Surveillance of tick-parasitized voles, mice and roe deer in Germany: Arboviral infection rates in relation to population densities and host characteristics

Dissertation

Submitted in partial fulfilment of the requirements for the degree of
Doctor of Forest Sciences (Dr. forest)

Faculty of Forest Sciences and Forest Ecology,
Georg-August-Universität Göttingen

Submitted by
Christian Kiffner
Born in Lübeck, Germany

Göttingen, 08/11/2010

Committee in charge:

1. Prof. Dr. Stefan Schütz
2. PD Dr. Stephan Neumann
3. Prof. Dr. Christian Ammer

Day of oral examination: 27/08/2010

Acknowledgements

The presented dissertation had not been possible without the support of numerous people. First of all, I would like to thank Prof. Schütz for providing a liberal working atmosphere at the *Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management* and for being the main supervisor of this thesis. Thank you very much for your trust, interest and support. I sincerely thank the co-referees PD Dr. Neumann and Prof. Ammer for reviewing this thesis.

This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance). I thank Dr. Ferdinand Rühe for employing me in this project, for providing academic freedom and critical comments. The dissertation was mainly embedded in the 'arbo-virus network' (AVN), led by Professor Frank Hufert. Thank you very much for initiating and for coordinating the research network and for the associated funding.

Several people helped realising the research presented in the following dissertation. First of all, I would like to sincerely thank all co-authors of the chapters who contributed in many ways to the success of the chapters: Matthias Alings, Peter Hagedorn, Christina Lödige, Matthias Niedrig, Ferdinand Rühe, Philipp Schomaker, Rainer Ulrich (representative for the multi-authored chapter 10), Torsten Vor, and Walter Zucchini. Especially, I would like to thank Torsten Vor for being a very cooperative and enjoyable colleague in this project and for planning the majority of the logistics involved in the data collection in southern Hesse. Thank you very much! Professor Walter Zucchini provided clear and valuable guidance in statistical issues and was therefore a great help. I would also like to thank you for convincing me to use the statistical software package *R*!

For acquiring the data presented in this dissertation, considerable effort and time was required for fieldwork in southern Hesse and to a lesser degree around Göttingen. In Hesse, Family Krämer provided a warm, hospital atmosphere, great catering and a piece of home in their Pension "Gästehaus Margarethe". Successful data collection had not been feasible without the help of numerous people. The names of the numerous helpers are listed in the relevant chapters. Fieldwork often required long working hours and working at night under all possible weather conditions. I would like to thank you all for your endurance, commitment, reliability and sense of humour during this project! In particular, I would like to highlight the efforts of Martin Scholz, who provided a large fraction of the roe deer examined for this thesis and was a helpful and enjoyable companion during several fieldwork trips in Hesse. Thank you!

In the frame of this Ph.D. study, I also spent one week at the Friedrich-Löffler-Institute in Riems for dissecting rodents. I sincerely thank Rainer Ulrich and Matthias Schlegel for having a good time at the FLI and for introducing me to the science of dissecting rodents under high safety regulations. Thank you also for rapid and uncomplicated data sharing.

For almost the entire Ph.D. period, Philipp Schomaker shared the same office room with me. It was a great pleasure to share thoughts, to discuss academic and non-academic problems and to find solutions for the emerging issues. Additionally, Philipp provided a considerable amount of GIS work necessary for the success of this thesis. Thank you very much and all the best for your thesis and your future.

Michael Ksinsik is thanked sincerely for being a friendly and humorous colleague during the field work trips and during the time at the Department. Unfortunately, he passed away in a tragic car accident early 2010. He will remain in my memory as an extraordinary personality.

The period of almost three years required for this dissertation was not always easy and involved personal and academic difficulties, tensions, conflicts, highly variable work loads and a lot of travelling. My apologies especially to Britta and all friends, for being occasionally stressed, troubled and for having little time.

I would like to thank the countless people who were interested and discussed with me about my PhD topic. These informal discussions were a great help for getting a new view on the relevant issues.

During the last three years I also worked on “non-tick issues” from my “former life” as a Master student. It was a great pleasure to work with Dr. Matthias Waltert on African wildlife issues. This fruitful co-operation was a considerable enrichment and an enjoyable way of learning how to publish jointly.

I would like to thank my mother Veronika for educating me to an independent person, for always being truly interested in my activities and for being there when needed in the past 30 years. My father Erhard is thanked for his interest, for the numerous medical papers provided and for his support during the past.

Last but not least I would like to highlight all the people not mentioned here but who contributed to the success of this dissertation.

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Article information and author's contribution

Chapter 1 – Introduction. Unpublished.
Authored by: Kiffner C

Chapter 2 – Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008. Published in: *International Journal of Health Geographics,* 9:42, doi: 10.1186/1476-072X-9-42.
Authored by: Kiffner C, Zucchini W, Schomaker P, Vor T, Hagedorn P, Niedrig M, Rühe F
CK designed the experiment, acquired ~70% of the data, performed the statistical analysis, interpreted the results and wrote the manuscript.

Chapter 3 – Surveillance of emerging arthropod-borne viruses in wildlife species in Germany. Published in: *Review of Forests, Wood products and Wood Biotechnology of Iran and Germany – Part II* (Eds. A.R. Kharazipour, C. Schöpper & C. Müller), 21-32. Available at: http://webdoc.sub.gwdg.de/univerlag/2008/kharazipour.pdf
Authored by: Kiffner C, Vor T, Rühe F
CK sampled ~50% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 4 – Tick burden on roe deer (*Capreolus capreolus*). Published in: *Experimental and Applied Acarology*, 51:405-417, doi: 10.1007/s10493-010-9337-0.
Authored by Vor T, Kiffner C, Hagedorn P, Niedrig M, Rühe F
CK sampled ~50% of the data, derived roe deer density estimates, analysed the data with TV and wrote parts of the manuscript (parts of the material and methods, results and discussion).

Chapter 5 – Abundance estimation of Ixodes (Acari: Ixodidae) ticks on roe deer (*Capreolus capreolus*). Published in: *Experimental and Applied Acarology*, 52:73-84, doi: 10.1007/s10493-010-9341-4.
Authored by: Kiffner C, Lödige C, Alings M, Vor T, Rühe F
CK sampled ~15% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 6 – Attachment site selection of ticks on roe deer (*Capreolus capreolus*). Published in: *Experimental and Applied Acarology*, doi: 10.1007/s10493-010-9378-4.
Authored by: Kiffner C, Lödige C, Alings M, Vor T, Rühe F
CK sampled ~15% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 7 – Body-mass or sex-biased tick parasitism in roe deer (*Capreolus capreolus*)? A GAMLSS approach. Accepted by *Medical and Veterinary Entomology*
Authored by: Kiffner C, Lödige C, Alings M, Vor T, Rühe F
CK sampled ~15% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 8 – Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. Published in: *Parasitology Research*, doi: 10.1007/s00436-010-2065-x
Authored by: Kiffner C, Vor T, Hagedorn P, Niedrig M, Rühe F
CK sampled ~15% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 9 – Tick-borne encephalitis virus antibody prevalence in roe deer (*Capreolus capreolus*) sera. Revised version submitted to *Medical and Veterinary Entomology*.
Authored by: Kiffner C, Vor T, Hagedorn P, Niedrig M, Rühe F
CK sampled ~50% of the data, analysed the data and wrote the draft of the manuscript.

Chapter 10 – Network "Rodent-borne pathogens": Monitoring of hantavirus infections in Germany (in German with English summary). Published in: *Beiträge zur Jagd- und Wildforschung* 34:229-250.
Authored by: Ulrich R, Schlegel M, Mertens M, Groschup MH, Schmidt-Chanasit J, Jacob J, Freise J, Pelz H-J, Wenk M, Thiel J, Triebenbacher C, Schex S, Plenge-Bönig A, Schmolz E, Kurth A, Krüger F, Ansoge H, Rühe F, Kiffner C, Gerwin W, Wegener W, Müller J, Bemman M, Wolf R, Otto L-F, Oehme R, Pfeffer M, Heckel G, Essbauer S
CK supplied data/ specimens, helped with the dissection of rodents and with the processing of probes and contributed to the manuscript.

Chapter 11 – Discussion and outlook. Unpublished.
Authored by: Kiffner C

Appendix I – Numerical response of ticks in relation to small rodent densities. Published 2009 in: *X International Jena Symposium on tick-borne diseases (formerly IPS) Prevention* (Eds. J. Süss & O. Kahl), 36.
Authored by: Kiffner C, Vor T, Rühe F
CK sampled ~50% of the data, analysed the data and wrote the draft of the manuscript.

Summary

This dissertation is focussed on the ecology of tick-borne encephalitis (TBE), an infectious disease of major medical significance. The thesis is organised as a series of 9 self-contained chapters, enframed by a general introduction to the topic and the research questions (Chapter 1) and a concluding general discussion (Chapter 11). Chapter 2 preludes the chapters 3-9. Using human incidence data from 140 counties in southern Germany over a period of 8 years, several abiotic and biotic factors contributing to the infection risk of TBE were identified in a spatial explicit, predictive framework. By controlling for unexplained spatial and temporal variation, the analysis indicated that type and amount of forest cover, and indexed population densities of roe deer (*Capreolus capreolus*) in the previous year were positively associated with TBE incidence in humans. An index of forest fragmentation and red deer (*Cervus elaphus*) and red fox (*Vulpes vulpes*) population densities in the previous year were negatively associated with TBE incidence in humans. Unexpectedly, spring warming, a suspected key driver for seasonal synchrony of immature ticks and thus for TBE transmission dynamics, was negatively associated with TBE incidence. The results are discussed with respect to their biological significance and the current resolution (county level) of the public health system is critically discussed. Building up from this analysis, the following chapters investigate specific issues on a finer scale using field data. Chapter 3 provides a brief overview of the study area in southern Hesse, where a large fraction of the data collection was carried out. This chapter presents the methods used and highlights the importance of reservoir studies for disentangling the often complex interactions of zoonotic vector-borne diseases. In Chapter 4, extrinsic and intrinsic factors affecting individual tick burdens (*Ixodes* spp. and *Dermacentor* spp.) on roe deer were examined. Controlling for season, spatial autocorrelation and individual correlates, the population size of roe deer appears to be positively correlated with nymphal *Ixodes* spp. tick burdens which lends further support to the notion that roe deer amplify tick densities. In Chapter 5, linear models are presented which allow rapid tick abundance estimations on individual roe deer. For estimating larval burdens, counts can be restricted to the front legs, for nymphal ticks, counts can be restricted to the head and for estimating burdens of adult ticks, counts can be restricted to the neck. The presented models explain considerable parts of the observed variation and thus display reliable alternatives to total body counts. Chapter 6 is focussed on attachment site selection of ticks. Feeding site niches and interstadial feeding site overlap were quantified with standard ecological indices and the distribution of ticks was tested against the 'ideal free distribution' hypothesis. Levels of inter- and intrastadial aggregation were relatively high and ticks of the same stage appear to attract each other which contradicts the 'ideal free distribution' hypothesis. The results are discussed with respect to

pathogen transmission. Chapter 7 presents a flexible modelling approach which also models the dispersion of a distribution as a function of dependent variables. Similar to Chapter 4, two basic questions (1) 'Is tick parasitism in roe deer sex biased' and (2) 'Does tick parasitism in roe deer scale with body mass?' are addressed. The results are largely in concordance with those of Chapter 4 and support the 'resource trade-off' hypothesis and provide only limited support for the 'sex-bias' hypothesis. Chapter 8 investigates the extrinsic and intrinsic factors affecting tick burdens on rodents. The study confirmed that yellow-necked mice (*Apodemus flavicollis*) experience higher *Ixodes* spp. tick burdens than bank voles (*Myodes glareolus*). Unexpectedly, roe deer densities did not explain the observed variation in individual tick burdens. The analyses provided only limited support for the 'sex-bias' hypothesis and highlight multiple correlates (e.g. vegetation cover, body mass, age) for individual tick burdens. Further on, the results suggest that the individual tick burden declined with increasing rodent population density i.e. ticks were diluted among many hosts. Co-feeding (feeding of nymphal and larval ticks on the same host) was correlated with spring temperature warming and mainly occurs in yellow-necked mice and rarely in bank voles. Also, the occurrence of *Dermacentor* spp. ticks was recorded and appeared to be positively associated with areas experiencing relatively warm temperatures during the vegetation period. In Chapter 9, roe deer sera were screened for TBE-antibodies and correlates for the presence of these antibodies were identified. The results suggest that the TBE virus mainly circulated in areas with high roe deer densities and that spatial autocorrelation was an important factor. Spring warming was not statistically associated with TBE virus antibody presence in roe deer. Among the individual correlates, hind foot length and number of female *Ixodes* spp. ticks were identified as best predictors. Yet, the interpretation of these characteristics is not unambiguous. More importantly, results of this chapter demonstrate how variable TBE virus presence in roe deer (and thus in ticks) can be within a county. This strongly suggests scaling down the resolution of public health units to ecologically meaningful units, e.g. from the county level to the forest patch level. Chapter 10 summarises the activities, research questions and preliminary results of the Network "Rodent-borne pathogens", mainly focusing on hanta virus research in Germany.

With regard to the ecology of TBE, the presented results reframe the importance of spring warming for the maintenance of TBE and suggest that other factors are instrumental in TBE virus transmission (e.g. species composition, presence of the virus in the system). Moreover, strong statistical support for the importance of roe deer densities has been found and it is highly recommended to explicitly identify the biological mechanism behind this association.

Zusammenfassung

Thema dieser Dissertation ist die Ökologie von Frühsommer-Meningo-Encephaltitis (FSME). Diese Arbeit besteht aus 9 eigenständigen Kapiteln, die von einer allgemeinen Einleitung und einer abschließenden Diskussion eingerahmt sind. Kapitel 2 bildet das Fundament für Kapitel 3-9. Mittels eines räumlichen Vorhersagemodells werden abiotische und biotische Faktoren identifiziert, die mit der FSME-Inzidenz in 140 Landkreisen Süddeutschlands über einen Zeitraum von 8 Jahren korreliert sind. Unter Berücksichtigung von unerklärter räumlicher und zeitlicher Variation, waren Waldart, Waldfläche und indizierte Rehwilddichte (*Capreolus capreolus*) im Vorjahr positiv mit der FSME-Inzidenz in der Bevölkerung korreliert. Ein Index der Waldfragmentierung, Rotwild- und (*Cervus elaphus*) Rotfuchsdichte (*Vulpes vulpes*) im Vorjahr waren negativ mit der FSME-Inzidenz assoziiert. Erstaunlicherweise ergab sich eine negative Beziehung zwischen FSME-Inzidenz und Frühjahrserwärmung. Frühjahrserwärmung gilt als wichtiger Faktor für saisonale Synchronität der beiden ersten Zeckenentwicklungsstufen. Dies wird wiederum als essentiell für Virus-Transmissionen angesehen. Die Ergebnisse werden hinsichtlich ihrer biologischen Bedeutung diskutiert und die derzeitige Auflösung des öffentlichen Gesundheitssystems wird kritisch diskutiert. Aufbauend auf dieser großräumigen Analyse, behandeln die folgenden Kapitel spezielle Fragestellungen mit einer feineren Auflösung. In Kapitel 3 wird das Untersuchungsgebiet in Südhessen und die angewandten Methoden vorgestellt und die Bedeutung von Reservoirstudien für das Verständnis von Zoonosen wird diskutiert. In Kapitel 4 werden Faktoren identifiziert, die den Zeckenbefall (*Ixodes* spp. und *Dermacentor* spp.) an Rehwild beeinflussen. Auffallend war vor allem ein positiver Zusammenhang zwischen Rehwilddichte und *Ixodes* spp. Nymphenbefall. In Kapitel 5 werden lineare Modelle vorgestellt, die, basierend auf der Zeckenzählung eines einzelnen Körperteils eines Rehes, zur Schätzung des Gesamtzeckenbefalls genutzt werden können. Die zu untersuchenden Körperteile für die jeweilige Zeckenentwicklungsstufe sind: Vorderläufe-Larven, Kopf-Nymphen, Hals-adulte Zecken. Die vorgestellten Modelle erklären einen hohen Anteil der beobachteten Varianz und sind daher eine Alternative zu Zeckenzählungen auf dem gesamten Rehkörper. Kapitel 5 hat die Wahl des Anhaftungsortes von Zecken auf Rehen zum Thema. Nischenbreite und Nischenüberlappung der Anhaftungsorte (Körperteile) der jeweiligen Zeckenentwicklungsstadien wurden mit ökologischen Standardindizes quantifiziert und die Verteilung der Zecken wurde gegen die Hypothese der „idealen freien Verteilung“ getestet. Zecken derselben und verschiedener Entwicklungsstufen wählten in hohem Maße dieselben Körperteile, so dass es lokal zu großen Ansammlungen kam. Die Ergebnisse widersprechen der „idealen freien Verteilung“ und haben womöglich Implikationen für die Übertragung von

Pathogenen. In Kapitel 7 wird ein innovativer Modellierungsansatz angewendet wobei zusätzlich zum Mittelwert die Dispersion einer Verteilung modelliert wird. Ähnlich wie in Kapitel 4 werden die Fragen „Ist der Zeckenbefall an Rehwild geschlechterspezifisch?“ und „Steigt der Zeckenbefall mit dem Körpergewicht des Wirtstieres?“ beantwortet. Die Ergebnisse bestätigen die Befunde aus Kapitel 4; für die „Körpergewicht“-Hypothese gibt es starke Hinweise während es nur bedingte Hinweise zur „Geschlechtsunterschieds“-Hypothese gibt. In Kapitel 8 werden extrinsische und intrinsische Faktoren quantifiziert, die den Zeckenbefall an Nagetieren beeinflussen. Die Studie bestätigt zunächst, dass Gelbhalsmäuse (*Apodemus flavicollis*) stärker mit *Ixodes* spp. Zecken parasitiert sind als Rötelmäuse (*Myodes glareolus*). Entgegen der Erwartung trugen variable Rehwilddichten nicht zur Aufklärung des variablen Zeckenbefalls bei Nagetieren bei. Ebenso liefern die Analysen wenig Bestätigung für die „Geschlechtsunterschieds“-Hypothese, zeigen aber dass viele Faktoren mit dem individuellen Zeckenbefall korreliert sind (Bodenbedeckung in der Krautschicht, Körpergewicht, Alter). Zudem wird gezeigt, dass der individuelle Zeckenbefall mit zunehmender Nagerpopulationsdichte abnimmt. Gleichzeitiges Vorhandensein von Larven und Nymphen wurde insbesondere auf Gelbhalsmäusen beobachtet und war positiv mit der Frühjahrserwärmung korreliert. Ebenso konnte das Vorhandensein von *Dermacentor* spp. Zecken festgestellt werden. Das Auftreten dieser Zeckenart war lokal gehäuft und wies eine positive Beziehung zu der Temperatur während der Vegetationsperiode auf. In Kapitel 9 werden Faktoren untersucht, die das Vorhandensein von FSME-Antikörpern in Rehwildseren erklären. Während räumliche Autokorrelation und Rehwilddichten (positive Beziehung) in dem Modell enthalten sind, gibt es keinen statistisch gesicherten Zusammenhang zwischen FSME-Antikörper Prävalenz und Frühjahrserwärmung. Zusätzlich wurden Hinterfußlänge (positive Beziehung) und Anzahl der weiblichen *Ixodes* spp. Zecken (negative Beziehung) als zusätzliche Prädiktoren für das Vorhandensein von FSME-Antikörpern identifiziert. Die Analysen zeigen zudem wie heterogen das Auftreten von FSME innerhalb eines Landkreises sein kann und geben Anlass, die geographische Auflösung von Einheiten des öffentlichen Gesundheitswesens zu überdenken. In Kapitel 10 wird das Netzwerk „Nagetier-übertragene Pathogene“ vorgestellt, das sich vorwiegend mit Fragestellungen zu Hantavirusinfektionen beschäftigt. In Hinblick auf die Ökologie des FSME-Virus wird die Bedeutung der Frühjahrserwärmung relativiert was darauf hinweist, dass andere Faktoren von stärkerer relativer Bedeutung für das Zirkulieren des FSME-Virus sind. Zudem gibt es starke Hinweise für den Einfluss von hohen Rehwilddichten auf die Häufigkeit von FSME positiven Zecken. Die funktionale Ursache für diesen Zusammenhang sollte genauer erforscht werden.

Chapter 1 - Introduction

Emerging infectious diseases

Emerging infectious diseases (EIDs) are a major threat to public health and pose a considerable burden on national and global economies (Binder et al. 1999; Morens et al. 2004). In the year 1998, approximately 13.3 million people died from symptoms caused by infectious diseases (WHO 1999). Among the known, ca. 1,400 human-pathogenic disease agents, approximately 60% are classified as zoonoses, i.e. infectious diseases which are transmitted from animals to humans (Woolhouse and Gowtage-Sequeria 2005; Jones et al. 2008). The majority of zoonoses (ca. 72%) originate from wildlife species (Jones et al. 2008) which has severe biological implications: (1) many wildlife species are reservoirs of pathogens that threaten domestic animal and human health; (2) wildlife EIDs pose a threat to the conservation of global biodiversity (Daszak et al. 2000). Albeit the term 'emerging infectious disease' might be over- and/or misused due to its socio-political and funding implications and technical advances have facilitated diagnostics (Telford and Goethert 2004), there is overwhelming evidence supporting the increasing frequency of EID events in the world (Jones et al. 2008; Daszak et al. 2000). Consequently, this research field gained growing relevance both in the scientific and public agenda. The growing importance of EIDs fostered research not only in the field of pathogenesis and clinical diagnostics but also in the field of 'ecology of infectious diseases' (see e.g. the recent books by Collinge and Ray 2006; Ostfeld et al. 2008). EIDs are characterised by considerable heterogeneity both in space and time (e.g. Guernier et al. 2004; Jones et al. 2008). In order to understand and finally to predict the spatial and temporal occurrence of specific EIDs, several main drivers have come into the focus of the academic community: (1) global climate warming (e.g. Harvell et al. 2002), (2) land-use / land cover changes (e.g. Patz et al. 2004) and (3) changes in species diversity (e.g. Keesing et al. 2006). The known and yet unknown synergisms and interactions among these factors, potential feedback loops between these factors and disease occurrence and the diversity of pathogen-systems, however, complicate identifying general ecological principles that underlie the dynamics of disease systems (e.g. Ostfeld et al. 2008).

In Germany, infectious diseases received increasing public and political attention which finally resulted in the enacting of the 'Law for the prevention and abatement of infectious diseases in humans' in the year 2001 (German: 'Gesetz zur Verhütung und Bekämpfung von Infektionskrankheiten beim Menschen') (Bundesministerium der Justiz 2001). According to this law, several infectious diseases are notifiable, i.e. they have to be reported to a federal public health institution. One of these notifiable diseases, tick-borne encephalitis (TBE) will be in the focus of this thesis. Tick-borne disease systems contain at least three components:

(1) the tick, (2) the pathogen and (3) the reservoir host(s). Given the importance of each of these components, they will be introduced in the following pages.

Ticks

Ticks are taxonomically grouped in the Arthropod clade (Table 1-1). Within the class of Arachnida they form the sub-class Acari (together with mites). Morphologically, ticks differ from the usually smaller mites by the presence of the hypostome which anchors the mouth to the host and by the presence of the Haller's organ which is located on the first pair of legs (Hillyard 1996). In the order Ixodida, three families are described: (1) Ixodidae (hard ticks), (2) Argasidae (soft ticks) and (3) Nuttalliellidae (Horak et al. 2002).

Table 1-1. Taxonomic overview of ticks (following Horak et al. 2002)

Clade: **Arthropoda**

Class: **Arachnida**

Sub-class: **Acari**

Order: **Ixodida**

Family	**Ixodidae**	**Argasidae**	**Nuttalliellidae**
Genus	*Amblyomma*	*Argas*	*Nuttalliella*
	Anomalohimalaya	*Carios*	
	Bothriocroton	*Ornithodorus*	
	Cosmiomma	*Otobius*	
	Dermacentor		
	Haemaphysalis		
	Hyalomma		
	Ixodes		
	Margaropus		
	Nosomma		
	Rhipicentor		
	Rhipicephalus[1]		

[1]The five *Boophilus* species are now grouped in the genus *Rhipicephalus* (Murrel et al. 2000; 2001; Murrell and Barker 2003).

Worldwide, more than 850 tick species are described (Horak et al. 2002) and efforts are made in order to clarify the taxonomy and nomenclature of ticks (Barker and Murrell 2004; Guglielmone et al. 2009). The family Nuttalliellidae contains the monospecific genus *Nuttalliella*. Within the Argasidae-family, 183 species in four genera (*Argas*, *Carios*, *Ornithodorus*, *Otobius*) are described. The Ixodidae is the species richest family, comprising 241 species in the genus *Ixodes* and 442 species in the genera *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Margaropus*, *Nosomma*, *Rhipicentor* and *Rhipicephalus*[1] (Horak et al. 2002). In north-west Europe, 26 tick species are known to occur (Hillyard 1996). In Germany, ticks are mainly represented by *Ixodes ricinus* (Linnaeus 1758) which constitute ca. 90% of the ticks found in forest ecosystems (Cornely and Schultz 1992; Hillyard 1996). *Ixodes ricinus* ticks are exophilic (i.e. the host seeking stages occur in the open/vegetation) and have a very wide range of feeding hosts; this species has been reported to parasitize ca. 300 species (Süss 2008). Several other, closely related species (e.g. *Ixodes canisuga*, Johnston 1849; *Ixodes hexagonus*, Leach 1815; *Ixodes ventalloi* Gil Collado, 1936) also attach on domestic and wild mammals in Germany (Beichel et al. 1996; Hillyard 1996). These tick species are endophilic (i.e. mainly quest below the soil surface) and are rather specialised in certain hosts (*Ixodes canisuga*: red fox, mustelidae, feral dogs and cats; *Ixodes hexagonus*: rodents, hedgehogs, mustelidae, domestic dogs and cats; *Ixodes ventalloi*: rabbits) (Cornely and Schultz 1992; Gothe et al. 1977; Hillyard 1996; Christian 2002). Other tick species are specialised in parasitizing birds [e.g. pigeons: *Argas reflexus*, Fabricius 1758 (Dautel et al. 1994; 2009); seabirds: *Ixodes uriae*, White 1852 (Hillyard 1996)], bats [e.g. *Ixodes vespertilionis*, Koch 1844; *Ixodes simplex*, Neumann 1906*; Argas vespertilionis* Latreille 1802; (Walter and Kock 1985)] or mainly parasitize domestic animals such as sheep and dogs [e.g. *Dermacentor marginatus*, Sulzer 1776 (Liebisch and Rahman 1976)]. In some regions of Germany, however, the tick *Dermacentor reticulatus (*Koch, 1844*)* a vector of pathogens of veterinary and human health importance (Estrada-Pena and Jongejan 1999), is of significance. It seems that this species expands its range in Germany (Dautel et al. 2006; Menn 2006) and was also found in some locations of the study area in southern Hesse.

Ixodid ticks are recognised by their distinct mouthparts and by their scotum which is an unflexible shield restricted to the anterior parts of larvae, nymphs and females but covers the entire dorsal surface of males (Hillyard 1996). Ticks undergo three distinct life stages: larva, nymph and adult (male and female) (Figure 1-1). Larvae have only three pairs of legs, nymphs and adults have four pairs of legs. Each tick stage seeks a host in order to take a

blood meal. Males of *Ixodes ricinus* usually do not gorge, whereas male *Dermacentor reticulatus* take a small blood meal (Immler 1973; Hillyard 1996).

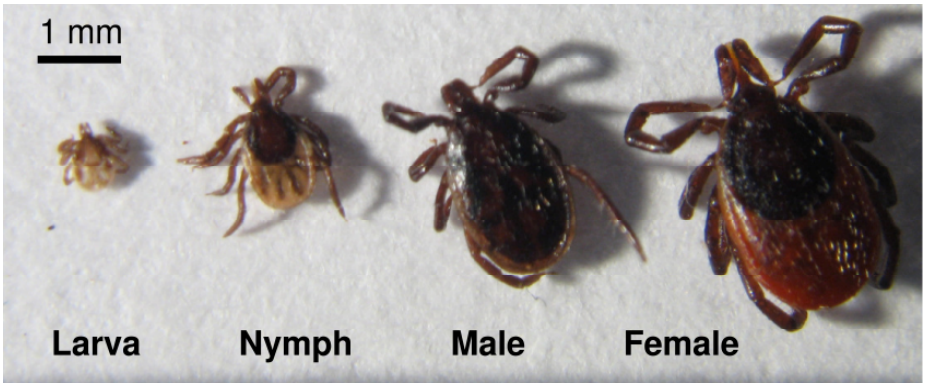


Figure 1-1 Life stages of *Ixodes ricinus* (Photo: Christian Kiffner).

The life cycle of *Ixodes ricinus* can be described as follows. After hatching, the larva seeks host number 1 (usually a small mammal, bird or reptile), attaches, feeds on it and drops off the host. On the ground, the six-legged larva moults into an eight-legged nymph. The nymph feeds on host number 2, drops off and develops into an adult tick. If it develops into a female, it feeds on host number 3, mates, drops off the host and deposits its eggs before it dies. Depending on conditions [development rates of the life stages are temperature-dependent (Randolph 2004) and availability of hosts might be a further limiting factor], the life cycle of *Ixodes ricinus* takes 2-6 years, typically 3 years (Hillyard 1996). In temperate regions such as Germany, ticks have to cope with marked seasonality in environmental conditions. In *Ixodes ricinus*, the inter-stadial periods between two or more stages may be delayed in the onset of development. This is called 'morphogenetic diapause', which is mainly triggered by photoperiod (Belozerov 1998). Most newly emerged ticks delay their questing activity into the next vegetation period, which is called 'behavioural diapause' (Randolph 2004). Host perception is facilitated by the Haller's organ, located on the dorsal surface of the tarsus of the first pair of legs. When questing for hosts, ticks expose their front legs in the air. The sensory pits and bristle-like sensilla of the Haller's organ are able to detect temperature, humidity, carbon-dioxide concentration, ammonia, aromatic chemicals, pheromones and vibrations (Holsher et al. 1980; Homsher et al. 1988; Steullet and Guerin 1994; Hillyard 1996). Once on the host, ticks wander around and search for an appropriate feeding site. Initially, ticks puncture the epidermis (usually without causing pain) with their chelicerae and then gradually insert their hypostome in the lesion. Via the groove of the hypostome, ticks excrete a 'cocktail' of substances from their salivary glands in the lesion (enzymes; vasodilators to increase blood-flow; anti-inflammatory, anti-haemostatic and immunosuppressive

substances). One of the main effects of this ‘cocktail’ is to inhibit the host inflammatory response. Among their immunomodulatory mechanisms is the ability to subvert the cytokine network at the feeding site by secreting cytokine binding molecules. The injection of tick saliva may also amplify infection with pathogenic agents (Hillyard 1996; Bowman and Sauer 2004; Brossard and Wikel 2004; Nuttall and Labuda 2004; Peterkova et al. 2008). The other main purpose of their excretions is to create a feeding pool. Ticks suck up the blood and exudate from the feeding pool via the groove of the hypostome into the gut. Periods of excreting saliva and sucking alternate and are interspersed by inactive phases. Ticks remain attached until they are fully engorged which usually takes several days: larva 2-6 days, nymph 3-8 days and female adults 6-12 days. Ixodid females can duplicate their unfed body mass by 80-120 times but only mated females can engorge completely. Mating of adult ticks may take place on or off the host. After feeding, ticks detach, drop off the host and digest their blood meal. Larvae and nymphs moult to the next stage. Females produce eggs (*Ixodes ricinus*: 2000-3000), deposit them in a single load and then die (Hillyard 1996). Ixodid ticks spent ~90% of their life off the host which they spent in the vegetation or in the shelter of the host. Off the host, they maintain their water balance by having (1) a slow metabolic rate and (2) an integument and (3) tracheal system that minimises water loss, (4) by absorbing water with their saliva and (5) by seeking humid habitats (Hillyard 1996; Bowman and Sauer 2004).

The tick species of interest, *Ixodes ricinus* and *Dermacentor reticulatus* differ with respect to their life history as summarised in the following table:

Table 1-2. Key aspects of *Ixodes ricinus* and *Dermacentor reticulatus* (based on: Hillyard 1996; Kahl et al. 1992; Jongejan and Uilenberg 2004; Randolph 2004; Menn 2006; Süss 2008).

	Ixodes ricinus	*Dermacentor reticulatus*
Habitat	Prefers rather humid habitats	Also found in dry habitats
Questing behaviour	All stages predominantly exophilic	Larvae and nymphs mainly endophilic, adults exophilic
Host associations	More than 300 host species incl. rodents, reptiles, birds, small- to large-sized mammals	Found on numerous species; host size ranges from rodents to cattle, occasionally found on birds
Life cycle	Three-stage tick, life cycle takes 2-6 (mean 3) years; min. temperature for questing: larvae: ~10°C, nymphs and adults: ~7°C. Time from engorged larva to moulted nymph: 2-3 months	Three-stage tick, lifecycle usually completed within 1 year; questing activity of adult ticks starts after snowmelt. Time from engorged larva to moulted nymph: 1 month
Activity peak of adults	April-June, second peak possible in autumn	March-June, second peak possible in autumn
Main associated pathogens (in central Europe)	*Anaplasma phagocytophilum* *Babesia divergens* *Babesia microti* *Borrelia burgdorferi* *Rickettsia helvetica* Tick-borne encephalitis virus	*Babesia caballi* *Babesia canis* *Rickettsia slovaka* (*Borrelia spp.*) (Tick-borne encephalitis virus)

Tick-borne pathogens with particular reference to tick borne encephalitis virus

Several aspects of the tick biology favour their role as disease vectors: (1) firm attachment to the host, (2) long feeding process, (3) wide host range, (4) dispersal ability (facilitated by hosts such as wide-ranging mammals and birds), (5) adaptability (to modified habitats and domestic animals), (6) longevity, (7) high reproductive potential, (8) resistance to starvation, (9) salivary glands which return excess fluid back to the host during feeding, (10) ability to suppress or evade the host's immune response, (11) infectious agents do not affect the tick itself, (12) transovarial transmission (pathogen can be transmitted via the ovary glands to the next generation), (13) transstadial transmission (pathogenic agent survives each moulting process) and (14) co-feeding transmission (pathogen can be transmitted among ticks feeding on the same host without systemic infection of the host) (Hillyard 1996; Randolph et al. 1996; Labuda and Nuttall 2004). Ticks transmit a greater variety of pathogenic agents (protozoa, rickettsiae, bacteria and viruses), than any other arthropod vector group (Jongejan and Uilenberg 2004). The most significant tick-borne diseases occurring in central Europe, their associated causative agents, vector and reservoir species and clinical manifestations in humans and animals are summarised in Table 1-3. The most widespread tick-borne pathogen of medical significance is probably the Lyme spirochaete. Although it might cause severe and chronic disease in humans, its symptoms are usually not lethal. The other protozoan and bacterial agents rarely cause symptoms in humans and are mainly of veterinary significance (Jongejan and Uilenberg 2004).

Table 1-3. Overview of tick-borne pathogens with a focus on central Europe

Disease	Causative agent	Primary vector species	Reservoir species	Clinical manifestation in humans	Clinical manifestation in animals	References
Lyme borreliosis	Gram-negative bacteria:			Often asymptomatic; disease may process in three (early localised, early disseminated and late disseminated lyme disease) phases. Symptoms include: Erythema migrans, acrodermatitis chronica atrophicans, lymphocytoma, arthritis, facial palsy, meningitis, peripheral radiculoneuropathy, atrioventricular block	Often asymptomatic. Similar symptoms as in humans observed in domestic and wild animals	Kurtenbach et al. 2002; Piesman and Gern 2004
	Borrelia burgdorferi s.l.	*Ixodes hexagonus & Ixodes ricinus*	Hedgehog			
	Borrelia garinii	*Ixodes ricinus*	Birds			
	Borrelia valaisiana	*Ixodes ricinus*	Birds			
	Borrelia burgorferi s.s.	*Ixodes ricinus*	Rodents & Birds			
	Borrelia afzelii	*Ixodes ricinus*	Rodents			
	Borrelia garinii serotype 4	*Ixodes ricinus*	Rodents			
Tick-borne encephalitis	Flavivirus: Tick-borne encephalitis virus. European subtype present in Germany, (Siberian and Far Eastern subtype not found in Germany)	Mainly *Ixodes ricinus*	Forest rodents (*Apodemus* spp, *Myodes glareolus*), role of larger animals not clear	Often asymptomatic or mild fever. First phase: influenza-like symptoms (disease might stop after first phase) Second phase: meningitis, meningoencephalitis, meningoencephalomyelitis or meningoencephaloradiculitis; disease may be fatal (<2% fatality in adults)	Largely asymptomatic in domestic and wild animals but not well studied; Monkeys (e.g. *Macaca sylvanus*) developed similar symptoms as humans, domestic dogs may show similar symptoms as humans	Mansfield et al. 2009; Süss et al. 2007
Babesiosis	Protozoan piroplasms:			Most cases of babesia infection are asymptomatic or include mild fevers. Haemolysis, resulting in haemolytic anaemia and jaundice. Anoxia and toxic effects which might lead to organ failure.	Often leads to severe symptoms, can be fatal if untreated	Kappmeyer et al. 1999; Gray et al. 2010; Malandrin et al. 2010
	Babesia microti	*Ixodes ricinus*?	Meadow vole?			
	Babesia divergens	*Ixodes ricinus*?	Cattle			
	Babesia venatorum	*Ixodes ricinus*?	Deer			
	Babesia divergens-like	Unknown	Unknown			
	Babesia capeoli	*Ixodes ricinus*	Roe deer			
	Babesia caballi	*Dermacentor reticulatus*	Horses			
	Babesia canis	and *D. marginatus*	Dogs			

Table 1-3 continued. Overview of tick-borne pathogens with a focus on central Europe

Disease	Causative agent	Primary vector species	Reservoir species	Clinical manifestation in humans	Clinical manifestation in animals	References
Granulocytic Anaplasmosis	Gram-negative bacteria of the family *Anaplasmataceae* *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila, E. equi*)	*Ixodes ricinus*	Vertebrates, especially deer species likely to play a major role in transmission	Undifferentiated febrile illness, Anorexia, arthralgies, nausea, cough, atypical pneumonitis, Leucopenia. Thrombocytopenia. May be severe.	Infections found in sheep, goats, cattle, horses, dogs, cats, deer, wild boar, foxes, rodents, rabbits, birds. Causes cytoplasmatic inclusions in phagocytes and severe neutropenia Symptoms may include: high fever, decrease in milk production, anorexia, depression, lethargy. cough, nasal discharge, diarrhea, abortion, mastitis. Symptoms may be unapparent, mild to severe (depending on species and individuals). Seldom fatal.	Parola et al. 2005; Stuen 2007
Rickettsia infection	Gram-negative bacteria of the family *Rickettsiaceae* *Rickettsia slovaka*	*Dermacentor reticulatus* and *D. marginatus*	*Dermacentor reticulatus* and *D. marginatus*	Fever and Rash rare. Typical eschar on the scalp with cervical nodes. Usually mild symptoms. Fatal perimyocarditis and sarcoidosis related to *R. helvetica* infection have been reported. Rash and eschar rarely observed	Infections with *R. slovaka* and *R. helvetica* can result in rickettsiemia in both domestic and wild animals.	Parola et al. 2005
	Rickettsia helvetica	*Ixodes ricinus*	*Ixodes ricinus,* (both agents: role of vertebrates unclear)			

Tick-borne viruses belong to 6 virus families (Asfarviridae, Bunyaviridae, Flaviviridae, Orthomyxoviridae, Reoviridae, Rhabdoviridae) whereas each family is characterized by a unique genome organization and replication strategy. The major pathogenic agent considered in this thesis is the tick-borne encephalitis virus (TBEV), which belongs to the family Flaviviridae, genus *Flavivirus*. This arbovirus (arthropod-borne virus: a heterogenous group of ca. 500 vertebrate viruses transmitted by haematophagous arthropods in which they replicate) belongs to the mammalian tick-borne flavivirus group, also known as TBEV serocomplex (Labuda and Nuttall 2004; Mansfield et al. 2009). TBEV is a small (diameter: 40-60nm), lipid enveloped virus having a spherical structure. Its genome consists of a single-stranded, positive sense RNA [ss(+)RNA] of approximately 11kb. The genome RNA is infectious and can produce virus progeny in susceptible cells (Mansfield et al. 2009 and references therein). For more details of the viral genome, specific structural proteins and their functions, and the viral genome replication consider the review by Mansfield et al. (2009).

Based on serological and genomic features, three subtypes of TBEV are differentiated in the literature: European, Siberian and Far Eastern TBEV. In Germany, only the European subtype occurs and is mainly transmitted by *Ixodes ricinus*. The Far Eastern subtype is distributed in Russia, China and Japan, and the Siberian subtype is found in Russia, the Baltics and Finland; both viruses are primarily transmitted by *Ixodes persulcatus* (Gritsun et al. 2003; Labuda and Nuttall 2004; Jääskeläinen et al. 2006; Mansfield et al. 2009). Clinical symptoms of TBE were first described in Austria in 1931 and the virus was first isolated in Russia in 1937 (Schneider 1931; Gritsun et al. 2003; Labuda and Nuttall 2004). The Far Eastern virus subtype of TBEV causes severe disease in humans with a mortality probability that can reach 50%. The Siberian subtype virus is known to cause chronic infections in humans (Labuda and Nuttall 2004). Disease associated with the European subtype is comparatively less severe and mortality is usually very low. A detailed description of the clinical manifestation of this disease is given in the introductions of Chapter 2 and 3. Within the European subtype, several virus strains have been identified (Hypr, Neudoerfl, Salem, AS33) which differ in their genomic structure and which are likely to differ in the pathogenicity (Kupca et al. 2010).

There are different ways how humans may acquire TBEV. Most human infections are acquired via a bite from a TBEV-infected tick. The primary vector ticks are *Ixodes ricinus* (European subtype) and *Ixodes persulcatus* (Far Eastern and Siberian subtype), but other species such as *Dermacentor reticulatus*, *Dermacentor marginatus* and *Rhipicephalus appendiculatus* are also capable of transmitting this virus; these tick species are, however, regarded as secondary vectors (Randolph et al. 1996). Nymphal *Ixodes ricinus* are responsible

for the majority of tick bites in humans and are thus probably the most important tick stage transmitting TBEV and other tick-borne diseases (Vassalo and Perez-eid 2002). Alimentary infection via consumption of unpasteurized milk and milk products from infected animals (goats and cattle) and inhalation or injections of infectious material (such as crushed infected ticks) are other possible infections pathways (Dumpis et al. 1999; Gritsun et al. 2003; Kriz et al. 2009; Mansfield et al. 2009). During the tick bite by an infected tick, the virus is transmitted via the excreted saliva whereas the local skin site of tick feeding is an important focus of viral replication early after TBE virus transmission by ticks (Labuda et al. 1996).

The maintenance of TBEV in nature is facilitated by several transmission routes (see Figure 1-2) whereas there is considerable debate as to which degree each of the transmission routes contributes to the basic reproduction number of TBEV in nature (e.g. Hartemink et al. 2008):

(1) Transstadial transmission: TBEV is maintained in the tick as it develops to the next stage, i.e. an infected larva (nymph) develops into an infected nymph (adult) (Hillyard 1996).

(2) Transovarial transmission: female ticks transmit TBEV to their offspring. This transmission path has been shown to be very important for long term virus circulation in a natural focus (Danielova et al. 2002).

(3) Viraemic transmission: Animals may develop a viraemia [e.g. bank vole *Myodes glareolus* (Chunikhin and Kurenkov 1979), red fox *Vulpes vulpes* (Rieger et al. 1999), roe deer *Capreolus capreolus* (Gerth et al. 1995)] if infected with TBEV. Other ticks feeding on this individual during the phase of viraemia (usually lasting for only a few days) might acquire the virus via the bloodmeal.

(4) Non-viraemic or co-feeding transmission: A pathogenic agent is transmitted among ticks feeding simultaneously on the same host without a systemic infection of the host animal. Ticks of the species *Ixodes ricinus, Ixodes persulcatus, Dermacentor marginatus, Dermacentor reticulatus* and *Ripicephalus appendiculatus* can transmit the TBE-virus via this pathway. *Borrelia burgdorferi* spirochaetes can also be transmitted among ticks via this transmission route (Randolph et al. 1996).

(5) Sexual transmission. The virus is exchanged when adults mate (Mansfield et al. 2009)

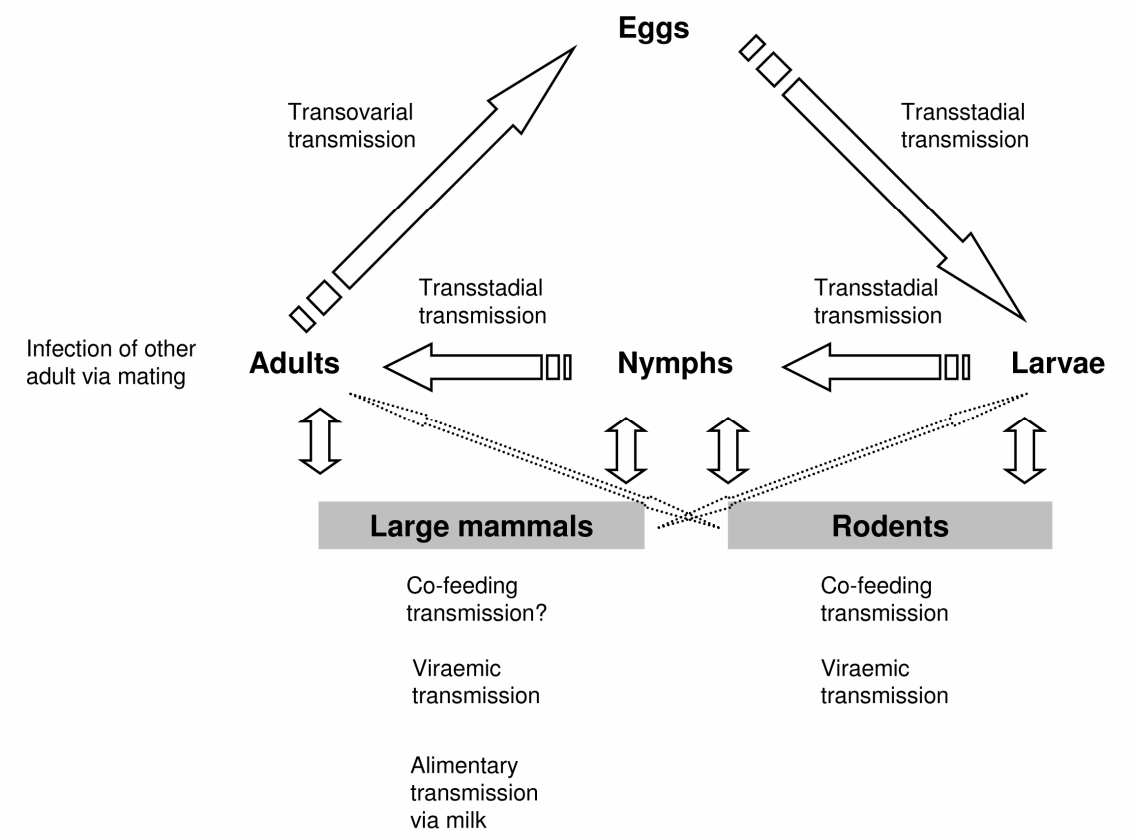


Figure 1-2 Transmission routes of tick-borne encephalitis virus within the life cycle of *Ixodes ricinus* (adapted from Mansfield et al. 2009).

Reservoir and host species

While many ticks such as *Ixodes ricinus* feed on a wide variety of hosts (Hillyard 1996, Süss 2008), some host species are especially important for ticks and may enhance tick densities. High tick densities (Europe: *Ixodes ricinus;* East coast of USA*: Ixodes scapularis*) are associated with high deer densities (Europe: especially *Capreolus capreolus*; East coast of USA: mainly *Odocoileus virginianus*) (Europe: Jensen et al. 2000; Harrison et al. 2010; East coast of USA: Daniels et al. 1993; Rand et al. 2004). Also, forest rodents have been shown to be crucial for bloodmeals of larval ticks. Successful blood meals are fundamental for further development of the larvae and hence rodent densities in $year_t$ are positively correlated with nymph densities in $year_{t+1}$ (Ostfeld et al. 2006; Rosà et al. 2007). Other host species such as (opossums *Didelphis virginian*, squirrels *Sciurus carolinensis*, both native to North-America), however, are very effective in removing and destroying attached ticks. This effective grooming behaviour might actually reduce tick densities considerably (Keesing et al. 2009). This suggests that host species show a different competence with regard to allowing successful blood meals.

Concerning the transmission of a pathogenic agent, the term 'Reservoir competence' is often used. This expression contains at least three components: (1) How susceptible is the host individual when bitten by an infected vector? (2) How does the pathogen persist and multiply in the host? And (3) how effective is the infected host in transmitting the disease agent to feeding vectors (Richter et al. 2000). Usually, the single components of 'Reservoir competence' can only be disentangled and quantified under controlled conditions where host individuals are challenged with infected ticks (e.g. Labuda et al. 1993; Jones et al. 1997). Measuring 'Reservoir competence' in a field-based study rather estimates 'realized reservoir competence'. This incorporates the history of exposure of the host to infected vectors (LoGiudice et al. 2003). For the *Ixodes scapularis*-Lyme disease system, LoGiudice et al. (2003) measured every host species' contribution to the number of larval ticks fed and infected. Combining these relative contributions with data on host abundance, the authors parameterised models which aim at estimating the density of infected nymphs, the key ecological risk factor for acquiring Lyme disease. The models indicate that some species act as dilution hosts (i.e. they have a high population density, feed many ticks but have a low reservoir competence and hence keep the density of infected nymphs low). These finding have considerable implications since species composition (relative abundance of high competent host vs. relative abundance of low competent host species) in a given ecosystem is

thus a crucial component affecting the transmission potential of this pathogen (LoGiudice et al. 2003).

Unfortunately, reservoir competence has not been tested for the wide range of potential host species in the TBE enzootic cycle. Successful transmission of the TBE-virus has been observed in forest rodent species such as *Apodemus* spp., *Myodes glareolus* (Labuda et al. 1993; Labuda et al. 1996). Despite several claims that deer are incompetent with regard to TBE-virus transmission (e.g. Hartemink et al. 2008; Mansfield et al. 2009), to my knowledge no experiments have been conducted to verify this important issue. Only if we are able to estimate the contributions of different species to overall rates of transmission we will be able to predict actual disease risk (Brunner et al. 2008).

Aims of this study

"Main objective of the project is to study relationships of vole, mouse and roe deer abundances to their arboviral infection rates, to their infestation with ticks and to the tick infection rates with arboviruses in Germany" (Rühe 2007). In the course of the project several problems occurred which interfered with the main objective of the study. The main issues were (1) delayed and missing data on infection in sampled specimens (no virus/ antibody data from rodents caught in 2009 provided yet) and (2) no analysis (yet) of sampled ticks due to missing funds. Since these issues became apparent relatively early, I decided to set up two side projects: First, I intended to analyse the spatial pattern of TBE on a large geographical scale in order to identify landscape-scale correlates for TBE. Secondly, since patterns of tick-parasitism are essentially important for virus transmission, I set up a project aiming at quantifying patterns of tick-parasitism on roe deer in detail in the area around Göttingen.

Overall, the objectives of this study can be summarized as follows:

- Which broad-scale factors are correlated with TBE incidence in humans?
- Which extrinsic and intrinsic factors influence individual tick-burdens on roe deer?
- How can one quickly estimate the individual tick burden on roe deer?
- Which are the preferred feeding sites of the different tick life-stages and to what extent do feeding sites of the life stages overlap spatially and temporarily?
- Which extrinsic and intrinsic factors influence individual tick-burdens on bank voles and yellow-necked mice?
- Which extrinsic and intrinsic factors influence the presence of TBE antibodies in roe deer sera?

References

Barker SC, Murrell A (2004) Systematics and evolution of ticks with a list of valid genus and species names. Parasitology 129:S15-S36

Beichel E, Petney TN, Hassler D, Brückner M, Maiwald M (1996) Tick infestation patterns and prevalence of *Borrelia burgdorferi* in ticks collected at a veterinary clinic in Germany. Vet Parasitol 65:147-155

Belozerov, VN (1998). Role of two-step photoperiodic reaction in the control of development and diapause in the nymphs of *Ixodes persulcatus*. Russ J Zool 2:414–418

Binder S, Levitt AM, Sacks JJ, Hughes JM (1999) Emerging infectious diseases: Public health issues for the 21st century. Science 284:1311-1313

Bowman AS, Sauer JR (2004) Tick salivary glands: function, physiology and future. Parasitology 129:S67-S81

Brossard M, Wikel SK (2004) Tick immunobiology. Parasitology 129:S161-S176

Brunner JL, LoGiudice K, Ostfeld RS (2008) Estimating reservoir competence of *Borrelia burgdorferi* hosts: prevalence and infectivity, sensitivity and specificity. J Med Entomol 45:139-147

Bundesministerium der Justiz (2001) Gesetz zur Verhütung und Bekämpfung von Infektionskrankheiten beim Menschen. http://www.gesetze-im-internet.de/ifsg/index.html. Cited 25 Mar 2010

Christian A (2002) Zeckenbefall am Baummarder in Mecklenburg. Abh Ber Naturkundemus Görlitz 74:15-20

Chunikhin SP, Kurenkov VB (1979) Viraemia in *Clethrionomys glareolus* – a new ecological marker of tick-borne encephalitis virus. Acta Virol 23:257-260

Collinge SK, Ray C (2006) Disease ecology: Community structure and pathogen dynamics. Oxford University Press, Oxford, UK

Cornely M, Schultz U (1992) The tick fauna of eastern Germany / Zur Zeckenfauna Ostdeutschlands. Angew Parasitol 3:173-83

Danielova V, Holubova J, Pejcoch M, Daniel M (2002) Potential significance of transovarial transmission in the circulation of tick-borne encephalitis virus. Fol Parasitol 49:323-325

Daniels TJ, Fish D, Schwartz I (1993) Reduced abundance of *Ixodes scapularis* (Acari: Ixodidae) and Lyme disease risk by deer exclusion. J Med Entomol 30:1043–1049

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science 287:443-449

Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E (2006) Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Ricketsia* sp RpA4. J Med Microbiol 296(S156):149-156

Dautel H, Kahl O, Knülle W (2009) The soft tick *Argas reflexus* (F.) (Acari, Argasidae) in urban environments and its medial significance in Berlin (West). J Appl Entomol 111:380-390

Dautel H, Kahl O, Scheurer S, Knülle W (1994) Seasonal activities of the pigeon tick *Argas reflexus* (Acari: Argasidae) in Berlin, Germany. Folia Parasitol 41:155-160

Dumpis U, Crook D, Oksi J (1999) Tick-borne encephalitis. Clin Infect Dis 28:882-890

Estrada-Pena A, Jongejan F (1999) Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. Exp Appl Acarology 23:685-715

Gerth HJ, Grimshandl D, Stage B, Doller G, Kunz C (1995) Roe deer as sentinels for endemicity of tick-borne encephalitis virus. Epidemiol Infect 115:355-365

Gothe R, Stendel W, Holm R (1977) Zum Vorkommen von *Ixodes canisuga* Johnston, 1848, in Deutschland, ein Beitrag zur *Ixodes*-Fauna. Z Parasitenk 53:123-128

Gray J, Zintl A, Hildebrandt A, Hunfeld K-P, Weiss L (2010) Zoonotic babesiosis: Overview of the disease and novel aspects of pathogen identity. Ticks and Tick-borne Diseases 1:3-10.

Gritsun TS, Lashkevich VA, Gould EA (2003) Tick-borne encephalitis. Antiviral Res 57:129-146

Guernier V, Hochberg ME, Guégan J-F (2004) Ecology drives the worldwide distribution of human diseases. PloS Biol 2(6):0740-0746

Guglielmone AA, Robbins RG, Apanskevich DA, Petney TN, Estrada-Pena A, Horak IG (2009) Comments on controversial tick (Acari: Ixodida) species names and species described or resurrected from 2003 to 2008. Exp Appl Acarol 48:311-327

Harrison A, Scantlebury M, Montgomery WI (2010) Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. Oikos: doi: 0.1111/j.1600-0706.2009.18072.x

Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: defining R_0 for tick-borne diseases. Am Nat 171:743-754

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158-2162

Hillyard P (1996) Ticks of North-West Europe. The Dorset Press, Dorchester

Holsher KH, Gearhart HL, Barker RW (1980) Electrophysiological responses of three tick species to carbon dioxide in the laboratory and in the field. Ann Entomol Soc Am 73:288-292

Homsher, PJ, Keirans JE, Robbins RG, Irwin-Pinkley L, Sonenshine DE (1988) Scanning electron microscopy of ticks for systematic studies: structure of Haller's organ in eight species of the subgenus Sternalixodes of the genus Ixodes (Acari:Ixodidae). Med Entomol 25:348-353

Horak IG, Camicas JL, Keirans JE (2002) The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names. Exp Appl Acarol 28:27-54

Immler RM (1973) Untersuchungen zur Biologie und Ökologie der Zecke *Dermacentor reticulatus* (FABRICUS, 1794) (Ixodidae) in einem endemischen Vorkommensgebiet. Mitt Schweiz Entomol Ges 46:1-70

Jääskeläinen AE, Tikkakoski T, Uzcategui NY, Alekseev AN, Vaheri A, Vapalahti O (2006) Siberian subtype tickborne encephalitis virus, Finland. Emerg Infect Dis 12:1568-1571

Jensen PM, Hansen H, Frandsen F (2000) Spatial risk assessment for Lyme Borreliosis in Denmark. Scand J Infect Dis 32:545-550

Jones DJ, Gaunt M, Hails RS, Laurenson K, Hudson PJ, Reid H, Henbest P, Gould EA (1997) Transmission of louping ill virus between infected and uninfected ticks co-feeding on mountain hares. Med Vet Entomol 11:172-176

Jones KE, Patel NG, Levy MA Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451:990-994

Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3-S14.

Kahl O, Janetzki C, Gray JS, Stein J, Bauch RJ (1992) Tick infestation rates with Borrelia: *Ixodes ricinus* versus *Haemaphysalis concinna* and *Dermacentor reticulatus* in two locations in eastern Germany. Med Vet Entomol 6:363-366

Kappmeyer LS, Perryman LE, Hines SA, Baszler TV, Katz JB, Hennager SG, Knowles DP (1999) Detection of equine antibodies to *Babesia caballi* by recombinant *B. caballi* rhoptry-associated protein 1 in a competitive-inhibition enzyme-linked immunosorbent assay. J Clin Microbiol 37:2285–2290

Keesing F, Brunner J, Duerr S, Killilea M, LoGiudice K, Schmidt K, Vuong H, Ostfeld RS (2009) Hosts as ecological traps for the vector of Lyme disease. Proc R Soc B 276:3911-3919.

Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. Ecol Lett 9:485-498.

Kriz B, Benes C, Daniel M (2009) Alimentary transmission of tick-borne encephalitis in the Czech Republic (1997-2008). Epidemiol Mikrobiol Imunol 58:98-103

Kupca, AM, Essbauer S, Zoeller G, de Mendonca P, Brey R, Rinder M, Pfister K, Spiegel M, Doerrbecker B, Pfeffer M, Dobler G (2010) Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. Ticks and tick-borne diseases 1:44-51

Kurtenbach K, De Michelis S, Etti S, Schäfer SM, Seswell H-S, Brade V, Kraiczy P (2002) Host association of *Borrelia burgdorferi* sensu lato – the key role of host complement. Trends Microbiol 10:74-79

Labuda M, Austyn JM, Zuffova E, Kozuch O, Fuchsberger N, Lysy J, Nuttall PA (1996) Importance of localized skin infection in tick-borne encephalitis virus transmission. Virology 219:357-366

Labuda M, Jones LD, Williams T, Danielova V, Nuttall PA (1993) Efficient transmission of tick-borne encephalitis virus between cofeeding ticks. Journal of Medical Entomology 30:295-299

Labuda M, Nuttall PA (2004) Tick-borne viruses. Parasitology 129:S221-S245.

Liebisch A, Rahman MS (1976) Prevalence of ticks *Dermacentor marginatus* (Sulzer, 1776) and *Dermacentor reticulatus* (Fabricius, 1794) and their importance as vectors of diseases in Germany. Tropenmed Parasitol 27: 393-404

LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. PNAS 100:567-571

Malandrin L, Jouglin M, Sun Y, Brisseau N, Chauvin A (2010) Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): Isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. Int J Parasitol 40:277-284.

Mansfield KL, Johnson N, Phipps LP, Stephenson, Fooks AR, Solomon T (2009) Tick-borne encephalitis – a review of an emerging zoonosis. J Gen Virol 90:1781-1794

Menn B (2006) Untersuchungen zur Verbreitung und Ökologie von *Dermacentor* spec. (Ixodidae, Acari) in Deutschland. Diploma thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

Morens DM, Folkers GK, Fauci AS (2004) The challenge of emerging and re-emerging infectious diseases. Nature 430:242-249

Murrell AN, Barker SC (2003) Synonymy of *Boophilus*, Curtice 1891 with *Rhipicephalus*, Koch 1844 (Acari: Ixodidae). Syst Parasitol 56:169-172

Murrell AN, Campbell JH, Barker SC (2000) Phylogenetic analysis of the rhipicephaline ticks indicates that the genus *Rhipicephalus* is paraphyletic. Mol Phylogenet Evol 16:1-7

Murrell AN, Campbell JH, Barker SC (2001) Recurrent gains and losses of large (84–109 bp) repeats in the rDNA internal transcribed spacer 2 (ITS2) of rhipicephaline ticks. Insect Mol Biol 10: 587-596

Nuttall PA, Labuda M (2004) Tick-host interactions: saliva-activated transmission. Parasitology 129:S177-S189.

Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F (2006) Climate, deer, rodents and acorns as determinants of variation in Lyme-disease risk. PLoS Biol 4:e145

Ostfeld RS, Keesing F, Eviner VT (2008) Infectious disease ecology: Effects of ecosystems on disease and of disease on ecosytems. Princeton University Press, Princeton, USA

Parola P, Davoust B, Raoult, D (2005) Tick- and flea-borne rickettsial emerging zoonoses. Vet Res 36:469-492

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, Wolfe ND, Kilpatrick AM, Foufopoulos J, Molyneux D, Bradley DJ, and Members of the Working Group on Land Use Change and Disease Emergence (2004) Unhealthy Landscapes: Policy Recommendations on Land Use Change and Infectious Disease Emergence. Environ Health Persp 112:1092-1098

Peterkova K, Vancova I, Hajnicka V, Slovak M, Simo L, Nuttal PA (2008) Immunomodulatory arsenal of nymphal ticks. Med Vet Entomol 22:167-171.

Piesman J, Gern L (2004) Lyme borreliosis in Europe and North America. Parasitology 129:S191-S220

Rand PW, Lubelczyk C, Holman MS, Lacombe EH, Smith RP (2004) Abundance of *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated offshore island, endemic for Lyme disease. J Med Entomol 41:779–784.

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129:S37-S65.

Randolph SE, Gern L, Nuttall PA (1996) Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission. Parasitol Today 12:472-479

Richter D, Spielman A, Komar N, Matuschka FR (2000) Response to Dr. Randolph and Drs. Gern and Humair. Emerg Infect Dis 6:659–662

Rieger MA, Nubling M, Müller W, Hasselhorn HM, Hofmann F, TBE Foxes Study Group (1999) Foxes as indicators for TBE endemicity – a comparative serological investigation. Int J Med Microbiol (previously Zentralblatt für Bakteriologie) 289:610-618

Rosà R, Pugliese A, Ghosh M, Perkins SE, Rizzoli A (2007) Temporal variation of Ixodes ricinus intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics. Vector-Borne Zoonotic Dis 7:285–295

Rühe F (2007) Surveillance of tick-parasitised voles, mice and roe deer in Germany: Arboviral infection rates in relation to population densities and host characteristics (Project no. 6). In: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance (Ed. F. Hufert), Proposal to BMBF, p. 67

Schneider H (1931) Über epidemische akute *Meningitis serosa*. Wr Klin Wochenschrift 44:350-352

Steullet P, Guerin PM (1994) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus-I of the tick *Amblyomma variegatum* Fabricius (Ixodidae). 2. Receptors within the Hallers organ capsule. J Comp Physiol A 174:27-38

Stuen S (2007) *Anaplasma phagocytophilum* – the most widespread tick-borne infection in animals in Europe. Vet Res Commun 31:79-84

Süss J (2008) Zecken, Was man über FSME und Borreliose wissen muss. Heinrich Hugendubel Verlag, Kreuzlingen/ München, Germany

Süss J, Gelpi E, Klaus C, Bagon H, Liebler-Tenorio EM, Budka H, Stark B, Müller W, Hotzel H (2007) Tickborne encephalitis in naturally exposed monkey (*Macaca sylvanus*). Emerg Infect Dis 13:905-907

Telford SR, Goethert HK (2004) Emerging tick-borne infections: rediscovered and better characterized, or truly 'new'? Parasitology 129:S301-S327

Vassalo M, Perez-eid C (2002) Comparative behavior of different life cycle stages of *Ixodes ricinus* (Acari: Ixodidae) to human-produced stimuli. J Med Entomol 39:234-236

Walter G, Kock D (1985) Records of *Ixodes vespertilionis*, *I. simplex* and *Argas vespertilionis* (Ixodoidea: Ixodidae, Argasidae) from German bats (Chiroptera). Z Parastenkd 71:107-111

WHO (1999) Leading causes of death. http://www.who.int/infectious-disease-peport/pages/graph1.html. Cited 25 Mar 2010

Woolhouse ME, Gowtage-Sequeria S (2005) Host range and emerging and re-emerging pathogens. Emerg Infect Dis 11:1842–1847

Chapter 2 – Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008

INTERNATIONAL JOURNAL OF HEALTH GEOGRAPHICS

RESEARCH Open Access

Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008

Christian Kiffner[1*], Walter Zucchini[2], Philipp Schomaker[1], Torsten Vor[1], Peter Hagedorn[3], Matthias Niedrig[3], Ferdinand Rühe[1]

Abstract

Background: Tick-borne encephalitis (TBE) virus can cause severe symptoms in humans. The incidence of this vector-borne pathogen in humans is characterised by spatial and temporal heterogeneity. To explain the variation in reported human TBE cases per county in southern Germany, we designed a time-lagged, spatially-explicit model that incorporates ecological, environmental, and climatic factors.

Results: We fitted a logistic regression model to the annual counts of reported human TBE cases in each of 140 counties over an eight year period. The model controlled for spatial autocorrelation and unexplained temporal variation. The occurrence of human TBE was found to be positively correlated with the proportions of broad-leafed, mixed and coniferous forest cover. An index of forest fragmentation was negatively correlated with TBE incidence, suggesting that infection risk is higher in fragmented landscapes. The results contradict previous evidence regarding the relevance of a specific spring-time temperature regime for TBE epidemiology. Hunting bag data of roe deer (*Capreolus capreolus*) in the previous year was positively correlated with human TBE incidence, and hunting bag density of red fox (*Vulpes vulpes*) and red deer (*Cervus elaphus*) in the previous year were negatively correlated with human TBE incidence.

Conclusions: Our approach suggests that a combination of landscape and climatic variables as well as host-species dynamics influence TBE infection risk in humans. The model was unable to explain some of the temporal variation, specifically the high counts in 2005 and 2006. Factors such as the exposure of humans to infected ticks and forest rodent population dynamics, for which we have no data, are likely to be explanatory factors. Such information is required to identify the determinants of TBE more reliably. Having records of TBE infection sites at a finer scale would also be necessary.

Background

Tick-borne encephalitis (TBE) is the most important flavivirus infection of the central nervous system in Europe and Russia. The annual number of cases is estimated to be as high as 10,000 in Russia and about 3,000 in European countries [1-5]. Severe TBE infections caused by European virus strains typically take a biphasic course: After a short incubation period (usually 7-14 days, with extremes of 4-28 days), the first (viraemic) phase presents as an uncharacteristic influenza-like illness lasting 2-4 days (range 1-8 days) with fever, malaise, headache, myalgia, gastrointestinal symptoms, leukocytopenia, thrombocytopenia and elevated liver enzymes, and is often followed by a symptom-free interval of about one week (range 1-33 days). The second phase of TBE occurs in 20-30% of infected patients and is marked by four clinical features of different severity (meningitis, meningoencephalitis, meningoencephalomyelitis or meningoencephaloradiculitis) and the appearance of specific antibodies in the serum and cerebrospinal fluid. This is usually the time when patients with high fever and severe headache seek medical advice. The fatality rate in adult patients is less than 2%. However, severe courses of TBE infection with higher mortality and long-lasting sequelae, often affecting the patient's quality of life, are correlated with increased age [6-9].

* Correspondence: ckiffne@gwdg.de
[1]Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany
Full list of author information is available at the end of the article

As with most zoonotic diseases [10], TBE incidence in humans is characterised by considerable temporal (Figure 1) and spatial (Figure 2) heterogeneity [11]. The main determinant of infection risk is the density of infected ticks, i.e. the product of pathogen prevalence in the ticks and tick density [12]. *Ixodes ricinus*, the main vector of TBE virus (TBEV), has three distinct stages, larval, nymphal and adult ticks [13] whereas nymphs are responsible for the majority of tick bites in humans [14]. The *prevalence* of TBEV in (nymphal) ticks depends on a combination of factors; the basic reproduction number (R_0) of TBEV, which crucially depends on the occurrence of non-viraemic virus transmission between ticks co-feeding, especially on forest rodents [15-17]. Furthermore, the persistence of TBEV depends on a threshold value between the density of competent (e.g. yellow-necked mice *Apodemus flavicollis*) and incompetent hosts (e.g. red deer *Cervus elaphus*) [18]. Possibly resulting from the species-specific reservoir competence, peaks in rodent populations in a given year are positively correlated with TBE incidence in humans in the succeeding year [19]. Co-feeding of larval and nymphal ticks on rodents critically depends on seasonal activity synchrony of these immature tick stages. Since Randolph et al.'s study [20], which related larval and nymphal synchrony to a certain temperature regime in autumn, subsequent research attention has been directed towards a specific temperature regime during spring time [11] causing the seasonal synchrony of immature ticks. Tick *density* in a given landscape is primarily determined by the availability of suitable forest habitat [21]. The findings of Allan et al. [22], relating to Lyme borreliosis risk, suggest that forest fragmentation might affect epidemiological risk. Tick density is further influenced by the abundance of host species such as roe (*Capreolus capreolus*) and red (*Cervus elaphus*) deer [23,24] which feed large numbers ticks in all stages of development [25,26]. Since estimates of wildlife population densities do not exist at the appropriate temporal and spatial resolution, we used hunting bag statistics as proxy for density. We also used hunting bag data of red fox (*Vulpes vulpes*), which was found to be positively correlated (with a time lag of one year) with TBE incidence in humans in Sweden [27]. The explanatory variables investigated in this paper include hunting bag data for red deer, roe deer and red fox, land cover, spring warming increase, and an index of forest fragmentation. Specifically we apply statistical tests, which take account of spatial correlation, to assess whether these factors are related to TBE incidence in humans.

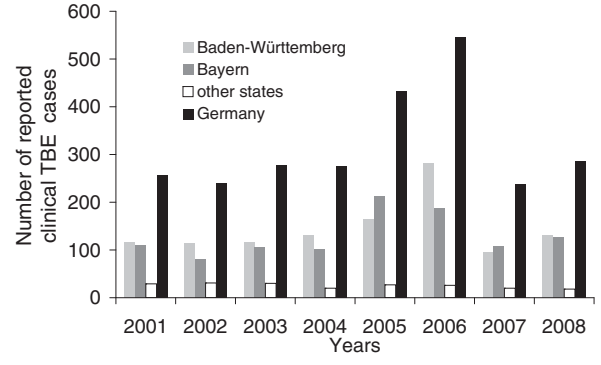


Figure 1 Number of reported human TBE infections in Germany from 2001-2008. Data source: Robert-Koch Institut: SurvStat, http://www3.rki.de/SurvStat. Accessed: 2/04/2009.

Methods

Dependent variable

Annual symptomatic TBE infections in humans registered by the patients' place of permanent residence (county level) for the period 2001-2008 were obtained from the data base of the Robert Koch-Institut (SurvStat, http://www3.rki.de/SurvStat, 11/02/2009). We included the human population size for each county and year (provided by the federal statistical bureau) in our model. For a given county in a given year we modelled p, the proportion of reported clinical TBE cases y out of n inhabitants, assuming that TBE incidence in humans and infection risk are closely correlated.

A model for the TBE-count data

Since our dependent variable consists of counts of successes (y) and failures (n-y) in n trials, we used a generalized linear model (GLM) with binomial error distribution, i.e. a logistic regression model [28]. The statistical package *R* [29] was used to estimate parameters of the model and to compute the test statistics. We model the probability that a person in a given county, and in a given year, is infected as a function of the explanatory variables.

Explanatory variables

Forest type and forest cover

Information on forest type and forest cover for each county was obtained from the CORINE dataset with a spatial resolution of 100 meters [30]. We used the most recent available land cover information (year 2000) for all years, assuming that forest type and cover did not change substantially over this relatively short time period. For each county, we computed the proportion of coniferous, broad-leafed and mixed forest area.

Forest fragmentation

Given the relative coarse resolution of the CORINE dataset, we used the 'largest patch index' (largest forest patch/total forest area) to approximate the level of forest fragmentation for each county [31].

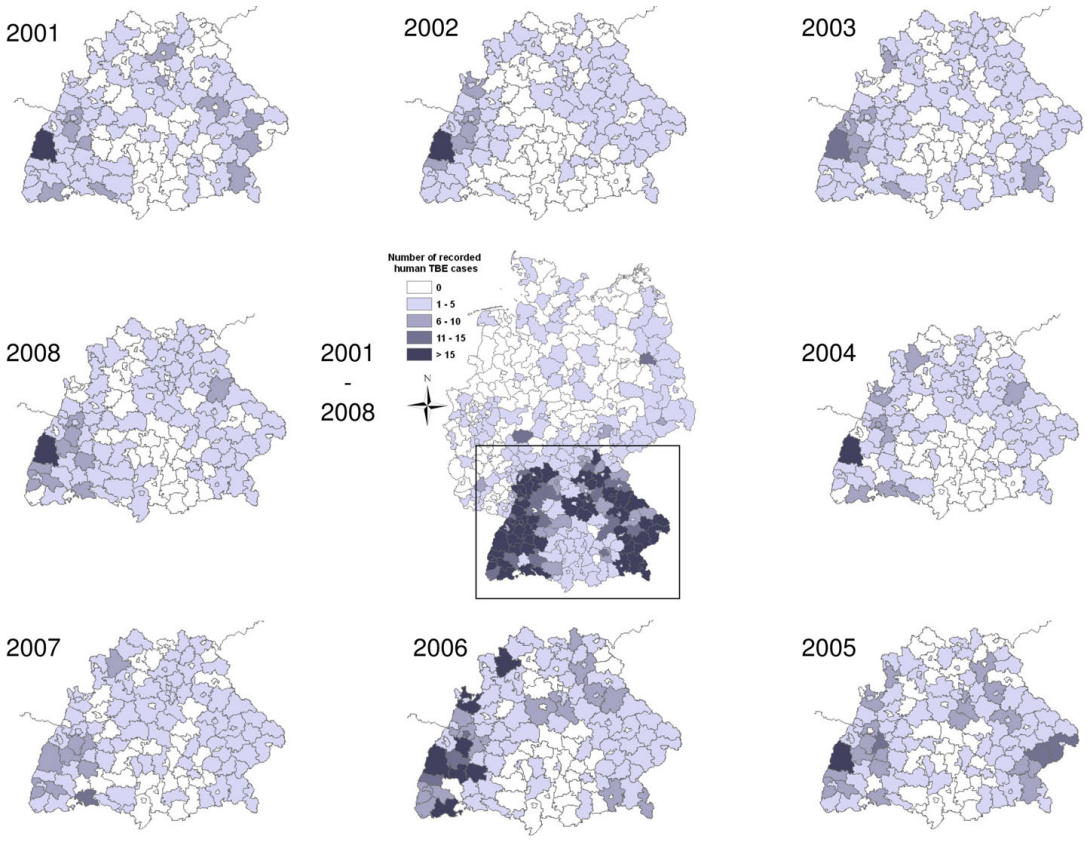


Figure 2 Spatial and temporal variation in reported numbers of human TBE infections from 2001-2008. The central map shows the distribution of TBE infection of entire Germany for the entire study period. Maps depicting the annual distribution of human TBE infections for the study area (States of Baden-Württemberg and Bavaria) are arranged clockwise around the central map. Data source: Robert-Koch Institut: SurvStat, http://www3.rki.de/SurvStat. Accessed: 02/04/2009.

Spring warming

We extracted mean diurnal monthly interpolated temperatures from 30 arc s resolution climate surface maps based on the 1950-2000 period [32]. These data describe the spatial variation of temperatures which seems reasonable for our research question, given that temporal variation of temperatures is unlikely to explain the temporal variation of TBE incidence [33,34]. Data preprocessing included a validity check of the temperature data and a re-projection to the WGS84 cartographic system in GIS ArcView version 9 (ESRI, Redlands, CA, USA). Mean interpolated temperatures (in °C) were computed for each county, for the months of January, February, March and April. From these we computed the temperature increase in spring as the mean temperature increase between February and April corrected for the mean temperature of January [11].

Hunting bag data

We obtained annual hunting statistics of game species from wildlife authorities of both states (Baden-Württemberg and Bavaria) for the period 2000/2001-2007/2008. To deal with the unequal sizes of the 140 counties, we standardised the hunting bag counts by dividing them by area available for wildlife, the latter being approximated by the area covered by agriculture and forest. For Bavaria, hunting bag data on roe deer were not available for the period 2000/2001. Considering the minor temporal variation in roe deer hunting bags, we used the roe deer bag data of the period 2001/2002 also for the missing period. In Bavaria, roe deer hunting is based upon a three years management plan but hunting bags are approximately evenly realised among years. In Baden-Württemberg, roe deer hunting is based on yearly management plans. Management plans for carnivores are not in place.

Unexplained spatial and temporal variation

Spatial autocorrelation [35] was incorporated by using the proportion of TBE cases per inhabitant in the neighbouring counties as an explanatory variable in the model. To control for unexplained temporal variation, we included each year as fixed factor (with year 2001 as reference) in the model. Summary statistics of dependent and explanatory variables are provided in Table 1.

Table 1 Summary statistics of dependent and explanatory variables for a model explaining the spatio-temporal variation of tick-borne encephalitis in southern Germany from 2001-2008

Dependent variable	Mean	SD	Min.	Max.
Number of reported TBE infections in humans	1.95	3.26	0.00	39.00
County population size (in units of 10,000)	16.54	14.10	2.86	131.20
Explanatory variables				
Proportion of forest cover/county				
Broad-leafed forest	0.05	0.06	0	0.28
Coniferous forest	0.18	0.13	0	0.56
Mixed forest cover	0.09	0.07	0	0.36
Forest fragmentation				
Connectivity (largest forest patch/entire forest cover)	0.19	0.14	0	0.74
Hunting bag in previous year per km^2 hunting area				
Red deer *Cervus elaphus*	0.09	0.30	0	6.77
Roe deer *Capreolus capreolus*	4.46	1.30	0.76	10.10
Red fox *Vulpes vulpes*	2.17	0.91	0	17.86
Climatic variables				
Spring warming: Temperature increase from February-April (°C) corrected for mean January temperature	9.25	1.39	6.07	12.59
Unexplained spatial variation				
Total human TBE cases in all neighbouring counties per inhabitants of those counties	0.00013	0.00014	0	0.00091
Unexplained temporal variation				
Each year was entered as a fixed factor in the model	-/-	-/-	-/-	-/-

Note that the hunting bag of roe deer in the counties of Bavaria is the average annual offtake of three years.

Modelling procedure

Before fitting a full model, we tested the effect of single covariates on the probability of TBE infections in humans after correcting for unexplained spatial and temporal variation (Table 2). We tested for multicollinearity between the explanatory variables (Table 3) by computing the condition number. For model checking the deviance residuals were computed and displayed using GRASS GIS [36].

Results

Preliminary analyses

After correcting for unexplained spatial and temporal variation, five of the eight candidate variables were significantly ($p < 0.05$) related with TBE incidence in humans (Table 2). The candidate variables showed rather weak correlations, resulting in a condition number of 6.5, which indicates that multicollinearity is not of concern [37]. The final model was fitted using all candidate variables.

Full model

The full model suggested that all candidate variables were significantly associated with TBE infection risk in humans (Table 4). The proportion of forest cover was positively correlated with probability of TBE infection; the effect of coniferous forest cover was stronger than that of broad-leafed and mixed forest cover (Figure 3a-c). When tested on its own, the proportion of broad-leafed forest cover was not significant (Table 2).

TBE risk was significantly negatively associated with the forest fragmentation index. This suggests that,

Table 2 Relationships between single variables and the probability of tick-borne encephalitis infections in humans after correcting for spatial autocorrelation and unexplained temporal variation as tested by a logistic regression model

Parameter	Algebraic sign of coefficient estimate	Significance level
Proportion broad-leafed forest	-	0.10
Proportion coniferous forest	+	< 0.001
Proportion mixed forest	+	< 0.001
Connectivity	+	0.59
Red deer hunting bag	+	< 0.01
Roe deer hunting bag	+	< 0.001
Red fox hunting bag	-	0.24
Spring warming	-	< 0.001

Table 3 Correlation matrix (Pearson product-moment correlation coefficient) between variables potentially explaining the probability of tick-borne encephalitis infections in humans in southern Germany

Parameter	Proportion broad-leafed forest	Proportion coniferous forest	Proportion mixed forest	Largest forest patch index	Red deer hunting bag	Roe deer hunting bag	Red fox hunting bag
Proportion coniferous forest	-0.53						
Proportion mixed forest	0.33	-0.11					
Largest patch index	-0.13	0.17	-0.15				
Red deer hunting bag	-0.09	0.27	0.14	-0.06			
Roe deer hunting bag	-0.03	0.06	0.21	-0.34	-0.07		
Red fox hunting bag	0.20	-0.17	0.13	-0.17	-0.06	0.12	
Spring warming	-0.44	0.32	-0.33	-0.16	0.16	-0.02	-0.38

taking all other variables as fixed, TBE risk in humans would decline if forest cover were more continuous (Figure 3d). Admittedly, we did not detect a significant relationship between the forest fragmentation index and TBE risk if tested without other explanatory variables (Table 2).

Wildlife population densities (measured indirectly as described above) were significantly correlated with TBE risk in humans in the following year (Figure 3e-g). The correlation with roe deer density was positive but those with red fox and red deer were negative. Except in the case of red deer, all coefficients in the full model have the same sign as those in the corresponding restricted models. TBE risk was significantly correlated with temperature increase during the spring months (Figure 3h) both in the restricted and in the full model (Tables 2, 4). Unexpectedly, the correlation is negative.

Table 4 Parameter estimates of variables derived from the full logistic regression model aiming at explaining the spatio-temporal variation in human tick-borne encephalitis infections in southern Germany from 2001-2008

Parameter	Estimate	Standard error	z-value
Intercept	-11.94***	0.32	-37.30
Proportion broad-leafed forest	2.21***	0.47	4.71
Proportion coniferous forest	4.38***	0.23	18.69
Proportion mixed forest	3.21***	0.32	10.04
Largest patch index	-0.58**	0.21	-2.76
Red deer hunting bag	-0.27*	0.11	-2.74
Roe deer hunting bag	0.21***	0.02	10.42
Red fox hunting bag	-0.21***	0.04	-4.97
Spring warming	-0.14***	0.02	-6.09
Spatial autocorrelation	817.75***	211.47	3.87
Year 2002	-0.16	0.10	-1.63
Year 2003	-0.02	0.10	-0.18
Year 2004	-0.09	0.09	-0.98
Year 2005	0.42***	0.08	4.92
Year 2006	0.64***	0.08	7.81
Year 2007	-0.30**	0.10	-2.93
Year 2008	-0.01	0.09	-0.13

(***P-value < 0.001; **P-value < 0.01; *P-value < 0.05).

There is strong evidence of spatial autocorrelation (Figure 3i) and, unfortunately, also of unexplained temporal heterogeneity. The incidence of TBE infections varied substantially from year to year (see Figure 1) peaking in 2005 and 2006. Of course we would have wished that the model could explain these peaks in terms of the explanatory variables considered here. As is evident in Table 4 we were unable to account for this temporal heterogeneity with the covariates available to us.

With the above limitations in mind, the model (Null deviance = 3580.0 on 1119 degrees of freedom; residual deviance = 2494.8 on 1101 degrees of freedom) explained a large fraction of the observed variance (Cox-Snell-R^2 = 0.96, Nagelkerke's R^2 = 0.65). A histogram of the deviance residuals of the model (Figure 4) indicates that the distribution of the residuals is somewhat skewed, which is due to the discrete nature of the response; about 40% of the observed counts are equal to zero, and almost 75% of them are two or less. The spatial distribution of the residuals (Figure 5) varied considerably from year to year. There was a slight tendency to underestimate the TBE incidence in counties with few reported cases (south central part of the study area) and to overestimate the incidence in counties with many reported cases (western, northern and eastern regions of the study area) (Figures 2 and 5).

Discussion

The model explaining TBE incidence in southern Germany contains eight variables (three related to forest cover, one related to forest fragmentation, three related

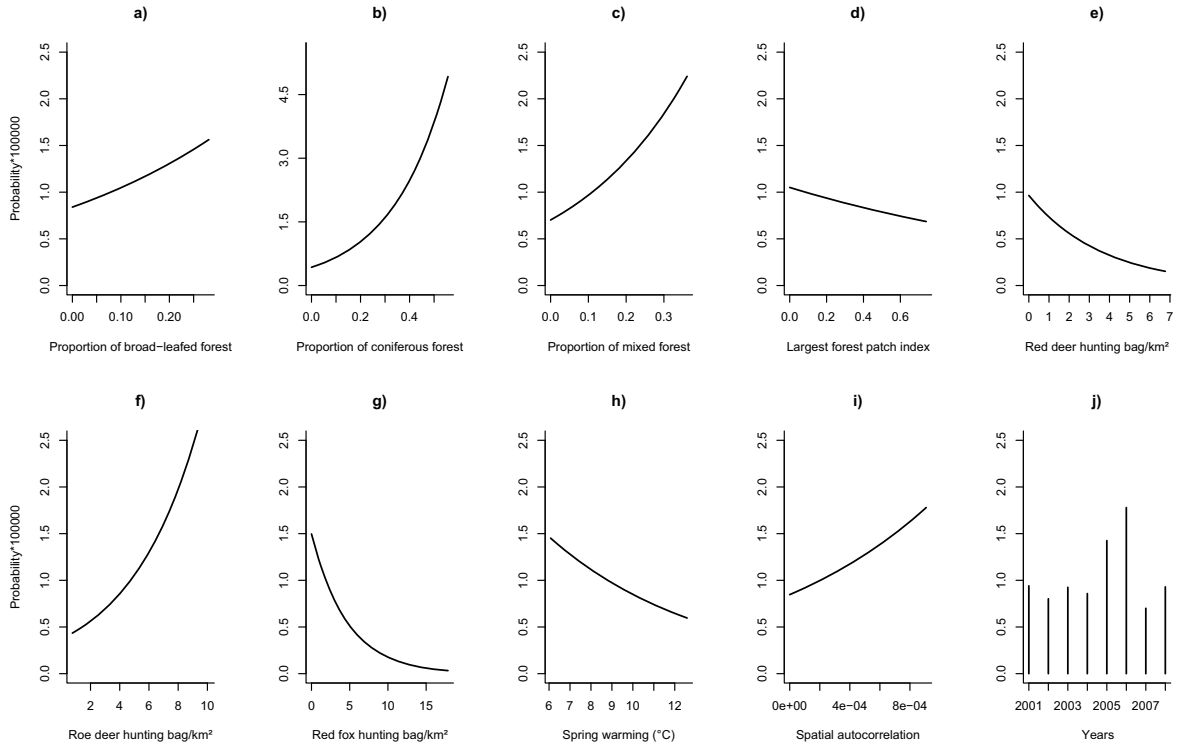


Figure 3 Relative effects of significant variables of the final model explaining the variation of human TBE infections in southern Germany. The effect of single variables on the probability of TBE incidence was estimated by predicting the full model on the entire range of the target variable. Non-target variables entered the model prediction with their mean value and using the year 2001 as reference. For a definition of the variables shown in a) - j), see Table 1.

to wildlife species population dynamics and one related to climatic conditions during spring time) plus one variable each to account for unobserved spatial and temporal variation. With one exception (spring warming) the results are broadly in line with previous research, highlighting the range of factors influencing TBE transmission dynamics and thus infection potential in humans [33,34,38]. The fact that the factor "year" had to be included in the model constitutes a failure of the model. Other factors, especially those taking account of human activities, would need to be considered to explain the remaining and substantial temporal

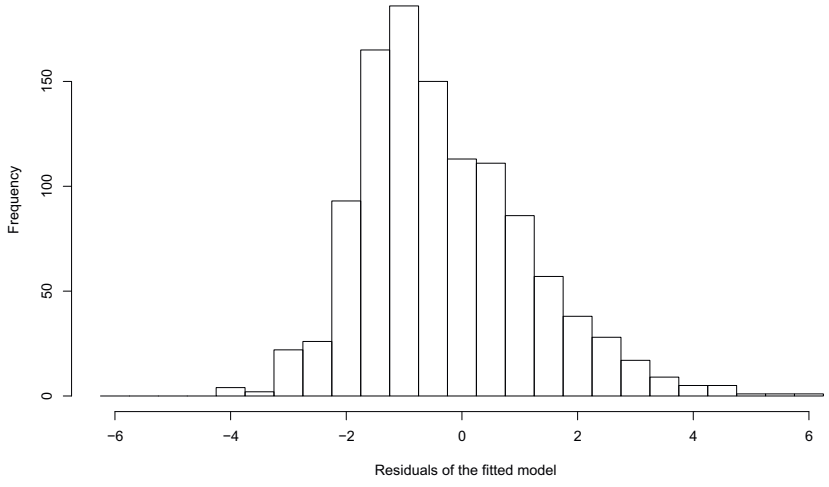


Figure 4 Histogram of the residuals of the final model explaining the variation of human TBE infections in southern Germany.

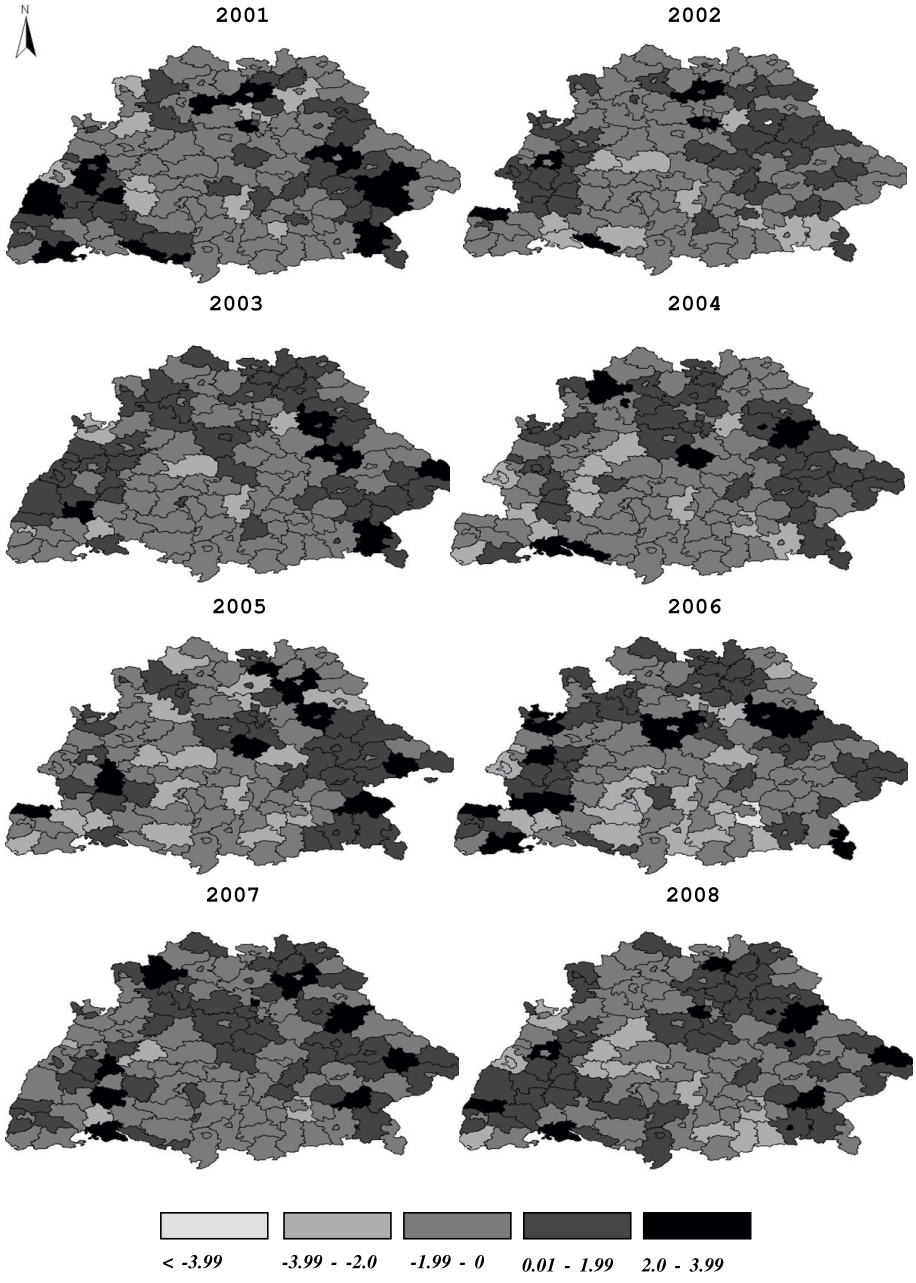


Figure 5 Spatial distribution of residuals derived from the negative binomial regression model on TBE infections in humans.

heterogeneity. The admittedly incomplete model does, however, consider the influence of several explanatory variables simultaneously, rather than one variable or a few variables.

The proposed model suggests that the proportion of coniferous forests had a stronger effect on TBE risk for humans compared to other forest types. Results from field studies provide equivocal results concerning the epidemiological risk [densities of (infected) ticks] according to forest type, but generally broad-leafed forest is considered as a suitable habitat for ticks. In North American forests, black-legged ticks (*Ixodes scapularis*) are more abundant in deciduous than in coniferous woodlands [39]. In Scotland, tick densities were highest in coniferous forests compared to deciduous woodland and pastures [40]. In Denmark, no differences in *Ixodes ricinus* tick densities between spruce (conifer) forests and non spruce forests were found [23]. It is, however, unlikely that forest type itself accounts directly for tick density patterns. Potentially, forest type

indirectly affects tick densities by providing appropriate moisture and host species for ticks or by sustaining more TBE competent reservoir species relative to incompetent ones [18,23,33], or forest types are unevenly frequented by humans.

In addition to forest extent, we hypothesized that forest fragmentation would enhance TBE risk either by increasing density of infected ticks [22] or by enhancing contact rates between humans and ticks. The effect of forest fragmentation was unclear. Despite showing weak correlations with other variables (Table 3), which indicates that it is not confounded with other variables, the sign of the coefficient in the simple model was the opposite of that in the full model. Yet, removing this variable from the full model did not alter the signs of other coefficients.

Hunting bag densities of three game species were found to be significantly associated with TBE risk in humans. Since hunting bags are subject to management plans (deer) and/or influenced by hunting effort (red fox), these observations should be regarded cautiously [41]. Yet, "important ecological questions simply have to be addressed on the right scale - which often means an uncomfortable large scale - even if that means a certain degree of imprecision" [42]. There is growing empirical evidence that roe deer populations increase the epidemiological risk of ticks [23], Lyme disease risk [43] and TBE risk [[33], [44], this study]. Regarding the role of red deer in determining TBE risk, our results are inconclusive (changing signs simple model vs. full model) but do not necessarily contradict findings from Italy, where a non-significant effect was found between red deer and TBE incidence in humans [33].

The negative relationship found in this study between red fox hunting bags and human TBE cases in the succeeding year is in contrast to a study in TBE endemic areas in Sweden, where this relationship was found to be positive [27]. These contradictory findings will need to be clarified in future studies on the specific role of foxes in the epidemiology of TBEV.

Unfortunately, reliable data on forest rodent dynamics are not available at the appropriate temporal and spatial scale, and could thus not enter our model. Further attempts should be made to test the hypothesis that high rodent (especially *Apodemus flavicollis*) densities translate into high nymphal densities in the following year [45] and hence to elevate TBE incidence in humans [19]. Such a finding might be of use as an effective early warning system for public health.

Unexpectedly, the direction of the regression coefficient of the spring warming variable is not in line with the claimed importance of simultaneous activity of larval and nymphal ticks for TBE maintenance [11]. That theory suggests that fast warming in spring is required to allow nymphs (critical temperature ~7°C) and larvae (critical temperature ~10°C) to feed synchronously [16] and to transmit pathogens among the tick population [15]. However, it should be emphasised that spring warming has so far been used only to explain the distribution of TBE foci and not the variable incidence.

As in most epidemiological studies, we based our analysis on the assumption that TBE incidence is strongly correlated with the epidemiological risk of TBE (i.e. the density of infected nymphs). Although the model provides a reasonable fit, some potentially important factors were not investigated due to lack of data. Included here are differing virulence of TBE, variable exposure of humans to ticks, differing immune responses, demography and TBE immunisation coverage. E.g. Kimmig et al. [46] found discrepancies in spatial distribution of clinical TBE cases and prevalence of TBE antibodies in sera of persons at risk. In addition, demographic analyses indicate that men above the age of 35 years are disproportional represented (Figure 6) among the persons with clinical TBE symptoms, which might be related to higher exposure risk to ticks and/or to differing immune responses [47]. Furthermore, locations associated with the counts available to us are the patients' place of permanent residence, and not the place of infection. Although it is likely that most infections occur in the infected person's home county, exceptions in this respect are clearly a potential source of bias. Due to data availability this analysis is based on a rather coarse scale [cf. 42]. Counties do not necessarily represent homogenous units with respect to environmental and ecological conditions. Generally forests, animal communities and interpolated temperatures are very heterogeneous within a given county. Thus any analysis based on values that have been averaged over variable conditions cannot be expected to yield precise results.

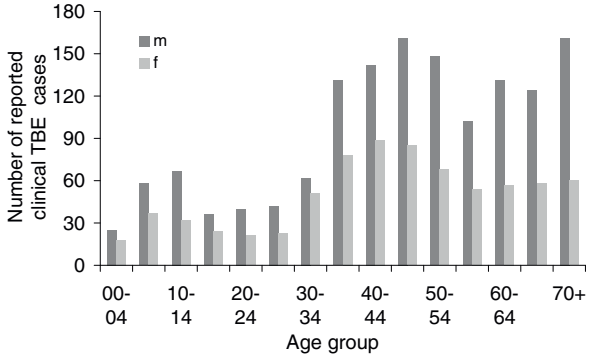


Figure 6 Distribution of reported TBE cases in Baden-Württemberg and Bavaria by age group and sex (m = male, f = female). Data source: Robert-Koch Institut: SurvStat, http://www3.rki.de/SurvStat. Accessed: 02/04/2009.

Consequential we strongly recommend recording the suspected places of TBE infection as accurately as possible i.e. at the resolution of the forest patch.

Conclusions

Our modelling approach focussed on ten biotic and abiotic factors possibly influencing the epidemiological risk of TBE. Except for the influence of spring warming, the results are in line with previously published findings. The presented approach might therefore be useful for predicting at least the potential direction, and approximate magnitude of TBE risk, as a function of ecological change (land cover change, wildlife population dynamics). To make further progress, a higher spatial resolution is needed in order to make more reliable interferences about the relationships between eco-environmental factors and TBE incidence. Moreover, neither this analysis nor the in-depth analyses of yearly temperature variability [33,34,48] could entirely explain the temporal dynamics of tick abundance and TBE in the past decade. This underlines previous notions that besides the epidemiological risk, the contact rate probability between infected ticks and humans is of crucial importance and should be incorporated in models for tick-borne diseases [11,43,48].

Acknowledgements

This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).The Bavarian State Ministry of Alimentation, Agriculture and Forestry and the Wildlife Research Unit of the State Baden-Württemberg kindly provided hunting bag data. Data on human population were kindly supplied by the German Federal Statistical Office. We sincerely thank F. Herschleb for assistance in digitalising hunting bag data and H. Reinecke for generating the map of the residuals. We thank four anonymous reviewers whose comments greatly improved previous drafts of this manuscript.

Author details

[1]Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany. [2]Institute for Statistics and Econometrics, Georg-August-University Göttingen, Platz der Göttinger Sieben 5, 37073 Göttingen, Germany. [3]Centre for Biological Safety, Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany.

Authors' contributions

CK designed the experiment, acquired the data, performed the statistical analysis, interpreted the results and wrote the manuscript. WZ designed the experiment, performed the statistical analysis, contributed to the interpretation of the results and the writing of the MS. PS performed the GIS analyses. FR acquired the data, contributed to the interpretation of the results and the writing of the MS. TV, PH and MN contributed to the interpretation of the results and the writing of the MS. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 6 April 2010 Accepted: 13 August 2010
Published: 13 August 2010

References

1. Pugliese A, Beltramo T, Torre D: **Emerging and re-emerging viral infections in Europe.** *Cell Biochem Funct* 2007, **25(1)**:1-13.
2. Günther G, Haglund M: **Tick-borne encephalopathies: epidemiology, diagnosis, treatment and prevention.** *CNS Drugs* 2005, **19(12)**:1009-32.
3. Charrel RN, Attoui H, Butenko AM, Clegg JC, Deubel V, Frolova TV, Gould EA, Gritsun TS, Heinz FX, Labuda M, Lashkevich VA, Loktev V, Lundkvist A, Lvov , Mandl CW, Niedrig M, Papa A, Petrov VS, Plyusin A, Randolph S, Süss J, Zlobin VL, de Lamballerie X: **Tick-borne virus diseases of human interest in Europe.** *Clin Microbiol Infect* 2004, **10(12)**:1040-55.
4. Gritsun TS, Lashkevich VA, Gould EA: **Tick-borne encephalitis.** *Antiviral Res* 2003, **57(1-2)**:129-46.
5. Süss J: **Tick-borne encephalitis in Europe and beyond - the epidemiological situation as of 2007.** *Euro Surveill* 2008, **13(26)**, pii=18916.
6. Holzmann H: **Diagnosis of tick-borne encephalitis.** *Vaccine* 2003, **21(Suppl 1)**:S36-40.
7. Kunze U, Baumhackl U, Bretschneider R, Chmelik V, Grubeck-Loebenstein B, Haglund M, Heinz F, Kaiser R, Kimmig P, Kunz C, Kunze M, Mickiene A, Misic-Majerus L, Randolph S, Rieke B, Stefanoff P, Süss J, Wimmer R, International Scientific Working Group on Tick-borne encephalitis: **The Golden Agers and Tick-borne encephalitis: Conference report and position paper of the International Scientific Working Group on Tick-borne encephalitis.** *Wiener Med Wochenschr* 2005, **155(11-12)**:289-94.
8. Kaiser R: **Tick-borne encephalitis (TBE) in Germany and clinical course of the disease.** *Int J Med Microbiol* 2002, **291(Suppl 33)**:58-61.
9. Mickiene A, Laiskonis A, Günther G, Vene S, Lundkvist A, Lindquist L: **Tickborne encephalitis in an area of high endemicity in Lithuania: disease severity and long-term prognosis.** *Clin Infect Dis* 2002, **35(6)**:650-658.
10. Jones E, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P: **Global trends in emerging infectious diseases.** *Nature* 2008, **451**:990-994.
11. Randolph SE, Sumilo D: **Tick-borne encephalitis in Europe: dynamics of changing risk.** *Emerging Pests and Vector-borne Diseases in Europe* Wageningen, University PublishersTakken W, Knols B 2007, 187-206.
12. Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F: **Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk.** *PLoS Biol* 2006, **4(6)**:e145.
13. Randolph SE: **Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors.** *Parasitology* 2004, **129(Suppl 1)**:S37-S65.
14. Vassalo M, Perez-eid C: **Comparative behavior of different life cycle stages of *Ixodes ricinus* (Acari: Ixodidae) to human-produced stimuli.** *J Med Entomol* 2002, **39**:234-236.
15. Labuda M, Nuttal PA, Kozuch O, Eleckova E, Zuffova E, Sabo A: **Non-viraemic transmission of tick-borne encephalitis virus: a mechanism for arbovirus survival in nature.** *Experientia* 1993, **49**:802-805.
16. Randolph SE, Gern L, Nutall PA: **Co-feeding ticks: Epidemiological significance for tick-borne pathogen transmission.** *Parasitol Today* 1996, **12(12)**:472-479.
17. Hartemink NA, Randolph SE, Davis SA, Heesterbeek : **The basic reproduction number for complex disease systems: defining R_0 for tick-borne infections.** *Am Nat* 2008, **171(8)**:743-754.
18. Rosà R, Pugliese A: **Effects of tick population dynamics and host densities on the persistence of tick-borne infections.** *Math Biosci* 2007, **208**:216-240.
19. Zeman P, Benes C: **A tick-borne encephalitis ceiling has moved upwards during the last 30 years: possible impact of global warming?** *Int J Med Microbiol* 2004, **293(Suppl 37)**:48-54.
20. Randolph SE, Green RM, Peacey MF, Rogers DJ: **Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data.** *Parasitology* 2000, **121**:15-23.
21. Racz GR, Ban E, Ferenczi E, Berencsi G: **A simple spatial model to explain the distribution of human tick-borne encephalitis cases in Hungary.** *Vector-Borne Zoonot* 2006, **6**:369-378.
22. Allan BF, Keesing F, Ostfeld RS: **Effect of forest fragmentation on lyme disease risk.** *Conserv Biol* 2003, **17(1)**:267-272.
23. Jensen PM, Hansen H, Frandsen F: **Spatial risk assessment for Lyme borreliosis in Denmark.** *Scand J Infect Dis* 2000, **32**:545-550.
24. Gilbert L: **Altitudinal patterns of tick and host abundance: a potential role for climate change in regulating tick-borne diseases?** *Oecologia* 2010, **162**:217-225.

25. Kiffner C, Lödige C, Alings M, Vor T, Rühe F: **Abundance estimation of Ixodes ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*).** *Exp Appl Acarol* 2010, **52**:73-84.
26. Vor T, Kiffner C, Niedrig M, Hagedorn P, Rühe F: **Tick burden on European roe deer (*Capreolus capreolus* L.).** *Exp Appl Acarol* 2010, **51**:405-417.
27. Haemig PD, Lithner S, Sjöstedt de Luna S, Lundkvist Å, Waldenström J, Hansson L, Arneborn M, Olsen B: **Red fox and tick-borne encephalitis (TBE) in humans: Can predators influence public health?** *Scand J Infect Dis* 2008, **40**:527-532.
28. Dobson AJ, Barnett AG: **An introduction to generalized linear models.** Boca Raton, Chapman & Hall/CRC 2008.
29. R Development Core Team: **R: A language and environment for statistical computing.** R Foundation for Statistical Computing, Vienna, Austria 2008 [http://www.R-project.org].
30. Anonymous: **CORINE Land Cover.** Federal Environmental Agency, German Aeospace Center 2004.
31. Haines-Young R, Chopping M: **Quantifying landscape structure: a review of landscape indices and their application to forested landscapes.** *Prog Phys Rev* 1996, **20**:418-445.
32. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A: **Very high resolution interpolated climate surfaces for global land areas.** *Int J Climat* 2005, **25**:1965-1978.
33. Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R: **Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy.** *PloS ONE* 2009, **4**:e4336.
34. Sumilo D, Asokliene L, Bormane A, Vasilenko V, Golovljova I, Randolph SE: **Climate change cannot explain the upsurge of tick-borne encephalitis in the Baltics.** *PLoS ONE* 2007, **6**:e500.
35. Wimberly MC, Baer AD, Yabsley MJ: **Enhanced spatial model for predicting the geographic distributions of tick-borne pathogens.** *Int J Health Geogr* 2008, **7**:15.
36. **GRASS Development Team: Geographic Resources Analysis Support System (GRASS) Software,I 2010.** Open Source Geospatial Foundation [http://grass.osgeo.org].
37. Mason CH, Perreault WD: **Collinearity power and interpretation of multiple regression analysis.** *J Marketing Res* 1991, **28**:268-280.
38. Estrada-Pena A: **Tick-borne pathogens, transmission rates and climate change.** *Front Biosci* 2009, **14**:2674-2780.
39. Ginsberg HS, Zhioua E, Mitra S, Fischer J, Buckley PA, Verret F, Underwood HB, Buckley FG: **Woodland type and spatial distribution of nymphal *Ixodes scapularis* (Acari : Ixodidae).** *Environ Entomol* 2004, **33(5)**:1266-1273.
40. Walker AR, Alberdi MP, Urquhart KA, Rose H: **Risk factors in habitat of the tick *Ixodes ricinus* influencing human exposure to *Ehrlichia phagocytophila* bacteria.** *Med Vet Entomol* 2001, **15**:40-49.
41. Ranta E, Lindström J, Lindén H, Helle P: **How reliable are harvesting data for analyses of spatio-temporal population dynamics?** *Oikos* 2008, **117**:1461-1468.
42. May RM: **Crash test for the real.** *Nature* 1999, **398**:371-372.
43. Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, Vanwambeke SO, Lambin EF: **Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium.** *Int J Health Geogr* 2007, **6**:15.
44. Hudson PJ, Rizzoli A, Rosà R, Chemini C, Jones LD, Gould EA: **Tick-borne encephalitis virus in northern Italy: molecular analysis, relationships with density and seasonal dynamics of *Ixodes ricinus*.** *Med Vet Entomol* 2001, **15**:304-313.
45. Rosà R, Pugliese A, Ghosh M, Perkins SE, Rizzoli A: **Temporal variation of *Ixodes ricinus* intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics.** *Vector-Borne Zoonotic Dis* 2007, 285-295.
46. Kimmig P, Oehme R, Backe H: **Epidemiologie der Frühsommer-Meningoenzephalitis (FSME) und Lyme-Borreliose in Südwestdeutschland.** *Ellipse* 1998, **14(4)**:95-105.
47. Sheldon BC, Verhulst S: **Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology.** *TREE* 1996, **11**:317-321.
48. Randolph SE, Asokliene L, Avsic-Zupanc T, Bormane A, Burri C, Gern L, Golovljova I, Hubalek Z, Knap N, Kondrusik M, Kupca A, Pejcoch M, Vasilenko V, Zygutiene M: **Variable spikes in tick-borne encephalitis incidence in 2006 independent of variable tick abundance but related to weather.** *Parasites & Vectors* 2008, **1**:44.

doi:10.1186/1476-072X-9-42
Cite this article as: Kiffner *et al.*: Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008. *International Journal of Health Geographics* 2010 **9**:42.

Chapter 3 – Surveillance of emerging arthropod-borne viruses in wildlife species in Germany

Surveillance of emerging arthropod-borne-viruses in wildlife species in Germany

C. Kiffner, T. Vor, and F. Rühe

Georg-August-University Göttingen, Faculty of Forest Sciences and Forest Ecology,
Büsgen-Institute, Forest Zoology and Forest Conservation incl. Wildlife Biology and Hunting Sciences, Büsgenweg 3, 37077 Göttingen, Germany

E-Mail: ckiffne@gwdg.de

Abstract

In recent years, infection rates of tick borne encephalitis (TBE) in humans have increased considerably in Germany. In forest ecosystems, deer (*Capreolus capreolus* and *Cervus elaphus*) and especially rodents (*Apodemus spp.*, *Microtus spp.* and *Myodes spp.*) could be important reservoirs for TBE and for other arthropod-borne ("arbo-") viruses.

The main objective of this project is to analyse whether infection rates of host species are density dependent, dependent on individual characteristics or influenced by climatic or habitat factors. Therefore, we aim at investigating the relationships of vole, mouse and deer abundances and physical conditions to their virus infection rates, to their infestation with ticks, to the tick infection rates and to climatic and habitat factors.

Sampling of ticks, voles, mice and roe deer, recording data on individual hosts and on indices of rodent population density is carried out in nine forest departments in late spring, mid-summer and early autumn in each of the three project years, respectively. Deer population densities are estimated in early spring. Ticks and blood from roe deer, rodents and ticks from rodents are sampled simultaneously at the same field sites. Number of ticks per host animal and age, sex and physical condition of the sampled roe deer are recorded in the field.

We dispatch the samples to our network partners for virus screening. By using presence/absence data of arbo-viruses in the studied animals and cell types, infection risks of cell types, voles, mice and deer will be calculated in relation to the local population density, the recorded biometric characteristics of the individual hosts, habitat structure and climatic factors of the study area. In this paper, we present preliminary results of the first sampling session.

Introduction

Arbo-viruses are transmitted to humans via an arthropod vector. In central Europe, the tick species *Ixodes ricinus* (L.) is an important vector of Tick Borne Encephalitis Virus (TBEV) (Family: *Flaviridae*) and several other viruses. TBEV can cause Tick Borne Encephalitis in infected persons: The majority (60-70 %) of humans bitten by an infected tick does not show symptoms whereas 20-30 % of bitten people show flu-like symptoms. Approximately 10% of infected humans suffer from meningitis and meningoencephalitis, which may result in permanent neurological deficits (3-10 %) and in death of infected persons (1-2 %) (Gold *et al.* 1992). Effective vaccination against TBEV is available but no specific treatment exists to prevent damages in the central nervous system (Gold *et al.* 1992, Pöllabauer *et al.* 2003, 2007). Since the aetiology of about more than 50 % of

aseptic meningo-encephalitis cases is unclear, other neglected arbo-viruses [Eyach Virus, Tribec Virus, Lipovnik Virus, Erve Virus (Family: *Reoviridae*) and Tahyna Virus (Family: Bunyaviridae)] are suspected to cause this syndrome in humans.
In Germany, TBE is subject to registration at the federal Robert-Koch-Institute (Infektionschutzgesetz 2000) which allows specific monitoring of both geographic range and numbers of infections in humans.
Since the implementation of the infection-prevention-law in 2000, a significant increase in human infections per 100 000 inhabitants with TBEV was observed (from 2001 to 2006): $y = 0.0694x - 138.69$; $R^2=0.7649$; $P=0.023$ (calculated from Robert Koch-Institut 2007a). This can mainly be explained by increasing infection rates in high risk areas[1] and only to a lesser extent by range expansion of the virus (Robert Koch-Institut, 2007b).
Due to the increase of infection rates and the absence of specific treatments, means of preventing infections with the focal viruses are essential for reducing encephalitis related diseases and mortality. To formulate effective preventive measures, one clearly requires detailed knowledge of the ecology of the viruses in reservoir populations and of the specific mechanisms which drive infection risks in both wildlife host species and humans.

Reservoir studies

The focal arboviruses (TBE, Eyach, Tribec, Lipovnik, Erve and Tahyna Virus) propagate in vertebrate hosts. During viremia they can be transferred to other host individuals by bloodsucking ticks (Figure 1).
This horizontal virus transmission can be influenced by numerous factors, e.g. the ecology of different host species, tick ecology and sociology of humans, and is thus very complex. In this study, we mainly focus on the ecology of host species. However, the aims are numerous: (i) to identify specific reservoir hosts, (ii) to determine the area in which the specific virus is endemic, (iii) to define relative infection risks of wildlife host individuals and ultimately (iv) to define relative infection risks of humans. Specifically, we want to investigate whether infection risks of wildlife species with arbo-viruses are (a) dependent on population density of the specific host species or vector (b) dependent on individual characteristics of host species or (c) influenced by habitat, weather or climatic conditions.
In this paper we briefly present the research project including the applied methods and give an overview on the first sampling session which took place in September 2007.

[1] High risk areas are defined as: Administrative district in which the number of recorded TBE cases within the period 2002-2006 was significantly ($p<0,05$) higher than the number of cases one would expect at an incidence of one case per 100000 inhabitants (Robert Koch-Institut 2007b).

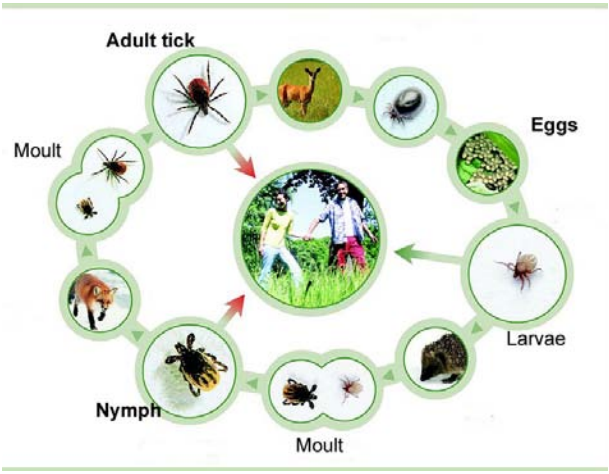


Figure 1: Life cycle of the tick Ixodes ricinus. (Figure adapted from www.zecken.de)

Figure 2: Location of the study area indicated by a larger red dot. The smaller red dots indicate study sites of our network partners. Figure adapted from: www.deutschland.de/images/aeb/karte.de.gif

Material and Methods

The project started in July 2007 and will run for at least three years. The field data sampling is scheduled as follows: Estimation of deer density will be carried out during one week in March. In May, July and September we will spend two weeks each in the study region to sample rodents and ticks on rodents and estimate local

rodent densities. During the same period, we will also collect blood and ticks from deer killed by hunters.

Study sites

We selected nine different forest departments which are located in three different forest districts in the southern part of Hesse. The forests are located within high risk areas for TBEV (Robert Koch-Institute 2007b). Mean size of the study sites is 1150 ha (range: 520 – 1710 ha). In all nine forest departments, roe deer (*Capreolus capreolus*) is abundant. In three forest departments, red deer (*Cervus elaphus*) is also common.

Rodent trapping

In each forest department, we placed two trapping grids (Figure 3) on randomly selected intersections of a systematic 1 km x 1 km grid which we superimposed over the entire study area. We defined the coordinates of the intersection as the position of the southern- and western-most trap. Each grid was established by placing 36 Sherman live traps in a 50 m x 50 m square, whereas perpendicular and horizontal distances between neighbouring traps were 10 m. We used fresh apple to bait rodents and placed hay, chipped wood or foliage into the traps to provide nesting material and to enhance survival of trapped mice. Trapping grids were operated for four consecutive nights and were controlled once a day.

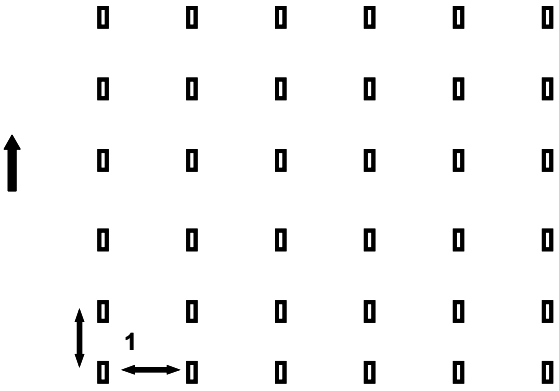


Figure 3: Schematic presentation of a trapping grid showing the relative position of 36 Sherman live traps.

Figure 4: Bank vole (Myodes glareolus). Photo: Christian Kiffner

Caught rodents were collected and sampled according to standardised protocols: Investigators wearing rubber gloves controlled the grid lines and transfer rodents from the trap into a plastic bag, labelled the bag with the grid and trap station number and transported the rodents to a central processing station. When handling the rodents, investigators wore rubber gloves, respirators and face shields to avoid infections with Hantavirus (Family: Bunyaviridae) (Mills *et al.* 1999). Rodents were identified to species level and then killed by filling the bag with CO_2. Eventually trapped non-target animals were set free immediately. A standardised protocol was then used to collect the following data: capture number, trap station number, species, age (subadult, adult), mass (g), length of body (cm), length of tail (cm), reproductive status (males: position of testes, females: description of nipples) and presence of wounds. All rodents were then carefully screened for ticks by combing the fur with a bug comb and by intensively searching the ears and other exposed sites of each individual. All detected ticks were transferred in sterile tubes which were labelled with the unique capture number of the rodent. Ticks were then stored at 4°C and rodents in dry ice at -80°C.

Density estimation of rodents

In contrast to virus reservoir studies in North America where mark-recapture-studies are commonly used (Mills *et al.* 1999) we applied a removal method because the entire bodies of rodents are required to detect viruses in different cell types.
Rodent density will be estimated by the "refined-100-trap-night-index" which corrects for non-functioning traps (Krüger, 2002). Simultaneously, we will apply the "catch-per-unit-effort" method (Borchers *et al.* 2002) and compare both methods with each other.

Deer Sampling

During the field study periods, local hunters were instructed to take blood samples from the fresh carcass. Deer and blood samples of deer were stored in six different central cold storages of the forest districts which were controlled by the research team once a day. The head and neck of each deer carcass was intensively screened for tick infestation by two observers for a maximum of 30 minutes Ticks were removed with tweezers and tick-hooks and were collected - separated by host individual, tick development stage and sex of adult ticks - in sterile tubes which were then stored at 4 °C.
We estimated the age of roe deer by tooth wear (Mysterud & Østbye, 2006). Blood samples of hunter killed deer were centrifuged for ten minutes at 2500 rotations per minute (rpm). Thereafter, the supernatant (blood serum) was pipetted in a sterile and properly labelled tube. The blood serum was stored at 4 °C until serologic screening.

Figure 5: Sampling ticks from a roe buck (Capreolus capreolus). Photo: Martin Scholz

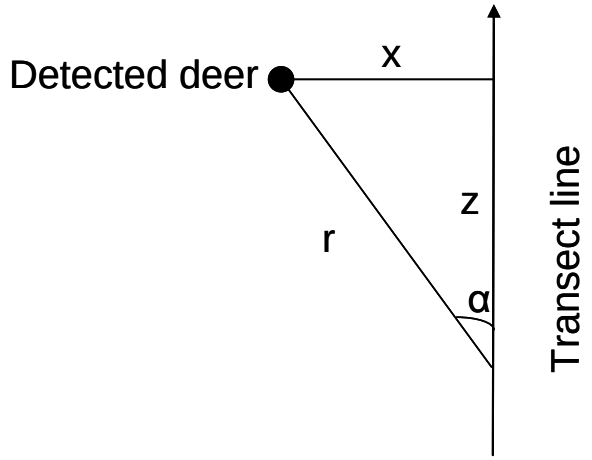


*Figure 6: Computing perpendicular distances of line transect data: When measuring the distance r and the angle a, one can compute the perpendicular distance by x = r * sin (a) (Buckland et al. 2001).*

Estimation of deer density

We intend to estimate both roe deer and red deer densities at the study sites by conducting line transect surveys at night. In early march of each year (2008, 2009 & 2010), we will drive along 20 systematically arranged transects in each forest department. With the help of spotlights - and possibly a thermal imager - two observers will search for deer on and next to the transect (Ward et al. 2004). Apart from recording species, sex and age class of the observed species, we will measure the perpendicular distance between the initial position of the deer and the transect (Buckland *et al.* 2001) to model detection functions of deer species. By incorporating covariates such as observer, vegetation type, study site, deer species and time of the observation we aim at modelling site, species, observer, time and vegetation specific detection functions (Buckland *et al.* 2004). Modelling of detection functions and density estimates will be performed by the software package DISTANCE 5.0, Release 2 (Thomas *et al.* 2006).

Weather and environmental factors

We will assess weather and climatic data such as precipitation and temperature of the current and previous year from meteorological stations of the German weather service and relate these data to population dynamics of rodents and abundance of ticks on host species and in cooperation with the Robert Koch-Institute and local health authorities to infection rates in humans. Additionally, we will acquire data on habitat structure, acorn and beech-nut yield of previous and current years from the forest administration and relate these data to tick, rodent and deer population dynamics (Ostfeld *et al.* 2006).

Preliminary Results

Rodents

During the first sampling period in September 2007, we operated 2628 trapping nights (one trapping grid was operated for five nights). In approximately 9 % of the trapping nights, traps were malfunctioning, leading to a total of 2412 corrected trap nights. Pooling all 18 trapping grids, we collected 97 rodents, giving a mean trapping success of 4 %, whereas trapping success was highly variable between locations ranging from 0 to 21 rodents per trapping grid.
We collected a total of 377 ticks from the caught rodents, whereas 99 % of all ticks were in the larval stage and only 1 % in the nymph stage. Only four rodents carried a single nymph each. On average, larval tick load per rodent was 5 ticks whereas the range of larval infestation ranged from 0 to 24 larvae per individual rodent.

Deer

During the first field data sampling period we investigated a total of 23 roe deer and 1 red deer of which we obtained blood samples and collected ticks.

Table 1: Tick distribution on roe deer (n=23). Total number of collected ticks, separated by development stage and attachment sites of ticks and mean tick infestation rates per roe deer including standard deviation (SD).

	Larva	Nymph	Adult ♀	Adult ♂	Total
Total number on head	45	432	65	26	568
Mean (SD) per animal on head	2 (6)	19 (11)	3 (4)	1 (1)	15 (14)
Total number on neck	38	17	115	38	208
Mean (SD) per animal on neck	2 (7)	1 (1)	5 (5)	2 (2)	9 (11)
Total number on head & neck	83	449	180	64	776
Mean (SD) per animal on head & neck	4 (13)	20 (11)	8 (7)	3 (3)	34 (27)

Average tick burden amounted to 34 ticks per roe deer whereby variability among individual hosts was high (Table 1). More than 55% of all collected ticks were nymphs attached to the head of the deer and overall nymphs made up to 58% of all collected ticks. Surprisingly, the sex ratio of adult ticks was strongly (three folds) skewed towards female ticks.

Laboratory Analysis

Up to date, no serologic or microbiological analyses of the samples have been completed. Rodents are stored in the Virology of the Clinical Centre of the University of Göttingen at -80 °C and ticks of rodents and deer as well as blood of deer are stored at 4 °C. Samples will be processed at the Institute of

Microbiology of the Bundeswehr, Munich, in the Virology of the Clinical centre of the University Göttingen and at the Robert Koch-Institute, Berlin.

Outlook

Upon receiving the data on virus infections in the host species (rodents and deer), we will conduct a logistic regression to identify significant variables which might explain virus infection rates of the vectors and host species. Logistic regression models investigate the influence of multiple factors on the distribution of dichotomous properties (Hosmer & Lemeshow 2000).
In this particular case, we will explore whether infection rates of individual rodents and deer are influenced by the population density of the host species, by the tick burden of different tick development stages on the specific host, the tick infestation rates with the specific viruses, by the species, the age, the sex or the condition of the host.
Similarly we will analyse factors influencing tick infestation rates with the viruses (presence and population densities of virus specific wildlife hosts), and underlying factors which might drive both population dynamics of host species (e.g. availability of food resources) and tick vectors (habitats, weather conditions, climate change) of the focal viruses.

Acknowledgements

We thank the Federal Ministry of Education and Research for financial support (BMBF-1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance). We sincerely acknowledge K. Ehlmann, M. Ksinsik, C. Lödige and M. Scholz for supporting the team during the field sampling period.

References

Borchers, D.L., Buckland, S.T. & Zucchini, W. (2002): Estimating Animal Abundance – closed populations. Springer-Verlag, London, UK.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., Borchers, D.L., Thomas, L. (2001): Introduction to Distance Sampling. Oxford University Press. UK.

Buckland, S.T., Anderson, D.R., Burnham, K.P., Laake, J.L., Borchers, D.L., Thomas, L. (2004): Advanced Distance Sampling. Oxford University Press. UK.

Gold, R., Wiethölter, H., Rihs, I., Löwer, J. & Kappos, L. (1992): Frühsommer-Meningoencephalitis-Impfung. *Deutsche Medizinische Wochenschrift* 117: 112-116.

Hosmer, D.W. & Lemeshow, S 2000. Applied logistic regressions. 2nd edition. John Wiley & Sons, New York – Chichester – Brisbane – Toronto – Singapore.

http://www.deutschland.de/images/aeb/karte de.gif

http://www.zecken.de

http://www.ruwpa.st-and.ac.uk/distance/

Infektionsschutzgesetz (2000): Gesetz zur Verhütung und Bekämpfung von Infektionskrankheiten beim Menschen. http://www.gesetze-im-internet.de/bundesrecht/ifsg/gesamt.pdf. Accessed on 06/11/2007.

Krüger, F. (2002): Zur zeitlichen Prognose, räumliche Verteilung und Heilungsdynamik von durch Wühlmäuse (Erdmaus, *Microtus agrestis* L., Rötelmaus, *Clethrionomys glareolus* Schreb. und Feldmaus, *Microtus arvalis* Pallas) verursachten Nageschäden an Forstpflanzen. PhD thesis. University Göttingen, Germany.

Mills, J.N., Yates, T.L., Ksiazek, T.G., Peters, C.J. & Childs, J.E. (1999): Long-Term studies of Hantavirus Reservoir Populations in the Southwestern United States: Rationale, Potential, and Methods. *Emerging Infectious Diseases* 5 (1), 95-101.

Mysterud, A. & Østbye E. 2006. Comparing simple methods for ageing roe deer *Capreolus capreolus*: are any of them useful for management? *Wildlife Biology* 12, 101-107.

Ostfeld, R.S., Canham, C.D., Oggenfuss, K., Winchcombe, R.J. & Keesing, F. (2006): Climate, Deer, Rodents and Acorns as Determinants of variation in lyme-disease risk. *PloS Biology* 4 (6), 1058-1068.

Pöllabauer, E.M.,Löw-Baselli, A., Pavlova, B.G., Mai, I., Peschel, N., Fritsch, S., Maritsch, F., Behre, U., Konior, R., Dorner, F., Barrett, P.N. & Ehrlich, H.J. (2003): FSME-Immun Junior: Immunogenität und Verträglichkeit in klinischen Studien und in der Praxis. *Ellipse* 19 (2): 37-38.

Pöllabauer, E.M., Fritsch, Dvorak, T., Loew-Baselli, A., Pavlova, B.G., Cil, I., Maritsch, F. & Ehrlich, H.J. (2007): Seropersistenz von Frühsommer-Meningoenzephalitis (FSME)-Antikörpern und Immunantwort nach FSME-Impfung bei Kindern und Jugendlichen. *Ellipse* 23 (1): 7-8.

Robert Koch-Institut (2007a): SurvStat, http://www3.rki.de/SurvStat. Accessed on 06/11/2007.

Robert Koch-Institute (2007b): FSME: Risikogebiete in Deutschland. *Epidemologisches Bulletin* 15:129-135.

Thomas, L., Laake, J.L., Strindberg, S., Marques, F.F.C., Buckland, S.T., Borchers, D.L., Anderson, D.R., Burnham, K.P., Hedley, S.L., Pollard, J.H., Bishop, J.R.B. & Marques, T.A. (2006). Distance 5.0. Release 2. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK.

Ward, A.I., White P.C.L. & C. H. Critchley (2004) Roe deer *Capreolus capreolus* behaviour affects density estimates from distance sampling surveys. *Mammal Review* 34 (4), 315-319.

Chapter 4 – Tick burden on European roe deer (*Capreolus capreolus*)

Exp Appl Acarol
DOI 10.1007/s10493-010-9337-0

Tick burden on European roe deer (*Capreolus capreolus*)

Torsten Vor · Christian Kiffner · Peter Hagedorn · Matthias Niedrig · Ferdinand Rühe

Received: 27 August 2009 / Accepted: 9 January 2010

Abstract In our study we assessed the tick burden on roe deer (*Capreolus capreolus* L.) in relation to age, physical condition, sex, deer density and season. The main objective was to find predictive parameters for tick burden. In September 2007, May, July, and September 2008, and in May and July 2009 we collected ticks on 142 culled roe deer from nine forest departments in Southern Hesse, Germany. To correlate tick burden and deer density we estimated deer density using line transect sampling that accounts for different detectability in March 2008 and 2009, respectively. We collected more than 8,600 ticks from roe deer heads and necks, 92.6% of which were *Ixodes* spp., 7.4% *Dermacentor* spp. Among *Ixodes*, 3.3% were larvae, 50.5% nymphs, 34.8% females and 11.4% males, with significant seasonal deviation. Total tick infestation was high, with considerable individual variation (from 0 to 270 ticks/deer). Adult tick burden was positively correlated with roe deer body indices (body mass, age, hind foot length). Significantly more nymphs were found on deer from forest departments with high roe deer density indices, indicating a positive correlation with deer abundance. Overall, tick burden was highly variable. Seasonality and large scale spatial characteristics appeared to be the most important factors affecting tick burden on roe deer.

Keywords *Ixodes* · *Dermacentor* · *Capreolus capreolus* · Vector borne diseases · Encephalitis · Lyme disease

Introduction

European roe deer (*Capreolus capreolus* L.) are very common all over Europe. Their distribution ranges from southern Spain to northern Scandinavia, to the Ural mountains in Russia and to scattered populations in Turkey, Israel and Jordan (Linnell et al. 1998). As generalist

T. Vor (✉) · C. Kiffner · F. Rühe
Wildlife Biology and Game Management Team, Department of Forest Zoology and Forest Conservation, Büsgen-Institute, Georg-August-University Göttingen, Göttingen, Germany
e-mail: tvor@gwdg.de

P. Hagedorn · M. Niedrig
Robert Koch-Institute, Centre for Biological Safety (ZBS 1), Berlin, Germany

herbivores, roe deer are able to feed on a wide variety of plants and thus live in several kinds of habitats. Many of these habitats are also occupied by certain tick species (mostly *Ixodes* spp. (Latreille, 1795) and *Dermacentor* spp. (Koch, 1844)) and roe deer are important hosts for ticks (Jensen et al. 2000; Walker et al. 2001; Rizzoli et al. 2007, Zeman and Pecha 2008). The ticks may profit from roe deer social behaviour and diurnal activities. Adult roe deer are territorial in spring and summer, offering a constant and reliable blood supply for tick development. High density roe deer populations are very common in Central Europe and are becoming more and more common in other parts of Europe (Andersen et al. 1998). Territorial behaviour includes chasing away subadult or subdominant individuals, providing the chance for ticks to be distributed quickly and effectively over long distances. Roe deer are known to migrate more than 100 km (Linnell et al. 1998), distances up to a few kilometres are usual for large parts of continental Europe. The preference of roe deer for dense vegetation and their diurnal rhythm of feeding and resting phases make them to easily accessible hosts for questing ticks. Roe deer have also learned to deal with and in many cases to profit from humans and their activities. It is one of the most important hunting game in Europe. Roe deer, along with other mammals (i.e. small rodens and dogs, Silaghi et al. 2008), are therefore important vectors for ticks and human tick borne diseases (TBDs). At a large spatial scale, a positive relationship between Lyme disease incidence in humans and roe deer density has been shown (Linard et al. 2007). Tick borne encephalitis (TBE) incidence in humans is also statistically associated with roe deer density (Rizzoli et al. 2009). Data on the role of deer on the transmission of TBDs are equivocal, however. For some of the tick-borne pathogens (for a review see Jongejan and Uilenberg 2004) roe deer are competent hosts, and for others roe deer might provide a cofeeding platform (Randolph et al. 1996; Kimura et al. 1995; Bruno et al. 2000). Although there is evidence for a correlation between deer and tick densities (Carpi et al. 2008), there is surprisingly little information on the tick burden of roe deer in general and its variation according to individual deer characteristics. Knowing the links might contribute to understand and to quantify the risk of tick borne diseases. It is also unknown how many ticks can be supported by roe deer. In general there is a lack of data on the effects of tick-borne diseases on roe deer. Some researchers state that roe deer are not susceptible to TBDs like tick borne encephalitis (TBE, Labuda et al. 2002; Hartemink et al. 2008; Rizzoli et al. 2009) or Lyme disease (Hartemink et al. 2008; Pugliese and Rosà 2008). Malandrin et al. (in press) could recently identify *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer blood and could clearly separate this *Babesia* species from others. *Babesia capreoli* can be fatal for roe deer, but does not pose a threat to either humans or livestock.

In this paper we aimed at testing the following hypotheses for each development stage/ sex of *Ixodes* and *Dermacentor* ticks and for the combined *Ixodes* and *Dermacentor* tick burden:

1. tick burden underlies seasonal variation
2. tick burden is influenced by individual characteristics of roe deer (sex, age, body mass, hind foot length)
3. tick burden reflects roe deer density

Materials and methods

Study area

Ticks from roe deer were collected in nine different forest departments located in three different forest districts in South Hesse, Germany (Fig. 1). Site characteristics are

Fig. 1 Location of the study sites in Southern Hesse, Germany

Table 1 Characteristics of the study sites

	FD Dieburg	FD Lampertheim	FD Beerfelden
Altitude a.s.l. (m)	125–300	95–110	360–470
Mean annual temperature (°C)	9.4	9.5	7.6
Mean annual precipitation (mm)	715	695	1,136

FD forest district; climate data as averages from 1961–1990, provided by the Deutscher Wetterdienst (www.dwd.de)

summarised in Table 1. The forests are located within high risk areas for TBE (Robert Koch-Institute 2007). Mean size of the study sites is 1150 ha (range: 520–1,710 ha). In all nine forest departments, roe deer are abundant. Annual hunting bags ranged in the hunting season 2007/2008 between 23 and 60 roe deer per forest department (3.5–6.7/100 ha). Roe deer density indices computed after distance sampling in early spring at each of the study sites (see "Estimating roe deer density indices") ranged from 2.4–9.1 roe deer /100 ha. Even within forest districts roe deer densities are quite variable, reflecting different habitat types, hunting regimes and possibly interspecific competition with other ungulate species. In three forest departments in the forest district Beerfelden, red deer (*Cervus elaphus* L.) with a hunting bag of 1.4–2.8 heads/100 ha in 2007/2008 is also common.

Tick sampling and assessment of roe deer data

In September 2007 ($n = 23$), May 2008 ($n = 47$), July 2008 ($n = 9$), September 2008 ($n = 23$), May 2009 ($n = 37$) and July 2009 ($n = 3$) we sampled during 10 days each period ticks from 142 (= n_{total}) hunter killed roe deer. Deer were stored in six different central cold storages of the forest districts which were maintained by the research team

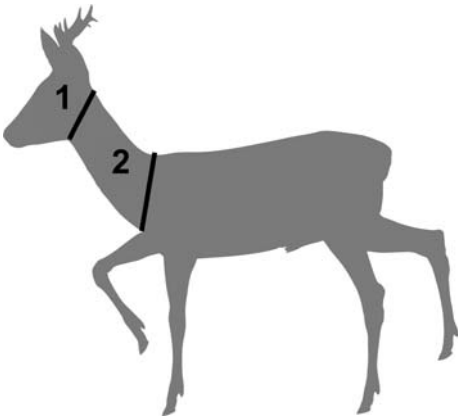


Fig. 2 Screened parts of roe deer (1 = head, 2 = neck). Drawing: W. Tambour

once a day. For 20 deer, the heads or the carcasses were removed by the hunters prior to investigation. They could only partly be screened (either head or neck). Two observers intensively investigated 138 necks and 126 heads from a total of 142 roe deer for tick infestation for a maximum time of 30 min each (Fig. 2). A preliminary study showed that roe deer from deciduous and coniferous forests in the region were mostly infested with ticks on their heads and necks. Ticks were removed with tweezers and tick-hooks and were collected in sterile tubes, separated by host individual, tick development stage and sex of adult ticks, and then stored at −80°C. As we collected thousands of ticks and some tick species are very similar, we separated them in the field at the genus level between *Ixodes* spp. and *Dermacentor* spp. ticks. Keys for identification in the lab were used to confirm the field separation. All randomly chosen and identified ticks were either *Ixodes ricinus* (L.) or *Dermacentor reticulatus* (Fabricius).

We estimated the age of roe deer by tooth wear (Mysterud and Østbye 2006), weighed them, and measured their hind foot length (HFL, Zannèsse et al. 2006). Furthermore, we assessed obvious health problems or physical damage through visual examination of the whole carcasses and by remarks from the hunters.

Estimating roe deer density indices

We estimated relative densities of roe deer using line transect methodology (Buckland et al. 2001) and subsequent analyses with the software package Distance 5 Release 2 (Thomas et al. 2006). In early March 2008 and 2009, we drove a fixed circuit in each forest area (mean ± SD: 18.3 ± 3.3 km); each circuit was driven twice on consecutive nights. We conducted each count with three persons: one person driving slowly ($\sim$6–12 km h^{-1}) and observing animals on the transect line and two persons sitting on the top of the vehicle scanning both sides of the transect line with handheld spotlights (12 V, 55 W). In order to model detection functions, we estimated the perpendicular distance between the initial position of the deer and the transect using the cosine function (Buckland et al. 2001). We measured sighting distances with a laser rangefinder and sighting angles with a compass.

Acknowledging that this approach violates some of the distance sampling assumptions [i.e. transects are not distributed randomly, perfect detection on the line not given due to evasive behaviour of roe deer (Ward et al. 2004) or due to avoidance of roads by roe deer], we consider our estimates not as absolute density but as indices which allow comparisons

of roe deer densities among different forest areas and years. Because the number of roe deer sightings/forest area/year was low (mean: 15.8 ± 6.4 SD), we pooled roe deer sightings according to the predominant terrain ('hilly' vs. 'flat') of the forest area. Based on AIC-values, these pooled detection functions performed better than forest area specific detection functions. We discarded the largest 5% of the distances and used half-normal key function with cosine series expansion to fit the detection functions. Using these stratum-specific detection functions and the size-bias regression method to estimate cluster size, we estimated area and year specific roe deer densities.

Predictive models for tick burden on roe deer

In order to explain the variation of ticks, we applied generalised linear models (GLM, Dobson and Barnett 2008) in SPSS (Version 17.0). Each model was fitted using a negative binomial error distribution (Shaw et al. 1998; Carpi et al. 2008) of the response variables 'total *Ixodes* larvae', 'total *Ixodes* nymphs', 'total *Ixodes* females', 'total *Ixodes* males', 'total *Ixodes*' and 'total *Dermacentor*'. We did not subdivide *Dermacentor* data due to rare occurrence. We tested effects of the study sites (forest department), sampling month, roe deer density, sex, age (in months), hind foot length (cm) and disembowelled body mass (kg). Univariate relationships between two variables were further tested by Kendall's Tau. Differences between variable values were tested by using the Mann–Whitney *U*-test.

Results

Overall tick burden

In total we collected 8,611 ticks from roe deer. Tick numbers ranged from 0 to 270 ticks per deer (head and neck only, Table 2), with an average of 65 ticks. 92.6% belonged to the *Ixodes* genus, 7.4% to the *Dermacentor* genus. We found all tick stages (Fig. 3a, b). Most of the ticks were nymphs (50.5%), followed by females (34.8%) males (11.4%), and larvae (3.3%). Most of the attached (feeding or questing) ticks were found on the roe deer's heads (61%).

Individual variation

Adult tick burden was positively correlated with roe deer body indices such as body mass, age and hind foot length, with significantly more adult ticks on older and heavier animals with higher hind foot length (Table 3). Overall, investigated male roe deer carried more ticks than female roe deer. This result was, however, biased by the different hunting seasons of male (from May to October) and female roe deer (in May, only yearlings, and from September to January). In September, when both males and females were hunted, there were no significant differences in tick burden (38 vs. 31 ticks/deer, on average; $P < 0.05$, Mann–Whitney *U*-Test).

Spatial and seasonal variation

The variation between total tick infestations was not significant on the forest district level with 59–85 ticks/deer, on average. However, total larvae burden on roe deer was

Table 2 Tick numbers on roe deer

	N	Minimum	Maximum	Mean	Standard error
Ixodes larvae head	126	0	54	1.3	0.5
Ixodes nymphs head	126	0	200	30.4	2.8
Ixodes females head	126	0	27	5.0	0.5
Ixodes males head	126	0	9	1.7	0.2
Total *Ixodes* head	126	0	229	38.5	3.1
Dermacentor larvae head	126	0	19	0.2	0.2
Dermacentor nymphs head	126	0	46	1.3	0.5
Dermacentor females head	126	0	27	0.6	0.3
Dermacentor males head	126	0	24	0.5	0.2
Total *Dermacentor* head	126	0	72	3.4	1.0
Total tick burden head	126	0	232	41.9	3.4
Ixodes larvae neck	137	0	35	0.7	0.3
Ixodes nymphs neck	138	0	48	1.4	0.4
Ixodes females neck	138	0	146	15.5	1.6
Ixodes males neck	138	0	51	5.0	0.6
Total *Ixodes* neck	138	0	184	22.6	2.3
Dermacentor larvae neck	138	0	4	0.1	0.0
Dermacentor nymphs neck	138	0	8	0.1	0.1
Dermacentor females neck	138	0	19	0.4	0.2
Dermacentor males neck	138	0	13	0.3	0.1
Total *Dermacentor* neck	138	0	33	1.5	0.4
Total tick burden neck	138	0	184	24.1	2.4
Total *Ixodes* larvae	122	0	78	2.2	0.8
Total *Ixodes* nymphs	122	0	201	31.9	2.9
Total *Ixodes* females	122	0	149	20.9	1.9
Total *Ixodes* males	122	0	58	7.1	0.8
Total *Dermacentor* larvae	122	0	19	0.3	0.2
Total *Dermacentor* nymphs	122	0	46	1.5	0.5
Total *Dermacentor* females	122	0	35	0.9	0.4
Total *Dermacentor* males	122	0	25	0.7	0.3
Ixodes total	122	0	265	62.1	4.3
Dermacentor total	122	0	82	3.4	1.1
Ticks total	122	0	270	65.4	4.6

N indicates number of investigated roe deer

significantly ($P < 0.05$, Kendall's Tau) higher in Dieburg than in Beerfelden and Lampertheim (Table 3). Although the factor 'Forest Department' had no direct effect on combined tick burden, significantly more nymphs were found on deer from forest departments with high roe deer density indices (RDI, Table 3), reflecting a positive correlation with deer abundance.

Roe deer were highly infested with ticks in May, whereas tick burden was lowest in September (Table 4). This was obvious for the total tick burden and the *Ixodes* genus. However, *Dermacentor* infestation was highest in July.

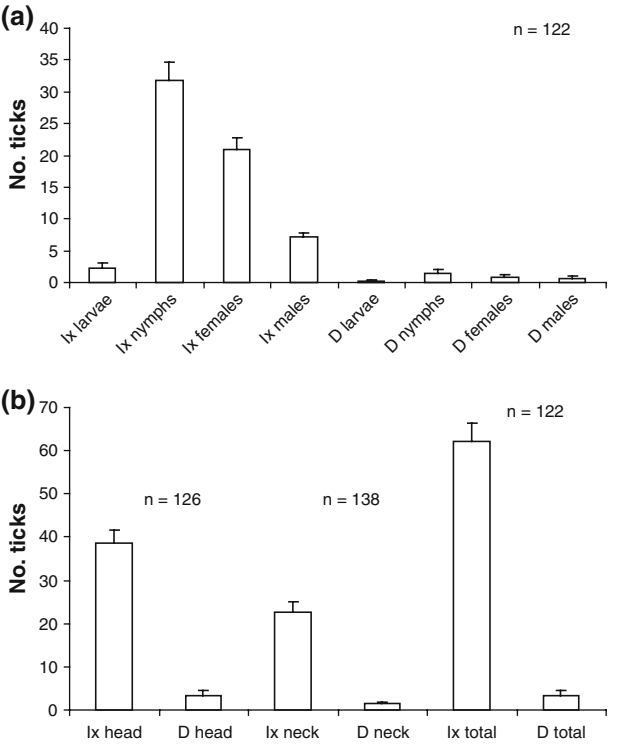


Fig. 3 Numbers of *Ixodes* (Ix) and *Dermacentor* (D) larvae, nymphs, females and males (**a**), and total *Ixodes* and *Dermacentor* numbers on roe deer heads vs. necks (**b**). Figures are given as means + standard errors; n = numbers of investigated roe deer

Table 3 Unifactorial relationships of habitat and individual characteristics to roe deer tick burden

	District[1]	Departm.[2]	Month[3]	Age[4]	Sex[5]	Body mass[6]	HFL[7]	RDI[8]
Ix. larvae	*	NS	NS	NS	NS	NS	NS	NS
Ix. nymphs	NS	NS	**	NS	NS	NS	NS	(+0.239)**
Ix. male	NS	NS	***	(+0.241)***	***	(+0.375)***	(+0.257)***	NS
Ix. female	NS	NS	***	(+0.167)*	***	(+0.340)***	(+0.293)***	NS
Ix. total	NS	NS	NS	NS	***	(+0.132)	NS	NS
D. larvae	NS	NS	NS	NS	NS	NS	NS	NS
D. nymphs	NS	NS	NS	NS	*	NS	NS	NS
D. male	NS	NS	NS	NS	*	NS	NS	NS
D. female	NS	NS	NS	NS	*	NS	NS	NS
D. total	NS	NS	*	NS	**	NS	*	NS

Ix., *Ixodes*, D., *Dermacentor,* NS, not significant ($P \geq 0.05$, Mann–Whitney U-Test for [1,2,3,5]; Kendall's Tau for [4,6,7,8]); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; correlation coefficients in parentheses

[1] Forest district, $n = 3$; [2] forest department, $n = 9$; [3] month of deer kill; [4] estimated age; [5] significant differences indicate higher values for male roe deer; [6] disembowelled body mass; [7] hind foot length; [8] roe deer density index

Table 4 Seasonal variation of roe deer tick burden

Month		All ticks	*Ixodes*	*Dermacentor*
May ($N_{\text{roe deer}} = 69$)	Mean tick numbers	84.9[a]	81.1[a]	3.8[b]
	Standard deviation	52.5	50.7	12.4
July ($N_{\text{roe deer}} = 11$)	Mean tick numbers	65.4[ab]	52.3[b]	13.1[a]
	Standard deviation	62.3	41.1	23.7
September ($N_{\text{roe deer}} = 42$)	Mean tick numbers	33.5[b]	33.4[c]	0.0[c]
	Standard deviation	22.3	22.3	0.2

In month mean tick numbers denoted with different letters are significantly different ($P < 0.05$, Mann–Whitney *U*-Test)

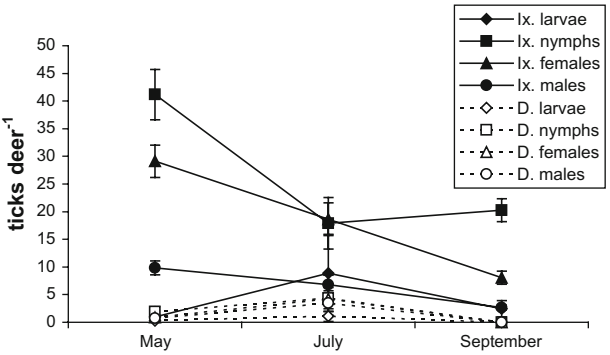


Fig. 4 Seasonal variation of tick burden on roe deer. Data are given as means with standard error, $n_{\text{May}} = 69$, $n_{\text{July}} = 11$, $n_{\text{September}} = 42$

In May and September, most of the *Ixodes* ticks were nymphs, whereas in July the number of female *Ixodes* was slightly higher (Fig. 4). The number of male *Ixodes* was consistently about 1/3 of the number of females. *Ixodes* larvae burden peaked in July, as well as for all *Dermacentor* stages.

Predictive modelling

Computing single factor correlations (see "Spatial and seasonal variation") does not account for ecological interaction of factors and is thus not helpful for general prediction of tick burden on roe deer. We therefore use generalised linear models (GLM) with the response variables '*Ixodes* larvae', '*Ixodes* nymphs', '*Ixodes* males', '*Ixodes* females', '*Ixodes* total', '*Dermacentor* total' and 'ticks total', the categorical variables 'forest district', 'month', 'roe deer sex' and the covariates 'age', 'hind foot length (HFL)', 'body mass' and 'roe deer density index (RDI)'(Table 5).

Larval *Ixodes* tick burden varied significantly on the forest district level and by season. Roe deer age was negatively correlated with larval burden. *Ixodes* nymph burden showed a significant seasonality and was positively correlated with roe deer density indices. Adult *Ixodes* burden was positively correlated with roe deer body mass; female burden showed significant seasonality, however, male burden did not. Overall, *Ixodes* burden was only significantly affected by season. Total *Dermacentor* burden showed significant variation at

Table 5 Generalized linear models for explaining the variation of tick burdens on roe deer for each *Ixodes* stage and sex, totalled *Ixodes*, totalled *Dermacentor* and totalled ticks using site, month and roe deer characteristics ($n = 122$) as potential factors

	Ix. larvae	Ix. nymphs	Ix. ad. male	Ix. ad. female	Ix. total	D. total	Ticks total
Model Sig.[1]	0.000*	0.003*	0.000*	0.001*	0.076	0.000*	0.068
Const. Term	0.078	0.232	0.630	0.522	0.094	0.856	0.123
District[2]	0.000*	0.591	0.059	0.205	0.251	0.000*	0.386
Month[3]	0.000*	0.004*	0.050	0.007*	0.005*	0.000*	0.005*
Sex[4]	0.887	0.508	0.478	0.683	0.479	0.110	0.582
Age[5]	0.001* (−0.048)	0.779	0.753	0.560	0.344	0.021* (−0.028)	0.244
HFL[6]	0.274	0.829	0.489	0.703	0.470	0.170	0.577
Body mass[7]	0.719	0.270	0.001* (+0.224)	0.010* (+0.159)	0.406	0.751	0.409
RDI[8]	0.590	0.049* (+0.203)	0.858	0.783	0.288	0.000* (−2.648)	0.345

Significant effects are denoted with asterisks. Regression coefficients are given in brackets, if applicable
Ix. ad., adult *Ixodes,* D., *Dermacentor*; [1] Model significance, Omnibus-test; [2] forest district [3] month of deer kill; [4] roe deer sex; [5] estimated roe deer age; [6] roe deer hind foot length; [7] mass of eviscerated roe deer body; [8] roe deer density index

the spatial (Forest District) and temporal (month) scale, and was negatively correlated with roe deer density indices and roe deer age.

The variables 'sex' and 'roe deer hind foot length' had no significant effect on tick burden.

Discussion

The general tick burden of roe deer in the study area was high. Overall, tick burden on roe deer appears to be highly variable with seasonality being the major factor explaining the variation of tick burden. Considering that we only sampled the deer's heads and necks, the reported tick abundance per roe deer reflects ca. 60% of the total burden. In another study we found the deer's head and neck to account for 47.28 % (SE ± 3.55) and 13.29% (±1.74) of the total Ixodes burden respectively (Kiffner et al. in press).

Modelling tick burden mainly revealed seasonal and spatial variation. It appears that individual host characteristics do not have the expected high effects on tick burden. However, *Ixodes* larvae and total *Dermacentor* numbers were negatively correlated with deer age, indicating a preference for younger hosts. We hypothesize that this is caused by behavioural differences (younger deer have longer resting phases, especially as fawns) and by the thinner skin of younger animals. Adult tick burden is positively correlated with the roe deer body mass, being in accordance with the results of the parasite-host metaanalysis done by Poulin and George-Nascimento (2007). As the male tick burden is mostly triggered by the female burden, effects on both sexes are almost the same. The negative influence of the roe deer density index on total *Dermacentor* burden might be explained by spatial differences in *Dermacentor* distribution, coincidently overlapping with also highly significant forest district effects. The sex of roe deer does not have any significant effect on tick parasitism. Schalk and Forbes (1997) generally found small differences in parasitism

between the sexes of mammals. Schmidtmann et al. (1998) reported male biased tick parasitism (*Ixodes scapularis*) on white-tailed deer (*Odocoileus virginianus*). This deer species, however has a more pronounced sexual size dimorphism than roe deer (Geist 1998) and thus male-biased parasitism might actually be an artefact of sexual size dimorphism.

We did not observe any specific sign of deer health problems caused by high tick infestation. At least for Lyme borreliosis and TBE, roe deer are not competent hosts (Labuda et al. 2002; Hartemink et al. 2008; Pugliese and Rosà 2008; Rizzoli et al. 2009). However, roe deer *are* competent reservoirs for *Anaplasma phagocytophilum* causing a febrile disease (Silaghi et al. 2008), and for *Babesia capreoli* (Malandrin et al. in press).

Carpi et al. (2008) screened parts of the forelegs of roe deer from Northeastern Italy for tick infestation. They found very high tick numbers as well (up to 388 *Ixodes ricinus*/deer), although almost 90% were larvae. As we could also find all life stages of ticks (larvae, nymphs and adults) feeding on the same individual and sometimes aggregated very closely together (<1 cm), the chance of co-feeding (Randolph 2004) and TBE virus transmission from infected nymphs to larvae or even females to nymphs and larvae should be further considered. In spite of roe deer being a non-competent host for TBE, it is already being used as an ideal and easily available sentinel animal for TBE distribution using serological investigation (Gerth et al. 1995; Labuda et al. 2002; Carpi et al. 2008).

Walker et al. (2001) removed ticks from roe deer forelegs. Similar to our study, months with the highest larvae infestation were July and August (mean $\sim$58 larvae per roe deer leg) and most nymphs were collected in May (mean $\sim$16 nymphs per leg). The seasonal variation of tick densities in general and of tick development stages questing or feeding on roe deer followed the marked seasonality in tick population dynamics including diapauses and in environmental conditions in temperate zones (MacLeod 1939; Lees and Milne 1951; Gray 1971; Kalsbeek and Frandsen 1996). In our study *Ixodes* nymph, female and male numbers on roe deer peaked in May, whereas most larvae were counted in July. This corresponds well with data presented by Randolph (2004) for *Ixodes ricinus*, although she could show a very high interannual variability as well.

As far as we know, only one further publication reports on roe deer infestation with *Dermacentor* ticks (Dautel et al. 2006). In their study *D. reticulatus* was found on 23 deer out of 721 deer from all over Germany. Most of the infested animals were red deer (*Cervus elaphus*) and only a few roe deer. Their results showed that *D. reticulatus* is much more common in Germany than previously known. In Hungary this tick species is also expanding its geographic range, and *D. reticulatus*-borne diseases (e.g. tularemia and tick-borne lymphadenopathy) are a concern in the region (Sréter et al. 2005). *Dermacentor reticulatus* is better known as a vector for the pathogens *Babesia canis* and *Ehrlichia canis*, causing diseases mainly in dogs (Ogden et al. 2000). They can also be vector for *Coxiella burnetii* causing Q fever in domestic animals and even in humans (Movila et al. 2006), and for *Rickettsia* (Dautel et al. 2006). *Borrelia* have been found in *D. reticulatus* (Kahl et al. 1992), but this tick species is apparently not an effective vector for *Borrelia* (Jongejan and Uilenberg 2004). In our study roe deer were most infested with *Dermacentor* ticks in July. The high risk potential for several diseases and their activity in mid-summer when people frequently enter the forests for recreation make further studies on the ecology of this tick species and the role of wildlife for its population dynamics indispensable.

The question whether roe deer abundance enhances tick abundance and the subsequent risk for tick borne diseases can not be satisfactorily answered. The fact that we found a significant positive correlation between *Ixodes* nymph numbers on roe deer and roe deer density indices does not prove to be an obligate relationship, since other factors (e.g. small

rodent densities as more important hosts for larvae, site conditions or habitat structures) could be more important. The same factors could also explain the increasing TBE risk in recent years which correlate with increasing roe deer densities in the Italian Alps (Rizzoli et al. 2009), as both could have been made possible by other factors and the following distribution of both ticks and roe deer in higher altitudes. Carpi et al. (2008) concluded that tick infestation of roe deer is very site specific, but not necessarily dependent on roe deer densities. This might even explain the negative correlation of roe deer density indices and *Dermacentor* numbers on roe deer in our study. An exclusively positive influence of roe deer densities on ticks has rarely been shown. Jensen et al. (2000) found a positive correlation of *Ixodes* nymph density and roe deer abundance. Ostfeld et al. (2006) could only confirm a weak effect of white tailed deer (*Odocoileus virginianus*) abundance on *Ixodes scapularis* larvae and state that there is a clear decoupling of stage specific abundances. Walker et al. (2001) even found negative correlations between densities of roe deer and nymphs. Gray et al. (1992) showed that the availability of deer as hosts has a major impact on tick densities, but even small numbers of deer can maintain very large tick populations (Wilson et al. 1984; Robertson et al. 2000). A white-tailed deer reduction by almost 50% had no apparent effect on questing *Ixodes scapularis* numbers (Jordan et al. 2007).

All roe deer of our study were culled in forested areas. However, home ranges of roe deer are relatively large (10—more than 200 ha (Lovari and San José 1997; Mysterud 1999)) and usually cover more than one habitat type under Central European conditions, thus impeding the identification of habitat specific effects on roe deer tick burden with our dataset.

To better predict tick densities we suggest including more specific habitat characteristics such as soil moisture and grinding vegetation cover.

Acknowledgments This study is funded by the German Federal Ministry of Education and Research (BMBF) within the "Research on Zoonotic Infectious Diseases" programme, "Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance" (grant no. 1363120) and greatly supported by the State Forest Administration of Hesse. We are also grateful to S. Bauling, K. Ehlmann, M. Ksinsik, A., C. and L. Lödige, A.-L. Schäfer, E. Rühe and M. Scholz for their help during field work, and Kenneth Elgersma for editing the English.

References

Andersen R, Duncan P, Linnell JDC (1998) The European roe deer: the biology of success. Scandinavian University Press, Oslo

Bruno P, Bruno G, Peréz-Eid C (2000) Detection of spirochaetes of *Borrelia burgdorferi* complexe in the skin of cervids by PCR and culture. Eur J Epidemiol 16:869–873

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001) Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press, Oxford, UK

Carpi G, Cagnacci F, Neteler M, Rizzoli A (2008) Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. Epidemiol Infect 136:1416–1424

Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E (2006) Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. Int J Med Microbiol 296(Suppl. 1):149–156

Dobson AJ, Barnett AG (2008) An introduction to generalized linear models, 3rd edn. Chapman & Hall/CRC, Boca Raton
Geist V (1998) Deer of the world: their evolution, behavior, and ecology, stackpole books. Mechanicsbarg, USA, p 421
Gerth HJ, Grimshandl D, Stage B, Döller G, Kunz C (1995) Roe deer as sentinels for endemicity of tick-borne encephalitis virus. Epidemiol Infect 115:355–365
Gray JS (1971) The development and seasonal activity of the tick *Ixodes ricinus*: a vector of *Lyme Borreliosis*. Rev Med Vet Ent 79:323–333
Gray JS, Kahl O, Janetzki C, Stein J (1992) Studies on the ecology of Lyme disease in a deer forest in County Galway, Ireland. J Med Entomol 29:915–920
Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: Defining *R*0 for tick-borne infections. Am Nat 171:743–754
Jensen PM, Hansen H, Frandsen F (2000) Spatial risk assessment for *Lyme borreliosis* in Denmark. Scan J Infect Dis 32:545–550
Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3–S14
Jordan RA, Schulze TL, Jahn MB (2007) Effects of reduced deer density on the abundance of *Ixodes scapularis* (Acari: Ixodidae) and Lyme disease incidence in a northern New Jersey endemic area. J Med Entomol 44:752–757
Kahl O, Janetzki C, Gray JS, Stein J, Bauch RJ (1992) Tick infection rates with *Borrelia*: *Ixodes ricinus* versus *Haemaphysalis concinna* and *Dermacentor reticulates* in two locations in eastern Germany. Med Vet Entomol 6:363–366
Kalsbeek V, Frandsen F (1996) The seasonal activity of *Ixodes ricinus* ticks in Denmark. Anzeiger für Schädlingskunde (J Pest Sci) 69:160–161
Kiffner C, Lödige C, Alings M, et al (in press) Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). Exp Appl Acarol
Kimura K, Isogai E, Isogai H et al (1995) Detection of Lyme disease spirochetes in the skin of naturally infected wild sika deer (Cervus nippon yesoensis). Appl Environ Microbiol 61:1641–1642
Koch-Institute Robert (2007) FSME: risikogebiete in Deutschland. Epidemiol Bull 15:129–135
Labuda M, Elecková E, Licková M, Sabó A (2002) Tick-borne encephalitis virus foci in Slovakia. Int J Med Microbiol 291:43–47
Lees AD, Milne A (1951) The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L.). Parasitology 41:189–208
Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V et al (2007) Determinants of the geographic distribution of Puumula virus and *Lyme borreliosis* infections in Belgium. Int J Health Geo 6:15. doi: 10.1186/1476-072x-6-15
Linnell JDC, Wahlström K, Gaillard JM (1998) From birth to independence: birth, growth, neonatal mortality, hiding behaviour and dispersal. In: Andersen R et al (eds) The European roe deer: the biology of success. Scandinavian University Press, Oslo, pp 257–284
Lovari S, San José C (1997) Wood dispersion affects home range size of female roe deer. Behav Proc 40:239–241
MacLeod J (1939) The seasonal and annual incidence of the sheep Tick, *Ixodes ricinus*, in Britain. Bull Entomol Res 30:103–118
Malandrin L, Jouglin M, Sun Y, Brisseau N, Chauvin A (in press) Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. Int J Parasitol. doi: 10.1016/j.ijpara.2009.08.008
Movila A, Uspenskaia I, Toderas I, Melnic V, Conovalov J (2006) Prevalence of *Borrelia burgdorferi sensu lato* and *Coxiella burnetii* in ticks collected in different biocenoses in the Republic of Moldova. Int J Med Microbiol 296(Suppl. 1):172–176
Mysterud A (1999) Seasonal migration pattern and home range of roe deer (*Capreolus capreolus*) in an altitudinal gradient in southern Norway. J Zool 247:479–486
Mysterud A, Østbye E (2006) Comparing simple methods for ageing roe deer *Capreolus capreolus*: are any of them useful for management? Wildl Biol 12:101–107
Ogden NH, Cripps P, Davison CC, Owen G, Parry JM, Timms BJ, Forbes AB (2000) The ixodid tick species attaching to domestic dogs and cats in Great Britain and Ireland. Med Vet Entomol 14:332–338
Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F (2006) Climate, deer, rodents and acorns as determinants of variation in lyme-disease risk. PloS Biol 4:1058–1068
Poulin R, George-Nascimento M (2007) The scaling of total parasite biomass with host body mass. Int J Parasitol 37:359–364

Pugliese A, Rosà R (2008) Effect of host populations on the intensity of ticks and the prevalence of tick-borne pathogens: how to interpret the results of deer exclosure experiments. Parasitology 135:1531–1544

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129:37–65

Randolph SE, Gern L, Nuttall PA (1996) Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parastitol Today 12:472–479

Rizzoli A, Neteler M, Rosà R, Versini W, Cristofolini A, Bregoli M, Buckley A, Gould EA (2007) Early detection of tick-borne encephalitis virus spatial distribution and activity in the province of Trento, northern Italy. Geospatial Health 2:169–176

Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R (2009) Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. PLoS ONE 4:e4336. doi:10.1371/journal.pone.0004336

Robertson JN, Gray JS, Stewart P (2000) Tick bite and *Lyme borreliosis* risk at a recreational site in England. Eur J Epidemiol 16:647–652

Schalk G, Forbes MR (1997) Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon. Oikos 78:67–74

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Silaghi C, Gilles J, Höhle M, Fingerle V, Just FT, Pfister K (2008) *Anaplasma phagocytophilum* infection in *Ixodes ricinus*, Bavaria, Germany. Em Inf Dis 14:972–974

Sréter T, Széll Z, Varga I (2005) Spatial distribution of *Dermacentor reticulatus* and *Ixodes ricinus* in Hungary: evidence for change? Vet Parasitol 128:347–351

Thomas L, Laake JL, Strindberg S, Marques FFC, Buckland ST, Borchers DL, Anderson DR, Burnham KP, Hedley SL, Pollard JH, Bishop JRB, Marques TA (2006) Distance 5.0. Release 2. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK. http://www.ruwpa.st-and.ac.uk/distance/

Walker AR, Alberdi MP, Urquhart KH, Rose H (2001) Risk factors in habitats of the tick *Ixodes ricinus* influencing human exposure to *Ehrlichia* phagocytophila bacteria. Med Vet Entomol 15:40–49

Ward AI, White PCL, Critchley CH (2004) Roe deer *Capreolus capreolus* behaviour affects density estimates from distance sampling surveys. Mam Rev 34:315–319

Wilson ML, Levine JF, Spielman A (1984) Effect of deer reduction on abundance of the deer tick (*Ixodes dammini*). Yale J Biol Med 57:697–705

Zannèsse A, Baïsse A, Gaillard J-M, Hewison AJM, Saint-Hillaire K, Toïgo C, van Laere G, Morellet N (2006) Hind foot length: an indicator for monitoring roe deer populations at a landscape scale. Wildl Biol Bull 34:351–358

Zeman P, Pecha M (2008) Segregation of genetic variants of *Anaplasma phagocytophilum* circulating among wild ruminants within a Bohemian forest (Czech Republic). Int J Med Microbiol 298(Suppl. 44):203–210

Chapter 5 – Abundance estimation of *Ixodes* (Acari: Ixodidae) ticks on roe deer (*Capreolus capreolus*)

Exp Appl Acarol
DOI 10.1007/s10493-010-9341-4

Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*)

Christian Kiffner · Christina Lödige · Matthias Alings · Torsten Vor · Ferdinand Rühe

Received: 23 November 2009 / Accepted: 30 January 2010

Abstract Despite the importance of roe deer as a host for *Ixodes* ticks in central Europe, estimates of total tick burden on roe deer are not available to date. We aimed at providing (1) estimates of life stage and sex specific (larvae, nymphs, males and females, hereafter referred to as tick life stages) total *Ixodes* burden and (2) equations which can be used to predict the total life stage burden by counting the life stage on a selected body area. Within a period of 1½ years, we conducted whole body counts of ticks from 80 hunter-killed roe deer originating from a beech dominated forest area in central Germany. Averaged over the entire study period (winter 2007–summer 2009), the mean tick burden per roe deer was 64.5 (SE ± 10.6). Nymphs were the most numerous tick life stage per roe deer (23.9 ± 3.2), followed by females (21.4 ± 3.5), larvae (10.8 ± 4.2) and males (8.4 ± 1.5). The individual tick burden was highly aggregated ($k = 0.46$); levels of aggregation were highest in larvae ($k = 0.08$), followed by males ($k = 0.40$), females ($k = 0.49$) and nymphs ($k = 0.71$). To predict total life stage specific burdens based on counts on selected body parts, we provide linear equations. For estimating larvae abundance on the entire roe deer, counts can be restricted to the front legs. Tick counts restricted to the head are sufficient to estimate total nymph burden and counts on the neck are appropriate for estimating adult ticks (females and males). In order to estimate the combined tick burden, tick counts on the head can be used for extrapolation. The presented linear models are highly significant and explain 84.1, 77.3, 90.5, 91.3, and 65.3% (adjusted R^2) of the observed variance,

C. Kiffner (✉) · C. Lödige · M. Alings · T. Vor · F. Rühe
Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany
e-mail: ckiffne@gwdg.de

C. Lödige
e-mail: christinaloedige@hotmail.com

M. Alings
e-mail: efrafa@web.de

T. Vor
e-mail: tvor@gwdg.de

F. Rühe
e-mail: fruehe@gwdg.de

Published online: 04 March 2010

respectively. Thus, these models offer a robust basis for rapid tick abundance assessment. This can be useful for studies aiming at estimating effects of abiotic and biotic factors on tick abundance, modelling tick population dynamics, modelling tick-borne pathogen transmission dynamics or assessing the efficacy of acaricides.

Keywords Ectoparasite · Negative binomial distribution

Introduction

Ticks of the *Ixodes ricinus* (L.) complex (hereafter *Ixodes* ticks) are vectors of numerous arthropod-borne pathogens of medical and veterinary importance. These include *Anaplasma phagocytophilum* (causative agent of granulocytic anaplasmosis in humans, tick-borne fever in sheep and canine ehrlichiosis in dogs), *Babesia divergens* (causative agent of babesiosis in humans and redwater fever in cattle), *Babesia venatorum* (causative agent of babesiosis in humans), *Rickettsia helvetica* (causative agent for diffuse symptoms in humans), *Borrelia burgdorferi* (causative agent of Lyme disease) and tick-borne encephalitis virus (causative virus of tick-borne encephalitis) (for a review see Jongejan and Uilenberg 2004).

Roe deer (*Capreolus capreolus*) are important host species for *Ixodes* ticks in central Europe and might be important for the population dynamics of ticks. However, the role of roe deer for pathogen dynamics is largely unknown (e.g. *Anaplasma phagocytophilum*). For most of the tick-borne pathogens roe deer are believed to be dead-end or dilution hosts (e.g. *Borrelia burgdorferi,* tick-borne encephalitis virus), but deer potentially provide a platform for non-systemic pathogen transmission among co-feeding ticks (Jaenson and Tälleklint 1992; Matuschka et al. 1993; Kimura et al. 1995; Randolph et al. 1996; Bruno et al. 2000; Schmid and Ostfeld 2001).

Despite the importance of roe deer as host for *Ixodes* ticks and hence for associated tick-borne pathogens in central Europe, few studies have investigated patterns of tick infestation on roe deer (Matuschka et al. 1993; Carpi et al. 2008). However, none of these studies aimed at estimating the total tick burden per roe deer individual, one of the most essential parameters for describing this host-parasite system. Studies on tick burdens on deer could be used for estimating effects of abiotic and/or biotic factors on tick densities (Carpi et al. 2008), or for assessing the efficacy of acaricide applications (Rand et al. 2000; Fish and Childs 2009; Pound et al. 2009). Furthermore, estimates of tick abundance on deer are useful for realistically parameterising models describing tick population dynamics and possibly tick-borne pathogen transmission dynamics (Randolph et al. 1996; Ogden et al. 1997; Randolph 2004; Hartemink et al. 2008).

Previous studies aiming at estimating tick burdens of domestic and/or wild mammals (Barnard and Morrison 1985; Bloemer et al. 1988; Barnard et al. 1989; Fourie and van Zyl 1991; Fourie et al. 1991; L'Hostis et al. 1994; Fourie and Kok 1995; Mathee et al. 1997; Ogden et al. 1998; Schmidtmann et al. 1998) used whole body counts on dead or live animals. Some of these studies provided simple linear models which can be used to predict total tick burdens based on tick counts on one or a few distinct host body parts (Barnard and Morrison 1985; Bloemer et al. 1988; Barnard et al. 1989; Fourie and van Zyl 1991; L'Hostis et al. 1994; Mathee et al. 1997). The term "density" of ticks which had been used by previous authors (Barnard and Morrison 1985; Bloemer et al. 1988; Barnard et al. 1989; Fourie and van Zyl 1991; L'Hostis et al. 1994; Mathee et al. 1997) described their estimates of tick abundance as density estimates. Here we use the terms tick "abundance" or

"burden" as density is clearly defined as individuals/unit area, i.e. ticks/cm^2 (Sutherland 1996; Borchers et al. 2002). Neither previous authors nor we can provide this measure without estimating the surface area of parts or the entire host species.

Here, we conducted total tick counts on hunter killed roe deer in order to provide (1) estimates of stage/sex specific total *Ixodes* burden, and (2) equations which can be used to predict the total life stage burden by counting the number of ticks per life stage/sex on a selected body area.

Materials and methods

We opportunistically sampled 91 hunter-killed roe deer in the forested region east and northeast of Göttingen (centred at, 51°32′2″N, 9°56′8″), central Germany during regular hunting activities over a period lasting from winter 2007 to summer 2009 (November–December 2007 $n = 20$ roe deer; May–June 2008 $n = 20$; July–August 2008, $n = 18$; November–December 2008 $n = 14$; May–June 2009 $n = 13$; July–August $n = 6$).

The study area is dominated by mixed deciduous forests. The predominant tree species are European beech (*Fagus sylvatica*), Norway maple (*Acer pseudoplatanus*), European ash (*Fraxinus excelsior*) and sessile oak (*Quercus petraea*). The sites are often characterized by shallow limestone plateaus with rendzina soils and haplic luvisols, rich in nutrient supply, but rather poor in water supply during dry periods in summer. Some forest stands however are located on sandstone with sandy and loamy cambisols and these soils are characterized by lower nutrient contents but higher water availability, and are often covered by pure Norway spruce (*Picea abies*) stands. Depending on nutrient, water and light availability the ground cover with tree seedlings and herbaceous species is heterogeneous. Tree diameter or age classes vary on a small scale, providing enough shelter and nutrition for roe deer throughout the whole study area. The altitude above sea level ranges from 151 to 400 m, the mean annual rainfall is 780 mm (370 mm in the growing season), and the average annual temperature is 7.8°C (Petritian et al. 2007).

Roe deer carcasses were disembowelled by the hunters and stored in cooling chambers at 2–8°C until examination. Within 16 h on average (SE: ± 2.5 h) after roe deer individuals had been shot, each carcass was examined by two observers wearing latex gloves. The carcass was divided into 6 distinct parts (head, neck, front legs, hind legs, sternum & abdomen and rest of the body, Fig. 1). The roe deer skin was systematically inspected and palpated to detect all ticks. Sites heavily infested were consecutively searched and palpated by both persons. All ticks were removed from each body part with forceps. These ticks were immediately counted and recorded according to life stage and sex (larvae, nymphs, males and females, hereafter referred to as tick life stages). Finally, they were transferred to sampling tubes and stored at −20°C. All removed ticks belong to the *Ixodes ricinus* complex; no *Dermacentor* spp. (Koch 1844) were identified during this study.

Statistical analysis

For each tick life stage and roe deer body part we calculated mean (μ) tick numbers in absolute and relative terms (Table 1) and the corresponding standard errors (±SE). Since patterns of macroparasite burdens on wildlife hosts usually follow the negative binomial distribution which is described by μ and the inverse measure of aggregation (k), we also calculated k for the absolute tick burdens (Shaw et al. 1998):

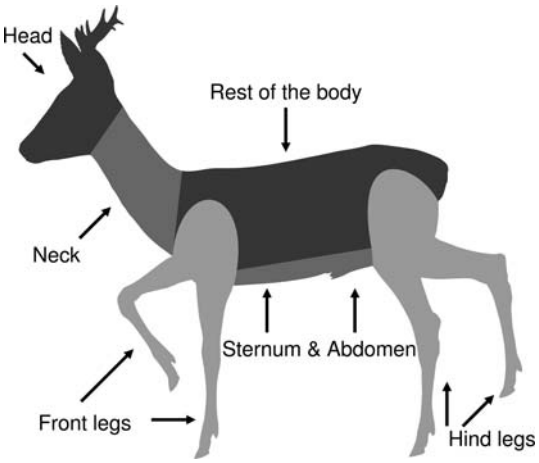


Fig. 1 Sketch outline of the tick collection sites on a roe deer (*Capreolus capreolus*) buck. Drawing: W. Tambour/J. Seelig

$$k = \frac{\mu^2}{s^2 - \mu}$$

where s^2 is the variance of the sample. Small values ($k < 1$) indicate a high level of aggregation, while k values > 5 indicate randomness (Crawley 2005).

In order to provide equations which can be used to estimate tick burdens based on counts of selected body parts, we pooled all tick counts and calculated correlations (Kendall's *T*) between tick life stage abundance on one body part and life stage abundance on other body parts and the total number of this life stage on the entire roe deer carcass (Table 2). For each tick life stage, we selected the body part correlating most strongly with the total number of this life stage on the entire roe deer and fitted a linear regression model:

$$y = a + b\,(x)$$

where y is the total number of the life stage on the entire roe deer, a the intercept, b the slope of the regression and x the number of the tick life stage on the selected body part. We used SPSS 17.0 (SPSS Inc.) and R 2.51 (R Development Core Team) for analysing the data.

Results

In total, we sampled ticks from 91 roe deer. Eleven heads of roe deer had been removed by hunters prior to investigation, resulting in 80 complete tick-roe deer data sets. In sum we collected 5,159 ticks of which 862 were larvae, 1,912 were nymphs, 1,714 were females and 671 were males. The mean tick burden per roe deer was 64.49 ($\pm$10.62), and the tick burden was highly aggregated ($k = 0.46$).

On average, nymphs were the most numerous tick life stage on roe deer (23.90 $\pm$ 3.21), followed by females (21.43 $\pm$ 3.47), larvae (10.78 $\pm$ 4.18) and males (8.39 $\pm$ 1.52). The range of life stage specific tick burden per roe deer was wide: 0–255 larvae, 0–167 nymphs, 0–157 females, 0–72 males and 0–582 ticks combined. Levels of aggregation were highest in larvae ($k = 0.08$), followed by males ($k = 0.40$), females ($k = 0.49$) and nymphs ($k = 0.71$).

Table 1 Mean number (±SE) and percentage (±SE) of ticks of each life stage/sex in each body zone and on the entire roe deer body

Life stage	Head	Neck	Rest of the body	Sternum & abdomen	Front legs	Hind legs	Entire body
Larvae	1.44 ± 0.51 ($n = 80$)	1.01 ± 0.41 ($n = 91$)	0.91 ± 0.34 ($n = 91$)	0.77 ± 0.29 ($n = 91$)	4.75 ± 2.4 ($n = 91$)	1.41 ± 0.73 ($n = 91$)	10.78 ± 4.18 ($n = 80$)
Larvae (%)	12.94 ± 3.17% ($n = 80$)	2.22 ± 0.71% ($n = 80$)	1.69 ± 0.53% ($n = 80$)	1.98 ± 0.75% ($n = 80$)	21.50 ± 3.94% ($n = 80$)	3.43 ± 1.29% ($n = 80$)	
Nymphs	16.86 ± 2.21 ($n = 80$)	1.51 ± 0.53 ($n = 91$)	0.36 ± 0.17 ($n = 91$)	0.58 ± 0.14 ($n = 91$)	4.32 ± 1.07 ($n = 91$)	1.13 ± 0.24 ($n = 91$)	23.9 ± 3.21 ($n = 80$)
Nymphs (%)	63.26 ± 4.24% ($n = 80$)	2.30 ± 0.55% ($n = 80$)	0.47 ± 0.20% ($n = 80$)	1.55 ± 0.43% ($n = 80$)	11.04 ± 2.08% ($n = 80$)	2.63 ± 0.52% ($n = 80$)	
Females	5.15 ± 0.92 ($n = 80$)	10.52 ± 1.89 ($n = 91$)	1.35 ± 0.37 ($n = 91$)	0.94 ± 0.22 ($n = 91$)	2.87 ± 0.47 ($n = 91$)	2.93 ± 0.39 ($n = 91$)	21.43 ± 3.47 ($n = 80$)
Females (%)	18.77 ± 2.71% ($n = 80$)	24.01 ± 2.91% ($n = 80$)	1.89 ± 0.45% ($n = 80$)	3.53 ± 0.92% ($n = 80$)	13.29 ± 2.33% ($n = 80$)	14.76 ± 2.46% ($n = 80$)	
Males	1.91 ± 0.34 ($n = 80$)	4.82 ± 0.91 ($n = 91$)	0.82 ± 0.25 ($n = 91$)	0.55 ± 0.2 ($n = 91$)	0.77 ± 0.15 ($n = 91$)	1.1 ± 0.2 ($n = 91$)	8.39 ± 1.52 ($n = 80$)
Males (%)	14.71 ± 2.57% ($n = 80$)	24.09 ± 3.35% ($n = 80$)	2.42 ± 0.63% ($n = 80$)	1.66 ± 0.47% ($n = 80$)	7.88 ± 2.29% ($n = 80$)	9.24 ± 2.37% ($n = 80$)	
Ticks	25.36 ± 3.23 ($n = 80$)	17.86 ± 3.19 ($n = 91$)	3.45 ± 0.87 ($n = 91$)	2.83 ± 0.63 ($n = 91$)	12.7 ± 3.63 ($n = 91$)	6.57 ± 1.21 ($n = 91$)	64.49 ± 10.62 ($n = 80$)
Ticks (%)	47.28 ± 3.55% ($n = 80$)	13.29 ± 1.74% ($n = 80$)	1.53 ± 0.30% ($n = 80$)	2.36 ± 0.45% ($n = 80$)	14.70 ± 2.09% ($n = 80$)	8.33 ± 1.57% ($n = 80$)	

The number (n) of investigated roe deer (parts) varies because heads of 11 roe deer were not available for investigation

Table 2 Correlations (Kendall's tau) between numbers of tick life stages/tick sex on one body part with tick numbers of the same life stage/sex on other body parts and on the entire roe deer body

		Head	Neck	Rest of the body	Sternum & abdomen	Front legs	Hind legs
Neck-Larvae	Kendall's tau	0.558					
	P-value	<0.001					
	n	80					
Rest of the body-Larvae	Kendall's tau	0.472	0.778				
	P-value	<0.001	<0.001				
	n	80	91				
Sternum & abdomen-Larvae	Kendall's tau	0.474	0.643	0.776			
	P-value	<0.001	<0.001	<0.001			
	n	80	91	91			
Front legs-Larvae	Kendall's tau	0.445	0.585	0.581	0.491		
	P-value	<0.001	<0.001	<0.001	<0.001		
	n	80	91	91	91		
Hind legs-Larvae	Kendall's tau	0.45	0.444	0.565	0.673	0.439	
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	
	n	80	91	91	91	91	
Entire body-Larvae	Kendall's tau	0.69	0.628	0.598	0.58	**0.817**	0.553
	P-value	<0.001	<0.001	<0.001	<0.001	**<0.001**	<0.001
	n	80	80	80	80	**80**	80
Neck-Nymphs	Kendall's tau	0.494					
	P-value	<0.001					
	n	80					
Rest of the body-Nymphs	Kendall's tau	0.23	0.306				
	P-value	0.014	0.002				
	n	80	91				
Sternum & abdomen-Nymphs	Kendall's tau	0.302	0.318	0.422			
	P-value	0.001	0.001	<0.001			
	n	80	91	91			
Front legs-Nymphs	Kendall's tau	0.456	0.39	0.276	0.347		
	P-value	<0.001	<0.001	0.003	<0.001		
	n	80	91	91	91		
Hind legs-Nymphs	Kendall's tau	0.408	0.441	0.272	0.391	0.527	
	P-value	<0.001	<0.001	0.005	<0.001	<0.001	
	n	80	91	91	91	91	
Entire body-Nymphs	Kendall's tau	**0.883**	0.554	0.28	0.385	0.584	0.5
	P-value	**<0.001**	<0.001	0.003	<0.001	<0.001	<0.001
	n	**80**	80	80	80	80	80
Neck-Females	Kendall's tau	0.686					
	P-value	<0.001					
	n	80					
Rest of the body-Females	Kendall's tau	0.494	0.571				
	P-value	<0.001	<0.001				
	n	80	91				

Table 2 continued

		Head	Neck	Rest of the body	Sternum & abdomen	Front legs	Hind legs
Sternum & abdomen-Females	Kendall's tau	0.254	0.359	0.337			
	P-value	0.007	<0.001	<0.001			
	n	80	91	91			
Front legs-Females	Kendall's tau	0.557	0.524	0.492	0.328		
	P-value	<0.001	<0.001	<0.001	<0.001		
	n	80	91	91	91		
Hind legs-Females	Kendall's tau	0.474	0.5	0.372	0.334	0.505	
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	
	n	80	91	91	91	91	
Entire body-Females	Kendall's tau	0.764	**0.818**	0.532	0.407	0.671	0.606
	P-value	<0.001	**<0.001**	<0.001	<0.001	<0.001	<0.001
	n	80	**80**	80	80	80	80
Neck-Males	Kendall's tau	0.663					
	P-value	<0.001					
	n	80					
Rest of the body-Males	Kendall's tau	0.552	0.559				
	P-value	<0.001	<0.001				
	n	80	91				
Sternum & abdomen-Males	Kendall's tau	0.415	0.452	0.503			
	P-value	<0.001	<0.001	<0.001			
	n	80	91	91			
Front legs-Males	Kendall's tau	0.452	0.579	0.499	0.419		
	P-value	<0.001	<0.001	<0.001	<0.001		
	n	80	91	91	91		
Hind legs-Males	Kendall's tau	0.416	0.505	0.478	0.381	0.436	
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	
	n	80	91	91	91	91	
Entire body-Males	Kendall's tau	0.749	**0.851**	0.577	0.553	0.587	0.559
	P-value	<0.001	**<0.001**	<0.001	<0.001	<0.001	<0.001
	n	80	**80**	80	79	80	80
Neck-Ticks	Kendall's tau	0.673					
	P-value	<0.001					
	n	80					
Rest of the body-Ticks	Kendall's tau	0.52	0.61				
	P-value	<0.001	<0.001				
	n	80	91				
Sternum & abdomen-Ticks	Kendall's tau	0.539	0.544	0.52			
	P-value	<0.001	<0.001	<0.001			
	n	80	91	91			
Front legs-Ticks	Kendall's tau	0.6	0.596	0.575	0.579		
	P-value	<0.001	<0.001	<0.001	<0.001		
	n	80	91	91	91		

Table 2 continued

		Head	Neck	Rest of the body	Sternum & abdomen	Front legs	Hind legs
Hind legs-Ticks	Kendall's tau	0.593	0.64	0.495	0.571	0.615	
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	
	n	80	91	91	91	91	
Entire body-Ticks	Kendall's tau	**0.849**	0.772	0.593	0.616	0.704	0.699
	P-value	**<0.001**	<0.001	<0.001	<0.001	<0.001	<0.001
	n	**80**	80	80	80	80	80

The strongest correlations between tick life stages on one body part and entire life stage burden are highlighted in bold

Abundance estimation

Life stage specific tick numbers on one body part were significantly correlated with life stage specific tick numbers on all other body parts and with the entire life stage burden (Table 2).

For *Ixodes* larvae, the counts on the front legs correlated most strongly with the entire larvae burden (Table 2). Fitting a linear regression to these data (Fig. 2a) resulted in a significant predictive model ($F = 419$, DF $= 1, 78$, $P \ll 0.001$) which explains a considerable amount of the observed variance (adjusted $R^2 = 0.841$). Figures in brackets indicate the standard error of each regression coefficient.

$$\text{Larvae on entire roe deer} = 3.38(\pm 1.71) + 1.41(\pm 0.07) \times \text{larvae on front legs}$$

For *Ixodes* nymphs, the tick count on the head was chosen as predictor for the entire nymph burden (Table 2). The fitted linear relationship for these data (Fig. 2b) was highly significant ($F = 270.7$, DF $= 1, 78$, $P \ll 0.001$) and explained ca. 77% of the variance (adjusted $R^2 = 0.773$).

$$\text{Nymphs on entire roe deer} = 2.27(\pm 2.02) + 1.28(\pm 0.08) \times \text{nymphs on head}$$

For the adult ticks, the tick counts on the neck were selected to predict the total female and male tick abundance (Table 2). For female ticks (Fig. 2c), a linear model ($F = 752.8$, DF $= 1, 78$, $P \ll 0.001$) explained ca. 90% of the variance (adjusted $R^2 = 0.905$):

$$\text{Females on entire roe deer} = 5.87(\pm 1.21) + 1.61(\pm 0.06) \times \text{females on neck}$$

For male ticks (Fig. 2d), the corresponding model ($F = 827.3$, DF $= 1, 78$, $P \ll 0.001$) explained ca. 91% of the variance (adjusted $R^2 = 0.913$):

$$\text{Males on entire roe deer} = 1.86(\pm 0.50) + 1.57(\pm 0.05) \times \text{males on neck}$$

For all ticks combined, tick counts on the head appeared to be the best predictor for total tick burden (Table 2). This relationship (Fig. 3) was also described with a linear model ($F = 149.3$, DF $= 1, 78$, $P \ll 0.001$) and explained ca. 65% of the variance (adjusted $R^2 = 0.653$):

$$\text{Ticks on entire roe deer} = -3.06(\pm 8.35) + 2.66(\pm 0.22) \times \text{Ticks on head}$$

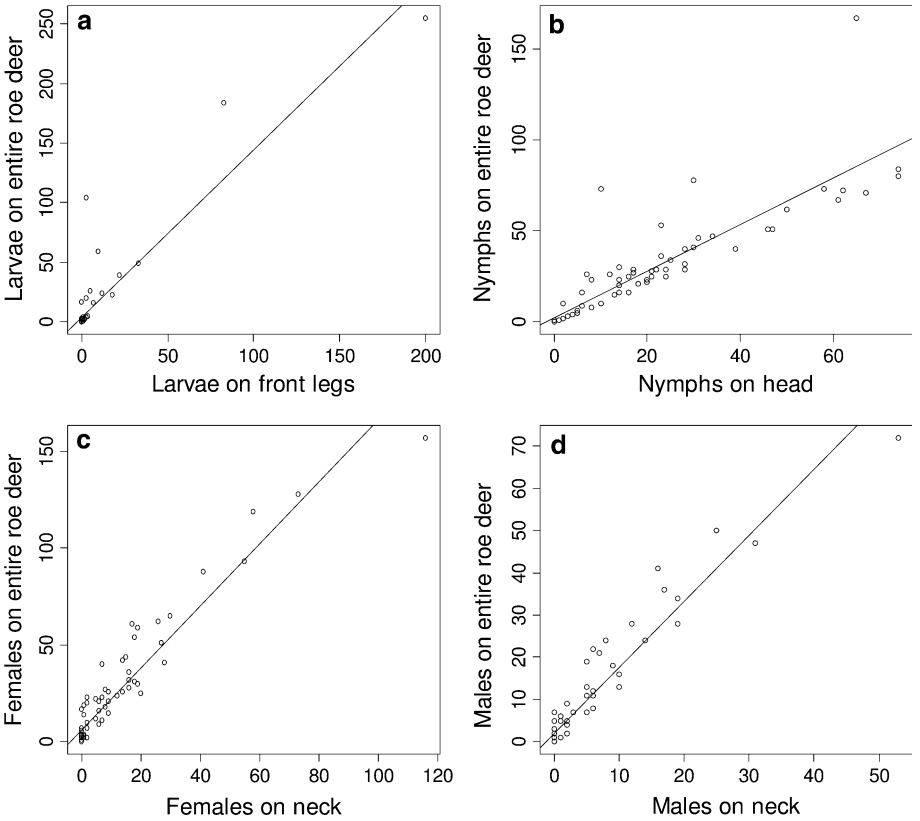


Fig. 2 Linear regression plots showing the relationship between number of larvae (**a**), nymphs (**b**), females (**c**) and males (**d**) on one body part and predicted number of each tick life stage on the entire roe deer body

Discussion

Based on entire body counts of 80 hunter-killed roe deer we present life stage specific tick burdens of roe deer and linear models which can be used to extrapolate tick burdens on roe deer based on tick counts on selected body parts.

Although the tick examination was carried out with extraordinary diligence, we cannot exclude that some (i.e. larval) ticks were missed (MacIvor et al. 1987). It is however unlikely that ticks detached prior to the investigation; detached ticks were never observed in trays underneath the carcasses. A few ticks, especially in the abdomen region may have been missed because parts of the abdomen hide had been removed by the hunter during the disembowelling of the carcass. Thus, reported tick numbers should be regarded as minimum numbers.

It is difficult to compare *Ixodes* tick burdens of roe deer with those of other host species such as sheep (Ogden et al. 1998), due to the considerable effect of season on tick parasitism (Randolph 2004). Nevertheless, tick numbers on roe deer in the summer months can be characterised as very high (up to 582 ticks/individual). Over the year, roe deer are parasitized by all four *Ixodes* life stages/sexes, whereas adult ticks combined (females 33%

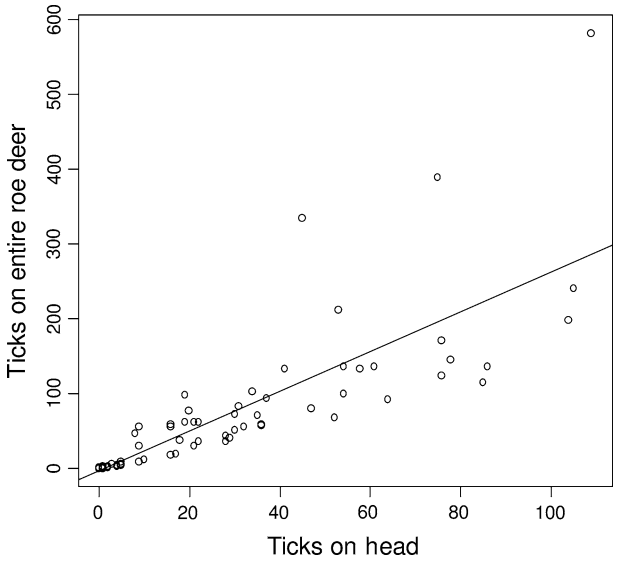


Fig. 3 Linear regression plot showing the relationship between the number of ticks (all life stages combined) attached on the head and predicted number of ticks on the entire roe deer body

of all ticks + males 13% of all ticks) make up ~46% of the total tick burden. Nymphs are very abundant on roe deer as well, whereas larvae appear to be extremely aggregated on few individual deer ($k = 0.08$). This extreme level of aggregation is probably a consequence of the strong seasonality in larvae activity (Randolph et al. 1999) and the spatial aggregation of questing larvae ticks in the vegetation.

Estimating life stage abundance based on restricted counts

For assessing abundance of nymphs and adult *Ixodes* ticks, it is sufficient to sample the head and the neck of roe deer. For best approximating larvae burden, additional tick counts on the front legs are required. However, larvae counts on the head offer the second best equation for extrapolating larvae burden ($F = 72.3$, DF = 1, 78, $P \ll 0.001$, adjusted $R^2 = 0.474$):

$$\text{Larvae on entire roe deer} = 2.53(\pm 3.18) + 5.74(\pm 0.67) \times \text{larvae on head}$$

It is thus reasonable to restrict tick counts to the head and the neck in order to estimate life stage specific tick burdens on roe deer.

Whenever possible, tick life stage/sex specific counts and models should be applied for estimating total tick burdens of roe deer. The model based on the combined tick count (Fig. 3) shows a rather large variance and the model's intercept is associated with a large margin of error, thus questioning the reliability of this model.

The presented tick life stage or sex specific models show good fits and explain a large amount of the observed variance in tick parasitism and thus offer a robust basis for rapid tick abundance assessment.

Acknowledgments Representative for all hunters and forest rangers involved we would like to thank J. Berger, D. Birke, E. Kreysern, W. Mänz, M. Scholz, and R. Zietlow for making the roe deer available for this study. We sincerely thank K. Hammer, H. Lippe, A. and M. Lödige, M. Scholz and several other students for their help during the field work. We thank B. Wilson for linguistic improvements of the manuscript. This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).

References

Barnard DR, Morrison RD (1985) Density estimators for populations of the lone star tick, *Amblyoma americanum* (Acari: Ixodidae), on pastured beef cattle. J Med Entomol 22:244–249

Barnard DR, Morrsion RD, Ervin T (1989) Sites of attachment and density assessment in *Amblyoma americanum* (Acari: Ixodidae) on nursing beef calves. Exp Appl Acarol 6:245–252

Bloemer SR, Zimmermann RH, Fairbanks K (1988) Abundance, attachment sites, and density estimators of lone star ticks (Acari: Ixodidae) infesting white tailed deer. J Med Entomol 25:295–300

Borchers DL, Buckland ST, Zucchini W (2002) Estimating animal abundance: closed populations. Springer, London

Bruno P, Bruno G, Peréz-Eid C (2000) Detection of spirochaetes of *Borrelia burgdorferi* complexe in the skin of cervids by PCR and culture. Eur J Epidemiol 16:869–873

Carpi G, Cagnacci F, Neteler M et al (2008) Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. Epidemiol Infect 136:1416–1424

Crawley MJ (2005) Statistics: an introduction using R. Wiley, The Atrium, Southern Gate, Chichester, West Sussex

Fish D, Childs JE (2009) Community-based prevention of Lyme disease and other tick-borne diseases through topical application of acaricide to white-tailed deer: background and rationale. Vector-Borne Zoonotic Dis 9:357–364

Fourie LJ, Kok DJ (1995) A comparison of *Ixodes rubicundus* infestations on Friesian and Bonsmara cattle in South Africa. Exp Appl Acarol 19:529–531

Fourie LJ, van Zyl JM (1991) Interspecific variations in attachment sites and density assessment in female *Ixodes rubicundus* (Acari: Ixodidae) on domestic and natural hosts. Exp Appl Acarol 13:1–10

Fourie LJ, Horak IG, van Zyl JM (1991) Sites of attachment and intraspecific infestation densities of the brown paralysis tick *Rhipicephalus punctatus* on Angora goats. Exp Appl Acarol 12:243–249

Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: defining R_0 for tick borne infections. Am Nat 171:743–754

Jaenson TG, Tälleklint L (1992) Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. J Med Entomol 29:813–817

Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3–S14

Kimura K, Isogai E, Isogai H et al (1995) Detection of Lyme disease spirochetes in the skin of naturally infected wild sika deer (*Cervus nippon yesoensis*). Appl Environ Microbiol 61:1641–1642

L'Hostis M, Diarra O, Seegers H (1994) Sites of attachment and density assessment of female *Ixodes ricinus* (Acari: Ixodidae) on dairy cows. Exp Appl Acarol 18:681–689

MacIvor KM, Horak IG, Holton KC et al (1987) A comparison of live and destructive sampling methods of determining the size of parasitic tick populations. Exp Appl Acarol 3:131–143

Mathee S, Meltzer DGA, Horak IG (1997) Sites of attachment and density assessment of ixodid ticks (Acari: Ixodidae) on impala (*Aepyceros melampus*). Exp Appl Acarol 21:179–192

Matuschka FR, Heiler M, Eiffert H et al (1993) Diversionary role of hoofed game in the transmission of Lyme disease spirochetes. Amer J Trop Med Hyg 48:693–699

Ogden NH, Nuttal PA, Randolph SE (1997) Natural Lyme disease cycles maintained via sheep by co-feeding ticks. Parasitology 115:591–599

Ogden NH, Hailes RS, Nuttall PA (1998) Interstadial variation in the attachment sites of *Ixodes ricinus* ticks on sheep. Exp Appl Acarol 22:227–232

Petritian AM, von Lüpke B, Petritan IC (2007) Effects of shade on growth and mortality of maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) saplings. Forestry 80:397–412
Pound JM, Miller JA, George JE et al (2009) The United States department of agriculture's northeast area-wide tick control project: summary and conclusions. Vector-Borne Zoonotic Dis 9:439–448
Rand PW, Lacombe EH, Holman MS et al (2000) Attempt to control ticks (Acari: Ixodidae) on deer on an isolated island using ivermectin-treated corn. J Med Entomol 37:126–133
Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129:S37–S65
Randolph SE, Gern L, Nuttall PA (1996) Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parastitology Today 12:472–479
Randolph SE, Miklisová D, Lysy J et al (1999) Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118:177–186
Schmid KA, Ostfeld RS (2001) Biodiversity and the dilution effect in disease ecology. Ecology 82:609–619
Schmidtmann ET, Caroll JF, Watson DW (1998) Attachment site patterns of adult blacklegged ticks (Acari: Ixodidae) on white-tailed deer and horses. J Med Entomol 35:59–63
Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610
Sutherland WJ (ed) (1996) Ecological census techniques: a handbook. Cambridge University Press, Cambridge

Chapter 6 – Attachment site selection of ticks on roe deer, *Capreolus capreolus*

Exp Appl Acarol
DOI 10.1007/s10493-010-9378-4

Attachment site selection of ticks on roe deer, *Capreolus capreolus*

C. Kiffner · C. Lödige · M. Alings · T. Vor · F. Rühe

Received: 3 March 2010 / Accepted: 10 June 2010

Abstract The spatio-temporal attachment site patterns of ticks feeding on their hosts can be of significance if co-feeding transmission (i.e. from tick to tick without a systemic infection of the host) of pathogens affects the persistence of a given disease. Using tick infestation data on roe deer, we analysed preferred attachment sites and niche width of *Ixodes* ticks (larvae, nymphs, males, females) and investigated the degree of inter- and intrastadial aggregation. The different development stages showed rather consistent attachment site patterns and relative narrow feeding site niches. Larvae were mostly found on the head and on the front legs of roe deer, nymphs reached highest densities on the head and highest adult densities were found on the neck of roe deer. The tick stages feeding (larvae, nymphs, females) on roe deer showed high degrees of intrastadial spatial aggregation, whereas males did not. Male ticks showed large feeding site overlap with female ticks. Feeding site overlap between larval-female and larval-nymphal ticks did occur especially during the months May–August on the head and front legs of roe deer and might allow pathogen transmission *via* co-feeding. Tick density, niche width and niche overlap on roe deer are mainly affected by seasonality, reflecting seasonal activity and abundance patterns of ticks. Since different tick development stages occur spatially and temporally clustered on roe deer, transmission experiments of tick-borne pathogens are urgently needed.

C. Kiffner (✉) · C. Lödige · M. Alings · T. Vor · F. Rühe
Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany
e-mail: ckiffne@gwdg.de

C. Lödige
e-mail: christinaloedige@hotmail.com

M. Alings
e-mail: efrafa@web.de

T. Vor
e-mail: tvor@gwdg.de

F. Rühe
e-mail: fruehe@gwdg.de

Published online: 29 June 2010

Keywords Co-feeding · Competition · Heterogeneity · Host-parasite interaction · Niche overlap · Reverse density-dependence · Tick-borne pathogens

Introduction

For several tick-host associations, it is well known that ticks apparently prefer certain feeding sites (e.g. Nelson et al. 1975; Randolph 1975; Barnard and Morrison 1985; Bloemer et al. 1988; Barnard et al. 1989; Fourie and van Zyl 1991; Fourie et al. 1991; L'Hostis et al. 1994; Fourie and Kok 1995; Mathee et al. 1997; Ogden et al. 1998; Schmidtmann et al. 1998).

In central Europe, roe deer (*Capreolus capreolus*) are probably the most important free-living host for adult ticks of the *Ixodes ricinus* complex. Yet, only few studies have investigated patterns of tick infestation on roe deer (Matuschka et al. 1993; Carpi et al. 2008; Vor et al. 2010; Kiffner et al. in press), and none of them explicitly investigated the spatio-temporal distribution of ticks on roe deer individuals. Knowledge about the most infested body parts is, however, crucial for designing acaricide-treated devices to control ticks feeding on deer, a control strategy that turned out to be effective in lowering densities of free-living ticks *(Ixodes scapularis* and *Amblyoma americanum*) in the north-eastern USA (e.g. Fish and Childs 2009; Pound et al. 2009).

Besides having a direct effect on the host by inducing a considerable blood loss which might reduce overall fitness of the host (Pfäffle et al. 2009), ticks of the *Ixodes ricinus* complex are vectors of several bacterial (e.g. *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Rickettsia helvetica*), protozoan (e.g. *Babesia capreoli*, *Babesia divergens*, *Babesia venatorum*) and viral (e.g. Louping-ill virus, tick-borne encephalitis virus) pathogens of medical and veterinary importance (Jongejan and Uilenberg 2004; Malandrin et al. 2010). There is a growing body of evidence that, at large scales, numbers of infected ticks (tick-borne encephalitis virus: Hudson et al. 2001) or numbers of human infections (*Borrelia burgdorferi*: Linard et al. 2007; tick-borne encephalitis virus: Rizzoli et al. 2009) are positively correlated with the density of roe deer. The interpretation of this correlation is, however, not unambiguous: Roe deer have been shown to amplify tick densities (e.g. Jensen et al. 2000; Walker et al. 2001), but considering the tick-borne pathogens of major human health importance such as *Borrelia burgdorferi* and tick-borne encephalitis virus, they are believed to be dead-end or dilution (Ostfeld and Keesing 2001) hosts. However, it is unclear whether roe deer and other ungulates do provide a platform for non-systemic pathogen transmission among co-feeding ticks (Randolph 2008; Jaenson and Tälleklint 1992; Matuschka et al. 1993; Kimura et al. 1995; Bruno et al. 2000), but it can not be excluded as a possibility (e.g. Gern et al. 1998). If co-feeding transmission does occur between ticks feeding on roe deer, intra- and interstadial aggregation of ticks may enhance co-feeding transmission and might therefore be of considerable interest for models aiming at quantifying basic reproduction numbers of tick-borne pathogens (e.g. Hartemink et al. 2008).

In this paper, we aimed at estimating the preferred feeding sites and tested the ideal free distribution hypothesis at the host level (Fretwell and Lucas 1970), i.e. we investigated whether feeding site selection by ticks is density dependent. This hypothesis predicts that most animals (here ticks) should be found in preferred habitats (in the tick-host context: preferred body parts) and spill over to less preferred habitats when animal density is high (Sutherland 1996). Further on, we aimed at estimating the niche width (i.e. the range of feeding sites) of the different life stages (larvae, nymphs, adults) and sexes (female, male)

feeding on roe deer and investigated the degree of niche overlap between different stages/sexes. Ultimately, we investigated whether indices of niche width and overlap are seasonal and whether they depend on the size (body mass) of the host.

Materials and methods

Tick sampling

We opportunistically sampled 80 hunter-killed roe deer from forests around the city of Göttingen (centred at, 51°32′2″N, 9°56′8″, radius of ca. 20 km) in central Germany during regular hunting activities. The study area is dominated by mixed deciduous forests comprising mainly of European beech (*Fagus sylvatica)*, Norway maple (*Acer pseudoplatanus)*, European ash (*Fraxinus excelsior)* and sessile oak (*Quercus petraea)*. The geology of the region is characterized by shallow limestone plateaus with rendzina soils and haplic luvisols, whereas some forest stands grow on sandstone with sandy and loamy cambisols. The altitude above sea level ranges from 151 to 400 m, the mean annual rainfall is 780 mm, and the average annual temperature is 7.8°C (Petritian et al. 2007). Data collection was stratified into 6 distinct sampling seasons: November–December 2007, $n = 20$; May–June 2008, $n = 18$; July–August 2008, $n = 12$; November–December 2008, $n = 14$, May–June 2009, $n = 10$ and July–August 2009, $n = 6$. Roe deer carcasses were disembowelled by the hunters and stored in cooling chambers at 2–8°C until examination. Within 16 h on average (SE: ±2.5 h) after roe deer individuals had been shot, each carcass was examined by two observers wearing latex gloves. The carcass was divided into 6 distinct parts (head, neck, sternum & abdomen, rest of the body, front legs and hind legs, Fig. 1). The roe deer skin was systematically inspected and palpated to detect all ticks. Sites heavily infested were consecutively searched and palpated by both persons.

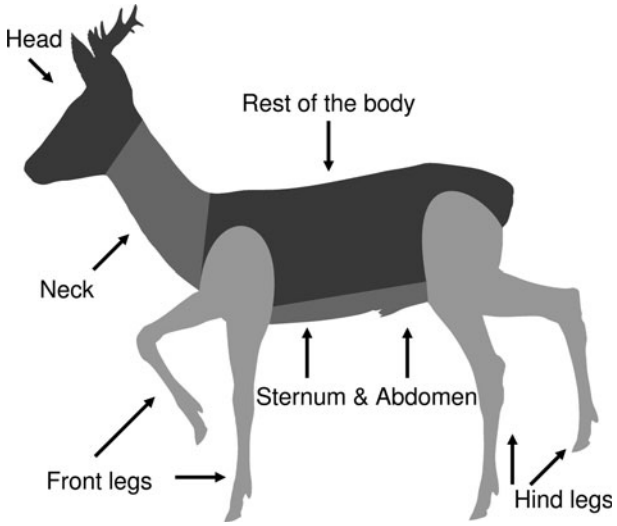


Fig. 1 Sketch outline of the tick collection sites on a roe deer (*Capreolus capreolus*) buck. Drawing: W. Tambour, J. Seelig

Table 1 Mean proportional surface area (±SE) of roe deer body parts

	Head	Neck	Sternum & Abdomen	Rest of the body	Front legs	Hind legs
>2 years	0.12 (±0.01)	0.06 (±0.00)	0.08 (±0.00)	0.33 (±0.01)	0.17 (±0.02)	0.25 (±0.01)
<2 years	0.12 (±0.01)	0.05 (±0.01)	0.10 (±0.01)	0.28 (±0.02)	0.20 (±0.01)	0.26 (±0.02)

Sample size for each age class is $n = 3$. For a delineation of the body parts, see Fig. 1

All ticks were removed from each body part with forceps. These ticks were immediately counted and recorded according to life stage and sex (larvae, nymphs, males and females). Finally, they were transferred to sampling tubes and stored at −20°C. All removed ticks belong to the *Ixodes ricinus* complex; for a more detailed description of the study site and the tick collection see Kiffner et al. (in press). In order to relate the number of ticks to the surface area of each body part, we estimated the surface area of body parts of six roe deer individuals (3 individuals <2 year, 3 individuals >2 years) using basic geometric measurements. Since the absolute surface areas varied considerably among individuals, we used proportional data (Table 1) and allocated these relative measurements to each investigated roe deer individual.

Data analysis

We analysed the relative tick density (i.e. number of ticks of each stage or sex/relative surface area) of each body part with the nonparametric Friedman test in order to provide a density ranking of the body parts infested by ticks. We also analysed whether the relative use of the preferred attachment site varied as a function of the abundance of the considered tick life stage/sex.

To investigate the feeding site specialisation of each tick stage/sex, we calculated Levins index of niche breadth, using each body part as a resource state.

$$B = \frac{1}{\sum p_j^2}$$

where B is the index of niche breadth, p_j is the proportion of ticks collected from body part j (Levins 1968). Since B does not follow a normal distribution (B reaches a maximum value if ticks of one life stage/sex were distributed homogenously on all body parts and reaches a minimum value if all ticks of this life stage/sex were concentrated on one body part), we standardised B to B_S using the formula:

$$B_S = \frac{B - 1}{n - 1}$$

where n is the number of body parts (Hurlbert 1978). This standardised index ranges from 0 to 1 (wide niche). Cases with a zero-count of a specific tick life stage/sex were omitted. Using generalised linear models, we tested whether B_S varied with sampling season and with body mass (mass of disembowelled body with head in kg) of the host individual.

In order to investigate the overlap in site selection among the different tick life stages and sexes, we calculated a niche overlap index for all tick life stage/sex combinations following Pianka (1973).

$$O_{xy} = \frac{\sum_n p_{jx} \times p_{jy}}{\sqrt{\sum p_{jx}^2 \times \sum p_{jy}^2}}$$

where O_{xy} is the index of niche overlap between tick life stage/sex x and tick life stage/sex y, p_{jx} the proportional relative density of tick life stage/sex x on body part j (relative density of this life stage/sex on body part j divided by the total number of this life stage/sex on the entire roe deer), p_{jy} the proportional relative density of tick life stage/sex y on body part j and n the number of body parts (i.e. 6). This index is a symmetrical measure, ranging from 0 (no body parts used in common) to 1 (complete overlap in body part selection). Roe deer individuals with a zero-count of a specific tick life stage/sex were treated as 0 (i.e. no overlap in resource use).

Analogously to the analyses of B_S, we ran generalised linear models to test whether O_{xy} is affected by sampling season and host body mass. All calculations were performed for the entire study period and for the 6 distinct sampling seasons. Indices of niche breadth and niche overlap were calculated with *Microsoft Excel*, statistical tests were performed with *R* (R Development core team) and *SPSS 17.0* (SPSS Inc.).

Results

Preferred attachment sites

Larvae showed a slightly inconsistent pattern of feeding site selection (Table 2, Fig. 2) across the study period. In two sampling seasons (November–December 2008, May–June 2009), we found no significant (Friedman test $P > 0.05$) density differences among body parts, and during one sampling period we found no larvae at all (November–December 2007). For the remaining sampling periods, the results of the Friedman test indicate significant (all $P < 0.05$), but differing density rankings. In May–June 2008 and July–August 2008, the front legs showed the highest larvae density, followed by the head, whereas during the season July–August 2009, larvae density was highest on the head, followed by the front legs.

Nymphs showed a clear pattern of attachment site selection, at least for the top-ranked body part. During all seasons, significantly highest densities of nymphs were observed on the roe deer's head. The second highest density was usually observed on the front legs (except for May–June 2009: neck).

Male ticks also exhibited significant density differences between roe deer body parts, except for the winter months ($P > 0.05$). The highest male tick density was usually found on the roe deer's neck, followed by its head. Similarly, female ticks reached highest densities on the neck, followed by the head. Again, except for the winter months, female densities were highly significantly different between the distinct body parts.

Testing the ideal free distribution

The proportion of tick life stages on the preferred body (i.e. the top ranked body part for the entire study period, Table 2) part increased significantly (larvae: Kendall's $\tau = 0.60$, $P < 0.001$, nymphs: $\tau = 0.42$, $P < 0.001$, females: $\tau = 0.58$, $P < 0.001$) or did not change significantly (males: $\tau = -0.05$, $P = 0.575$) with increasing absolute abundance of the same tick life stage/sex (Fig. 3; all correlations $n = 80$).

Table 2 Ranking (Friedman test) of tick densities on different roe deer body parts (Rest = Rest of the body, St. & Ab. = Sternum & Abdomen) by sampling period and for the entire study period

Nov.–Dec. 2007		May–June 2008		July-Aug. 2008		Nov.–Dec. 2008		May–June 2009		July–Aug. 2009		Study period	
Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank
Larvae													
–	–	Front legs	4.86	Front legs	4.67	Head	3.68	Head	4.30	Head	4.83	Front legs	4.05
–	–	Head	3.56	Head	3.63	Neck	3.46	Neck	4.05	Front legs	4.17	Head	3.76
–	–	St. & Ab.	3.31	Hind legs	3.46	Rest	3.46	Front legs	3.70	Neck	3.83	Neck	3.46
–	–	Neck	3.22	Neck	3.08	St. & Ab.	3.46	St. & Ab.	3.45	St. & Ab.	3.17	St. & Ab.	3.36
–	–	Hind legs	3.17	Rest	3.08	Front legs	3.46	Rest	2.85	Rest	2.50	Hind legs	3.23
–	–	Rest	2.89	St. & Ab.	3.08	Hind legs	3.46	Hind legs	2.65	Hind legs	2.50	Rest	3.14
Nymphs													
Head	4.35	Head	5.89	Head	5.92	Head	5.64	Head	5.70	Head	5.58	Head	5.42
Front legs	3.45	Front legs	4.47	Front legs	4.33	Front legs	3.21	Neck	4.55	Front legs	3.83	Front legs	3.78
Neck	3.30	Neck	3.39	Hind legs	3.04	Neck	3.04	St. & Ab.	3.25	Neck	3.50	Neck	3.36
Rest	3.30	Hind legs	2.72	St. & Ab.	3.00	Rest	3.04	Front legs	3.25	St. & Ab.	3.08	St. & Ab.	3.03
St. & Ab.	3.30	St. & Ab.	2.58	Neck	2.71	St. & Ab.	3.04	Hind legs	2.65	Hind legs	2.83	Hind legs	2.97
Hind legs	3.30	Rest	1.94	Rest	2.00	Hind legs	3.04	Rest	1.60	Rest	2.17	Rest	2.46
Males													
Front legs	3.75	Neck	4.75	Neck	5.67	Head	3.64	Neck	6.00	Neck	5.50	Neck	4.58
Head	3.45	Head	3.92	Head	3.96	Neck	3.64	Head	5.00	Head	5.17	Head	3.99
Neck	3.45	Hind legs	3.56	Front legs	3.21	Rest	3.43	St. & Ab.	2.80	Front legs	3.08	Front legs	3.29
Rest	3.45	Front legs	3.22	Hind legs	3.04	St. & Ab.	3.43	Hind legs	2.65	St. & Ab.	2.92	Hind legs	3.24
St. & Ab.	3.45	St. & Ab.	2.81	St. & Ab.	2.63	Front legs	3.43	Front legs	2.55	Hind legs	2.58	St. & Ab.	3.06
Hind legs	3.45	Rest	2.75	Rest	2.50	Hind legs	3.43	Rest	2.00	Rest	1.75	Rest	2.84

Table 2 continued

Nov.–Dec. 2007		May–June 2008		July-Aug. 2008		Nov.–Dec. 2008		May–June 2009		July–Aug. 2009		Study period	
Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank	Body part	Mean rank
Females													
Front legs	3.80	Neck	5.56	Neck	5.21	Front legs	3.82	Neck	6.00	Neck	6.00	Neck	4.66
Hind legs	3.75	Head	3.83	Head	4.83	Head	3.79	Head	4.80	Head	4.83	Head	4.13
Head	3.68	Hind legs	3.61	Front legs	3.50	Neck	3.61	Front legs	3.20	Hind legs	3.33	Front legs	3.53
St. & Ab.	3.38	Front legs	3.39	St. & Ab.	3.29	Hind legs	3.50	Hind legs	3.00	Front legs	2.92	Hind legs	3.39
Neck	3.20	St. & Ab.	2.83	Hind legs	2.67	Rest	3.14	Rest	2.00	St. & Ab.	2.25	St. & Ab.	2.94
Rest	3.20	Rest	1.78	Rest	1.50	St. & Ab.	3.14	St. & Ab.	2.00	Rest	1.67	Rest	2.35
Ticks													
Head	4.53	Head	5.72	Head	5.25	Head	5.57	Neck	5.60	Neck	5.67	Head	5.22
Front legs	3.75	Neck	5.11	Neck	5.08	Front legs	3.57	Head	5.20	Head	5.17	Neck	4.35
Hind legs	3.50	Front legs	3.61	Front legs	4.00	Neck	3.18	Front legs	3.20	Front legs	3.67	Front legs	3.65
St. & Ab.	3.18	Hind legs	2.75	St. & Ab.	2.83	Hind legs	3.11	St. & Ab.	2.60	Hind legs	2.83	Hind legs	2.95
Neck	3.03	St. & Ab.	2.47	Hind legs	2.50	Rest	2.79	Hind legs	2.60	St. & Ab.	2.58	St. & Ab.	2.78
Rest	3.03	Rest	1.33	Rest	1.33	St. & Ab.	2.79	Rest	1.80	Rest	1.08	Rest	2.05

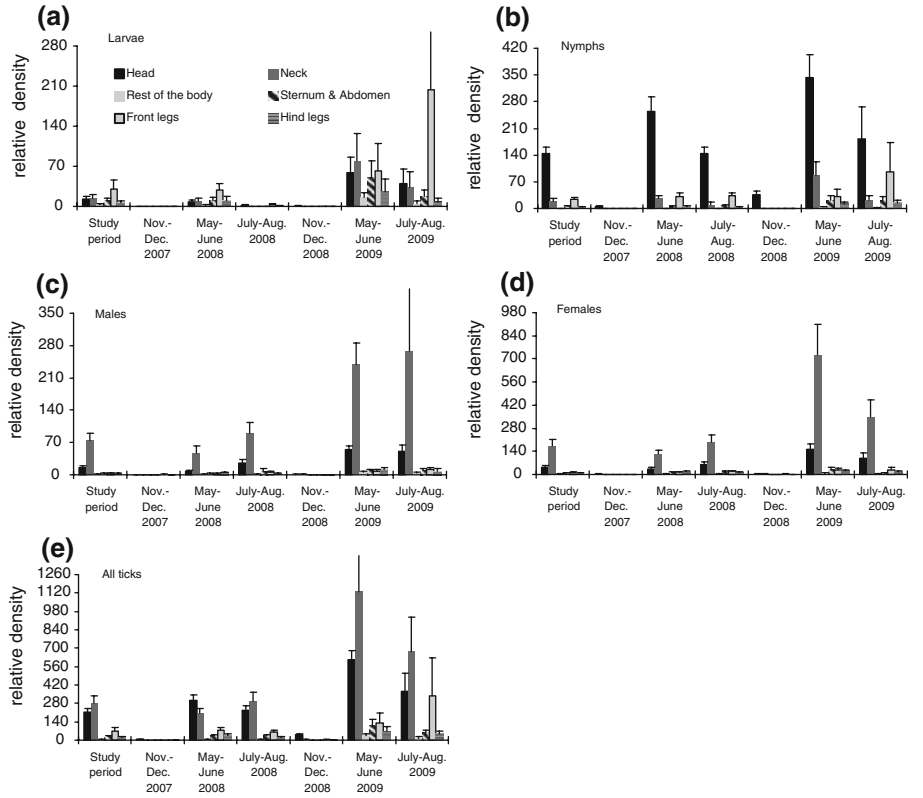


Fig. 2 The seasonal pattern of mean relative density (number/proportional surface area of each body part) of (**a**) larvae, (**b**) nymphs, (**c**) male, (**d**) female and (**e**) all ticks (*Ixodes ricinus* complex) attached to roe deer (*Capreolus capreolus*) from the forests of Göttingen, Germany. *Note* the different scales on the *y-axes*

Niche breadth

Across the study period, nymphs were most specialised in their feeding site selection, followed by larvae, males and females (Table 3). Niche indices of larvae and nymphs, however, did not differ significantly from each other (paired *t*-test: $t = 0.276$, df $= 34$, $P = 0.784$). Differences in niche indices were significantly different between larvae and males ($t = -2.209$, df $= 32$, $P = 0.034$), larvae and females ($t = -3.951$, df $= 33$, $P < 0.001$), nymphs and males ($t = -3.407$, df $= 46$, $P = 0.01$), nymphs and females ($t = -7.288$, df $= 55$, $P < 0.001$), and between males and females ($t = -4.397$, df $= 47$, $P < 0.001$). Compared to the niche breadth in the winter months 2008, niche breadth of larvae was significantly larger in May–June 2009 (Table 4). Relative to the same reference season, nymphal niche breadth was significantly larger in all other seasons except for the winter months 2007. Niche breadth of male ticks was significantly larger in May–June 2009, July–August 2008 and 2009 compared to the niche breadth in November–December 2008; niche breadths in other sampling seasons were not significantly different to the reference season. Niche breadth of males was positively associated with host body mass. Female tick niche breadth was also larger during the May–June and July–August seasons compared to the reference season.

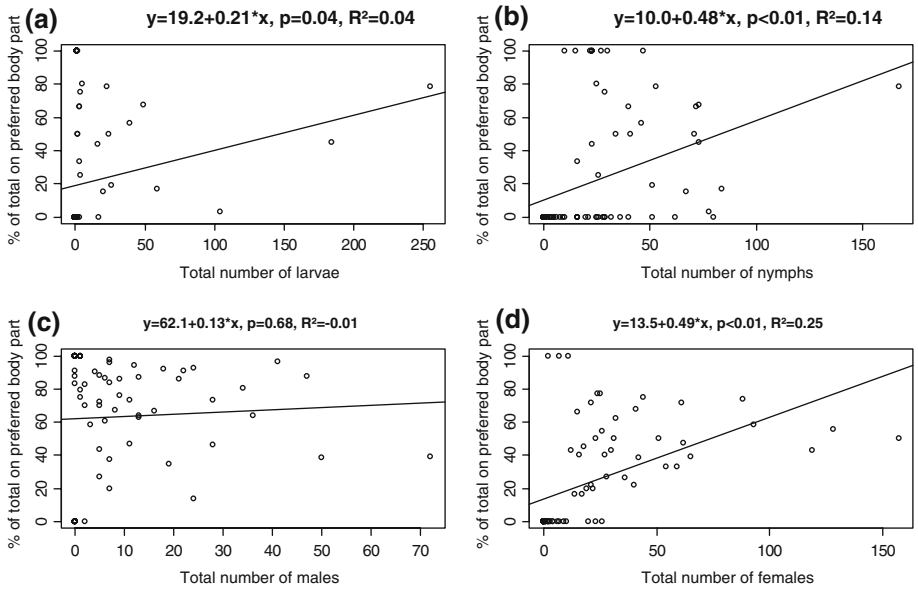


Fig. 3 Use of preferred attachment sites by the four different tick life stages/sexes in relation to total abundance of the same tick life stage/sex on the entire roe deer ($n = 80$)

Spatial niche overlap

Interstadial niche overlap was observed for all tick development/sex combinations (Table 5). During the entire study period, it was highest among adult ticks, followed by nymphs-females, larvae-nymphs, nymphs-males, larvae-males and larvae-females. Larvae-nymph overlap was usually highest in May–June (both years) and in July–August 2009 (Table 6). Overlap between males and larvae and larvae and females roughly followed the same pattern as larvae-nymph overlap. Spatial overlap between nymph-male and female-male ticks was generally higher during the summer months (May–June and July–August) than during the winter months. Spatial female-male tick overlap was positively associated with roe deer body mass.

Discussion

Feeding site selection and intraspecific aggregation

Overall, ticks were highly aggregated on the roe deer host. Averaged over the study period, roe deer heads were most heavily infested with ticks (Table 2); 54% (SE ± 3) of the total tick burden was found on only 12% (SE ± 1) of the roe deer surface area.

For all sampling seasons combined, tick densities of body parts differed significantly; the pattern of tick density ranking was, however, only consistent for the most (head) and least (rest of the body, i.e. the flanks and the dorsal part of the roe deer) infested body part. The intermediate ranked body parts showed some variation in the density ranking from sampling season to sampling season. The observed distribution patterns and the relative narrow niche widths (Table 3) suggest that each tick life stage apparently selects for

Table 3 Standardised index of niche breadth ($B_S \pm$ SE) for each tick stage/sex on roe deer (*Capreolus capreolus*) estimated for each sampling period and for the entire study period

	Nov.–Dec. 2007	May–June 2008	July–Aug. 2008	Nov.–Dec. 2008	May–June 2009	July–Aug. 2009	Study period
Larvae	No larvae found	0.09 (±0.03)	0.13 (±0.05)	0	0.40 (±0.08)	0.22 (±0.19)	0.18 (±0.17)
Nymphs	0	0.14 (±0.01)	0.16 (±0.01)	0.01 (±0.00)	0.15 (±0.06)	0.29 (±0.18)	0.11 (±0.11)
Males	0	0.22 (±0.02)	0.27 (±0.03)	0	0.34 (±0.04)	0.26 (±0.18)	0.24 (±0.15)
Females	0.07 (±0.03)	0.41 (±0.02)	0.42 (±0.02)	0.11 (±0.01)	0.29 (±0.04)	0.39 (±0.12)	0.31 (±0.16)

Table 4 Parameter estimates of generalised linear models explaining the variance of niche breadth of each tick development stage/sex on roe deer (*Capreolus capreolus*)

	Larvae	Nymphs	Males	Females
Intercept	−0.052	−0.125	−0.502	0.016
May–June 2008	0.068	0.131**	0.287	0.280***
May–June 2009	0.379*	0.144**	0.421*	0.168*
July–August 2008	0.111	0.161**	0.379*	0.306***
July–August 2009	0.203	0.287***	0.343*	0.267**
November–December 2007	No larvae found	0.005	0.037	−0.056
Body mass	0.022	0.008	0.027*	0.007

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

certain body parts albeit there are minor derivations from season to season and year to year (cf. Table 2). Larvae appear to prefer mainly the head and the front legs of roe deer. This pattern seems to be roughly similar in *Ixodes ricinus* larvae feeding on sheep which apparently prefer the head and distal limbs of their hosts (Ogden et al. 1998). Nymphs showed a narrow feeding site niche and had strong preferences for the head and the front legs, again similar to feeding sites of nymphs parasitizing sheep (Ogden et al. 1998). Nymphs were especially clumped on the outside and inside (haired) parts of the roe deer ears. Adult ticks (males and females) apparently prefer the neck and the head which also appears similar in ticks feeding on sheep (Ogden et al. 1998). Adult *Ixodes scapularis* feeding on white-tailed deer (*Odocoileus virginianus*) also prefer feeding on the head (incl. the ears) and the neck of their hosts (Schmidtmann et al. 1998). Clearly, any host-targeted tick control device (e.g. Fish and Childs 2009; Pound et al. 2009) should focus the acaricide agent application on these body parts. Feeding site selection and consequently, niche width and niche overlap varied considerably by season but also by year. Ultimately, these variations are mainly due to the strong seasonality in activity patterns of the different stages (Fig. 4).

Numerous factors might influence the observed spatial aggregation of tick life stages/sexes among which season and year (this paper), but also host/body part characteristics like skin thickness, humidity, blood circulation and de-ticking by grooming behaviour of the host might play an important role (L'Hostis et al. 1994; Ogden et al. 1998). The observed attachment patterns suggest that there is some sort of active selection involved. Contradicting the ideal free distribution hypothesis (Fig. 3), number of ticks feeding on the preferred body part increased significantly (and did not change significantly in males) with increasing abundance of this life stage/sex on the entire roe deer. These results are in line with propositions that aggregation of *Ixodes ricinus* ticks are caused by pheromone excreted by conspecifics (e.g. Sonenshine 2006; Healy and Bourke 2008) whereas this remains to be further tested for *Ixodes ricinus*. For the tick *Rhipicephalus appendiculatus*, gregarious feeding turned out to be beneficial (increased blood feeding rate, reduced time to mating and repletion) for female ticks (Wang et al. 2001). Gregariousness might also be beneficial for *Ixodes* ticks that attach on the host in order to feed, seeming to be an evolutionary stable strategy. That gregarious feeding might be beneficial is further supported by the fact that apparent intrastadial attraction only occurs in those tick stages that actually feed (larvae, nymphs, females) on the roe deer blood but not in males which ultimately seek a female tick for reproduction. The quest for a mate might explain the strong overlap between the female and male ticks which mainly occurs on the neck of the

Table 5 Pianka's index of spatial niche overlap on roe deer (*Capreolus capreolus*) for each tick stage/sex combination estimated for each sampling period and for the entire study period

	Nov.–Dec. 2007	May–June 2008	July–Aug. 2008	Nov.–Dec. 2008	May–June 2009	July–Aug. 2009	Study period
Larvae-Nymphs	0	0.39 (±0.09)	0.19 (±0.09)	0.07 (±0.07)	0.63 (±0.13)	0.58 (±0.19)	0.25 (±0.04)
Larvae-Males	0	0.27 (±0.09)	0.04 (±0.03)	0	0.47 (±0.12)	0.28 (±0.12)	0.15 (±0.03)
Larvae-Females	0	0.29 (+0.07)	0.06 (±0.02)	0	0.44 (±0.12)	0.29 (±0.10)	0.15 (±0.03)
Nymphs-Males	0	0.28 (±0.07)	0.31 (±0.07)	0.07 (±0.07)	0.43 (±0.07)	0.34 (±0.11)	0.20 (±0.03)
Nymphs-Females	0.04 (±0.04)	0.32 (±0.05)	0.43 (±0.08)	0.15 (±0.09)	0.43 (±0.07)	0.42 (+0.11)	0.26 (±0.03)
Males-Females	0.08 (±0.06)	0.62 (±0.10)	0.88 (±0.06)	0.09 (±0.07)	0.97 (±0.02)	0.83 (±0.10)	0.50 (±0.05)

Table 6 Parameter estimates of generalised linear models explaining the variance of spatial niche overlap among tick development stage/sex combinations on roe deer (*Capreolus capreolus*) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

	Larvae-Nymphs	Larvae-Males	Larvae-Females	Nymphs-Males	Nymphs-Females	Males-Females
Intercept	−0.248	0.090	0.083	−0.022	0.011	−0.284
May–June 2008	0.271*	0.281**	0.301***	0.279***	0.153	0.490***
May–June 2009	0.527***	0.477***	0.448***	0.429***	0.268*	0.863***
July–August 2008	0.107	0.043	0.059	0.312***	0.279**	0.799***
July–August 2009	0.479**	0.332**	0.310**	0.373***	0.205	0.785***
November–December 2007	−0.089	0.001	0.001	0.000	−0.113	−0.011
Body mass	0.023	−0.006	−0.006	0.001	0.010	0.025*

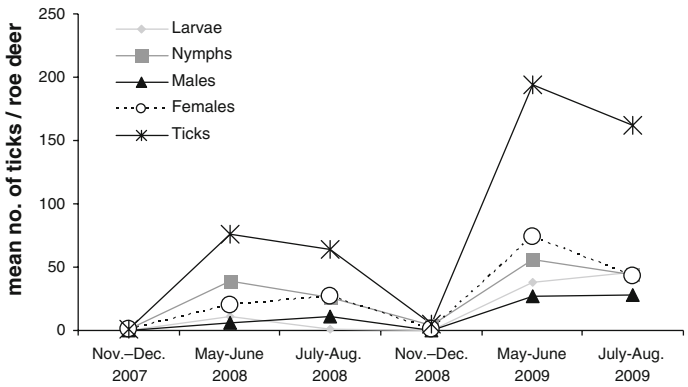


Fig. 4 Seasonal pattern of mean numbers of larval, nymphal, male, female and all ticks (*Ixodes ricinus* complex) attached to roe deer (*Capreolus capreolus*) from the forests of Göttingen, Germany

roe deer. It is notable that niche width of males and female-male overlap are correlated with body mass of the host. This might be due to the positive relationship between adult tick burden and age and body mass of roe deer (Vor et al. 2010). However, until now tick burden on roe deer appears to follow rather unpredictable patterns apart from seasonality and thus impedes straight forward identification of potential super-spreaders (cf. Perkin et al. 2003).

Interstadial aggregation

Next to intraspecific aggregation, interstadial attachment site overlap is of considerable interest as this might facilitate pathogen transmission from one feeding development stage to another (i.e. larvae, nymphs and females). Therefore, larvae-nymph, larvae-female and nymph-female feeding aggregations are of special importance. Of these, niche overlap was greatest between nymphs and females and larvae and nymphs. Nymph-female feeding overlap mainly takes place on the head of the roe deer. Here, distances between the two stages are however relatively large, because most nymphs are located on the pinna whereas

most larvae are attached around the muzzle and the eyes. Larvae-female overlap also mainly takes place on the roe deer's head, whereas both stages feed in close proximity to each other, suggesting that pathogen transmission could be relatively efficient. Overall, niche overlap was significantly affected by season, reflecting seasonal activity peaks of the development stages (Fig. 4) observed during our study. In areas, where larvae and nymphs show even stronger seasonal synchrony (e.g. Randolph et al. 1999, 2000), co-feeding between these immature tick stages might take place, especially on the head and front legs of roe deer, where the two stages feed side by side. For tick-borne encephalitis virus, co-feeding transmission has been observed in forest rodent species such as *Apodemus* spp., *Myodes glareolus* (Labuda et al. 1993, 1996); for *Borrelia burgdorferi* co-feeding transmission has been demonstrated for ticks feeding on several rodent species, medium sized mammals, sheep (*Ovis aries*) and several bird species (Gern and Rais 1996; Ogden et al. 1997; Gern et al. 1998). Without a systematic infection of the host, Louping-ill virus can be transmitted from tick to tick feeding on hares (*Lepus timidus*), sheep, red grouse (*Lagopus lagopus scoticus*), but not by ticks feeding on red deer (*Cervus elaphus*), rabbits (*Oryctolagus cuniculus*) and rodents (*Apodemus sylvaticus, Myodes glareolus*) (Jones et al. 1997; Gilbert et al. 2000). In the absence of experiments testing co-feeding pathogen transmission on roe deer, we strongly recommend conducting such co-feeding transmission experiments for different tick-vectored pathogens.

Acknowledgments We would like to sincerely thank J. Berger, D. Birke, E. Kreysern, W. Mänz, M. Scholz, and R. Zietlow and all involved hunters and forest rangers for providing the roe deer. We thank K. Hammer, H. Lippe, A. and M. Lödige and M. Scholz for their help during the field work. This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).

References

Barnard DR, Morrison RD (1985) Density estimators for populations of the lone star tick, *Amblyoma americanum* (Acari: Ixodidae), on pastured beef cattle. J Med Entomol 22:244–249

Barnard DR, Morrsion RD, Ervin T (1989) Sites of attachment and density assessment in *Amblyoma americanum* (Acari: Ixodidae) on nursing beef calves. Exp Appl Acarol 6:245–252

Bloemer SR, Zimmermann RH, Fairbanks K (1988) Abundance, attachment sites, and density estimators of lone star ticks (Acari: Ixodidae) infesting white tailed deer. J Med Entomol 25:295–300

Bruno P, Bruno G, Peréz-Eid C (2000) Detection of spirochaetes of *Borrelia burgdorferi* complexe in the skin of cervids by PCR and culture. Eur J Epidemiol 16:869–873

Carpi G, Cagnacci F, Neteler M, Rizzoli A (2008) Tick infestation on roe deer in relation to geographic and remotely sensed climatic variables in a tick-borne encephalitis endemic area. Epidemiol Infect 136:1416–1424. doi:10.1017/S0950268807000039

Fish D, Childs JE (2009) Community-based prevention of Lyme disease and other tick-borne diseases through topical application of acaricide to white-tailed deer: background and rationale. Vector-Borne Zoonotic Dis 9:357–364. doi:10.1089=vbz.2009.0022

Fourie LJ, Kok DJ (1995) A comparison of *Ixodes rubicundus* infestations on Friesian and Bonsmara cattle in South Africa. Exp Appl Acarol 19:529–531

Fourie LJ, van Zyl JM (1991) Interspecific variations in attachment sites and density assessment in female *Ixodes rubicundus* (Acari: Ixodidae) on domestic and natural hosts. Exp Appl Acarol 13:1–10

Fourie LJ, Horak IG, van Zyl JM (1991) Sites of attachment and intraspecific infestation densities of the brown paralysis tick *Rhipicephalus punctatus* on Angora goats. Exp Appl Acarol 13:243–249

Fretwell SD, Lucas HL (1970) On territorial behaviour and other factors influencing habitat distribution in birds. I. Theoretical development. Acta Biotheor 19:16–36

Gern L, Rais O (1996) Efficient transmission of *Borrelia burgdorferi* between cofeeding *Ixodes ricinus* ticks (Acari: Ixodidae). J Med Entomol 33:189–192
Gern L, Estrada-Pena A, Frandsen F, Gray JS, Jaenson TGT, Jongejan F, Kahl O, Mehl R, Nuttall PA (1998) European reservoir hosts of *Borrelia burgdorferi* sensu lato. Zbl Bakt 287:196–204
Gilbert L, Jones LD, Hudson PJ, Gould EA (2000) Role of small mammals in the persistence of Louping-ill virus: field survey and tick co-feeding studies. Med Vet Entomol 14:277–282
Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: defining R_0 for tick-borne infections. Am Nat 171:743–754. doi:10.1086/587530
Healy JAE, Bourke P (2008) Aggregation in the tick *Ixodes ricinus* (Acari: Ixodidae): use and reuse of questing vantage points. J Med Entomol 45:222–228
Hudson PJ, Rizzoli A, Rosà R, Chemini C, Jones LD, Gould EA (2001) Tick-borne encephalitis virus in northern Italy: molecular analysis, relationships with density and seasonal dynamics of *Ixodes ricinus*. Med Vet Entomol 15:304–313
Hurlbert SH (1978) The measurement of niche overlap and some relatives. Ecology 59:67–77
Jaenson TG, Tälleklint L (1992) Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. J Med Entomol 29:813–817
Jensen PM, Hansen H, Frandsen F (2000) Spatial risk assessment for lyme borreliosis in Denmark. Scand J Infect Dis 32:545–550. doi:10.1080/003655400458857
Jones LD, Gaunt M, Hails RS, Laurenson K, Hudson PJ, Reid H, Henbest P, Gould EA (1997) Transmission of Louping ill virus between infected and uninfected ticks co-feeding on mountain hares. Med Vet Entomol 11:172–176
Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129(Suppl):S3–S14. doi:10.1017/S0031182004005967
Kiffner C, Lödige C, Alings M, Vor T, Rühe F (in press) Abundance estimation of Ixodes ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). Exp Appl Acarol. doi:10.1007/s10493-010-9341-4
Kimura K, Isogai E, Kamewaka Y, Nishikawa T, Ishii N, Fujii N (1995) Detection of Lyme-disease spirochetes in the skin of naturally infected wild Sika-deer (*Cervus nippon yesoensis*) by PCR. Appl Environ Microbiol 61:1641–1642
L'Hostis M, Diarra O, Seegers H (1994) Sites of attachment and density assessment of female *Ixodes ricinus* (Acari: Ixodidae) on dairy cows. Exp Appl Acarol 18:681–689
Labuda M, Nuttall PA, Kozuch O, Eleckova E, Zuffova E, Williams T, Sabo A (1993) Non-viraemic transmission of tick-borne encephalitis virus: a mechanism of arbovirus survival in nature. Experientia 49:802–805
Labuda M, Austyn JM, Zuffova E, Kozuch O, Fuchsberger N, Lysy I, Nuttall PA (1996) Importance of localized skin infection in tick-borne encephalitis virus transmission. Virology 219:357–406
Levins R (1968) Evolution in changing environments: some theoretical explanations. Princeton University Press, Princeton
Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, Vanwambeke SO, Lambin F (2007) Determinants of the geographic distribution of Puumula virus and Lyme borreliosis infections in Belgium. Int J H Geogr 6:15. doi:10.1186/1476-072x-6-15
Malandrin L, Jouglin M, Sun Y, Brisseau N, Chauvin A (2010) Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): Isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. Int J Parasitol 40:277–284. doi:10.1016/j.ijpara.2009.08.008
Mathee S, Meltzer DGA, Horak IG (1997) Sites of attachment and density assessment of ixodid ticks (Acari: Ixodidae) on impala (*Aepyceros melampus*). Exp Appl Acarol 21:179–192
Matuschka FR, Heiler M, Eiffert H, Fischer P, Lotter H, Spielman A (1993) Diversionary role of hoofed game in the transmission of Lyme disease spirochetes. Amer J Trop Med Hyg 48:693–699
Nelson WA, Keirans JE, Bell JF, Clifford CM (1975) Host-ectoparasite relationship. J Med Entomol 12:143–166
Ogden NH, Nuttal PA, Randolph SE (1997) Natural Lyme disease cycles maintained via sheep by co-feeding ticks. Parasitology 115:591–599
Ogden NH, Hailes RS, Nuttall PA (1998) Interstadial variation in the attachment sites of *Ixodes ricinus* ticks on sheep. Exp Appl Acarol 22:227–232
Ostfeld R, Keesing F (2001) Biodiversity and disease risk: the case of Lyme disease. Conserv Biol 14:722–728
Perkin SE, Cattadori IM, Tangliapietra V, Rizzoli AP, Hudson PJ (2003) Empirical evidence for key hosts in persistence of a tick-borne disease. Int J Parasitol 33:909–917. doi:10.1016/S0020-7519(03)00128-0
Petritian AM, von Lüpke B, Petritan IC (2007) Effects of shade on growth and mortality of maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) saplings. Forestry 80:397–412. doi:10.1093/forestry/cpm030

Pfäffle M, Petney T, Elgas M, Skubulla J, Taraschewski H (2009) Tick-induced blood loss leads to regenerative anaemia in the European hedgehog (*Erinaceus europaeus*). Parasitology 136:443–452. doi:10.1017/S0031182009005514

Pianka ER (1973) The structure of lizard communities. Annu Rev Ecol Evol S 4:53–74

Pound JM, Miller JA, George JE, Fish D, Caroll JF, Schulze TL, Daniels TJ, Falco RC, Stafford KC III, Mather TN (2009) The United States Department of Agriculture's Northeast Area-Wide Tick Control Project: summary and conclusions. Vector-Borne Zoonotic Dis 9:439–448. doi:10.1089=vbz.2008.0200

Randolph SE (1975) Patterns of distribution of the tick *Ixodes trianguliceps* Birula on its hosts. J Anim Ecol 44:451–474

Randolph SE (2008) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129(Suppl):S37–S65. doi:10.1017/S0031182004004925

Randolph SE, Miklisova D, Lysy J, Rogers DJ, Labuda M (1999) Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118:177–186

Randolph SE, Green RM, Peacy MF, Rogers DJ (2000) Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. Parasitology 121:15–23

Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R (2009) Forest Structure and roe deer abundance predict tick-borne encephalitis risk in Italy. PLoS ONE 4:e4336. doi:10.1371/journal.pone.0004336

Schmidtmann ET, Caroll JF, Watson DW (1998) Attachment site patterns of adult blacklegged ticks (Acari: Ixodidae) on white-tailed deer and horses. J Med Entomol 35:59–63

Sonenshine DE (2006) Tick pheromones and their use in tick control. Annu Rev Entomol 51:557–580. doi:10.1146/annurev.ento.51.110104.151150

Sutherland WJ (1996) From individual behaviour to Population Biology. Oxford University Press, Oxford

Vor T, Kiffner C, Hagedorn P, Niedrig M, Rühe F (2010 online only) Tick burden on European roe deer (*Capreolus Capreolus* L.). Exp Appl Acarol. doi:10.1007/s10493-010-9337-0

Walker AR, Alberdi MP, Urquhart KH, Rose H (2001) Risk factors in habitats of the tick *Ixodes ricinus* influencing human exposure to *Ehrlichia phagocytophila* bacteria. Med Vet Entomol 15:40–49

Wang H, Hails RS, Cui WW, Nuttal PA (2001) Feeding aggregation of the tick *Rhipicephalus appendiculatus* (Ixodidae): benefits and costs in the contest with host responses. Parasitology 123:447–453. doi:10.1017/S0031182001008654

Chapter 7 – Body-mass or sex-biased tick parasitism in roe deer (*Capreolus capreolus*)? A GAMLSS approach

TITLE PAGE

Body-mass or sex-biased tick parasitism in roe deer (*Capreolus capreolus*)? A GAMLSS approach

C. Kiffner[1*], C. Lödige[1], M. Alings[1], T. Vor[1] and F. Rühe[1]

[1]Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany

Running title: Modelling tick burden on roe deer

[*]Corresponding Author: Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany. Tel: +49 551 393583. Fax: +49 551 3922089. e-mail: ckiffne@gwdg.de

e- mail C. Lödige: christinaloedige@hotmail.com

e- mail M. Alings: efrafa@web.de

e- mail T. Vor: tvor@gwdg.de

e- mail F. Rühe: fruehe@gwdg.de

Abstract. Macroparasites feeding on wildlife hosts follow skewed distributions for which basic statistical approaches are of limited use. To predict *Ixodes* spp. tick burden on roe deer, we applied Generalized Additive Models for Location, Scale and Shape (GAMLSS) which allow incorporating a variable term describing the dispersion. We analysed tick burden of 78 roe deer, sampled in a forest region of Germany over a period of 20 months. Assuming a negative binomial error distribution and controlling for ambient temperature, we analysed whether host sex and body mass affected individual tick burdens. Models for larval and nymphal tick burden included host sex, with male hosts being more heavily infested than female ones. However, the influence of host sex on immature tick burden was associated with wide standard errors (nymphs) or the factor was marginally significant (larvae). Adult tick burden was positively correlated with host body mass. Overall, if controlled for host body mass and ambient temperature, there is weak support for sex-biased parasitism in this system. Compared to models which assume linear relationships, GAMLSS provided a better fit. Adding a variable dispersion term improved only one of the four models. Yet, the potential of modelling dispersion as a function of other variables appears promising for larger host-parasite datasets.

Key words. Aggregation, Cervidae, ecto-parasite, *Ixodes ricinus*, parasitism, seasonality, tick-borne disease, sex-bias

Introduction

A central task of parasitological research is the identification of those individuals that harbour the majority of the parasites. Most host-parasite including host-pathogen systems are characterised by heterogeneities among individuals with regard to the probability of being exposed to, being infected with and to further transmit parasites or pathogens (Lloyd-Smith *et al.*, 2005). This individual heterogeneity results in highly skewed parasite distributions within their host populations (Shaw *et al.*, 1998). In several of these biological systems, key host groups which contribute disproportional to the parasite burden or to the spread of a pathogen had been identified (e.g. Poulin, 1996; McCurdy *et al.*, 1998; Perkins *et al.*, 2003; Ferrari *et al.*, 2004). These key groups often represent ~20% of the entire population and account for a large fraction (~80%) of the parasite burden or pathogen transmission, which is often referred to as the '20/80 Rule' (Woolhouse *et al.*, 1997). Recent investigations indicated that parasitism is often sex-biased with males being more heavily infested than females, likely caused by either the immunosuppressive effect of testosterone and/or sex-specific host behaviour favouring exposure to parasites (Poulin, 1996; Zuk & McKean, 1996; McCurdy *et al.*, 1998; Ferrari *et al.*, 2004). However, as parasite abundance/biomass is scaled with host body mass (Poulin & George-Nascimento, 2007), the susceptibility of males to parasitism might solely reflect their greater body size (Moore & Wilson, 2002) and should thus be taken into account in models aiming to predict parasite abundance. There is empirical evidence that parasitism is positively correlated with mortality rates in free ranging mammal populations (e.g. Moore & Wilson, 2002). Thus factors affecting parasitism might also affect vital rates and ultimately population dynamics of wildlife populations.

Approaches to disentangle effects of single variables are often associated with some limitations. Conventional approaches often assume linear relationships between a linear predictor and the response variable and in the case of count data, often assume a constant dispersion parameter (Shaw *et al.*, 1998). The dispersion of parasites within the host population is, however, of central interest in parasitological and infectious disease research (Lloyd-Smith *et al.*, 2005). In contrast to General Additive (GAM) or General Linear models (GLM), Generalized Additive Models for Location, Scale and Shape (GAMLSS) allow modelling not only the mean but also other parameters of the chosen distribution (such as the dispersion) of the response variable as linear parametric and/or additive non-

parametric functions of explanatory variables (Rigby & Stasinopoulos, 2005; Stasinopoulos & Rigby, 2007). In this paper we use this approach for identifying and quantifying factors affecting individual parasite burden in a roe deer-tick system of central Germany. Since *Ixodes ricinus*, the dominant tick in central Europe (Hillyard, 1996) shows a distinct seasonal activity pattern whereas the probability that a tick actively quests for a host is largely influenced by ambient temperature (Randolph, 2004), attempts to identify additional risk factors necessarily need to control for ambient temperature. In doing so, we tested whether tick burden of individual roe deer was affected by host sex and/or host body mass and discuss the results with respect to the biological significance and the methodological approach.

Materials and Methods

Tick sampling and data pre-processing

We opportunistically sampled hunted roe deer from forests east and northeast of Göttingen (centred at, 51° 32′ 2″ N, 9° 56′ 8″, radius of ca. 20 km), central Germany during regular hunting activities over a period lasting from winter 2007 to summer 2009 (overall 78 individuals with complete data). Open seasons for adult does and bucks only partly overlapped. In order to get a more balanced sample, we also sampled ticks from accidentally killed roe deer (e.g. roadkills) of the opposite sex during non-overlapping hunting periods. The study area is dominated by mixed deciduous forests; the altitude above sea level ranges from 151 to 400 m, the mean annual rainfall is 780 mm, and the average annual temperature is 7.8°C (Petritan *et al*., 2007). We counted the total number of ticks found on the entire body of the roe deer (stage and sex specific) and used these figures as response variables in the modelling approach. Due to the large number of ticks (larvae=862, nymphs=1903, males=670, females=1708), we did not identify each individual tick to the species level. However, sample individuals determined to species level were all identified as *Ixodes ricinus*. For a more detailed description of the study site and the tick collection see Kiffner *et al*. (2010). Disembowelled roe deer carcasses were weighed to the nearest 500 g using either a spring scale or a beam balance. According to Poulin & George-Nascimento (2007), body mass was converted by raising it to the power of 0.75 because this conversion would lead to a physiologically expected linear relationship with parasite

biomass. Since we were mainly interested in the stage specific tick abundance, we made no efforts to convert tick burden into parasite biomass. We calculated ambient temperature [average mean temperature of the three days before and the day the roe deer was shot- a period that corresponds well to the average attachment duration of the studied tick-species (Hillyard 1996)] from data of a weather station located in the study area (www.wetterstation-goettingen.de).

Statistical modelling approach

Due to the flexibility of GAMLSS, several model selection steps are required. The link function (log) for both mean and dispersion was kept constant throughout the procedures. The 'RS' algorithm (explained in detail in Rigby & Stasinopoulos, 2005) was employed to fit the model using maximum likelihood estimation. Initially, we tested whether the distribution of the response variables were better described by a Poisson or a negative binomial distribution (Shaw *et al*., 1998). Based on values of the Akaike Information Criterion, the negative binomial distribution (type I) described all tick-datasets better than a Poisson distribution and hence used. As the tick burden is mainly influenced by ambient temperature, we always kept the variable temperature in the models and considered a cubic spline (cs) function with varying degrees of freedom to model the relationship between mean tick burden and temperature. Using the 'find.hyper'-function, which selects the degrees of freedom based on values of the Generalised Akaike Information Criterion (GAIC, penalty term k=2.5), we selected the appropriate degrees of freedom. Since we also assumed heterogeneity in the dispersion, we attempted to estimate it as a cubic spline function of the ambient temperature. Degrees of freedom were again chosen based on GAIC-values. Due to the data transformation based on biological theory, we expected that body mass should increase linearly with parasite burden. We fitted full models (temperature+host sex+ body mass) and more simple models (temperature+one additional explanatory variable) and used the sample size corrected AIC_C-value (Burnham & Anderson 2002) to select the most parsimonious model. Finally, we compared the GAMLSS approach with a conventional GLM approach. All calculations were performed with the GAMLSS-package in *R* (R Development Core Team, 2005).

Results

Larval, nymphal, female and male ticks were present on 45, 81, 77 and 60% of the sampled hosts, respectively. Mean tick abundance per host was 11 larvae, 24 nymphs, 22 females and 9 males. The data indicated that the distribution of ticks on roe deer was considerable over-dispersed with variance/mean ratios ranging from 22 (males)-130 (larvae) and *k*-values (the inverse measure of aggregation) of 0.09 (larvae), 0.73 (nymphs), 0.50 (females) and 0.41 (males). All tick life stages showed a non-linear, positive relationship with ambient temperature (Fig.1) with few counts at low temperatures. Tick abundance appeared to peak at ambient temperatures of ca. 14°C and then to decline with further temperature increase. Thus, all models describing individual tick burdens contained a non-linear function describing the influence of ambient temperature.

Using an information-theoretic approach based on AIC_C-values (Table 1), the best models for the immature tick life stages (larvae and nymphs) additionally contained the sex of the roe deer host, with male roe deer individuals being slightly more heavily infested than female roe deer. The larvae model was, however, rather imprecise with regard to the standard error of the intercept (Table 2) and the standard error estimates for the factor sex (Fig.2(A)). In the model for nymphs, the factor host sex was only marginally significant whereas the intercept was insignificant (Table 2), suggesting a cautious handling of these predictions (Fig.2(C), (D)). The models selected for the adult ticks contained ambient temperature and the converted body mass however, it did not contain the sex of the host individual (Table 1). Body mass correlated positively with numbers of male and female ticks whereas this relationship was stronger (Table 2) for male ticks compared to female ticks (Fig.2(E)-(H)). Overall, the models lend only limited support to the sex-bias hypothesis and rather support the hypothesis that individuals with more body mass can support more parasites.

Compared to a model with a constant dispersion term, the nymph model which included a temperature dependent dispersion term was improved considerably (ΔAIC_C=16.4). Fits of the other models were reduced when including a variable dispersion parameter (ΔAIC_C ~10), probably because the terms for the variable dispersion parameter were insignificant (Table 3). Compared to conventional GLM approaches which assume linear or squared relationships and assume a constant variance, the GAMLSS approach provided an improved fit to the data (Table 4), with ΔAIC_C values ≥28.

Overall, the presented models showed an adequate fit to the data. The residuals of the larvae model showed a mean near zero (0.0691), their variance approximated one (0.987) and their coefficient of kurtosis was close to three (3.301). This was also true for the residuals of the nymph model (mean= -0.054, variance= 0.9960, kurtosis= 3.510), of the male model (mean= 0.060, variance= 1.001, kurtosis= 2.744) and for the residuals of the female model (mean= 0.037, variance= 0.9740, kurtosis= 2.289). Thus, the residuals of the final models were approximately normally distributed. Graphically, this is underlined as all observations, except for a few larvae counts, were within the 'acceptance' region inside the two elliptic curves of the worm-plots (Fig.3(A)-(D)).

Discussion

Similar to other parasites and infectious disease agents (Shaw *et al.*, 1998; Altizer et al., 2006), *Ixodes* ticks showed a considerable temperature driven abundance pattern (Randolph, 2004). The observed non-linear relationship between tick activity/abundance and temperature with peaks at intermediate temperatures largely reflects the bi-modal seasonal activity pattern of *Ixodes ricinus* which is characterised by activity peaks in early summer and autumn and a reduced activity during the hot mid-summer months when dehydration might be a limiting factor (Randolph, 2004).

By controlling for the non-linear effect of temperature with a cubic spline function, there is only weak indication that host sex affects immature tick burden on roe deer. Adult tick burden was not affected by host sex and correlated positively with host body mass.

Immunosuppresion, exposure bias or body mass?

Several authors (e.g. Schulze *et al.*, 1984; Kitron *et al.*, 1992; Schmidtmann *et al.*, 1998) reported male biased tick parasitism in another cervid, the white-tailed deer (*Odocoileus virginianus*). This deer species, however, shows a more pronounced sexual size dimorphism in adults than roe deer (von Raesfeld, 1985; Whitehead, 1993; Geist, 1998) and thus male-biased parasitism might essentially be an effect of sexual size dimorphism. Furthermore, male-biased tick burden was only observed in fall, during the rutting season (Schulze *et al.*, 1984; Schmidtmann *et al.*, 1998). In male deer, the rutting season usually coincides both with increasing movements (e.g. Flint & Krzywinski, 1997) and with

elevated testosterone levels (e.g. Bubenik *et al.*, 1983; Blottner *et al.*, 1996) which impedes inferring correlation or even causality to one of the factors. However, in red deer (*Cervus elaphus*) no significant relationship was found between testosterone level and tick burden [genera *Hyalomma* and *Rhipicephalus* (Malo *et al.*, 2009)] which might favour the 'exposure' hypothesis over the 'immunosuppresion' hypothesis for explaining slightly male-biased tick burden in roe deer found in this study. In a different study area in southern Germany, Vor *et al.* (2010) found no evidence for male-biased tick parasitism on roe deer. The present study also reveals only slight effects of sex on immature tick burden only, and broadly confirms that host sex *per se* plays a rather minor role for parasitism (Schalk & Forbes, 1997).

Effects of tick parasitism

Tick parasitism might affect roe deer both in a direct and an indirect way. High tick burdens might induce anaemia which in turn might reduce individual fitness (Tälleklint & Jaenson 1997; Pfäffle *et al.*, 2009). Adult tick burden scaled with host body mass, whereas the scaling factor was higher in male than in female ticks, which is consistent with findings by Vor *et al.* (2010). The scaling factor is below the expected value of 1 (Poulin & George-Nascimento, 2007) which is not surprising, as the expected value is considered as a maximum value and is based on total parasite biomass which, for roe deer might include additional ecto-parasite species (von Raesfeld, 1985). .High levels of tick infections might enhance the infection probability of tick-borne diseases and thus might involve indirect costs for the host individual. Among the tick-borne disease agents (Jongejan & Uilenberg, 2004), at least *Anaplasma phagocytophilum* (Woldehiwet, 2010), and *Babesia* parasites (Malandrin *et al.*, 2009) naturally infect free ranging cervids in Europe. These infections may cause severe clinical symptoms in roe deer (*A. phagocytophilum*: severe febrile reaction, bacteraemia and leukopenia; *B. divergens*-like: anaemia and severe morbidity) and may, in the case of *B. divergens*-like parasites be fatal (Müller & Rapp, 1971; Langton *et al.*, 2003). Based on findings from our study, increased body mass in roe deer might thus be associated with a potentially high direct and indirect cost.

The modelling approach

The conventional negative binomial additive model, which fits a constant scale parameter, is not flexible enough for the data of the nymph model. Here, the variable dispersion parameter clearly improved the model fit. In contrast, for the models explaining larval and adult tick burden, inclusion of a flexible dispersion parameter reduced the fit compared to models with a constant dispersion parameter. Likely, the reduction of the fit is caused by low sample size relative to the degrees of freedom which are required to model the relationship between temperature and the dispersion factor (e.g. using AIC instead of AIC_C only reduces the fit by $\Delta AIC_C \sim 1$).

In the case of count data, the proposed approach allows modelling a flexible dispersion parameter which is likely to be influenced by the same parameters which influence the mean of a distribution (Shaw *et al.*, 1998). A further main advantage of the GAMLSS approach is that it adequately models the non-linear relationship between a driving factor and parasite activity/abundance. This might be essential in situations in which parasitic or infectious disease systems are subject to dramatic or disruptive changes (e.g. Hudson *et al.*, 2006; Bompangue *et al.*, 2009). In such cases, GAMLSS offer a straightforward and relatively easy approach which is superior to conventional (e.g. Wilson et al., 1996) modelling approaches.

Acknowledgements

Acting as representative for all hunters and forest rangers we would like to thank J. Berger, D. Birke, E. Kreysern, W. Mänz, M. Scholz, and R. Zietlow for making the roe deer available for this study. We sincerely thank K. Hammer, H. Lippe, A. and M. Lödige, M. Scholz and several other students for their help during the field work. We sincerely thank W. Zucchini for recommending the GAMLSS procedure and for helpful comments on an earlier draft. Further more, we would like to thank two anonymous reviewers for constructive comments and J. Parisi for improving the English. This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).

References

Altizer, S., Dobson, A., Hudson, P., Pascual, M. & Rohani, P. (2006) Seasonality and the dynamics of infectious diseases. *Ecology Letters*, **9**, 467-484.

Blottner, S., Hingst, O., Meyer, H.H.D. (1996) Seasonal spermatogenesis and testosterone production in roe deer (*Capreolus capreolus*). *Journal of Reproduction and Fertility*, 108, 299-305.

Bompangue, D., Giraudoux, P., Piarroux, M., Mutombo, G., Shamavu, R., Sudre, B., Mutombo, A., Mondonge, V. & Piarroux, R. (2009) Cholera Epidemics, War and Disasters around Goma and Lake Kivu: An Eight-Year Survey. *PLoS Neglected Tropical Diseases*, **3**: e436.

Bubenik, G.A., Bubenik, A.B., Schams, D. & Leatherland, J.F. (1983) Circadian and circannual rhythms of LH, FSH, Testosterone (T), Proloctin, Cortisol, T_3 and T_4 in plasma of mature, male white-tailed deer. *Comparative Biochemistry and Physiology A- Physiology*, 76, 37–45.

Burnham, K.P. &Anderson, D.R. (2002) Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer Verlag, New York USA

Ferrari, N., Cattadori, I.M., Nespereira, J., Rizzoli, A. & Hudson, P.J. (2004) The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse *Apodemus flavicollis*. *Ecology Letters*, **7**, 88–94.

Flint, A.P.F. & Krzywinski, A. (1997) Sex differences in time budgeting in roe deer during the rut. *Acta Theriologica*, **42**, 313-320.

Geist, V. (1998) *Deer of the world: their evolution, behaviour, and ecology*. Stackpole Books, Mechanicsburg, USA.

Hillyard, P.D. (1996) *Ticks of north-west Europe*. Dorset Press, Dorchester, UK.

Hudson, P.J., Cattadori, M., Boag, B. & Dobson, A.P. (2006) Climate disruption and parasite-host dynamics: patterns and processes associated with warming and the frequency of extreme climatic events. *Journal of Helminthology*, **80**, 175-182.

Jongejan, F. & Uilenberg, G. (2004) The global importance of ticks. *Parasitology*, **129**, S3-S14.

Kiffner, C., Lödige, C., Alings, M., Vor, T. & Rühe, F. (2010) Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology*, doi: 10.1007/s10493-010-9341-4.

Kitron, U., Jones, C.J., Bouseman, J.K., Nelson, J.A. & Baumgartner, D.L. (1992) Spatial analysis of the distribution of *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Ogle County, Illinois. *Journal of Medical Entomology*, **29**, 259-266.

Langton, C., Gray, J.S., Waters, P.F. & Holman, P.J. (2003) Naturally acquired babesiosis in a reindeer (*Rangifer tarandus tarandus*) herd in Great Britain. *Parasitology Research*, **89**, 194–198.

Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E. & Getz, W.M. (2005) Superspreading and the effect of individual variation on disease emergence. *Nature*, **438**, 355–359.

Malandrin, L., Jouglin, M., Sun, Y., Brisseau, N. & Chauvin, A. (2010) Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): Isolation, cultivation, host specificity, molecular characterisation and differentiation from *Babesia divergens*. *International Journal for Parasitology*, **40**, 277-284.

Malo, A.F., Roldan, E.R.S., Garde, J.J., Soler, A.J., Vicente, J., Gortazar, C. & Gomendio, M. (2009) What does testosterone do for red deer males? *Proceedings of the Royal Society B-Biological Sciences*, **276**, 971-980.

McCurdy, D.G., Shutler, D., Mullie, A. & Forbes, M.R. (1998) Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. *Oikos*, **82**, 303-312.

Moore, S.L. & Wilson, K. (2002) Parasites as a viability cost of sexual selection in natural populations of mammals. *Science*, **297**, 2015-2018.

Müller, B. & Rapp, J. (1971) Babesiose als Todesursache bei einem Rehkitz. *Tierärztliche Umschau*, **6**, 314–315.

Petritan, A.M., von Lüpke, B. & Petritan, I.C. (2007) Effects of shade on growth and mortality of maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) saplings. *Forestry*, **80**, 397–412.

Perkins, S.E., Cattadori, I.M., Tagliapietra, V., Rizzoli, A.P. & Hudson, P.J. (2003) Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology*, **33**, 909-917.

Pfäffle, M., Petney, T., Elgas, M., Skuballa, J. & Taraschewski, H. (2009) Tick-induced blood loss leads to regenerative anaemia in the European hedgehog (*Erinaceus europeaus*). *Parasitology*, **136**, 443-452.

Poulin, R. (1996) Sexual inequalities in helminth infections: a cost of being males? *American Naturalist*, **147**, 287–295

Poulin, R. & George-Nascimento, M. (2007) The scaling of total parasite biomass with host body mass. *International Journal for Parasitology*, **37**, 359-364.

R Development Core Team (2005): *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org

Randolph, S.E. (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology*, **129**, S37-S65.

Rigby, R.A. & Stasinopoulos, D.M. (2005) Generalized additive models for location, scale and shape (with discussion). *Applied Statistics*, **54**, 507–554.

Schalk, G. & Forbes, M.R. (1997) Male biases in parasitim of mammals: effects of study type, host age, and parasite taxon. *Oikos*, **78**, 67-74.

Schmidtmann, E.T., Carroll, J.F. & Watson, D.W. (1998) Attachment-site patterns of adult blacklegged ticks (Acari: Ixodidae) on white-tailed deer and horses. *Journal of Medical Entomology*, **35**, 59-63.

Schulze, T.L., Lakat, M.F., Bowen, G.S., Parkin, W.E. & Shisler, J.K. (1984) *Ixodes dammini* (Acari: Ixodidae) and other ixiodid ticks collected from white-tailed deer in New Jersey, USA. 1. Geographical distribution and its relation to selected environmental and physical factors. *Journal of Medical Entomology*, **21**, 741-749.

Shaw, D.J., Grenfell, B.T. & Dobson, A.P. (1998) Patterns of macroparasite aggregation in wildlife host populations. *Parasitology*, **117**, 597–610.

Stasinopoulos, D.M. & Rigby, R.A. (2007) Generalized Additive Models for Location Scale and Shape (GAMLSS) in R. *Journal of Statistical Software*, **23(**7), 1-46.

Stuen, S. (2007) *Anaplasma phagocytophylum* - the most widespread tick-borne infection in animals in Europe. *Veterinary Research Communications*, **31**, 79-84.

Tälleklint, L. & Jaenson, T.G.T (1997) Infestation of mammals by Ixodes ricinus ticks (Acari: Ixodidae) in south-central Sweden. *Experimental and Applied Acarology*, **21**, 755-771.

von Raesfeld, F. (1985) *Das Rehwild: Naturgeschichte, Hege u. Jagd*. Parey Verlag, Berlin, Germany.

Vor, T., Kiffner, C., Hagedorn, P., Niedrig, M. & Rühe F. (2010) Tick burden on European roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology*, doi: 10.1007/s10493-010-9337-0.

Whitehead, G.K. (1993) *The Whitehead Encyclopdia of Deer*. Swan Hill Press, Shrewsbury, UK.

Wilson, K., Grenfell, B.T. & Shaw, D.J. (1996) Analysis of aggregated parasite distributions: A comparison of methods. *Functional Ecology*, **10**, 592-601.

Woldehiwet, Z. (2010) The natural history of *Anaplasma phagocytophylum*. *Veterinary Parsitology*, **167**, 108-122.

Woolhouse, M.E.J., Dye, C., Etard, F.-F., Smith, T., Charlowood, J.D., Garnett, G.P., Haga, P., Hii, J.K.L., Ndhlovu, Quinnel, R.J., Watts, C.H., Chandiwana, S.K. & Anderson, R.M. (1997) Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. *Proceedings of the National Academy of Sciences of the USA*, **94**, 338-342.

Zuk, M. & McKean, K.A. (1996) Sex differences in parasite infections: Patterns and processes. *International Journal for Parasitology*, 26, 1009-1023.

Figures

Fig. 1. Mean larval (A), nymphal (B), male (C) and female (D) tick (*Ixodes* spp.) burden of roe deer (*Capreolus capreolus*) from the forests of Göttingen, Germany modelled as a cubic spline smoothing function of ambient temperature.

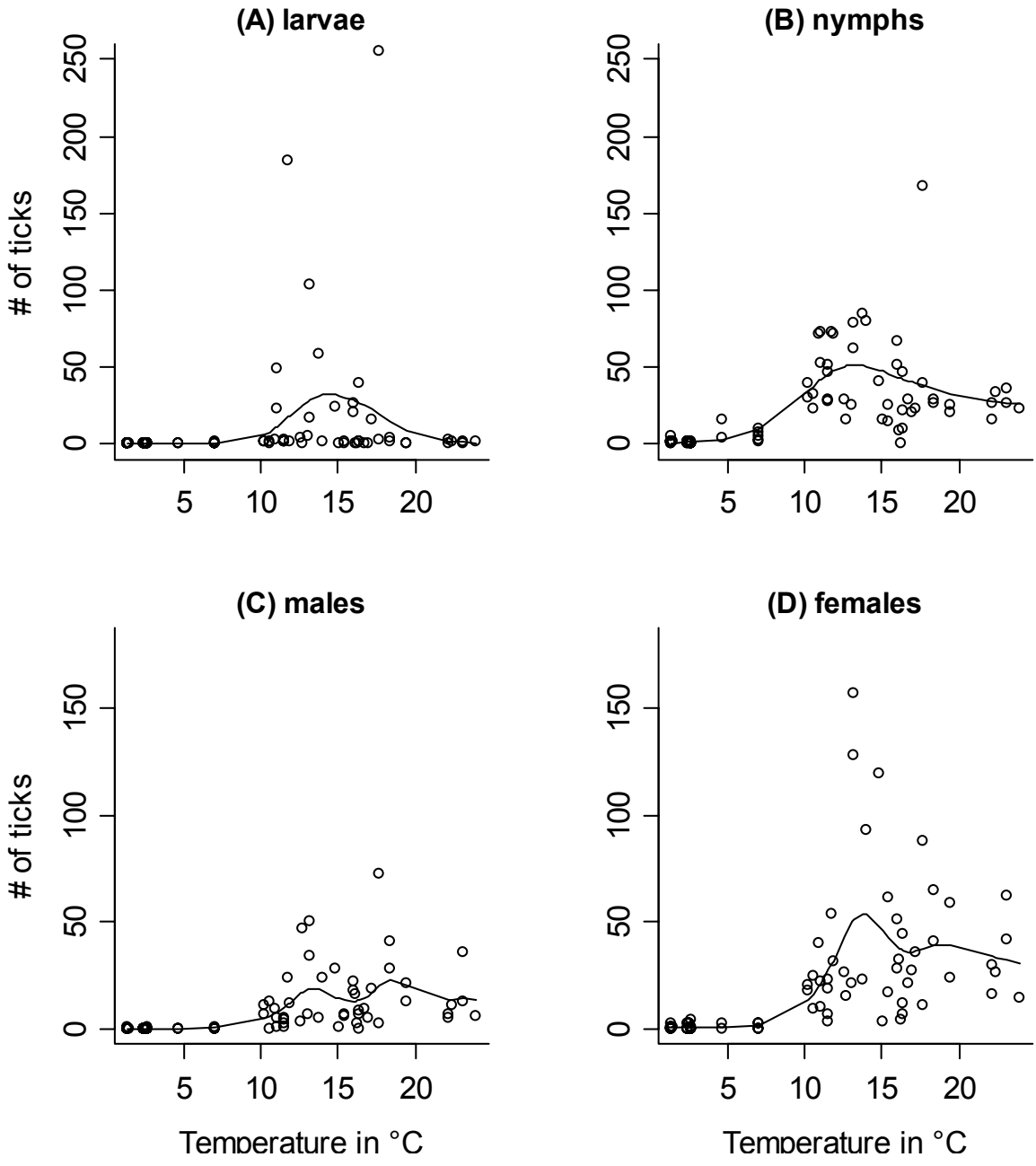


Fig. 2. Additive plots for the partial effects of the larval (A)-(B), nymphal (C)-(D), male (E)-(F) and female (G)-(H) tick model, respectively. See Table 3 for the parameter estimates. Dashed lines indicate standard errors.

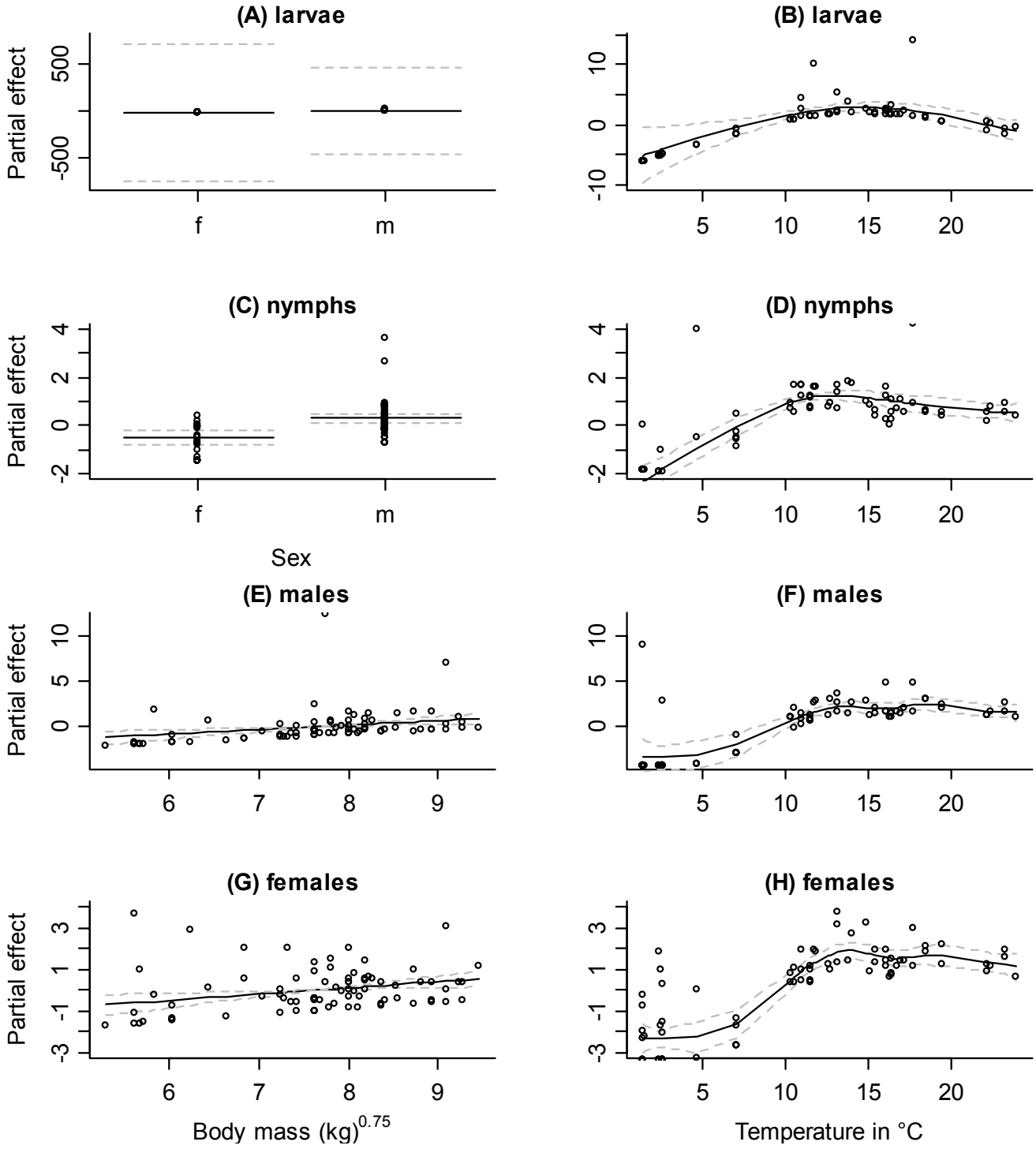
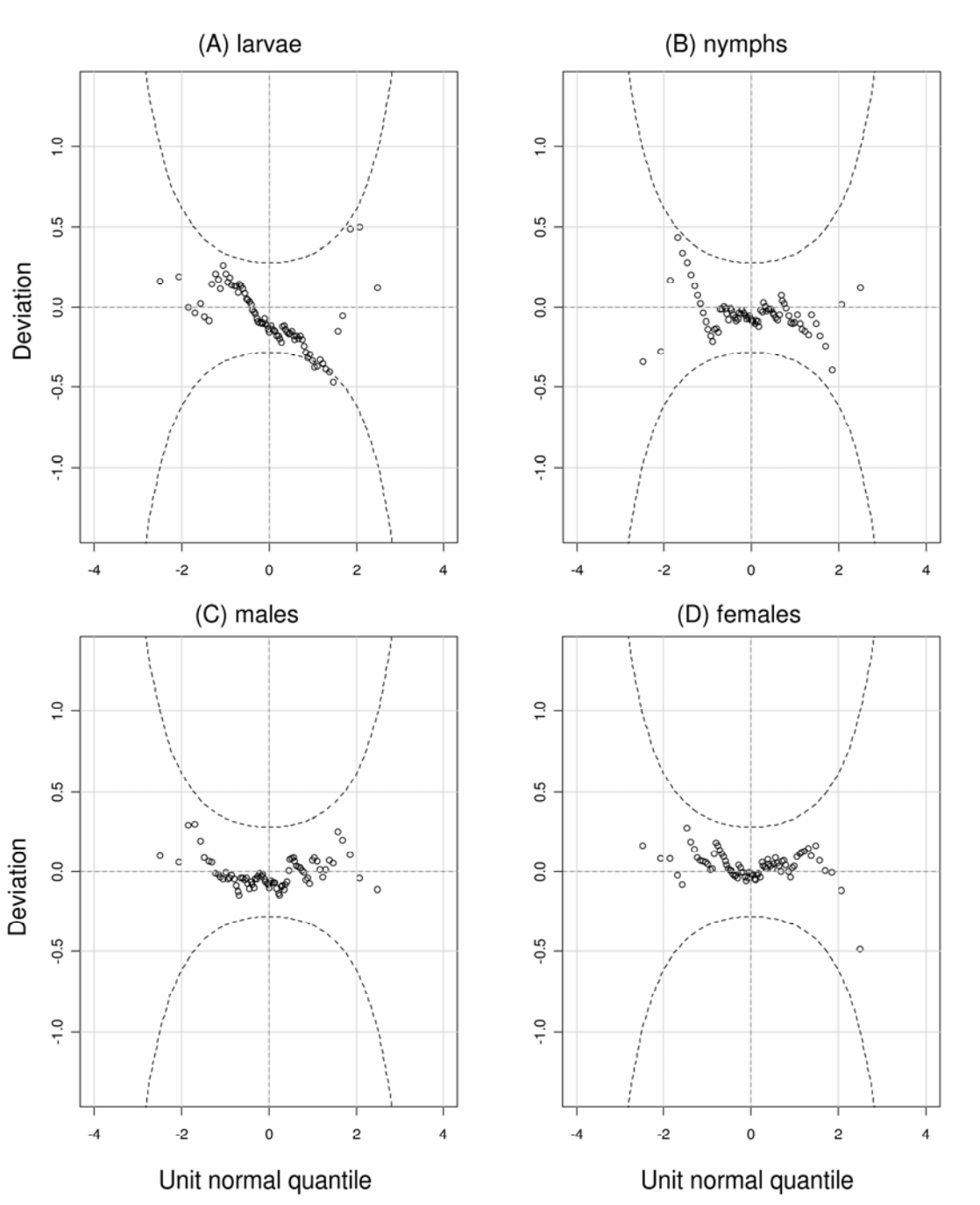


Fig. 3. Worm-plots (de-trended QQ-plots) for the larval (A), nymphal (B), male (C) and female (D) tick model, respectively.



Tables

Table 1. Degrees of freedom (DF) and AIC_C-values for the full models and simplified models explaining tick burden on roe deer. Explanatory variables were ambient temperature, host body mass $(kg)^{0.75}$ (BM) and host sex (Sex). The selected models are highlighted in bold. All models include an additive term for the dispersion (see Tab.3).

	Larvae		Nymphs		Males		Females	
Model	DF	AIC_C	DF	AIC_C	DF	AIC_C	DF	AIC_C
y = Sex+BM+cs(Temperature)	9.1	302.0	12.3	521.4	10.4	374.1	9.9	507.4
y = Sex+cs(Temperature)	**8.1**	**299.2**	**11.3**	**520.2**	11.4	376.7	10.9	509.2
y = BM+cs(Temperature)	8.1	312.1	11.3	526.5	**11.4**	**369.7**	**10.9**	**503.8**
y = cs(Temperature)	7.1	309.7	10.3	525.3	10.4	374.1	9.9	507.4

Table 2. Parameter estimates of the selected models explaining mean tick burden on roe deer. Significance codes: '<0.1,*<0.05, **<0.01, ***<0.001. All models include an additive term for the dispersion (see Tab.3).

Model	Intercept (±Standard error)	Formula and degrees of freedom Temperature (±Standard error)	Host sex (male compared to female)	Body mass$^{0.75}$
Larvae	-20.9433(±598.9420)***	cs(Temperature, df = 2.1) 0.3064(±0.05321)***	18.4125(±598.9416)***	Not included
Nymphs	0.2851(±0.5240)	cs(Temperature, df = 2.8) 0.1592(±0.0500)**	0.7982(±0.4152)'	Not included
Males	-6.3331(±2.3456)**	cs(Temperature, df = 5.4) 0.2967(±0.0936)**	Not included	0.5088(±0.2289)*
Females	-2.6812(±0.8562)***	cs(Temperature, df = 4.9) 0.2287(±0.0140)***	Not included	0.3044(±0.1074)***

Table 3. Parameter estimates for the dispersion terms of the selected models. Significance codes: '<0.1,*<0.05, **<0.01, ***<0.001.

Model	Intercept (±Standard error)	Formula and degrees of freedom Temperature (±Standard error)
Larvae	1.3690 (±1.3780)	cs(Temperature, df = 1) -0.0173(±0.0859)
Nymphs	0.9814(±0.5410)'	cs(Temperature, df = 3.5) 0.1095(±0.04375)*
Males	0.0554(±1.4690)	cs(Temperature, df = 1) -0.0232±(0.0933)
Females	-0.7641(±1.0998)	cs(Temperature, df = 1) 0.0125(±0.0734)

Table 4. Degrees of freedom (DF) and AIC_C -values for a range of models explaining tick burden on roe deer. In GLM (1) temperature was entered as a linear predictor in a conventional linear model (GLM), in GLM (2) temperature was entered as a squared term in a conventional GLM. The explanatory variables entered in each model were temperature (all models; in the GAMLSS approach modelled as a function of a cubic spline with specified degrees of freedom and including an additive term for modelling the dispersion as a cubic spline of temperature), sex of roe deer (larvae and nymph model only) and body $mass^{0.75}$ (male and female model only).

	Larvae		Nymphs		Males		Females	
	DF	AIC_C	DF	AIC_C	DF	AIC_C	DF	AIC_C
GLM (1)	4	336.0	4	583.0	4	397.7	4	543.9
GLM (2)	4	349.9	4	599.0	4	421.7	4	572.0
GAMLSS	8.1	299.2	11.3	520.2	11.4	369.7	10.9	503.8

Chapter 8 – Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany

Parasitol Res
DOI 10.1007/s00436-010-2065-x

ORIGINAL PAPER

Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany

Christian Kiffner · Torsten Vor · Peter Hagedorn · Matthias Niedrig · Ferdinand Rühe

Received: 16 July 2010 / Accepted: 10 September 2010

Abstract Identifying factors affecting individual vector burdens is essential for understanding infectious disease systems. Drawing upon data of a rodent monitoring programme conducted in nine different forest patches in southern Hesse, Germany, we developed models which predict tick (*Ixodes* spp. and *Dermacentor* spp.) burdens on two rodent species *Apodemus flavicollis* and *Myodes glareolus*. Models for the two rodent species were broadly similar but differed in some aspects. Patterns of *Ixodes* spp. burdens were influenced by extrinsic factors such as season, unexplained spatial variation (both species), relative humidity and vegetation cover (*A. flavicollis*). We found support for the 'body mass' (tick burdens increase with body mass/age) and for the 'dilution' hypothesis (tick burdens decline with increasing rodent densities) and little support for the 'sex-bias' hypothesis (both species). Surprisingly, roe deer densities were not correlated with larvae counts on rodents. Factors influencing the mean burden did not significantly explain the observed dispersion of tick counts. Co-feeding aggregations, which are essential for tick-borne disease transmission, were mainly found in *A. flavicollis* of high body mass trapped in areas with fast increase in spring temperatures. Locally, *Dermacentor* spp. appears to be an important parasite on *A. flavicollis* and *M. glareolus*. *Dermacentor* spp. was rather confined to areas with higher average temperatures during the vegetation period. Nymphs of *Dermacentor* spp. mainly fed on *M. glareolus* and were seldom found on *A. flavicollis*. Whereas *Ixodes* spp. is the dominant tick genus in woodlands of our study area, the distribution and epidemiological role of *Dermacentor* spp. should be monitored closely.

C. Kiffner (✉) · T. Vor · F. Rühe
Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen,
Büsgenweg 3,
37077 Göttingen, Germany
e-mail: ckiffne@gwdg.de

P. Hagedorn · M. Niedrig
Centre for Biological Safety, Robert Koch-Institut,
Nordufer 20,
13353 Berlin, Germany

Introduction

Rodents are important hosts for the immature stages of hard ticks, and when taking a blood meal, ticks may transmit a range of tick-borne disease agents of medical and veterinary significance. In central Europe, ticks of the *Ixodes ricinus* complex can be infected with and subsequently transmit pathogens such as *Borrelia burgdorferi* spirochaetes, gram-negative bacteria of the family *Anaplasmataceae* and *Rickettsiaceae* and tick-borne encephalitis virus (Kurtenbach et al. 2002; Labuda and Nuttall 2004; Parola et al. 2005). Ticks of the genus *Dermacentor*, especially *D. reticulatus*, appear to expand their range in Germany (Dautel et al. 2006). Ticks of this genus are competent vectors of *B. burgdorferi*, rickettsia bacteria and tick-borne encephalitis virus and may also transmit protozoan piroplasms (Kahl et al. 1992; Randolph et al. 1996; Jongejan and Uilenberg 2004).

A key element for quantifying transmission rates of these pathogens is the vector burden on host individuals. Specifically, not only the mean per capita burden but also the level of aggregation within the population affects the intrinsic growth rate R_0 of a pathogen (Woolhouse et al. 1997; Hartemink et al. 2008). Tick-host systems and indeed most host–parasite systems are characterised by heterogeneities with respect to the probability of hosts being exposed to parasites and in

Published online: 28 September 2010

turn to spread these among the population (Shaw et al. 1998; Lloyd-Smith et al. 2005). Parasite distributions within their host populations are generally most adequately described by a negative binomial distribution, characterised by the mean (μ) and a dispersion parameter (σ) (Shaw et al. 1998). The level of parasite aggregation is of crucial importance because the intrinsic growth rate of a pathogen R_0 increases with the degree of parasite aggregation (Woolhouse et al. 1997). The observed level of aggregation is often in accordance with the '80/20' rule, where a fraction of approximately 20% of the population is responsible for approximately 80% of the disease transmission (Woolhouse et al. 1997; Perkins et al. 2003). Identifying characteristics of this rather small proportion of hosts which are responsible for the majority of disease spread is a central task for parasitological research and for designing effective control mechanisms (Woolhouse et al. 1997; Perkins et al. 2003; Lloyd-Smith et al. 2005). For the two tick-borne disease agents of major medical importance, *B. burgdorferi*, and especially for tick-borne encephalitis virus, co-feeding transmission (i.e. the pathogen transmission from infected nymphs to naïve larvae, all feeding on the same host and not involving amplification within the host) is of major importance for the maintenance of the pathogen in the tick population (Jones et al. 1987; Randolph et al. 1996; Hartemink et al. 2008). Thus, identification of factors affecting (1) mean tick burden, (2) level of aggregation and (3) the simultaneous presence of larval and nymphal ticks on a single host is central for understanding transmission dynamics of tick-borne diseases.

Several studies have investigated the patterns of tick parasitism in forests of Europe. Most studies largely focussed on single or few factors affecting individual mean tick burden in rodent populations such as seasonality (Radda 1968; Radda et al. 1969; L'Hostis et al. 1996; Randolph et al. 1999), extrinsic spatial factors such as habitat type or structure (Boyard et al. 2008; Paziewska et al. 2010), microclimate (Randolph and Storey 1999) or intrinsic features such as rodent species, sex, age and body mass (Randolph 1975; Nilsson and Lundqvist 1978; Matuschka et al. 1991; Humair et al. 1993; Perkins et al. 2003; Harrison et al. 2010). Recently, the abundance of larger ungulates such as roe deer (*Capreolus capreolus*) which are key hosts for adult ticks (Vor et al. 2010; Kiffner et al. 2010) has been hypothesised to contribute to increased tick densities (Gilbert 2010) and consequently to increased tick-borne disease incidence in humans (Linard et al. 2007; Rizzoli et al. 2009). However, effects of variable deer densities on individual tick burdens of rodents have rarely been tested (but see Harrison et al. 2010). Furthermore, high rodent densities might 'dilute' individual burdens since the available ticks might be spread across many hosts (Schmidt et al. 1999).

Given the wide range of factors potentially affecting individual tick burden, simultaneous testing of these variables is needed. Even if sufficient data are available, conventional statistical approaches are often inappropriate as they usually assume a constant aggregation level (Shaw et al. 1998; but see Brunner and Ostfeld 2008). In order to analyse a data set of tick burdens (*Ixodes* spp. and *Dermacentor* spp.) on yellow-necked mice (*Apodemus flavicollis*) and bank vole (*Myodes glareolus*), the two dominant rodent species in central European woodlands, we adopted a flexible modelling approach, general linear models for location, scale and shape (GAMLSS, Stasinopoulos and Rigby 2007). This statistical framework allows modelling of the mean and the dispersion parameter of a negative binomial distribution as a function of explanatory variables. In this framework, we test whether (1) external abiotic (e.g. season) factors influenced individual tick burdens; (2) external biotic factors, such as relative humidity, temperature, vegetation cover, roe deer and rodent density affected individual tick loads; and (3) intrinsic factors such as sex, age or body mass were correlated with tick burdens. Additionally, we tested whether the dispersion parameter varied with those parameters that influenced mean burdens.

In order to identify variables of categories (1)–(3) which might predict co-feeding, we used a statistical framework with binomial structure following Perkins et al. (2003). Additionally, we tested whether co-feeding of larvae and nymphs were correlated with the increase in spring temperature relative to the mean temperature of January. Fast temperature increase in spring time is thought to be the main driver for the seasonal synchrony of larvae and nymphs and thus for co-feeding aggregations (Randolph and Sumilo 2007).

Materials and methods

Study sites

We selected nine different forest patches (mean size 1,150 ha, range: 520–1,710 ha) in three forest districts (Beerfelden, Dieburg, Lampertheim) in the southern part of Hesse, Germany. The forest districts were located in counties (Bergstraße and Odenwaldkreis, Darmstadt-Dieburg and Bergstraße, respectively) defined as risk areas for TBEV (Robert Koch-Institute 2007).

Small mammal trapping

We conducted repeated rodent trapping in the first weeks of September 2007; May, July and August 2008; and May, July and August 2009. In each forest patch, we established two trapping grids on randomly selected intersections of a

superimposed 1 km×1 km grid. Each of the 18 trapping grids consisted of 36 Sherman live traps placed systematically in a 50 m×50 m square (10-m inter-trap distance). We used fresh apple parts to bait rodents and placed hay into the traps to provide nesting material. We operated trapping grids for four consecutive nights and controlled the traps every morning. We transferred caught rodents in a plastic bag and identified them to species level based on morphological traits. We released non-target animals (e.g. *Soricidae*) immediately and euthanized rodents with CO_2. The trapping and euthanizing protocol was authorised by the responsible authority. Overall, we caught 270 rodents, whereas bank voles *M. glareolus* (143 individuals) and yellow-necked mice *A. flavicollis* (106 individuals) combined represented 92% of all captures. Other species [*A. sylvaticus* (n=15), *Microtus agrestis* (n=4), *M. arvalis* (n=1), and *Mus musculus* (n=1)] were captured infrequently and were not considered for the statistical analyses. For each rodent, we assessed basic biometric characteristics such as sex (male/female), age (sub-adult, adult) and body mass. We carefully screened each rodent for ticks by combing the fur and by intensively searching the ears, head, throat, toes and tail. All detected ticks were removed using forceps and transferred in sterile tubes and stored at −80°C. Ticks were determined to genus (*Dermacentor* spp. (Koch 1844) and *Ixodes* spp. (Latreille 1795)) and stage (larvae and nymphs, no adults were found on rodents). Sample individuals determined to species level belonged to *I. ricinus* (Linneaus 1758) and *Dermacentor reticulatus* (Fabricius 1794). Since a rodent removal protocol was necessary for further virus screening (TBEV and Hantavirus) of the rodents (see, e.g. Ulrich et al. 2009), we approximated rodent density as number of individuals per 100 corrected trap nights (rodents per 100CTN). Trapping effort was corrected for closed traps without captures or captures of non-target animals. We calculated three different density indices: a mice density index (*A. flavicollis*, *Apodemus sylvaticus* and *M. musculus* captures per 100CTN), a vole density index (*M. glareolus*, *M. agrestis* and *Microtus arvalis* captures per 100CTN) and a rodent density index (all rodent species combined per 100CTN). During each trapping session, we visually estimated the percentage of vegetation cover in the herb layer in four categories: 0–24%, 25–49%, 50–74% and 75–100%.

Climatic data

At each trapping grid, we placed a weather data logger (Thermo/Hygro Button 23, Maxim Integrated Products, Inc., Sunnyvale, USA) at a tree trunk near the forest floor and with minimal exposition to solar radiation to record relative humidity and temperature. Since data loggers were not operated for the entire study period and frequently failed to store data, it was not possible to relate relative humidity and temperature to the grid- and time-specific individual tick burden. Hence, we calculated average temperature and relative humidity for the vegetation period 2009 (1 March–30 September) for each trapping grid. According to Randolph and Sumilo (2007), we estimated the spring temperature increase from February to April 2009 corrected by the mean temperature of January 2009.

Roe deer density estimation

We estimated densities of roe deer using line transect methodology (Buckland et al. 2001) and analysed the data with the software package Distance 5 Release 2 (Thomas et al. 2010). In early March 2008 and 2009, we drove a fixed circuit (mean length ± SD 18.3±3.3 km) in each forest area. We repeated each circuit in one of the following nights. We counted roe deer with three persons; one person driving the car slowly (6–12 km h^{-1}) and screening for animals on the transect line and two persons scanning both sides of the transect line with handheld spotlights. We measured sighting distances with a laser rangefinder and sighting angles with a compass. Considering that we used forest roads and hence that transects were not distributed randomly, our estimates should be regarded as density indices. However, these indices allow comparisons of roe deer densities among different forest areas and years. Because the numbers of roe deer sightings forest per area per year were low (mean 15.8±6.4 SD), we pooled roe deer sightings according to the predominant terrain of the forest area. Based on Akaike's information criterion (AIC) values, these pooled detection functions indicated a better fit than forest-area-specific detection functions. We discarded the largest 5% of the distances and used half-normal key function with cosine series expansion to fit the detection functions. Using these stratum-specific detection functions and the size-bias regression method to estimate cluster size, we estimated area- and year-specific roe deer densities. Because mean roe deer densities in the nine forest patches remained remarkably stable between 2008 and 2009 (Kendalls tau 0.93, p<0.001, n=9), we used the 2008 estimate also for the year 2007.

Modelling approach

For predicting host-species- and tick-genus-specific models of individual larval burdens, we ran several general additive models for location, scale and shape, defining the distribution as negative binomial (NBD type I) (Shaw et al. 1998). The modelling procedure was performed with the 'gamlss' package (Stasinopoulos and Rigby 2007) implemented in R (R Development Core Team 2005). Similar to Brunner and Ostfeld (2008), we used a stepwise forward model selection

procedure. We started with the most basic extrinsic factors (seasonality, forest district) potentially influencing mean (μ) larval tick burdens. Then, always selecting the model with the lowest Akaike's information criterion corrected for sample size (AIC_c), we tested whether inclusion of further extrinsic variables (climatic variables, vegetation cover in the shrub layer, roe deer density and rodent density) improved the models (Burnham and Anderson 2002). Further on, we tested whether intrinsic individual characteristics of rodents (sex, age and body mass) improved the model fit. Based on the selected model explaining the mean larval burden, we tested whether addition of a variable dispersion parameter (σ) enhanced the model fit. Since the level of aggregation is likely to be correlated with μ (Shaw et al. 1998), we tested whether σ was affected by those variables explaining μ. We used logistic regression, to test which factors affected presence of nymphal ticks on rodent individuals. Analogous to the larval models, we used a stepwise forward model selection procedure.

Results

Larval burden

Larval *Ixodes* spp. burdens on *A. flavicollis* (mean=19, range=0–129, SD=22) were on average higher (Mann–Whitney *U* test, $z=-8.96$, $p<0.001$) than on *M. glareolus* (mean=6, range=0–86, SD=14). Almost all (98%, 104/106) *A. flavicollis* individuals were parasitized with at least one *Ixodes* spp. larvae while the larval *Ixodes* spp. prevalence in *M. glareolus* was 68% (98/144). Larval ticks were highly aggregated. In *A. flavicollis*, 20% of the most infested individuals harboured 56% of the entire larval *Ixodes* spp., and in *M. glareolus* the same proportion fed 81% *Ixodes* spp. larvae.

For predicting larval *Ixodes* spp. burdens on *A. flavicollis*, the model selection procedure provided most support for model K (Table 1). This model suggested that larval burdens were influenced by sampling month, with mean larval burdens being highest in July and lower in May and September (Fig. 1). Mean larval burdens were higher in the forest district Beerfelden compared to the forest districts of Dieburg and Lampertheim and slightly declined with increasing relative humidity during the vegetation period. Individual burdens were also associated with vegetation cover in the shrub layer whereas stands with ≥25% vegetation cover were associated with higher tick burdens compared to stands with <25% vegetation cover. There was statistical support that mean larval burden of *A. flavicollis* decreased with increasing rodent density. Further on, adult *A. flavicollis* showed higher larval burdens than sub-adult conspecifics. There was no support for including a variable dispersion parameter. Models which included dispersion parameters either as a function of month, forest district, relative humidity, vegetation cover, indexed rodent density or host age had poorer fits (based on AIC_C, models not shown) compared to model K without a variable dispersion parameter. Model K suggested the use of a constant dispersion parameter (−0.67, SE±0.14).

In order to explain variation in larval *Ixodes* spp. burdens on *M. glareolus*, we found most support for model N (Table 1). Similar to the *A. flavicollis–Ixodes* spp. larvae model, this model suggested including the sampling month and the forest district as explanatory variables (Fig. 2). Individual burden declined with increasing indexed vole densities. Among the intrinsic factors, host age (adult > sub-adult) and body mass were positively correlated with mean larval burdens. Again, models with variable dispersion parameters performed worse than model N with a constant (0.21, SE±0.17) dispersion parameter.

Larval *Dermacentor* spp. burdens were not statistically different (Mann–Whitney *U*, $z=-0.97$, $p=0.333$) between *A. flavicollis* (mean=3, range=0–88, SD=12) and *M. glareolus* (mean=3, range=0–100, SD=13). Prevalence of larval *Dermacentor* spp. was slightly higher in *A. flavicollis* (20%, 21/106) than in *M. glareolus* (15%, 21/144). The larval *Dermacentor* spp. was highly aggregated whereas the 20% of the most infested *A. flavicollis* and *M. glareolus* individuals fed 100% of the counted *Dermacentor* spp. larvae.

Models for explaining larval *Dermacentor* spp. burden on *A. flavicollis* indicated no significant effect of sampling month, and thus models did not include this variable. Mean larval *Dermacentor* spp. burden on *A. flavicollis* was best explained by model M (Table 2). Once more, this model indicated an effect of forest district (Fig. 3). Further on, the model suggested that *Dermacentor* spp. burden were positively correlated with the average temperature during the vegetation period and that individual burdens were higher in male *A. flavicollis* compared to female conspecifics. Models incorporating a variable dispersion parameter (either modelled as a function of forest district, temperature or host sex) had higher AIC_C values, and we thus favoured the model with a constant dispersion parameter (2.30e+00, SE±2.80e–01).

For predicting larval *Dermacentor* spp. burdens on *M. glareolus*, model D was selected (Table 2). Several model combinations were not possible due to redundant factor combinations. The selected model suggested similar effects (forest district and average temperature) as the larval *Dermacentor* spp. model for *A. flavicollis*, except that it did not include host sex as an explanatory variable (Fig. 4). Also, for this model, a constant dispersion parameter was considered (1.92, SE±0.29).

Table 1 Support for models explaining mean larval tick burdens (*Ixodes* spp.) on yellow-necked mice (*A. flavicollis*) and bank vole (*M. glareolus*)

Model	*Apodemus flavicollis*					*Myodes glareolus*				
	Model letter	*P*	AIC_C	Δ_i	w_i	Model letter	*P*	AIC_C	Δ_i	w_i
Individual characteristics										
μ is a function of age, sex and body mass	Q	15	804.72	3.11	0.09	Q	11	677.17	1.05	0.16
μ is a function of sex and body mass	P	14	808.59	6.97	0.01	P	10	678.43	2.31	0.08
μ is a function of sex and age	O	14	804.06	2.44	0.12	O	10	677.27	1.15	0.15
μ is a function of age and body mass	N	14	802.08	0.46	0.32	*N*	*10*	*676.12*	*0.00*	*0.27*
μ is a function of sex	M	13	815.93	14.32	0.00	M	9	689.77	13.65	0.00
μ is a function of body mass	L	13	805.94	4.33	0.05	L	9	677.71	1.59	0.12
μ is a function of age	*K*	*13*	*801.61*	*0.00*	*0.41*	K	9	676.55	0.43	0.22
Best model from below	J	12	813.60	11.99	0.00	I	8	691.02	14.90	0.00
Rodent density										
μ is a function of rodent density	J	12	813.60	11.99	0.00	J	8	696.27	20.15	0.00
μ is a function of vole density	I	12	815.00	13.39	0.00	I	8	691.02	14.90	0.00
μ is a function of mice density	H	12	813.77	12.16	0.00	H	8	699.34	23.22	0.00
Best model from below	F	11	813.68	12.07	0.00	B	7	697.12	21.00	0.00
Roe deer density										
μ is a function of roe deer density	G	12	814.51	12.90	0.00	G	8	698.11	21.99	0.00
Best model from below	F	11	813.68	12.07	0.00	B	7	697.12	21.00	0.00
Vegetation										
μ is a function of vegetation cover	F	11	813.68	12.07	0.00	F	10	700.33	24.21	0.00
Best model from below	C	8	814.94	13.33	0.00	B	7	697.12	21.00	0.00
Climatic factors										
μ is a function of relative humidity and temperature	E	9	817.33	15.71	0.00	E	9	701.68	24.56	0.00
μ is a function of temperature	D	8	817.42	15.81	0.00	D	8	698.66	22.54	0.00
μ is a function of relative humidity	C	8	814.94	13.33	0.00	C	8	699.36	23.24	0.00
Best model from below	B	7	815.67	14.06	0.00	B	7	697.12	21.00	0.00
Spatial factors										
μ is a function of forest district	B	7	815.67	14.06	0.00	B	7	697.12	21.00	0.00
Best model from below	A	5	819.54	17.93	0.00	A	5	714.21	38.09	0.00
Seasonal dynamics										
μ is a function of season	A	5	819.54	17.93	0.00	A	5	714.37	37.62	0.00

Models are grouped from bottom (simple models) to top (more complex). We first addressed the fundamental extrinsic factors season and forest district and then included further extrinsic factors (climatic factors, vegetation, roe deer density and rodent density) and intrinsic factors (age, body mass and sex). In each case, the best model from the set below was chosen based on minimum AIC_c values. *P* indicates the number of parameters used for fitting each model, Δ_i is the difference in AIC_c and w_i is the AIC_c weight based on all models (Burnham and Anderson 2002). The models with most support are in italics

Nymphal burden

Ixodes spp. nymphs were found on 16% (17/106) of captured *A. flavicollis* with a maximum of eight nymphs on one individual. Prevalence of nymphs on *M. glareolus* was 10% (15/144) whereas one individual was infested by 25 nymphs.

A. flavicollis individuals parasitized by *Ixodes* spp. nymphs had higher average larval *Ixodes* spp. burdens (mean=47±SE 9) compared to individuals without nymphs (13±1). Individuals with an *Ixodes* spp. nymph also fed more *Dermacentor* spp. larvae (12±6) than individuals without a nymph (1±5).

We observed similar patterns in *M. glareolus*: Individuals parasitized by an *Ixodes* spp. nymph fed on average more *Ixodes* spp. larvae (22±6) than conspecifics not feeding nymphs (4±1). The same individuals, however, fed similar numbers of *Dermacentor* spp. larvae (2±1) compared to individuals without *Ixodes* spp. nymphs (3±1).

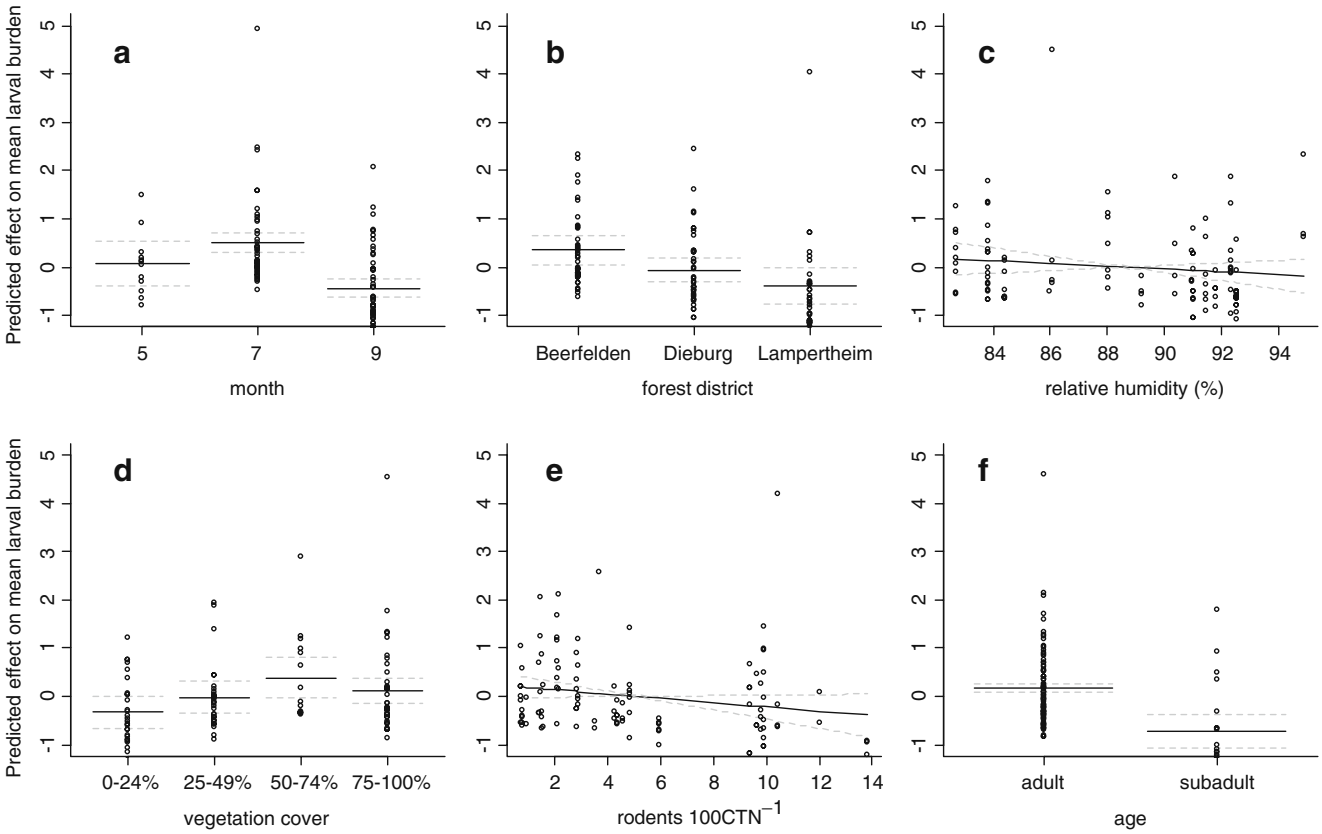


Fig. 1 The effect of **a** month, **b** forest district, **c** relative humidity during the vegetation period, **d** vegetation cover in the shrub layer, **e** indexed rodent density and **f** host age on the mean larval (*Ixodes* spp.) burden on yellow-necked mice (*A. flavicollis*) as predicted by model K (Table 1). *Dashed lines* indicate standard errors

The model selection approach suggested that model P (Table 3) best explained prevalence of *Ixodes* spp. nymphs on *A. flavicollis*. The logistic regression model provided support that presence of *Ixodes* spp. nymphs on *A. flavicollis* was associated with sampling month (July > May, p=0.025, September > May, p=0.71) and forest district (Lampertheim < Beerfelden, p=0.0048; Dieburg < Beerfelden, p=0.18) and that it was positively correlated with spring warming rate (coefficient=1.33, p=0.031) and body mass (coefficient=0.14, p=0.04) of individual yellow-necked mice.

Presence of *Ixodes* spp. nymphs on *M. glareolus* was best explained by model M (Table 3). This model suggested that forest district (Dieburg < Beerfelden, p=0.39, Lampertheim < Beerfelden, p=0.46), temperature during the vegetation period (coefficient=9.56, p=0.43) and indexed vole density (coefficient=−0.18, p=0.14) affected the probability that a nymphal *Ixodes* spp. was present on a bank vole. Yet all model parameters were insignificant (p values > 0.05), suggesting cautious treatment of this model.

Prevalence of nymphal *Dermacentor* spp. was very low. Since only 2% (2/106) of the captured *A. flavicollis* was parasitized by *Dermacentor* spp. nymphs, we did not analyse this host-tick system statistically. The two infested yellow-necked mice infested with a *Dermacentor* spp. nymph tended to have higher *Ixodes* spp. larvae burdens (57±45 vs. 18±2) and *Dermacentor* spp. burdens (39±1 vs. 2±1) than conspecifics without a *Dermacentor* spp. nymph. Six percent (9/144) of the captured *M. glareolus* individuals showed a prevalence of *Dermacentor* spp. nymphs, whereas the maximum per capita count was three nymphs. Individuals parasitized by *Dermacentor* spp. nymphs fed similar numbers of *Ixodes* spp. ticks (7±6 vs. 6±1) but on average higher numbers of *Dermacentor* spp. larvae (40±10 vs. 1±1) compared to conspecifics not feeding a nymph. The selected model N (Table 4) explaining presence of nymphal *Dermacentor* spp. on *M. glareolus* should be regarded conservatively. In this model, only one variable, indexed mice density (showing a positive correlation), reached statistical significance (p=0.01). Other variables in the model (forest district, relative humidity, host age and host body mass) were insignificant (p>0.05).

Discussion

After controlling for season and unexplained spatial variation (forest district entered as factor), we found that

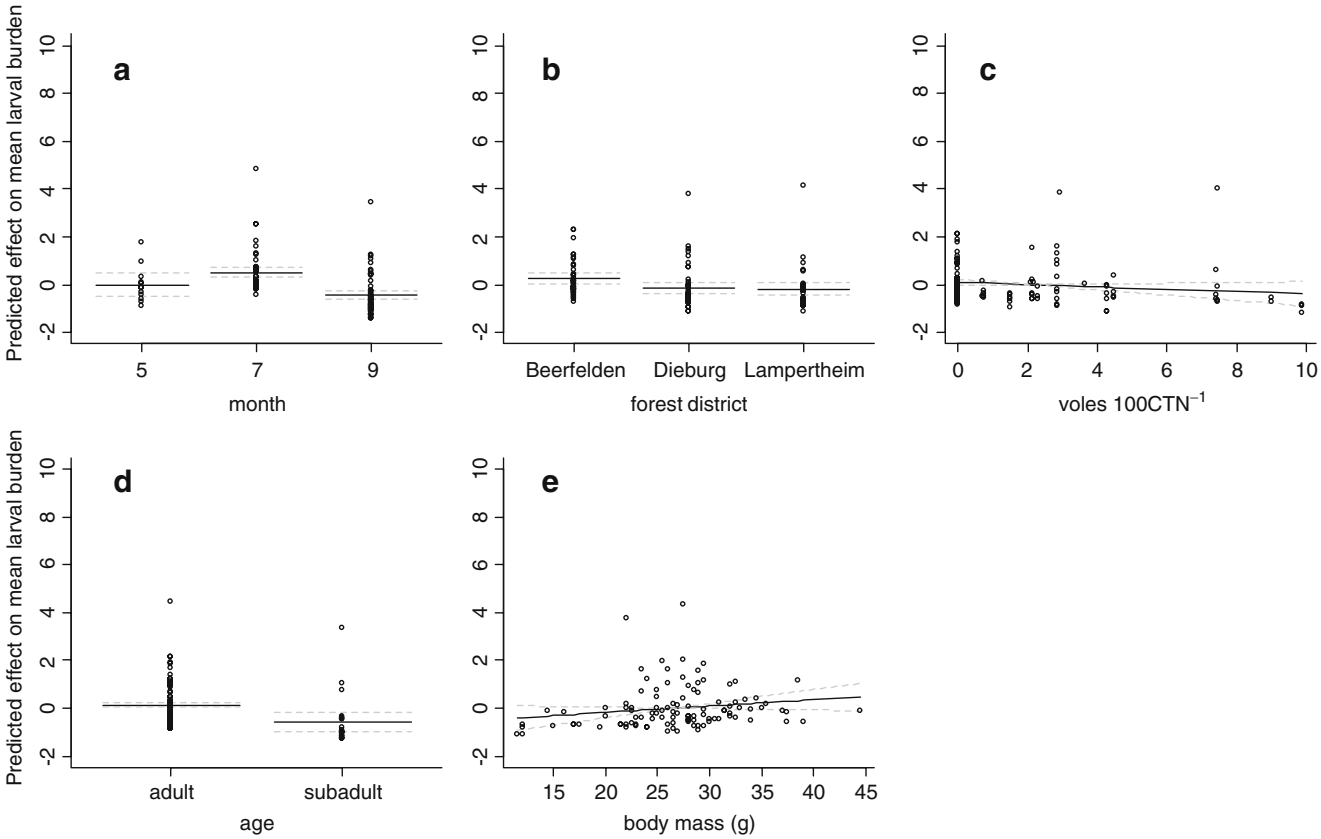


Fig. 2 The effect of **a** month, **b** forest district, **c** indexed vole density, **d** host age and **e** host body mass on the mean larval (*Ixodes* spp.) burden on bank vole (*M. glareolus*) as predicted by model N (Table 1). *Dashed lines* indicate standard errors

several extrinsic and intrinsic factors influence the investigated rodent hard-tick systems.

Factors affecting mean *Ixodes* spp. larvae burdens

It was expected that larval *Ixodes* spp. burdens were higher in *A. flavicollis* than in *M. glareolus* (cf. Matuschka et al. 1991; Humair et al. 1993; Boyard et al. 2008; Paziewska et al. 2010) because *M. glareolus* may acquire resistance against *I. ricinus*, the dominant tick species in central Europe (Dizij and Kurtenbach 1995). Highest mean larval burdens were observed in July, whereas we cannot exclude that larval activity might have peaked in June (cf. Randolph 2004). Mean infestation levels of *A. flavicollis* were also influenced by relative humidity during the vegetation period and by vegetation cover. The effect of relative humidity appears inconclusive: On the one hand, *Ixodes* ticks require high relative humidity [e.g. they are inactive at relative humidity <70% (Aeschlimann 1972)]; on the other hand, we observed that larval tick burden slightly declined with increasing relative humidity. This finding is in contrast to tick feeding experiments (Randolph and Storey 1999) but in accordance with results from a field study where the numbers of larval *Ixodes* spp. were also higher under drier conditions (82–89% vs. 91–98% relative humidity) (Boyard et al. 2008). To complicate this issue, vegetation cover (which is usually positively correlated with relative humidity on the forest floor) affected the mean number of larval *Ixodes* spp. The underlying factors causing these apparently contradictory findings need further experimental clarification. Unambiguously, our findings lend support to the 'dilution hypothesis' (Schmidt et al. 1999; Brunner and Ostfeld 2008), i.e. with increasing rodent densities, the mean per capita larval burden of *A. flavicollis* declines. Further on, adult individuals had higher levels of infestations than sub-adults, which is in line with other studies of tick–rodent systems (Brunner and Ostfeld 2008; Harrison et al. 2010), given the probable correlation between age and body mass (cf. Brunner and Ostfeld 2008).

Mean infestation levels of *M. glareolus* with *Ixodes* spp. larvae were affected by similar variables also affecting infestation levels of *A. flavicollis*. The 'dilution effect' was, however, related to indexed vole density (vs. combined rodent density index in *A. flavicollis*). Further on, adult bank voles and heavier bank voles fed on average more larval *Ixodes* spp. than younger and lighter conspecifics which largely supports the 'body size' hypothesis (Harrison et al. 2010).

Table 2 Support for models explaining mean larval tick burdens (*Dermacentor* spp.) on yellow-necked mice (*A. flavicollis*) and bank vole (*M. glareolus*)

Model	*Apodemus flavicollis*					*Myodes glareolus*				
	Model letter	*P*	AIC_C	Δ_i	w_i	Model letter	*P*	AIC_C	Δ_i	w_i
Individual characteristics										
M is a function of age, sex and body mass	Q	8	242.49	3.93	0.05	–[b]				
M is a function of sex and body mass	P	8	240.86	2.30	0.10	–[b]				
M is a function of sex and age	O	8	240.27	1.71	0.14	–[b]				
M is a function of age and body mass	N	8	246.00	7.44	0.01	–[b]				
M is a function of sex	*M*	*7*	*238.56*	*0.00*	*0.32*	–[b]				
M is a function of body mass	L	7	243.94	5.38	0.02	F	7	245.52	2.15	0.19
M is a function of age	K	7	243.70	5.14	0.02	E	7	245.21	1.84	0.22
Best model from below	D	6	242.33	3.77	0.05	D	6	243.37	0.00	0.56
Rodent density										
M is a function of rodent density	J	7	244.51	5.95	0.02	–[b]				
M is a function of vole density	I	7	243.47	4.91	0.03	–[b]				
M is a function of mice density	H	7	242.42	3.86	0.05					
Best model from below	D	6	242.33	3.77	0.05	D	6	243.37	0.00	0.56
Roe deer density										
M is a function of roe deer density	G	7	243.97	5.41	0.02					
Best model from below	D	6	242.33	3.77	0.05	D	6	243.37	0.00	0.56
Vegetation										
M is a function of vegetation cover	F	9	242.59	4.03	0.04					
Best model from below	D	6	242.33	3.77	0.05	D	6	243.37	0.00	0.56
Climatic factors										
M is a function of relative humidity and temperature	E	7	244.40	5.84	0.02	–[b]				
M is a function of temperature	D	6	242.33	3.77	0.05	*D*	*6*	*243.37*	*0.00*	*0.56*
M is a function of relative humidity	C	6	243.43	4.87	0.03	C	6	252.23	8.86	0.01
Best model from below	B	5	242.45	3.89	0.05	B	5	250.36	6.99	0.02
Spatial factors										
M is a function of forest district	B	5	242.45	3.89	0.05	B	5	250.36	6.99	0.02
Best model from below	–/–[a]					–/–[a]				
Seasonal dynamics										
M is a function of season	A	5	242.35	3.79	0.05	A	5	256.98	13.61	0.00

Models are grouped from bottom (simple models) to top (more complex). We first addressed the fundamental extrinsic factors season and forest district and then included further extrinsic factors (climatic factors, vegetation, roe deer density and rodent density) and intrinsic factors (age, body mass and sex). In each case, the best model from the set below was chosen based on minimum AIC_c values. *P* indicates the number of parameters used for fitting each model, Δ_i is the difference in AIC_c and w_i is the AIC_c weight based on all models (Burnham and Anderson 2002). The models with most support are in italics

[a] The effect of season was not significant ($p>0.10$) and hence was not included in further models

[b] Redundant combination of variables

In both rodent species, we found no support for the 'sex-bias' hypothesis, which is in contrast to similar tick–rodent systems in Europe (Harrison et al. 2010; Boyard et al. 2008) or in the USA (e.g. Brunner and Ostfeld 2008). Further on, it was unexpected that roe deer densities were not correlated with *Ixodes* spp. larval counts on forest rodents (cf. Gilbert 2010). Potentially, the failure to detect a significant effect of roe deer density was due to the different scales at which roe deer density estimation and rodent trapping were conducted. Moreover, the range of roe deer density indices (2.0–9.9 deer per square kilometre) might not be wide enough to detect a significant relationship between roe deer density and *Ixodes* larvae density.

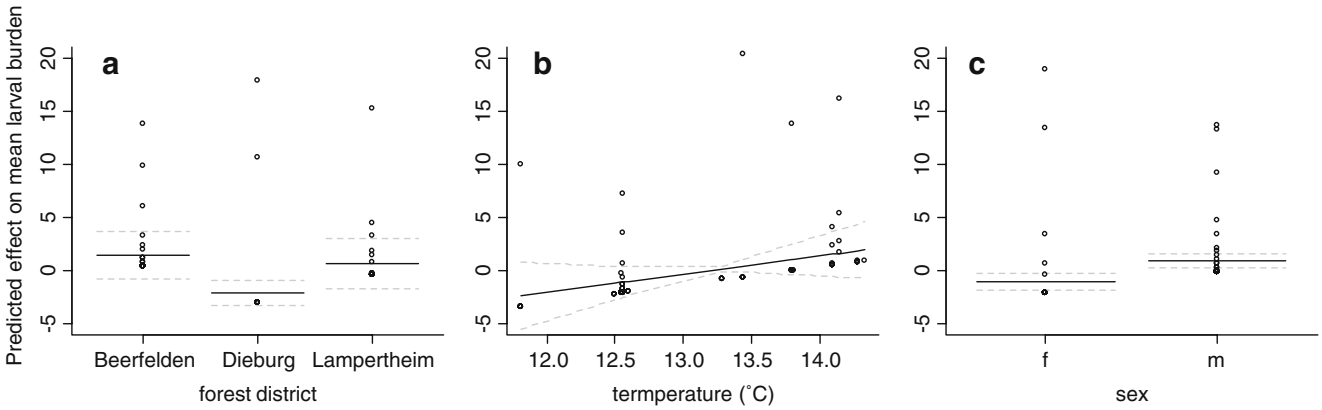


Fig. 3 The effect of **a** forest district, **b** average temperature during the vegetation period and **c** host sex on the mean larval (*Dermacentor* spp.) burden on yellow-necked mice (*A. flavicollis*) as predicted by model M (Table 2). *Dashed lines* indicate standard errors

Factors affecting mean *Dermacentor* spp. larvae burdens

Dermacentor spp. ticks were found in all forest districts but in one rodent trapping grid in the Rhine valley, this tick species was very abundant, supporting the hypothesis that *Dermacentor* spp. is associated with and/or expands along rivers (Bullová et al. 2009; Zygner et al. 2009). The relative abundance of *Dermacentor* spp. varied considerably at small scale whereas ticks of this genus appear to prefer areas with higher average temperatures; this might be important for potential further expansion of *Dermacentor* species and associated diseases with respect to climate change scenarios. In contrast to other studies which identified *M. glareolus* as the main host species (e.g. Randolph et al. 1999; Paziewska et al. 2010), we did not detect an apparent preference for a certain rodent species. Male *A. flavicollis*, which usually have a relative large home range (Schwarzenberger and Klingel 1994), were disproportionally infested with larval *Dermacentor* spp.

Dispersion of larval ticks

Larval ticks were highly aggregated on their rodent hosts, and in the case of *Ixodes* spp. the observed patterns broadly confirmed the '20/80' rule (Woolhouse et al. 1997). In *Dermacentor* spp., the level of aggregation was even more pronounced, possibly due to the spatial clumping of these ticks. Whereas we found several factors affecting the variation in mean infestation levels, we failed to detect variables affecting the level of aggregation. This was rather disappointing since the level of aggregation is as important as the mean infestation level. Our approach suggests that other undocumented variables might influence the dispersion of larval ticks in rodent populations. Recent findings suggest that individual space use, which is not necessarily correlated with attributes such as sex, age or host density, affects the distribution of ticks among their hosts (Boyer et al. 2010). This would offer a mechanistic explanation for differences in mean tick loads but also for different aggregation levels. Incorporating individual space use of hosts as an explanatory variable is, however, not feasible in a removal study and would necessitate a capture–recapture design.

Factors affecting co-feeding

Overall, we found very few nymphs infesting forest rodents. The typical rodent individual infested with an *Ixodes* spp. nymph was an *A. flavicollis* of high body mass, captured in July. Co-feeding of *Ixodes* spp. ticks in *M. glareolus* appeared to be a rather erratic event. We found empirical evidence that the spring warming rate was positively correlated with co-feeding presence. A fast increase in spring temperatures relative to January temperatures

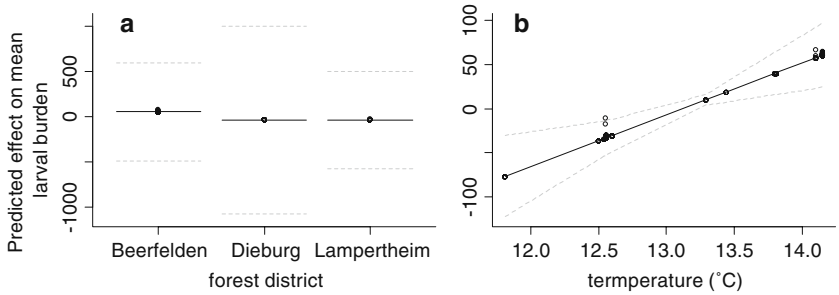


Fig. 4 The effect of **a** forest district and **b** average temperature during the vegetation period on the mean larval (*Dermacentor* spp.) burden on bank vole (*M. glareolus*) as predicted by model D (Table 2). *Dashed lines* indicate standard errors. Note the different scales on the *y-axes*

Table 3 Support for models explaining the presence (¤) of nymphal ticks (*Ixodes* spp.) on yellow-necked mice (*A. flavicollis*) and bank vole (*M. glareolus*)

Model	*Apodemus flavicollis*					*Myodes glareolus*				
	Model letter	*P*	AIC_C	Δ_i	w_i	Model letter	*P*	AIC_C	Δ_i	w_i
Individual characteristics										
¤ is a function of age, sex and body mass	U	9	83.18	2.86	0.06	U	8	92.96	4.38	0.01
¤ is a function of sex and body mass	T	8	80.85	0.53	0.18	T	7	91.31	2.73	0.03
¤ is a function of sex and age	S	8	83.44	3.12	0.05	S	7	90.85	2.27	0.03
¤ is a function of age and body mass	R	8	82.55	2.23	0.08	R	7	90.76	2.18	0.03
¤ is a function of sex	Q	7	82.70	2.38	0.07	Q	6	90.46	1.88	0.04
¤ is a function of body mass	*P*	*7*	*80.32*	*0.00*	*0.24*	P	6	89.20	0.62	0.08
¤ is a function of age	O	7	83.23	2.91	0.06	O	6	88.69	0.11	0.10
Best model from below	C	6	83.12	2.80	0.06	M	5	88.58	0.00	0.10
Rodent density										
¤ is a function of rodent density	N	7	85.09	4.77	0.02	N	5	89.23	0.65	0.07
¤ is a function of vole density	M	7	85.17	4.85	0.02	*M*	*5*	*88.58*	*0.00*	*0.10*
¤ is a function of mice density	L	7	85.22	4.90	0.02	L	5	90.66	2.08	0.04
Best model from below	C	6	83.12	2.80	0.06	E	4	88.81	0.22	0.09
Roe deer density										
¤ is a function of roe deer density	K	7	85.30	4.98	0.02	K	5	90.82	2.24	0.03
Best model from below	C	6	83.12	2.80	0.06	E	4	88.81	0.22	0.09
Vegetation										
¤ is a function of vegetation cover	J	9	89.53	9.21	0.00	J	7	92.23	3.65	0.02
Best model from below	C	6	83.12	2.80	0.06	E	4	88.81	0.22	0.09
Climatic factors										
¤ is a function of spring warming rate, relative humidity and temperature	I	8	85.77	5.45	0.02	I	6	92.07	3.49	0.02
¤ is a function of humidity and temperature	H	7	89.32	9.00	0.00	H	5	89.97	1.39	0.05
¤ is a function of spring warming rate and temperature	G	7	83.52	3.20	0.05	G	5	90.00	1.42	0.05
¤ is a function of spring warming rate and relative humidity	F	7	85.40	5.08	0.02	F	5	91.27	2.69	0.03
¤ is a function of temperature	E	6	87.30	6.98	0.01	E	4	88.81	0.22	0.09
¤ is a function of relative humidity	D	6	87.84	7.52	0.01	D	4	89.15	0.56	0.08
¤ is a function of spring warming rate	C	6	83.12	2.80	0.06	C	4	91.07	2.48	0.03
Best model from below	B	5	85.65	5.33	0.02	B	3	89.20	0.62	0.08
Spatial factors										
¤ is a function of forest district	B	5	85.65	5.33	0.02	B	3	89.20	0.62	0.08
Best model from below	A	3	88.64	8.32	0.00	–/–[a]				
Seasonal dynamics										
¤ is a function of season	A	3	88.64	8.32	0.00	A	3	98.91	10.33	0.00

Models are grouped from bottom (simple models) to top (more complex). We first addressed the fundamental extrinsic factors season and forest district and then included further extrinsic factors (climatic factors, vegetation, roe deer density and rodent density) and intrinsic factors (age, body mass and sex). In each case, the best model from the set below was chosen based on minimum AIC_c values. *P* indicates the number of parameters used for fitting each model, Δ_i is the difference in AIC_c and w_i is the AIC_c weight based on all models (Burnham and Anderson 2002). The models with most support are in italics

[a] The effect of season was not significant ($p>0.10$) and hence was not included in further models

promotes seasonal synchrony of larval and nymphal activity peaks (Randolph and Sumilo 2007). Rodents that fed at least one nymph were also disproportionally infested with larval ticks, being in accordance with Perkins et al. (2003). In contrast to Perkins et al. (2003), our data do not suggest that "sexually mature males of high body mass" were mainly feeding nymphs. With our data (which only contain few co-feeding aggregations), we only revealed an effect of body

Table 4 Support for models explaining the presence (¤) of nymphal ticks (*Dermacentor* spp.) on bank vole (*M. glareolus*)

Model	*Myodes glareolus*				
	Model letter	*P*	AIC_C	Δ_i	w_i
Individual characteristics					
¤ is a function of age, sex and body mass	Q	8	34.30	2.23	0.12
¤ is a function of sex and body mass	P	7	36.02	3.95	0.05
¤ is a function of sex and age	O	7	38.23	6.16	0.02
¤ is a function of age and body mass	*N*	*7*	*32.07*	*0.00*	*0.36*
¤ is a function of sex	M	6	36.26	4.19	0.04
¤ is a function of body mass	L	6	34.31	2.24	0.12
¤ is a function of age	K	6	36.02	3.95	0.05
Best model from below	H	5	34.14	2.07	0.13
Rodent host density					
¤ is a function of rodent density	J	5	35.40	3.33	0.07
¤ is a function of vole density	I	5	36.34	4.27	0.04
¤ is a function of mice density	H	5	34.14	2.07	0.13
Best model from below	C	4	41.08	9.01	0.00
Roe deer density					
¤ is a function of roe deer density	G	5	43.18	11.11	0.00
Best model from below	C	4	41.08	9.01	0.00
Vegetation					
¤ is a function of vegetation cover	F	7	42.15	10.08	0.00
Best model from below	C	4	41.08	9.01	0.00
Climatic factors					
¤ is a function of relative humidity and temperature	E	5	43.18	11.11	0.00
¤ is a function of temperature	D	4	41.36	9.29	0.00
¤ is a function of relative humidity	C	4	41.08	9.01	0.00
Best model from below	B	3	44.84	12.77	0.00
Spatial factors					
¤ is a function of forest district	B	3	44.84	12.77	0.00
Best model from below	–/–[a]				
Seasonal dynamics					
¤ is a function of season	A	3	58.91	26.84	0.00

Models are grouped from bottom (simple models) to top (more complex). We first addressed the fundamental extrinsic factors season and forest district and then included further extrinsic factors (climatic factors, vegetation, roe deer density and rodent density) and intrinsic factors (age, body mass and sex). In each case, the best model from the set below was chosen based on minimum AIC_c values. *P* indicates the number of parameters used for fitting each model, Δ_i is the difference in AIC_c and w_i is the AIC_c weight based on all models (Burnham and Anderson 2002). The models with most support are in italics

[a] The effect of season was not significant ($p>0.10$) and hence was not included in further models

mass. Potentially, drawing upon a larger sample size would identify also further intrinsic variables such as host sex. Given a longer time horizon and a larger sample size of tick-infested rodents, it would be interesting to test the effect of time lags and to test explicitly whether high rodent densities in a given year translate into high nymph densities in the following year (Ostfeld et al. 2006; Rosa et al. 2007).

Presence of *Dermacentor* spp. nymphs was also rather inconsistent. Nymphs of this genus were predominantly found on *M. glareolus*, being in accordance with Paziewska et al. (2010). Only one variable (indexed mice density) was statistically associated with nymphal presence. Given the fast life cycle of *D. reticulatus* (and *D. marginatus*) (Hillyard 1996) and the strong association between larvae and rodents, high rodent densities during spring time might boost nymphal *Dermacentor* spp. densities in early summer. As an analogue to the *Ixodes* spp. system, an advanced study drawing upon a larger sample size of rodents infested with *Dermacentor* spp. nymphs should explicitly test the effect of spring rodent density on the prevalence/abundance of nymphs at a later stage (i.e. during early summer).

Conclusion

Multiple factors appear to influence tick burdens on forest rodent species. We provide evidence for the ‘dilution’ and for the ‘body size’ hypotheses but find little support for the ‘sex-bias’ hypothesis. Co-feeding aggregations which are essential for tick-borne disease transmission (especially tick-borne encephalitis virus) were mainly found in yellow-

necked mice of high body mass trapped in areas showing a fast increase in spring temperatures. Whereas *Ixodes* spp. is the dominant tick genus in woodlands of our study area, *Dermacentor* spp. is locally very abundant. Its occurrence and its epidemiological role should be monitored closely.

Acknowledgements We thank the administrative staff of the forest districts Beerfelden, Dieburg and Lampertheim for granting access to their forests. The 'Regierungspräsidium Darmstadt' kindly authorised the rodent capture and handling protocol. For assistance in controlling the trapping grids we thank S. Bauling, the late M. Ksinsik, A., C. and M. Lödige, M. Scholz, E. Rühe, A.-L. Schäfer and N. Schäfers. This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).

References

Aeschlimann A (1972) *I. ricinus*, Linne, 1758 (Ixodoidea; Ixodidae). Essai préliminaire de synthèse sur la biologie de cette espèce en Suisse. Acta Trop 29:321–340

Boyard C, Vourc'h G, Barnouin J (2008) The relationship between *Ixodes ricinus* and small mammal species at the woodland–pasture interface. Exp Appl Acarol 44:61–76

Boyer N, Réale D, Marmet J, Pisanu B, Chapuis J-L (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. J Anim Ecol 79:538–547

Brunner JL, Ostfeld RS (2008) Multiple causes of variable tick burdens on small mammal hosts. Ecology 89:2259–2272

Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001) Introduction to distance sampling: estimating abundance of biological populations. Oxford University Press, Oxford

Bullová E, Lukáň M, Stanko M, Petko B (2009) Spatial distribution of *Dermacentor reticulatus* tick in Slovakia in the beginning of the 21st century. Vet Parasitol 165:357–360

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Dautel H, Dippel C, Oehme R, Hartelt K, Schettler E (2006) Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of Rickettsia sp RpA4. J Med Microbiol 296(S156):149–156

Dizij A, Kurtenbach K (1995) *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. Parasite Immunol 17:177–183

Gilbert L (2010) Altitudinal patterns of tick and host abundance: a potential role for climate change in regulating tick-borne diseases? Oecologia 162:217–225

Harrison A, Scantlebury M, Montgomery WI (2010) Body mass and sex biased parasitism in wood mice *Apodemus sylvaticus*. Oikos. doi:10.1111/j1600-0706.2009.18072.x

Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: defining R_0 for tick-borne diseases. Am Nat 171:743–754

Hillyard PD (1996) Ticks of north-west Europe. Dorset, Dorchester

Humair PF, Turrian MN, Aeschlimann A, Gern L (1993) *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. Folia Parasitol 40:65–70

Jones LD, Davies CR, Steele GM, Nuttall PA (1987) A novel mode of arbovirus transmission involving a nonviremic host. Science 237:775–777

Jongejan F, Uilenberg G (2004) The global importance of ticks. Parasitology 129:S3–S14

Kahl O, Janetzki C, Gray JS, Stein J, Bauch RJ (1992) Tick infestation rates with Borrelia: *Ixodes ricinus* versus *Haemaphysalis concinna* and *Dermacentor reticulatus* in two locations in eastern Germany. Med Vet Entomol 6:363–366

Kiffner C, Lödige C, Alings M, Vor T, Rühe F (2010) Abundance estimation of Ixodes ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). Exp Appl Acarol 52:73–84. doi:10.1007/s10493-010-9341-4

Kurtenbach K, De Michelis S, Etti S, Schäfer SM, Seswell H-S, Brade V, Kraiczy P (2002) Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. Trends Microbiol 10:74–79

Labuda M, Nuttall PA (2004) Tick-borne viruses. Parasitology 129: S221–S245

L'Hostis M, Dumon H, Fusade A, Lazareff S, Gorenflot A (1996) Seasonal incidence of *Ixodes ricinus* ticks (Acari: ixodidae) on rodents in western France. Exp Appl Acarol 20:359–368

Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, Vanwambeke SO, Lambin EF (2007) Determinants of the geographic distribution of Puumala virus and Lyme borreliosis infections in Belgium. Int J Health Geogr 6:15

Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM (2005) Superspreading and the effect of individual variation on disease emergence. Nature 438:355–359

Matuschka F-R, Fischer P, Musgrave K, Richer D, Spielman A (1991) Hosts on which *Ixodes ricinus* most abundantly feed. Am J Trop Med Hyg 44:100–107

Nilsson A, Lundqvist L (1978) Host selection and movements of *Ixodes ricinus* (Acari) larvae on small mammals. Oikos 31:313–322

Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing (2006) Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLoS Biology 4:e145

Parola P, Davoust B, Raoult D (2005) Tick- and flea-borne rickettsial emerging zoonoses. Vet Res 36:469–492

Paziewska A, Zwolińska L, Harris PD, Bajer A, Siński E (2010) Utilisation of rodent species by larvae and nymphs of hard ticks (Ixodidae) in two habitats in NE Poland. Exp Appl Acarol 50:79–91

Perkins SE, Cattadori IM, Tagliapietra V, Rizzoli AP, Hudson PJ (2003) Empirical evidence for key hosts in persistence of a tick-borne disease. Int J Parasitol 33:909–917

Radda A (1968) Populationsstudien an Rötelmäusen (*Clethrionomys glareolus* Schreber, 1780) durch Markierungsfang in Niederösterreich. Oecologia 1:219–235

Radda A, Pretzmann, Steiner HM (1969) Bionomische und ökologische Studien an österreichischen Populationen der Gelbhalsmaus (*Apodemus flavicollis*, Melchior 1834) durch Markierungsfang. Oecologia 3:351–373

Randolph SE (1975) Patterns of distribution of the tick *Ixodes trianguliceps* Birula on its hosts. Anim Ecol 44:451–474

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology* 129(Suppl):S37–S65

Randolph SE, Storey K (1999) Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. J Med Entomol 36:741–748

Randolph SE, Sumilo (2007) Tick-borne encephalitis in Europe: dynamics of changing risk. In: Takken W, Knols B (eds) Emerging pests and vector-borne diseases in Europe. University Publishers, Wageningen, pp 187–206

Randolph SE, Gern L, Nuttall PA (1996) Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. Parasitol Today 12:472–479

Randolph SE, Miklisová D, Lysy RDJ, Labuda M (1999) Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. Parasitology 118:177–186

R Development Core Team (2005) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

R Koch-Institute (2007) FSME: risikogebiete in Deutschland. Epidemiol Bull 15:129–135

Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R (2009) Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. PloS ONE 4:e4336

Rosa R, Pugliese A, Ghosh M, Perkins SE, Rizzoli A (2007) Temporal variation of *Ixodes ricinus* intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics. Vector Borne Zoonotic Dis 7:285–295

Schmidt KA, Ostfeld RS, Schauber EM (1999) Infestation of *Peromyscus leucopus* and *Tamias striatus* by *Ixodes scapularis* (Acari: Ixodidae) in relation to the abundance of hosts and parasites. J Med Entomol 36:749–757

Schwarzenberger T, Klingel H (1994) Telemetrische Untersuchungen zur Raumnutzung und Aktivitätsrhytmik freilebender Gelbhalsmäuse, *Apodemus flavicollis* Melchior, 1834. Z Säugetierkunde 60:33–40

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Stasinopoulos DM, Rigby RA (2007) Generalized Additive Models for Location Scale and Shape (GAMLSS) in R. Journal of Statistical Software 23:1–46

Thomas L, Buckland ST, Rexstad EA, Laake JL, Strindberg S, Hedley SL, Bishop JRB, Marques TA, Burnham KP (2010) Distance Software: design and analysis of distance sampling surveys for estimating population size. J Appl Ecol 47:5–14

Ulrich R, Schlegel M, Mertens M, Groschup MH, Schmidt-Chanasit J, Jacob J, Freise J, Pelz H-J, Wenk M, Thiel J, Triebenbacher C, Schex S, Plenge-Bönig A, Schmolz E, Kurth A, Krüger F, Ansoge H, Rühe F, Kiffner C, Gerwin W, Wegener W, Müller J, Bemman M, Wolf R, Otto L-F, Oehme R, Pfeffer M, Heckel G, Essbauer S (2009) Netzwerk "Nagetier-übertragene Pathogene": monitoring von Hantavirus-Infektionen in Deutschland. Beitr Jagd- Wildforsch 34:229–250

Vor T, Kiffner C, Hagedorn P, Niedrig M, Rühe F (2010) Tick burden on European roe deer (*Capreolus capreolus*). Exp Appl Acarol 51:405–417. doi:10.1007/s10493-010-9337-0

Woolhouse MEJ, Dye C, Etard J-F, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JLK, Ndhlovu PD, Quinnel RJ, Watts CH, Chandiwana SK, Anderson RM (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. PNAS 94:338–342

Zygner W, Górski P, Wedrychowith H (2009) New localities of *Dermacentor reticulatus* tick (vector of *Babesia canis canis*) in central and eastern Poland. Pol J Vet Sci 12:549–555

Chapter 9 – Tick-borne encephalitis virus antibody prevalence in roe deer (*Capreolus capreolus*) sera

TITLE PAGE

Tick-borne encephalitis virus antibody prevalence in roe deer (*Capreolus capreolus*) sera

C. Kiffner [1*], T. Vor [1], P. Hagedorn[2], M. Niedrig[2] and F. Rühe[1]

[1]Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany

[2] Centre for Biological Safety, Robert Koch-Institut, Nordufer 20, 13353 Berlin, Germany

Running title: TBE virus antibody prevalence in roe deer

[*]Corresponding Author: Department of Forest Zoology and Forest Conservation incl. Wildlife Biology and Game Management, Büsgen-Institute, Georg-August-University Göttingen, Büsgenweg 3, 37077 Göttingen, Germany. Tel: +49 551 393583. Fax: +49 551 3922089. e-mail: ckiffne@gwdg.de

e- mail T. Vor: tvor@gwdg.de

e- mail P. Hagedorn: hagedornp@rki.de

e- mail M. Niedrig: niedrigm@rki.de

e- mail F. Rühe: fruehe@gwdg.de

Abstract.

In order to identify variables associated with the presence of tick-borne encephalitis virus, we conducted a serological survey of roe deer (*Capreolus capreolus*) in three forest districts of southern Hesse, Germany. Overall, 24/105 (22.9%) of the sera were positive (≥1:10 plaque reduction neutralisation test). Using a logistic regression, we found that spatial autocorrelation, indexed roe deer density (positive correlation), hind foot length of the tested roe deer (positive correlation) and infestation with female *Ixodes* spp. ticks (negative correlation) predicted the probability of TBE virus antibody presence in individual roe deer sera. Spring temperature increase and host sex were rejected as explanatory variables.We found considerable differences in TBE virus seroprevalence (50.0% vs. 17.6%) between two forest districts located in the same county; this finding questions the current county-resolution of public health recordings. Given the high seroprevalence of roe deer and the considerable explanatory power of our model, our approach appears suitable to delineate science-based risk maps at the scale of a forest patch and to abandon the current human incidence per county criterion. Importantly, using roe deer as sentinels would eliminate the inherent bias of risk maps based on human incidence (varying levels of immunisation and exposure of humans).

Key words. *Ixodes ricinus*, host-pathogen interaction, risk mapping, tick-borne pathogens

Introduction

Tick-borne encephalitis (TBE) is an important flavivirus infection of the central nervous system in Europe and Siberia which can cause severe symptoms in humans. In European countries, approximately 3,000 human cases are recorded annually. Whereas the main vector *Ixodes ricinus* is distributed widely in central Europe, the occurrence of TBE virus infections is associated with considerable spatial and temporal heterogeneity (Gritsun *et al.*, 2003; Randolph & Sumilo, 2007; Süss, 2008; Kiffner *et al*., 2010a). In Germany, human infections with tick-borne encephalitis virus are notifiable. Between the year 2001 and 2008, the mean annual number of reported human infections was 319 with a range of 238-546 annual infections. Geographically, infections with the TBE virus are largely confined to the southern part of Germany (SurvStat, http://www3.rki.de/SurvStat, 11/02/2009).

Several transmission routes are known for the tick-borne encephalitis virus, yet there is considerable evidence that co-feeding transmission (i.e. virus transmission among ticks feeding on the same host without a systemic infection of the host individual) is a prerequisite for the maintenance of the virus in the host (e.g. rodent) and vector (tick) population (Labuda *et al*., 1993; Hartemink *et al.*, 2008). Essentially, seasonal activity overlap of the immature tick life stages (larvae and nymphs) is required for simultaneous feeding of larval and nymphal ticks on a host individual serving as co-feeding platform (e.g. yellow-necked mice, *Apodemus flavicollis*). Recently, it has been shown that a fast increase in spring temperatures corrected for mean temperature of January facilitates the required overlap in larval and nymphal tick activity. The observed spatial pattern of spring warming/ occurrence of co-feeding has been shown to be closely associated with the observed occurrence of TBE in central Europe which lends support for the importance of co-feeding for the maintenance of the TBE-virus (Randolph *et al.*, 2000; Randolph & Sumilo, 2007). Additional to large scale climatic conditions which explain the patchy distribution of the TBE virus in Eurasia, there is increasing evidence that roe deer (*Capreolus capreolus*) densities are statistically associated with the circulation of the TBE virus (Rizzoli *et al*., 2009; Kiffner *et al*., 2010a). Yet, this positive relationship has only been found at a relatively large scale and has not been confirmed at the scale of the forest patch.

Here, we aim at identifying factors which determine that TBE virus circulates actively in selected forest areas in TBE virus risk areas in southern Hesse, Germany. Since the prevalence of the TBE

virus is very low in the tick population (Randolph, 2004) and thus very expensive to assess, we tested the sera of hunter-killed roe deer for the presence of TBE virus antibodies. Because roe deer are widely distributed, heavily parasitized by ticks (Kiffner *et al.*, 2010b, Vor *et al.*, 2010) and develop TBE virus antibodies (Labuda *et al.*, 2002), they are useful sentinels for the magnitude of tick-borne encephalitis virus circulation (Gerth *et al.*, 1995; Skarphédinsson et al., 2005). Additional to identifying extrinsic correlates (spring warming, roe deer density) for the presence of TBE virus antibodies in roe deer sera, we tested whether TBE virus antibody presence was associated with intrinsic features (e.g. sex, body mass, hind foot length as a measure of body size, age, tick burden) of roe deer individuals. These intrinsic characteristics might be useful proxies for explaining previous or actual exposure of roe deer to TBE virus positive ticks.

Materials and Methods

Study area and sample collection

The study area comprised three forest districts (Beerfelden, Dieburg, Lampertheim) of southern Hesse, Germany (Figure 1). The forest districts were located in counties (Beerfelden: Bergstraße and marginally overlapping with Odenwaldkreis; Dieburg: Darmstadt-Dieburg; Lampertheim: Bergstraße) defined as risk areas for TBE virus (Robert Koch-Institute, 2007). From 2007 to 2009, 22 human TBE infections were recorded in these counties. The counties differed with respect to the number of recorded human TBE cases/ incidence: Bergstraße 19 cases (incidence: 2.65/100,000 inhabitants), Odenwald 3 cases (0.51/100,000 inhabitants) and Darmstadt-Dieburg 0 cases (http://www3.rki.de/SurvStat, 23/06/2010).

In each forest district, roe deer from three different forest patches were sampled during regular hunting activities. Hunters were instructed to take a blood sample from the thorax in a sterile tube when disembowelling the carcass. Blood samples were labelled so that they could be allocated to the appropriate carcass, stored at 2-8°C and pre-processed within 24 hours. Samples were centrifuged at ca. 3,000 rpm for ca. 10 minutes. The supernatant was transferred in a sterile tube and stored at -80°C until it was tested in the lab. Samples were collected in September 2007, 2008 and 2009, May 2008 and 2009 and July 2008 and 2009, during 10 days each.

Serologic testing

All sera were analysed for TBE virus specific antibodies by a modified plaque reduction neutralisation test (NT), as described previously for Yellow Fever virus (Reinhardt *et al.*, 1998). All serum samples were analysed twice and in twofold dilutions (range 1:10 to 1:160 NTs for the final dilution). The plaques caused by lysis by infected cells were counted, and the 90% NT was calculated. According to the study of Niedrig *et al.* (1999) NTs of ≥1:10 were defined as reactive, and those of <1:10 were defined as nonreactive, which was considered as negative.

Explanatory variables

For each roe deer we assessed the following parameters: age (based on tooth wear), sex, body mass (disembowelled) and hind foot length (Zannèse *et al*. 2006). Additionally, we quantified the tick burden on the neck and head of each individual. Among the extrinsic factors, we estimated the roe deer density in each forest patch using line transect sampling in 2008 and 2009 (Vor *et al.*, 2010). Because mean roe deer densities in the forest patches remained almost constant between 2008 and 2009 (Kendalls tau: 0.93, $p<0.001$, $n=9$), we used the 2008 estimate for the year 2007 as well. Considering that we counted deer along roads and hence that transects were not distributed randomly, our density estimates should be regarded as indices. As a climatic factor, we estimated the spring temperature increase from February to April corrected for the January temperature (Randolph & Sumilo, 2007). For this purpose, we installed weather data loggers (Thermo/Hygro Button 23, Maxim Integrated Products, Inc., Sunnyvale, USA) in two randomly selected locations of each forest patch. The buttons were installed at the trunk base of a tree (ca. 5 cm above the soil surface) and were not exposed to direct solar radiation. Because data loggers were not operated for the entire study period we estimated the mean spring temperature increase according to Randolph & Sumilo (2007) for each forest patch for the year 2009.

Statistical analysis

Since we were interested in factors affecting the presence of TBE virus antibodies in roe deer sera (i.e. the response variable being TBE virus antibody presence in each serum) we fitted a logistic regression to our data. As a correction factor for unexplained spatial variation, we included the forest district as a constant factor in our model. Building up from this simple model, we investigated whether adding extrinsic variables (spring warming, roe deer density index) improved the model's performance. Then, we investigated whether addition of intrinsic variables (sex, age, body mass, hind foot length) further improved the model. Finally, we investigated whether the individual tick load additionally improved the model. For each step, we used the sample sized corrected Akaike's Information criterion (AIC_c) to select the most parsimonious model from the set of candidate models (Burnham & Anderson, 2002). Modelling was performed with the GAMLSS package, implemented in *R* (R Development Core Team, 2005; Rigby & Stasinopoulos, 2005).

Results

Overall, we collected 105 roe deer sera. Of these, 24 (22.9%) were found to be TBE virus antibody positive. The mean antibody titre of the positive sera was 1:41 (range 1:10-1:185). In the northernmost forest district (Dieburg), 5 out of 34 (14.7%) roe deer sera were positive. In the Rhine-valley (forest district Lampertheim), 9 out of 51 (17.6%) sera were positive. We found the highest proportion of TBE antibody prevalence in the forest district Beerfelden; here 10 out of 20 samples (50.0%) were tested positive (Table 1).

The modelling selection process suggested that forest district (Dieburg vs. Beerfelden, P=0.004; Lampertheim vs. Beerfelden, P=0.046) was significantly associated with TBE virus antibody presence in roe deer sera. Further on, there was support to include indexed roe deer density (P=0.108), roe deer hind foot length (P=0.007) and the female *Ixodes* spp. burden (P=0.057) as variables in the model explaining TBE virus antibody presence in roe deer sera (Table 2, Figure 2); adding these variables improved the model's fit compared to the simple model containing forest district only. Indexed roe deer density and hind foot length of tested roe deer were positively associated whereas the number of female *Ixodes* spp. infesting the tested individual was negatively associated with the probability of TBE virus antibody presence in roe deer sera. The model fit was reasonable and the model explained

31.6% of the observed variance (Nagelkerke's R^2). Unexpectedly, spring warming did not enter the model. Since climate usually shows strong spatial autocorrelation and hence the factor "forest district" might have blurred potential effects of variable climate, we tested the effect of the spring warming variable separately. This univariate logistic regression model also suggested that spring warming was not significantly associated with TBE virus antibody presence in roe deer sera (P=0.882).

Discussion

By relating seroprevalence of TBE virus antibodies in roe deer to climatic and ecological factors we identified two extrinsic factors associated with the circulation of the TBE virus in forests of southern Hesse, Germany. Additionally, we identified two intrinsic variables (roe deer hind foot length and roe deer infestation with female *Ixodes* ticks) which were associated with the probability of TBE virus antibody presence in individual deer. Among the extrinsic factors, we found support that spatial proximity (as indicated by the factor "forest district") is influential for TBEV circulation. This finding might represent some unknown factor(s) specific to each forest district. Among these undocumented variables, host species composition (relative abundance of competent vs. non-competent hosts) (LoGiudice *et al.*, 2003; Keesing *et al.*, 2006; Rosà & Pugliese, 2007), and factors affecting tick-host ratios might be important (Randolph, 2004; Rosà & Pugliese, 2007).

Second, we found moderate support (marginal significance but improving model fit) that high roe deer densities favour TBE virus circulation. This is in line with previous studies who found positive correlations between densities of TBE virus-infected ticks (Hudson *et al.*, 2001), numbers of human TBE virus infections (Rizzoli *et al.*, 2009; Kiffner *et al.*, 2010a) and indexed roe deer densities. There is clear a line of evidence that roe deer support tick populations feeding large numbers of all tick stages and thus enhance tick densities (e.g. Jensen *et al.*, 2000; Vor *et al.*, 2010). While the competence of roe deer as a reservoir for TBE virus has – to our knowledge- never been explicitly tested, ticks are spatially highly clustered on roe deer. This aggregated feeding might facilitate co-feeding transmission (Kiffner *et al.*, 2010c).

It was unexpected that spring warming (Randolph & Sumilo, 2007) was not statistically associated with TBE virus antibody presence in roe deer. Notably, spring warming has only been associated with

the presence of the TBE virus and not with the varying prevalence of TBE virus infection. However, Rizzoli *et al.* (2007) found strong support that autumnal cooling was significantly related to TBE virus presence in goats in northern Italy. The underlying mechanism of spring warming and autumnal cooling is that these specific climatic regimes favour seasonal synchrony of larval and nymphal ticks which is a prerequisite for co-feeding transmission. The main difference between these two climatic settings is that spring warming offers a more mechanistic explanation for seasonal synchrony compared to autumnal cooling (Randolph & Sumilo, 2007). Albeit we found evidence that spring warming supports co-feeding of immature ticks on forest rodents (Kiffner *et al*., 2010d), our finding suggests that spring warming is not necessarily a predictor for varying TBE virus prevalence in sentinel animals. .

Amongst the individual correlates for TBE virus presence, we found most support for hind foot length representing body size of roe deer and female *Ixodes* spp. burden. In contrast to Gerth *et al.* (1995) we did not detect a significant effect of roe deer sex on the probability of TBE virus antibody presence.

Hind foot lenght was statistically the best explanation for previous exposure to TBE virus positive ticks. Possible explanations might be (1) that long-legged deer have larger skin surfaces which offer larger feeding areas for ticks and (2) increased movements of larger roe deer due to their dominance and territorial behaviour (Linnell *et al*., 1998). Larger territories and thus higher movement rates could enhance the risk for contact with TBE virus positive ticks

Interestingly, the number of female *Ixodes* spp. ticks infesting the roe deer was negatively associated with TBE virus antibody prevalence. With all due caution, this relationship could indicate a reduced tick-infection susceptibility of roe deer after a TBE virus infection. We can only cautiously speculate that this might be caused by a possible immune response of the roe deer triggered by the TBE virus infection.

Given the limited number of counties (n=2) in which TBE virus antibody presence in roe deer sera was studied , it is difficult to relate seroprevalence in roe deer with TBE virus infection incidence in humans. More importantly within a given county, prevalence of TBE virus antibodies in roe deer can vary considerably in space. In the western part of the county Bergstraße (forest district Lampertheim) we estimated a seroprevalence of 17.6% whereas in the eastern part (forest district Beerfelden) we

found a seroprevalence of 50.0%. This is a crucial aspect because the definition of TBE risk areas is still based on the political unit "county" (Robert Koch-Institute, 2007). Our data clearly indicate that TBE virus circulation can be highly heterogeneous within a given county and we thus suggest a more sophisticated definition of TBE virus risk areas. If human infections should still be the basis for defining risk areas, we propose that TBE virus risk areas should be based on locations of infections at the forest patch level. A more sensible system would be to extent the presented approach of using roe deer sera -which can conveniently be obtained from local hunters- as sentinels. The high prevalence of TBE virus antibodies in roe deer sera as compared to antibody prevalence in other sentinel species and compared to the prevalence of TBE virus in ticks (Table 3), clearly advocates the use of roe deer as sentinels. The high percentage of TBE virus antibody-positive roe deer indicates that roe deer are a sensitive sentinel for TBE virus even in low endemic areas. By relating the probability of TBE virus antibody presence in roe deer to climatic and ecological factors we gained a well-fitted model explaining a large proportion of the observed variance. Thus, our approach contributes to a determination of TBE virus risk areas that is unbiased by human TBE virus vaccinations, human demography and variable human exposure to ticks and might hence substitute the present risk maps that are based on human TBE incidence.

Acknowledgements

We sincerely thank the forest officers and all participating hunters of the forest districts Beerfelden, Dieburg and Lampertheim for supporting this study. We sincerely thank K. Ehlmann, the late M. Ksinsik, A., C. and M. Lödige, E. Rühe, A.-L. Schäfer, N. Schäfers, M. Scholz, S. Schnitzler, S. Bauling for their help during the field work and two anonymous reviewers for their helpful comments. We thank P. Bußmann and A.Teichmann for excellent technical laboratory work. This study was funded by a research grant from the Federal Ministry of Education and Research (BMBF grant 1363120: Emerging arthropod-borne-viral infections in Germany: Pathogenesis, diagnostics and surveillance).

References

Burnham, K.P. &Anderson, D.R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. Second edition. Springer Verlag, New York USA

Hartemink, N.A., Randolph, S.E., Davis, S.A. & Heesterbeek, J.A.P (2008) The basic reproduction number for complex disease systems: defining R_0 for tick-borne infections. *American Naturalist*, **171(8)**, 743-754.

Gerth, H.J., Grimshandl, D., Stage B., Doller G. & Kunz, C. (1995) Roe deer as sentinels for endemicity of tick-borne encephalitis-virus. Epidemiol Infect, 115, 355-365.

Gritsun, T.S., Lashkevich V.A. & Gould, E.A. 2003 Tick-borne encephalitis. *Antiviral Research*, **57(1-2)**, 129-46.

Hudson, P.J., Rizzoli, A., Rosà, R., Chemini, C., Jones, L.D. & Gould, E.A. (2001) Tick-borne encephalitis virus in northern Italy: molecular analysis, relationships with density and seasonal dynamics of *Ixodes ricinus*. *Medical and Veterinary Entomology*, **15**, 304-313.

Jensen, P.M., Hansen, H. & Frandsen, F, (2000) Spatial risk assessment for Lyme borreliosis in Denmark. *Scandinavian Journal of Infectious Diseases*, **32**, 545-550.

Keesing, F., Holt, D. & Ostfeld, R. (2006) Effects of species diversity on disease risk. *Ecology Letters*, **9**, 485-498.

Kiffner, C., Zucchini, W., Schomaker, P., Vor, T., Hagedorn, P., Niedrig, M., Rühe, F. (2010a) Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008. *International Journal of Health Geographics*, **9**, 42.

Kiffner, C., Lödige, C., Alings, M., Vor, T. & Rühe, F. (2010b) Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology*,**52**, 73-84.

Kiffner, C., Lödige, C., Alings, M., Vor, T. & Rühe, F. (2010c) Attachment site selection of ticks on roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology*, doi: 10.1007/s10493-010-9378-4.

Kiffner, C., Vor, T., Hagedorn, P., Niedrig, M., Rühe, F. (2010d). Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. *Parasitology Research*, doi: 10.1007/s00436-010-2065-x.

Klaus, C., Hoffmann, B., Hering, U., Mielke, B., Sachse, K., Beer, M. & Süss, J. (2010) Tick-borne encephalitis (TBE) virus prevalence and virus genome characterization in field-collected ticks (*Ixodes ricinus*) from risk, non-risk and former risk areas of TBE, and in ticks removed from humans in Germany. *Clinical Microbiology and Infection*, **16**, 238–244.

Kupča, A.M., Essbauer, S., Zoeller, G., de Mendonça, P, Brey, R., Rinder, M., Pfister, K., Spiegel, M., Doerrbecker, B., Pfeffer, M. & Dobler, G. (2010) Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. *Ticks and Tick-borne Diseases*, **1**, 44-51.

Labuda, M., Eleckova, E., Lickova, M. & Sabó, A. (2002) Tickborne encephalitis virus foci in Slovakia. *International Journal of Medical Microbiology*, **291**, 43-47.

Labuda, M., Jones, L.D., Williams, T., Danielova, V. & Nuttal, P.A. (1993) Efficient transmission of tick-borne encephalitis virus between cofeeding ticks. *Journal of Medical Entomology*, **30**, 295-299.

Linnell, J.D.C., Wahlström, K. & Gaillard, J.M. (1998) From birth to independence: Birth, growth, neonatal mortality, hiding behaviour and dispersal. *The European roe deer: the biology of success* (ed. by R. Andersen, P. Duncan & J.D.C. Linnell), pp. 257-284. Scandinavian University Press, Oslo.

LoGuidice, K., Ostfeld, R.S., Schmidt, K.A. & Keesing, F. (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 567-571.

Niedrig, M., Lademann, M., Emmerich, P. & Lafrenz, M. (1999) Assessment of lgG antibodies against yellow fever virus after vaccination with 17D by different assays: neutralization test, haemagglutination inhibition test, immunofluorescence assay and ELISA. *Tropical Medicine & International Health*, **4**, 876-71.

R Development Core Team (2005): *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org.

Randolph, S.E., Green, R.M., Peacey, M.F. & Rogers, D.J. (2000) Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. *Parasitology*, **121,** 15-23.

Randolph, S.E. (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology*, **129**, S37-S65.

Randolph, S.E. & Sumilo, D. (2007) Tick-borne encephalitis in Europe: dynamics of changing risk. *Emerging Pests and Vector-borne Diseases in Europe* (ed. by W. Takken & B. Knols), pp. 187-206. University Publishers, Wageningen.

Reinhardt, B., Jaspert, R., Niedrig, M., Kostner, C. & L'age-Stehr J. (1998) Development of viremia and humoral and cellular parameters of immune activation after vaccination with yellow fever virus strain 17D: A model of human flavivirus infection. *Journal of Medical Virology*, **56**, 159-67.

Rieger, M.A., Nübling, M., Müller, W., Hasselhorn, H.-M., Hoffmann. F., & the "TBE in foxes study group". *Zentralblatt für Bakteriologie*, **289**, 610-618.

Rigby, R.A. & Stasinopoulos. D.M. (2005) Generalized additive models for location, scale and shape (with discussion). *Applied Statistics*, **54**, 507–554.

Rizzoli, A., Neteler, M., Rosà, R., Versini, W., Cristofolini, A., Bregoli, M., Buckley, A. & Gould, E.A. (2007) Early detection of tick-borne encephalitis virus spatial distribution and activity in the province of Trento, northern Italy. *Geospatial Health*, **2**, 169-176.

Rizzoli, A., Hauffe, H.C., Tagliapietra, V., Neteler, M. & Rosà, R. (2009) Forest Structure and Roe Deer Abundance Predict Tick-Borne Encephalitis Risk in Italy. *PLoS ONE,* **4**, e4336. doi:10.1371/journal.pone.0004336.

Robert Koch-Institute (2007): FSME: Risikogebiete in Deutschland. *Epidemiologisches Bulletin*, **15**, 129-135.

Rosà, R., Pugliese, A. (2007) Effect of tick population dynamics and host densities on the persistence of tick-borne infections. *Mathematical Biosciences*, **208**, 216-240.

Skarphédinsson, S., Jensen P.M., Kristiansen K. (2005) Survey of tickborne infections in Denmark. *Emerging Infectious Diseases*, **11(7)**, 1055-1061.

Suess, J., Klaus, C., Diller, R., Schrader, C., Wohanka, N. & Abel, U. (2006) TBE incidence versus virus prevalence and increased prevalence of the TBE virus in Ixodes ricinus removed from humans. *Interantional Journal of Medical Microbiology*, **296 (Suppl.1)**, 63-68.

Süss, J. (2008) Tick-borne encephalitis in Europe and beyond – the epidemiological situation as of 2007. *Euro Surveillance*, **13 (26)**, pii=18916.

Vor, T., Kiffner, C., Hagedorn, P., Niedrig, M. & Rühe F. (2010) Tick burden on European roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology*, **51**, 405-417.

Zannèse, A., Baïsse, Gaillard, J.-M., Hewison, A.J.M., Saint-Hilaire, K., Toïgo, C., van Laere, G. & Morellet, N. (2006) Hind foot length: An indicator for monitoring roe deer populations at a landscape scale. *Wildlife Society Bulletin*, **34**, 351-358.

Figures

Fig. 1. Location of the study area in southern Hesse, Germany (left). The approximate position of the forest patches (white circles, forest size not to scale) within the counties is given in the detail map (right). The capital letters denote the forest district (B: Beerfelden, D: Darmstadt, L: Lampertheim).

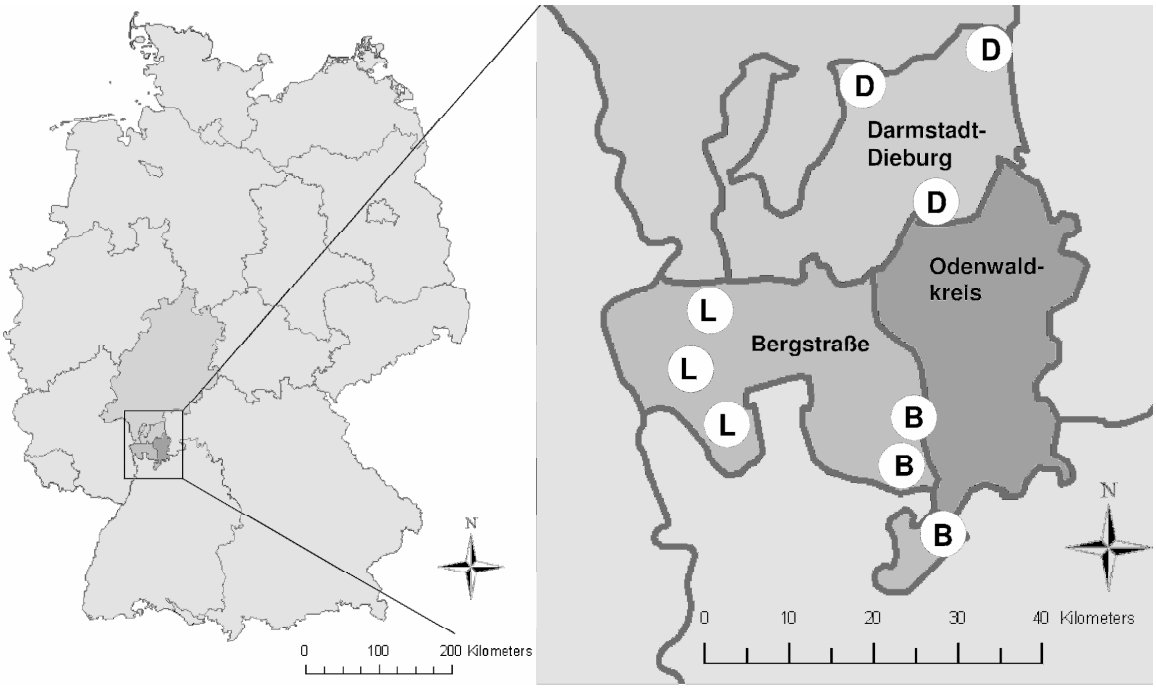
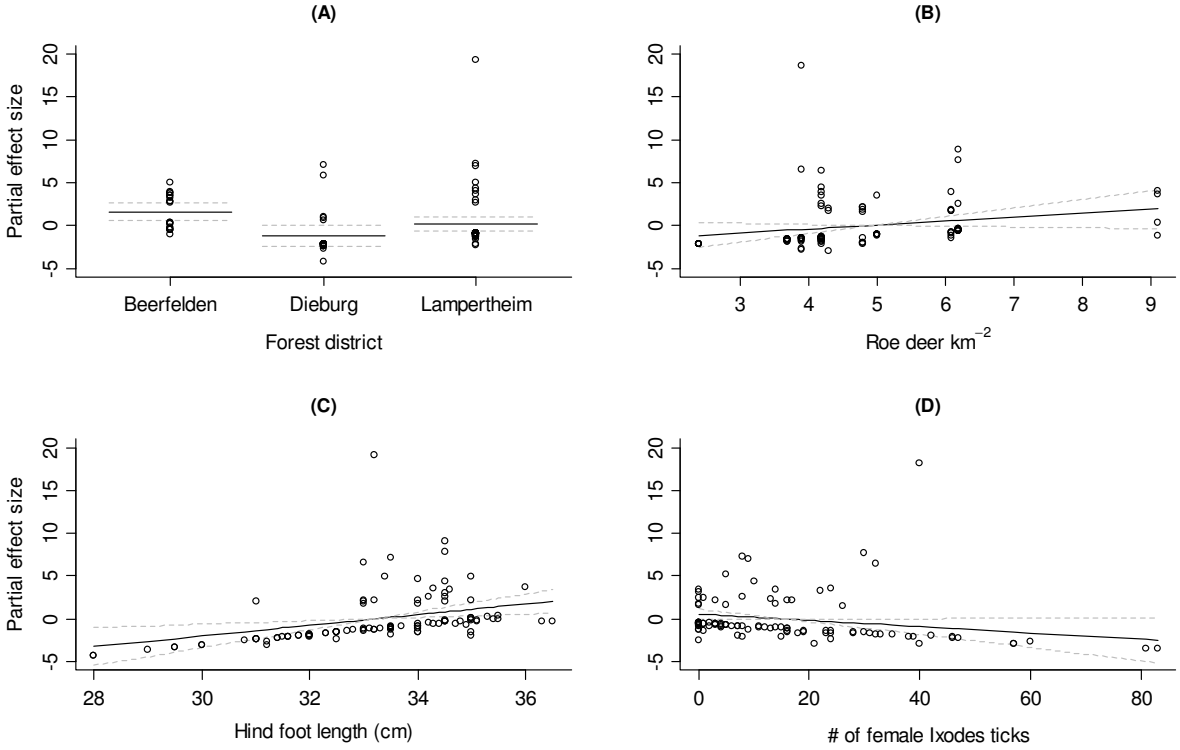


Fig. 2. Partial effect size (depicted as the linear predictor of the logistic regression model U) of variables explaining TBE virus antibody presence in roe deer (*Capreolus capreolus*) sera collected in three forest districts of southern Hesse, Germany, as derived from model U (Table 1).



Tables

Table 1. Summary statistics of TBE virus presence in roe deer sera and associated extrinsic explanatory variables stratified by forest district.

Forest District	TBE virus antibody prevalence	Range of spring warming (°C)	Range of roe deer density indices (deer/km²)
Beerfelden	50.0% (sample size=20)	12.9-13.4	4.3-6.1
Dieburg	14.7% (sample size=34)	10.9-14.7	2.4-9.1
Lampertheim	17.6% (sample size=51)	14.2-14.3	3.7-5.0

Table 2. Support for models explaining TBE virus presence in roe deer (*Capreolus capreolus*) sera. Models are grouped from bottom (simple models) to top (more complex). We first addressed the fundamental extrinsic factors season and forest district and then included further extrinsic factors (climatic factors, roe deer density index), intrinsic factors (age, body mass, hind foot length, sex) and tick burden. In each case, the best model from the set below was chosen based on minimum sample size corrected Akaike's information criterion values (AIC_c). *P* indicates the number of parameters used for fitting each model, Δ_i is the difference in AIC_c and w_i is the AIC_c weight based on all models (Burnham and Anderson 2002). The model with most support is highlighted in bold.

Model	Model letter	*P*	AIC_C	Δ_i	w_i
Tick burden					
*Dermacentor (*larvae and nymphs and males and females combined)	V	6	105.16	3.97	0.06
Ixodes females	**U**	**6**	**101.19**	**0.00**	**0.40**
Ixodes nymphs	T	6	105.86	4.67	0.04
Ixodes larvae	S	6	104.01	2.82	0.10
Best model from below	G	5	103.64	2.45	0.12
Individual characteristics					
Age and body mass and sex and hind foot length	R	8	108.58	7.39	0.01
Body mass and sex and hind foot length	Q	7	107.39	6.20	0.02
Age and sex and hind foot length	P	7	107.53	6.34	0.02
Age and body mass and hind foot length	O	7	107.29	6.10	0.02
Age and body mass and sex	N	7	108.08	6.89	0.01
Sex and hind foot length	M	6	105.45	4.26	0.05
Body mass and hind foot length	L	6	105.68	4.49	0.04
Body mass and sex	K	6	108.43	7.24	0.01
Age and hind foot length	J	6	105.74	4.55	0.04
Age and sex	I	6	113.64	12.45	0.00
Age and body mass	H	6	106.88	5.69	0.02
Hind foot length	G	5	103.64	2.45	0.12
Sex	F	5	111.43	10.24	0.00
Body mass	E	5	106.60	5.41	0.03
Age	D	5	111.39	10.20	0.00
Best model from below	C	4	109.23	8.04	0.01
Roe deer density					

Roe deer density index	C	4	109.23	8.04	0.01
Best model from below	A	3	109.88	8.69	0.01
Climatic factors					
Spring warming rate	B	4	111.89	10.70	0.00
Best model from below	A	3	109.88	8.69	0.01
Spatial factors					
Forest district	A	3	109.88	8.69	0.01

Table 3. Comparison of TBE virus prevalence in ticks (as determined by real-time-PCR) and prevalence of TBE virus antibodies (as determined by plaque reduction neutralisation test or ELISA) in sentinel animals in selected areas of central Europe.

Location	**Tick species/ sentinel species**	**Mean prevalence (n=sample size)**	**Reference**
Burgenland, Austria	*Ixodes ricinus* and *Dermacentor* spp..collected in vegetation	0% (n=306)	Dobler *et al.*, 2008
Northern Bavaria, Germany	*Ixodes ricinus* collected in vegetation	0. 2% (n=2150)	Kupča *et al*., 2010
Saxony-Anhalt, Germany	*Ixodes ricinus* collected in vegetation	0% (n=2202)	Klaus *et al.*, 2010a
Mecklenburg-Western Pomerania, Germany	*Ixodes ricinus* collected in vegetation	0% (n=651)	Klaus *et al.*, 2010a
Salem, Germany	*Ixodes ricinus* collected in vegetation	0% (n=294)	Klaus *et al.*, 2010b
Thuringia, Germany	*Ixodes ricinus* collected in vegetation and from deer	0% (n=1075)	Klaus *et al.*, 2010a
Bavaria, Germany	*Ixodes ricinus* collected from humans	8.8% (n=561)	Suess *et al*., 2006
Bavaria, Germany	*Ixodes ricinus* collected from humans	1.3% (n=239)	Klaus *et al.*, 2010a
Salem, Germany	Barbary macaque (*Macaca sylvanus*)	1.1 % (n=283)	Klaus *et al.*, 2010b
Trento, Italy	Goat (*Capra* spp.)	12.0%; range: 0-41.9% (n=459)	Rizzoli *et al*., 2007
South-western Germany	Red fox (*Vulpes vulpes*)	34.2% (n=79)	Rieger *et al*., 1999
Eastern Black Forest, Germany	Red fox (*Vulpes vulpes*)	9.8% (n=153)	Rieger *et al*., 1999
Odenwald, Germany	Red fox (*Vulpes vulpes*)	10.0% (n=50)	Rieger *et al*., 1999
Taunus, Germany	Red fox (*Vulpes vulpes*)	2.0% (n=50)	Rieger *et al*., 1999
Brandenburg, Germany	Red fox (*Vulpes vulpes*)	0% (n=86)	Rieger *et al*., 1999
North-eastern France	Red fox (*Vulpes vulpes*)	1.8% (n=55)	Rieger *et al*., 1999
Southern Germany	Roe deer (*Capreolus capreolus*)	43% (n=192)	Gerth *et al*., 1995
Denmark	Roe deer (*Capreolus capreolus*)	8.7% (n=237)	Skarphédinsson *et al*., 2005
Salem, Germany	Sheep (*Ovis aries*)	9.0% (n=100)	Klaus *et al.*, 2010b

Chapter 10 – Network “Rodent-borne pathogens”: Monitoring of hantavirus infections in Germany (in German with English summary)

Beiträge zur
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forschung · 34

Rainer G. Ulrich, Mathias Schlegel, Marc Mertens, Martin H. Groschup, Greifswald – Insel Riems; Jonas Schmidt-Chanasit, Anita Plenge-Bönig, Hamburg; Jens Jacob, Hans-Joachim Pelz, Münster; Jona Freise, Oldenburg; Matthias Wenk, Eberswalde; Jörg Thiel, Gotha; Cornelia Triebenbacher, Freising; Erik Schmolz, Andreas Kurth, Berlin; Frank Krüger, Ferdinand Rühe, Christian Kiffner, Göttingen; Hermann Ansorge, Görlitz; Werner Gerwin, Cottbus; Wolfgang Wegener, Köln; Jörg Müller, Grafenau; Margrit Bemmann, Schwerin; Ronny Wolf, Leipzig; Lutz-Florian Otto, Pirna; Rainer Oehme, Stuttgart; Martin Pfeffer, Leipzig; Gerald Heckel, Bern; Susanne Schex, Sandra S. Essbauer München

Netzwerk „Nagetier-übertragene Pathogene“: Monitoring von Hantavirus-Infektionen in Deutschland

Schlagworte/key words: Rodents, network, pathogens, hantavirus, Germany, endemic regions, prevalence

1. Netzwerk „Nagetier-übertragene Pathogene“ in Deutschland: Zielstellungen

Weltweit versterben jährlich mindestens 13 Millionen Menschen in Folge von Infektionskrankheiten (WHO, 1998). Von den bekannten rund 1.400 humanpathogenen Krankheitserregern sind ca. 60 % Erreger von Zooanthroponosen (Woolhouse und Gowtage-Sequeria, 2005; Jones et al., 2008). Bei Zooanthroponosen, im Folgenden als Zoonosen bezeichnet, handelt es sich um Infektionskrankheiten, bei denen der Erreger vom Tier auf den Menschen übertragen wird. In den vergangenen Jahren haben zoonotische Erkrankungen auch in Deutschland eine erhöhte Aufmerksamkeit erfahren. Mit Inkrafttreten des Gesetzes zur Verhütung und Bekämpfung von Infektionskrankheiten beim Menschen (Infektionsschutzgesetz, IfSG) im Jahr 2001 und der damit verbundenen Einführung der Meldepflicht für humane Infektionen mit bestimmten Zoonoseerregern wird eine bessere Erfassung dieser Erkrankungen ermöglicht. Bei einer größeren Zahl der gemäß IfSG erfassten Krankheiten spielen Nagetiere eine Rolle als Reservoirwirt (Tabelle 1). Das Wissen zur geographischen Verbreitung und Häufigkeit dieser Erreger in ihren natürlichen Reservoirwirten ist demgegenüber jedoch sehr gering. So fehlen insbesondere Langzeitdaten zu Dynamik und Übertragungs- und Migrationsprozessen in Nagetier-Populationen und deren Einfluss auf Prävalenz und molekulare Veränderungen der Zoonoseerreger.

Für Untersuchungen zu den genannten Fragestellungen wurde das Netzwerk „Nagetier-übertragene Pathogene“ initiiert, das eine Plattform für eine interdisziplinäre Zusammenarbeit von Arbeitsgruppen unterschiedlichster Expertise darstellt (Ulrich et al., 2009a). Die angestrebte intensive Zusammenarbeit von Zoologen, Ökologen, Virologen, Mikrobiologen, Parasitologen, Genetikern, Epidemiologen,

Tabelle 1 Übersicht über Nagetier- und Kleinsäuger-assoziierte Zoonoseerreger, die im Rahmen des Netzwerkes untersucht werden.

Erreger	Taxonomie: Familie (Genus)	Erregertyp	Vektoren	Erkrankung	Zahl der gemeldeten Fälle 2001–2009[a]	Partner im Netzwerk	Organprobe für Untersuchungen im Netzwerk
A- Vektor-vermittelte Übertragung							
FSME-Virus	*Flaviviridae (Flavivirus)*	(+) RNA-Virus	Zecken	FSME	2.738	G. Dobler, München; M. Niedrig, Berlin; J. Süss, Jena	Milz, Gehirn, Leber, Transudat
Borrelia spp.	*Spirochaetaceae (Borrelia)*	Gram-negative Bakterien	Zecken	Lyme-Borreliose	37.607[d]	F.-R. Matuschka, D. Richter, Berlin; V. Fingerle, Oberschleißheim	Ohren
Francisella tularensis	*Francisellaceae (Francisella)*	Gram-negative Bakterien	Blutsaugende Arthropoden	Tularämie	70	W. Splettstößer, München; R. Grunow, Berlin	Leber, Milz
Coxiella burnetii[b]	*Coxiellaceae (Coxiella)*	Gram-negative intrazelluläre Bakterien	Zecken	Q-Fieber	2.199	K. Henning, Wusterhausen; M. Runge, Hannover	Leber
Anaplasma (Ehrlichia) phagocytophilum	*Rickettsiaceae (Anaplasma)*	Gram-negative intrazelluläre Bakterien	Zecken	Anaplasmose/ Ehrlichiose	n.m.	K.-P. Hunfeld, Frankfurt/Main	Milz
Rickettsia spp.	*Rickettsiaceae*	Gram-negative intrazelluläre Bakterien	Zecken	Rickettsiose	n.m.	S. Essbauer/S. Schex, München	Ohren, Transudat
B – Nagetier-vermittelte Übertragung							
Hantaviren	*Bunyaviridae (Hantavirus)*	(-) RNA-Virus, 3 Segmente	–	HFRS/NE	3.282	R.G. Ulrich, Riems; S. Essbauer, München; J. Schmidt-Chanasit, Hamburg; B. Klempa, Berlin/Bratislava; D.H. Krüger, Berlin	Lunge, Herz, Gehirn, Transudat

Erreger	Taxonomie: Familie (Genus)	Erregertyp	Vektoren	Erkrankung	Zahl der gemeldeten Fälle 2001–2009[a]	Partner im Netzwerk	Organprobe für Untersuchungen im Netzwerk
LCM-Virus	*Arenaviridae (Arenavirus)*	(-) RNA-Virus, 2 Segmente	–	LCM	n.m.	S. Günther, J. Schmidt-Chanasit, Hamburg	Milz, Transudat
Kuhpockenvirus	*Poxviridae (Orthopoxvirus)*	dsDNA-Virus	–	Hautläsionen	n.m.	A. Nitsche, Berlin; B. Hoffmann, Riems; S. Essbauer, München; P. Kinnunen, Helsinki	Lunge, Leber, Transudat
Leptospira spp.	*Spirochaetaceae (Leptospira)*	Gram-negative Bakterien	–	Leptospirose	580	K. Nöckler, Berlin; H.C. Scholz, München; M. Pfeffer, Leipzig	Niere
Brucella spp.	*Brucellaceae (Brucella)*	Gram-negative Bakterien	–	Brucellose	239	H.C. Scholz, München; F. Melzer, Jena; K. Nöckler, Berlin	Leber
C – unbekannter Übertragungsweg/Lebensmittel-übertragene Erreger							
Hepatitis E-Virus[c]	nicht klassifiziert *(Hepevirus)*	(+) RNA-Virus	–	Hepatitis E	466	R. Johne, Berlin; R.G. Ulrich, Riems	Leber, Transudat
Escherichia coli (EHEC und weitere darmpathogene *E. coli* und deren resistente Stämme)	*Enterobacteriaceae (Escherichia)*	Gram-negative Stäbchenbakterien	–	HUS, HC	HUS: 591 EHEC/ STEC: 8.604	L.H. Wieler, S. Günther, K. Heidemanns, Berlin	Darm, Kot

a Quelle: RKI SurvStat, http://www3.rki.de/SurvStat, Datenstand: 19.8.2009
b Nagetiere spielen bei der Übertragung wahrscheinlich eine untergeordnete Rolle.
c serologische Nachweise von HEV-spezifischen Antikörpern bei Nagetieren, aber Rolle der Nagetiere als Reservoir ist unklar.
d nur in einigen Bundesländern meldepflichtig

Abkürzungen:
FSME, Frühsommer-Meningo-Enzephalitis
HFRS, hämorrhagisches Fieber mit renalem Syndrom
NE, Nephropathia epidemica
LCM, Lymphozytäre Choriomeningitis
EHEC, enterohämorrhagische *Escherichia coli*
STEC, Shiga-Toxin bildende *E. coli*
HUS, hämolytisch urämisches Syndrom
HC, hämorrhagische Colitis
n.m., nicht meldepflichtig

Forstwissenschaftlern und Klimaforschern mit Klinikern der Human- und Veterinärmedizin soll zu einem besseren Verständnis der komplexen Wechselbeziehungen zwischen Krankheitserregern, Reservoirwirten, Vektoren und Prädatoren und dem Auftreten von Infektionen beim Menschen beitragen. Die Aktivitäten der Partner des Netzwerkes umfassen gegenwärtig vier Schwerpunkte: (i) Untersuchungen zu unterschiedlichen Aspekten der Nagetierbiologie und deren Zusammenhang mit der Verbreitung von Zoonoseerregern betreffen vor allem Fragen zur Ökologie, Paläozoologie und Nagetierphylogenie sowie Populationsdynamik und Nagetier-Bekämpfung (Resistenzentwicklung). Zukünftig sollen diese Studien durch Untersuchungen zur Immun- und Populationsgenetik und Verhaltensbiologie der Nagetiere ergänzt werden. (ii) Molekular- und seroepidemiologische Studien zu Nagetier-assoziierten Zoonoseerregern (siehe Tabelle 1) beinhalten vor allem Untersuchungen zu den möglichen Ursachen des gehäuften Auftretens humaner Infektionen und Monitoringstudien in ausgewählten geografischen Regionen. Die Untersuchungen dienen letztendlich auch der Identifizierung und molekularen Charakterisierung der verschiedenen Erreger (Viren, Bakterien und Parasiten). Zu den in die Untersuchungen einbezogenen Erregern zählen solche mit einem den Hantaviren ähnlichen Übertragungsweg über Aerosole (Arenaviren und Leptospiren), solche mit Übertragungswegen, bei denen Haus- und Nutztiere eine Rolle spielen (Kuhpockenviren) aber auch durch Arthropoden übertragene Erreger (Frühsommer-Meningo-Enzephalitis-Virus, Rickettsien und Borrelien). Daneben werden Pathogene betrachtet, die über Lebensmittel oder einen bisher nicht genauer bekannten Übertragungsweg, bei dem Nagetiere aber möglicherweise als Reservoir dienen, verbreitet werden (Hepatitis E-Virus und verschiedene bakterielle Durchfallerreger). Schließlich dient das Netzwerk der Suche nach neuen Pathogenen, die als Modellobjekte für verwandte human- und tiermedizinisch relevante Erreger dienen könnten. So wurden beispielsweise bei der Untersuchung von Nagetieren und anderen Kleinsäugern eine große Zahl neuer Herpesviren entdeckt (Ehlers et al., 2007, 2008). Die umfangreichen Kooperationsbeziehungen des Netzwerkes sollten zukünftig auch eine passive Surveillance bei Nagetieren und anderen Kleinsäugern erlauben. (iii) Einen dritten Schwerpunkt bilden Prävalenzstudien in Risikogruppen. So wird beispielsweise gegenwärtig eine Waldarbeiterstudie im Land Brandenburg durchgeführt, an der 10 wissenschaftliche Einrichtungen beteiligt sind und in der die Seroprävalenz für 15 verschiedene Erreger ermittelt wird. Zu diesem Zweck wurden im Jahr 2008 von insgesamt 563 Beschäftigten aller 10 Forstämter des Landes Brandenburg Serumproben gewonnen. (iv) Neben den wissenschaftlichen Zielsetzungen des Netzwerkes stellt die Öffentlichkeitsarbeit einen weiteren wichtigen Schwerpunkt der Aktivitäten dar. Diese beinhaltet unter anderem Veröffentlichungen zur Aufklärung von Berufsgruppen wie Waldarbeitern, Jägern und Schädlingsbekämpfern, die durch bestimmte Zoonoseerreger, wie Hantaviren, besonders gefährdet sind (Ulrich et al., 2005, 2006a, 2007). Kürzlich wurde auch in einer gemeinsamen Aktivität von Robert Koch-Institut (RKI), Julius Kühn-Institut (JKI), dem Konsiliarlaboratorium für Hantaviren am Institut für medizinische Virologie (IMV) der Charité und dem Friedrich-Loeffler-Institut (FLI) das Merkblatt „Wie vermeide ich Hantavirusinfektionen“ aktualisiert und dort auch Ansprechpartner für Fragen zu Hantavirusinfektionen bei Mensch und Nagetier sowie zur Nagetierbekämpfung benannt (siehe Homepage des FLI: http://www.fli.bund.de/1235.html).

2. Organisationsstruktur der Untersuchungen im Netzwerk

Den bisherigen Schwerpunkt der Untersuchungen stellen Studien an wildlebenden Nagetieren dar. So bildeten von der Landesforstanstalt Eberswalde koordinierte Monitoringfänge von forstschädlichen Nagetieren im Land Brandenburg den Ausgangspunkt für die Etablierung des Netzwerkes. Aus diesen Monitoringfängen wurden dem Netzwerk seit dem Jahr 2001 mehr als 1.000 Nagetiere zur Verfügung gestellt. Inzwischen werden durch weitere forstliche Einrichtungen in Mecklenburg-Vorpommern, Thüringen, Sachsen-Anhalt, Bayern, Sachsen und Hessen sowie dem Büsgen-Institut (BI)

der Universität Göttingen Nagetiere aus Monitoringfängen und von anderen Fangaktivitäten zur Verfügung gestellt (Abb. 1). Im Rahmen des Arbeitskreises „Mäuse im Forst“ besteht ein regelmäßiger intensiver Informationsaustausch zwischen dem Netzwerk und den forstlichen Einrichtungen. Einen zweiten Schwerpunkt von Wildnagetierfängen bilden aufsuchende epidemiologische Untersuchungen an Wohn- und anderen potentiellen Expositionsorten von Hantavirus-Patienten insbesondere in Regionen mit häufigen humanen Infektionen und in Ausbruchsregionen. Die Planung der entsprechenden Fänge erfolgt hierbei in enger Zusammenarbeit mit niedergelassenen Ärzten, lokalen und regionalen Gesundheitsämtern und dem RKI. Neben eigenen Fängen des FLI und des Instituts für Mikrobiologie der Bundeswehr (IMB) werden diese Studien durch das JKI, das Niedersächsische Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES), das Bernhard-Nocht-Institut für Tropenmedizin (BNI) und das Gesundheitsamt Köln unterstützt. Die Fangaktivitäten beinhalteten bisher vor allem ländliche Regionen, aber auch in verschiedenen Städten wie Köln und Aachen wurden bereits Nagetiere für Untersuchungen im Netzwerk gefangen (Abb. 2A, B). Neben

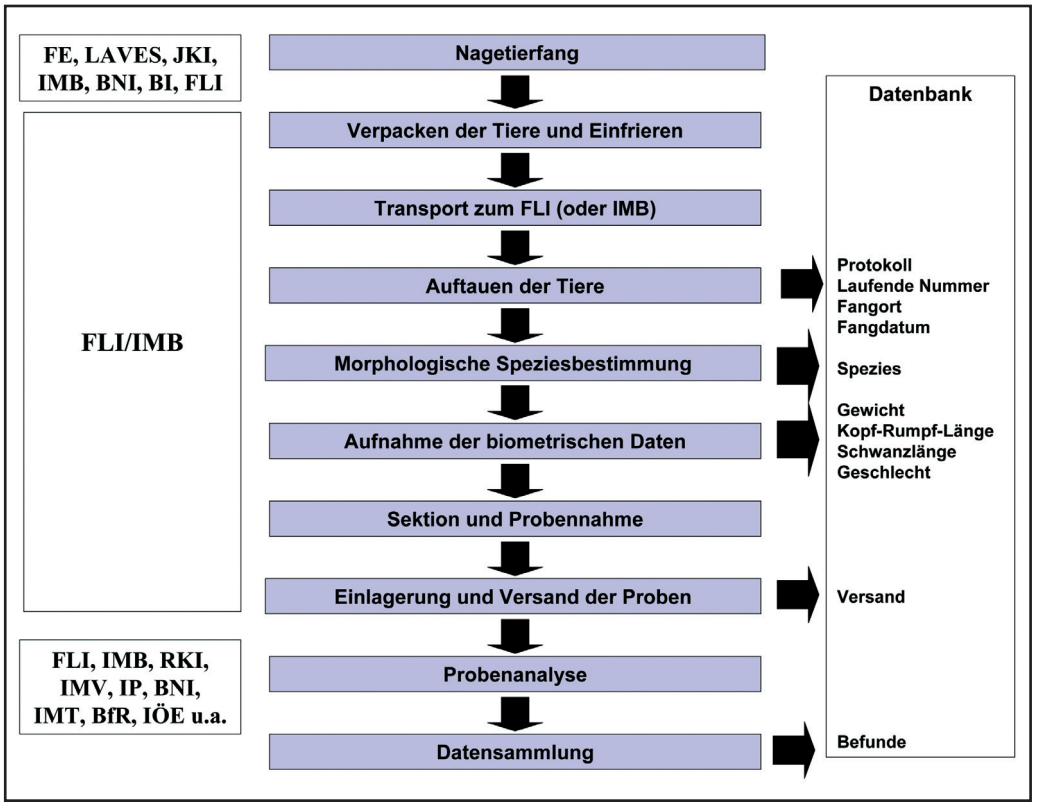

Abb. 1 Ablaufschema der Nagetier-Untersuchungen im Netzwerk.
Abkürzungen: FE, forstliche Einrichtungen in verschiedenen Bundesländern; LAVES, Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg; JKI, Julius Kühn-Institut, Münster; IMB, Institut für Mikrobiologie der Bundeswehr, München; BNI, Bernhard-Nocht-Institut für Tropenmedizin, Hamburg; BI, Büsgen-Institut, Göttingen; FLI, Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Wusterhausen und Jena; RKI, Robert Koch-Institut, Berlin; IMV, Charité – Universitätsmedizin Berlin, Institut für Medizinische Virologie; IP, Charité – Universitätsmedizin Berlin, Institut für Pathologie, Abteilung Parasitologie, Berlin; IMT, Freie Universität Berlin, Institut für Mikrobiologie und Tierseuchen, Berlin; BfR, Bundesinstitut für Risikobewertung, Berlin; IÖE, Institut für Ökologie und Evolution, Universität Bern, Bern, Schweiz

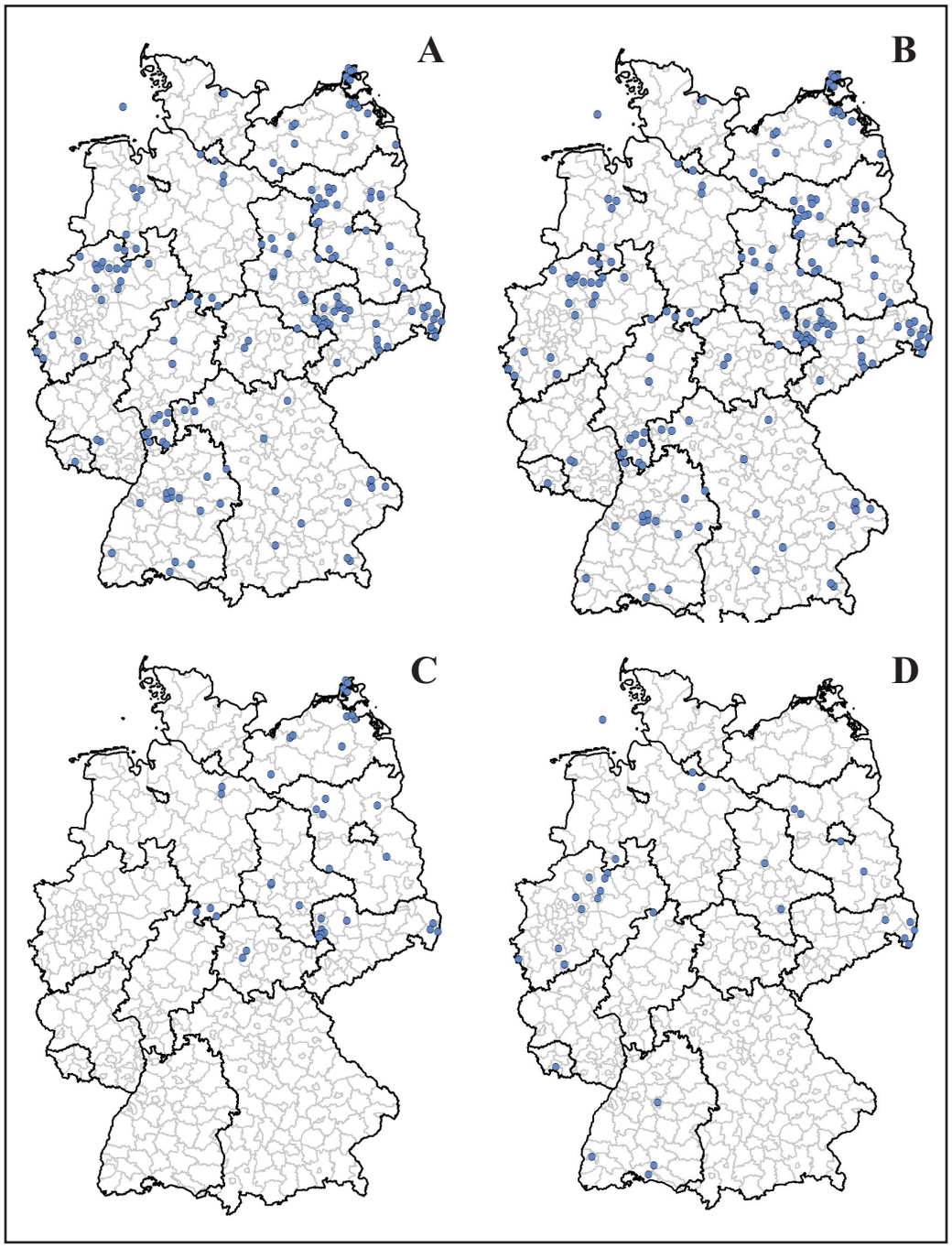

Abb. 2 A-D Bisherige Fangorte von Nagetieren insgesamt (A), Rötelmäusen (Myodes glareolus; B), Brandmäusen (Apodemus agrarius; C) und kommensalen Nagetieren (Mus musculus/domesticus, Rattus spp.; D) in Deutschland und Probenentnahmeorte von Wanderratten (Rattus norvegicus) in der Stadt Hamburg (E).
Die Abbildungen A, B, C und D wurden freundlicher Weise von Petra Kranz und Heike Kubitza (Wusterhausen) angefertigt.

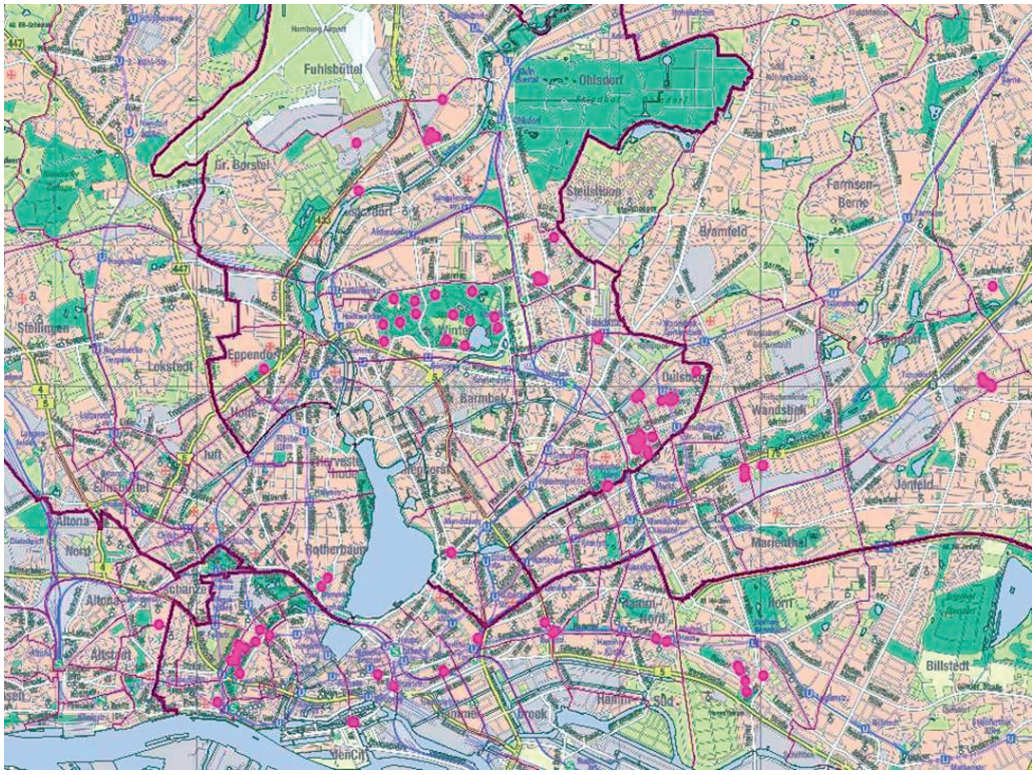

Abb. 2 E Stadt Hamburg

den genannten Institutionen wird das Netzwerk gegenwärtig auch durch eine große Zahl weiterer Institutionen und einzelner Kolleginnen und Kollegen durch Bereitstellung von Nagetierproben unterstützt.

Insgesamt stehen uns bisher ca. 7.500 Kleinsäuger aus 14 verschiedenen Bundesländern (Abb. 2A) von Fängen des FLI, IMB, BNI und deren Kooperationspartnern zur Verfügung. Darunter befinden sich ca. 2.100 Rötelmäuse (*Myodes glareolus,* Abb. 2B), ca. 2.100 Gelbhals- und Waldmäuse (*Apodemus flavicollis*, *A. sylvaticus*), ca. 300 Brandmäuse (*A. agrarius;* Abb. 2C), ca. 1.200 Feld- und Erdmäuse (*Microtus arvalis*, *M. agrestis*) sowie einzelne weitere Nagetiere und andere Kleinsäuger. Die bisherigen Nagetierfänge geben auch erste Hinweise auf die gegenwärtige westliche Verbreitungsgrenze der Brandmaus in Niedersachsen und Hessen (Abb. 2C).

Neben den Untersuchungen an Wildnagetieren sollen zukünftig auch kommensale Nagetiere, wie Hausmaus (*Mus musculus*/*M. domesticus*) und Wanderratte (*Rattus norvegicus*), stärker in die Untersuchungen einbezogen werden. Dazu wird gegenwärtig eine zusätzliche Anbindung des Netzwerkes an weitere Forschungsverbünde vollzogen. So hat beispielsweise das behördliche Hamburger Institut für Hygiene und Umwelt für die Hafenmetropole zur Verbesserung der Kontrolle der Stadtratten ein Geodaten gestütztes Monitoring der urbanen Populationen von *R. norvegicus* entwickelt.

In die Datenbank gehen alle Rattenmeldungen aus der Bevölkerung sowie Eigenermittlungen ein. Ein Datenbestand von etwa 3.000 Ratten-Vorkommen pro Jahr wird so systematisch und vergleichend einer Auswertung und einer geographischen Darstellung sowie zukünftig auch einer Geodaten-bezogenen Analyse unterzogen.

Das Monitoring wird für die Probennahme bei Tieren zum Zwecke der Untersuchung auf Zoonoseerreger im Netzwerk sowie auf Genmutationen, die bei Nagetieren eine Resistenz gegen

Antikoagulantien vermitteln, genutzt. Hierbei erfährt das Netzwerk auch Unterstützung durch verschiedene Schädlingsbekämpfer in Deutschland.

Die Untersuchungsergebnisse tragen zur Aufklärung der Verbreitung resistenter Ratten und Hausmäuse sowie zur Entwicklung einer Resistenzmanagementstrategie bei (Pelz et al., 2007; Pelz & Freise, 2009). Bisher sind dem Netzwerk ca. 170 Wanderratten und ca. 280 Hausmäuse aus verschiedenen Bundesländern zur Verfügung gestellt worden (Abb. 2D, E).

Die Untersuchungen zu Resistenzen gegen Antikoagulantien können im Bereich der Seuchenbekämpfung besondere Bedeutung erlangen, da das IfSG beim Auftreten von gesundheitsgefährdenden Schadnagetieren behördliche Bekämpfungsmaßnahmen vorschreibt (IfSG § 17). Dafür dürfen nur staatlich auf Wirksamkeit geprüfte und gelistete Rodentizide verwendet werden (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2008).

Das Umweltbundesamt führt solche Wirksamkeitsprüfungen durch und entwickelt entsprechende Testmethoden. Für die Prüfungen werden Wildstammzuchten von Hausratten (*R. rattus*), Wanderratten (*R. norvegicus*) und Hausmäusen (*M. musculus*) gehalten. Tiere aus diesen Zuchten werden zudem Netzwerkpartnern für Untersuchungen auf neue Pathogene zur Verfügung gestellt. Momentan wird verstärkt mit Partnern aus dem Netzwerk an dem Aufbau von Rötelmaus- und Feldmauszuchten und der Entwicklung entsprechender Prüfsysteme gearbeitet.

Für ein gehäuftes Auftreten von Kuhpockenvirusinfektionen bei Patienten in Nordrhein-Westfalen, Bayern und Niedersachsen in den Jahren 2008 und 2009 wurde erstmalig der direkte Kontakt zu Virus-infizierten Heim- und Liebhabertieren, sogenannten „Schmuseratten" (*R. norvegicus* forma *domestica*), verantwortlich gemacht (Becker et al., 2009).

Zwischen Februar 2008 und März 2009 verstarben über 50 % der nachgewiesenen Kuhpockenvirus-infizierten Ratten. Die Herkunft der Ratten wurde über Zootierhändler zu deutschen Großhändlern zurückverfolgt, die die Tiere aus dem europäischen Ausland bezogen. Dabei wurden von einem verdächtigten ausländischen Großhändler innerhalb von 4 Monaten über 120.000 Ratten nach Deutschland exportiert. Wegen der großen Bedeutung für die Übertragung von Zoonoseerregern sollen zukünftig auch solche Tiere in Untersuchungen des Netzwerkes einbezogen werden.

Um eine möglichst breite Untersuchung der Nagetiere auf verschiedene bekannte Zoonoseerreger und neue Pathogene zu ermöglichen, erfolgt eine zentrale Erfassung und Sektion der Tiere am FLI (bzw. IMB; Abb. 1).

Dazu werden die Nagetiere nach dem Fang zunächst bei den entsprechenden Partnern eingefroren. Zu jedem Tier erfolgt die Erfassung von Fangort und Fangdatum. Die Überführung der Tiere zum FLI (bzw. IMB) erfolgt im gefrorenen Zustand, die Sektion und Entnahme der Organproben nach einem Standardprotokoll. Für serologische Untersuchungen wird von jedem Tier aus der Brusthöhle Transudat entnommen. Organproben für Erreger-spezifische RT-PCR- und PCR-Untersuchungen beinhalten Herz, Lunge, Leber, Milz, Niere, Ohren und Gehirn (Tabelle 1).

Für spezielle Fragestellungen wird auch noch anderes Gewebe wie Darm, Lymphknoten oder Ganglien entnommen. Um mitochondriale Genloci und Resistenzgenpolymorphismen zu analysieren, werden Schwanzstücke der Tiere verwendet. Während die Hantavirus-Untersuchungen am FLI und in Zusammenarbeit mit IMB, BNI und IMV durchgeführt werden, werden weitere Viren, Bakterien und Parasiten und die Antikoagulantienresistenz bei anderen Netzwerkpartnern untersucht (Abb. 1 und Tabelle 1).

Die Zentralisierung von Sektion, Probensammlung, Probenversand und Dokumentation ermöglicht in Zukunft eine Kombination von Daten zu verschiedenen Nagetier-assoziierten Krankheitserregern sowie zu biologischen und populationsgenetischen Markern der Nager. Um entsprechende Zusammenhänge aufdecken zu können, soll eine Datenbank aufgebaut werden.

Zur weiteren Ausgestaltung der Zusammenarbeit im Netzwerk fand im November 2008 erstmalig ein Workshop der Netzwerkpartner am FLI statt, der zukünftig im Zweijahresrhythmus durchgeführt werden soll (siehe Homepage des FLI: http://www.fli.bund.de).

3. Hantaviren in Deutschland

Hantavirus-Infektionen können beim Menschen zu einer fiebrigen Erkrankung mit Nierenfunktionsstörungen führen, die als Hämorrhagisches Fieber mit renalem Syndrom (HFRS) bezeichnet wird. Die meisten Fälle des HFRS in Deutschland werden durch das *Puumalavirus* (PUUV) hervorgerufen. Diese Erkrankungen zeigen meist eher milde und moderate Verläufe und werden als *Nephropathia epidemica* bezeichnet. Häufig verlaufen die Infektionen mit einer Grippe-ähnlichen Symptomatik und werden deshalb nicht als Hantavirus-Infektion diagnostiziert (Ulrich et al., 2004).

In Deutschland gibt es mindestens drei verschiedene Hantaviren (siehe Tabelle 2). Das von der Rötelmaus (*Myodes glareolus*, vormals *Clethrionomys glareolus*) übertragene PUUV scheint deutschlandweit verbreitet zu sein (Ulrich et al., 2009b). Humane PUUV-Infektionen sind in Deutschland durch umfangreiche seroepidemiologische und molekularbiologische Studien bereits seit Anfang der 1990er Jahre belegt (Pilaski et al., 1991, 1994; Zöller et al., 1995). Insbesondere intensive Untersuchungen bei Patienten während der Ausbrüche in den Jahren 2004 und 2007 führten zur molekularen Charakterisierung von PUUV-Sequenzen (Schilling et al., 2007; Hofmann et al., 2008). Serologische Untersuchungen unter Verwendung des Neutralisationstests zeigten das Vorkommen von *Dobrava-Belgrad-Virus* (DOBV) in Patienten und Risikogruppen aus Nord- und Nordostdeutschland (Meisel et al., 1998; Mentel et al., 1999; Sibold et al., 2001). Bisher

Tabelle 2 Übersicht über die in Deutschland vorkommenden Hantaviren, deren Humanpathogenität, potentielle Nagetier-Reservoire und bisherige Nachweise in Nagetierreservoiren in Deutschland

Reservoirwirt*				**Hantavirus-Art**	**Nachweis in Nagern in Deutschland**	**Human-pathogenität**
Ordnung	Familie	Unterfamilie	Art			
Rodentia	Cricetidae	Arvicolinae	Rötelmaus (*Myodes glareolus*)	PUUV	BW, BY, HE, NI, NW, SH, ST	HFRS/NE
			Feld- und Erdmaus (*Microtus arvalis, M. agrestis*)	TULV	BB, BW, HE, NI, ST, TH	HFRS**
	Muridae	Murinae	Brandmaus *(Apodemus agrarius)*	DOBV-Aa	BB, MV, NI	HFRS
			Wanderratte *(Rattus norvegicus)*	SEOV	NW	HFRS (?)***

Erläuterungen zu Tabelle 2:

HFRS, Hämorrhagisches Fieber mit renalem Syndrom; NE, *Nephropathia epidemica* (milde Form des HFRS, verursacht durch PUUV); PUUV, *Puumalavirus*; TULV, *Tulavirus*; DOBV, *Dobrava-Belgrad-Virus*; DOBV-Aa, *A. agrarius*-assoziiertes Dobrava-Belgrad-Virus; SEOV, *Seoulvirus*; BW, Baden-Württemberg; BY, Bayern; HE, Hessen; NI, Niedersachsen; NW, Nordrhein-Westfalen; SH, Schleswig-Holstein; ST, Sachsen-Anhalt; BB, Brandenburg; TH, Thüringen; MV, Mecklenburg-Vorpommern.

* Taxonomie nach Wilson und Reeder (2005).

** bisher wurde nur von einem Patienten mit TULV-Infektion berichtet.

*** trotz eines Hinweises zum Vorkommen des SEOV in Wanderratten bisher kein Nachweis von humanen SEOV-Infektionen.

Daten entnommen aus: Heiske et al., 1999; Essbauer et al., 2006, 2007a,b; Hofmann et al., 2008; Klempa et al., 2003; Pilaski et al., 1991, 1994; Schilling et al., 2007; unsere unveröffentlichten Daten.

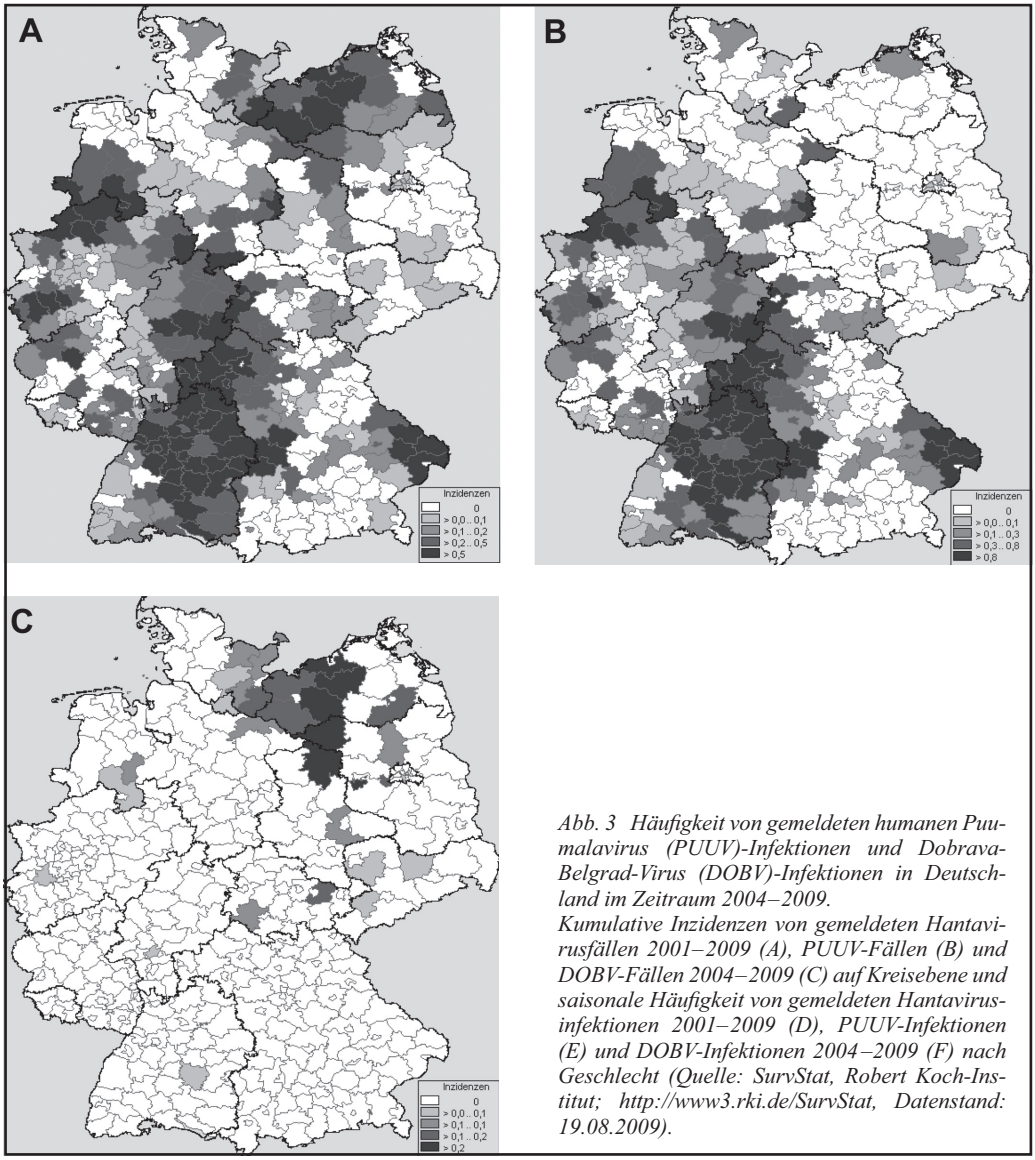

Abb. 3 Häufigkeit von gemeldeten humanen Puumalavirus (PUUV)-Infektionen und Dobrava-Belgrad-Virus (DOBV)-Infektionen in Deutschland im Zeitraum 2004–2009.
Kumulative Inzidenzen von gemeldeten Hantavirusfällen 2001–2009 (A), PUUV-Fällen (B) und DOBV-Fällen 2004–2009 (C) auf Kreisebene und saisonale Häufigkeit von gemeldeten Hantavirusinfektionen 2001–2009 (D), PUUV-Infektionen (E) und DOBV-Infektionen 2004–2009 (F) nach Geschlecht (Quelle: SurvStat, Robert Koch-Institut; http://www3.rki.de/SurvStat, Datenstand: 19.08.2009).

liegt jedoch nur eine kurze DOBV-Sequenz aus einem Patienten aus Nordostdeutschland vor (Klempa et al., 2004). Zum Vorkommen von Infektionen mit einem dritten, möglicherweise nicht oder nur sehr gering humanpathogenen Hantavirus, dem *Tulavirus* (TULV), gibt es in Deutschland bisher nur sehr wenige Daten (Klempa et al., 2003; Ulrich et al., 2004).

Die geografische Verbreitung und Häufigkeit von humanen Hantavirus-Infektionen ist durch große seroepidemiologische Studien (Zöller et al., 1995) und die Meldung von klinischen Fällen seit Einführung des IfSG im Jahr 2001 gut belegt. So wurden in Deutschland im Zeitraum 2001–2009 insgesamt 3.282 Fälle gemeldet (Robert-Koch-Institut: SurvStat, http://www3.rki.de/SurvStat, Datenstand 19.08.2009), von denen die meisten auf die Bundesländer Baden-Württemberg (1.716), Bayern (517), Nordrhein-Westfalen (480) und Niedersachsen (229)

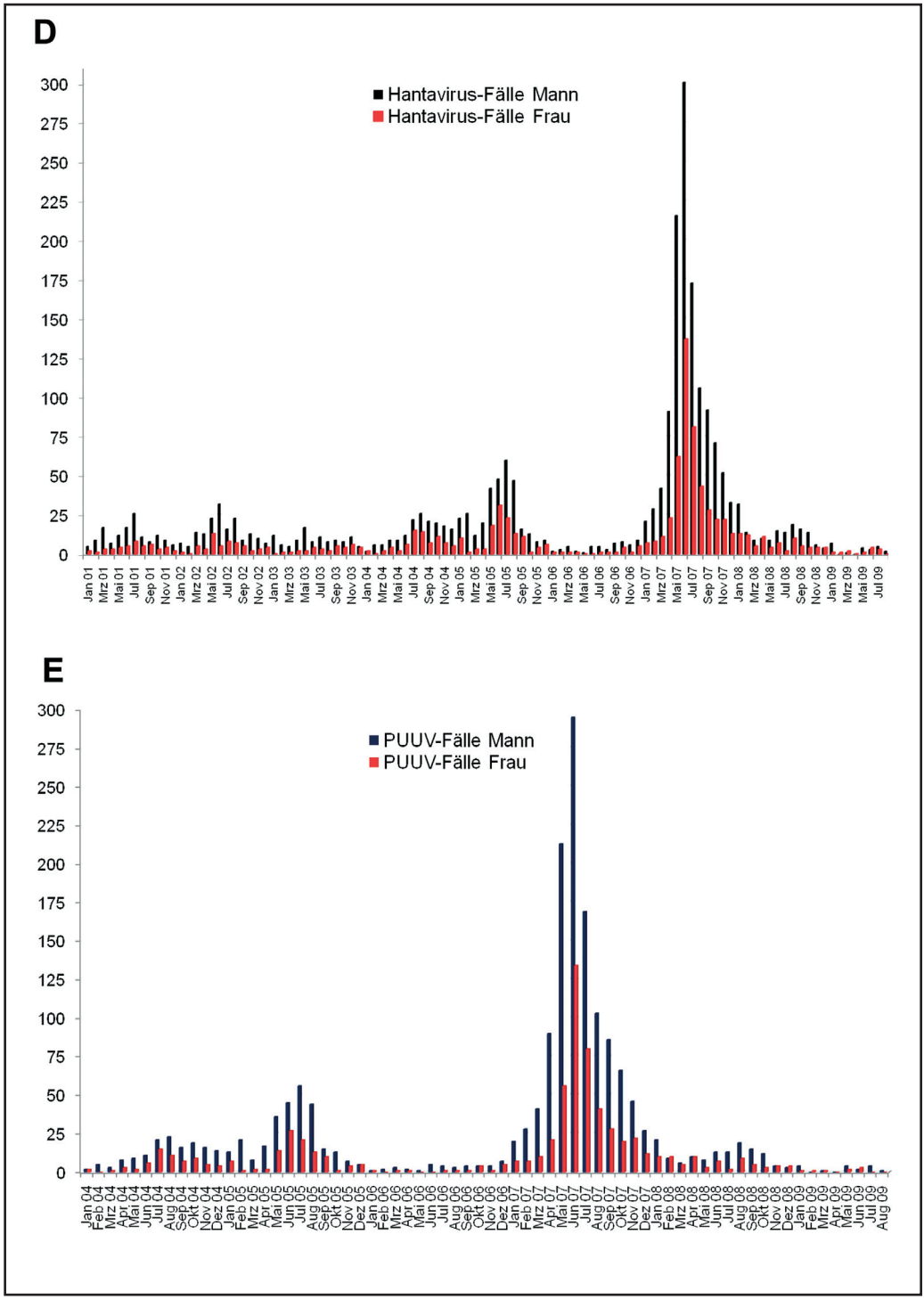
D
Hantavirus-Fälle Mann
Hantavirus-Fälle Frau
E
PUUV-Fälle Mann
PUUV-Fälle Frau

Abb. 3 D/E

entfielen (Abb. 3A). Die Mehrzahl der gemeldeten Fälle in den Jahren 2004–2009 betreffen PUUV-Infektionen (2.509 von 2.726, 92 %; Abb. 3B), während nur ein kleiner Teil auf Infektionen mit dem DOBV, das sehr wahrscheinlich ausschließlich in Nord- und Nordostdeutschland vorkommt, zurückgeführt wird (Abb. 3C).

Während in den Jahren 2001 bis 2004 und im Jahr 2006 etwa 70–240 klinische Fälle registriert wurden, ist im Jahr 2005 und insbesondere im Jahr 2007 ein starker Anstieg der Zahl der gemeldeten Fälle auf 447 bzw. 1.688 verzeichnet worden (Abb. 3D).

Eine genauere Betrachtung der Saisonalität der Zahl der gemeldeten Fälle zeigt für die Gesamtheit der Hantavirus-Fälle (Abb. 3D) und gesondert für die PUUV-Fälle (Abb. 3E) einen deutlichen Sommerpeak. Obgleich die Zahl der gemeldeten DOBV-Fälle gegenwärtig noch zu niedrig ist, scheint das Auftreten von humanen DOBV-Infektionen ein anderes Saisonalitätsmuster zu zeigen (Abb. 3F). Die Zahl aller gemeldeten Hantavirus-Fälle betrifft zu 72,4 % Männer (Abb. 3D). Bei PUUV-Infektionen entfielen im Zeitraum 2004–2009 71,7 % auf Männer (Abb. 3E); bei DOBV-Infektionen sind im gleichen Zeitraum 72,7 % für Männer gemeldet worden (Abb. 3F).

Potentielle Hantavirus-Reservoire in Deutschland sind die Rötelmaus (*M. glareolus*), Feldmaus (*M. arvalis*) sowie Brand- und Gelbhalsmaus (*A. agrarius* und *A. flavicollis*; siehe Tabelle 2).

Zu Beginn unserer Untersuchungen war eine PUUV-Sequenz aus einer Rötelmaus aus Nordrhein-Westfalen und drei TULV-Sequenzen aus Feldmäusen aus Brandenburg bekannt (Heiske et al., 1999; Klempa et al., 2003). Der Reservoirwirt des DOBV in Deutschland war nicht identifiziert. Die Ähnlichkeit einer DOBV-Sequenz aus einem HFRS-Patienten aus Norddeutschland mit DOBV-Sequenzen aus Brandmäusen aus anderen Teilen Europas wies auf einen Ursprung des Virus aus der Brandmaus hin (Klempa et al., 2004).

In Deutschland vorkommende kommensale Nagetiere wie Wanderratte (*R. norvegicus*) und Hausmaus (*M. musculus*), Wildnagetiere wie der Bisam (*Ondatra zibethicus*) aber auch In-

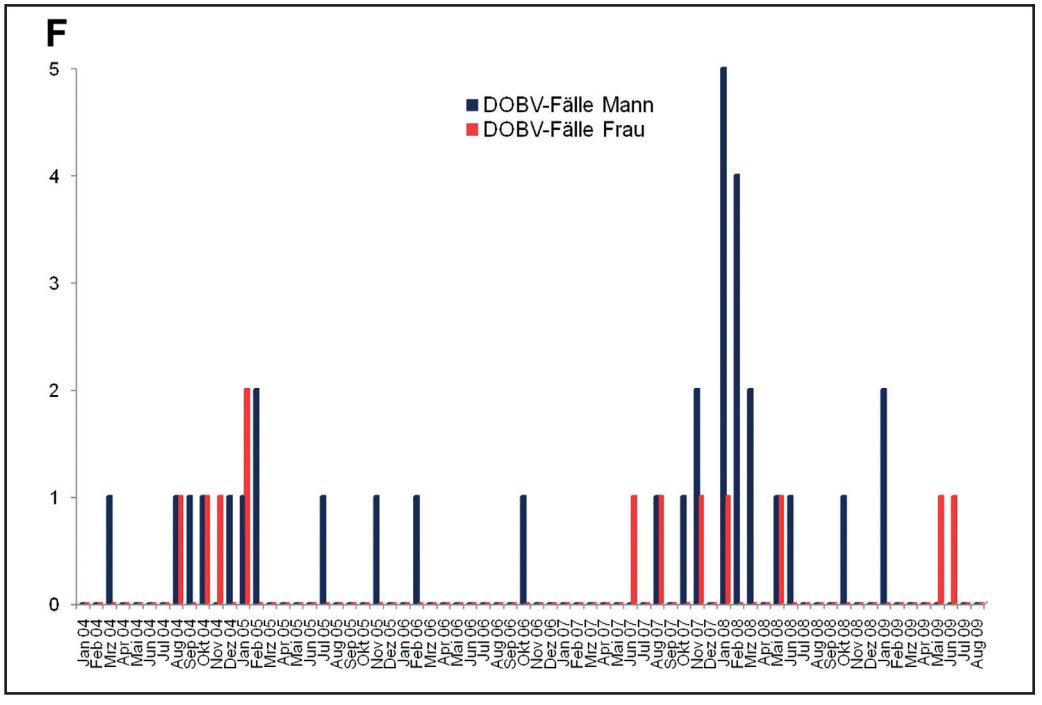

Abb. 3 F

sektenfresser wie Spitzmäuse (Soricidae) und Maulwürfe (Talpidae) könnten weitere Hantaviren beherbergen (Pilaski et al., 1991; Vahlenkamp et al., 1998; Kang et al., 2009).
Aus diesem Kenntnisstand ergeben sich eine Reihe von Fragen, die in der ersten deskriptiven Phase der Netzwerk-Untersuchungen beantwortet werden sollen (Tabelle 3). Zukünftig sollen die Untersuchungen im Rahmen des Netzwerkes verstärkt auch evolutionsbiologischen und epidemiologischen Fragestellungen gewidmet werden (Tabelle 4).

Tabelle 3 Fragestellungen für gegenwärtige Untersuchungen zu Hantaviren in Reservoirwirten

Fragestellung
Was sind die Nagetier-Reservoire von DOBV und TULV in Deutschland?
Wie ist die geografische Verbreitung von Hantavirus-Infektionen in Wildnagetieren in Deutschland?
Wie ist die Häufigkeit von Hantavirus-Infektionen bei Nagetieren in Ausbruchs- und Nichtausbruchsregionen und in Zeiten von Ausbrüchen und während Nicht-Ausbruchszeiten?
Wie stark unterscheiden sich die Stämme von PUUV, TULV und DOBV in den verschiedenen Regionen Deutschlands?
Gibt es in Deutschland Spitzmaus-assoziierte Hantaviren?
Kommen in Deutschland bei kommensalen Nagetieren und Neozoa Hantaviren vor?
Gibt es Interaktionen zwischen Hantaviren und anderen Nagetier-assoziierten Erregern?

Tabelle 4 Fragestellungen für zukünftige epidemiologische und evolutionsbiologische Untersuchungen

Fragestellung
Wie kann die gegenwärtige Verbreitung von Nagetieren und der mit ihnen assoziierten Hantaviren erklärt werden?
Wie und durch welche Evolutionsfaktoren bedingt verändern sich Hantaviren und andere Nagetier-assoziierte Krankheitserreger?
Wie ist die Divergenz der PUUV-, DOBV- und TULV-Sequenzen in Deutschland zu erklären?
Welche Auswirkungen hat die Nagetierbekämpfung auf die Durchseuchung mit Hantaviren?
Was sind die Ursachen von Hantavirus-Ausbrüchen?
Wodurch werden Massenvermehrungen von Nagetierreservoiren hervorgerufen und lassen sich diese vorhersagen?
Welche klimatischen und Habitat-Faktoren beeinflussen die Verbreitung der Erreger innerhalb und zwischen Nagetier-Populationen und die Übertragung auf den Menschen?
Welchen Einfluss haben Nagetier-Prädatoren auf die Epidemiologie von Hantavirus-Infektionen?
Gibt es genetische Faktoren bei den Nagetieren, die die Infektion mit Hantaviren und anderen Erregern begünstigen oder behindern?
Wie häufig treten Spillover-Infektionen auf?
Kommt es bei Spillover-Infektionen zur Ausbildung von Krankheitssymptomen und könnte das der Ausgangspunkt für die Entwicklung neuer Tiermodelle sein?

4. Methodisches Vorgehen bei Hantavirus-Untersuchungen in Reservoir-Nagetieren

Zur Beantwortung der in Tabelle 3 aufgeführten Fragestellungen werden alle Tiere nach einem Standardprotokoll untersucht. Zunächst erfolgt ein serologisches Screening der Nagetiere unter Verwendung eines indirekten Immunglobulin G (IgG)-ELISA auf der Basis des jeweiligen „homologen" rekombinanten Hantavirus-Antigens (Razanskiene et al., 2004). Somit werden Rötelmäuse mit PUUV-Antigen, *Apodemus*-Mäuse mit DOBV-Antigen und *Microtus*-Mäuse mit TULV-Antigen untersucht (Essbauer et al., 2006; unsere unveröffentlichten Daten). Zur Verbesserung der diagnostischen Sensitivität wurde kürzlich zur Verwendung eines Nukleokapsidproteins eines PUUV-Stammes aus Niederbayern übergegangen. Für die Untersuchung von Rattenproben werden *Hantaanvirus*- und/oder *Seoulvirus*-Antigene (Schmidt et al., 2005) eingesetzt. In PUUV-Ausbruchsregionen werden zur Ermittlung möglicher Spillover-Infektionen zusätzlich alle Nichtreservoirarten, wie beispielsweise die Gelbhalsmaus, auch mit dem (heterologen) PUUV-Antigen untersucht. Bei Arvicolinen werden zur serologischen Untersuchung PUUV- und TULV-Antigene verwendet. Bei allen anderen Nagetieren, wie z.B. der Hausmaus, werden serologische Untersuchungen unter Verwendung aller drei Antigene bzw. weiterer Antigene durchgeführt. Für die Entwicklung serologischer Testverfahren zum Nachweis Hantavirus-spezifischer Antikörper in Insektenfressern, wie Spitzmäusen, werden gegenwärtig entsprechende Kontrollseren und Sekundärantikörper hergestellt.

Parallel zum serologischen Screening werden die Lungengewebeproben aller Tiere von Fangorten mit serologisch positiven Tieren mit einer L-Segment-spezifischen Pan-RT-PCR (Klempa et al., 2006) getestet. Möglicherweise kann diese Screeningmethode zukünftig durch eine Echtzeit-RT-PCR-Methode ersetzt werden. Bei allen in diesem Screening positiven Gewebeproben werden im Anschluss S- und M-Segment-spezifische RT-PCR-Analysen durchgeführt, um die Sequenzen der kompletten Nukleokapsidprotein- und Glykoprotein-kodierenden Regionen aufzuklären. Parallel zu den RT-PCR-Analysen sollen bei den Pan-L-Segment-RT-PCR-positiven Tieren auch Anzuchtversuche unter Verwendung von Vero E6-Zellen durchgeführt werden.

5. Untersuchungen zur geografischen Verbreitung von Hantaviren in Reservoirwirten in Deutschland

Untersuchungen zur geografischen Verbreitung und Wirtsassoziation der drei in Deutschland vorkommenden Hantavirus-Arten, PUUV, TULV und DOBV, konzentrierten sich nicht nur auf Ausbruchs- und Endemiegebiete in Baden-Württemberg, Bayern, Nordrhein-Westfalen und Niedersachsen, sondern schlossen auch Nagetiere aus Mecklenburg-Vorpommern, Brandenburg, Sachsen-Anhalt, Sachsen, Thüringen, Hessen, Schleswig-Holstein und Rheinland-Pfalz ein (Abb. 2A-C).

In allen genannten Ausbruchsregionen wurde bei den gefangenen Rötelmäusen serologisch und/oder molekularbiologisch eine hohe PUUV-Prävalenz beobachtet (Essbauer et al., 2006, 2007a,b; unsere unveröffentlichten Daten, siehe Tabelle 2). So wurde bei Rötelmäusen, die während des Ausbruchs 2007 in fünf Landkreisen von Baden-Württemberg gefangen worden sind, eine Seroprävalenz von etwa 20 bis 76 % beobachtet (unsere unveröffentlichten Daten). Ähnlich hohe Prävalenzen wurden während des Ausbruchs 2007 bei Nagetieren aus zwei Landkreisen Unterfrankens (Main-Spessart und Aschaffenburg) und einer ländlichen Region nahe Münster nachgewiesen (unsere unveröffentlichten Daten).

Bei Untersuchungen in anderen Regionen konnten PUUV-infizierte Rötelmäuse in Sachsen-Anhalt nachgewiesen werden (unsere unveröffentlichten Daten). Bei weiteren Studien anderer Arbeitsgruppen sind auch PUUV-positive Rötelmäuse in der Nähe von Lübeck und Koblenz gefunden worden (Schilling et al., 2007). Trotz der zum Teil geringen Prävalenz in einigen Regionen ist insgesamt von einer großen geografischen Verbreitung des PUUV in Deutschland auszugehen (Ulrich et al., 2009b).

TULV-Infektionen scheinen in *Microtus*-Mäusen in Deutschland ebenfalls weit verbreitet zu

sein. So konnten TULV-Infektionen in Nagetieren aus Mecklenburg-Vorpommern, Brandenburg, Sachsen-Anhalt, Niedersachsen, Baden-Württemberg und Bayern nachgewiesen werden (unsere unveröffentlichten Daten). Serologische Untersuchungen zum Vorkommen von DOBV zeigten DOBV-reaktive Antikörper bei Brandmäusen im Landkreis Lüneburg und bei Brand- und Gelbhalsmäusen aus verschiedenen Landkreisen Mecklenburg-Vorpommerns und Brandenburgs (siehe Tabelle 2; unsere unveröffentlichten Daten).

7. Monitoring von Hantavirus-Infektionen in Nagetier-Reservoiren

Um Veränderungen in der Hantavirus-Durchseuchung in Rötelmaus-Populationen zu verfolgen, bestand ein zweiter Schwerpunkt der Untersuchungen zu Hantaviren in der Etablierung eines Monitorings von Hantavirus-Infektionen bei Nagetieren an ausgewählten Fangorten. Dazu wurden in ländlichen Regionen Niederbayerns, im Landkreis Osnabrück, in der Stadt Köln und am Truppenübungsplatz Heuberg 2004, 2005 bzw. 2007 Longitudinalstudien begonnen. Diese Untersuchungen zeigten ein stabiles Vorkommen des PUUV in den lokalen Rötelmaus-Populationen. Bei 30 % der in Niederbayern in den Jahren 2004 und 2005 gefangenen Rötelmäuse wurden serologisch und molekularbiologisch PUUV-Infektionen nachgewiesen (Essbauer et al., 2006; unsere unveröffentlichten Daten).

Im Kölner Stadtwald wurden bei einer ersten Untersuchung aufgrund gehäuft aufgetretener humaner PUUV-Infektionen von April bis Juni 2005 Rötelmäuse gefangen, von denen mehr als 50 % PUUV-positiv waren (Abb. 4A; Essbauer et al., 2007a,b).

Nach Durchführung einer Nagetier-Bekämpfungsmaßnahme im Juli/August 2005 (Ulrich et al., 2006b) wurden im Dezember 2006/Januar 2007, Oktober/November 2007 und November 2008 erneut Rötelmäuse gefangen. Die serologische Analyse der Rötelmäuse zeigte Antikörperprävalenzen zwischen ca. 19 und 29 % (Abb. 4A; unsere unveröffentlichten Daten). Im Durchschnitt aller bisher analysierten Tiere waren deutlich mehr männliche Tiere (47,4 %) als weibliche Tiere (30,8 %) von einer PUUV-Infektion betroffen (Abb. 4A; unsere unveröffentlichten Daten).

In einem Waldgebiet im Landkreis Osnabrück wurden bei in den Jahren 2005, 2007 und 2008 gefangenen Rötelmäusen PUUV-reaktive Antikörper nachgewiesen, während im Jahr 2006 bei keiner der 6 analysierten Rötelmäuse PUUV-reaktive Antikörper gefunden wurden (Abb. 4B; unsere unveröffentlichten Daten). Im Gegensatz zu den Untersuchungen in Köln zeigte sich kein deutlicher Unterschied in der Seroprävalenz zwischen Männchen (23,1 %) und Weibchen (22,6 %; Abb. 4B; unsere unveröffentlichten Daten).

Seit dem Jahr 2007 sind die Untersuchungen im Landkreis Osnabrück auf weitere Fangorte ausgedehnt worden. Bei Rötelmäusen von allen drei analysierten Fangorten wurden sowohl 2007 als auch 2008 PUUV-infizierte Rötelmäuse nachgewiesen (unsere unveröffentlichten Daten).

Der Truppenübungsplatz Heuberg in Baden-Württemberg wird seit dem Jahr 2007 jährlich beprobt. Hier lag die PUUV-Prävalenz bei Rötelmäusen im Jahr 2007 je nach Beprobungs-Standort bei bis zu 52 % mit einem Durchschnitt für alle 17 Fangorte von 17 %. Im darauffolgenden Jahr war keine der drei an zwei weiter beprobten Hantavirus-Hotspots gefangenen Rötelmäuse positiv (unsere unveröffentlichten Daten).

Neben dem hier beschriebenen PUUV-Monitoring in Rötelmäusen wird auch das Vorkommen von DOBV in *Apodemus*-Mäusen und TULV in *Microtus*-Mäusen an ausgewählten Fangorten weiter verfolgt.

8. Neue Monitoringprojekte zu Hantaviren und anderen Nagetier-assoziierten Zoonoseerregern

Neben den oben genannten Longitudinalstudien sind vor kurzem weitere Monitoringprojekte in Hessen, Bayern und Brandenburg begonnen worden. Im Rahmen eines vom Bundesministerium für Bildung und Forschung geförderten Zoonose-Verbundprojektes zum Zecken-übertragenen Frühsommer-Meningoenzephalitis (FSME)-Virus werden vom Arbeitsbereich

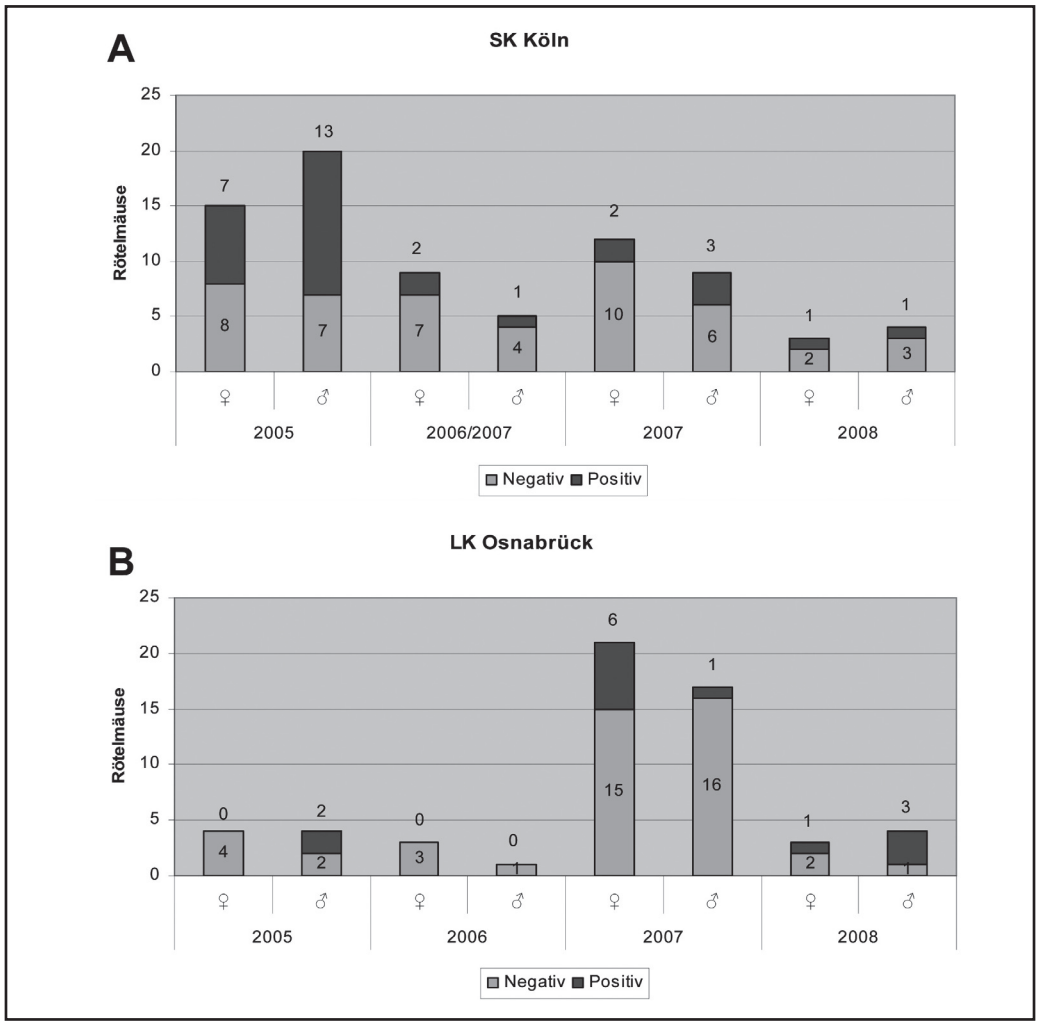

Abb. 4 Serologische Ergebnisse von Puumalavirus-Monitoringuntersuchungen im Stadtkreis (SK) Köln (A) und im Landkreis (LK) Osnabrück (B).
Transudate aller Rötelmäuse wurden mittels eines indirekten Immunglobulin G-Enzymimmunoassays (EIA) unter Verwendung des in Hefe exprimierten Nukleokapsidproteins eines Puumalavirus (PUUV)-Stammes aus Niederbayern (PUUV-Bava) auf das Vorhandensein Hantavirus-reaktiver Antikörper untersucht. Dabei wurde jede Probe jeweils in einem Doppelansatz mit spezifischem Antigen und in bovinem Serumalbumin-haltigem Puffer untersucht. Die Differenz der Mittelwerte der jeweiligen optischen Dichte (OD) bei einer Wellenlänge von 450 nm ergab den finalen OD-Wert jeder einzelnen Probe. Als Negativkontrolle wurde ein Transudat einer serologisch und RT-PCR-negativen Rötelmaus eingesetzt. Ein Serum von einer mit rekombinantem PUUV-Nukleokapsidprotein immunisierten Labormaus diente als Positivkontrolle für den Test. Zur Bewertung der EIA-Ergebnisse wurden obere und untere Grenzwerte definiert: Der obere Grenzwert wurde aus dem doppelten OD-Mittelwert der Negativkontrolle errechnet (ca. 0,1), während sich der untere Grenzwert aus dem einfachen OD-Mittelwert der Negativkontrolle ergab (ca. 0,05). Eine untersuchte Probe wurde als „positiv" bewertet, wenn der berechnete OD-Wert größer war als der obere Grenzwert. Als „negativ" wurde das EIA-Ergebnis gewertet, wenn der OD-Wert der Probe kleiner als der untere Grenzwert war. Bei einem finalen OD-Wert zwischen unterem und oberem Grenzwert wurde die Probe als „fraglich" eingeschätzt. Alle positiven und fraglichen Testergebnisse wurden in einem zweiten EIA überprüft. Die Endauswertung der Ergebnisse erfolgte nach einem kürzlich ausführlich beschriebenen Protokoll (Mertens et al., 2009).

Wildbiologie und Jagdkunde der Universität Göttingen seit September 2007 in FSME-Endemiegebieten im hessischen Teil des Odenwaldes und in der angrenzenden Rheinebene Untersuchungen zu Populationsdichten und individuellen Merkmalen von Reservoirwirten (Nagetieren und Rehen) und Zeckenvektoren (*Ixodes* spp., *Dermacentor* spp.), zu deren Durchseuchung mit dem FSME-Virus sowie zum Parasitierungsgrad der Reservoirwirte durchgeführt. An 18 Waldstandorten werden Nagetier- und Zeckendaten sowie Klimadaten erfasst und in den betreffenden Förstereien geschossene Rehe beprobt. Die gesammelten Nagetiere, Zecken und Blutplasmaproben der Rehe werden den Arbovirus-Netzwerkpartnern und dem Koordinator des Netzwerkes „Nagetier-übertragene Pathogene“ zu weiteren Untersuchungen übergeben. Deren Befunde über die Durchseuchung von Wirten und Zecken mit dem FSME-Virus werden anschließend dem Arbeitsbereich Wildbiologie und Jagdkunde mitgeteilt, der sie zu seinen zuvor erhobenen Resultaten in Beziehung setzt. Auf diese Weise sollen Zusammenhänge zwischen Wirts- und Vektorenmerkmalen, Durchseuchungsgraden, Klima- und Standortfaktoren erkannt und Übertragungswahrscheinlichkeiten für das FSME-Virus abgeschätzt werden (Rühe, 2007; Kiffner et al., 2009; Vor et al., 2009).

In Bayern läuft seit August 2008 eine als Drittmittelprojekt durch das Bayerische Staatsministerium für Gesundheit, Umwelt und Verbraucherschutz geförderte Studie „zum Vorkommen Nagetier-übertragener Zoonosen entlang eines Klimagradienten im Nationalpark Bayerischer Wald“. Diese Studie ist eines von acht Projekten des Netzwerkes „VICCI“ (Vector-Borne Infectious Diseases in Climate Change Investigation) im Forschungsverbund „Gesundheitliche Folgen des Klimawandels in Bayern“ (http://www.bayceer.uni-bayreuth.de/vicci/).

In diesem VICCI-Teilprojekt werden im Nationalpark Bayerischer Wald und in daran angrenzenden Gebieten bis in die Donau-Auen in verschiedenen Höhenlagen (300 –1.400 m üNN) Mäuse mit Lebendfallen gefangen. Im Nationalpark existiert an über 350 Beprobungspunkten ein umfangreiches longitudinales Datenmaterial bezüglich Klima, Niederschlag, Vegetation, Artenvielfalt etc. Von diesen Punkten wurden insgesamt 22 Fangorte für Wildmäuse ausgewählt und mittels GPS genau lokalisiert.

Organe und Blutproben der gefangenen Mäuse werden mittels serologischer und molekularbiologischer Methoden auf Hantaviren und Rickettsien untersucht. Ziel dieser Studie ist es, am Beispiel von Hantaviren und Rickettsien die Prävalenz der Erreger und genetischen Typen in Wildmäusen auf einem Höhen- und damit auch Klimagradienten im Bayerischen Wald festzustellen, diese Erreger in betroffenen Tieren zu quantifizieren und ihre Verteilung in verschiedenen Organen zu beurteilen. Die Ergebnisse der Untersuchungen der Erreger in den Wildmäusen sollen mit den im Nationalpark bereits vorhandenen Daten statistisch ausgewertet werden.

Ein weiterführendes Ziel ist, aus den gewonnenen Daten eine prospektive Risikoabschätzung unter anderem für durch Hantaviren und/oder Rickettsien verursachte Erkrankungen geben zu können.

Das Umweltbundesamt hat für die Jahre von 2009 bis 2012 im Rahmen der UFOPLAN – Vorhaben des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit ein Forschungsvorhaben vergeben, in dem mit Netzwerkpartnern (JKI und FLI) der Zusammenhang von Klimawandel und der Verbreitung Hantavirus-übertragender Nagetiere untersucht werden soll.

Es gibt Hinweise darauf, dass Klimaänderungen einen Einfluss auf die Populationsdichte und -stärke von Nagetieren haben können. So ergab eine Analyse von Klimadaten der Jahre 1995 bis 2007 aus Belgien einen Zusammenhang zwischen spezifischen Klimaereignissen (warmer Sommer, im darauf folgenden Jahr warmer Herbst), die offenbar die Populationsentwicklung von Rötelmäusen begünstigen, und der Häufigkeit des Auftretens von Hantavirus-Erkrankungen beim Menschen (Tersago et al., 2009).

Nach Mastjahren erreichen Rötelmäuse Populationsspitzen. Solche Jahre, in denen das Nahrungsangebot für die Mäuse (Eicheln und Bucheckern) stark ansteigt, kommen regelmäßig vor. Die Frequenz des Vorkommens von Mastjahren hat offenbar klimabedingt von ursprünglich alle 6 Jahre (im Zeitraum von 1895 bis 1929) auf

alle 2 bis 3 Jahre (im Zeitraum von 1974 bis 2006) zugenommen (Övergaard et al., 2007).
Im Rahmen des Forschungsvorhabens werden die Populationsgrößen der Nagetiere und deren Entwicklung vom JKI an 4 Standorten in Deutschland untersucht, von denen 2 Klima-Extremstandorte sind. Alle Standorte liegen in Regionen mit bekannten Hantavirus-Endemiegebieten.
Das zu untersuchende Artenspektrum umfasst Rötelmäuse, Brandmäuse und Feldmäuse. Die gefangenen Tiere sollen vom FLI auf ihre Durchseuchung mit Hantaviren untersucht, die entsprechende Hantavirus-Art identifiziert und die Erregerlast in ausgewählten Organen bestimmt werden. Die so gewonnenen Daten zu Nagetierpopulationen und deren Durchseuchung mit Hantaviren werden auf Korrelationen zwischen Nagetierhäufigkeiten und Durchseuchungsraten, Klimafaktoren und Abundanz der Nagetiere sowie Abundanzen der verschiedenen Nagetierarten (Vergleich Rötelmaus/Feldmaus) geprüft. Die Ergebnisse sollen die zuständigen Behörden in die Lage versetzen, entsprechende Gefährdungslagen frühzeitig erkennen zu können.
Der Sonderforschungsbereich/Transregio 38 der Brandenburgischen Technischen Universität (BTU) Cottbus, der Technischen Universität München und der Eidgenössischen Technischen Hochschule Zürich beschäftigt sich seit Mitte 2007 in einem künstlich geschaffenen Wassereinzugsgebiet im Braunkohlentagebau Welzow-Süd, südlich von Cottbus, mit Prozessen der Ökosystementwicklung (Gerwin et al., 2009).
Die 6 ha große Fläche stellt einen repräsentativen Landschaftsausschnitt dar und blieb einer nicht gelenkten Eigenentwicklung überlassen. Es wird versucht, Struktur-Prozess-Interaktionen für das Initialstadium der Ökosystemgenese abzuleiten. In Ergänzung dieser bereits laufenden Studie wurde im April 2009 eine gemeinsame Langzeitstudie der BTU Cottbus, des Senckenberg-Museums für Naturkunde Görlitz und des Friedrich-Loeffler-Instituts (FLI) begonnen, die sich den Migrationsprozessen von Nagetieren und den mit ihnen assoziierten Krankheitserregern widmen soll.
Das Ziel dieser Untersuchungen besteht insbesondere darin aufzuklären, wie sich Gründereffekte in einer Nagetier-Population auf die molekulare Evolution der mit ihnen assoziierten Krankheitserreger auswirken.
Die hier vorgestellten Longitudinalstudien in verschiedenen geografischen Regionen werden zukünftig wichtige Erkenntnisse zu Evolutionsprozessen bei Hantaviren und anderen Nagetier-assoziierten Zoonoseerregern und den zugrunde liegenden Mechanismen in Nagetier-Populationen liefern.

Danksagung

Die Autoren möchten sich ganz herzlich bei allen Partnern des Netzwerkes „Nagetier-übertragene Pathogene“ und weiteren Kooperationspartnern bedanken.
Der Dank gilt insbesondere Heike Kubitza, Martina Steffen und Dörte Kaufmann für die langjährige Unterstützung der Untersuchungen und Kirsten Tackmann und Roswitha Mattis für die Ausarbeitung der Standardsektionsprotokolle. Die Untersuchungen im Labor von R.G. Ulrich werden durch das Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV), Forschungsvorhaben 07HS027, finanziell unterstützt.
Susanne Schex wird im Rahmen des VICCI-Projektes durch das Bayerische Staatsministerium für Umwelt und Gesundheit gefördert. Jonas Schmidt-Chanasit und Mathias Schlegel danken dem Förderverein des Friedrich-Loeffler-Instituts und der Paul und Ursula Klein-Stiftung sowie Stefan Richter und Ragnar Kinzelbach für die Unterstützung.

Zusammenfassung

Mit der Einführung des Infektionsschutzgesetzes und der damit verbundenen Meldepflicht für ausgewählte humane Infektionskrankheiten ist die Kenntnis der Häufigkeit und geografischen Verbreitung von Infektionen mit Nagetier-assoziierten Zoonoseerregern deutlich verbessert worden. Im Gegensatz dazu ist zur Variation von geografischer Verbreitung und Häufigkeit von Infektionen in Nagetier- und anderen Kleinsäuger-Reservoiren und über die zugrunde liegenden molekularen und Po-

pulations-basierten Mechanismen sehr wenig bekannt. Aus diesem Grund wurde in Deutschland das Netzwerk „Nagetier-übertragene Pathogene“ etabliert, das interdisziplinäre Untersuchungen zu Prozessen in Nagetier- und Kleinsäuger-Populationen und den damit assoziierten Zoonoseerregern sowie deren Einfluss auf die Häufigkeit humaner Infektionen mit diesen Erregern unterstützt. In Zusammenarbeit mit einer Vielzahl von Kooperationspartnern wurden bisher ca. 7.500 Nagetiere und andere Kleinsäuger in 14 Bundesländern gesammelt.

Ein Standardprotokoll für die zentrale Sektion der Tiere und die nachfolgende Dokumentation von biometrischen Daten und Ergebnissen der serologischen und molekularbiologischen Untersuchungen ermöglicht zukünftig eine umfassende Beurteilung der Durchseuchung von Reservoirtieren mit den verschiedenen Zoonoseerregern.

Ein Monitoring von Hantaviren in Nagetieren wurde in einer Reihe von Bundesländern initiiert: Baden-Württemberg, Bayern, Nordrhein-Westfalen, Niedersachsen, Mecklenburg-Vorpommern, Brandenburg, Sachsen, Sachsen-Anhalt, Thüringen, Schleswig-Holstein, Hessen, Rheinland-Pfalz.

Insgesamt wurde eine breite geographische Verbreitung des *Puumalavirus* (PUUV) in Rötelmäusen und des *Tulavirus* in *Microtus*-Mäusen dokumentiert. *Dobrava-Belgrad-Virus*-positive *Apodemus*-Mäuse wurden bisher ausschließlich in Brandenburg, Mecklenburg-Vorpommern und Niedersachsen gefunden. In den Hantavirus-Ausbruchsgebieten in Baden-Württemberg, Bayern, Nordrhein-Westfalen und Niedersachsen wurde bei Rötelmäusen eine hohe PUUV-Prävalenz beobachtet. Initiale Longitudinalstudien in Nordrhein-Westfalen (Stadt Köln), Bayern (Niederbayern) und Niedersachsen (ländliche Region bei Osnabrück) zeigten ein stabiles Vorkommen des PUUV in den Rötelmaus-Populationen.

Neben den Untersuchungen zu Hantaviren ist auch mit Studien zum Vorkommen von anderen Nagetier-assoziierten Zoonoseerregern, wie FSME-Virus, Leptospiren und Borrelien, begonnen worden. Die begonnenen Longitudinalstudien werden Schlussfolgerungen zur molekularen Evolution von Hantaviren und anderen Nagetier-assoziierten Erregern und zu Veränderungen in deren Häufigkeit und Verbreitung in Reservoirwirten ermöglichen. Diese Untersuchungen werden zukünftig eine verbesserte Risikoabschätzung für die Gefährdung der Bevölkerung ermöglichen.

Summary

Network “Rodent-borne pathogens”: Monitoring of hantavirus infections in Germany

Since the introduction of the German Federal Infection Protection Act (Infektionsschutzgesetz) we gained substantial knowledge regarding the prevalence and geographical occurrence of notifiable rodent-borne diseases. Despite these descriptive data on the human cases, we are currently lacking necessary data on the abundance and geographical distribution of the infections in reservoir rodent hosts.

Further, variations in the population dynamics of rodents and other small mammals are poorly explored with regard to their driving forces, and the consequences for the prevalence and distribution of a given pathogen within the rodent community. In order to shed light on the processes within the rodent populations leading to clusters of human infections, we have recently established the interdisciplinary network „Rodent-borne pathogens“ in Germany. Together with the partners of the network a total of about 7,500 rodents and other small mammals were collected in fourteen Federal States.

A standard procedure including a standardised protocol for necropsy and documentation of biometric data was developed which will facilitate the interpretation of prevalence data of each zoonotic pathogen within the various reservoir hosts. Monitoring for hantaviruses in rodent hosts was initiated in twelve German states (Baden-Wuerttemberg, Bavaria, North Rhine-Westphalia, Lower Saxony, Mecklenburg-Western Pomerania, Brandenburg, Saxony, Saxony-Anhalt, Thuringia, Schleswig-Holstein, Hesse, Rhineland-Palatinate).

While a broad geographical distribution was found for both *Puumala virus* (PUUV) in bank voles and *Tula virus* in *Microtus* mice, *Dobrava-Belgrade virus*-positive *Apodemus* mice were thus far only found in north-east Germa-

ny, i.e., Brandenburg, Mecklenburg-Western Pomerania and Lower Saxony. In regions with clusters of human hantavirus cases in Baden-Wuerttemberg, Bavaria, North Rhine-Westphalia and Lower Saxony, the prevalence of PUUV in the corresponding bank vole populations was higher than in other regions of our study area. The first results of longitudinal studies in North Rhine-Westphalia (City of Cologne), Bavaria (Lower Bavaria) and Lower Saxony (close to the city of Osnabrück) revealed a continuing presence of PUUV in the respective vole populations.

Besides hantaviruses, similar studies for the detection of other rodent-associated zoonotic pathogens such as tick-borne encephalitis virus, *Leptospira* and *Borrelia* were initiated. These long-term studies will lead to a better understanding of the molecular evolution of hantaviruses and other zoonotic rodent-borne pathogens and of the fluctuations in abundance and geographic distribution of their reservoir hosts. Hence, we are aiming to understand the mechanisms leading to clusters of human cases, which are required for robust risk assessment and the development of suitable prevention strategies.

Literatur

Becker, C.; Kurth, A.; Hessler, F.; Kramp, H.; Gokel, M.; Hoffmann, R.; Kuczka, A. & Nitsche, A. (2009): Kuhpocken bei Haltern von Farbratten – Ein nicht immer sofort erkanntes Krankheitsbild. – Dt. Ärztebl. **19**: 329–334.

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2008): Bekanntmachung der geprüften und anerkannten Mittel und Verfahren zur Bekämpfung von tierischen Schädlingen nach §18 Infektionsschutzgesetz. – Bundesgesundheitsbl. **51**: 1220–1238.

Ehlers, B.; Kuchler, J.; Yasmum, N.; Dural, G.; Schmidt-Chanasit, J.; Jäkel, T.; Matuschka, F.-R.; Richter, D.; Essbauer, S.S.; Hughes, D.J.; Summers, C.; Bennett, M.; Stewart, J.P. & Ulrich, R.G. (2007): Identification of novel rodent herpesviruses, including the first gammaherpesvirus of *Mus musculus*. – J. Virol. **81**: 8091–8100.

Ehlers, B.; Dural, G.; Yasmum, N.; Lembo, T.; de Thoisy, B.; Ryser-Degiorgis, M.-P.; Ulrich, R.G. & McGeoch, D.J. (2008): Novel mammalian herpesviruses and lineages within the *Gammaherpesvirinae*: Cospeciation and interspecies transfer. – J. Virol. **82**: 3509–3516.

Essbauer, S.S.; Schmidt, J.; Conraths, F.J.; Friedrich, R.; Koch, J.; Hautmann, W.; Pfeffer, M.; Wölfel, R.; Finke, J.; Dobler, G. & Ulrich, R.G. (2006): A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. – Epidemiol. Infect. **134**: 1333–1344.

Essbauer, S.S.; Schmidt-Chanasit, J.; Madeja, E.L.; Wegener, W.; Friedrich, R.; Petraityte, R.; Sasnauskas, K.; Jacob, J.; Koch, J.; Dobler, G.; Conraths, F.J.; Pfeffer, M.; Pitra, C. & Ulrich, R.G. (2007a): Nephropathia epidemica outbreak in a metropolitan area, Germany. – Emerg. Infect. Dis. **13**: 1271–1273.

Essbauer, S.S.; Schmidt-Chanasit, J.; Madeja, E.L.; Wegener, W.; Friedrich, R.; Koch, J., Conraths, F.J., Pfeffer, M., Ulrich, R.G. & Dobler, G. (2007b): Aufklärung von ungewöhnlichen Krankheitsausbrüchen: Zum Ausbruch von Puumala Virus-bedingter Nephropathia epidemica in einer deutschen Großstadt. – Wehrmed. Mschr. **51**: 325–329.

Gerwin, W.; Raab, T.; Biemelt, D.; Bens, O. & Hüttl, R.F. (2009): The artificial water catchment "Chicken Creek" as an observatory for critical zone processes and structures. – Hydrol. Earth Syst. Sci. Discuss. **6**: 1769–1795.

Heiske, A.; Anheier, B.; Pilaski, J.; Volchkov, V.E. & Feldmann, H. (1999): A new *Clethrionomys*-derived hantavirus from Germany: evidence for the distinct genetic sublineages of Puumala viruses in Western Europe. – Virus Res. **61**: 101–112.

Hofmann, J.; Meisel, H.; Klempa, B., Vesenbeckh, S.M.; Beck, R.; Michel, D.; Schmidt-Chanasit, J.; Ulrich, R.G.; Grund, S.; Enders, G. & Krüger, D.H. (2008): Molecular epidemiology of a large hantavirus outbreak in Germany, 2007. – Emerg. Infect. Dis. **14**: 850–852.

Jones, K.E.; Patel, N.G.; Levy, M.A.; Storeygard, A.; Balk, D.; Gittleman, J.L.; Daszak, P. (2008): Global trends in emerging infectious diseases. – Nature **451**: 990–993.

Kang, H.J.; Bennett, S.N.; Sumibcay, L.; Arai, S.; Hope, A.G.; Mocz, G.; Song, J.W.; Cook, J.A.; Yanagihara, R. (2009): Evolutionary insights from a genetically divergent hantavirus harbored by the European common mole (*Talpa europaea*). PLoS One **4**: e6149.

Kiffner, C.; Vor, T. & Rühe, F. (2009): Numerical response of ticks in relation to small rodent densities. – X. International Jena Symposium on tick-borne diseases (formerly IPS) 2009, J. Süss & O. Kahl eds. Abstracts: 36.

Klempa, B.; Meisel, H.; Räth, S.; Bartel, J.; Ulrich, R.G. & Krüger, D.H. (2003): Occurrence of renal and pulmonary syndrome in a region of North-East Germany where Tula hantavirus circulates. – J. Clin. Microbiol. **41**: 4894–4897.

Klempa, B.; Schütt, M.; Auste, B.; Ulrich, R.G.; Meisel, H. & Krüger, D.H. (2004): First molecular identification of human Dobrava virus infection in Central Europe. – J. Clin. Microbiol. **42**: 1322–1325.

Klempa, B.; Fichet-Calvet, E.; Lecompte, E.; Auste, B.; Aniskin, V.; Meisel, H.; Denys, C.; Koivogui, L.; ter Meulen, J. & Krüger, D.H. (2006): Hantavirus in African wood mouse, Guinea. – Emerg. Infect. Dis. **12**: 838–840.

Meisel, H.; Lundkvist, Å.; Gantzer, K.; Bär, W.; Sibold, C. & Krüger, D.H. (1998): First case of infection with hantavirus Dobrava in Germany. Eur. J. Clin. Microbiol. Infect. Dis. **17**: 884–885.

Mentel, R.; Bordihn, N.; Wegner, U.; Wendel, H. & Niklasson, B. (1999): Hantavirus Dobrava infection with pulmonary manifestation. – Med. Microbiol. Immunol. **188**: 51–53.

Mertens, M.; Wölfel, R.; Ullrich, K.; Yoshimatsu, K.; Blumhardt, J.; Römer, I.; Esser, J.; Schmidt-Chanasit, J.; Groschup, M.H.; Dobler, G.; Essbauer, S.S. & Ulrich R.G. (2009): Seroepidemiological study in a *Puumala virus* outbreak area in South-East Germany. – Med. Mircobiol. Immunol. **198**: 83–91.

Övergaard, R.; Gemmel, P. & Karlsson, M. (2007): Effects of weather conditions on mast year frequency in beech (*Fagus sylvatica* L.) in Sweden. – Forestry **80**: 555–565.

Pelz, H.-J.; Rost, S. & Müller, C.R. (2007): DNA-based field monitoring of warfarin resistance in rats (*Rattus norvegicus*). – Int. J. Pest Management **53**: 281–284.

Pelz, H.-J. & Freise, J. (2009): Antikoagulantien-Resistenz bei kommensalen Nagern. – Mitt. Julius Kühn-Institut **421**: 68–75.

Pilaski, J.; Ellerich, C.; Kreutzer, T.; Benik, W.; Lewandowski, B.; Lang, A.; Autenrieth, I.B. & Vanek, E. (1991): Endemisches Vorkommen des Hämorrhagischen Fiebers mit renalem Syndrom (HFRS) in der Bundesrepublik Deutschland. – Z. ärztl. Fortbild. (Jena) **85**: 869–874.

Pilaski, J.; Feldmann, H.; Morzunov, S.; Rollin, P.E.; Ruo, S.L.; Lauer, B.; Peters, C.J. & Nichol, S.T. (1994): Genetic identification of a new Puumala virus strain causing severe haemorrhagic fever with renal syndrome in Germany. – J. Infect. Dis. **170**: 1456–1462.

Razanskiene, A.; Schmidt, J.; Geldmacher, A.; Ritzi, A.; Niedrig, M.; Lundkvist, Å.; Krüger, D.H.; Meisel, H.; Sasnauskas, K. & Ulrich, R.G. (2004): High yields of stable and highly pure nucleocapsid proteins of different hantaviruses can be generated in the yeast *Saccharomyces cerevisiae*. – J. Biotechnol. **111**: 319–333.

Rühe, F. (2007): Zeckenparasitismus bei Wühlmäusen, Mäusen und Rehen in Deutschland: Untersuchung arboviraler Infektionsraten in Beziehung zu Populationsdichten und individuellen Merkmalen der Wirtstiere. Zoonosen-Forschung: Gemeinsame Herausforderung für Veterinär- und Humanmedizin. – Tagungsband zum BMBF-Workshop am 24./25. September 2007 in Berlin: 33.

Schilling, S.; Emmerich, P.; Klempa, B.; Auste, B.; Schnaith, E.; Schmitz, H.; Krüger, D.H.; Günther, S. & Meisel, H. (2007): Hantavirus outbreak in Germany: Limitations of routine serological diagnostics and clustering of virus sequences of human and rodent origin. – J. Clin. Microbiol. **45**: 3008–3014.

Schmidt, J.; Jandrig, B.; Klempa, B.; Yoshimatsu, K.; Arikawa, J.; Meisel, H.; Niedrig, M.; Pitra, C.; Krüger, D.H. & Ulrich, R.G. (2005): Nucleocapsid protein of cell culture-adapted Seoul virus strain 80–39: Analysis of its encoding sequence, expression in yeast and immuno-reactivity. – Virus Genes **30**: 37–48.

Sibold, C.; Ulrich, R.G.; Labuda, M.; Lundkvist, Å.; Martens, H.; Schütt, M.; Gerke, P.; Leitmeyer, K.; Meisel, H. & Krüger, D.H. (2001): Dobrava hantavirus causes hemorrhagic fever with renal syndrome in central Europe and is carried by two different *Apodemus* mice species. – J. Med. Virol. **63**: 158–167.

Tersago, K.; Verhagen, R.; Servais, A.; Heyman, P.; Ducoffre, G. & Leirs, H. (2009): Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. – Epidemiol. Infect. **137**: 250–256.

Ulrich, R.G.; Meisel, H.; Schütt, M.; Schmidt, J.; Kunz, A.; Klempa, B.; Niedrig, M.; Kimmig, P.; Pauli, G.; Krüger, D.H. & Koch, J. (2004): Verbreitung von Hantavirusinfektionen in Deutschland. – Bundesgesundheitsbl. – Gesundheitsforsch. – Gesundheitsschutz **47**: 661–670.

Ulrich, R.G; Essbauer, S.S.; Wenk, M. & Koch, J. (2005): Gefahr für den Jäger? – Pirsch – Magazin für Jagd und Natur **18**: 20–21.

Ulrich, R.G.; Essbauer, S.S.; Schmidt, J.; Schütt, M.; Koch, J.; Conraths, F.J.; Pelz, H.-J. & Wenk, M. (2006a): Zunehmende Gefährdung durch Nagetier-übertragene Hantaviren? AFZ – Der Wald **2**: 90–94.

Ulrich, R.G.; Essbauer, S.S.; Wenk, M.; Schmidt, J.; Pelz, H.-J.; Jacob, J.; Wegener, W.; Madeja, E.L.; Bender, U.; Bradt, K.; Quast, H.; Koch, J.; Groschup, M.; Conraths, F.J.; Dobler, G. & Mettenleiter, T.C. (2006b): Zoonoseforschung: Hantaviren und Netzwerk „Nagetier-übertragene Pathogene“. – Pest Control News **33**: 6–9.

Ulrich, R.G.; Koch, J.; Schmidt-Chanasit, J.; Mertens, M.; Pelz, H.-J.; Jacob, J.; Madeja, E.L.; Quast, H.; Freise, J.; Groschup, M.H.; Conraths, F.J.; Dobler, G.; Bradt, K.; Wegener, W. & Essbauer, S.S. (2007): 2005, ein Jahr der Hantaviren – Quo vadis? Der Hygieneinspektor. – Umwelt- und Infektionshygiene **9**: 61–68.

Ulrich, R.G.; Heckel, G.; Pelz, H.-J.; Wieler, L.H.; Nordhoff, M.; Dobler, G.; Freise, J.; Matuschka, F.-R.; Jacob, J.; Schmidt-Chanasit, J.; Gerstengarbe, F.W.; Jäkel, T.; Süss, J.; Ehlers, B.; Nitsche, A.; Kallies, R.; Johne, R.; Günther, S.; Henning, K.; Grunow, R.; Wenk, M.; Maul, L.C.; Hunfeld, K.-P.; Wölfel, R.; Schares, G.; Scholz, H.C.; Brockmann, S.O.; Pfeffer, M. & Essbauer, S.S. (2009a): Nagetiere und Nagetier-assoziierte Krankheitserreger – das Netzwerk „Nagetier-übertragene Pathogene“ stellt sich vor. – Bundesgesundheitsbl. – Gesundheitsforsch. – Gesundheitsschutz **52**: 352–369.

Ulrich, R.G.; Schlegel, M.; Schmidt-Chanasit, J.; Jacob, J.; Freise, J.; Pelz, H.-J.; Mertens, M.; Wenk, M.; Büchner, T.; Masur, D.; Sevke, K.; Meier, M.; Thiel, J.; Triebenbacher, C.; Buschmann, A.; Lang, J.; Löhr, P.W.; Allgöwer, R.; Borkenhagen, P.; Schröder, T.; Endepols, S.; Heidecke, T.; Stodian, I.; Hueppop, O.; Hornung, M.; Fiedler, W., Krüger, F.; Rühe, F.; Gerstengarbe, F.-W.; Pfeffer, M.; Wegener, W.; Bemmann, M.; Ohlmeyer, L.; Wolf, R.; Gehrke, A.; Heidecke, D.; Stubbe, M.; Zoller, H., Koch, J.; Brockmann, S.O.; Heckel, G. & Essbauer, S.S. (2009b): Hantaviren und Nagetiere in Deutschland: Das Netzwerk „Nagetier-übertragene Pathogene“. – Mitt. Julius-Kühn-Institut **421**: 76–92.

Vahlenkamp, M.; Müller, T.; Tackmann, K.; Löschner, U.; Schmitz, H. & Schreiber, M. (1998): The muskrat (*Ondatra zibethicus*) as a new reservoir for Puumala-like hantavirus strains in Europe. – Virus Res. **57**: 139–150.

Vor, T.; Kiffner, C. & Rühe, F. (2009): Tick burden on roe deer (*Capreolus capreolus* L.). – X. International Jena Symposium on tick-borne diseases (formerly IPS) 2009, J. Süss & O. Kahl eds. Abstracts: 97.

WHO (1998): http://www.who.int/infectious-disease-peport/pages/graph1.html.

Woolhouse, M.E. & Gowtage-Sequeria, S. (2005): Host range and emerging and reemerging pathogens. – Emerg Infect Dis **11**: 1842–1847.

Zöller, L.; Faulde, M.; Meisel, H.; Ruh, B.; Kimmig, P.; Schelling, U.; Zeier, M.; Kulzer, P.; Becker, C.; Roggendorf, M.; Bautz, E.K.F.; Krüger, D.H. & Darai, G. (1995): Seroprevalence of hantavirus antibodies in Germany as determined by a new recombinant enzyme immunoassay. – Eur. J. Clin. Microbiol. Infect. Dis. **14**: 305–313.

Anschrift des korrespondierenden Autors:

PD Dr. Rainer G. Ulrich

Friedrich-Loeffler-Institut
Bundesforschungsinstitut für Tiergesundheit,
Institut für neue und neuartige Tierseuchenerreger

Südufer 10
D-17493 Greifswald – Insel Riems

Tel.: 038351-7159;
Fax: 038351-7192
E-Mail: rainer.ulrich@fli.bund.de

Chapter 11 – Discussion and outlook

Factors affecting tick burdens

In this thesis, several factors affecting individual tick burdens had been identified (e.g. Chapter 4, 7, 8). Some of these relationships are complex, e.g. high roe deer densities appear to boost tick densities, yellow-necked mice densities also amplify nymphal tick densities in the next year (e.g. Appendix I) whereas rodents have lower individual burdens when rodents are numerous (i.e. they dilute larvae). Interestingly, results from this thesis provide little support for the 'sex-bias parasitism hypothesis'. Recent approaches related parasite burdens to individual movements (Boyer et al. 2010). This approach offers considerable potential to elucidate the causal mechanisms behind the observed variation in parasite burdens. Clearly, fast and accurate estimations of individual parasite burdens (e.g. Chapter 5) are required for this approach which necessitates a (non-lethal) capture-recapture protocol.

The ecology of tick-borne encephalitis

The results from this thesis support previous notions that many factors contribute to the epidemiology of tick-borne encephalitis (e.g. Randolph and Sumilo 2007; Randolph 2008; Süss 2008). One of the findings was that spring warming was positively correlated with synchronous feeding of nymphal and larval ticks on rodents (Chapter 8) which is thought to be a necessary prerequisite for co-feeding transmission of tick-borne encephalitis virus (Randolph and Sumilo 2007) and thus for the basic growth number of this pathogen (Hartemink et al. 2008). In contrast to this, Chapter 2 and Chapter 9 suggest that spring warming was negatively correlated with number of human TBE infections (Chapter 2) or not statistically associated with TBE antibody prevalence in roe deer (Chapter 9). These apparently contradictory findings do not necessarily reject the importance of spring warming/ seasonal synchrony of immature ticks for the maintenance of the TBE-virus in forest ecosystems. Rather, these findings strongly suggest that other factors might be of significance.

The most obvious factor might be that the TBE-virus has to be present in the system. If the pathogen is not present in the system, all environmental and ecological factors potentially contributing to its transmission (spring warming, high number of competent hosts, high density of tick vectors) are simply ineffective. The strong effects of spatial autocorrelation in the TBE distribution papers (Chapter 2 & 9) strongly support this obvious but possibly overlooked reality. This finding also suggests that transovarial (from adult tick to offspring) virus transmission might be of considerable importance for the maintenance of the virus.

Additionally, this thesis lends considerable support for the hypothesis that populations of large wildlife hosts (here roe deer) contribute to the observed variation of tick-borne pathogens (Hudson et al. 2001; Linard et al. 2006; Randolph 2008; Rizzoli et al. 2009). Because relatively strong statistical associations between roe deer densities and TBE incidence in humans and/or TBE prevalence in sentinel species were found in this thesis (e.g. Chapter 2 & 9) and in the literature (Hudson et al. 2001; Rizzoli et al. 2009), it would be worth investigating the biological (mechanistic) pattern that causes this relationship (e.g. do deer only act as tick amplifier or are they also a platform for virus transmission?). I would recommend investigating the following aspects in more detail which would allow a causal interference between roe deer densities and TBE incidence (cf. Plowright et al. 2008):

(1) Are roe deer incompetent TBE reservoir hosts with regard to systemic TBE transmission? How competent are other wildlife species with regard to TBEV transmission?
(2) Does co-feeding transmission of TBE-virus on roe deer occur given the narrow spatial and temporal feeding aggregation of different tick life stages (cf. Chapter 6)?

Answers from these questions would necessitate experiments in which roe deer (and possibly other wildlife species) are challenged with TBEV (e.g. Jones et al., 1997). Results from these proposed studies could then be used in conjunction with assessments of animal community structure of forest ecosystems aiming at estimating the entomological risk (i.e. density of infected nymphs) posed by infected ticks given different species compositions (relative abundance of competent vs. non-competent hosts). Such a study has been conducted for Lyme disease (LoGiudice et al. 2003). For TBE, similar models have been parameterised (Rosà and Pugliese 2007) but these models are based on simulations and on assumptions which have not been tested rigorously (see e.g. reservoir competence of roe deer as discussed in Chapter 6).

Thus, an interesting starting point would be to estimate the density of TBEV-infected questing nymphal ticks (TBEV prevalence in nymphs x nymph density) in different forest ecosystems over a long time horizon and relate this key factor to climatic variations and to changes in relative host species composition (cf. Ostfeld et al. 2006). Given the relatively narrow variation in TBE prevalence in ticks that rarely exceeds 1%, density of infected nymphs is expected to be merely a function of tick density (Randolph et al. 2008). There is considerable evidence that rodent densities in one year are correlated with nymph densities in the following year (Ostfeld et al. 2006; Rosà et al. 2007). Probably because of the rodent species specific variation in tick burdens (cf. Chapter 8), yellow-necked mice densities have been shown to be positively correlated with nymph densities in the following year (Appendix

1). Albeit these investigations are preliminary and based upon a very short time series, this finding supports the hypothesis that variation in rodent densities are key drivers of the variable epidemiological risk posed by ticks (Ostfeld et al. 2006). Thus, reliable predictions of rodent population dynamics (e.g. as a function of mast events) or regular and systematic monitoring of rodent population dynamics (Ostfeld et al. 2006) would possibly enable one to predict the nymphal tick density in advance (e.g. two years after a mast event, one year after a peak in yellow-necked mice). Hence, studies aiming at understanding the relative contribution of food availability, competition (e.g. with large herbivores) and predation to rodent population dynamics might be essential for further understanding and prediction of tick-borne and rodent-borne diseases (cf. Chapter 10).

In this thesis I mainly investigated and discussed the environmental and ecological factors affecting TBE infection potential. The other side of the coin, probably just as or even more important, are sociological factors affecting exposure to (infected) ticks by humans. These include risk behaviours such as leisure (e.g. hunting, walking) or professional (e.g. timber and non-timber forest products extraction) activities in the forest (Linard et al 2006; Randolph 2008; Randolph et al. 2008). Clearly, the exposure of humans to ticks is central for infections with tick-borne diseases. Yet, the human exposure to infectious agents is difficult to quantify and would rather demand a sociological research approach.

Tick control

Probably the most ambitious efforts to reduce tick populations were conducted in the United States of America (USA). In the 1980s an integrated tick control strategy was implemented. The simultaneous reduction of deer populations, thinning of forests and area wide application of acaricides reduced tick populations below a pest-threshold level (Barnard et al. 1988). The negative perception of and possible adverse effects on other invertebrates of acaricides, and the required substantial effort of this strategy, however, hampered an area wide application of this method. Later on, a less invasive control strategy was implemented successfully in the USA. In this approach, bait stations (corn is offered) are distributed widely in the forests. Deer (mainly white-tailed deer *Odocoileus virginianus*) feeding on these stations are treated with acaricides via a roller system ("4-Poster"-device) (Fish and Childs 2009). Albeit being less expensive and having fewer side-effects than area-wide application of acaricides (Pound et al. 2009) this strategy is still cost-intensive. Further on, its effectiveness has not been tested for ticks infesting deer in a European setting. Roe deer are characterized by territorial behaviour and thus show a rather even distribution within forests (von Raesfeld 1985) which

would potentially necessitate a high number of baited acaricide-stations and thus very high associated costs. Recent developments combine pheromones or the compounds thereof (which regulate assembly, aggregation and mating behaviour) with acaricides. These combined devices are very effective in controlling tick numbers when applied to tick-infested vegetation or to tick-infested animals (Sonenshine 2006).

These cited approaches are, however, not much in line with an ecosystem management paradigm (e.g. Puettmann and Ammer 2009). A more system-oriented approach would be to adjust wildlife (especially roe deer) species densities by appropriate wildlife management practices (e.g. effective culling, banning of supplementary feeding). Forest ungulate densities have increased considerably in the past decades (e.g. *Cervus elaphus*: Milner et al. 2006; *Capreolus capreolus*: Burbaite and Csányi 2009) and there is considerable evidence that these increased ungulate densities have not only adverse effects on the regeneration, species composition and economic revenues of forest ecosystems (e.g. Ammer 1996; Allombert et al. 2005), but that at least roe deer densities also elevate the epidemiological risk posed by ticks (e.g. this thesis).

A further system-oriented approach would be to adjust silvicultural practices so that the habitat is less suitable for host species (rodents and deer) and for ticks. For rodents and ticks, silvicultural concepts which create only small gaps when harvesting or thinning timber stands are likely to reduce the habitat suitability for rodents and for roe deer (e.g. Krüger 2003; Kuijper et al. 2009) mainly be reducing the quantity of available forage and shelter (i.e. for rodents) by creating an unsuitable light regime for the ground vegetation. On the other hand, this suggested practice might create a favourable microclimate for ticks. In essence, effects of different silvicultural measures should thus be explicitly tested in an experimental field study.

An additional approach would be to study the ecology of forest roadsides (i.e. places where humans usually enter a forest and get into contact with ticks). Understanding this "roadside epidemiology" offers considerable potential to alter roadside conditions (e.g. by removing edge vegetation) so that roadsides are less suitable for ticks (Haemig et al. 2008).

Personal protection

Given that tick control strategies and ecosystem-based interventions are unlikely (1) to be implemented area-wide in Germany, (2) to have an immediate effect and (3) to eliminate ticks and tick-borne pathogens entirely if implemented, personal protection measures are highly recommended. For TBE, the most effective measure is vaccination (Gold et al. 1992). The best example for the success of TBE vaccination programmes comes from Austria. Here, a

considerable reduction of human TBE cases was achieved by an effective vaccination campaign initiated in the 1980s. Nowadays, 88% of the total population have a history of TBE vaccination (Heinz et al. 2007). Despite variable TBE spikes and a rather increasing tendency of TBE incidence in humans in other European countries (e.g. Randolph et al. 2008) TBE incidence in Austria remains at a low, rather constant level which can be merely attributed to the high vaccination coverage and the effectiveness of the vaccine. It has been estimated that about 2800 human TBE cases were prevented by vaccination in the years 2000-2006 (Heinz et al. 2007). Currently, there is no vaccine available for European Lyme diseases spirochaetes but is currently in pre-clinical development (www.intercell.com/main/forvaccperts/products/lyme-borreliosis-vaccine/). Therefore, and regarding the transmission potential of additional tick-borne pathogens, strategies to avoid contact with ticks e.g. by using effective repellents (del Fabro and Nazzi 2008) are advisable.

References

Allombert S, Gaston A, Martin J-L (2005) A natural experiment on the impact of overabundant deer on songbird populations. Biol Cons 126:: 1-13

Ammer C (1996) Impact of ungulates on structure and dynamics of natural regeneration of mixed mountain forests in the Bavarian Alps. For Ecol Manage 88:43-53

Barnard DR, Mount GA, Koch HG Haile DG, Garris GI (1988) Management of the Lone Star Tick in Recreation areas. Agriculture Handbook 682, Gainsville, USA. pp. 1-33

Boyer N, Réale D, Marmet J, Pisanu B, Chapuis J-L (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. J Anim Ecol 79:538-547

Burbaite L, Csányi S (2009) Roe deer population and harvest changes in Europe. Estonian J Ecol 58: 169-180

Del Fabro S, Nazzi F (2008) Repellent effect of sweet basil compounds on *Ixodes ricinus* ticks.Exp Appl Acarol 45:219-228

Fish D, Childs JE (2009) Community-based prevention of Lyme disease and other tick-borne diseases through topical application of acaricide to white-tailed deer: Background and Rationale. Vector-Borne Zoonotic Dis 9:357-364

Gold R, Wiethölter H, Rihs I, Löwer J, Kappos L (1992) Frühsommer-Meningoencephalitis-Impfung. D Med Wschrift 117:112-116

Haemig PD, Waldenström J, Olsen B (2008) Roadside ecology and epidemiology of tick-borne diseases. Scand J Inf Dis 40:853-858

Hartemink NA, Randolph SE, Davis SA, Heesterbeek JAP (2008) The basic reproduction number for complex disease systems: defining R_0 for tick-borne diseases. Am Nat 171:743-754

Heinz FX, Holzmann H, Essl A, Kundi M (2007) Field effectiveness of vaccination against tick-borne encephalitis. Vaccine 25:7559-7567

Hudson PJ, Rizzoli A, Rosà R, Chemini C, Jones LD, Gould EA (2001) Tick-borne encephalitis virus in northern Italy: molecular analysis, relationships with density and seasonal dynamics of *Ixodes ricinus*. Med Vet Entomol 15: 304-313

Jones DJ, Gaunt M, Hails RS, Laurenson K, Hudson PJ, Reid H, Henbest P, Gould EA (1997) Transmission of louping ill virus between infected and uninfected ticks co-feeding on mountain hares. Med Vet Entomol 11:172-176

Kuijper DPJ, Cromsigt JPGM, Churski M, Adam B, Jedrzejewska B, Jedrzeweski W (2009) Do ungulates preferentially feed in forest gaps in European temperate forests? For Ecol Manageme 258:1528-1535

Krüger F (2002) Zur zeitlichen Prognose, räumliche Verteilung und Heilungsdynamik von durch Wühlmäuse (Erdmaus, *Microtus agrestis* L., Rötelmaus, *Clethrionomys glareolus* Schreb. und Feldmaus, *Microtus arvalis* Pallas) verursachten Nageschäden an Forstpflanzen. PhD thesis. University Göttingen, Germany

Linard C, Lamarque P, Heyman P, Ducoffre G, Luyasu V, Tersago K, Vanwambeke, Lambin EF (2007) Determinants of the geographic distribution of Puumala virus and Lyme borreliosi infections in Belgium. Int J H Geogr 6:15

LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. PNAS 100:567-571

Milner JM, Bonenfant C, Mysterud A, Gaillard JM, Csanyi S, Stenseth NC, (2006) Temporal and spatial development of red deer harvesting in Europe: biological and cultural factors. J Appl Ecol 43:721–734

Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F (2006) Climate, deer, rodents and acorns as determinants of variation in Lyme-disease risk. PLoS Biology 4: e145

Plowright RK, Sokolow SH, Gorman ME, Daszak P, Foley JE (2008) Casual interference in disease ecology: investigating ecological drivers of disease emergence. Front Ecol Environ 6:420-429

Pound JM, Miller JA, George JE, Fish D, Carroll JF, Schulze TL, Daniels TJ, Falco RC, Stafford III KC, Mather TN (2009) The United States Department of Agriculture's northeast area-wide tick control project: Summary and Conclusions. Vector-Borne Zoonotic Dis 9:439-448

Puettmann KJ, Ammer C (2007) Trends in American and European regeneration research under the ecosystem management paradigm. Eur J For Res 126:1–9

Randolph SE, Asokliene L, Avsic-Zupanc T, Bormane A, Burri C, Gern L, Golovljova I, Hubalek Z, Knap N, Kondrusik M, Kupca A, Pejcoch M, Vasilenko V, Zygutiene M (2008) Variable spikes in tick-borne encephalitis incidence in 2006 independent of variable tick abundance but related to weather. Parasites & Vectors 1:44

Randolph SE (2008) Dynamics of tick-borne disease systems: minor role of recent climate change. Rev Sci Tech Off Int Epiz 27: 367-381

Randolph SE, Sumilo D (2007) Tick-borne encephalitis in Europe: dynamics of changing risk. Emerging Pests and Vector-borne Diseases in Europe (ed. by W. Takken & B. Knols), pp. 187-206. University Publishers, Wageningen

Rizzoli A, Hauffe HC, Tagliapietra V, Neteler M, Rosà R (2009) Forest Structure and Roe Deer Abundance Predict Tick-Borne Encephalitis Risk in Italy. PLoS ONE 4:e4336. doi:10.1371/journal.pone.0004336

Rosà R, Pugliese A (2007) Effect of tick population dynamics and host densities on the persistence of tick-borne infections. Math Biosc 208:216-240

Rosà R, Pugliese A, Ghosh M, Perkins SE, Rizzoli A (2007) Temporal variation of *Ixodes ricinus* intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics. Vector-Borne Zoonotic Dis 7:285–295

Sonenshine DE (2006) Tick pheromones and their use in tick control. Annu Rev Entomol 51:557-580

Süss J (2008) Zecken, Was man über FSME und Borreliose wissen muss. Heinrich Hugendubel Verlag, Kreuzlingen/ München, Germany

von Raesfeld F. (1985) Das Rehwild: Naturgeschichte, Hege u. Jagd. Parey Verlag, Berlin, Germany

Appendix I – Numerical response of ticks in relation to small rodent densities

V 12 Numerical response of ticks in relation to small rodent densities

Christian Kiffner, Torsten Vor, Ferdinand Rühe

Wildlife Biology and Game Management Team, Dept. of Forest Zoology, Büsgen-Institute, Georg-August-University Göttingen, Germany

The risk of humans to be bitten by a tick is mainly a function of nymphal density and activity. We hypothesized that high rodent densities in year t translate in high nymph densities in the following year t+1, because rodents are crucial hosts for the early life stages of the vector ticks. To test this hypothesized numerical response of ticks, we established 18 permanent plots in forests of southern Hesse, Germany.

We monitored population fluctuations of 4 rodent species (*Apodemus flavicollis, A. sylvaticus, Myodes glareolus*, and *Microtus agrestis*) in September 2007 and in May, July, and September 2008 using a standardised capture protocol. We also estimated densities of all tick (*Ixodes* spp.) life stages in March, May, July, and September 2008 by dragging a 1-m^2 blanket along 4 transects, each 50 m in length. Ticks on the blanket were counted and removed after completing each transect.

All 4 rodent species showed considerable seasonal and annual fluctuations in density (as measured by captures per 100 corrected trap nights). Adult tick densities were highest in May 2008, and larval and nymphal densities were highest in July 2008.

We found a moderately strong relationship (Kendall's t=0.404, *P*=0.023, n=18) between the density index of all rodent species combined in September 2007 and *Ixodes* nymph density in July 2008. On a species level, only the density of *A. flavicollis* was significantly associated with the next year's nymphal density (Kendall's t=0.393, *P*=0.036, n=18). Associations between other small rodent species and the next year's nymphal or adult tick densities were insignificant but combined rodent density of the previous year was correlated with the next year's larvae density (Kendall's t=0.353, *P*=0.046, n=18).

These results suggest that the hypothesis is supported in regard to *A. flavicollis*. Yet, for developing a predictive model, more time series and additional data (especially weather) are required.

Appendix II – Curriculum vitae and publication list

Curriculum vitae:

Name:	Christian Kiffner
Date of birth:	06/05/1980
Place of birth:	Lübeck
Citizenship:	German

Education

06/1999	Abitur, Thomas-Mann-Schule, Lübeck

Scientific background

01/07/2007-30/06/2010	Scientific assistant (wiss. Angestellter), Dept of Forest Zoology and Forest Conservation incl. Wildlife Biology and Wildlife Management, Georg-August-Universität Göttingen. BMBF-funded project: "Surveillance of tick-parasitized voles, mice and roe deer in Germany: Arboviral infection rates in relation to population densities and host characteristics".
01/01/2007-30/06/2007	Scientific assistant (wiss. Hilfskraft), Dept of Forest Zoology and Forest Conservation incl. Wildlife Biology and Wildlife Management, Georg-August-Universität Göttingen.
15/08/2006-31/12/2006	Scientific assistant (wiss. Hilfskraft), Research Institute of Forest Ecosystems and Forestry, Rhineland-Palatinate
01.10.2000-23/03/2004	Studies in „Tropical and International Forestry", Faculty of Forest Sciences and Forest Ecology, Georg-August-Universität Göttingen. Master degree with distinction.
15/02/2004-16/06/2004	Studies in "International Nature Conservation" (MSc), Lincoln University, New Zealand.
01/10/2000-05/02/2004	Studies in „Forestry and Forest Ecology", Faculty of Forest Sciences and Forest Ecology, Georg-August-Universität Göttingen.

Internships and work abroad

Internships in Germany:	Stadtwald Lübeck; Baden-Württembergische Bank Stuttgart
Internships overseas:	Wilderness Africa Trust, Zimbabwe; MTO Forestry LtD., South Africa; Consejo Superior de Investigationes scientificas, Spain.
Consultancy:	Eco-consulting group/GTZ, Katavi National Park, Tanzania (08/2006-12/2006).

Reviewed articles (ISI-listed):

1. **Kiffner C.**, Zucchini W., Schomaker P., Vor T., Hagedorn P, Niedrig M. & Rühe F. (revised manuscript in review): Determinants of tick-borne encephalitis in counties of southern Germany, 2001-2008. *International Journal of Health Geographics* 9, 42 (doi: 10.1186 /1476-072X- 9-42)
2. **Kiffner C.,** Vor T., Hagedorn P, Niedrig M. & Rühe F. (in review): Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. *Parasitology Research* (doi: 10.1007/s00436-010-2065-x)
3. **Kiffner C.**, Lödige C., Alings M., Vor T. & Rühe F. (2010): Attachment site selection of ticks on roe deer, *Capreolus capreolus*. *Experimental and Applied Acarology* (doi: 10.1007/s10493-010-9378-4)
4. **Kiffner C.,** Lödige C., Alings M., Vor T. & Rühe F. (2010): Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). *Experimental and Applied Acarology* 52, 73-84 (doi: 10.1007/s10493-010-9341-4).
5. Vor T., **Kiffner C.**, Hagedorn P., Niedrig M. & Rühe F. (2010): Tick burden on European roe deer (*Capreolus capreolus* L.). *Experimental and Applied Acarology* 51, 405-417 (doi: 10.1007/s10493-010-9337-0)
6. Waltert M., Chuwa M. & **Kiffner C.** (2009): An assessment of the puku (*Kobus vardonii* Livingstone 1857) population at Lake Rukwa, Tanzania. *African Journal of Ecology* 47, 688-692 (doi: 10.1111/j.1365-2028.2008.01024.x).
7. Waltert M., Meyer B. & **Kiffner C.** (2009): Habitat availability, hunting or poaching: what affects distribution and density of large mammals in western Tanzanian woodlands? *African Journal of Ecology* 47, 737-746 (doi: 10.1111/j.1365-2028.2009.01080.x).
8. **Kiffner C.** (2009): Coincidence or evidence: Was the sabretooth cat Smilodon social? *Biology Letters* 5(4), 561-562 (doi:10.1098/rsbl.2009.0008).
9. **Kiffner C.**, Meyer B., Mühlenberg M. & Waltert M. (2009): Plenty of prey, few predators: what limits lions in Katavi National Park, western Tanzania? *Oryx* 43(1), 52-59 (doi: 10.1017/S0030605307002335).
10. **Kiffner C.,** Meyer B., Waltert M. & Mühlenberg M. (2008): Response of lions (*Panthera leo* LINNAEUS 1758) and spotted hyaenas (*Crocuta crocuta* ERXLEBEN 1777) to sound playbacks. *African Journal of Ecology* 46(2), 223-226 (doi: 10.1111/j.1365-2028.2007.00813.x).
11. Rühe F., Ksinsik M. & **Kiffner C.** (2008): Conversion factors in carnivore scat

analysis: Sources of bias. *Wildlife Biology* 14(4), 500-506 (doi: 10.2981/0909-6396-14.4.500).

12. **Kiffner C.,** Roessiger E., Trissl O., Schulz R. & Ruehe F. (2008): Probability of recent bark stripping damage by Red Deer (*Cervus elaphus*) on Norway Spruce *(Picea abies*) in a low mountain range in Germany – a preliminary analysis. *Silva Fennica* 42(1), 125-134.

Articles in review:

13. **Kiffner C.**, Lödige C., Alings M., Vor T. & Rühe F. (accepted): Body-mass or sex-biased tick parasitism in roe deer (*Capreolus capreolus*)? A GAMLSS approach. *Medical and Veterinary Entomology*
14. **Kiffner C.,** Vor T., Hagedorn P, Niedrig M. & Rühe F. (in review): Tick-borne encephalitis virus antibody prevalence in roe deer (*Capreolus capreolus*) sera. *Medical and Veterinary Entomology*

Articles in books:

1. **Kiffner C.**, Vor T. & Rühe F. (2008): Surveillance of emerging arthropod-borne-viruses in wildlife species in Germany. In: Review of Forests, Wood products and Wood Biotechnology of Iran and Germany – Part II (Eds. A.R. Kharazipour, C. Schöpper & C. Müller), 21-32.

Not-ISI listed articles:

1. **Kiffner, C.** (2008, printed in 2010): African lions and the trophy hunting dilemma. *Endangered Species UPDATE* 25(1), 3-8.
2. Ulrich R., Schlegel M., Mertens M., Groschup M.H., Schmidt-Chanasit J., Jacob J., Freise J., Pelz H.-J., Wenk M., Thiel J., Triebenbacher C., Schex S., Plenge-Bönig A., Schmolz E., Kurth A., Krüger F., Ansoge H., Rühe F., **Kiffner C.**, et al. (2009): Netzwerk "Nagetier-übertragene Pathogene": Monitoring von Hantavirus-Infektionen in Deutschland. *Beiträge zur Jagd- und Wildforschung* 34, 229-250.
3. **Kiffner C.** & Waltert M. (2009): Wildlife Population assessments in the Katavi Ecosystem. *Miombo. The newsletter of the Wildlife Conservation Society of Tanzania (WCST)*, 34, 1, 3-4, 8.
4. **Kiffner C.** (2002): Wild Success – Umfurudzi Wildlife Survey. *Wildlife Zimbabwe*, 108(2002/2), 8.