T., Koenen, F., Madder, M., Pascucci, I. (2013). Association of environmental traits with the geographic ranges of ticks (Acari: Ixodidae) of medical and veterinary importance in the western Palearctic. A digital data set. Exp. Appl. Acarol. 59, 351–366.

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Storch, D., Konvicka, M., Benes, J., Martinková, J., Gaston, K. J. (2003). Distribution patterns in butterflies and birds of the Czech Republic: separating effects of habitat and geographical position. J. Biogeogr. 30, 1195–1205.

# Ticks in Romania and Recent Trends of Spread

L. Chitimia-Dobler

*Institute of Diagnosis and Animal Health, Bucharest, Romania*

Romania is located in the transition zones of Central, Eastern and Southern Europe which create many different ecological niches such as coastal shores, medium and high altitude mountains and plains. In Romania actually 25 different species of ixodid ticks have been described in Romania. They include ticks from five genera, among them *Ixodes* (11 species), *Dermacentor* (2 species), *Haemaphysalis* (5 species), *Rhipicephalus* (4 species) and *Hyalomma* (3 species). These descriptions and the knowledge on the geographical distribution are mainly based on the work of Feider (1965) and two recent updates (2011, 2012). However, few studies are done on the recent geographical distribution and host preferences of Romanian ixodid ticks so far. These data are essential for the surveillance of tick pathogens of veterinary and medical importance.

Ticks were sampled in 25 of the 41 districts of Romania by flagging and sampling from domestic and wild animals and determined morphologically. The found data were compared with the available data of geographical distribution and host preferences in Romania.

A total of three species of *Ixodes* (*Ix. ricinus*, *Ix. crenulatus, Ix. apronophorus*), the two species of *Dermacentor (De. marginatus, De. reticulatus*), four species of *Haemaphysalis (Ha. punctata, Ha. concinna, Ha. sulcata, Ha. parva*), three species of *Rhipicephalus (Rh. sanguineus, Rh. annulatus, Rh. bursa*) and two species of *Hyalomma (Hy. scupense, Hy. marginatum*) could be identified and geographically located.

Comparing our results with the available data on the distribution of ixodid ticks in Romania shows that tick species identified have a larger distribution as mentioned before in Romanian literature. At least for *De. reticulatus* a clear spread into new districts could be shown during the last 8 years. *Rhipicephalus bursa* could be detected again in areas already described by Feider, 50 years ago. *Dermacentor reticulatus* could be detected for the first time in Romania on wild boar (*Sus scrofa*), on foxes (*Vulpes vulpes*) and on humans. *Ixodes apronophorus* was described for the first time on dogs and *Hy. marginatum* nymphs were detected for the first time on humans and on dogs.

Our data clearly show that the geographic distribution of tick species is not static but changing during time. The changes might be caused by misidentification, sampling outside of the respective tick activity or on the wrong host or ecological niche or by a real invasion of tick species into new areas of distribution. The knowledge on the distribution and of the invasion of tick species into new areas forms the basic for correct diagnosis, treatment and prophylaxis of tick-borne pathogens in humans and animals.

# Dynamics of a TBE Focus over a Period of 6 Years

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Tick-borne encephalitis (TBE) is the most important tick-borne virus disease in humans in Central Europe. The causative agent is a virus of the mammalian tick-borne group in the genus Flavivirus of the family Flaviviridae. TBE virus is circulating in so called natural foci which are small locations where the TBE virus can be detected in ticks and in mammalian hosts (small mammals). These natural foci are scattered over large areas in Central Europe. Human TBE cases are appearing with a large range of total numbers in many countries of Europe. So far it is not clear what might be the reasons for these fluctuations of annual human TBE cases. So far it is not known what ecological factors determine these natural foci. Also the tick population and the prevalence of TBE virus in the ticks has not been followed over a longer period to understand more about the dynamics of TBE virus circulation in the natural focus.

In order to characterize the dynamics of the tick population and to identify climatic or other ecological factors which might influence the tick populations and the prevalence of TBE virus in ticks, a natural focus of TBE virus in Eastern Bavaria was chosen since 2009 sampled every month for ticks. The sampling was conducted by flagging and under comparable conditions. Two hours before dawn sampling was started using the same way and also sampling for a time of 90 to 105 minutes and also by the same person. Ticks were counted in total and also separated into different stages and adults also in different sexes and were pooled in 10 animals (larvae, nymphs) or five animals (adults). Tick pools were crushed using a Bio101 matrix A. Total nucleic acid was extracted from ticks using a MagNA Pure automatic extraction machine. Tick pools were tested by real time-RT-PCR (Schwaiger & Cassinotti, 2003). For positive tick pools the E gene and the NS2a gene were sequenced to confirm the real time PCR result.

Between 2009 and July 2014 a total of 9004 ticks were collected and tested for TBE virus. All ticks were examined morphologically and identified as *Ixodes ricinus*. No clear periodicity of the tick populations could be detected over 6 years of testing. In 2009 we found a high number of nymphs and adults, while in 2010 the numbers of nymphs and adults were very low. In 2011 the numbers of nymphs increased to comparable values as in 2009 but the adult tick stages remained on a low level. In 2012 the number of nymphs was low, but high number of adults were detected. In 2013 the numbers of nymphs increased again while the number of adult ticks remained stable at the level of 2012. In 2014 we found similar numbers of all stages of ticks like in 2013. In 2012 and 2013 an unusual second peak of tick activity and numbers in autumn could be detected. A total of 23 positive pools were detected by PCR during the period studied. Positive ticks were found in all months from March to September, but at any year only two to four of all months samples resulted in TBE virus positive ticks. Assuming that only one tick per pool was positive an overall infection rate of 0.26% was detected. Looking at the particular tick stages no positive larvae were detected. The prevalence in the nymphal stage ticks was 0.23%. The overall positivity rate of female adult ticks was 0.25% and of adult male ticks was 0.29%. However calculating the infection rates of the particular single sampling activities the TBE virus prevalence rates for nymphs ranged from 0.3 to 2.5% while the adult prevalence rates ranged from 1.5 to 3.7%.

A comparison of the tick dynamics with weather parameters showed no clear correlation between tick populations and the monthly maximum or minimum temperatures or to the precipitation. Also the winter precipitation or temperatures seem to have no impact on the tick population of the following spring and summer period.

After six years of tick sampling in one TBE focus no clear periodicity of tick numbers nor of virus prevalence rates could be detected. Also the weather parameters did not show to have any effect on the tick populations. Also the TBE virus prevalence rates in ticks seem to have a random range. In total the adult stages seem to be infected to a higher proportion than nymphal stages. The negative results for larvae maybe resulted in the very low numbers of collected larvae. The current results imply that the tick populations and prevalence of TBE virus in tick populations seem to correlate with human cases. Other factors may be responsible for the large annual ranges of human TBE cases.

# Monitoring *Ixodes ricinus* Questing Activity on Field Plots over Several Years – Implications on our Knowledge of the Life Cycle and the Phenology of the Tick

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The hard tick *Ixodes ricinus* is the most common vector tick of the causative agents of Lyme borreliosis (LB) and tick-borne encephalitis (TBE) in large parts of Europe. Knowledge of the seasonal questing activity of different life stages of *I. ricinus* and its interference by environmental factors is crucial for estimating the circulation patterns of tick-borne pathogens. Flagging or dragging has been the standard method to determine the level of questing of *I. ricinus* populations and its seasonal course. Although flagging seems a simple methodology and is basically effective for collecting questing larvae, nymphs, and adults of *I. ricinus*, it has also certain drawbacks. For example, flagging is far less effective and sometimes even impossible when it is raining, when there is wet substrate, strong wind, darkness, and thick or thorny underbrush. A promising alternative is the use of field plots where engorged ticks are deployed and kept under field conditions. They develop in those plots to the following life stage and get active sooner or later after the moult. Vertically set wooden rods allow ticks of each postembryonic life stage to leave the leaf litter and climb the tips where they can be counted as a measure of questing activity. Observation and counting of questing ticks on such plots is possible independently from weather and time of the day. We have used this robust, quasi-natural monitoring system in different parts of Germany for several years. This system allowed us to monitor tick activity with regard to short-term weather events, and also year-to-year variations of questing tick abundance could be detected. This was particularly distinct in the year 2012, when tick activity was very low in many parts of Germany, as were LB and TBE incidences. Moreover, because the developmental history of all ticks in those plots is known, it is also possible to learn more about the seasonal timing of the tick life cycle. The used system allows us to obtain data on the seasonal time when individual ticks quest for the very first time after their previous moult.

Based on our results of the seasonal timing and duration of various developmental phases of *I. ricinus* (including periods of development, questing, and dormancy), we conclude that the life cycle of the species in Central Europe under natural conditions lasts 4–6 years, which is distinctly longer than so far estimated.

Since 2011 the study has been performed on behalf of the Federal Environment Agency within the UFOPLAN FKZ 3711 48402 and 3713 48 402 and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety.

# ZUP – Zecken, Umwelt, Pathogene Baden-Württemberg. An Interdisciplinary Project to Study the Ecology of Ticks as Vectors of Pathogens in Baden-Württemberg, Germany

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Ticks are the main vectors of disease to humans and animals in Europe. The presence of tick-borne diseases in a region is affected by a variety of factors. Thus, for example, ecological and micro-and macroclimatic factors influence the abundance of ticks and their hosts. Although many of these factors are already known and a lot has been published about ticks and tick-borne diseases in the last decades, there has been no long-term study in Central Europe in which the influences of environmental factors, the population dynamics of the vertebrate hosts of ticks, the ticks themselves and the pathogens they transmit have been well documented. Without information about these factors, however, it is impossible to describe and understand changes in the abundance and spread of ticks and tick-borne diseases of humans correctly. The same applies to the development and introduction of appropriate prevention and control strategies. The project Z(ecken) U(mwelt) P(athogene) Baden-Württemberg (ticks, environment, pathogens) is an interdisciplinary project, in which experts from different fields work together to investigate the ecology of ticks and tick-borne diseases in Baden-Württemberg. Our goal is to find out which abiotic (climate, weather, soil) and biotic (hosts, vegetation) factors play a role in the distribution and dynamic of ticks and the pathogens they transmit.

# *In vitro* Cultivation and Development of Diagnostic Tools for the Emerging Pathogen Borrelia miyamotoi

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*Borrelia miyamotoi* was first described in 1995 (1) in Japan. It belongs to the relapsing-fever group of spirochetes but is transmitted by ixodid ticks and occurs sympatrically with *B. burgdorferi* s.l. in Asia, Europe and USA. In 2011, human cases of *B. miyamotoi* infection and associated disease were reported in Russia (2) suggesting that this species represents an emerging human pathogen. More recently, human infection with *B. miyamotoi* were also reported from USA and Europe. *Borrelia* are known to be fastidious bacteria and some strains of this species have proven particularly difficult to adapt to culture conditions. Here, we report a method for successful long-term *in vitro* cultivation of *B. miyamotoi* from Japan and the USA. The type and quantity of serum as well as the atmosphere were critical for successful *in vitro* cultivation. Maximum density of bacteria reached $2.5x10^7$/ml.

We have also developed a screening PCR for *B. miyamotoi* in *Ixodes* and potentially human material. This duplex real-time PCR can be used for simultaneous detection of *B. burgdorferi* s.l. and *B. miyamotoi*. Tick samples from a recreational area near Bonn and from Bavaria were tested. Real-time PCR results were confirmed by Sanger sequencing of housekeeping genes. Results indicate a prevalence of *B. miyamotoi* in *Ixodes ricinus* from these regions of about 1-2 %.

**References:**

(1) Fukunaga et al., 1995. Genetic and Phenotypic Analysis of *Borrelia miyamotoi* sp. nov., Isolated from the Ixodid Tick Ixodes persulcatus, the Vector for Lyme Disease in Japan, Int J System Bacteriol 45(4), 804-810

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# Abundances of *Ixodes ricinus* and Prevalences of *Borrelia sp.* in the Nature Reserve Siebengebirge, Germany

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In the Siebengebirge, a forested hilly nature reserve and popular local recreation region near to Bonn, Germany, ticks were collected 2012 to 2014 in three different plant communities possessing different plant densities to compare the abundances of *Ixodes ricinus* and prevalences of *Borrelia* sp. with data obtained 1987/89 and 2001, 2003, 2007 and 2008 at the same locations. Mild winter months in 2007/2008 and 2013/2014 and extended autumn seasons have favoured most likely the host-seeking activity of ticks at the site with high plant densities. However, presumably late cold periods in winter 2011/2012 and strong precipitation in summer induced opposite effects. Total prevalences of *B. burgdorferi* s.l. in questing ticks decreased from 2007 to 2013. Considering *B. miyamotoi* for the first time, in 2013 about 2.6% of the ticks were infected. In the genospecies determinations of *Borrelia* s.l., *B. valaisiana* and *B. garinii*, which are associated with birds, were most prominent in the Siebengebirge from 2001-2013. *B. lusitaniae*, detected for the first time in the nature reserve in 2007, was also present in 2013. Infections of ticks with multiple *Borrelia* genospecies increased in the Siebengebirge during the last century and for the first time triple *Borrelia* infections were detected in 2007. This phenomenon requires more detailed investigations of the methodology because in 2013 rarely double infections were found. In conclusion, tick densities and the *Borrelia* composition in ticks have changed considerably in the last 20 years in the Siebengebirge.

Since 2012 the study has been performed on behalf of the Federal Environment Agency within the UFOPLAN FKZ 3711 48402 and 3713 48 402 and funded by the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety.

# Lyme-Borreliosis – Aspects on the Diagnosis and the Obligation to Report

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A major challenge for the diagnosis of Lyme borreliosis (LB) is the heterogeneity of the spirochetes that cause the disease. Meanwhile at least 20 assured and proposed genospecies were described and combined in the *Borrelia (B.) burgdorferi* sensu lato complex. Of these, five species – namely *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. bavariensis*, and *B. spielmanii* – are assured human pathogenic, while another three – *B. valaisiana*, *B. lusitaniae*, and *B. bissettii* - were only rarely associated with human disease. The major diagnostic tools for laboratory diagnosis of LB include cultivation, PCR, and – most important - detection of specific antibodies. Serology should be performed as stepwise diagnostic – screening with a highly sensitive immunoassay, that should be confirmed only in case of reactivity by an immunoblot. While in the early phase of the disease detection of specific IgM is important, in the late phase high IgG-values and a broad spectrum of specific IgG-bands is in the front. Only IgM-positivity even argues against late disease. Problems for serology include a diagnostic gap in the early phase of the disease, missing parameters for activity and therapy control and missing gold standard. Culture and PCR should not be used for screening but only for unclear cases, e.g. dermatological symptoms with high suspicion for LB but negative serology. A positve result should always be specified by adequate methods, that usually allows species identification. Most appropriate diagnostic materials include synovia/synovialis, skin and CSF. Several recent studies indicate that the chemocine CXCL13 when measured from CSF is a highly sensitive and specific parameter for diagnosis of early neuroborreliosis that even allows control of therapy.

Reasons for reporting of a disease include prevention of person to person transmission or control by vaccination – the public health system can do something, prevent citizens from disease. What can be expected from reporting LB? Primarily epidemiological data regarding incidence, higher awareness of the citizens and higher awareness, education and training of physicians.

On the other hand there is forseeable underreporting, education and training of physicians is not the task for notfication, so far there is no vaccine and if so there is no emergency scenario to prevent citizens from LB in realtime and reliable case definitions exist only for Em, Neuroborreliosis and Lyme arthritis. If the obligation to report is reasonable for LB is still a matter of controversial discussion.

## Vegetation vs. Vertebrates

# Activity Patterns of *Ixodes ricinus* on Vegetation and on Small Mammal Hosts in Baden-Wuerttemberg Forest Areas

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The phenology of ticks is the result of a complex network of interactions between abiotic and biotic factors. But it is not only the evaluation of the relative contributions of influencing variables to the resulting tick population and their dynamics, but also the simple analysis of tick activity patterns in the field which poses problems for scientists.

*Ixodes ricinus* is the most abundant and medically important tick species in Central Europe. Of particular interest for the transmission of tick-borne pathogens (TBP) to humans is the instar that causes most cases of infestation: the nymphs of *I. ricinus*.

To predict nymphal densities in the area of interest and thereby estimating the future risk for humans of acquiring an infection with TBP it is important to correctly assess the stock and activity patterns of larvae in the preceding year.

An easily accessible way to collect *I. ricinus* in the field is by dragging a cotton cloth over a defined area of vegetation.

However, tick larvae usually occur highly aggregated in nests - which can dramatically influence the assessment of the actual larval density.

Here we compare the seasonal dynamics of juvenile *I. ricinus* questing on vegetation with the infestation patterns of *I. ricinus* on small mammals in four different forest areas in Baden-Wuerttemberg, highlighting the differences between these two sampling methods.

# *Dermacentor reticulatus* – Host Preferences of Immatures and their Role as Vectors in the Hardtwald, Karlsruhe, Germany

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*Dermacentor reticulatus* is a widely distributed tick throughout much of Europe with a rapidly expanding range, making past statements on restricted habitat use difficult to interpret. It is associated with a variety of pathogens and although humans are rarely bitten, it might play an important role in the maintenance of pathogens in zoonotic cycles. Studies about the ecology of *D. reticulatus* are rare and more investigations on the host-tick relationship are required.

The results presented here are a part of a larger interdisciplinary project, studying the ecology of ticks, mainly *Ixodes ricinus*, as vectors of pathogens in Baden-Württemberg, Germany.

The study took place from May – October 2012 and March – October 2013 in the Hardtwald, north of Karlsruhe, Germany. As a mixed forest built on drift sand and inland dunes, the Hardtwald is unique for this region. Small mammals were trapped using Longworth live traps, arranged in a 40 x 40m grid patch with 25 traps per patch. In total we examined 4,800m² with a total of 75 traps. Trapping took place once a month on two consecutive nights. Caught mammals were weighed, identified to species, examined for tick infestation, marked with toe tattoos and released after the examination. All collected ticks were identified to life history stage and species and tested for the prevalence of *Borrelia burgdorferi* s.l., *Rickettsia* spp., *Babesia* spp. and tick-borne encephalitis virus.

In 2012 257 small mammals were trapped, in 2013 only 84, with the majority being the yellow-necked mouse *Apodemus flavicollis* (2012: 63.8%; 2013: 71.4%) and the bank vole *Myodes glareolus* (2012: 30%; 2013: 21.4%). The dominant tick species found on the hosts was *Ixodes ricinus* (2012: 1,260 (77.3%); 2013: 1,507 (85.9%)), followed by *D. reticulatus* (2012: 347 (21.3%); 2013: 246 (14%)) and other *Ixodes* spp (2012: 22 (1.4%), 2013: 2 (0.11%)). In both years, abundance and prevalence of *D. reticulatus* was significantly higher on *M. glareolus* compared to *A. flavicollis*, indicating clear host preferences. Additionally *D. reticulatus* does not seem to be important as a vector for *B. burgdorferi* s.l., however high prevalence of *Rickettsia* spp. in both larvae and nymphs were found.

# Abundance and Species Diversity of Rodent-attached Ectoparasites Trapped in Differently Structured Habitats in Germany

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Small mammals serve as main hosts for the development and the distribution of several blood-sucking ectoparasites such as ticks, fleas and mites. The aim of this study was to investigate the ectoparasite species composition and their abundance on rodents trapped in different habitats in order to evaluate the small mammals' role in the ectoparasites' development and maintenance.

In 2012 and 2013 rodents were captured at three differently structured study sites. The different locations are a forest site south of Augsburg, an urban study site in the city centre of Regensburg, both located in Bavaria, and a recultivated brown coal pit region in Leipzig, Saxony. Fleas, mites and ticks were collected from captured rodents and morphologically determined.

Altogether 631 rodents were collected: 243 (139 *Myodes glareolus*, 99 *Apodemus flavicollis*, 5 *Sorex coronatus*) at the sylvatic site, 36 (*A. sylvaticus*) at the urban site and 352 (257 *M. glareolus*, 79, *A. flavicollis*, 4 *A. agrarius*, 2 *Mustela nivalis*, 7 *Microtus agrestis*, 1 *Microtus arvalis*, 1 *Talpa europaea*, 1 *S. araneus*) at the renatured site. A total of 5656 ectoparasites, which belong to 27 different species, were collected from 508 of these small mammals. 3028 ectoparasites were collected at the recultivated site, 2326 at the sylvatic and 302 at the urban site. Altogether 1164 mites (mostly Laelapidae) belonging to 14 different species, 1101 fleas (mostly Hystrichopsyllidae**)** also belonging to 14 different species and 3391 ticks (Ixodidae) of three different species, were collected. The highest ectoparasite species diversity was detected at the recultivated site with 21 species. In comparison to this finding, 18 species were detected at the forest site and 11 species at the urban site. At the sylvatic site the avarage ectoparasite burden was 9.6 ectoparasites per rodent while the average ectoparasite burden was 8.6 at the recultivated and 8.4 at the urban site per rodent. The high species diversity of ectoparasites and the high abundance on small mammals underline their essential role as hosts for several different ectoparasite species and their developmental stages. The particular allocation of ectoparasite species on their small mammal host species as well as their abundance will be subject to further analyses.

# Vaccinomics: Understanding Tick-Host-Pathogen Interactions for Vaccine Development

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Ticks and the pathogens that they transmit have co-evolved, resulting in a complex relationship complementary to both the tick host and pathogen. The infection and transmission cycle of tick-borne pathogens is perfectly coordinated with the tick feeding cycle, and ticks can harbor high pathogen levels without impacting their biology. Tick-borne diseases continue to emerge and/or spread to new areas of the world posing increasing threat to human and animal health. The limitations of acaricide control have been realized, most notably by the selection of acaricide resistant ticks. New approaches for tick control are dependent on defining molecular interactions between hosts, ticks and pathogens to allow for discovery of key molecules that could be tested in vaccines for intervention of tick-pathogen cycles. Tick vaccines offer the important advantages of being a cost-effective and environmentally friendly alternative with a dual effect reducing tick infestations and preventing ticks from transmitting disease-causing pathogens. Tick antigens studied thus far have demonstrated multiple impacts when used in a vaccine including reductions in (a) tick infestations and fertility, (b) tick pathogen infection, (c) tick vector capacity for pathogen transmission and (d) tick response to pathogen infection. However, commercialization of tick vaccines has not advanced since the first BM86-based vaccines were registered in the early 1990s. The challenge of developing improved tick vaccines arises from the need to understand the complex molecular relationship between vertebrate hosts, ticks and pathogens which requires a systems biology approach that will allow for discovery of key molecules that mediate tick and pathogen success. A vaccinomics approach could then be used to identify and fully characterize candidate protective antigens and validate vaccine formulations, including development of effective screening platforms and algorithms for analysis and validation of data produced by the systems biology approach to tick research. New candidate protective antigens will most likely be identified by focusing on abundant proteins with relevant biological function in tick feeding, reproduction, development, immune response, subversion of host immunity and pathogen transmission. Tick vaccines that affect both tick infestations and pathogen transmission could then be developed and used to vaccinate human and animal populations at risk for disease prevention and also reservoir host species in order to reduce tick infections and their vector capacity for pathogens that affect human and animal health worldwide.

# Tick Control – A Challenge in the Fields of Health and Environmental Protection

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Ticks are of outstanding importance as hematophagous ectoparasites of animals and man and especially as vectors of pathogens like TBE-virus, *Borrelia* spp., *Rickettsia* spp., *Babesia* spp., *Anaplasma phagocytophilum*. In the year 2013, in Germany were registered 442 new cases of human infections with the TBE-virus and 7834 new cases of Borrelia infections in 8 of the 16 German Federal States that are reporting (1). Data on human TBE in Europe are summarized by the European Center for Disease Control (2). The ixodid tick species *Ixodes ricinus, Dermacentor reticulates* and *Rhipicephalus sanguineus* and the argasid tick species *Argas reflexus* have been the main target organisms in Central Europe for prevention or control measures.

*I. ricinus* as the most important vector in Central Europe is highly abundant in many ecosystems where the use of acaricides is inappropriate due to the effect on non-target organisms (spiders, insects, food chain). Individual protection measures against tick infestations are the use of repellents (see presentations Dautel et al., Büchel et al.) or acaricides that are applied to animals (see presentation Mencke et al.), the wearing of acaricide-impregnated clothes by persons with a professionally increased risk of tick infestation, and in general the adaptation of clothing and the direct searching for ticks on the body after visits of tick habitats.

The permanently high number of new cases of infection with tick-transmitted pathogens requires the improvement of measures for tick bite prevention and tick control. Therefore, the validation and practical implementation of new methods is necessary to achieve a regional reduction of tick populations in natural foci of tick-borne infections. The most promising procedures to reduce tick populations in compliance with health and environment protection are the development of anti-tick vaccines (see presentation de la Fuente) and methods of biological tick control (see presentations Wassermann et al., Steidle et al.) if the latter is realizable under practical conditions. Tick traps that contain tick pheromones as attractants showed limitations in the effective time and range. The direct or indirect acaricide treatment of wild hosts is problematic in the field of environment protection and promotes the development of acaricide resistant tick strains.

Indoor populations of the pigeon tick *Argas reflexus* and the brown dog tick *Rhipicephalus sanguineus* can be eradicated directly and in consideration of the survivability of the tick species. Officially approved tick eradication measures are required according to the German Protection against Infection Act (IfSG). Former efficacy tests of chemical products (biocides) to control *A. reflexus* in our laboratory resulted in the recommendation of an effective combination of diazinon and pyrethrum as active ingredients (3). In 2010 diazinon was excluded from the inclusion in Annex I to Biocidal Directive 98/8/EC for product-type 18. Effective products or treatments for the eradication of soft and hard tick populations in indoor conditions are still lacking in the publication according to §18 of the German Protection against Infection Act (3; current publications available at www.bvl.bund.de). A potentially effective active ingredient against *A. reflexus* is alphacypermethrin. This pyrethroid, among a row of tested formulations against the bed bug *Cimex lectularius,* led to the eradication of the test animals within short exposure times on different types of surfaces.

The plenary discussion at the end of this workshop day serves the identification of essential gaps in the research of hard tick control measures and of options of a hard tick reduction in the environment with the focus on practicability.

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# Biocidal Product Authorisation – General Procedure and Outlook for Insecticides/Acaricides and Repellents

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Biocidal products are intended to repel, harm or kill organisms such as bacteria, arthropods or rodents with the aim to protect materials (e. g. wood or house facades) and to preserve hygiene in domestic houses and facilities. As these substances are intended to harm or kill target organisms, the probability of unintended harm to human health and the environment is given. Therefore, the review programme for existing active substances started in 2004, a European Union (EU)-wide strategy to assess active substances with a biocidal mode of action. The goal of this programme is to ensure that the use of biocidal products is safe for human health and the environment. The legal basis, the Biocidal Products Directive (98/8/EEC), was published in 1998. In 2012 the Biocidal Products Regulation (BPR) EU No. 528/2012 was published and replaced the directive in September 2013.

The authorisation of biocidal products consists of two parts – first the active substance has to be approved and included in a union list. Subsequently, applications for authorisation of biocidal products which contain substances on the list can be submitted to EU Member States. If the risk assessment for human health and the environment shows no risk for the applied use(s), the products will be authorised . Until all active substances are assessed, approved and included (or not) in the union list, biocidal products which contain notified substances can remain on the market.

What is the part of the UBA in the authorisation process? For active substances and subsequently biocidal products a risk for the environment has to be ruled out during the assessment. The Environmental Risk Assessment (ERA) compares a predicted environmental concentration (PEC) with a concentration, for which it is assumed, that it causes no effects on non-target organisms in the environment (Predicted No Effect Concentration – PNEC). If the quotient of both, the "Risk Quotient", is smaller than 1, the biocidal product poses no risk for the environment. Besides the classical risk assessment other negative factors have to be ruled out for active substances. The BPR gives exclusion criteria – substances which fulfill these criteria shall not be included in the union list. As an example for the environment, substances which are persistent (P), bioaccumulative (B) and toxic (T), so called PBT substances, shall be phased out and substituted with substances with less negative effects.

According to the Biocidal Product Regulation, biocidal products are divided into 22 product types (PTs), which can be summarised into 4 main groups: Disinfectants (main group 1), Preservatives (main group 2), Pest control (main group 3) and other biocidal products (main group 4). Products against arthropods (PT 18) and Repellents and Attractants (PT 19) fall into main group 3. For this group 21 (PT 18) and 5 (PT 19) active substances are currently included in the union list (cf. BAuA homepage, 08/2014) and the product authorization for PT 18 and PT 19 has already started. Until now an authorisation was granted for 25 biocidal products in PT 18 and three products in PT 19 (cf. BAuA homepage, 08/2014). These repellants contain the active substance DEET (N,N- diethyl-meta-toluamide) and two of the products are intended to be used against ticks (Ixodidae) in addition to mosquitoes.

# Efficacy Testing of *Ixodes ricinus* Tick Repellents: Comparison of Two Test Protocols for Human Subject Trials

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The hard tick *Ixodes ricinus* is the main vector of causative agents of tick-borne human diseases in Europe. Personal protection against tick bites is an important means to prevent such diseases and repellents applied to human skin are frequently used by the public. According to the Biocides Directive 98/8 EC, repellents marketed for human use in the EU need registration, which in turn requires evaluation and documentation of efficacy. However, up to date no technical guidelines are provided for evaluation of repellent efficacy.

During the past years, we performed numerous repellent assays with ticks, including human subject trials. For the latter, two protocols are available: the standard method of the US Environmental Protection Agency (EPA) used for regulatory purposes in the US, and the procedure used by the Stiftung Warentest (StiWa) and K-Tipp, a German and Swiss consumer care organisation, respectively. Although the designs of the assays seem to be similar, the ticks running through the tests have to perform dissimilar tasks and the question arises whether or not these assays produce comparable results.

We, therefore, directly compared both protocols in two trials. In the first one, we examined two repellents: Autan®, based on 20 % Picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid-1-methylpropyl ester), and ZeckWeck, based on 12.5 g/100 g Citriodiol™ (main compound: p-menthane-3,8-diol). In a second one, three repellents were investigated: Anti Brumm® naturelle, based on 20 % Citriodiol™ (main compound: p-menthane-3,8-diol), G090141, based on 20 % EBAAP (ethyl buthyl acetyl aminopropionate), and G090152 based on 10 % decanoic acid (capric acid) were compared.

The EPA assay indicated a significantly higher repellency of products containing Icaridin and EBAAP than the StiWa test, while no significant difference between assays could be detected for the remaining products. Additionally, the protection times were significantly longer (up to four hours) when determined according to EPA than to StiWa for three of the products. Also, significantly less ticks initially walked onto the repellent-treated skin when tested according to EPA than to StiWa in three products. Thus, the StiWa protocol appears to pose higher demands on a repellent than the EPA method. It remains open whether the degree of protection determined by such trials corresponds with the protection in the field, i.e. under real life conditions.

# Repellent Efficacy of DEET, Icaridin, and EBAAP against *Ixodes ricinus* and *Ixodes scapularis* Nymphs (Acari, Ixodidae)

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The European castor bean tick (*I. ricinus L;* Acari: Ixodidae) and the American blacklegged tick, (*I. scapularis Say;* Acari: Ixodidae) are important vectors carrying a variety of microorganisms potentially harmful to animals and humans. In Europe and the USA, more than an estimated 100.000 and 20.000 humans, respectively, develop Lyme Borreliosis (LB) as a consequence of tick bites. As there is no vaccine against LB available, prevention of tick-borne diseases in humans primarily depends on personal protection measures including the use of repellents. The most widely used repellents are based on quite a small number of active ingredients. These include DEET (N,N-diethyl-3-methylbenzamide), the most frequently used repellent worldwide, Icaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester)), and EBAAP (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester). However, studies that have directly compared efficacies of these repellents against ticks are not available as yet. In this investigation, the repellent efficacies of ethanolic solutions of 10% EBAAP, 10% Icaridin, and 20% DEET in human subject trials against host-seeking nymphs of *I. ricinus* and *I. scapularis* were evaluated. Tests were carried out according to the US-EPA standard protocol with repellents being applied to the forearm of ten volunteers. Application of 20% DEET resulted in median complete protection times (CPT; Kaplan-Meier median) between 4 and 4.5 hours, while 10% EPAAB yielded CPTs of 3.5 to 4 hours against *I. ricinus* and *I. scapularis*, respectively. Significant differences were neither found between the efficacies of the two repellents nor between the two species tested. In contrast, 10% Icaridin yielded a median CPT of 5 hours against nymphs of *I. scapularis*, being significantly shorter than against *I. ricinus* ($p < 0.01$), where the CPT lasted 8 hours. Based on these studies, EBAAP and Icaridin are efficacious alternatives to the widely used DEET in their repellent activity against nymphs of the two *Ixodes* ticks with Icaridin demonstrating particularly promising results against *I. ricinus*. Future research should investigate which potencies these substances do have in repelling adult *Ixodes* ticks and/or other tick species.

This study was performed on behalf of the Federal Environment Agency, contract number 27962.

# The Effects of *Metarhizium anisopliae* and *Steinernema carpocapsae* on Different Developmental Stages of *Ixodes ricinus*

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Ticks (Ixodida) are important vectors for many pathogens and are of public health and veterinary importance. The focus of the present study was to test natural antagonists against the most common tick species in Europe *Ixodes ricinus* [1] and to study their possible use as biological control agents. The tested antagonists are *Steinernema carpocapsae*, an entomopathogenic nematode, and the fungal species *Metarhizium anisopliae*. The efficiency of those antagonists was investigated under laboratory as well as under field conditions.

*Steinernema carpocapsae* was able to reduce the number of nymphs as well as adult ticks up to 27% or 19% respectively. Three different concentrations of *Metarhizium anisopliae* blastospores were tested against different developmental stages of *Ixodes ricinus*. The highest concentration of $10^7/cm^2$ reduced the number of unfed nymphs and adult ticks significantly. 85% of the engorged nymphs were killed under field conditions and only few engorged larvae were able to develop into nymphs.

The results of the experiments revealed that especially *Metarhizium anisopliae* was able to reduce *Ixodes ricinus* significantly. The potential of these antagonists as possible biological control agents are finally discussed.

# The Biology of the Chalcid Wasp *Ixodiphagus hookeri* and its Suitability for the Biological Control of Ticks in Europe

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The chalcid wasp *Ixodiphagus hookeri* is a highly specialized parasitoid of ticks. It lays its eggs in the larval or nymphal stages of ticks. When the parasitized nymph starts feeding the development of the wasps begins. During this process the engorged tick gets eaten from the inside by the wasp larvae, leaving only an empty shell in which the wasp larvae pupate. After some weeks adult wasps hatch out of the tick shell.

When we started our research with *I. hookeri* only two sites in Germany were known to harbor wasps, one in Berlin and one in the Lueneburg Heath. Today many other sites are known. This indicates that the wasps are widely distributed in Germany and occur at sites where a larger density of ticks is present. In bioassays we could show that *I. hookeri* most likely searches for bigger mammals like boars and deer to parasitize the ticks feeding on these animals. It seems smaller mammals like mice or voles are not used for host finding.

The suitability of *I. hookeri* for the biological control of ticks is also researched in other countries. In Kenia scientists were able to reduce the tick density in a herd of cattle by 95%. We want to find out whether the wasps can also be used for the control of ticks in Central Europe.

# Phylo-Geography of Tick-Borne Encephalitis Virus in Central Europe

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Tick-borne encephalitis (TBE) is the most important tick-borne virus disease in humans in Central Europe. The causative agent is a virus of the mammalian tick-borne group in the genus *Flavivirus* of the family Flaviviridae. Recent studies on the phylogeny of TBE virus and related tick-borne flaviviruses show that TBE virus evolved in Asia and divided into two branches, a eastern branch containing the Far Eastern subtype of TBE virus and the Siberian subtype(s) of TBE virus and an eastern branch which finally evolved into the European subtype of TBE virus and Louping ill virus and its subtypes.

It is so far not clear how the European subtype of TBE virus evolved and how it spread over Eastern and Central Europe to reach its current area of distribution. So far also no efforts were conducted to sub-type the European subtype of TBE virus and finally to sub-classify into genetic clades or genotypes to clarify the geographic distribution of this subtype and also to construct possible ways of spread.

In an effort to identify the TBE virus strains circulating in Central Europe we so far sequenced the E genes of more than 100 TBE virus strains and compared the sequences with 12 TBE virus E gene sequences which were available in the data base. The TBE virus strains were originating from Germany, Austria, Czech Republic, Slovak Republic, Poland and Russia. Most of the strains were isolated or amplified directly from infected ticks. Some of the strains were isolated in cell culture and finally low cell culture passages were sequenced using amplification by conventional PCR and Sanger sequencing.

The 115 analyzed E genes of TBE viruses could be distinguished in a total of 12 genotypes. Some of the genotypes could be only detected in single TBE foci or in TBE natural foci close to each other (regional distribution). Some other genotypes included strains which were originally located over whole countries (e.g. Austria, Czech Republic) or even were distributed over the whole European continent (e.g. Germany, Czech Republic, Poland, Russia). Although the total differences in E gene homology were high (> 98%) viruses from each focus could be distinguished from viruses from other foci. Earlier analyses on the chrono-phylogeny of TBE virus strains show that the TBE virus separated in two different genetic branches already more that 350 years ago. The close proximity of TBE virus strains from the two branches show that the TBE virus was introduced into Germany and maybe other areas by multiple importations. So far, it cannot be understood how this introduction might have happened. However the current data on the geographic distribution of TBE virus in Central Europe imply that it did not spread along man-made routes like it seems to be in Russia. More phylogenetic data are needed to finally get a complete picture of the distribution of TBE virus and then again try to establish models on the spread of TBE virus in Europe.

# Evolution of TBE Virus in a TBE focus over a Period of 5 Years

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Tick-borne encephalitis (TBE) is the most important tick-borne virus disease in humans in Central Europe. The causative agent is a virus of the mammalian tick-borne group in the genus *Flavivirus* of the family Flaviviridae. Morphologically, TBE virus is a singled strand RNA virus of positive polarity with a genomic size of about 11.000 nucleotides. As RNA virus , it replicates with an RNA-dependant RNA polymerase which is an enzyme lacking the proof reading function. Therefore RNA viruses exhibit high nucleotide variabilities ("quasi species"). So far no data exist on the genetic stability of TBE virus during the time.

In order to characterize the genetic stability and variability of TBE virus over time we sequenced the whole genomes of a total of 11 TBE virus isolates. All virus isolates were isolated from the same TBE focus in Eastern Bavaria. TBE virus strains were isolated from PCR positive ticks (*Ixodes ricinus*) in Vero cells over a period of 5 years (2009 to 2013). Sequencing was done using next generation sequencing from low passage isolates after Trizol extraction of viral RNA from cell culture supernatants.

The eleven virus strain genomes showed a high genetic homology of 99.88 to 100%. Using the master sequence of a strain of 2009 the virus strains could be distinguished in a total of 27 nucleotide exchanges resulting in only 7 amino acid changes in the whole genomes. Mutations were found in all structural and no-structural virus proteins. There seem to be some hyper variable regions in the E protein, in the NS2a protein and in the 3' terminal of the NS5 protein. We detected one virus strain (HM 329/11), which was completely identical to the master sequence of strain HM 475/09. The other strains showed from 5 to 10 nucleotide exchanges resulting in one amino acid change (6 strains) two amino acid changes (2 strains) or even 3 amino acid changes (one strain). Looking at the phylogenetic analysis of the whole genomes there seems to be a minor undirected evolution of the virus strains which is completely different from the evolution seen in other flaviviruses like Dengue virus. So far it is unclear what stabilizes the genome in a way that also minor changes of the genome may be fatal for the resulting virus variant. The cellular factors of the tick cells or of the mammalian cells which narrow the genetic repertoire of TBE virus in a way like detected here have to be elucidated.

# Case of Human Granulocytic Anaplasmosis ex Scotland

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Human Granulocytic Anaplasmosis (HGA) is a tick-borne disease caused by *Anaplasma phagocytophilum,* an obligate intracellular Gram-negative bacterium that infects granulocytes. The usual clinical presentation includes nonspecific fever, chills, headache, and myalgia. Infection is more often mild or asymptomatic, but severe systemic complications can occur requiring intensive care. The rate of fatality is estimated at 0.5% to 1%.

HGA was first described in 1932 in Scotland as the causative agent of tick-borne fever in sheep but is as yet largely unknown both to European healthcare practitioners and to the general public due to the absence of documented clinical cases in several countries of Europe like UK and Germany. Although some clinical cases are described, the number of cases lags a lot behind the cases in the USA. This cannot be explained by the prevalence of the pathogen in ticks or the exposition of the people to it since the prevalence rate in the European vector for *Anaplasma phagocytophilum I. ricinus* seems to be with 3% nearly as high as in the American vectors *I. scapularis* and *I. pacificus* with a median prevalence of 4.7%.Moreover the median seroprevalence rate in European human is with 6.2% notable high whereupon it reach up to 21% in some studies. In comparison the seroprevalence in USA reaches 36% in endemic areas. In Europe most clinical cases are reported from Slovenia, Sweden and Poland.

In August 2013, an immunocompetent 40 year-old male came down with fever (about 39°C) and other nonspecific symptoms such as malaise, myalgia and severe headache three days after becoming aware of bites by several ticks while on a hiking vacation in Scotland. Three *I. ricinus* nymphs were removed from the patient's legs directly after their discovery, stored in a plastic container and sent for later analysis at the Consultant Laboratory for Tick-borne Encephalitis in Berlin, Germany. When the patient returned to Germany, five days after the onset of symptoms and eight days after tick removal, a first blood sample was drawn (sample 1) and the bite sites were swabbed with a sterile cotton bud. The patient was defervescent by that time; malaise and other symptoms persisted and a doxycycline course was started. All symptoms then subsided within two days, and the patient recovered completely.

A second blood sample was drawn 28 days after tick removal (sample 2). A complete blood count analysis was performed on both samples, and all parameters were within the reference range except a moderate increase of lactate dehydrogenase (248 U/l) compared to standard defaults (< 245 U/l) that was observed in the first sample. No significant changes in routine serum parameters were observed between samples 1 and 2.

DNA from whole blood samples and swabs was extracted (QiAmp DNA Blood Mini Kit) and tested for *A. phagocytophilum*, *Babesia* spp., *Borrelia* spp. and *Rickettsia* spp. using commercially available kits (rapidSTRIPE Anaplasma Assay, rapidSTRIPE Babesia Assay, rapidSTRIPE Borrelia Assay, rapidSTRIPE Rickettsia Assay; all Analytik Jena AG, Jena, Germany). DNA extracted from blood and swabs was negative for all the tested pathogens.

Following taxonomic identification of the tick specimens, DNA/RNA was extracted (blackPREP Tick DNA/RNA Kit, Analytik Jena AG) and likewise tested for the above-mentioned tick-borne pathogens. Two ticks out of the three tested positive for *A. phagocytophilum*. All ticks were negative for *Babesia* spp., *Borrelia* spp. and *Rickettsia* spp.

Indirect immunofluorescence (IIF) assays (Focus Diagnostic, Cypress, California, USA) performed on the paired serum samples revealed an increase of the *A. phagocytophilum*-specific IgM titer from 1:20 five days after the onset of symptoms to 1:80 twenty days later, while the specific IgG titer rose from a high level of 1:800 to a titer higher than 1:3,200 over this period.

The presence and fourfold increase of *A. phagocytophilum*-specific IgM and IgG antibodies in paired serum samples confirmed the diagnosis of HGA in accordance with the Centers for Disease Control and Prevention (CDC) criteria. As described previously for several cases of HGA, blood counts were normal while serum lactate dehydrogenase was elevated. The diagnosis was further corroborated by the detection of *A. phagocytophilum* DNA in two of the three ticks removed from the patient's skin. PCR amplification failed to detect *A. phagocytophilum* DNA in the patient's blood, consistent with previous studies documenting frequent non-detection of *A. phagocytophilum* DNA in whole blood and significant drop in PCR positivity after the acute phase of illness.

HGA is not usually reported in Scotland like in the rest of Europe. Compared with the USA the number of clinical cases reported in Europe is low. To explain this contrast the presence of genetically and biologically differences are discussed. In order to explain the present antibody titers in population and ruminants  cross reactivity of the serological test should be considered.

# Prevalence of *Rickettsia spp.* in Tanzanian Ticks

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Tick-borne rickettsioses are a major medical problem on the African continent. However, especially in central and Eastern Africa few data are yet available on the *Rickettsia* species and the prevalence of *Rickettsia* species in ticks and on the importance of particular tick species as vectors for rickettsiae. In a previous serological study an IgG antibody prevalence rate of 67% against rickettsiae of the spotted fever group could be found in a population in Southwestern Tanzania. In order to identify possible *Rickettsia* species and their respective tick vectors ticks were sampled in the Mbeya region in Southwestern Tanzania either directly from farm animals (cattle, sheep goat) or by flagging from the vegetation.

A total of 260 ticks were tested. Ticks were identified morphologically and part of them was also identified using molecular identification. Each single tick was crushed and the nucleic acid was extracted using a MagNA Pure extraction automate. Single ticks were tested for rickettsiae using a panRick RT-PCR. Positive ticks were further tested to identify the Rickettsia species using multi locus sequence typing (MLST) of *ompA*, *ompB*, *src4* and 23S RNA genes.

We identified 12 species of ticks belonging to the genera *Rhipicephalus* (7 species) *Amblyomma* (one species), *Hyalomma* (3 species) and *Haemaphysalis* (one species). Most ticks collected from farm animals could be identified as *Amblyomma variegatum* or as *Rhipicephalus microplus* (former *Boophilus microplus*). Flagging the vegetation was not very successful. Only 8 ticks were collected within 1.5 hours of flagging in and around Mbeya city. Two species, *Rhipicephalus sanguineus* (7 ticks) and *Rhipicephalus bursa* (one tick) were collected. Except *Rhipicephlaus bursa* (one tick rickettsia negative) and *Rhipicephalus decoloratus* (22% (11/50) of ticks tested Rickettsia positive) all other tick species found showed prevalence rates for rickettsiae of higher than 50%. Except the species where only few ticks were available, *Rhipicephalus microplus* showed a positive rate of 66% and *Amblyomma variegatum* showed a positive rate of 88%. Two species of rickettsiae were detected, *Rickettsia africae* and *Rickettsia massiliae*. While most positive ticks contained *Rickettsia africae*, two isolates of *Rickettsia massiliae* (one from *Rhipicephalus evertsi*, one from *Haemaphysalis ellipticus*) could be also detected.

This is the most southern and eastern detection of *Rickettsia massiliae* and extends the known area of distribution of this rickettsial species for several thousands of kilometers to south and east in Africa. Also this *Rickettsia massiliae* was detected for the first time in the tick species *Haemaphysalis ellipticus*. Both detected rickettsial species are known of human pathogenicity. While *Rickettsia africae* is the etiological agent of African tick bite fever, *Rickettsia massiliae* is known to cause a severe form of spotted fever similar to the clinics of Mediterranean spotted fever.

# Evaluation of DNA Extraction Methods from Blood Samples Applied on FTA Cards for Molecular Diagnosis of Tick-Borne Pathogens

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An essential step in the molecular detection of tick-borne pathogens in blood is the extraction of DNA. When cooled storage of blood under field conditions prior to DNA extraction in a dedicated laboratory is not possible, the storage of blood on filter paper forms a promising alternative. Biological samples applied to Flinders Technology Associates (FTA) cards can be stored at room temperature. In addition, potential pathogens such as viruses or bacteria become inactivated on FTA cards, making the samples safe to handle. We here report the evaluation of different DNA extraction methods from blood spotted on FTA cards, to determine the best procedure for subsequent molecular diagnosis of tick-borne pathogens by PCR and the Reverse Line Blot hybridization assay (RLB).

A tenfold serial dilution series of bovine blood infected with *Babesia bovis, Theileria mutans* or *Anaplasma marginale* was made by diluting them with uninfected bovine blood. A proportion of 125 µl was spotted in quadruplicate on FTA Classic cards (Whatman). After air drying, 3 mm diameter discs were punched out using a Harris Micro-Punch (Whatman). The samples were subsequently prepared for analysis using five different protocols: (I) preparation of FTA discs for PCR using FTA purification reagent following Whatman Protocol BD08, (II) DNA extraction using a standard phenol-chloroform-isoamyl alcohol (PCI) protocol, (III) saponin washing followed by PCI extraction (Tani et al., 2008), (IV) DNA extraction using the NucleoSpin Tissue kit (Macherey-Nagel) as per the manufacturer's support protocol and (V) washing of discs using FTA purification reagent, followed by elution using Chelex 100 resin as previously described for the detection of *Trypanosoma* spp. (Ahmed et al., 2011). For comparison purposes, DNA was extracted from 200 µl of each blood sample using the Nucleospin Blood kit (Macherey-Nagel) in a final elution volume of 100 µl. Following DNA extraction, PCRs targeting the 16S rRNA gene of *Anaplasma* and *Ehrlichia* species and the 18S rRNA gene of *Babesia* and *Theileria* spp. were performed, followed by gel electrophoresis and RLB as previously described (Matjila et al., 2008).

PCR/RLB assays for the detection of tick-borne pathogens from FTA cards showed the best results when the FTA purification reagent in combination with Chelex 100 resin was used. The detection limit increased when more discs were used as starting material for DNA extraction. However, the sensitivity of the assays in which the DNA extracted from 16 discs was used was still 10-fold lower in comparison to DNA prepared from 200 µl whole blood. Differences in the starting amount of blood between both methods may explain these results. The RLB assay was 10-fold more sensitive than agarose gel analysis.

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# Canine Vector Borne Diseases (CVBD): 2006 – 2014 an Initiative to Exchange Interdisciplinary Scientific Information and Increase Awareness of Vector Transmitted Diseases in Animals and Humans.

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Parasitic arthropods are of both human and veterinary importance with regards to their primary and secondary impact on the health and well-being of humans and animals alike. From the ecto-parasites ticks and fleas are referred to as the predominant parasitic arthropods. Interaction between ticks and their hosts is characterized by the blood feeding behaviour and capability to function as vector for the transmission of pathogens. Ticks harbour and transmit pathogens from a wider range of organisms, including viruses, bacteria or protozoa. The capability of vector-transmission is, compared to the direct impact (with one exception for tick saliva intoxication), of greater importance from a veterinary and medical, resp. public health perspective. The diseases caused by these pathogens are classified under the terms: tick-borne diseases (TBD), canine vector-borne diseases (CVBD), feline vector-borne diseases (FVBD) or in general term metazoonoses.

Ticks and mosquitoes are the two large groups of blood-feeders that play an important role in veterinary medicine as well as in public health. Ticks however transmit a greater variety of infectious organisms than mosquitoes or any other group of blood-sucking arthropods, especially as it has been confirmed that individual ticks may harbour more than one pathogen. Today in small animal clinics worldwide a large variety of ectoparasiticides are available specifically for the use on companion animals. Besides the proven effectiveness of their insecticidal and acaricidal properties, the view today has shifted especially in canine and increasingly in feline medicine, from solely ectoparasite control towards prevention of canine vector borne-diseases (CVBDs). Pathogens transmitted by acarids and insects are increasingly recognized as a major threat to companion animals and especially dogs may be exposed to a variety of pathogens. Besides infestation with ticks only and depending on the endemic area, it is common that different ectoparasites feed on the same mammalian hosts; a very common combination in southern European countries is e.g. ticks and sand flies. Thus prevention of tick attachment as well as blood-feeding of flying insects is today in focus of parasitologists, internists and clinicians.

In the beginning of the new millennium, ectoparasites have stepped back into the spotlight, after years of neglecting their ability impacting the health of humans and animals alike. Research efforts towards acarology and entomology had faced a lack of investment for decades, however are back in the research interest given the increasing interest in vector borne diseases (VBDs). Alongside, the veterinary medicinal markets are saturated by insecticides and acaricides based on various active ingredients and in several convenient formulations, such as spot-on's and collars. The veterinary medicinal products available are in contrast to the human pharmaceutical field, with no registered pharmaceuticals available in the latter. However it is also a reality, that although there are well-established veterinary medicinal products available, the compliance of regular use to prevent arthropod attachment on pet animals throughout the tick season is limited. Combining all these aspects, the necessity was born, to intensify the efforts on all levels in relation to VBD. A scientific meeting was initiated in 2006 named the **'International Canine Vector-Borne Diseases Symposium'**. It was the aim to intensify the interaction between disciplines working in the field of vector borne diseases and thus to increase the know-how in the general public, the medical professions in particular, but also among pet owners. Following its inauguration in 2006, the

conference was held annually, with the participating members forming the **'CVBD World Forum'**. The CVBD World Forum summarized their initiative within the following statement: *"The CVBD World Forum is a working group of leading experts in natural sciences, veterinary and human medicine from Europe, North America, Latin America, Australia and Asia. It was founded during the 1st International CVBD Symposium in April 2006 in Billesley, UK, as a consequence of the increasing global threats through canine vector-borne diseases (CVBD). The main goal of the CVBD World Forum is to exchange knowledge and findings about ectoparasite-pathogen-host interaction as well as the characterization and assessment of the distribution of pathogens and vectors in order to increase awareness for the specific regional risks of CVBD and to foster preventative measures. The CVBD World Forum is supported by Bayer HealthCare, Animal Health."*

The various aspects on TBDs in the 10 years of the CVBD symposium will be presented.

# *In vitro* Feeding of *Dermacentor reticulatus*

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*Dermacentor reticulatus* is a three-host tick species which is widespread in temperate zones of Europe and a vector of *Babesia canis*. Research on this tick species may benefit from the possibility to feed *D. reticulatus in vitro,* as this could reduce the use of laboratory animals and facilitate applications such as the *in vitro* infection with pathogens or the *in vitro* screening of acaricides. The aim of this study was to realize the *in vitro* feeding for all life stages of *D. reticulatus* and to develop a technique comparable to the *in vivo* feeding concerning effort and rearing success. Hence two existing feeding techniques were adapted and optimized for feeding *D. reticulatus* and a new feeding technique was developed.

Tick chambers were built by gluing cellulose reinforced silicone membranes to one side of 65 mm long autoclavable glass tube (diameter 32 mm) (Krober and Guerin, 2007). Mechanical and olfactory attachment stimuli (a piece of mosquito netting, hair and odor extract of a host animal and tick faeces) were applied to the membrane, prior to placing ticks into the chamber. The tick chamber was closed by a stopper wrapped with organza fabric. Field-collected ticks were fed with heparinized (20 I.U./ml) cattle blood which was collected weekly at a slaughterhouse and stored at 4°C. Blood was supplemented with 2 mg/ml glucose, 51 mg/ml ATP and 5 µg/ml gentamycin and warmed in a waterbath at 38°C in 50 ml beakers, in which the tick chambers were placed. The blood was changed every 12 hours and the number of attached ticks was counted. Engorged ticks were collected from the tick chamber, weighed, and stored at 90% RH at room temperature in a desiccator. Under these conditions, attachment rates of 50 % and engorgement rates of 31 % were obtained. The average weight of engorged females using this system was 222 mg.

To avoid frequent collecting of blood at the slaughterhouse the possibility of utilizing blood stored at -20°C was investigated (Habedank and Hiepe, 1993). Ticks readily attached and ingested blood, but blood which was previously frozen showed an increased susceptibility for fungal infections, which hampered the engorgement of the ticks.

Since ticks harbour various endosymbionts, it was hypothesized that the addition of gentamycin to the blood meal may have negative effects on tick feeding and oviposition. Irradiation of blood could form an alternative method to sterilize blood. Thus the use of blood gamma-irradiated with 1 kGy was compared with the use of blood supplemented with gentamycin and without any antibiotics. Microbiological examinations confirmed the success of the irradiation: the collected slaughterhouse blood became nearly germfree. However, extensive hemolysis of the irradiated blood was observed, followed by an increased microbial growth which exceeded that of blood without antibiotics. The bacterial contamination restrained the engorgement and the reproduction success of female *D. reticulatus*.

Two additional *in vitro* feeding systems were evaluated for the artificial feeding of *D. reticulatus* ticks. One feeding system consisted of a tick chamber and a blood chamber made of glass (Bonnet et al., 2007). The chambers were separated from each other by a rabbit or mouse skin. A water circulation heated the blood to 37°C. Only a small proportion of adult ticks attached and engorged, and bacterial and fungal contamination of the skins occurred. The other feeding system was developed to automatize the tick feeding process and make the technique less laborious. The

above mentioned tube-shaped tick chambers were fitted in a flow-through chamber through which a defined amount of blood was pumped. The average weight of engorged females fed using this semi-automated system was 269 mg.

The in-vitro-feeding of larvae and nymphs of *D. reticulatus* was also investigated and proved to be challenging. Less than 1 % of the juveniles attached and engorged on silicone membranes. In the feeding system with mouse skins, a slightly higher proportion of juveniles engorged.

**Acknowledgements**

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# A Manipulation of Wound Healing Process by Tick Saliva

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Injury of skin initiates a cascade of reactions lead to restoration of tissue integrity and function. Cutaneous wound healing is a complex biological process characterized by four overlapping phases (haemostasis, inflammation, proliferation and remodelling), requires cellular interactions among a variety of cells and is provided and orchestrated by cytokines, chemokines and growth factors.

Chemokines have an important regulatory role in recruitment of leukocytes in to the site of injury and contribute to the regulation of epithelialisation, angiogenesis and tissue remodelling. Growth factors attract cells into the wound, stimulate their proliferation and they have a profound influence on extracellular matrix deposition. The physical process of tick attachment and protracted feeding involves a penetration of their mouthparts into the host skin and elicits a wound healing and immune responses. Modulation of these responses by ticks is critical for their survival. Suppression is mediated by molecules synthesised in the tick salivary glands and secreted in saliva. The effects of tick saliva on some important cytokines and chemokines have already been demonstrated. The spectrum of the anticytokine activities differs among tick species.

In this study we showed, that SGEs from ixodid tick species also manipulate wound healing response by targeting at least four different mammalian growth factors: transforming growth factor β1 (TGF-β1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) depending on tick species. Other growth factors involved in wound healing, such as epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF) were not affected by any of the SGEs. Cell proliferation is regulated by growth factors. We compared the effect of SGE from different tick species on cell morphology and proliferation. Three tick species with long hypostome – *Amblyomma variegatum*, *Ixodes ricinus* and *Hyalomma excavatum* – that appear bind PDGF also inhibited cell proliferation *in vitro* and induced changes in the morphology of different cell lines. These effects correlated with disruption of the actin cytoskeleton. By comparison, SGE of two species with short hypostome – *Dermacentor reticulatus* and *Rhipicephalus appendiculatus* – had no effect on either cell proliferation or morphology.

Growth factors are pleiotropic molecules with important role during many physiological processes. However, they are also involved in vascular and immunological diseases and cancer. Tick growth factor binding molecules may provide new tools as pharmacological inhibitors.

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# Assessment of Climate Change Impacts and Adaptation Needs in Germany

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The first German vulnerability assessment to climate change (Zebisch et al. 2005) was used as basis for the German Adaptation Strategy (DAS), accepted by the government in 2008. The DAS sets the frame for Germany's national adaptation process. For the progress report of the adaptation strategy in 2015 an actual and consistent assessment covering whole of Germany is needed and was commissioned by the Inter-ministerial Working Group on adaptation in the First Adaptation Action Plan in 2011. The vulnerability assessment will serve as the official evidence base for the development of the second Adaptation Action Plan as part of the progress report. It covers all 15 sectors of the DAS to identify spatial and thematic hot-spots for the prioritization of adaptation needs.

The network consists of 16 different public authorities, the "Netzwerk Vulnerabilität" and a scientific consortium. In a cooperative manner the scientists developed the methodology, collected the available knowledge, prepared the assessment, and worked with the scientific officers, who supported the scientists by their expert knowledge and by taking the normative decisions to focus the assessment on most relevant aspects.

In a first step of the vulnerability assessment, the climate change impacts, which were considered as important for Germany, were selected for further investigation. In the Human Health sector the impacts "Potential heat effects on population > 60 years", "Effects of changing ozone concentration", and "Changes in geographical extension of vectors" were selected. The potential future climate change impacts were quantified as far as possible by impact models or indicators based on regional climate projections or otherwise estimated by expert judgments. For the climate change impact "changes in geographical extension of vectors" no quantitative data was available for all of Germany for the near future (2031- 2050), therefore expert interviews were conducted. However, the other both impacts could be quantified based on proxy indicators for today and the near future.

This talk explains the political contest and the methodological approach of the German vulnerability assessment including climate change impacts on human health. It also shows which sources of information are useful for climate change impact assessments and how uncertainties are considered.

# Abstracts of Poster Presentations

# A New Map of Geo-Referenced Tick Locations in Germany

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A new distribution map of hard ticks based on geo-referenced locations in Germany is introduced. The map is a result of an ongoing scientific project with short title „Klimawandel und Verbreitung Krankheitserreger übertragender Schildzecken (FKZ 3713 48 402)“ on behalf of the German Federal Environmental Agency (Umweltbundesamt, Deutschland).

Exclusively geo-referenced locations were used in order to prepare a dataset applicable for the development of niche models. Recently, Estrada-Peña and colleagues provided a comparable digital dataset for various European tick species, which was taken here as a first guess. For Germany, however, this dataset is limited to *Ixodes ricinus* and a single location of *Hyalomma marginatum* found at the Swiss-German border. Therefore, a comprehensive literature study was performed resulting in 1.163 additional tick locations in Germany. These comprise 1.002 locations of *I. ricinus*, 75 locations of *Dermacentor marginatus*, 74 locations of *Dermacentor reticulatus*, 7 locations of *Haemaphysalis concinna*, 3 locations of *Ixodes trianguliceps* as well as the first records of *Ixodes frontalis* and *Hyalomma marginatum*. In addition to other methods used to extract digital data, a method for the digitalization of historical, i.e. hand drawn, charts was developed.

The resulting tick map depicts the most frequent species, *I. ricinus*, distributed in whole Germany, while the sheep tick *D. marginatus* was confirmed exclusively in the climatologically favored region of the Rhine valley. Most sampling sites of the ornate cow tick *D. reticulatus*, in German known as the Auwaldzecke, are located in Berlin and its vicinity. Additionally, several locations of the relict tick *Ha. concinna* were found in the Eastern part of Germany. Records of *I. trianguliceps* are sparse, probably caused by the low number of investigations on endophilic ticks. Finally, the tick map reveals data gaps in the Northwestern parts of Germany. Providing geo-referenced records for that region of Germany would be very useful. There are no data available for the brown dog tick *Rhipicephalus sanguineus* in Germany, but 2 locations in Eastern France, not far away from the German border.

Concluding it should be noted that this first digital tick map for Germany is still incomplete and might not mirror the true current distribution of various hard tick species in Germany. Colleagues are therefore invited to provide their so far unpublished hard tick records in digital form to improve the digital dataset as well as our knowledge on the distributions of hard tick species in Germany.

The study has been funded by the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (UFOPLAN FKZ 3713 48 402).

# Materials on the Biology of Preimaginal Phases of Ticks (Acari: Ixodidae) in the South of the European Part of Russia

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Data on the biology of tick larvae and nymphs with two- or three-host types of development, with birds and insectivorous serving as feeders for preimaginal phases are scanty in the literature. This is connected with certain problems arising during the work with such biological objects.

The results of epizootiological monitoring of the vectors of Crimean-Congo hemorrhagic fever in the south of the European part of Russia formed the basis for this report. Preimaginal phases of ticks were collected from wild and domestic birds, as well as from insectivorous (Eulipotyphla: Erinaceidae) in points of long-term observations in the territory of semi-desert landscapes. In 2012-2014, larvae and nymphs of ixodides of the three species: Hyalomma marginatum Koch, 1844, Haemaphysalis punctata Canestrini et Fanzago, 1877, Rhipicephalus rossicus Jakimov et Kohl-Jakimova, 1911 were collected.

According to literary data, H. marginatum has a one-year life cycle which takes place with the change of two hosts-feeders: the larva and the nymph feed on the same animal. In the conditions of the south of Russia imagoes of H. marginatum attack the feeder from March till June-July. The maximum numbers of adult ticks are noted in May. First larvae appear in June, nymphs - in June-July. Impuberal forms parasitize till autumn. Large animals serve as the feeders of imagoes, birds feeding on the earth, hedgehogs and hares serve as the feeders of preimaginal phases. During the period of observations imagoes of H. marginatum were not noted on birds, and preimaginal phases – on big animals.

The life cycle of H. punctata is one-year. They develop according to three-host type. The maximum numbers of imagoes are noted in March-May and in September-October. Depending on weather conditions of the year the periods of tick activity of may shift. Larvae start feeding in May, basically in July. Nymphs also appear at that time.

The life cycle of R. rossicus occupies not less than two years (Reznik, 1974). They develop according to three-host type. Imagoes parasitize from April till July, larvae – from April till September with the greatest peak in May, nymphs – from April till October with a peak in June-July. The feeders of imagoes and preimaginal phases serve a wide range of animals.

Imagoes and preimaginal phases of H. punctata and R. rossicus, unlike those of H. marginatum, do not show strict host specificity while choosing hosts for their size. R. rossicus attack birds very seldom.

As it seems to us, the main feeders for preimaginal phases of H. marginatum in the south of Russia are birds of Corvidae family (Corvidae: Corvus frugilegus L., C. cornix L., C. monedula L., Pica pica L.), especially rooks. According to ornithologists (Hohlov, 2008) only in the area of Stavropol Territory the numbers of rooks after nidicolous period made up 5-6 million individuals. Other species of animals which act as the feeders of premaginal phases of H. marginatum, are less numerous. On birds, larvae and nymphs of H. marginatum and H. punctata prefer to feed on the head and top departments of the neck, first filling ear apertures. On other parts of the body: thighs, under wings they are found very seldom. In 2012-2014 the abundance index (the number of ticks on the investigated object) of preimaginal phases of H. marginatum on birds of Corvidae family varied and has

made up 56.9-106.9, the abundance index of preimaginal phases of H. punctata was 13.1-48.7. In June-July, all examined birds are generally infested with ticks.

Very convenient objects for gathering of preimaginal phases are domestic turkeys as their head and a neck are practically without plumage and ticks are well visible. Besides, turkeys unlike other domestic birds, actively grass in nature, and migrate covering considerable distances. In different years of observation from several individuals to several hundred individuals (928) of preimaginal phases of H. marginatum and H. punctata were found on one bird simultaneously. The percent of infested birds and abundance indexes of ixodids in such a case depends on timeliness and regularity of acaricide treatments of livestock carried out by its owners.

Hedgehogs (Erinaceus roumanicus B., Hemiechinus auritus G.) are universal feeders for a large number both of ixodid species occurring in the south of Russia, and all phases of their development. In July, during the whole period of observations of hedgehogs, ixoids of three species: larvae and nymphs of H. marginatum, larvae and nymphs of H. punctata, imagoes, larvae and nymphs of R. rossicus are noted simultaneously. The abundance index of preimaginal phases of H. marginatum during the period of observation made up 9-123, the abundance index of preimaginal phases of H. punctata - 2-18.7, the abundance index of preimaginal phases of R. rossicus – 6.3-15.2, the abundance index of imagoes – 3.2-15.5. The preimaginal phases of ticks attach for feeding to body parts without spines (paws, paunch, muzzle, ears) and only 0.1 % of larvae and nymphs are found out on the spinal part of the animal. On the contrary, imagoes of R. rossicus are always found on the spinal part of the body covered with spines.

The findings gave us better knowledge of ecological and biological features of some species of ixodids. The results of monitoring can be used for prediction the numbers of ixodids – vectors of infections with natural foci in the south of the European part of Russia.

# Lyme Disease Ecology in British Wildlife

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**Aims:** To quantify the role of badgers (Meles meles) as reservoirs for Borrelia burgdorferi sensu lato (sl), the causal agent of Lyme borreliosis, and to explore the vector competency of badger-associated ticks for the pathogen.

**Methods and results:** We conducted a two year study to determine the prevalence and diversity of B. burgdorferi infecting badgers, ticks feeding on badgers, and questing ticks on badger habitat in Woodchester Park, Gloucestershire. We recorded a prevalence of infection in questing Ixodes ricinus nymphs of 5% and encountered all three B. burgdorferi sl genospecies that are common across southern Britain (B. afzelii, B. garinii and B. valaisiana). We detected only B. afzelii, at a prevalence of 6% in ear biopsies taken from badgers. Badgers were infested with three Ixodes tick species, but we detected B. burgdorferi s.l. only in I. ricinus.

**Conclusions:** Though three B. burgdorferi sl genospecies are in circulation at Woodchester Park, to date we have only detected infections due to B. afzelii in badgers, suggesting that is genospeces alone is able to exploit badgers as a reservoir host. Furthermore, B. burgdorferi sl was only encountered in I.ricinus ticks, suggesting this species is the most important vector for the pathogen in our study system.

**Significance of study:** Little is known about the relative importance of different UK wildlife species as reservoirs for B. burgdorferi sl, or of different Ixodes species present in the UK as its vector. Here we present evidence that supports the role of the badger is a competent reservoir host for B. burgdorferi and the role of I. ricinus as an important vector for the pathogen. An understanding of the natural cycle of B. burgdorferi is important if we are to effectively control Lyme borreliosis, a disease that is fast emerging in the UK.

# Molecular Evidence of Tick-Borne Diseases in *Dermacentor reticulatus* Ticks Collected from Dogs from Eastern Austria

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The ornate dog tick, *Dermacentor reticulatus*, has a wide distribution in Europe and Asia in areas that have a high humidity and have a moderate temperature. The preferential hosts of this tick are dogs and other (wild) carnivores but it can also be found on cattle, horses, deer and other hooved animals. *D. reticulatus* is of significant medical, veterinary and epidemiology importance as this tick is known to be a vector of several protozoa and bacterial pathogens including but not limited to *Babesia canis*, *B. caballi*, *Coxiella burnetti* and *Theileria equi*. This prospective study serves as an indication to which pathogens are currently found in eastern Austria.

To screen the ticks, the Reverse Line Blot (RLB) hybridization technique has been used, which is a technique that has the advantage of screening multiple ticks for the presence of DNA of multiple pathogens at once. Briefly, the RLB consists of a negatively charged nitrocellulose membrane that has up to 43 (geno)species specific oligonucleotides covalently bound to it with the use of an amino-linker attached to the 5' side of the oligonucleotide probe and through the use of a miniblotter. To screen for pathogen DNA, whole tick DNA extractions are subjected to several genus specific PCRs with biotin-labeled reverse primers. The resulting PCR products are then loaded perpendicularly to the bound oligonucleotide probes and detection takes place through the use of chemiluminescence resulting from horseradish peroxidase-streptavidin conjugate that has been bound to the biotin attached to the PCR product. For the genus specific PCRs the following targets were used: For *Anaplasma/Ehrlichia* spp., the 16S rRNA gene, for *Babesia/Theileria* spp. the 18S rRNA gene, for *Rickettsia* spp. the 23S-5S intergenic spacer and for *Borrelia* the 5S-23S intergenic spacer.

This study is following a previous study in which the screened ticks were collected from dogs that were allowed to walk daily in an area in the east of Austria during a time period of 11 months from February to December 2008. It was possible to detect DNA of pathogens within these ticks and there were positive signals for the Rickettsia "genus catch-all" oligonucleotide probe. After sequencing these PCR fragments, a new probe could be designed for *Rickettsia raoulti*.

# Tick-borne Pathogen Xenodiagnosis in Ticks Collected from Ruminants in Maban County, South Sudan

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Ticks (*n*=482) collected from 33 cows and 1 camel in March 2013 in a refugee camp in Maban County, South Sudan were identified and subsequently screened for the presence of *Anaplasma*, *Babesia*, *Ehrlichia*, *Rickettsia* and *Theileria* species by Reverse Line Blot hybridization (RLB). Identified tick species included *Amblyomma lepidum, Hyalomma excavatum, Hyalomma rufipes, Rhipicephalus (Boophilus) annulatus, Rhipicephalus (Boophilus) decoloratus, Rhipicephalus muhsamae, Rhipicephalus preatextatus, Rhipicephalus sanguineus,* and *Rhipicephalus turanicus*. DNA of zoonotic rickettsial pathogens, including *Rickettsia africae*, *R. aeschlimannii* and *Rickettsia massilliae*, as well as DNA from uncharacterized *Anaplasma* species, *Anaplasma marginale*, *Ehrlichia ruminantium*, *Theileria mutans, Theileria separata* and *Theileria velifera* were detected by PCR/RLB. These results indicate that pathogens of veterinary and zoonotic relevance are circulating in the tick and ruminant population in Maban County and imply that the local population is at risk for tick-borne rickettsial diseases.

The study was supported by Vétérinaires sans Frontières Germany and the Brazilian Ciência sem Fronteiras Program.

# OAKS: Optimization and Automation of Artificial Tick Feeding

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Since ticks require blood for their development, the use of experimental animals is often inevitable in tick and tick-borne disease research. An artificial feeding method for ticks could replace the use of experimental animals, or lead to a reduction thereof. However, all methods developed thus far result in poor feeding- and reproduction ratios in comparison to ixodid ticks fed on animals. They are also laborious, which has hampered the adaptation of these techniques. In this project, critical steps in the artificial tick feeding process are investigated in more detail.

Existing artificial tick feeding systems are evaluated concerning their performance for feeding five ixodid tick species which differ in their mouthpart length, life cycle, host finding strategy, reproduction strategy or host specificity. The influence of parameters relevant to the attachment and feeding process such as membrane composition, atmospheric conditions and blood composition/additives will be examined and their influence on tick feeding success will be statistically validated. Research applications as well as methods to simplify and automatize the artificial feeding are also under scrutiny.

Results from the OAKS-project (funding period: 2013-2016) will give insight into factors critical in the artificial feeding of ixodid ticks, with the aim of making this technique more accessible and attractive for scientists working on ticks and tick-borne pathogens.

The project is supported by the German Federal Ministry of Education and Research (BMBF).

# Molecular Detection and Genetic Characterization of the Crimean-Congo Hemorrhagic Fever Virus in Ticks from South Russia

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Crimean-Congo hemorrhagic fever (CCHF) is a dangerous tick-borne viral infection, endemic in the southern regions of the European part of Russia. The natural focus of Crimean-Congo-hemorrhagic fever virus (CCHFV) is located in the territory of South Federal District (SFD) and the North Caucasus Federal District (NSFD) of Russian Federation. From 1999 to August 2013 1654 cases of CCHF have been reported in the South of Russia with the mortality level 4.4 %.

1040 pools of ticks, belonging to the genus *Hyalomma*, *Rhipicephalus*, *Heamaphysalis*, *Dermacentor*, *Ixodes*, *Boophilus*, were tested for CCHFV by using commercial PCR Kit (*AmpliSens CCHF-FL*, InterLabService, Russia) in 2012-2013. CCHFV has been detected in 69 pools of ticks: *Hyalomma marginatum* (79.7 % of positive samples), *H. scupense* (13.1 %), *Rhipicephalus rossicus* (5.8 %), *Boophilus annulatus* (1.4 %). *Hyalomma marginatum is* the primary vector and reservoir of CCHFV in the south of Russia. 12.4 % of ticks *Hyalomma marginatum* were infected with CCHFV.

29 samples of ticks *Hyalomma marginatum* and *H. scupence*, that had shown positive results in PCR were taken for the further genetic characterization of CCHFV, circulating in the south of Russia. For these samples 29 partial CCHFV S-segment sequences (538 bp) have been obtained. Phylogenetic tree was performed in Mega 5.05 (Neighboor joining, algorithm Kimura- 2).

The results of phylogenetic analysis have showed that several viral variants are present within the endemic region in the south of Russia. Most of obtained sequences (for 25 samples) clustered in the genetic lineage "Europe-1" (V), with previously published CCHFV sequences from Russian Federation (ROS/TI28044, ROS/HUVLV-100, STV/29223). These sequences were divided in 2 subgroups: "Stavropol'-Rostov-Astrakhan'-1" (Va), close to strain STV/29223 and "Volgograd-Rostov-Stavropol'" (Vb), close to strains ROS/TI28044 and ROS/HUVLV-100. 4 sequences were not clustered with any of the previously described strain and formed a new group on the phylogenetic tree "Kalmykia" (VIII).

Geographical distribution of genetic variants of CCHFV was analyzed using the software ArcGIS 10.1. "Stavropol'-Rostov-Astrakhan'"(Va) strains were isolated from southern part of SFD and NSFD (south of Rostov region, Stavropol' region and Astrakhan' region), whereas "Volgograd-Rostov-Stavropol'" (Vb) isolates circulated in the northern part of SFD and NSFD (Rostov region and north of Stavropol' region). Other strains, belonging to genetic group "Kalmykia" form local foci of CCHFV in the Republic of Kalmykia in the central part of SFD.

The result of analysis of CCHFV genetic variants geographical distribution in the south of Russia, show formation and parallel evolution of two large overlapping foci of CCHFV: north and south. Migration of CCHFV variants between northern and southern foci is possible by carrying of infected ticks on cattle and birds.

# Genetic Typing of *Coxiella burnetti* Isolates from Separate Areas of the North Caucasus

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Tick pools collected in the Karachai-Cherkess Republic in 2013, in Stavropol Territory in 2013-2014, and in the Republic of Dagestan in 2014 served as test material for this study. Detection of *Coxiella burnetii* in test samples was carried out by real-time PCR using primers described by Howe *et al.* (2009). Genetic typing of positive samples (plasmid-based typing, MLVA, SNP typing) was carried out on field material without isolation of strains.

Plasmid types were determined using primers we developed. The overwhelming majority of tested samples (all samples from Stavropol Territory) contained the QpRS plasmid. Only four samples from the Republic of Dagestan and one sample from the Karachai-Cherkess Republic were found to contain the QpH1 plasmid. Samples with the QpH1 plasmid which were isolated in the area of Dagestan were found to contain the plasmid QpRS as well. Inclusion of such samples in research complicates further genetic typing.

MLVA typing was carried out using two panels with 17 different VNTR loci described by Arricau-Bouvery *et al.* (2006). In order to compare our findings with previously described MLVA genotypes we used the electronic resource http://mlva.u-psud.fr.

As a result, two new MLVA genotypes which were not described previously have been found out in the areas of Stavropol Territory and Republic Dagestan. They differed by allele sizes of the loci ms 31 and ms 36. The genotypes of strains F4 and R1140 isolated in the areas of France and Russia are most closely related to them, which, however, differ by six (ms 24, ms 26, ms 28, ms 30, ms 31, ms 36) and seven (ms 24, ms 26, ms 28, ms 30, ms 33, ms 31, ms 36) loci, respectively.

SNP genotypes were determined using primers described by Huijsmans *et al.* (2011).

All tested isolates, both from the area of Stavropol Territory, and from the area of the Republic of Dagestan had identical SNP genotype (769 A, 2287 A, 4439 A, 4557 A, 4844 C, 5423 G, 6025 G, 7078 T C, 7726 G, 7974 G) which differs from already known genotypes and is most closely related to the genotype 6 SNP described by Huijsmans *et al.* (2011).

Thus, while testing tick pools containing the causative agent of Q fever we revealed a variety of MLVA genotypes in the structure of *Coxiella burnetii* population in the area of the North Caucasus and showed the possibility of typing the causative agent directly in field material without isolation of strains.

# The Reverse Line Blot for *Borrelia* Detection and Discrimination of different Borrelia Genospecies

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**Introduction:** The most common tick transmitted microbe in the northern hemisphere is *Borrelia burgdorferi* sensu lato (further *Borrelia*), a complex which by now comprises 19 genospecies. Seven of them (*B. afzelii, B. garinii, B. bavariensis, B. burgdorferi* sensu stricto, *B. spielmanii, B. bisettii*, and *B. valaisiana*) are currently known to be associated with human disease. In this study ticks collected from different regions in Austria were screened for the presence of *Borrelia*.

**Method:** The reverse line blot (RLB) technique has been developed in order to detect multiple agents and/or genospecies out of one sample. With a miniblotter species-specific oligonucleotides are applied in lanes to a nitrocellulose membrane and are covalently bound due to labeling with a 5'terminal aminolinker. After performing a PCR with biotin-labeled primers the PCR products are applied perpendicularly to the membrane with the oligonucleotide probes. In a further step streptavidin labeled with horse radish peroxidase (HRP) binds to the biotin-labeled products which are hybridized to the specific probes and the reaction is visualized by using an enhanced chemiluminescence substrate (ECL) and an imaging system.

For *Borrelia* a PCR amplifying the specific 5S-23S intergenic-spacer region (IGS) followed by RLB was performed.

In the first part of this study new probes were designed and evaluated for *B. spielmanii*, *B. lusitaniae* and *B. valaisiana* and used for the tick screening in addition to the already published *Borrelia* probes designed by Rijpkema et al. (1995). The sensitivity of the PCR/RLB method was determined and found comparable to a nested PCR designed by Wilhelmson et al. (2010).

**Results:** A total of 217 *Ixodes ricinus* ticks (10 adults, 199 nymphs and 8 larvae) collected from different locations in Austria were screened for the presence of *Borrelia* DNA. Sixtythree (29%) of them tested positive by the reverse line blot. The most abundant *Borrelia* species detected within these ticks was *B. afzelii* with 47.3% (30/63) followed by *B. burgdorferi s.s.* with 42.9% (27/63) and *B. valaisiana* with 33.3% (21/63). *B. garinii* and *B. lusitaniae* were found only in 3.2% (2/63) and 1.6% (1/63), respectively. No *B. spielmanii* was found in the screening so far. The province with the highest number of positive ticks was Tyrol followed by Upper Austria, Salzburg and Lower Austria. Co-infections with different Borrelia strains were observed in 19% of all positive ticks. The most frequent one was a mixture with *B. burgdorferi s.s.* and *B. afzelii*.

**Conclusion:** The reverse line blot hybridization allows to detect and discriminate between different *Borrelia* genospecies as well as to uncover co-infections in a single sample. The method also allows to discover new subspecies within a genus. Our results show that *B. afzelii*, the predominant agent of the most common manifestation of Lyme borreliosis erythema migrans, was detected most frequently. However, *B. burgdorferi* s.s. and *B. valaisiana* were also detected in a high proportion which is interestingly not mirrored by the genospecies which were detected in specimens from Lyme borreliosis patients in Austria.

# iSpot Lyme: A Sensitive and Specific ELISPOT Assay for the Detection of Antigen-Specific T-Cell Response to *Borrelia burgdorferi*

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A novel T-cell based assay was developed for the detection of antigen-specific T-cell response to *Borrelia burgdorferi*. Using interferon gamma as a biomarker, we developed a new immunospot method (iSpot Lyme) to detect *Borrelia* antigen-specific memory T cells that were activated ex vivo by recombinant *Borrelia* antigens. The detection of antigen-specific T cells was significantly increased by a combination of antigens. To test this method as a potential clinical diagnostic tool, we performed a study with a cohort of *Borrelia*-positive patients and healthy controls. A cut-off value was determined by using Receiver Operating Characteristic (ROC) curve analysis. The iSpot assay has a significantly higher specificity (94%) and sensitivity (84%) compared with Western Blot analysis (sensitivity 23%). This assay is invaluable for discerning chronic from acute infections, and it allows us to connect the immunology of Lyme disease to chronic manifestations. In addition it is a unique tool for monitoring the efficacy of treatment. Absence of reliable and objective monitoring tools allows acute Lyme disease to become chronic, thus contributing to or worsening other clinical conditions. Furthermore this system is ideal for the determination of the state of other tick-borne diseases, e.g. Babesiosis.

# Strong Systemic Th2 Responses in Nematode Co-Infection do not Influence Susceptibility to Ticks and Lyme Diseases Spirochaetes

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Wild rodents serve as reservoir hosts for a number of tick-borne pathogens, such as Lyme disease spirochetes, infecting humans and companion animals (1, 2). Since parasitic nematodes of mice modulate host immune responses and affect the control of experimental bacterial and protozoan infections (3-5), we raised the hypothesis that natural co-infections with helminths alter the reservoir competence of wild rodents for ticks and tick-borne pathogens.

We conducted a field study to determine the most frequent parasites of periurban rodents in Berlin. In 2010/2011 a total of 257 mice and voles from six species were trapped at four study sites followed by a thorough parasitological examination. 56.6% of the rodents were infested by ticks and 71.6% harboured intestinal nematodes. Among the two species *Apodemus flavicollis* and *A. sylvaticus* (n=107), 22.4% were co-infected with *Ixodes ricinus* and *Heligmosomoides polygyrus*.

To survey this natural co-infection under controlled laboratory conditions we experimentally infected C57Bl/6 mice with both parasites to assess the influence of a frequent nematode infection (1) on the feeding success of tick larvae and *Borrelia afzelli*-infected nymphs, (2) on the reservoir competence of mice for tick-borne Lyme-disease spirochetes and (3) on immune responses of tick infested mice. Murine hosts experimentally co-infected with the nematode *H. polygyrus* and larval/nymphal *I. ricinus* ticks evoked substantially stronger systemic Th2 responses, measured by GATA-3 and IL-13 expression, than single infected mice. However, the systemic and local anti-tick Th2 responses were unaffected by the nematode infection and the feeding success, measured by number of engorged ticks, feeding duration, weight and moulting rate was comparable to nematode-free mice. In addition, an observed partial protection against repeated larval tick feeding was unaltered by nematode infection. Although the strong systemic Th2 responses in co-infected mice resulted in a trend of decreased systemic and local Th1 reactivity against *B. afzelii*, they failed to affect the transmission, replication and dissemination of the spirochete. Our study shows that a concurrent enteric infection with *H. polygyrus* does not affect the susceptibility of mice for ticks and tick-borne *B. afzelii*. Co-infections of wild rodents with these frequent macroparasites appear not to promote their reservoir function in the natural transmission cycle of Lyme disease spirochetes.

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# Av-PDI Protein, a Candidate for Anti-Tick Vaccine?

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Identification of antigens that evoke host immune response and block early phases of tick feeding is a major research goal in development of anti-tick vaccines and vaccines blocking transmission of tick-borne pathogens. We identified a sequence of *Amblyomma variegatum* protein disulfide isomerase (AvPDI), abundantly expressed in salivary gland cells of *A. variegatum* ticks. The structural and functional characteristics of PDI suggest that AvPDI could play an important role in protein folding and aggregation in ticks during their life cycle. By high specific polyclonal serum, we detected a great immunogenic potential of AvPDI and its homologue in other ticks. This attributes of AvPDI allocate it to candidates of multicomponent vaccine. We investigated the effects of immunization of laboratory mice with recombinant N-terminal AvD-GST fusion protein on feeding success, weight, metamorphosis and oviposition of *Ixodes ricinus, A. variegatum* and *Rhipicephalus appendiculatus* ticks. In addition, potential transmission blocking effects of AvD-GST were studied on the mouse - *I. ricinus* - *Borelia afzelii* model. Despite strong anti-AvD-GST antibody response in mice, we did not detect any significant effects of immunisation on feeding success, development/metamorphosis or oviposition of the studied tick species. Immunisation with AvD-GST did not impair transmission of *B. afzelii* spirochetes via infected *I. ricinus* nymphs to mice nor to nymphs subsequently feeding on hosts infested primarily with *Borrelia*-infected ticks.

Further studies are needed to elucidate the role of different AvPDI variants in tick feeding and pathogen transmission, involving other animal models, tick species and pathogens.

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# An Immunological Strategy for the Control of Poultry Mites

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The Poultry Red Mite (PRM) *Dermanyssus gallinae* causes high economic losses and is the most devastating parasite in poultry farming world-wide. Different chemical, physical and biological strategies try to control the expansion of PRM. However, a solution to this problem still has highest priority. Therefore, an immunological strategy is presented here, which could allow the protection of chicken from PRM. The first step of the development consisted in the isolation of proteins from PRM. Following, chicken were immunized with different PRM extracts. Then, IgY were extracted from eggs of the immunized chicken and a PRM *in vitro* blood-feeding assay was performed. This assay, which used fresh chicken blood spiked with IgY isolated from the differentially immunized animals enabled the detection of antibodies which led to PRM mortality. In the next step, individual proteins were isolated through 2D gel analysis combined with antibody analysis and used for a second immunization of chicken. Analysis through ELISA and western blots showed a high specific antibody production against PRM extracts. Subsequently, it was possible through proteomics to identify specific proteins as candidates for the production of antibodies which display anti-RVM activity. These results suggest a high potential of this strategy for the development of a vaccine against the poultry mite *Dermanyssus gallinae*.

# Tick identification workshop

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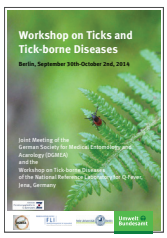
At the beginning of the course there will be an overview of current situation in tick taxonomy including areas of controversy. Literature and literature sources on tick identification guides, as well as an interactive program for training in tick morphology, will be provided (each participant should bring a stick to upload this material). The workshop will then provide participants with the opportunity examine and identify all life history stages of both argasid and ixodid ticks firstly to genus level and then, using certain key examples, to species level. Although emphasis will be placed on Central European genera and species, examples will also be provided for examination from Africa and the Middle East. Participants should bring a very fine forceps as well as a mounted needle.

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