RICKETTSIA SPP. IN FREE RANGING SMALL MAMMALS IN SOUTH-EASTERN GERMANY

SUSANNE YVONNE SCHEX

INAUGURAL-DISSERTATION zur Erlangung der **tiermedizinischen Doktorwürde**
der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2011

1st Edition 2011

Printed in Germany

édition scientifique
VVB LAUFERSWEILER VERLAG

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Umschlaggestaltung unter Verwendung einer Fotografie von Andreas Ebert

Aus dem

Department für Veterinärwissenschaften der Tierärztlichen Fakultät

der Ludwig-Maximilians-Universität München

Arbeit angefertigt unter Leitung von:

Univ.- Prof. Dr. Reinhard K. Straubinger, Ph.D.

Angefertigt am Institut für Mikrobiologie der Bundeswehr

(PD Dr. Sandra Essbauer)

Rickettsia spp. in free ranging small mammals in South-Eastern Germany

Inaugural-Dissertation

zur Erlangung der tiermedizinischen Doktorwürde

der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München

von

Susanne Yvonne Schex

aus München

München 2011

Gedruckt mit der Genehmigung der Tierärztlichen Fakultät
der Ludwig-Maximilians-Universität München

Dekan:	Univ.-Prof. Dr. Braun
Berichterstatter:	Univ.-Prof. Dr. Straubinger
Korreferent:	Univ.-Prof. Dr. Pfister

Tag der Promotion: 12. Februar 2011

To my family

TABLE OF CONTENTS

I. INTRODUCTION

Rodents and other small mammals are associated with several pathogens, such as RNA and DNA viruses, bacteria and parasites. These differ not only in their genetic organisation but also in their association with specific reservoir hosts, their geographical distribution and their transmission cycles. For many of those pathogens rodents act as a reservoir host (Mills & Childs, 1998; Haydon et al., 2002; Essbauer et al., 2009b; Ulrich et al., 2009). Rodent-borne and other zoonoses account for a significant proportion (60.3%) of Emerging Infectious Diseases (EID). Moreover, the majority of these infections (71.8%) originates from wildlife and increased significantly over time. More than 50% of EIDs are caused by bacteria (Jones et al., 2008).

Rickettsioses are recognized as emerging infections in several parts of the world (Parola & Raoult, 2001; Fournier & Raoult 2005; Parola et al., 2005; Brouqui et al., 2007; Rovery et al., 2008). These obligate intracellular bacteria are transmitted to humans and animals by blood-sucking arthropods such as insects (i.e. fleas) or arachnids (i.e. ticks and mites). Nearly half of the currently recognized rickettsioses have been discovered within the last two decades (Raoult & Roux, 1997). These included at least ten tick-transmitted rickettsioses characterised between 1993 and 2005 (Fournier & Raoult, 2005). Further, in the last few years, rickettsiae previously considered as non-pathogenic or mild turn out to implicate human disease (Blanco & Oteo 2006; Rovery et al., 2008). Moreover, several species, e.g. *R. raoultii* have been newly described (Mediannikov et al., 2008). Table 12 (Annex, p. 95) gives an exemplary overview about investigations regarding rickettsiae.

Other arthropod-borne pathogens such as *Borrelia* spp. or *Anaplasma* spp. (the latter also belonging to the order Rickettsiales) are known to exist in natural cycles involving rodents. Although the arthropod-related part of the rickettsial life cycle is widely agreed, the role of vertebrate reservoirs is still fairly unclear (Brouqui et al., 2007). In order to gather further knowledge on this subject, the VICCI Project (Vector borne

infectious diseases in climate change investigations) was established and funded by the Bavarian State Ministry of the Environment and Public Health (Bayerisches Staatsministerium für Umwelt und Gesundheit, StMUG). The present study was conducted in the scope of this project. One of its major aims was to investigate the role of rodents in the natural cycle of *Rickettsia* spp. in Germany. To the best of our knowledge, so far this is the first time an investigation concerning this aspect of rickettsiology is performed in Germany.

The present study focused on three aspects:

1) The occurrence of spotted fever group (SFG) rickettsiae in rodents in the National Park Bavarian Forest (syn. Bohemian Forest) and nearby areas in Lower Bavaria;

2) The prevalence and genetic diversity of SFG rickettsiae in those animals by means of

   a) molecular-biological analyses and sequencing

   b) serological analyses;

3) The assessment of predictors for a positive detection of rickettsial DNA in wild rodents.

The increase in the emergence and reemergence of rickettsial infections, coupled with recent characterisation of several proposed new species and the uncertainties in their natural life cycles make rickettsiology a truly engaging yet still fairly unexplored field of research. This study aims to contribute to the already widespread research on rodent-borne pathogens in Germany (as exemplified in the publication in Annex 4, p. 101). Furthermore, it aims to extend the state of knowledge on *Rickettsia* spp. occurring in Germany, their genetic characterisation and the possible role of wildlife reservoirs in their epidemiology.

II. LITERATURE REVIEW

1 Rodents as reservoir hosts

1.1 What is a reservoir host?

A natural reservoir is defined as the long-term host of a pathogen of an infectious disease. Previous definitions imply that the relevant infectious agent is nonpathogenic for the reservoir host or that it is carried as a subclinical and therefore asymptomatic and non-lethal infection. Haydon et al. (2002) propose that a reservoir is defined as one or more epidemiologically connected populations or environments in which the pathogen can be maintained permanently and from which infection is transmitted to the defined target population.

A significant coevolution is to be expected between reservoir host and pathogen (Zeier et al., 2005; Ulrich et al., 2009). Cospeciation and coevolution require a close association between two species. Pathogens may adapt by minimizing pathology in their reservoir hosts whilst causing pathologies in the incidental hosts. This is believed to be related with different immune systems, which respond strongly in order to eliminate the pathogen but contribute to host pathology (Calisher et al., 2008). In addition, pathogens that evolved with the reservoir species may have used cellular receptors and biochemical pathways, which are conserved in later evolved mammals, for replication. If these cellular receptors and pathways are preserved, they could contribute to the capacity for transmission of the agent (Calisher et al., 2006).

Knowledge about maintaining reservoir hosts is essential for disease prevention and control. In order to better understand reservoir ecology and its correlation to human or animal disease the following steps are essential:

1) Determination of the geographic distribution of the host;
2) Determination of the geographic range of the pathogen within the host range;

3) Determination of the regional distribution of the host and pathogen among the distinct habitat types;

4) Assessment of host-pathogen dynamics through prospective, longitudinal studies;

5) Development of an integrative time- and place-specific predictive model (Mills & Childs, 1998).

The following examples underline the key function of reservoir host surveillance in order to control infectious diseases. Only few European countries monitor wildlife reservoirs for zoonotic diseases, an overview is given on the website of the World Organisation for Animal Health (OIE) (World Animal Health Database, WAHID, 2010).

In the United Kingdom, tuberculosis was eradicated from large areas in the 1970s. Since the Wildlife and Countryside Act 1981, that put the European badger (*Meles meles*) under protection, *Mycobacterium bovis* infections in cattle have been rising once more (Palmer, 2007). The badger is considered a true maintenance host for *M. bovis*, with a regional tuberculosis prevalence as high as 20.5%. Infected badgers shed large numbers of *M. bovis* in saliva, urine and feces and can live 3 to 4 years following the first documented episode of shedding of *M. bovis* (Little et al., 1982). Transmission to cattle is thought to happen through inhalation of the pathogen from contaminated grass (Palmer, 2007). Little et al. (1982) showed that removing badgers from cattle farming areas resulted in a decline in bovine tuberculosis. The British Government is aiming to reduce tuberculosis in cattle by a combined strategy including culling and vaccination of badgers in the wild. Moreover, since March 2010, an injectable badger vaccine received Market Authorisation; an oral vaccination is currently being researched (Department for Environment, Food and Rural Affairs, 2010).

On the other hand, in New Zealand, Brushtail Possums are the main reservoir host for *M. bovis*. Infected possums exhibit abnormal behaviour such as rolling that attracts attention of cattle. These then get infected directly when having contact to the possums (Palmer, 2007). Attempts to eradicate possums have failed so far. In the USA, the main reservoir for

M. bovis is the White-Tailed Deer. Control measures aim to reduce deer density and monitor hunter-killed deer (Palmer, 2007). However, the only country with a known wildlife reservoir that was able to eradicate tuberculosis from cattle is Australia. There, the government established a control program including a substantial element of feral buffalo control, as the feral buffalo along with the feral pig is considered the main reservoir of bovine tuberculosis in Australia (Department for Environment, Food and Rural Affairs, 2010).

As for Europe, the wild boar is considered another important reservoir host not only for *M. bovis*, but also for other zoonotic agents such as *Brucella suis, Leptospira* spp*., Coxiella burnetii, Francisella tularensis, Yersinia pestis*, hepatis E virus (HEV), pseudorabies virus (PRV), porcine circovirus type 2 (PCV2), classical swine fever virus (CSFV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV) and parasites, e.g. *Trichinella* spp. and *Toxoplasma gondii* (reviewed in Meng et al., 2009). These infectious agents are transmissible to domestic pigs and other animal species including humans. The potential risk of pathogen transmission to humans and livestock is enhanced by increasing wild boar populations in some areas, increased chances of contact in suburban areas and further by hunting and consumption of wild boar meat.

An example for a successful concept for eradication of a disease of immense importance is rabies. Rabies is a viral disease maintained by multiple wildlife reservoirs such as foxes, bats and others. Oral vaccination strategies and surveillance programs were able to eradicate wildlife rabies in many European countries (Pastoret et al., 1999). In Zimbabwe, however, besides domestic dogs also jackals are a maintenance and source population of infections in humans. Since jackals can comprise all or part of a maintenance community independent of dogs, eliminating rabies will only be successful if jackal rabies was also controlled (Bingham et al., 1999a, b).

Being involved in transmission cycles of a large number of pathogens, rodents form a taxon of high interest in the research of zoonoses. Worldwide, rats and mice spread over 35 diseases (www.cdc.gov/rodents). Moreover, with about 2277 recognized species

(Wilson & Reeder, 2005) and their worldwide occurence (except for Antarctica) rodents are one of the most successful mammal taxa. In urban areas their population can increase to numbers 15 times higher than the human population (Battersby et al., 2002; Easterbrook et al., 2005; Bonnefoy et al., 2008). Due to enormous range of body size and weight (from a few grams up to 70 kg), complex social systems and different reproduction strategies (Wilson & Reeder, 2005) they form a highly diverse group. Their reproduction cycles are linked to regional, ecological and climatic factors and in favorable conditions can lead to 10-fold population increases (Lewellen & Vessey, 1998; Lambin et al., 2006). As a result, these population fluctuations can in turn lead to disease outbreaks (Schmaljohn & Hjelle, 1997). Worldwide distribution, rapid reproduction cycles and high adaptability to new habitats are characteristics that distinguish rodents from other vertebrate taxa and enable them to play an important role in the maintenance and transmission of pathogens.

1.2 Rodent-associated pathogens in Germany

The following paragraph gives an overview on the most important rodent-associated zoonoses in Germany; a summary is shown in Table 1 (p. 10). More details and examples of investigations conducted by the Bundeswehr Institute of Microbiology regarding these diseases are shown in the publication „Nagetier-übertragene Zoonosen: Beispiele aus Untersuchungen in Süd- und Westdeutschland“ (Annex 4, p. 101).

Leptospirosis is a disease caused by Gram-negative bacteria of the genus *Leptospira*, belonging to the family of spirochetes (Johnson, 1996). They are found worldwide and are currently divided into 16 species, seven of them being pathogenic for humans. Below species level, Leptospira are classified into over 250 serogroups due to antigen characteristics (Zoeller, 2009). The pathogen is transmitted via urine of persistent infected rodents; however, evidence for the carriage of Leptospira has been found in virtually all mammalian species examined (reviewed in Adler & de la Peña, 2010).

Francisella tularensis, the agent of Tularemia, is transmitted mainly by *Dermacentor* and *Ixodes* ticks but also by mosquitos (Ellis et al., 2002). However, a wide variety of rodents and other small mammals are involved in the environmental cycle (Boyce, 1975; Morner et al., 1992; Berdal et al., 1996; Tarnvik et al., 1996; Vorou et al., 2007). For instance, Kaysser et al. (2008) found prevalences up to 10% in rodents from German outbreak areas.

Borrelia burgdorferi sensu lato is a complex of bacteria of the order Spirochetales, causing Lyme Borreliosis, a disease in humans and animals, particularly in dogs (reviewed in Beugnet & Marié, 2009). *Borrelia* spp. are transmitted by ticks, at the same time rodents, e.g. *Apodemus* mice and *Myodes glareolus* act as important reservoir hosts, with a high proportion being seropositive for *B. burgdorferi* in Central Europe (Gern et al., 1998; Humair et al., 1999; Stefancíková et al., 2004; Gern, 2008). Also migratory birds act as reservoirs and long distance vectors of infected ticks (Vorou et al., 2007). An increase in incidence in humans has been noted in eastern Germany in the years 2002 and 2003 (Mehnert & Krause, 2005).

Further, Telford et al. (1996) showed that the agent of human granulocytic ehrlichiosis, *Anaplasma phagocytophilum* (formerly named *Ehrlichia phagocytophila*), an emerging rickettsial disease, is able to infect various rodent hosts, and that subadult deer ticks become infected and efficiently transmit infection to rodents. Transmission and propagation occurs in large mammals such as horses, ruminants, dogs and cats, small mammals serve as reservoirs (Vorou et al., 2007).

Moreover, also *Ehrlichia* spp., belonging to the family Anaplasmataceae, are transmitted by ticks from persistently infected ruminant, cervid and rodent hosts (Walker et al., 2004). *Ehrlichia chaffeensis* causes flu-like illness in humans, in more severe cases multisystemic disease, toxic shock-like syndrome, or meningitis. Canine Ehrlichiosis, caused by *Ehrlichia canis,* is a potentially fatal disease in dogs, infecting monocytes in the peripheral blood. It is transmitted by the brown dog tick (*Rhipicephalus sanguineus*). Other *Ehrlichia* spp. cause disease in ruminants, horses, dogs and humans (Selbitz, 2002).

Babesia spp. are protozoa of the order Piroplasmida and are transmitted by ixodid ticks. More than 100 *Babesia* species are known, infecting many types of mammalian hosts, mostly rodents and birds (Homer et al., 2000). *Babesia* spp. cause severe, sometimes malaria-like disease in humans, ruminants, horses and dogs with symptoms such as apathia, fever, anemia and ikterus (Eckert et al., 2005). Human babesiosis is predominantly caused by two *Babesia* spp.: by *B. divergens,* a bovine pathogen, in Europe and by *B. microti,* a rodent-borne piroplasm with the white-footed mouse (*Peromyscus leucopus*) as natural reservoir in North America (Homer et al., 2000). However, the first autochthonous European case of human *B. microti* infection was reported in Germany (Hildebrandt et al., 2007). In Europe, *B. microti* was found in yellow-necked mice (*A. flavicollis*) and bank voles (*M. glareolus*) with a prevalence of 16.2% (Beck et al., 2010).

Coxiella burnetii, the agent of Q-fever, is an obligate intracellular pathogen with a broad host range, including humans, cattle, sheep, goats and other domestic animals, birds and arthropods (Selbitz, 2002). Infection follows inhalation of aerosol particles derived from heavily infected birth products of sheep, goats and cattle, but *C. burnetii* is also shed in milk, urine and feces. Animals become infected by aerosol and by tick bites, in Europe particularly by *Dermacentor marginatus.* Epidemiology of *C. burnetii* includes a sylvatic cycle (arthropods, birds, mammals including rodents) and a domestic cycle (Selbitz, 2002).

At least 10 rodent species are considered maintenance and reservoir hosts in the ecology of tick-borne encephalitis (TBE) virus (Cerny, 1976). In particular, bank voles (*Myodes glareolus*) and *Apodemus* spp. are present in TBE infection foci and often infestated with *Ixodes ricinus* ticks, the main vector of this arbovirus. The infected tick can transmit the virus to a vertebrate host during feeding and is also able to pass on the virus to a non-infected tick during co-feeding at the same site on the host (Mansfield et al., 2009).

Human Cowpox virus (CPXV) infections are often related to contact with domestic cats, rather than cows. However, wild rodents are a natural reservoir (Chantrey et al., 1999; Essbauer et al., 2009a). In humans,

cowpox virus causes papular lesions with surrounding erythema, general malaise, headache and fever. Cats as well as rats often present papular, ulcerating lesions, some also present respiratory dysfunctions (Kuczka et al., 2009). Zoo and circus animals, especially elephants, seem to be highly susceptible to generalised CPXV infections (Kurth et al., 2008).

Hantaviruses are rodent-borne RNA viruses, belonging to the family *Bunyaviridae*. Hantaviruses are host-specific, thus, for instance, Puumala virus, the main hantavirus species in Europe, is associated with the bank vole (*Myodes glareolus*). This association is presumably caused by coevolution of Hantaviruses and rodent reservoirs (Zeier et al., 2005; Ulrich et al., 2009). In the specific host, the virus establishes a prolonged subclinical infection, with virus shedding via urine, feces, and saliva (Lee et al., 1981; Hutchinson et al., 1998; Vitullo et al., 1987). The virus is transmitted to humans and to other rodents mainly by inhalation of aerosolized virus (Mills & Childs, 1998). In humans, Puumala virus infections cause disease ranging from asymptomatic infection, mild influenza-like symptoms up to *Nephropathia epidemica* (NE) or even hemorrhagic fever with renal syndrome (HFRS), a feverish illness with kidney failure (Ulrich et al., 2004).

Table 1: Important rodent-associated pathogens in Germany (modified from Pfeffer et al., 2010)

Pathogen	**Family**	**Vector**	**human disease**	**Infections in domestic animals**
Leptospira spp.	Spirochaetacae	-	Leptospirosis	dogs, pigs, ruminants i.a.
Francisella tularensis	Francisellaceae	ticks	Tularemia	lagomorphs, monkeys i.a.
Borrelia spp.	Spirochaetacae	ticks	Lyme-Borreliosis	dogs, horses
Rickettsia spp.	Rickettsiaceae	ticks, fleas, mites	Spotted fever	dogs, cats
Babesia spp.	Babesiidae	ticks	Babesiosis	dogs, Bovinae
Anaplasma phagocytophilum	Rickettsiaceae	ticks	Anaplasmosis/ Ehrlichiosis	dogs, horses, ruminants
Coxiella burnetii	Coxiellaceae	ticks	Q-fever	i.a. ruminants, dogs, cats
Hantavirus	*Bunyaviridae*	-	HFRS/NE	n.d.
Cowpox virus (CPXV)	*Poxviridae*	-	Cowpox	cats, dogs, zoo animals, pet rats
TBE virus	*Flaviviridae*	ticks	Tick-borne encephalitis	small rumi-nants, dogs, monkeys

HFRS: hemorrhagic fever with renal syndrome;
NE: *Nephropathia epidemica*
n.d.: not determined
-: direct transmission without vector

2 Rickettsia

For Rickettsiae, pathogens that are transmitted by arthropods, knowledge about natural cycles and possible reservoir hosts is scarce. Regarding their phylogenetic close relationship to *Borrellia* spp. in the order Rickettsiales, it seems conceivable that similar cycles involving rodents and other small mammals could exist for *Rickettsia* spp. It is a major aim of the present study to evaluate the role of rodents in the natural cycle of Rickettsiae.

2.1 General aspects of Rickettsia

Taxonomy

Rickettsia spp. are a group of Proteobacteria, belonging to the order Rickettsiales (Figure 1); Gram-negative bacilli that have evolved in such close association with arthropod hosts that they are adapted to survive within the host cells. Because of their strictly intracellular growth, they can be cultivated only in viable eukaryotic host cells (e.g., in cell culture, embryonated eggs, or susceptible animals). Genetic studies support the endosymbiotic theory according to which mitochondria and related organelles developed from members of this group (Walker, 1996). At present, the order Rickettsiales includes the two families Anaplasmataceae and Rickettsiaceae, after the family Bartonellaceae and the species *Coxiella burnetii* (the agent of Q-fever) were removed based on new insights in nucleotide sequence data, following proposals of Brenner et al. (1993) and Dumler et al. (2001), respectively. Anaplasmataceae as well as Rickettsiaceae include several pathogens for humans and animals, such as *Anaplasma*, *Ehrlichia*, *Rickettsia* and *Orientia* species.

The genus *Rickettsia* consists of two antigenically defined groups: spotted fever group and typhus group. The formerly called scrub typhus rickettsiae belong to the separate genus *Orientia* and differ in lacking lipopolysaccharide, peptidoglycan and a slime layer (Walker 1996). The *rOmpB* protein is the immunodominant species of surface protein antigen for most of the rickettsiae (Roux & Raoult, 2000) and was found to be

4776 bp (Carl et al., 1990).

Taxonomy of Rickettsia

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Alphaproteobacteria
Order: Rickettsiales
Families: Anaplasmataceae, Holosporaceae, Rickettsiaceae
Tribes: Rickettsiae, *Orientia*
Genus: *Rickettsia*
Spotted fever group
Typhus group

Figure 1: Taxonomy of Rickettsia (www.ncbi.nlm.nih.gov/taxonomy)

Transmission

Rickettsioses are usually transmitted to humans by arthropods such as ticks, mites, fleas, louses or chiggers. Therefore, their geographic distribution is often determined by that of the infected arthropod. Within the rickettsia found in blood-feeding hosts, a range of transmission strategies is represented: horizontal, vertical (i.e. transovarial) and mixed transmission. In general, *Rickettsia* species that are pathogenic to vertebrates are transovarially transmitted to the next generation (Azad & Beard, 1998). Most rickettsia travel in the arthropod host from the gut to the haemocoel and then to the salivary glands. From there they may be horizontally transmitted to the vertebrate host. Vertical transmission appears to maintain the bacterial population when vertebrate hosts are scarce (Munderloh & Kurtti, 1995). Some rickettsia in blood-feeders such as *Rickettsia peacockii* in the tick *Dermacentor andersoni* appear to be transmitted only vertically not including a vertebrate host at all (Azad & Beard, 1998; Baldridge et al., 2004). A rather unusual form of transmission is presented in *R. prowazekii*, the epidemic typhus agent, that appears to be better adapted to its vertebrate host than its louse host (Azad & Beard, 1998) as it is pathogenic to the louse, generally killing it within two weeks, and is not transovarially transmitted. For tick-associated rickettsiae that are mainly maintained by transovarial transmission between tick generations, ticks can act both as a reservoir and a vector of the infection

(Vitale et al., 1989). Unlike the spotted fever group rickettsia, typhus rickettsia multiply in the epithelium of the intestinal tract of their arthropod vectors and are excreted in the feces (Perlman et al., 2006), and infection occurs via the dermis after scratching. They can also be transmitted by bite in case they gained access to the salivary glands of the arthropod.

Still, there are many unanswered questions about the various transmission modes of different *Rickettsia* species, which are closely linked to the question of their potential pathogenicity. So far, there are only few studies on this issue in Germany (Pluta, 2010).

Pathogenesis

Rickettsiae are important causes of human disease around the world (Walker, 1996; Fournier & Raoult, 2005; Brouqui et al., 2007). Diseases associated with Rickettsiales are for example: Rocky Mountain spotted fever (RMSF), murine typhus, sylvatic typhus, epidemic typhus, human monocytic ehrlichiosis, human granulocytic ehrlichiosis, rickettsialpox, boutonneuse fever and other spotted fevers.

From the portal of entry in the skin, rickettsiae spread via the bloodstream to infect the endothelium and sometimes the vascular smooth muscle cells. Rickettsia species enter their target cells, multiply by binary fission in the cytosol, and damage heavily parasitized cells directly (Walker, 1996), causing hyperplasia of endothelial cells and thrombus formation, which leads to obstruction of blood flow and escape of red blood cells into the surrounding tissue. Papules develope when inflammatory cells follow into the tissue. Beginning necrosis in the center of the papule causes the typical clinical sign of rickettsial infection, the eschar.

The pathologic effects of rickettsial diseases originate from the multifocal areas of endothelial injury and vasculitis with loss of intravascular fluid into tissue spaces (edema), resultant low blood volume, reduced perfusion of the organs, and disordered function of the tissues with damaged blood vessels (e.g., encephalitis, pneumonitis, and hemorrhagic rash) (Fournier & Raoult, 2005; Macaluso & Azad, 2005).

For immunodefense, T-lymphocyte-mediated immune mechanisms and cytokines, including gamma interferon and tumor necrosis factor alpha, play a more important role than antibodies (Walker, 1996). The treatment consists of doxycycline or other tetracyclines given over a period of at least one week (Centers for Disease Control and Prevention, 2000).

In dogs, *R. rickettsia* causes RMSF symptoms similar to those in humans (see below). Other tick-transmitted *Rickettsia* spp. are considered non-pathogenic for dogs and other domestic animals (Varela, 2003).

Diagnosis

Because of the rather unspecific symptoms (e.g. fever, headache, nausea, vomiting, muscle aches, rash) the diagnosis of Rickettsioses can sometimes be difficult. Rickettsial infections can be monitored by serological assays (e.g. immunofluorescence tests). However, IgM and IgG antibodies reactive with rickettsia may be undetectable during the first week of illness (Paddock et al., 1999).

Between the spotted fever group and the typhus group rickettsiae, an extensive antigenic cross-reaction exists, making immunofluorescence assays a less helpful tool to distinguish between the species (Ormsbee et al., 1978). Other serologic tools are the Weil-Felix test, complement fixation (CF), microagglutination test, latex agglutination, ELISA, and western immunoblot assays (La Scola & Raoult, 1997).

Molecular diagnosis of rickettsial infection is more sensitive and specific. Material of choice is a biopsy of an eschar. There are several commonly used genes for detection of rickettsial DNA such as the Rickettsia genus specific 17-kDa antigen gene, the 16S rRNA gene, the citrate synthase gene (gltA), and partial outer membrane proteins B and A (*ompB* and *ompA*) (Reif and Macaluso, 2009).

2.2 Relevant species of spotted fever group Rickettsia

More than 200 rickettsial species or proposed species exist in the spotted fever group (http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html, Benson et al., 2008). Those of veterinary or medical interest and those that are known to occur in Germany are presented in this chapter, their evidence in ticks and vertebrates is summarised in Table 2 (p. 18).

R. rickettsii

The agent of Rocky Mountain spotted fever (RMSF) is associated mainly with *Dermacentor andersoni* and *D. variabilis* but also with *Haemaphysalis* spp*., Amblyomma* spp., *Ixodes* spp*., Rhipicephalus sanguineus* and *Otobius lagophilus* (McDade & Newhouse, 1986). It occurs widespread throughout the United States of America, with most cases observed in the southern and southeastern regions (www.cdc.gov), limited by the geographic distribution of its arthropod hosts. In Brazil, the *R. rickettsii* caused disease is called Brazilian spotted fever, in Mexico fiebre manchada. Infected human individuals typically but not in every case present the triad of rash, fever and a history of tick bite. In addition, it can cause headache, myalgia, nausea, abdominal pain, a skin rash, and – in more severe cases – neurological problems, acute renal failure or meningoencephalitis. The mortality rate in humans is on average 1.4% (Chapman et al., 2006). In dogs, *R. rickettsia* also causes RMSF symptoms such as fever, rash, vasculitis and edema, decreased platelet numbers, joint swelling, myalgia and neorological abnormalities (Warner & Marsh, 2002; Otis et al., 2004).

R. conorii

Mediterranean spotted fever (MSF) is caused by *R. conorii* and endemic to the Mediterranean area, including northern Africa and southern Europe, with some cases observed also in Kenya, Somalia, South Africa, Turkey, Bulgaria and Ukraine (Rovery et al., 2008). Zhu et al. (2005) proposed the creation of the following subspecies because of serotypical, clinical and geographical differences: *R. conorii conorii*, *R. conorii caspia, R. conorii israelensis* and *R. conorii indica.* MSF is regarded as an emerging disease

with case numbers increasing in many countries in the last decade (reviewed in Rovery et al., 2008). Clinical features are similar to other spotted fevers, with the eschar sometimes being untypical and furuncle-like and rarely multiple. The mortality rate in humans can be as high as 32.3% (in Portugal 1997) and makes MSF at least as severe as RMSF (Rovery et al., 2008).

R. felis

R. felis was first identified in cat fleas (*Ctenocephalides felis*) by Adams et al. (1990), later also detected in other flea species and also in ticks and mites (Ishikura et al. 2003; Choi et al., 2007; Tsui et al., 2007; Oliveira et al., 2008) and is distributed nearly all over the world. The potential infection of both insects and acarines makes *R. felis* unique in the rickettsial family. Its prevalence in wild-caught arthropods ranges from 0.8 to 100% (depending on geographic location and arthropod species) with an average percentage of 25% (Reif & Macaluso, 2009). Like other rickettsial diseases, it can cause various symptoms in humans, such as fever, rash, headache, myalgia, eschar, visceral and neurological symptoms, put together as flea-borne spotted fever or cat flea typhus. Human cases have been reported in 12 countries worldwide, including Germany (Reif & Macaluso, 2009).

The cat flea is currently the only defined vector and reservoir as transmission of viable *R. felis* between mammals and arthropods has not been shown so far (Reif & Macaluso, 2009).

R. helvetica

R. helvetica was first isolated in 1979 from *I. ricinus* ticks in Switzerland (Burgdorfer et al., 1979) and afterwards found in France, Italy, Sweden, Slovenia, Portugal, Spain, Japan (reviewed in Blanco & Oteo, 2006; Fournier & Raoult, 2005) and also in Germany (Woelfel et al., 2006; Dobler & Woelfel, 2009). In 1999, it was implicated in a case of fatal perimyocarditis in a young human patient in Sweden (Nilsson et al., 1999), and involvement was proposed in a case of sarcoidosis 2002 (Nilsson et al., 2002) and in some cases of febrile illness (Fournier et al., 2000;

Fournier et al., 2004). Infections are present during the hot months and present with fever, headache, arthralgia and myalgia, but not with a cutaneous rash (Fournier et al., 2004).

R. slovaca

This SFG rickettsia is transmitted by *Dermacentor marginatus* ticks with the wild boar considered as main host (Blanco & Oteo, 2006) and was found in all European countries were those ticks were screened, including France, Portugal, Switzerland, former Yugoslavia, Slovakia, Ukraine, Armenia and Siberia (Sekeyova et al., 1998; Shpyonov et al., 2001). In Germany, Pluta et al. (2010) detected *R. slovaca* in five out of 666 *Dermacentor* ticks collected in Southern Germany and furthermore describe a clinical case of TIBOLA (Tick-borne lymphadenitis), the disease caused by *R. slovaca* in humans, which is also named DEBONEL (*Dermacentor*-borne necrosis erythemalymphadenopathy) and which is characterized by painful lymph nodes, sometimes fever, rarely cutaneous rash. However, the first case of clinical manifest human infection with *R. slovaca* was presented by Woelfel et al. (2009) in a 67-year-old female following a tick bite. Remarkably, only 50% of patients develop detectable antibodies, which may be evidence for a rather localized infection (Fournier & Raoult, 2005).

R. monacensis

A new rickettsia species, *R. monacensis*, was isolated from *I. ricinus* from Munich, Germany (Simser et al., 2002) and found to be the causative agent of two MSF-like human cases in Spain (Jado et al., 2007), proving its pathogenic potential for humans for the first time. Dobler et al. (2009) isolated and characterised two strains in southeastern Germany that grew interestingly enough at 28° C in cell culture but not at 37° C, which doubts the potential of growth and pathogenicity in organisms of these two strains.

Table 2: Evidence of *Rickettsia* spp. in ticks and vertebrates in Germany

	Species	**Evidence in ticks in Germany**	**Evidence in humans/ mammals in Germany**
Spotted fever group	*R. conorii spp.*	n.d.	n.d.
	R. africae	n.d.[2]	n.d.
	R. parkeri	n.d.[2]	n.d.
	R. sibirica spp.	n.d.[2]	n.d.
	R. slovaca	Rehacek et al., 1977; Pluta et al., 2009; Pluta et al., 2010	Woelfel et al., 2009; Pluta et al., 2009
	R. rickettsii	n.d.[2]	n.d.
	R. honei	n.d.[2]	n.d.
	R. heilongjangensis	n.d.[2]	n.d.
	R. japonica	n.d.[2]	n.d.
	R. montanensis	n.d.[2]	n.d.
	R. aeschlimanni	n.d.	n.d.
	R. raoultii	Pluta et al., 2010	n.d.
	R. rhipicephali	n.d.	n.d.
	R. massiliae	Dobler & Woelfel, 2009	n.d.
	R. helvetica	Hartelt et al., 2004 & 2008; Pichon et al., 2006; Woelfel et al., 2006; Silaghi et al., 2008; Hildebrandt et al., 2010	n.d.
	R. monacensis	Silaghi et al., 2008; Dobler et al., 2009; Dobler & Woelfel, 2009	n.d.
	R. felis	Gilles et al., 2008[1]; Dobler & Woelfel, 2009	n.d.
	R. australis	n.d.[2]	n.d.
	R. akari	n.d.[2]	n.d.
Typhus group	*R. typhi*	n.d.	n.d.
	R. prowazekii	n.d.	n.d.
Ancestral group	*R. canadensis*	n.d.[2]	n.d.
	R. belli	n.d.[2]	n.d.
	O. tsutsugamushi	n.d.[2]	n.d.

[1]detection in fleas;
[2]*Rickettsia* spp. with geographic distribution outside of Europe, thus not supposed to occur in Germany (Fournier & Raoult, 2005);
n.d.: not determined

III. MATERIALS AND METHODS

1 Rodent collection

In the years 2004 and 2005, rodents were collected in October, May and September at seven locations in the district of Lower Bavaria, including forests, pasture, blackberry bushes and farmland. Those rodents were collected for a study investigating Hantaviruses in Lower Bavaria and sampling areas were chosen according to the occurrence of Hantavirus-induced *Nephropathia epidemica* cases. Before trapping, a decoying method was conducted using plastic cups with apple pieces as bait located every 5 – 10 m, with 2 – 3x20 bait lines per location (Essbauer et al., 2006). Trapping (5 – 9 traps/area, 33 traps in total) was performed on sites with signs of rodent activities.

Sampling areas used for trapping in September and October 2008 belong to transects in the National Park Bavarian Forest. The Bavarian Forest National Park (24235 ha) is situated in the German part of the Bohemian Forest and is about 98% covered by forest (Elling et al., 1987). The region is characterised by montane and high montane areas. Four straight transects (Figure 2) were initiated following the altitudinal gradient for the BioKlim Project (Baessler et al., 2008), a project conducted by the National Park Authority that collects extensive data on macro- and microclimate, vegetation and different animal species living in this habitat. Those transects consist of about 400 research sites. A total of 22 of them were chosen for our study under two aspects:

(1) sites should cover an altitude gradient from 300 m up to 1400 m a.s.l. with one site every 100 m a.s.l.;

(2) sites are likely to be inhabited by a high rodent population, either because of dense vegetation, which offers coverage and green food or because of other sources of possibly rich food supply, for example beech trees (Figures 3, 5).

Sixteen Sherman live traps (Figure 4) were deployed in an 18 m^2 plot, one trap every three meter, decoyed with apple pieces (Golden Delicious) for

three consecutive nights. Twice a day the traps were checked for trapped animals. Captured animals were anesthetized by CO_2 exposure and killed humanely according to the German Animal Protection Act, after blood was drawn by heart punction. Rodents were dissected under BSL-2 conditions. Tissue samples as well as sera and transudates were deepfreezed until further use. Trapping site, species, sex, reproductive and physical conditions, as well as parasitic load were recorded (Figures 6, 7). Morphological species determination was conducted for a sampling ratio of ectoparasites found on 37 rodents.

To record microclimate data (temperature, humidity, dewpoint), datalogger (EL-USB-1, Lascar Electronics, UK) were permanently installed at every site.

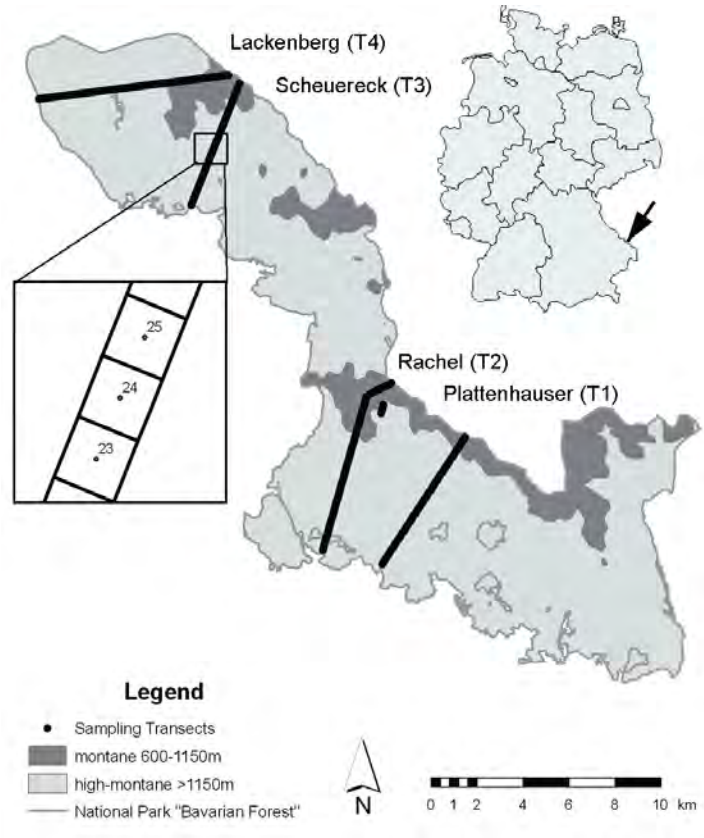

Figure 2: Sampling transects from the BIOKLIM Project in the National Park ‚Bavarian Forest' with indication of the boundaries of the montane and high montane zone (Baessler et al., 2008)

Figure 3: Spot Isa32, 317 m a.s.l. Dense vegetation consisting of blackberries, nettles, deciduous trees

Figure 4: Sherman live trap

Figure 5: Spot Els 8, 578 m a.s.l. Rocky area, vegetation consisting mainly of beech trees

Date	Site	time	weather	Temp°C	trap nr	mouse ID	species	sex	age	blood	Urine	Ektoparasites
04.08.	Isa 32	8:30	rain	15	F 123	DO 09/014	*M. glareolus*				--	--
					F125	DO 09/015	*A. flavicollis*	m	ad	2x	--	--
					F 121	DO 09/016	*M. glareolus*	m	juv	1x	--	1 flea
						DO 09/017	*M. glareolus*	m	juv	1x	--	--
	Igg 35	9:50	cloudy	15	neg	--	--	--	--	--	--	--
	Igg 33	10:40	cloudy	15	neg	--	--	--	--	--	--	--
	Sal 27	11:20	cloudy	17	neg	--	--	--	--	--	--	--
	Els 5	12:40	cloudy	15	F 48	DO 09/018	*A. flavicollis*	--	--		--	--
	Els 8	12:15	cloudy	15	neg	--	--	--	--	--	--	--
	Isa 32	13:45	cloudy	20	neg	--	--	--	--	--	--	--
05.08	Isa 32	8:30	sunny	16	F 124	DO 09/019	*M. glareolus*	m	juv	1x	--	--
			sunny		F 127	DO 09/020	*M. glareolus*	m	juv	1x	--	--
	Igg 35	9:10	sunny	16	neg	--	--	--	--	--	--	--
	Igg 33	9:45	sunny	19	neg	--	--	--	--	--	--	--
	Sal 27	10:35	sunny	21	neg	--	--	--	--	--	--	--
	Els 5	11:00	sunny	21	F 48	DO 09/021	*A. flavicollis*	m	ad	1x	--	2 ticks
					F 56	DO 09/022	*A. flavicollis*	m	ad	1x	x	--
					F 49	DO 09/023	*A. flavicollis*	m	ad	1x	--	--
						DO 09/024	*A. flavicollis*		ad	1x	--	--
	Els 8	11:45	sunny	22	neg	--	--	--	--	--	--	--
	Isa 32	14:00	sunny	24	neg	--	--	--	--	--	--	--
06.08.	Isa 32	8:20	sunny	19	F121	DO 09/025	*A. flavicollis*	f	ad	1x	--	--
	Igg 35	9:00	sunny	20	neg	--	--	--	--	--	--	--
	Igg 33	9:50	sunny	22	F 59	DO 09/026	*A. flavicollis*	m	ad	1x	--	--
			sunny		A25	DO 09/027	*A. flavicollis*	f	ad	1x	--	--
	Sal 27	10:50	sunny	24	neg	--	--	--	--	--	--	--
	Els 5	12:05	sunny	22		DO 09/028	*A. flavicollis*					
			sunny		Y24	DO 09/029	*A. flavicollis*					
					F 52	DO 09/030	*A. flavicollis*					
	Els 8	11:30	sunny	22	neg	--	--	--	--	--	--	--

Figure 6: Data collected when trapping animals

mouse ID	trapping date	trapping site	species	Sex	body length	length of hindfoot	body-tail length	body weight	heart [g]	liver [g]	spleen [g]	lung [g]	kidneys [g]	ears [g]	Serum	Urine	ectopara-sites	gonads [g]	comments
T2 09/001	28.5.09	Wsh	*M. glareolus*	f	10,60	1,70	14,10	24,63	0,23	0,85	0,08	0,21	0,39	0,14	x			0,17	
T2 09/002	3.6.09	T2 44	*Microtus agrestis*	f	9,3	1,6	12	33,11	0,16	1,24	0,18	0,17	0,82	0,19	x			5,51	pregnant
T2 09/003	3.6.09	T2 54	*M. glareolus*	f	11,00	1,70	15,00	31,03	0,30	1,87	0,18	0,16	1,04	0,14	x			1,17	pregnant early stage corneal defect
T2 09/004	4.6.09	T2 23	*M. glareolus*	m	10,80	1,60	15,60	30,15	0,18	1,58	0,33	0,26	0,62	0,32	x			1,44	o.s.
T2 09/005	5.6.09	T2 50	*M. agrestis*	m	12,00	1,80	15,40	39,82	0,14	1,41	0,24	0,32	0,69	0,23			5 fleas	1,42	
T2 09/006	9.6.09	T2 50	*M. glareolus*	m	7,80	1,50	11,20	13,00	0,29	0,94	0,15	0,02	0,44	0,08				<0,01	
T2 09/007	9.6.09	T2 73	*M. glareolus*	m	12,40	2,00	15,40	39,41	0,29	0,98	0,09	0,03	0,44	0,11		x	1 tick	1,12	
T2 09/008	10.6.09	T2 50	*M. glareolus*	m	7,20	1,60	11,00	12,11	0,44	1,55	0,33	0,20	0,69	0,19	x			0,09	
T2 09/009	10.6.09	Wsh	*M. glareolus*	f	10,50	1,90	14,50	38,19	0,28	2,78	0,24	0,29	0,79	0,21				5,81	pregnant 3 embryos tail fractured,
T2 09/010	25.8.09	T2 50	*M. glareolus*	f	9,60	1,70	14,50	24,58	0,47	1,46	0,15	0,17	1,43	0,11	x			0,20	pregnant
T2 09/011	26.8.09	T2 23	*M. glareolus*	f	9,30	1,70	14,50	26,73	0,33	1,74	0,21	0,30	0,64	0,12	x			0,08	
T2 09/012	26.8.09	Wsh	*M. glareolus*	f	11,00	1,60	13,90	31,20	0,42	1,48	0,20	0,29	0,58	0,14	x			4,89	left hindleg fractured
T2 09/013	26.8.09	T2 67	*Sorex araneus*	m	6,50	1,20	9,80	12,13	0,26	0,79	0,26	0,02	0,52	0,15	x			0,86	
T2 09/014	27.8.09	T2 50	*M. glareolus*	m	11,00	1,70	16,10	39,51	0,48	1,92	0,20	0,28	0,67	0,14				11,36	pregnant
T2 09/015	27.8.09	T2 54	*M. glareolus*	m	9,00	1,70	13,00	17,55	0,30	1,33	0,31	0,14	0,47	0,14	x			0,91	
T2 09/016	27.8.09	T2 67	*Sorex araneus*	m	7,40	1,00	11,20	11,34	0,17	0,67	0,21	0,21	0,31	0,08	x			0,64	
T2 09/017	8.9.09	T2 54	*M. glareolus*	m	11,40	2,00	16,50	26,62	0,23	1,30	0,58	0,58	0,52	0,19	x	x		1,84	white occlusion
T2 09/018	8.9.09	T2 67	*M. agrestis*	m	9,70	1,90	13,70	34,03	0,28	1,53	0,69	0,69	0,36	0,19	x	x		1,55	in liver
T2 09/019	8.9.09	T2 67	*M. agrestis*	f	9,50	1,80	13,00	26,64	0,33	1,29	0,33	0,33	0,28	0,10	x		1 flea	0,09	
T2 09/020	8.9.09	T2 67	*Sorex araneus*	f	5,90	1,30	10,20	7,22	0,08	0,64	0,18	0,18	0,30	0,03	x			<0,01	
T2 09/021	8.9.09	T2 73	*M. glareolus*	f	9,00	1,60	12,30	19,99	0,15	0,81	0,39	0,39	0,16	0,13	x			<0,01	
T2 09/022	8.9.09	T2 73	*Sorex araneus*	f	6,20	1,00	10,60	7,00	0,15	0,37	0,18	0,18	0,18	0,03	x	x		<0,01	
T2 09/023	8.9.09	T2 73	*M. glareolus*	f	9,00	1,60	11,60	17,95	0,19	0,89	0,32	0,32	0,24	0,13			4 ticks	0,02	left hindleg fractured
T2 09/024	9.9.09	T2 54	*M. glareolus*	m	9,20	1,70	13,00	19,03	0,19	1,05	0,45	0,45	0,24	0,11	x		1 flea 1 tic	0,84	
T2 09/025	9.9.09	T2 67	*M. glareolus*	m	10,50	1,70	13,50	29,37	0,32	1,60	0,55	0,55	0,35	0,17			1 flea	1,18	lower jaw injured
T2 09/026	10.9.09	T2 73	*Sorex minutus*	m	4,50	1,00	8,20	3,38	0,06	0,19		0,01	0,03	0,02	x			<0,01	
T2 09/027	10.9.09	T2 73	*Sorex araneus*	f	7,00	1,20	11,50	8,20	0,08	0,24	0,14	0,01	0,30	0,02				<0,01	

Figure 7: Data collected when dissecting animals

2 Immunofluorescence tests

For the investigation of sera and transudates we decided to use two different immunofluorescence tests. First, we used a commercial available test for detection of human IgG antibodies (*Rickettsia conorii* IFA IgG Antibody Kit, Fuller Laboratories RCG-120, Fullerton, California) that was modified for our purposes with a rabbit-anti-mouse conjugate. This test

was supposed to detect antibodies against *R. conorii*, a species that is not presumed to occur in Germany, but as it is a member of the spotted fever group we expected to detect antibodies against other SFG rickettsiae with this test. Additionally, we used an in-house test for the detection of antibodies against *R. helvetica*, also a SFG rickettsia, which is phylogenetically distinct from *R. conorii* and thus was supposed to detect those SFG rickettsial antibodies distinct from *R. conorii*. More details on the manufacture and conduction of these tests can be found in the publication (IV.5, p. 41).

3 DNA extraction

As the ear seemed to be the tissue of choice for detection of *Borrelia* DNA (Peavy et al., 1997; Kiessling, 2005), we decided to use this tissue for detection of rickettsia.

QIAamp DNA Mini Kit (Qiagen, Hilden Germany) was used for isolation of DNA from ear tissue samples. One ear per mouse was placed into a 1.5 ml Eppendorf tube, 180 µl ATL buffer and 20 µl proteinase K were added and the mixture was placed in a Thermomixer (Eppendorf, Germany) for about four hours, until the ear material was totally digested. The digesting process then was inhibited by putting the Eppendorf tubes into a 70° C water bath. After centrifugation the digested solution was put into a column, carefully leaving fur in the Eppendorf tube. The extraction process according to manufacturer's instruction followed. Nucleic acids from liver material were extracted using the QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. The elution volume was 100 µl. A tube not containing tissue material was added to every extraction process as quality control to ensure that no contamination had occurred during the extraction process.

4 Polymerase chain reaction for detection of Rickettsial DNA

4.1 Real-time PCR for detection of the gltA gene of *Rickettsia* spp.

For initial screening of rodent ear and liver tissue for rickettsial DNA a PanRickettsia real-time PCR established by Woelfel et al. (2008) was chosen. Woelfel et al. designed one set of primers and one TaqMan probe (Table 3) to amplify a 70-bp genome region of the citrate synthase (gltA) gene on the basis of all rickettsial gltA sequences available in GenBank. The following bacterial species were used for validation of the real-time PCR assay: *Rickettsia helvetica*, *R. honei*, *R. rickettsii*, *R. typhi*, *R. africae*, *R. conorii*, *R. mongolotimonae*, *R. IRS4*, and *R. felis* (Woelfel et al., 2008). A performance a little less successful is achieved with specimens of *R. typhi* (Woelfel, personal communication).

The reaction was carried out in a LightCycler 1.5 (Roche, Mannheim, Germany). Tables 4 and 5 show the reaction and cycling conditions used. Negative controls (DNA free extraction quality controls or sterile water) were always included.

All primers were ordered from TIB MOLBIOL, Berlin, Germany.

Table 3: Primers and Polymerase for real-time PCR detection of the gltA gene of *Rickettsia* spp.

Primer, Taq	Oligonucleotide sequence	Reference
PanRick_2_for	5'-ATAggACAACCgTTTATTT-3'	Woelfel et al., 2008
PanRick_2_rev	5'-CAAACATCATATgCAgAAA-3'	
PanRick_3_taq	5'-FAM-CCTgATAATTCgTTAgATTTTACCg–TMR-3'	

Table 4: Reaction conditions for PCR detection (gltA) of *Rickettsia* spp.

Reagent	**Volume in µl**
LC DNA Master Hybridization Probes	2.0
PanRick gltA2 taq (4 µM)	1.0
$MgCl_2$ (25 mM)	2.4
PanRick_2_for (10 µM)	1.0
PanRick_2_rev (10 µM)	1.0
UDG Mix (2U/µl)	0.2
DEPC-H_2O	7.4
Template DNA	5.0
Total volume	20.0

Table 5: Cycling conditions for PCR detection (gltA) of *Rickettsia* spp.

Parameter	**Temperature [°C]**	**Time [sec]**	**Slope**	**Acquisition mode**
Activation of Fast Start Taq DNA polymerase	95	600	20	none
Amplification (45 cycles)	95	40		none
	56	30	20	none
	72	30		single
Melting curve analysis	95	0	20	none
	45	30	20	none
	80	0	0.2	continuous
Cooling	40	30	20	none

4.2 PCR for the detection of the *ompB* genes

Samples tested positive in the PanRickettsia screening were further taken in a conventional PCR targeting the partial outer membrane protein B (*ompB*) by following the protocol published by Roux & Raoult (2000), using the primers 120-2788 (5'-AAACAATAATCAAGGTACTGT-3') and 120-3599 (5'-TACTTCCGGTTACAGCAAAGT-3'). For this PCR Platinum Taq Polymerase High Fidelity (Invitrogen, Carlsbad, California) was used, adding 5 µl of DNA to each reaction (Table 6). To avoid the appearance of unspecific amplification the protocol was modified with annealing steps (Table 7), as the annealing temperature during a polymerase chain reaction determines the specificity of primer annealing. Temperatures were gradually reduced in two degree steps from 60° C to 50° C (Touchdown) as it is described in Don et al. (1991).

Table 6: Reaction conditions for PCR detection (*ompB*) of *Rickettsia* spp.

Reagent	**Volume in µl**
10x High Fidelity PCR Buffer	5.0
High Fidelity Taq Polymerase	2.0
$MgSO_4$ (50 mM)	4.0
Primer 120-2788	2.5
Primer 120-3599	2.5
DNTP's	1.0
DEPC-H_2O	28.0
Template DNA	5.0
Total volume	50.0

Table 7: Cycling conditions for the detection of the *ompB* genes of *Rickettsia* spp. (modified after Roux & Raoult, 2000)

Cycle	**Step**	**Temperature [°C]**	**Duration [sec]**
Touchdown	Touchdown	95	180
2x each		**60**	30
		68	60
		95	180
		58	30
		68	60
		95	180
		56	30
		68	60
		95	180
		54	30
		68	60
		95	180
		52	30
		68	60
Cycle 1	Initial denaturation	95	180
1x			
Cycle 2	Denaturation	95	30
35x each	Annealing	50	30
	Extension	68	60
Cycle 3	Final extension	68	600
1x			

4.3 Mammalia species-specific 18S ribosomal RNA PCR

The possibility to amplify DNA in our study was assured by a universal human/ mammalian specific 18S PCR ahead of *Rickettsia* specific PCR amplification. For choice of sequences different human and mammalian 18S sequences were obtained from the Genbank (Mouse, rat, human,

rabbit, rat like hamster, horse, cattle, West European Hedgehog, Gray Short-tailed Opossum, Virginia Opossum), and aligned by ClustalW2 (Embl-EBI, www.ebi.ac.uk) (Annex 2, p. 96). The actual primer design was carried out by Gene Runner, Version 3.05, Hastings Software, Inc. (Table 8). Tables 9 and 10 show the reaction and cycling conditions used. Characteristic amplicons are 189 nt in length.

Table 8: Primers for universal mammalian-specific 18S ribosomal RNA gene PCR

Primers	**Oligonucleotide sequence**
18S fw	5'–ATTAGAGTGTTCAAAGCAGGC-3'
18S rev	5'-TGCTTTCGCTCTGGTCCGTC-3'

Table 9: Reaction conditions for universal mammalian-specific 18S ribosomal RNA gene PCR

Reagent	**Volume in µl**
LC-Fast Start Reaction Mix	2.0
$MgCl_2$ (25 mM)	1.2
Univ18S-fw (20pmol/µl)	0.8
Univ18S-rev (20pmol/µl)	0.8
DEPC-H_2O	13.2
Template DNA	2.0
Total volume	20.0

Table 10: Cycling conditions for universal mammalian-specific 18S ribosomal RNA gene PCR

Parameter	**Temperature [°C]**	**Time [sec]**	**Slope**	**Acquisition mode**
Activation of Fast Start Taq DNA polymerase	95	600	20	none
Amplification (45 cycles)	95	40		none
	56	30	20	none
	72	30		single
Melting curve analysis	95	0	20	none
	45	30	20	none
	80	0	0.2	continuous
Cooling	40	30	20	none

5 Agarose gel electrophoresis

Conventional PCR products were visualised under UV light after 1.5% agarose gel electrophoresis (1.5 g agarose/100 ml TAE Buffer) and subsequent staining with GelRed™ solution (Biotium Inc., Hayward, Canada), a fluorescent nucleic acid dye that is less toxic than ethidium bromide (Biotium Safety Report, 2008). For comparison a standardised DNA-Ladder was added to each electrophoresis.

6 DNA purification

Amplificates were purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden Germany) according to manufacturer's instruction.

7 Sequencing and sequence analysis

After purification, all rickettsial PCR products were sent off for sequencing to the GATC sequencing service (Konstanz, Germany) using corresponding specific primers. After evaluating the specificity of results with Chromas©Lite (www.technelysium.com.au/chromas_lite.html), sequence and phylogenetic analyses were performed using BioEdit (Version 7.0.0, Copyright ©1997-2004, Hall 1999). Sequence similarity searches were made, without the flanking primers, by BLAST analysis (www.ncbi.nlm.nih.gov.library.vu.edu.au/BLAST/). The obtained sequences were further analysed with each other and with GenBank sequences by multiple alignments (www.ebi.ac.uk/clustalw/index.html). The obtained sequences were submitted to Genbank. A phylogenetic tree (Figure 13, p. 36) was constructed using Bioedit, Neighbour Joining method, with *R. prowazekii* as outgroup, 1000 bootstraps, based on 770 nt fragment of *ompB*.

8 Statistical analysis

Data of trapped rodents were collected with Microsoft Excel® and evaluated with R 2.9.2 (R Core Development Team 2009). To come to an decision which parameters to chose for statistical analysis with R 2.9.2, preliminary tests for significance of several parameters such as species, sex or age were conducted using SPSS® version 12.0.1, SPSS Inc.,

Chicago, IL, USA, using chi-square test, student t-test including Levene's test for equal variance, Mann-Whitney test and Spearman rank correlation.

Serological assays were compared using concordance comparison and kappa-statistics. Statistical tests were performed for *ompB* PCR-positive samples for body size (head-body length), body weight and organ weights. Values of $p<0.05$ were regarded as significant. For the other variables we restrained from a formal test and provided only descriptive statistic. In our final test model with rickettsia-PCR positivity as dependent variable we included in the following sequence the predictors

1) as control variable the species,
2) occurrence of ectoparasites,
3) body size and
4) elevation.

We applied a sequential analysis of deviance table (Hastie & Pregibon, 1992). That is, the reductions in the residual deviance as each term of the formula added in turn are given in as the rows of a table, plus the residual deviances themselves. We first added the species to the model and then subsequently ectoparasites, body size and elevation. Due to the binomial dispersion of our dependent variable "Rickettsia-PCR positive" we applied the chi-square test.

IV. RESULTS

1 Trapping results

Trapping of small mammals with Sherman live traps was continued in the years 2009 and 2010 and resulted in a total of 690 trapped individuals, presented in Table 11. Screenings of animals for *Rickettsia* collected in 2009 and 2010 were not included in this thesis. Figure 8 shows a bank vole (*Myodes glareolus*), the species with highest trapping numbers. Figure 9 shows the distribution of species along the elevation gradient for small mammals collected in 2008. Species were determined morphologically and for some individuals by mitochondrial Cytochrom B-PCR and sequencing.

Morphological species determination of fleas found on 37 rodents revealed five species, predominantly *Ctenophthalmus agyrtes* (n=39/53). Other ectoparasites were mainly *Ixodes* ticks (nymphs and larvae). Their preferred body spots seemed to be the ears, the chin, and the tail base. The occurrence of ectoparasites was distributed among all rodent species and among all trapping sites.

Table 11: Trapping results for Lower Bavaria (2004/05) and National Park Bavarian Forest (2008-2010)

Species	**2004/05**	**2008**	**2009**	**2010**
yellow-necked mouse (*Apodemus flavicollis*)	21	23	47	164
wood mouse (*Apodemus sylvaticus*)	7	0	0	0
bank vole (*Myodes glareolus*)	47	15	71	260
brown vole (*Microtus agrestis*)	4	4	4	6
water vole (*Arvicola amphibius*)	1	0	0	0
house mouse (*Mus musculus*)	1	0	0	0
Eurasian pygmy shrew (*Sorex minutus*)	0	1	10	4
total	**81**	**43**	**132**	**434**

Figure 8: Bank vole (*Myodes glareolus*)

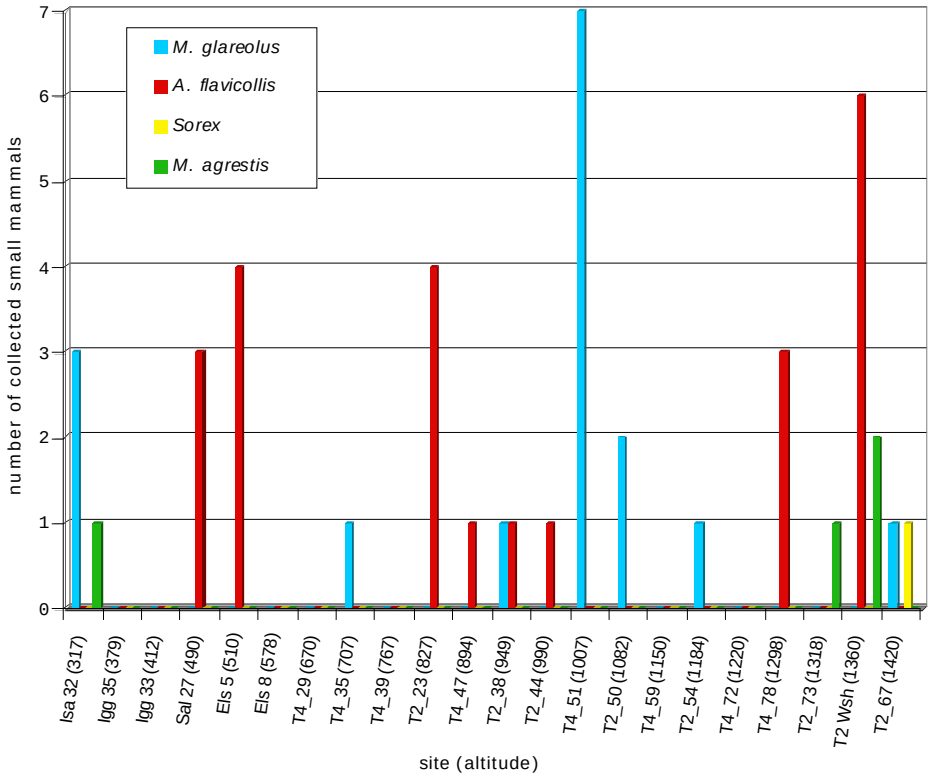

Figure 9: Distribution of trapped species along the altitude gradient

2 PCR results

The molecular-biological studies presented here resulted in the first detection of *Rickettsia* spp. in rodents by PanRickettsia real time-PCR. Screening of 119 DNA samples obtained from ear material revealed a prevalence of 7.6% (n=9/119) of rickettsial DNA. Screening of liver samples (n=46) revealed no amplification.

During amplification in the real-time PCR for detection of the rickettsial gltA gene, amplification later than cycle 40 was considered negative, however, some samples remained doubtful. Positive and doubtful samples were further processed to the conventional PCR targeting the *ompB* gene. Sample T2 08/018 and T2 08/021 have been chosen as examples for a strong fluorescent signal and high CT and for a low signal and low CT, respectively (Figure 10).

OmpB PCR revealed seven amplifications out of 9 further tested samples (Figure 11). More details on obtained results can be found in the publication (IV.5, p. 44).

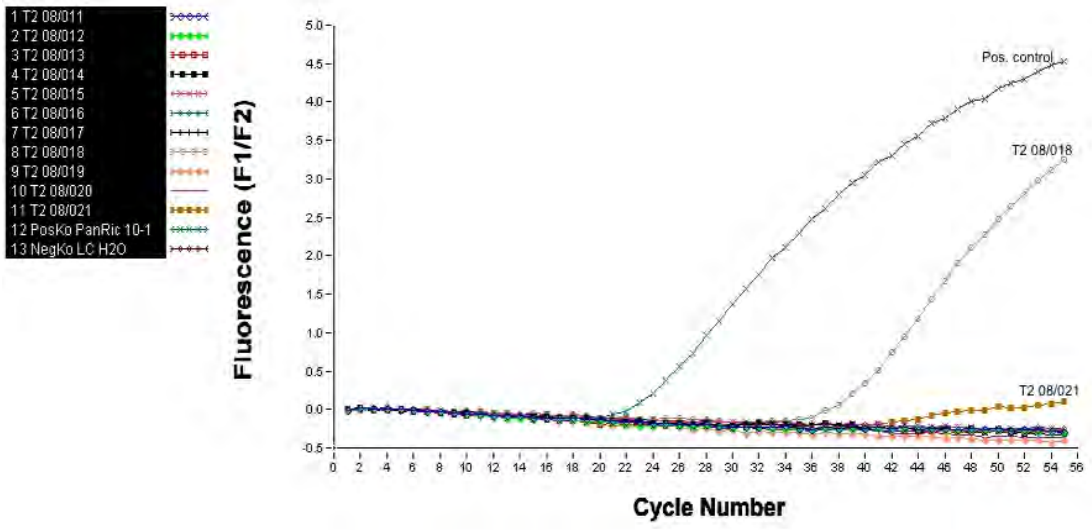

Figure 10: Results of Pan Rickettsia real-time PCR of ears with sample numbers T2 08/018 and T2 08/021 revealing signals

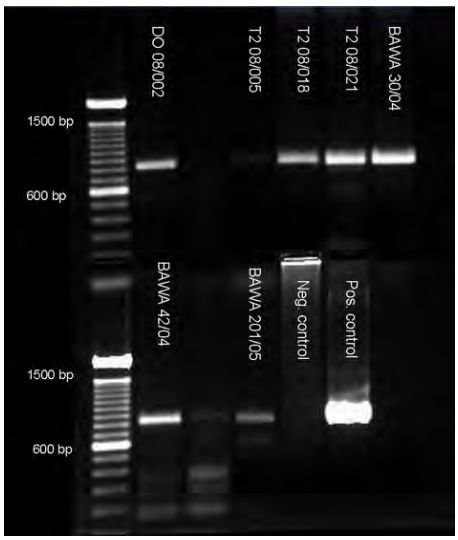

Figure 11: *OmpB* PCR products, showing amplification fragments of approx. 800 bp in length

3 IFT results

Serological investigations of rodent blood and transudates included two IFATs for detection of *R. conorii* and *R. helvetica* antibodies and revealed an overall seroreactivity of 28.1% (n=32/114). Results of IF-testing are presented in detail in the publication (IV.5, p. 45). Positive reactions appear as Rickettsial bodies exhibiting bright apple-green cytoplasmic fluorescence against a background of orange to red yolk sac matrix (Figure 12).

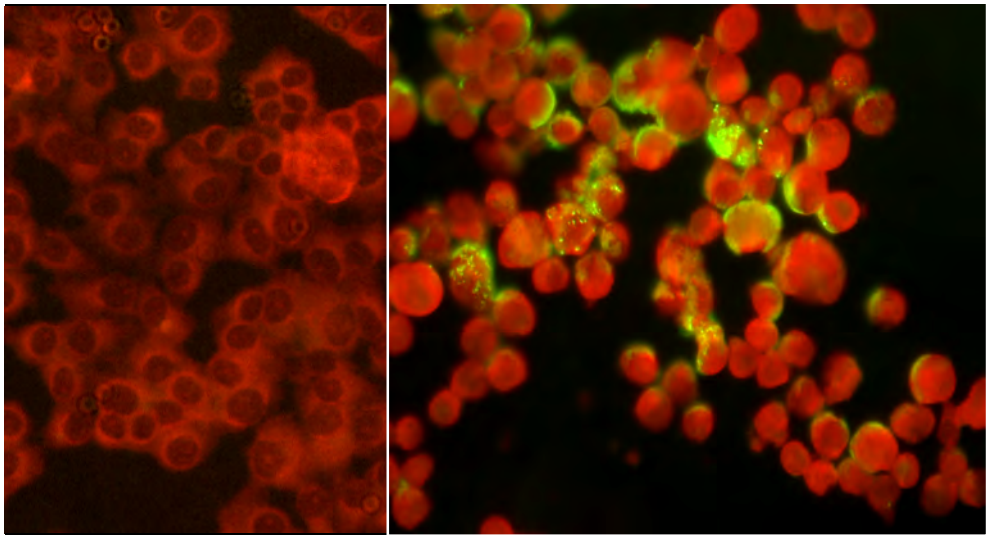

Figure 12: Images of IFTs. Left picture: negative sample with red colored Vero-76 cell nuclei stained with Evans blue. Right picture: positive sample with bright green specific fluorescence (dots in cytoplasma and between cells).

4 Sequence analysis

Successfully amplified *ompB* PCR amplificates were processed to sequencing. Sequence analyses resulted in five *R. felis* and two *R. helvetica ompB* nucleotide sequences that were 100% identical among each other and to the respective type species deposited in GenBank. Sequences were submitted to Genbank, with accession numbers GU324464-GU324470. Sequence alignments are presented in Annex 2 (p. 96). A phylogenetic tree shows clusters and relationships between own nucleotide sequences and those deposited in GenBank (Figure 13).

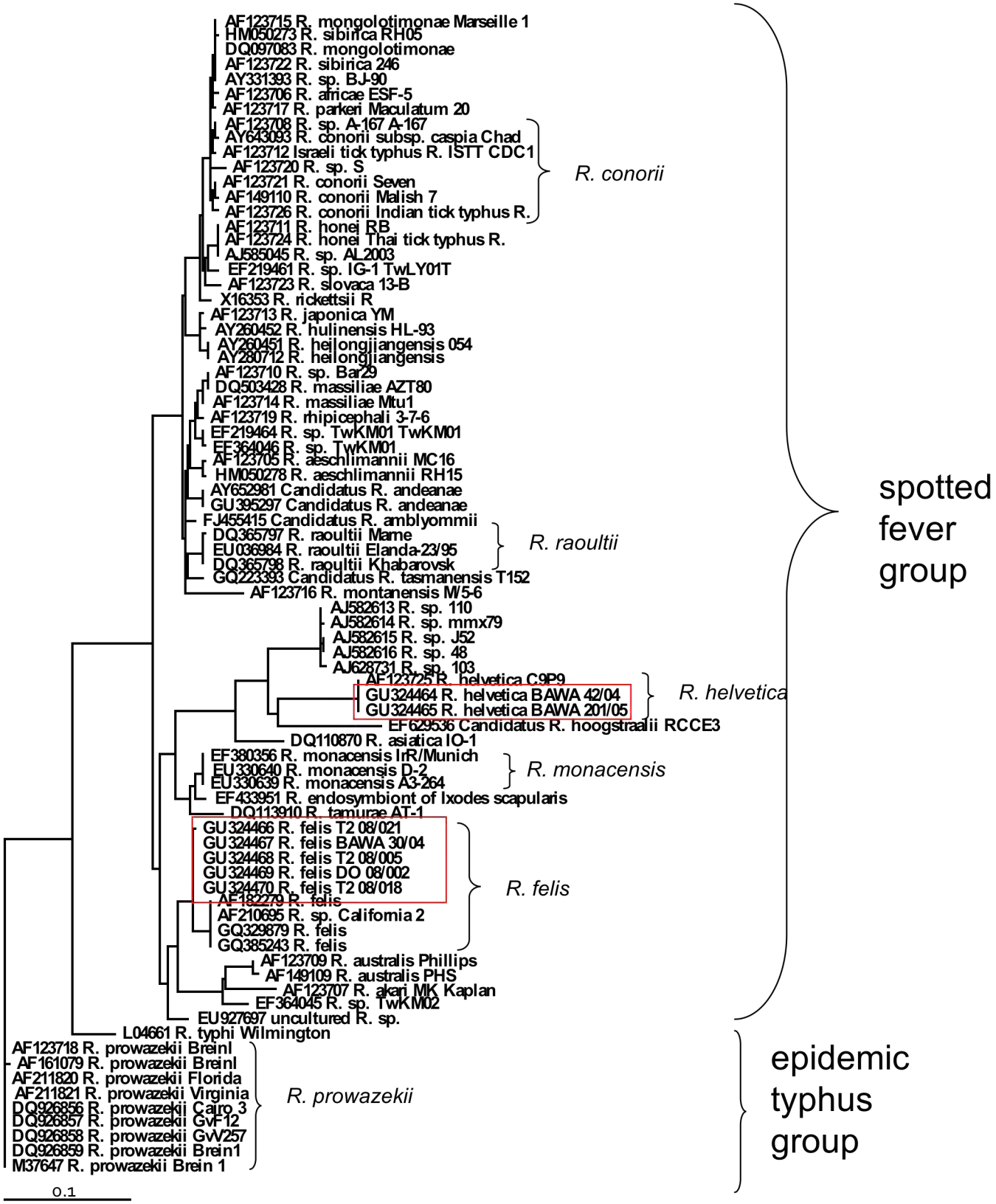

Figure 13: Phylogenetic tree, own sequences highlighted red. Clusters are designated as in Dobler & Woelfel, 2009

5 Publication

The results of both immunofluorescence tests, gltA real time PCR and *ompB* PCRs and statistical analyses as well as sequencing results were organised in a peer-reviewed publication. Further, the results of investigations regarding the comparability of sera and body fluids (transudates) were presented there. The publication was submitted to „Vector Borne Zoonotic Diseases“ and accepted in July 2010. It is available online since October 6, 2010 (Epub ahead of print).

Rickettsia spp. in Wild Small Mammals in Lower Bavaria, South-Eastern Germany

Susanne Schex,[1] Gerhard Dobler,[1] Julia Riehm,[1] Jörg Müller,[2] and Sandra Essbauer[1]

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 00, Number 00, 2010
[a] Mary Ann Liebert, Inc.
DOI: 10.1089/vbz.2010.0060

[1] Bundeswehr Institute of Microbiology, Munich, Germany.
[2] Nationalparkverwaltung Bayrischer Wald, Grafenau, Germany.

PDF available from:
http://www.liebertonline.com/doi/abs/10.1089/vbz.2010.0060

Rickettsia spp. in Wild Small Mammals in Lower Bavaria, South-Eastern Germany

Susanne Schex,[1] Gerhard Dobler,[1] Julia Riehm,[1] Jörg Müller,[2] and Sandra Essbauer[1]

1 Bundeswehr Institute of Microbiology, Munich, Germany.

2 Nationalparkverwaltung Bayrischer Wald, Grafenau, Germany.

Abstract

So far, data on the natural cycle of rickettsiae of the tick-borne spotted fever group (SFG) in Central Europe are barely available. Some studies showed the occurrence of different *Rickettsia* species in their arthropod vectors, but it is unclear which animals might have any kind of reservoir function. This survey was therefore set up to provide information on the occurrence of SFG rickettsiae in small mammals in Germany. A total of 124 rodents and insectivores were collected over a period of 3 years in Lower Bavaria, South-Eastern Germany. Screening for *Rickettsia* antibodies was performed using immunofluorescence with *Rickettsia conorii* and *R. helvetica* slides, and the comparability of sera and body fluids (transudates) was investigated in these assays. Further, real-time polymerase chain reaction (PCR) was used for screening of Rickettsial DNA in rodents and insectivores. Ear versus liver tissue was compared to evaluate the more suitable tissue for detection of specific DNA. Further, a new PCR targeting the 18S ribosomal nucleic acid was established as internal control. The results indicated that transudates are a sufficient alternative to proof infection in cases where no sera are available. Rickettsial DNA, that is, *Rickettsia felis* and *R. helvetica*, was found in seven animals with the ears proving to be a proper choice for PCR. Statistical analyses revealed that the presence of ectoparasites and the body size positively correlated with the occurrence of rickettsial DNA. Overall, our study suggests that rodents and other small mammals may act as reservoir hosts for Rickettsia. However, with the course of infection and its transmission in wild animals still unknown, further investigations

are needed to better understand the natural cycle of SFG rickettsiae.

Key Words: Ear—*ompB*—Reservoir—*Rickettsia*—Rodent—Serology.

Introduction

Rickettsiae are recognized as emerging infections in several parts of the world (Parola et al. 2005). The obligate intracellular bacteria are transmitted to humans and animals by blood-sucking arthropods such as fleas, ticks, and mites. The geographical distribution of *Rickettsia* species depends on the occurrence of their specific transmitting arthropod vector. In Europe, several *Rickettsia* species are endemic, such as *R. typhi, R. conorii, R. felis, R. helvetica, R. massiliae, R. sibirica, R. monacensis* and *R. slovaca* (Parola and Raoult 2001, Parola et al. 2005, Dobler and Wölfel 2009, Dobler et al. 2009). In Germany, however, the occurrence and geographical extension of rickettsial organisms is not known very well. In Southern Germany several studies found a widespread distribution of *R. helvetica* in *Ixodes ricinus* ticks (Pichon et al. 2006, Wölfel et al. 2006, Silaghi et al. 2008, Dobler et al. 2009). Investigation of more than 3500 ticks in Bavaria resulted in the detection of five *Rickettsia* species: *R. helvetica* (prevalence 4.8%), *R. felis* (0.4%), *R. monacensis* (0.6%), *R. sp. RpA4* (21%), and *R. massiliae* (1.7%) (Wölfel et al. 2006, Dobler and Wölfel 2009). All of these studies investigated the presence of *Rickettsia* spp. in their arthropod vectors, but only few data are available on the natural cycle of rickettsiae of the tick-borne spotted fever group (SFG) in Central Europe. To sustain a successful life cycle it is likely that wild animals act as natural reservoirs for *Rickettsia*. Up to now this fundamental part of life cycle of *Rickettsia* spp. is quite unclear, as—according to the literature—there are only very few studies focusing on rickettsiae in rodents and other small mammals in Central Europe to this date (Adjemian et al. 2008, Barandika et al. 2007, Spitalská et al. 2008). We report a screening of free-ranging small mammals for the presence of *Rickettsia* by serological and molecular biological techniques. To the best of our knowledge this is the first report of *Rickettsia* found in small mammals in Germany. In specific, this study had two major aims. First, we aimed to test the detectability of *Rickettsia* DNA in liver versus ears. In a previous study in South Germany we found that rodent ears seem to be an

adequate tissue to detect multiple *Borrelia* species in rodents (Essbauer and Kiessling, unpublished data) and tried these for the first time for *Rickettsia* screening. Second, we aimed to analyze several predictors for a positive detection of *Rickettsia* DNA in wild rodents. Due to the character as major vectors we hypothesized (1) a positive influence of ectoparasites on a positive detection of *Rickettsia*. Further, we hypothesized (2) that body size has a positive influence, because as higher the age (body size), the longer the susceptibility of an animal. Finally, we also hypothesized (3) that elevation of trapping site has a negative effect, because the activity and density of the major vector ticks might decrease with temperature along the elevation gradient.

Materials and Methods

Trapping of small mammals

Rodents were collected using Sherman live traps in October 2004 and May and September 2005 at seven locations in the district of Lower Bavaria, including forests, pasture, black-berry bushes and farmland (Fig. 1). Sampling areas were chosen according to the occurrence of Hantavirus-induced *Nephropathia epidemica* cases and trapped by a decoying method as described in Essbauer et al. (2006) and Mertens et al. (2009). In September and October 2008, trapping was performed at a total of 22 sites ranging from 300 up to 1400 m altitude (a.s.l.) in the German part of the Bohemian Forest. Sixteen Sherman live traps were deployed in an 18 m^2 plot, one trap every 3 m for three consecutive nights, and were checked twice a day. Animals were anesthetized by CO_2 exposure and killed humanely according to the German Animal Protection Act, after blood was drawn by heart puncture. Trapping site, species, sex, reproductive and physical conditions, including parasitic load, location of ectoparasites and also ectoparasite species, were recorded for each rodent.

Preparation of tissue samples

Rodents were dissected under BSL-2 conditions. Tissue samples (heart, lung, liver, spleen, kidneys, and ears) were aseptically removed. Extracted

viscera were put into Lysing matrix tubes A (MP Biomedicals), mixed with MEM supplemented with 3% fetal calf serum (Biochrom AG), and homogenized with the Fast Prep 120 instrument (Biogene) with a power of 5.0 m/s for 30 s.

Serological investigations

Rodent blood was centrifuged at 285 g for 10 min at 4°C. The derived sera were stored at -40°C until use. Sera were preliminary diluted 1:20 in phosphate-buffered saline (PBS), as this dilution proved to be the most suitable with regard to specificity and sensitivity for screenings in preliminary tests. As there were blood samples missing from several mice that could not be captured alive, the heart of each animal was rinsed with 1 mL PBS, with the resulting suspension (referred to as transudate) tested additionally or instead where no serum was available.

Sera and transudates of wild mice were screened with an in-house immunofluorescence test for detection of immunoglobulin G (IgG) antibodies against *R. helvetica. R. helvetica* (strain AS 819, own unpublished strain) was propagated on Vero E6 cell lines for 27 days at 34°C. Infected cells were trypsinated and centrifuged at 2000 g for 5 min. The pellet was resuspended in PBS and 10 µL each spotted on 10-well antigen slides (Biomérieux). After air-drying, cells were fixed with a 50% methanol/50% acetone (1:1) solution. Further, *R. conorii*, which is phylogenetically distinct from *R. helvetica* in the SFG, was used for immunofluorescence tests. It is also crossreactive with *Rickettsia* from the SFG but not endemic in Germany. Slides coated with *R. conorii*–infected cells were obtained from a commercial supplier (*Rickettsia conorii* immunofluorescence assay [IFA] IgG Antibody Kit; Fuller Laboratories RCG-120).

Both *Rickettsia* IFA were conducted with a polyclonal rabbit anti-mouse serum IgG/fluorescein isothiocyanate as a conjugate (dilution 1:20; Dako) together with Evans blue counterstaining. As positive/negative control served the controls provided by the commercial available kit as well as formerly positive tested mouse sera and PBS, respectively, were used. Slides were read by two independent examiners using a fluorescent

microscope.

Nucleic acid isolation

Nucleic acids (NA) were extracted using the QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer's instructions. This test was used because of investigations regarding RNA–viruses conducted in parallel. Moreover, it is comparable with other standard DNA extraction kits regarding the quality of the extracted NA.
For extraction of rickettsial DNA from ears, one ear per mouse in the aggregate was placed into a 1.5 mL Eppendorf tube and digested using the QIAamp DNA Mini Kit (Qiagen) as described in the instruction manual. If not analyzed immediately, the extracted NA were stored at -20°C.

Polymerase chain reactions

Rickettsial polymerase chain reactions.

A real-time (RT) polymerase chain reaction (PCR) targeting citrate synthase (*gltA*) was performed using the LightCycler FastStart DNA Master HybProbe System (Roche) for LightCycler 1.5 following the protocol published by Wölfel et al. (2008). An uracil–DNA–glycosylase (UDG) incubation step was added to prevent the re-amplification of carryover PCR products between reactions. Briefly, 0.5 U UDG Mix (Roche) was added to each reaction, and the protocol was modified including a preincubation step for UDG digest at 40°C for 10 min.
A conventional PCR was performed, targeting the partial outer membrane protein B (*ompB*) by following the protocol published by Roux and Raoult (2000). The protocol was then modified with annealing steps. Temperatures were gradually reduced in two-degree steps from 60°C to 50°C (touchdown). For this, Platinum Taq Polymerase High Fidelity (Invitrogen) was used, adding 5 µL of DNA to each reaction.

Mammalia species-specific PCRs.

To investigate if there was an inhibition of the PCR (internal control), we established a universal mammalian-specific 18S ribosomal RNA gene PCR ahead of *Rickettsia*-specific PCR amplification. For choice of

sequences, different mammalian 18S rRNA sequences were obtained from the GenBank (Table 1), and aligned by ClustalW2 (Embl-EBI, www.ebi.ac.uk) (Larkin et al. 2007). The actual primer design was carried out by Gene Runner (Version 3.05; Hastings Software, Inc.) resulting in the primers Univ18S-f1 5'-ATTAGAGTGTTCAAAGCAGGC-3' and Univ18S-r1 5'-TGCTTTCGCTCTGGTCCGTC-3', used in a concentration of 0.4 µM per reaction. Additionally, 2 µL of NA was amplified from livers and ears, using LightCycler FastStart DNA Master Sybr Green System (Roche) in a final volume of 20 µL according to the manufacturer's instructions. An UDG incubation step was conducted before PCR cycling at 40°C for 10 min and activation at 95°C for 10 min. DNA was amplified in 45 cycles for 40 s at 95°C, 30 s at 56°C, and 30 s at 72°C. Finally, a melting curve analysis (i.e., 95°C, 65°C, and 95°C) and cooling process of 30 s at 40°C followed.

In 2004/2005, for 23.4% (n=19/81, Hantavirus-positive animals of a previous study), and in 2008, for all animals species determination was confirmed by both PCR and subsequent sequencing of partial mitochondrial cytochrom B-(*mt*CytB) gene (Essbauer et al. 2006).

Table 1. Sequences obtained from the Genbank for 18s-rRNA Polymerase Chain Reaction primer design

Species	**Common name**	**Genbank no.**
Mus musculus	Mouse	NR 003278
Rattus norvegicus	Rat	M11188
Homo sapiens	Human	K03432
Oryctolagus cuniculus	Rabbit	X06778
Cricetulus griseus	Rat like hamster	DQ235090.1
Equus caballus	Horse	AJ311673.1
Bos taurus	Cattle	DQ222453.1
Erinaceus europaeus	West European hedgehog	AJ311675.1
Monodelphis domestica	Gray short-tailed opossum	AJ311676.1
Didelphis virginiana	Virginia opossum	AJ311677.1

Analysis of PCR products.

OmpB and mt*CytB* PCR products were loaded on a 1.5% agarose gel and analyzed by UV illumination. Amplificates were purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced by the GATC sequencing service (Konstanz) using corresponding specific primers. Sequence and phylogenetic analyses were performed using BioEdit (Version 7.0.0,

Copyright ©1997–2004) (Hall 1999).

Statistical analysis

Data of trapped rodents were collected and evaluated with R 2.9.2 (R Development Core Team 2009). Serological assays were compared using concordance comparison and kappa-statistics. Descriptive statistic was performed for *ompB*-PCR-positive samples for body size (head-body length), body weight, and organ weights. The number of these PCR-positive samples, that is, samples that could be sequenced for *Rickettsia* species determination, was low and restricted to seven animals in two rodent species (*Myodes glareolus* and *Apodemus flavicollis*). Therefore, we restricted our final analysis to these seven animals in our data set comprising 106 animals of the two dominant species. We selected three predictors only, because several of our variables were intercorrelated (e.g., body size and body length). For the other variables, we restrained from a formal test and provided only descriptive statistic. In our final test model with *Rickettsia*-PCR positivity as dependent variable, we included in the following sequence the predictors: (1) as control variable the species, (2) occurrence of ectoparasites, (3) body size, and (4) elevation. We applied a sequential analysis of deviance table (Hastie and Pregibon 1992). That is, the reductions in the residual deviance as each term of the formula is added in turn are given in as the rows of a table, plus the residual deviances themselves. We first added the species to the model and then subsequently ectoparasites, body size, and elevation. Due to the binomial dispersion of our dependent variable *Rickettsia*-PCR positive, we applied the chi-squared test.

Results

In 2004/2005 a total of 81 animals were trapped in Lower Bavaria: 21 yellow-necked mice (*A. flavicollis*), 7 wood mice (*Apodemus sylvaticus*), 47 bank voles (*M. glareolus*), 4 brown voles (*Microtus agrestis*), 1 water vole (*Arvicola amphibius*), and 1 house mouse (*Mus musculus*).

In 2008, a total of 43 animals were trapped in the Bohemian Forest: 15 bank voles (*M. glareolus*), 23 yellow-necked mice (*A. flavicollis*), 4 brown

voles (*M. agrestis*), and 1 Eurasian pygmy shrew (*Sorex minutus*). Table 2 summarizes trapped animals and available samples for all serological and molecular *Rickettsia* assays.

Table 2. Summary of investigated rodents and samples available for *Rickettsia* assays

A Lower Bavaria 04/05

Species	number	Serum available	Transudate available	ear available
A. amphibius	1	0	1	1
A. flavicollis	21	7	18	21
A. sylvaticus	7	1	6	7
M. agrestis	4	1	4	3
M. musculus	1	0	1	1
M. glareolus	47	20	41	43
Sum	**81**	**29**	**71**	**76**
B Bohemian Forest 2008				
A. flavicollis	23	13	23	23
M. agrestis	4	2	4	4
M. glareolus	15	7	15	15
Sorex minutus	1	1	1	1
Sum	**43**	**23**	**43**	**43**

Rickettsia *serology*

Of the 2004/2005 micromammalia, 23 out of 71 (32%; no serum or transudate available for 10 animals [Table 3]; animals listed in Table 4) tested rodents showed serological evidence of a past infection with rickettsiae. Among these are 30% (n=12/40) bank voles (*M. glareolus*), 21% (n=4/19) yellow-necked mice (*A. flavicollis*), 66.7% (n=4/6) wood mice (*A. sylvaticus*), and 50% (n=2/4) field voles (*M. agrestis*). In summary, 18.3% (n=13/71) of the samples showed a reaction in the *R. conorii* IF assay and 21.1% (n=14/71) against *R. helvetica*. There were five animals that seroreacted in both assays (Tables 3 and 4).

From small mammals collected in 2008, immunofluorescence tests for antibodies against *R. conorii* revealed 20.9% (n=9/43) reactive sera/transudates. For animals where both serum and transudate were available for testing (n=21), body fluids were tested in several dilution steps. For the rest of samples (n=22), the investigation was refined to just transudate. Serum titers ranged from 1:20 to 1:160 (DO 08/009, *M. agrestis*), with transudates showing positive reactions, ranging from

undiluted up to 1:16. Two animals with reactive sera were tested negative for transudates and two tested positive. In comparison, reactivity in the *R. helvetica* immunofluorescence was found in 10 samples from 10 rodents (23.3%), with serum titers ranging from undiluted up to 1:320 (DO 08/009, *M. agrestis*).

To evaluate and compare the results of the *R. helvetica* and the *R. conorii* IFA, we counted negative and seroreactive samples that revealed the same reactivity in both assays (in summary, 52 sera and 65 transudates). Of 52 tested serum samples, 39 reacted concordantly in the *R. helvetica* as well as in the *R. conorii* assay. Therefore, we obtained a high concordance. Nevertheless, the kappa value was rather low (k=0.35) and the confidence interval rather broad (0.05 to 0.66), due to the low and unequally distributed sample numbers. For transudates, 57 of 65 tested samples were concordant (k=0.43, confidence interval 0.06 to 0.80). In conclusion, with both IF assays, comparable results for *Rickettsia* antibody detection seemed to be obtained. Details of the serological results are summarized in Tables 3 and 4.

PCR results

Of the 2004/2005 micromammalia, 5.2% (n=4/76) tested samples were found positive in RT-PCR, targeting rickettsial *gltA* (i.e., one *M. glareolus* and three *A. flavicollis*). In 2008, 5 out of 43 (11.6%) micromammalia showed amplification, including 1 *M. glareolus*, 1 *M. agrestis*, and 3 *A. flavicollis*. In all PCR-positive animals, the ear was found positive with CTs between 36 and 50. RT-PCR of DNA extracted from liver tissues (n=46) showed that 100% for the 2008 sampling and three ear-positive animals for the 2004/2005 sampling had no positive results. Two ear DNA samples with CT >40 in the *gltA*-RT-PCR revealed no amplificate in the *ompB*-PCR. For the other seven samples *Rickettsia* species was determined using *ompB*-PCR, followed by sequencing and resulted in five *R. felis* and two *R. helvetica. R. felis* was found in four *A. flavicollis* (three adult males and one adult female, 2008) and in one *M. glareolus* (adult male, 2004).

Table 3. Overview of results of gltA-Real-Time Polymerase Chain Reaction and Rickettsia serology in small mammal samples from South-East Germany

			*Serology (transsudates, sera if available)**			
	gltA-*RT-PCR*		Anti-*R. conorii*		Anti-*R. helvetica*	
Species	**Pos/total**	**%**	**Pos/total**	**%**	**Pos/total**	**%**
A Lower Bavaria 2004/05						
A. amphibius	0/1	0	0/1	0	0/1	0
A. flavicollis	3/21	14.3	3/19	15.79	2/19	10.53
A. sylvaticus	0/7	0	2/6	33.33	4/6	66.66
M. agrestis	0/3	0	2/4	50.0	0/4	0
M. musculus	0/1	0	0/1	0	0/1	0
M. glareolus	1/43	2.3	6/40	15.0	9/40	22.5
Total	4/76	5.2	13/71	18.3	15/71	21.1
B Bohemian Forest 2008						
A. flavicollis	3/23	13.0	5/23	21.7	6/23	26.1
M. glareolus	1/15	6.7	3/15	20	3/15	20
M. agrestis	1/4	25.0	1/4	25	1/4	25
S. minutus	0/1	0	0/1	0	0/1	0
Total	5/43	11.6	9/43	20.9	10/43	23.3

**For detailed data of serological reactive sera of rodents, see Table 4.*
Pos, positive; RT-PCR, real-time polymerase chain reaction.

R. helvetica was found in one *A. flavicollis* and in one *M. glareolus* (both adult males, 2004/2005). Obtained *R. felis* or *R. helvetica ompB* nucleotide sequences were 100% identical among each other and to the respective type species deposited in GenBank. GenBank accession numbers are GU324464–GU324470. Results of the molecular biological screening of rodents are shown in Tables 3-5.

Ten percent of all DNA extracted from livers and ears were tested for signs of possible inhibition with the 18s rRNA Sybr Green PCR. Those 10% included animals of *M. glareolus*, *M. agrestis*, *A. flavicollis*, and *S. minutus*. As expected, all samples revealed no inhibition, proving that negative samples were not false negative.

Body conditions of PCR-positive animals

Descriptive statistics showed that there seem to be differences in body weight, body length, spleen, kidney, gonad, and liver weights for the seven partial *ompB*-sequenced animals (for details, see legend of Fig. 2), but not for seroreactive ones (data not shown).

Table 4: Overview of positive samples and comparison between gltA-rtPCR results, serum and transudate titers.

A) Lower Bavaria 2004/05				*Serology*			
			gltA-	Anti- *R. conorii*		Anti-*R. helvetica*	
Mouse No.	**Species**	**Sex**	rtPCR	**Serum**[a]	**Transudate**[b]	**Serum**[c]	**Transudate**[d]
BAWA 7/04	*A. sylvaticus*	m	neg	nt	neg	nt	1:8
BAWA 12/04	*A. flavicollis*	m	neg	neg	neg	1:80	neg
BAWA 16/04	*A. flavicollis*	f	neg	nt	undil	nt	neg
BAWA 18/04	*M. glareolus*	f	neg	neg	neg	1:80	undil
BAWA 19/04	*A. sylvaticus*	m	neg	neg	neg	1:20	neg
BAWA 20/04	*M. glareolus*	m	neg	neg	nt	1:20	nt
BAWA 26/04	*M. glareolus*	nd	neg	neg	neg	1:20	neg
BAWA 28/04	*A. flavicollis*	m	neg	1:20	neg	neg	neg
BAWA 30/04	*A. flavicollis*	m	pos	nt	neg	nt	neg
BAWA 37/04	*M. glareolus*	f	neg	neg	neg	1:20	neg
BAWA 42/04	*M. glareolus*	m	pos	nt	neg	nt	undil
BAWA 101/05	*M. agrestis*	m	neg	nt	undil	nt	neg
BAWA 151/05	*M. glareolus*	m	neg	nt	undil	nt	neg
BAWA 154/05	*M. glareolus*	f	neg	nt	1:8	nt	neg
BAWA 158/05	*A. flavicollis*	f	neg	nt	undil	nt	1:2
BAWA 159/05	*M. glareolus*	m	neg	nt	1:4	nt	1:4
BAWA 201/05	*A. flavicollis*	m	pos	nt	neg	nt	neg
BAWA 207/05	*M. glareolus*	f	neg	undil	undil	1:40	neg
BAWA 209/05	*A. sylvaticus*	f	neg	neg	1:2	neg	undil
BAWA 210/05	*A. flavicollis*	f	pos[e]	nt	neg	nt	neg
BAWA 211/05	*M. agrestis*	m	neg	undil	undil	neg	neg
BAWA 251/05	*M. glareolus*	f	neg	undil	neg	neg	neg
BAWA 258/05	*M. glareolus*	f	neg	nt	undil	nt	neg
BAWA 259/05	*A. sylvaticus*	f	neg	nt	1:4	nt	1:2
BAWA 261/05	*M. glareolus*	m	neg	neg	neg	1:80	undil
A) Bohemian Forest 2008				*Serology*			
			gltA-	Anti-*R. conorii*		Anti-*R. helvetica*	
Mouse No.	**Species**	**Sex**	rtPCR	**Serum**[f]	**Transudate**[g]	**Serum**[h]	**Transudate**[i]
T2 08/009	*A. flavicollis*	m	neg	nt	1:4	neg	neg
T2 08/018	*A. flavicollis*	f	pos	1:80	undil	1:40	neg
T2 08/021	*A. flavicollis*	m	pos	neg	neg	neg	neg
T2 08/014	*A. flavicollis*	f	neg	neg	neg	1:20	neg
T4 08/001	*A. flavicollis*	f	neg	1:80	undil	1:80	undil
DO 08/002	*A. flavicollis*	m	pos	neg	neg	neg	neg
DO 08/005	*A. flavicollis*	f (juv)	neg	neg	neg	1:20	neg
DO 08/008	*A. flavicollis*	m	neg	1:40	undil	1:40	neg
DO 08/009	*M. agrestis*	m	pos[e]	1:160	1:16	1:320	1:16
DO 08/001	*M.glareolus*	f	neg	neg	neg	1:20	neg
T2 08/005	*M.glareolus*	m	pos	neg	neg	neg	neg
T2 08/006	*M.glareolus*	f (juv)	neg	1:40	neg	undil	neg
T2 08/016	*M.glareolus*	m	neg	1:80	undil	1:40	undil
T4 08/010	*M. glareolus*	m	neg	nt	undil	nt	1:4

[a]13.8% (n=4/29*) of sera; [b]15.5% (n=11/71) of transudates; [c]32.0% (n=8/25*) of sera; [d]11.3% (n=8/71) of transudates; [e]no amplification in *ompB*-PCR possible; [f]27.3% (n=6/22) of sera; [g]16.3% (n=7/43) of transudates; [h]40.9% (n=9/22) of sera; [i]9.3% (n=4/43) of transudates; The asterisk (*) in the above footnotes indicates that numbers differ as for four sera there was not sufficient serum available for both assays. f, female; m, male; juv, juvenile; nt, not tested (no or insufficient serum); pos, positive; neg, negative; undil, undiluted serum; nd, not determined.

There seemed to be no difference in *Rickettsia* prevalence in *A. flavicollis* (n=44) and *M. glareolus* (n=58). After correcting for possible effects of species (Table 6), the parasites had a significant influence on the occurrence of *Rickettsia* (all of the *ompB*-PCR-positive animals carried ectoparasites). Beyond these effects even the body size as a surrogate for old animals was still significant (Table 6), while elevation had no additional effect on the occurrence of *Rickettsia*. Thereby, our analysis supports hypotheses 1 and 2, but hypothesis 3 has to be rejected.

Table 5: Summary of results of Rickettsia *ompB*-PCR and sequencing.

Mouse No.	Species, sex, age	Trapping site, altitude	*Rickettsia* species (partial *ompB*)
DO 08/002	*A. flavicollis*, male, adult	Grafenau, 510 m	*R. felis*
T2 08/005	*M. glareolus*, male, adult	Rachel, 949 m	*R. felis*
T2 08/018	*A. flavicollis*, female, adult	Rachel, 949 m	*R. felis*
T2 08/021	*A. flavicollis*, male, adult	Rachel, 827 m	*R. felis*
BAWA 30/04	*A. flavicollis*, male, adult	Glashütte, 804 m	*R. felis*
BAWA 42/04	*M. glareolus*, male, adult	Falkenstein, 701 m	*R. helvetica*
BAWA 201/05	*A. flavicollis*, male, adult	Regen, 532 m	*R. helvetica*
DO 08/009	*M. agrestis,* male, adult	Isar, 317 m	n.a.
BAWA 210/05	*A. flavicollis,* female, adult	Raimundsreuth, 804 m	n.a.

n.a., no amplificate in *ompB*-PCR.

Discussion

In this project we used a method of live trapping that has been widely field-tested in the last years to investigate rodent-associated pathogens (Ulrich et al. 2008, 2009). Aim of the study was to provide information on the occurrence of SFG rickettsiae in small mammals in southern Germany. To compare serum and transudates, we used two different immunofluorescence assays, one for *R. conorii* and one for the detection of *R. helvetica*. Antibody reactivity to the rickettsial antigen is group specific. A marked crossreactivity is seen in the IFA procedure between members of the SFG (e.g., *R. conorii*, *R. rickettsii*, *R. felis*, and *R. helvetica*) (Fuller Laboratories, *R. conorii* IFA Antibody Kit Instruction manual). Therefore, the commercial available *R. conorii* assay was used, although *R. conorii* itself is not expected to occur in Germany. However, the phylogenetic relation between *R. conorii* and *R. helvetica* is quite distant; hence, detection of both antibodies with only one assay could not be assured (Roux and Raoult 2000). We therefore conducted a specific *R.*

helvetica assay.

Twelve of 41 reactive sera/transudates (29.3%) reacted in both assays (Table 4). There was a small difference for the rodent samples between *R. helvetica* and *R. conorii* slides for IFA for 2008. Therefore, IFA screenings are a useful tool to assess past infections; however, they are not sufficient enough to differentiate *Rickettsia* species, offering future research potential.

So far nothing is known on *Rickettsia* IFA cut-off or even possible *Rickettsia*-neutralizing antibodies in wildlife rodent samples. Usually, screening of wild rodents is started with undiluted sera/transudates and after a specific reactivity a titration of sera/transudates is performed. We had at least four samples in 2004/2005 and six samples in 2008 with titers ≥1:40. Further, the comparison of antibody titers showed that in 9 out of 33 reactive samples both serum and transudate reacted (Table 4), that is, also confirming a specific reaction in IFA for low titers. Samples collected in 2008 showed higher reactivity and accordance than the 2004/2005 samples, which might be due to several freeze–thaw cycles of the older samples. Other studies showed the comparability of body fluids to serum in serological investigations: for the detection of antibodies against Trichinellosis in two enzyme immunoassays, tissue fluid of slaughtered swine showed equally effective as both blood and serum (Gamble and Patrascu 1996). Muscle transudate samples of pigs were also suitable in anti-*PRRSV* (porcine reproductive and respiratory syndrome virus) ELISA and IFAT tests (Molina et al. 2008). Moreover, the persistence of antibodies against *Microsporum canis* in decaying fox carcasses implied a positive serological result from blood or body fluid (Tryland et al. 2006). In rodents the comparability of serum and transudate was also shown for Hantavirus ELISA, Western blot, and IFA tests (Essbauer et al. 2006). However, more sampling should be conducted to further test this relationship. Finally, rinsed heart fluid was an acceptable alternative where no or only small amounts of serum are available. However, the best results are obtained with serum samples of live-captured animals.

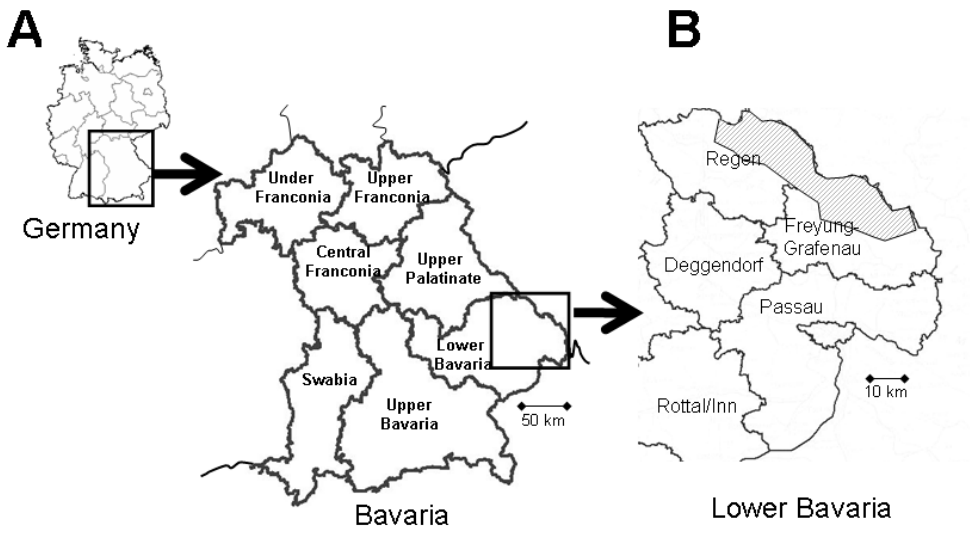

Figure 1: Geographic location of trapping sites in South-East Germany. **(A)** Schematic map of Germany (left) and Federal State of Bavaria (right). **(B)** Schematic map of administrative districts of Lower Bavaria. Shaded area shows most frequently used trapping sites, including the National Park Bohemian Forest.

Of seven PCR-positive samples (only the *ompB*-PCR-positive animals were considered securely positive), only two reacted in the IFAT for the detection of IgG antibodies. In 40 rodents, we found IgG antibody reactivity but in only two of these, rickettsial DNA. Other studies examining veterinary clinic cats infested with *R. felis*–infected cat fleas (*Ctenocephalides felis*) with and without fever were not able to amplify *R. felis* DNA in whole blood samples but found antibodies against *R. felis* (Hawley et al. 2007, Bayliss et al. 2009). Kidd et al. (2008) found *R. rickettsii* and *R. conorii* DNA in naturally and experimentally infected dogs, in some of them before seroconversion. Specific IgG antibodies are known to appear after 7 days the earliest, mostly after the onset of symptoms, and show a possible past infection. Cats that were exposed to fleas infected with *R. felis* seroconverted by 4 months post-exposure (Wedincamp and Foil 2000). Dogs experimentally infected with *R. rickettsii* showed an IgM antibody response between days 9 and 80 postinfection, and an IgG antibody response starting between days 22 and 28 postinfection and lasting until at least day 130 postinfection (Breitschwerdt et al. 1990). It is still unknown what amounts of rickettsiae are required for a vertebrate host to create a strong antibody response. As the time frame where bacteremia and antibody response overlap is usually quite short,

and taking more than one single blood sample per mouse is not possible for our study design, more samples of wild rodents are necessary to further evaluate these findings and to possibly find more animals where both antibodies and rickettsial DNA can be detected. Our findings raise questions about the duration of a rickettsial infection and whether it is persistent in rodents or not. This again queries whether mammals can be a reservoir host.

This study is ground breaking in that we find hints that small animals acted as reservoir hosts for *Rickettsia* as known for other pathogens like *Borrelia* spp. (Piesman and Gern 2004), *Bartonella* spp., or tick-borne encephalitis virus. Some studies found rickettsiae in arthropods collected from small mammals (Chen et al. 2002, De Sousa et al. 2006). However, there are only very few studies regarding rickettsiae in wild animals. In the Sierra Nevada one golden-mantled ground squirrel was found PCR positive for *R. rickettsii* (Adjemian et al. 2008), and in California three opossums were found positive for rickettsiae (Williams et al. 1992). In blood of rodents from Peru 2 out of 24 (8.3%) were found PCR positive for *R. felis* (Richards, personal communication). In our study sequencing of amplified *ompB* products revealed *Rickettsia* spp. infections in seven rodents. Two of them were *R. helvetica*, which were already described in ticks collected from rodents (Spitalská et al. 2008) and are known to be endemic in southern Germany (Pichon et al. 2006, Wölfel et al. 2006, Silaghi et al. 2008). Five of them belong to *R. felis*, a species that so far seemed to involve particularly cats and several flea species in its natural cycle (Reif and Macaluso 2009).

We herein also for the first time compare the suitability of wild rodent liver versus ear tissues for the detection of rickettsial DNA. Rickettsial DNA was detected in seven samples of rodent ears, whereas in no liver sample. Williams et al. (1992) found Rickettsial DNA in spleens of opossums. In another study DNA was taken from whole blood samples with 1 positive sample out of 89 (Adjemian et al. 2008). In a Spanish investigation, pools of tissues and ears were tested by multiplex PCR for evidence of SFG rickettsiae, with no positive result (Barandika et al. 2007). As we did not collect EDTA blood, we tried to test DNA isolated from blood cell pellets in serum separating tubes of the 43 animals trapped in 2008. However, we

did not get any positive PCR signal in these blood samples.

Our results suggest that ear tissue was more sensitive than liver tissue. A reason for that might be the sequestration of the Rickettsia organism in extremities of the body with a lower temperature like ears or tailtip. The optimum growth temperature for Rickettsia in vitro was shown to be at 32°C (Pornwiroon et al. 2006), which might explain the good growth in body extremities where also the immune response may be less effective. Another reason might be a more local infection without rickettsemia, a hint for that would be that ticks were often found in the ear region.

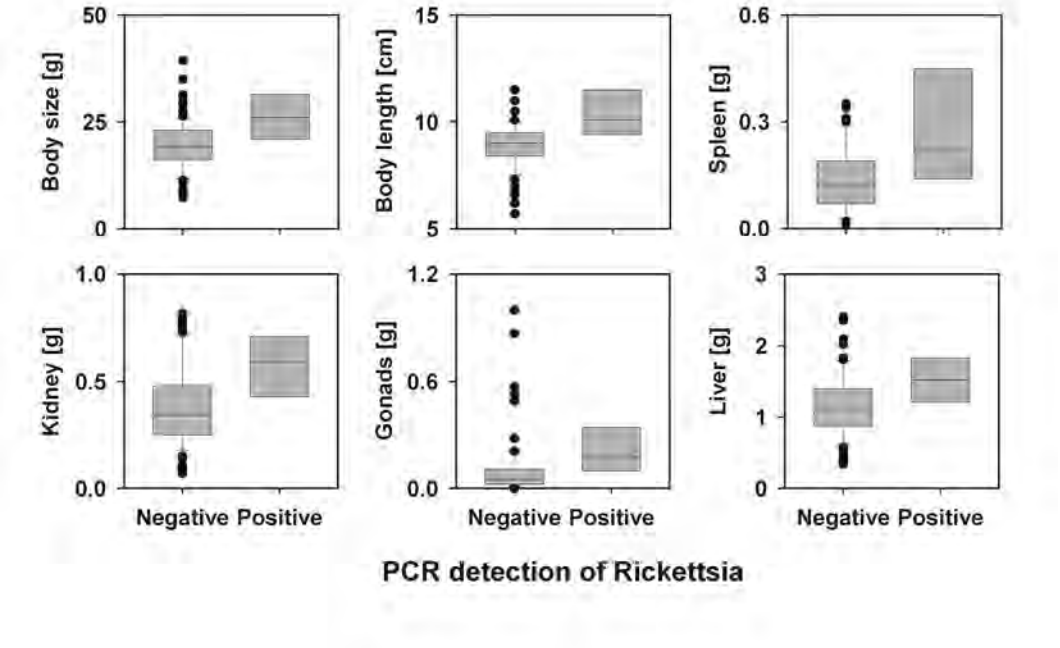

Figure 2: Box plots. Differences of mean body weight, mean body length, mean liver, spleen, kidney, and gonad weights to *ompB*-positive polymerase chain reaction (PC R) results. *OmpB*-PCR-positive animals (n=7) seem to have higher mean body size (26.4±5 g vs. 19.7±6.1 g) and length (11.3±3.6 cm vs. 8.6±1.2 cm), and higher liver (1.55±0.34 g vs. 1.15±0.44 g), spleen (0.27±0.15 g vs. 0.15±0.11 g), kidney (0.57±0.16 vs. 0.38±0.19 g), and gonad weights (0.22±0.15 g vs. 0.11±0.18 g) than PCR-negative animals (n=99).

We developed a new tool for internal control of tissue from wild mammals. Others describe the use of standard house-keeping genes like *GAPDH*, β-actin, β_2-microglobulin, cytochrom B, and as well rRNA (Livak and Schmittgen 2001). Selvey et al. (2001) reported that 18S rRNA expression offered superior consistency to that of β-actin under most circumstances and state that its expression appears to be a consistent measure of total

RNA in a sample throughout the cell cycle.

There are only very few studies that analyze data on rodents and their associated pathogens statistically as it was done for Cowpox virus infection by Essbauer et al. (2009). However, statistical analyses are useful to comprehend interrelations between infection courses of (reservoir-) hosts and ecological aspects. No formal sample size estimation was performed before the experiments, because our sampling of mice in a bad season was not evaluable and no literature about the expected effect was available. However, the sample size for the logistic model analysis is reasonable large to test the two parameters of interest and, obviously, the power was large enough to reject the hypothesis. As *Rickettsia* are transmitted by arthropods such as ticks, fleas, and mites, the ectoparasitic infestation of animals was of special interest. We quite often found *Ixodes* ticks (nymphs and larvae) on the investigated small mammals. Their preferred body spots seemed to be the ears, the chin, and the tail base, presumably because of the lack of fur there (data not shown). Fleas found consisted of five different species, mostly *Ctenophthalmus agyrtes*. It is conceivable that there was a correlation between *Rickettsia*-positive animals and their parasitic load.

There seemed to be a difference between body length, weight, and several organ weights. PCR-positive animals were heavier and taller, and their organs were usually heavier, which probably is a hint for mainly adult animals being infected. The older the animal, the more probable is an exposure to *Rickettsia* and *Rickettsia*-transmitting vectors. Differences in organ weights are also interesting, regarding the question whether infection with *Rickettsia* has clinical effects on small mammals. Clinical symptoms have been reported in dogs, for example, after infection with *R. rickettsii*, but not confirmedly in cats or small mammals. Infection with *R. felis* occurs in humans but has not been confirmed by isolation so far (Pérez-Osorio et al. 2008).

To summarize, we found that IF tests are a useful tool to investigate past infections with rickettsiae, but are unsuitable for further species differentiation. They can be used even with transudates, if there is no serum available, although results are less secure. We found two different rickettsial species, the more common *R. helvetica* (Dobler and Wölfel

2009) and *R. felis*, which has not been described in small mammals in Europe before. Rickettsial organisms can be detected best in mammals using ear tissue. The question whether rodents or other small mammals acted as reservoir hosts still needs to be answered. Although we found seroconverted animals and rickettsial DNA in their tissues, we do not know if there is a persistent infection and excretion. However, it is conceivable that infected mammals amplify the cycle by fleas feeding on their infected blood. For this reason, further research specific to this topic, including other rickettsial species endemic in Europe, is necessary.

Table 6: Results of sequentially generalized linear model with the binary dependent variable *Rickettsia ompB*-Polymerase Chain Reaction and four predictors.

Variable	**Deviance**	***p* value (chi-squared)**
Rodent species	2.852	0.091
Ectoparasites	6.460	**0.011**
Body size	4.938	**0.026**
Elevation	0.092	0.761

Significant *p*-values are shown in bold.

Acknowledgments

The authors gratefully acknowledge the excellent practical and technical assistance of H. Weber, R. Reiter, D. Eder, R. Terzioglu, D. Woll, and F. Goldberg. M. and D. Kiefer's support in species determination of fleas is highly appreciated. The authors also thank S. Speck for carefully reading the article, E. Seibold for help with statistical analysis, and H. Scholz for critical discussion. This project is supported by a grant from the Bavarian Ministry of Health, Vector-Borne Diseases in Climate Change Investigations (VICCI).

Disclosure Statement

No competing financial interests exist.

References

Adjemian, JD, Adjemian, MK, Foley, P, Chomel, BB, et al. Evidence of multiple zoonotic agents in a wild rodent community in the Eastern Sierra Nevada. J Wildl Dis 2008; 44:737–742.

Barandika, JF, Hurtado, A, García-Esteban, C, Gil, H, et al. Tick-borne

zoonotic bacteria in wild and domestic small mammals in Northern Spain. Appl Environ Microbiol 2007; 73:6166–6171.

Bayliss, DB, Morris, AK, Horta, MC, Labruna, MB, et al. Prevalence of *Rickettsia* species antibodies and *Rickettsia* species DNA in the blood of cats with and without fever. J Feline Med Surg 2009; 11:266–270.

Breitschwerdt, EB, Levy, MG, Davidson, MG, Walker, DH, et al. Kinetics of IgM and IgG responses to experimental and naturally acquired *Rickettsia rickettsii* infection in dogs. Am J Vet Res 1990; 51:1312–1316.

Chen, Z, Chen, M, Zhong, J, Bi, D. Using PCR/RFLP to detect spotted fever group rickettsia in ticks and rodents collected in Ninghua, Fujian province. Zhonghua Yu Fang Yi Xue Za Zhi 2002; 36:106–108.

De Sousa, R, Edouard-Fournier, P, Santos-Silva, M, Amaro, F, et al. Molecular detection of *R. felis, R. typhi*, and two Genotypes closely related to *Barthonella elizabethae*. Am J Trop Med Hyg 2006; 75:727–731.

Dobler, G, Essbauer, S, Wölfel, R. Isolation and preliminary characterisation of ‘‘*Rickettsia monacensis*’’ in south-eastern Germany. Clin Microbiol Infect 2009; 15 Suppl 2: 263–264.

Dobler, G, Wölfel, R. Typhus and other rickettsioses. Emerging infections in Germany. Dtsch Arztebl Int 2009; 106: 348–354.

Essbauer, S, Hartnack, S, Misztela, K, Kiessling-Tsalos, J, et al. Patterns of orthopox virus wild rodent hosts in South Germany. Vector Borne Zoonot Dis 2009; 9:301–311.

Essbauer, S, Schmidt, J, Conraths, FJ, Friedrich, R, et al. A new Puumala Hantavirus subtype in rodents associated with an outbreak of *Nephropathia epidemica* in South-East Germany in 2004. Epidemiol Infect 2006; 134:1333–1344.

Gamble, HR, Patrascu, IV. Whole blood, serum, and tissue fluids in an enzyme immunoassay for swine Trichinellosis. J Food Prot 1996; 59:1213–1217.

Hall, TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999; 41:95–98.

Hastie, TJ, Pregibon, D. Generalized linear models. In: Chambers, JM,

Hastie, TJ, eds. *Statistical Models* in S. Wadsworth & Brooks/Cole, Pacific Grove, CA 1992 , 6:195-2481992

Hawley, JR, Shaw, SE, Lappin, MR. Prevalence of *Rickettsia felis* DNA in the blood of cats and their fleas in the United States. J Feline Med Surg 2007; 9:258–262.

Kidd, L, Maggi, R, Diniz, PP, Hegarty, B, et al. Evaluation of conventional and real-time PCR assays for detection and differentiation of Spotted Fever Group Rickettsia in dog blood. Vet Microbiol 2008; 22;129:294–303.

Larkin, MA, Blackshields, G, Brown, NP, Chenna, R, et al. ClustalW and ClustalX version 2. Bioinformatics 2007; 23:2947–2948.

Livak, KJ, Schmittgen, TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25:402–408.

Molina, RM, Chittick, W, Nelson, EA, Christopher-Hennings, J, et al. Diagnostic performance of assays for the detection of anti-Porcine reproductive and respiratory syndrome virus antibodies in serum and muscle transudate ("meat juice") based on samples collected under experimental conditions. J Vet Diagn Invest 2008; 20:735–743.

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

Parola, P, Raoult, D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 2001; 32:897–928. Erratum: Clin Infect Dis 33:749.

Pérez-Osorio, CE, Zavala-Velázquez, JE, Arias León, JJ, Zavala- Castro, JE. *Rickettsia felis* as emergent global threat for humans. Emerg Infect Dis 2008; 14:1019–1023.

Pichon, B, Kahl, O, Hammer, B, Gray, JS. Pathogens and host DNA in *Ixodes ricinus* nymphal ticks from a German forest. Vector Borne Zoonot Dis 2006; 6:382–387.

Piesman, J, Gern, L. Lyme borreliosis in Europe and North America. Parasitology 2004; 129 Suppl:S191–S220.

Pornwiroon, W, Pourciau, SS, Foil, LD, Macaluso, KR. *Rickettsia felis* from cat fleas: isolation and culture in a tick-derived cell line. Appl Environ Microbiol 2006; 72:5589–5595.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2009. ISBN 3-900051-07-0. Available at www.R-project.org.

Reif, KE, Macaluso, KR. Ecology of *Rickettsia felis*: a review. J Med Entomol 2009; 46:723–736.

Roux, V, Raoult, D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (ompB). Int J Syst Evol Microbiol 2000; 50 Pt 4:1449–1455.

Selvey, S, Thompson, EW, Matthaei, K, Lea, RA, et al. Beta-actin—an unsuitable internal control for RT-PCR. Mol Cell Probes 2001; 15:307–311.

Silaghi, C, Gilles, J, Höhle, M, Pradel, I, et al. Prevalence of spotted fever group rickettsiae in *Ixodes ricinus* (Acari: Ixodidae) in southern Germany. J Med Entomol 2008; 45:948–955.

Spitalská, E, Boldis, V, Kostanová, Z, Kocianová, E et al. Incidence of various tick-borne microorganisms in rodents and ticks of central Slovakia. Acta Virol 2008; 52: 175–179.

Tryland, M, Handeland, K, Bratberg, AM, Solbakk, IT, et al. Persistence of antibodies in blood and body fluids in decaying fox carcasses, as exemplified by antibodies against *Microsporum canis*. Acta Vet Scand 2006; 48:10.

Ulrich, RG, Heckel, G, Pelz, HJ, Wieler, LH, et al. Rodents and rodent associated disease vectors: the network of ‘‘rodent carrying pathogens’’ introduces itself [German]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 2009; 52:352–369.

Ulrich, RG, Schmidt-Chanasit, J, Schlegel, M, Jacob, J, et al. Network „Rodent-borne pathogens“ in Germany: longitudinal studies on the geographical distribution and prevalence of Hantavirus infections. Parasitol Res 2008; 103 Suppl 1:S121– S129.

Wedincamp J, Jr., Foil, LD. Infection and seroconversion of cats exposed to cat fleas (*Ctenocephalides felis* Bouché) infected with Rickettsia felis. J Vector Ecol 2000; 25:123–126.

Williams, SG, Sacci JB, Jr., Schriefer, ME, Andersen, EM, et al. Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. J Clin Microbiol 1992; 30:1758–1762.

Wölfel, R, Essbauer, S, Dobler, G. Diagnostics of tick-borne rickettsioses in Germany: a modern concept for a neglected disease. Int J Med Microbiol 2008; 298 S1:368–374.

Wölfel, R, Terzioglu, R, Kiessling, J, Wilhelm, S, et al. *Rickettsia* spp. in *Ixodes ricinus* ticks in Bavaria, Germany. Ann N Y Acad Sci 2006; 1078:509–511.

Address correspondence to:
Sandra Essbauer
Bundeswehr Institute of Microbiology
Neuherbergstr. 11
D-80937 Munich
Germany
E-mail: sandraessbauer@bundeswehr.org

V. DISCUSSION

In the present study the occurence of SFG rickettsiae in rodents, their prevalence and genetic diversity was investigated by means of serologic tests and PCRs, with the major aim to brighten the question if there is a rodent reservoir for SFG rickettsiae in Germany. For this purpose, rodent blood as well as tissue samples were collected over a period of three years in the region of Lower Bavaria, in the year 2008 restricted to the National Park Bavarian Forest.

In this study, we tried to find statistically significant factors in sero- or PCR-positive individuals. Only few studies attempted to find a correlation between pathogen infestation and body condition, as for example for rodents carrying orthopox viruses (Essbauer et al., 2009a). Statistic tests such as chi-square test, student t-test, Mann-Whitney test or Spearman rank correlation were only used for preliminary screenings, since a Bonferroni correction and a reduction of the significance level needed to be used in order to maintain the familywise error rate. Therefore, we present descriptive tests only. In addition, as many aspects of our serologic test assays are unknown so far, statistical analyses of those were not included in this thesis.

Seroprevalence for *Rickettsia* spp. of tested serum samples was about one third (n=32/114); approximately 8% (n=9/119) of samples showed positive reaction in PanRickettsia real-time PCR. For comparison, prevalence of SFG rickettsiae in ticks in Southern Germany has been shown to vary between 3.5 to 6.2% for *R. helvetica* (Woelfel et al., 2006; Hartelt et al., 2004), 30.3% for *R. raoultii*, 0.75% for *R. slovaca* (Pluta et al., 2010), 1.7% for *R. massiliae*, 0.4% for *R. felis* and 0.6% for *R. monacensis* (Dobler & Woelfel, 2009). Interestingly, the results obtained from rodents in this study are considerably higher. At least for *R. felis* and *R. helvetica*, it could be concluded that rodent tissue is a promising tool for detection of Rickettsia, since the prevalence in ticks seems to be lower than in rodents. As rickettsiae are transmitted by arthropods, it is not surprising that infestation with parasites had a significant influence on the

occurrence of Rickettsia (all of the *ompB*-PCR-positive animals carried ectoparasites). In contrast to this, elevation had no additional effect on the occurrence of Rickettsia, presumably due to the fact that ectoparasites were found on rodents of every altitude. Detailed ectoparasitic investigations will be part of future studies.

As shown in Figure 9 (p. 32), we tried to find patterns of species distribution along the altitude gradient. However, trapped species apparently were distributed quite randomly, apart from *Sorex* spp., which could only be found in the highest trapping location. Presumably, this is correlated to habitat factors rather than to elevation itself. Following longitudinal studies will further investigate these aspects.

The phylogenetic tree (Figure 13, p. 36) shows the close relationship between our *R. helvetica* sequence and the *R. helvetica* sequence obtained by Roux and Raoult (2000). However, both are quite distinct to other Rickettsia species. The *R. felis* sequence obtained in this study clusters with *R. felis* sequences obtained in Australia and the USA.

Determination of host-pathogen dynamics and of possible reservoir hosts has always been one of the foci of rickettsial research. Ticks were considered not only as vectors but also as maintaining hosts. For *R. conorii conorii*, the causative agent of Mediterranean spotted fever (MSF), *Rhipicephalus sanguineus*, the brown dog tick, was thought to act as vector and reservoir (Blanc & Caminopetros, 1932) due to transovarial transmission. However, infection rates of *Rh. sanguineus* with *R. conorii conorii* were shown to be less than 15% (Raoult et al., 1993). *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever (RMSF) is transmitted by several tick species, and maintained in infected ticks by transovarial and transstadial transmission (Macaluso & Azad, 2005). However, Ricketts (1911) observed that less than 50% of experimentally infected *D. andersoni* female ticks transmitted *R. rickettsii* to their offspring, and Price (1954) found a transovarial transmission rate of only 30% in *D. variabilis* ticks, whereas Burgdorfer et al. (1967, 1975) observed 100% filial infection in *D. andersoni* and *D. variabilis* that were naturally infected. Considering these possible low passage rates it is, therefore, not very probable that ovarial passage is sufficient to maintain the natural cycles of *R. rickettsii*

and *R. conorii*. Furthermore, *R. rickettsii* and its acarine hosts do not seem to have established a fully effective symbiotic relationship, as some tick infections are incomplete, others can result in decreased fecundity, viability or even death of the tick (McDade & Newhouse, 1986).

Rickett's hypothesis of an additional maintenance of *R. rickettsii* seems to be far more reasonable. Vertebrate hosts provide a source for simultaneous feeding of ticks (infected and uninfected), serve as vehicles, are numerous, support a large number of ticks and – in case of small mammals – reproduce rapidly. Thus they offer many survival advantages for the rickettsial organism (McDade & Newhouse, 1986).

Furthermore, *Rickettsia* spp. have a restricted geographic range when compared to the transmitting ticks. Although the tick can be found worldwide, *R. conorii conorii* is endemic in much smaller regions. The same is true for the transmission of *R. rickettsii* by *Rh. sanguineus* in Arizona (Demma et al., 2005). One explanation might be that transovarial transmission may occur for a limited number of passages only and that the tick may only be the vector of the pathogen (Rovery et al., 2008). On the other hand other factors such as co-feeding on a vertebrate or maintenance in a rickettsemic vertebrate may play an essential role in the transmission cycle.

For *R. conorii conorii*, several mammals are proposed to act as reservoir hosts. Firstly, dogs serve as transport hosts as they carry ticks close to humans and can suffer from febrile illness related to *R. conorii conorii* infection (Solano-Gallego et al., 2006). Moreover, in certain zones of southern Europe, a correlation between the percentage of the canine population with antibodies to *R. conorii conorii* and the incidence of MSF in humans has been found (Herrero-Herrero, 1989). However, dogs are transient reservoirs due to a transient post-infection rickettsemia; and therefore do not seem to be an efficient reservoir for *R. conorii conorii* (Rovery et al., 2008). Secondly, antibodies to *R. conorii conorii* were found in wild rabbits and hares with prevalences up to 76.5% (Ruiz Beltran et al., 1992). Furthermore, Le Gac (1966) reported a sudden decrease in MSF cases on the French Mediterranean coast 1952 simultaneous with a lethal myxomatose outbreak that eradicated the local wild rabbit population. A

reemergence of this pathogen was also correlated with the reappearence of wild rabbits in 1967 (Rovery et al., 2008). Lastly, hedgehogs and other small rodents are also potential candidates for the reservoir of *R. conorii* since antibodies against rickettsiae have been detected in serum samples of these animals (Le Gac, 1966).

Antibodies to *R. rickettsii* were found in at least 53 species, belonging to the genera *Marsupialia, Insectivora, Rodentia, Carnivora* and *Artiodactyla* (McDade & Newhouse, 1986). In addition, *R. rickettsii* was isolated from *Microtus pennsylvanicus* (Gould & Miesse, 1954) and other small mammals such as rabbits, opossums, squirrels, voles and mice (Burgdorfer, 1977), suggesting that a broad variety of vertebrates are involved in the natural cycle of *R. rickettsii*.

In general, a natural cycle starts with the development of eggs from an infected female tick into infected larvae. These can then infect small rodents when feeding on them. After that, larvae develop into nymphs, these feed on medium-sized animals and thus can infect them too. Feeding on an infected animal can infect ticks of every stadium, although this depends on the magnitude and duration of its rickettsemia. The minimum level of required rickettsemia to infect 50% of *D. andersoni* larvae ranged between 10 and 100 guinea pig infectious doses (GPID) per 0.5 ml blood (Burgdorfer et al., 1966). Rickettsemia levels in turn depend on the susceptibility of the vertebrate. Some mammals turned out to be highly susceptible, such as Columbian ground squirrels, golden-mantled ground squirrels, chipmunks and meadow voles with GPIDs between 100 and 1000 for up to four days (Burgdorfer et al., 1966). On the other hand, less susceptible mammals such as bushy-tailed wood rats (Burgdorfer et al., 1966), cotton rats (Shirai et al., 1967) or dogs (Norment & Burgdorfer, 1984) develop much smaller or shorter rickettsemias or none at all. Rehácek et al. (1992) investigated the susceptibility of European rodent species (*M. glareolus, A. flavicollis, Mus musculus*) to rickettsiae (e.g. *Coxiella burnetii, R. conorii, R. slovaca* and *R. sibirica*) and found that for *R. conorii, R. slovaca* and *R. sibirica* a sharp increase of antibody levels, with high titres on days 4-6 and peak levels about day 11, was characteristic.

For *R. felis*, which is mainly transmitted by cat fleas, but was also detected in mites, no definitive mammalian host has been identified so far (Reif & Macaluso, 2009). To the authors knowledge this study is the first to suggest that rodents may also act as a reservoir host for *R. felis*. In addition to the results presented here, antibodies to *R. felis* were also found in fevrile and non-fevrile cats (Bayliss et al., 2009), dogs (Richter et al., 2002; Oteo et al., 2006), and opossums (Williams et al., 1992). Furthermore, in a laboratory experiment Wedincamp and Foil (2000) demonstrated that previously flea- and Rickettsia-naïve cats seroconverted within four months after infestation with *R. felis*-infected cat fleas. Although these studies show a horizontal transmission from flea to vertebrate, to the present moment, the transmission from infected vertebrate to arthropod has not yet been shown for *R. felis*. Nonetheless, Weinert et al. (2009) state that only occasional horizontal transmission from mammal to arthropod may be needed to maintain *R. felis* in nature.

For *R. helvetica*, the state of knowledge regarding a possible reservoir host is fragmentary or inexistent. Several studies found *R. helvetica* in ticks in Germany (Woelfel et al., 2006; Dobler & Woelfel, 2009), but no evidence has been found in rodents previously to this study (Hartelt et al., 2008).

In our study, we found seropositive animals and rickettsial DNA (*R. felis* and *R. helvetica*) in ear tissue. However, rickettsial DNA found in ear tissue does not necessarily implicate rickettsemia, as it may be associated with a local infection after a tick bite. The fact that ticks were often found in body regions not covered with fur such as the ear, the chin or the tail base may argue for that, as well as the fact that we could not amplify DNA from liver tissue. On the other hand, the lower body temperature in body extremities like the ear could be advantageous for Rickettsia and thus promote sequestration of the rickettsial organism, as their optimum growth temperature in vitro was shown to be at 32°C (Pornwiroon et al., 2006).

One of the potential limitations of this study was the type of blood samples collected. These consisted in serum samples only, which did not enable us to test full blood samples for rickettsial DNA. Therefore, we cannot draw a conclusion to a possible rickettsemia. Seropositive animals may only

develope limited, transient infections sufficient for the production of antibodies, but not sufficient to infect ticks and thus to maintain the rickettsial life cycle. Moreover, seropositivity indicates that infection has occurred, but is no evidence of direct or indirect transmission to the arthropod host, which, for being a reservoir, must exist. Furthermore the level of seroprevalence does not necessarily provide information as to whether the vertebrate is a maintenance host (Haydon et al., 2002). In addition, high seroprevalence at a single point in time may simply indicate an outbreak in the host population, rather than pathogen persistence (Van Bressem et al., 2001). The critical issue is the persistence of infection in the reservoir, which can only be determined through longitudinal studies. Nevertheless, the high rickettsial prevalence in rodents captured in our study over a period of three years suggests rickettsial persistence rather than a single outbreak.

Referring to Mills and Childs (1998), we were able to determine the possible rodent host species that occur in the region of Lower Bavaria and their distribution along an altitude gradient. Our data is a basis for further investigations on the geographic range of the pathogen within the host range and the regional distribution of the host and pathogen among the distinct habitat types. Further, our survey conducted over a period of three years is a starting point for longitudinal monitoring of host-pathogen dynamics.

Even if the question of rodents acting as a reservoir host or not cannot be answered definitively, the results of our study are of immense importance as we were able to detect DNA from *R. felis* and *R. helvetica* in small mammals in Germany for the first time. In contrast to the results of this study, several investigations screened different mammalian organ tissues or blood with no positive results at all: Yabsley et al. (2008) investigated dogs in Grenada for *Rickettsia* spp. and found them to be seronegative and PCR negative, however, it has to be noted that the primers they used targeted the *ompA* gene. Organ pools of 119 rodents (mainly *M. agrestis*) from Germany showed no positive result in PCR screening (Pluta et al., 2010), neither did organ pools including ear tissue from Spain, tested by multiplex PCR (Barandika et al., 2007). However, in the USA, rickettsial

DNA was found in three opossum spleens in California (Williams et al., 1992), and in a whole blood sample of one golden mantled ground squirrel in the Sierra Nevada (Adjemian et al., 2008). In a survey on rodents trapped on a military training base in Southern Germany, that was conducted at the Bundeswehr Institute of Microbiology, in three out of 221 rodents *Rickettsia* spp. DNA was detected by PCR, the seroprevalence was 11.8% (Publication Annex 4, p. 101). Two blood samples from rodents from Peru were found PCR-positive for Rickettsiae (Richards, personal communication). Apart from the latter, this study is the first to detect *R. felis* in rodent tissue, which is a quite thrilling result that several researchers received with immense interest. It is hoped that this study will act as a trigger for further investigations regarding the natural cycles of rickettsiae in Germany.

Furthermore, the molecular biological and serologic methods applied in this study were shown to be suitable for screening of rodent tissue and of sera as well as transudates, respectively. Thus they offer useful tools for diagnosis and further (longitudinal) research regarding rickettsia in humans as well as in vertebrate and arthropod hosts.

Although being most likely underdiagnosed, the incidence of rickettsial infections in Germany appears to be rather low so far, compared to other vector-borne diseases such as Lyme borreliosis or tick-borne encephalitis. However, Rickettsioses are considered as emerging diseases (Parola & Raoult, 2001; Fournier & Raoult 2005; Parola et al., 2005; Brouqui et al., 2007; Vorou et al., 2007; Rovery et al., 2008). Being arthropod-borne diseases, infection risks are determined by occurrence of the vector, which in turn is influenced by several factors such as climate, environment, and the presence of rodents and other small mammal reservoirs (Fritz, 2009). Climatic conditions are assumed to influence the distribution of ticks and tick-borne diseases, for example TBE. In the geographic range of TBE virus, a northern expansion was observed in recent years. However, socio-economic changes, agricultural activities, employment and income apparently are more important factors to affect the risk of TBE infection and exposition for humans (Randolph, 2008).

In a climatic warming scenario, ticks are likely to extend their northern and

altitude range in Europe (Lindgren et al., 2000; Gray et al., 2009; Pluta et al., 2010). For instance, until now the occurrence of *Dermacentor* ticks in Germany has been restricted to few areas. Due to increasing temperatures, their distribution is likely to be extended, and *Rickettsia* spp. might spread with them (Pluta et al., 2010). So far, research regarding these aspects is at a very early stage in Germany, however, the present study sets an important starting point for further investigations.

The influence of climate change on reservoir host abundance, such as rodents, is probably more complicated and still under discussion (Tersago et al., 2009). Also, socio-economic factors such as changes in human leisure behaviour in addition to an increase in outdoor activities could possibly amplify human exposure and likelihood of infections in the future. As there is no vaccination available, the only way to prevent rickettsial infection is to avoid contact to the vectors and reservoir hosts. Intensifying fundamental research regarding host associations and host adaptations of *Rickettsia* spp. is essential for risk assessment and disease prevention in humans, livestock and companion animals.

VI. CONCLUSION & OUTLOOK

In the present study, seroreactive small mammals and rickettsial DNA, belonging to the species *R. helvetica* and *R. felis*, were detected. The results are of enourmous importance as this is the first detection of rickettsial DNA in rodents in Germany. However, due to limitations of study design, the question of rodents acting as a reservoir could not be answered definitevely.

For this reason, further investigations are urgently needed, on the one hand to screen rodent sera over a longer period for possible rickettsemia and on the other hand to screen more ear samples for rickettsial DNA, also in other parts of the country, in order to obtain more reliable data and thus to finally answer the question if rodents and other small mammals play an important or maybe even essential role in maintaining rickettsial natural life cycles. The small mammals that were collected subsequently in the years 2009 and 2010 will be investigated to that effect in the future, e.g. by generating rickettsial isolates for further characterization.

In the scope of the VICCI project, the collected animals were also screened for Hantavirus prevalence. The year 2010 seems to be an outbreak year for human Puumala virus infections as well as for prevalence in rodents (to date about 18%, in comparison to about 3% in the recent years). Also regarding this aspect of immense importance for public health, investigations will be continued in the future.

More statistical analyses will be conducted by ecologists, cooperating with the National Park Bavarian Forest, in order to get more information about possible correlations between infection, body parameters and ecological factors such as habitat elevation or vegetation.

VII. SUMMARY

In recent years, several *Rickettsia* spp. have been detected in Germany for the first time, some of them pathogenic for humans. The present study investigated the prevalence of spotted fever group (SFG) rickettsiae in small mammals by serological and molecular biological means. In total, 124 rodents and insectivores were collected in the years 2004 and 2005 in Lower Bavaria and in 2008 in the National Park Bavarian Forest. Sera and transudates were tested in two immunofluorenscence assay tests, one commercially available for detection of antibodies against *R. conorii* and one in-house test for *R. helvetica* antibodies. DNA-extracts were taken from ear and liver tissue. Screening for *Rickettsia* spp. was performed with a real-time PCR targeting the citrate synthase gene (gltA), followed by conventional PCR detecting the *rOmpB* gene for all gltA positives. An 18S universal PCR was used as internal control. The overall prevalence for antibodies against SFG rickettsiae was 28.1%; 7.6% (n=9/119) samples showed positive reaction in PanRickettsia real-time PCR.

Sequence analysis of all *rompB* PCR products resulted in five *R. felis* and two *R. helvetica ompB* nucleotide sequences that were 100% identical among each other and to the respective prototypes deposited in GenBank.

Descriptive statistical tests revealed that prevalence of rickettsial DNA, but not seroprevalence were related to body weight, body length, spleen, kidney, gonad and liver weights. As expected, parasites had a significant influence, as well as body size as a surrogate for adult animals, in contrast to elevation.

With the first detection of two arthropod-borne *Rickettsia* spp. (*R. helvetica* and *R. felis*) in small mammals in Germany, this work is an important contribution and thus a starting point for further research regarding the life cycle of Rickettsiae and the reservoir role of small animals.

VIII. ZUSAMMENFASSUNG

In den letzten Jahren wurden in Deutschland einige zum Teil humanpathogene Rickettsienarten neu entdeckt. Die hier vorliegende Studie untersuchte mit molekularbiologischen und serologischen Methoden die Prävalenz von Rickettsien der Fleckfiebergruppe in Kleinsäugetieren. Insgesamt wurden in den Jahren 2004/2005 in Niederbayern und 2008 im Nationalpark Bayerischer Wald 124 Nagetiere und Insektivore gesammelt. Seren und Transudate wurden mit zwei Immunfluoreszenztests getestet, einem kommerziell erhältlichen zum Nachweis von Antikörpern gegen *R. conorii* und einem selbsthergestellten für Antikörper gegen *R. helvetica*. DNA wurde aus Ohr- und Lebermaterial extrahiert. Ein Screening aller Proben wurde mit einer real-time PCR mit Citratsynthase (gltA) als Zielgen durchgeführt, gefolgt von einer konventionellen PCR mit Zielgen *rompB* für alle Proben mit positiver Reaktion. Eine 18S universal PCR diente als interne Kontrolle. Insgesamt betrug die Antikörperprävalenz für Rickettsien der Fleckfiebergruppe 28.1%; 7.6% (n=9/119) der Proben zeigten eine positive Reaktion in der PanRickettsien real-time PCR.

Sequenzanalysen für alle *rompB*-PCR-Produkte ergaben fünf *R. felis* und zwei *R. helvetica ompB* Nukleotidsequenzen, die untereinander und im Vergleich zu den entsprechenden Genbank-Prototypen zu 100% identisch waren.

Deskriptive statistische Tests zeigten, dass die Prävalenz rickettsialer DNA, nicht aber die Seroprävalenz mit Körpergewicht, Körperlänge, Milz-, Nieren-, Gonaden- und Lebergewichten zusammenhängt. Wie erwartet, hatten Parasiten einen signifikanten Einfluss, genau wie Körpergröße als Surrogat für ausgewachsene Tiere, jedoch nicht die Höhe.

Mit der ersten Beschreibung von zwei von Arthropoden übertragenen Rickettsienarten (*R. helvetica* und *R. felis*) in Kleinsäugetieren in Deutschland liefert diese Arbeit einen wichtigen Beitrag und Ausgangspunkt für weitergehende Forschung zum Lebenszyklus von Rickettsien und zur Rolle von Kleinsäugetieren als potentielle Reservoirwirte.

IX. REFERENCES

Adams JR, Schmidtmann ET, Azad AF. Infection of colonized cat fleas (*Ctenocephalides felis* Bouché) with a rickettsia-like organism. Am J Trop Med Hyg. 1990; 43:400-409.

Adjemian JD, Adjemian MK, Foley P, Chomel BB, Kasten RW, Foley JE. Evidence of Multiple Zoonotic Agents in a Wild Rodent Community in the Eastern Sierra Nevada. J Wildl Dis 2008; 44(3):737-42.

Adler B, de la Peña Moctezuma A. Leptospira and leptospirosis. Vet Microbiol. 2010; 140(3-4):287-96.

Azad AF, Beard CB. Rickettsial pathogens and their arthropod vectors. Emerg Infect Dis 1998; 4:179–186.

Baessler C, Foerster B, Moning C, Mueller J. The BIOKLIM Project: Biodiversity Research between Climate Change and Wilding in a temperate montane forest–The conceptual framework. Waldoekologie, Landschaftsforschung und Naturschutz 2008; 7:21–33.

Baldridge GD, Burkhardt N, Simser JA, Kurtti TJ, Munderloh UG. Sequence and expression analysis of the ompA gene of *Rickettsia peacockii*, an endosymbiont of the Rocky Mountain wood tick, *Dermacentor andersoni*. Appl. Environ. Microbiol. 2004; 70:6628–6636.

Barandika JF, Hurtado A, García-Esteban C, Gil H, Escudero R, Barral M, Jado I, Juste RA, Anda P, García-Pérez AL. Tick-Borne Zoonotic Bacteria in Wild and Domestic Small Mammals in Northern Spain. Appl Environ Microbiol 2007; 73(19):6166-71.

Battersby SA, Parsons R, Webster JP. Urban rat infestations and the risk to public health. J Environm Health Res 2002; 1:57-65.

Bayliss DB, Morris AK, Horta MC, Labruna MB, Radecki SV, Hawley JR, Brewer MM, Lappin MR. Prevalence of *Rickettsia* species antibodies and

Rickettsia species DNA in the blood of cats with and without fever. J Feline Med Surg 2009; 11:266-270.

Beck R, Vojta L, Curković S, Mrljak V, Margaletić J, Habrun B. Molecular Survey of *Babesia microti* in Wild Rodents in Central Croatia. Vector Borne Zoonotic Dis. 2010 Jun 16. [Epub ahead of print]

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2008; 37 (Database issue):D26-31.

Berdal BP, Mehl R, Meidell NK, Lorentzen-Styr AM, Scheel O. Field investigations of tularemia in Norway. FEMS Immunol Med Microbiol 1996; 13:191–195.

Beugnet F, Marié JL. Emerging arthropod-borne diseases of companion animals in Europe. Vet Parasitol. 2009; 163(4):298-305.

Bingham J, Foggin CM, Wandeler AI, Hill FWG. The epidemiology of rabies in Zimbabwe. 1. Rabies in dogs (*Canis familiaris*). Onderstepoort J Vet Res 1999a; 66:1–10.

Bingham J, Foggin CM, Wandeler AI, Hill FWG. The epidemiology of rabies in Zimbabwe. 2. Rabies in jackals (*Canis adustus* and *Canis mesomelas*). Onderstepoort J Vet Res 1999b; 66:11–23.

Blanc G, Caminopetros J. Etudes épidémiologiques et expérimentales sur la fièvre boutonneuse, faites à l'Institut Pasteur d'Athènes. Arch Inst Pasteur Tunis. 1932: 343–94.

Blanco JR, Oteo JA. Rickettsiosis in Europe. Ann N Y Acad Sci. 2006; 1078:25-33.

Bonnefoy X, Kampen H, Sweeney K. Public Health significance of urban pests. WHO Regional Office for Europe, Copenhagen 2008.

Boyce JM. Recent trends in the epidemiology of tularemia in the United

States. J Infect Dis 1975; 131:197–199.

Brenner DJ, O'Connor SP, Winkler HH, Steigerwalt AG. Proposals to unify the genera bartonella and rochalimaea, with descriptions of *Bartonella quintana comb. nov*., *Bartonella vinsonii comb. nov*., *Bartonella henselae comb. nov*., and *Bartonella elizabethae comb. nov*., and to remove the family bartonellaceae from the order Rickettsiales. Int J Syst Bacteriol 1993; 43: 77-786.

Brouqui P, Parola P, Fournier PE, Raoult D. Spotted fever rickettsioses in southern and eastern Europe. FEMS Immunol Med Microbiol 2007; 49:2-12.

Burgdorfer W, Aeschlimann A, Peter O, Hayes SF, Philip RN. *Ixodes ricinus*: vector of a hitherto undescribed spotted fever group agent in Switzerland. Acta Trop 1979; 36:357-367.

Burgdorfer W, Brinton LP. Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. Ann NY Acad Sci 1975; 266:61-72

Burgdorfer W, Friedhoff KT, Lancaster JL. Natural history of tick-borne spotted fever in the USA. Bull WHO 1966; 35:149-53.

Burgdorfer W, Varma MGR. Trans-stadial and transovarial development of disease agents in arthropods. Ann Rev Entomol 1967; 12:347-76.

Burgdorfer W. Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever. Acta Trop 1977; 34:103-26.

Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: Important Reservoir Hosts of Emerging Viruses. Clin Microbiol Rev 2006; 19(3):531–545

Calisher CH, Holmes KV, Dominguez SR, Schountz T, Cryan P. Bats prove to be rich reservoirs for emerging viruses. Microbe 2008; 11:521-528.

Carl M, Dobson ME, Ching WM, Dash GA. Characterization of the gene encoding the protective paracrystalline-surface-layer protein of *Rickettsia prowazekii*: presence of a truncated identical homolog in *R. typhi*. Proc Natl Acad Sci USA 1990; 87:8237-8241.

Centers for Disease Control and Prevention. Consequences of delayed diagnosis of Rocky Mountain spotted fever in children—West Virginia, Michigan, Tennessee, and Oklahoma, May-July 2000. MMWR Morb Mortal Wkly Rep 2000; 49:885-888

Cerny V. Folia parasit 1976; 22:271.

Chantrey J, Meyer H, Baxby D, Begon M, Bown KJ, Hazel SM, Jones T, Montgomery WI, Bennett M. Cowpox: reservoir hosts and geographic range. Epidemiol Infect. 1999; 122(3):455-60.

Chapman AS, Murphy SM, Demma LJ, Holman RC, Curns AT, McQuiston JH. Rocky mountain spotted fever in the United States, 1997-2002. Ann N Y Acad Sci 2006; 1078:154-5.

Chen M, Fan MY, Bi DZ, Zhang JZ, Huang YP. Detection of *Rickettsia sibirica* in ticks and small mammals collected in three different regions of China. Acta Virol. 1998; 42(1):61-4.

Chen Z, Chen M, Zhong J, Bi D.2002: Using PCR/RFLP to detect spotted fever group rickettsia in ticks and rodents collected in Ninghua, Fujian province. Zhonghua Yu Fang Yi Xue Za Zhi. 2002; 36(2):106-8.

Choi YJ, Lee EM, Park JM, Lee KM, Han SH, Kim JK, Lee SH, Song HJ, Choi MS, Kim LS. Molecular detection of various rickettsiae in mites (acari: *trombiculidae*) in southern Jeolla Province, Korea. Microbiol Immunol 2007; 51:307-312.

Department for Environment, Food and Rural Affairs. Bovine Tuberculosis: The Government's approach to tackling the disease and consultation on a badger control policy. 2010 URL: www.defra.gov.uk/corporate/consult/tb-

control-measures/

Demma LJ, Traeger MS, Nicholson WL, Paddock CD, Blau DM, Eremeeva ME, Dasch GA, Levin ML, Singleton J Jr, Zaki SR, Cheek JE, Swerdlow DL, McQuiston JH. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N Engl J Med 2005; 353:587–94.

De Sousa R, Edouard-Fournier P, Santos-Silva M, Amaro F, Bacellar F, Raoult D. Molecular detection of *Rickettsia felis*, *Rickettsia typhi* and two genotypes closely related to *Bartonella elizabethae*. Am J Trop Med Hyg. 2006; 75(4):727-31.

Dobler G, Essbauer S, Woelfel R. Isolation and preliminary characterisation of ‚*Rickettsia monacensis*' in south-eastern Germany. Clin Microbiol Infect 2009; 15:263-264.

Dobler G, Woelfel R. Typhus and Other Rickettsioses - Emerging Infections in Germany. Dtsch Arztebl Int 2009; 106(20):348–354.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 1991; 19(14):4008.

Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, Rikihisa Y, Rurangirwa FR. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol 2001; 51:2145-65.

Easterbrook JD, Kaplan JB, Glass GE, Pletnikov MV, Klein SL. Elevated testosterone and reduced 5-HIAA concentrations are associated with wounding and hantavirus infection in male Norway rats. Horm Behav 2007; 52:474-481.

Easterbrook JD, Shields T, Klein SL, Glass GE. Norway rat population in Baltimore, Maryland, 2004. Vector Borne Zoonotic Dis 2005; 5(3):296-299.

Eckert J, Friedhoff KT, Zahner H, Deplazes P. Lehrbuch der Parasitologie für die Tiermedizin. 2005.

Elling W, Bauer E, Klemm G, Koch H. Klima und Boeden. Nationalparkverwaltung Bayerischer Wald 1987; 255.

Ellis J, Oyston PC, Green M, Titball RW. Tularemia. Clin Microbiol Rev 2002; 15:631-646.

Essbauer S, Schmidt J, Conraths FJ, Friedrich R, Koch J, Hautmann W, Pfeffer M, Woelfel R, Finke J, Dobler G, Ulrich R. A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. Epidemiol Infect. 2006; 134(6):1333–1344.

Essbauer S, Hartnack S, Misztela K, Kießling-Tsalos J, Baeumler W, Pfeffer M. Patterns of orthopox virus wild rodent hosts in South Germany. Vector-borne and zoonotic diseases 2009a; 9(3):301-311.

Essbauer S, Schex S, Splettstoesser W, Pfeffer M, Ulrich R, Seibold E, Dobler G, Woelfel R, Baeumler W. Nagetier-uebertragene Zoonosen: Beispiele aus Untersuchungen in Sued- und Westdeutschland. Mitt. Julius Kuehn-Inst. 421, 2009b; 37-48.

Fournier PE, Allombert C, Supputamongkol Y, Caruso G, Brouqui P, Raoult D. Aneruptive Fever Associated with Antibodies to *Rickettsia helvetica* in Europe and Thailand. J Clin Microbiol 2004; 42:816-818.

Fournier PE, Grunnenberger F, Jaulhac B, Gastinger G, and Raoult D. Evidence of *Rickettsia helvetica* infection in humans, eastern France. Emerg Infect Dis 2000; 6(4):389–392.

Fournier PE, Raoult D. Mediterranean Spotted Fever and Other Tick-

Borne Rickettsioses. in: Tick-Borne Diseases of Humans. Edited by Goodman JL, Dennis DT, Sonenshine D. Washington, 2005; 302-27

Fritz CL. Emerging tick-borne diseases. Vet Clin North Am Small Anim Pract. 2009; 39(2):265-78.

Gern L, Estrada-Peña A, Frandsen F, Gray JS, Jaenson TG, Jongejan F, Kahl O, Korenberg E, Mehl R, Nuttall PA. European reservoir hosts of *Borrelia burgdorferi sensu lato*. Zentralbl Bakteriol. 1998; 287(3):196-204.

Gern L. *Borrelia burgdorferi sensu lato*, the agent of lyme borreliosis: life in the wilds. Parasite. 2008; 15(3):244-7.

Gilles J, Just FT, Silaghi C, Pradel I, Passos LM, Lengauer H, Hellmann K, Pfister K. *Rickettsia felis* in fleas, Germany. Emerg Infect Dis 2008; 14(8):1294-6.

Gould DJ, Miesse ML. Recovery of a rickettsia of the spotted fever group from *Microtus pennsylvanicus* from Virginia. Proc Soc Exp Biol Med 1954; 85:558-61.

Gray JS, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis. 2009; 593232.

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999; 41:95–98.

Hannah MF, Bajic VB, Klein SL. Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. Brain Behav Immun 2008; 22:503-516.

Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. Identifying reservoirs of infection: a conceptual and practical challenge. Emerg Infect Dis 2002; 8(12):1468-73.

Hartelt K, Oehme R, Frank H, Brockmann SO, Hassler D, Kimmig P. Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. Int J Med Microbiol 2004; 293(37):86-92.

Hartelt K, Pluta S, Oehme R, Kimmig P. Spread of ticks and tick-borne diseases in Germany due to global warming. Parasitol Res 2008; 103(1):109-16.

Hastie TJ, Pregibon D. Generalized linear models. In: Statistical Models in S. Edited by Chambers JM, Hastie TJ. Wadsworth & Brooks/Cole, Pacific Grove, CA 1992; 6:195-248

Herrero-Herrero JI, Ruiz Beltran R, Martin-Sanchez AM, Garcia EJ. Mediterranean spotted fever in Salamanca, Spain. Epidemiological study in patients and serosurvey in animals and healthy human population. Acta Trop 1989; 46:335–50.

Hildebrandt A, Hunfeld KP, Baier M, Krumbholz A, Sachse S, Lorenzen T, Kiehntopf M, Fricke HJ, Straube E. First confirmed autochthonous case of human *Babesia microti* infection in Europe. Eur J Clin Microbiol Infect Dis. 2007; 26(8):595-601.

Hildebrandt A, Kraemer A, Sachse S, Straube E. Detection of *Rickettsia* spp. and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in a region of Middle Germany (Thuringia). Ticks and Tick-borne Diseases 2010; 1:52-56

Hinson ER, Shone SM, Zink MC, Glass GE, Klein SL. Wounding: the primary mode of Seoul virus transmission among male Norway rats. Am J Trop Med Hyg 2004; 70:310-317.

Homer MJ, Aguilar-Delfin I, Telford SR III, Krause PJ, Persing DH. Babesiosis. Clin Microbiol Rev 2000; 13: 451-469.

Humair PF, Rais O, Gern L. Transmission of *Borrelia afzelii* from *Apodemus mice* and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. Parasitology. 1999; 118(Pt 1):33-42.

Hutchinson KL, Rollin PE, Peters CJ. Pathogenesis of North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus*. Am J Trop Med Hyg 1998; 59:58-65.

Ishikura MS, Ando S, Shinagawa Y, Matsuura K, Hasegawa S, Nakayama T, Fujita H, Watanabe M. Phylogenetic analysis of spotted fever group rickettsiae based on GltA, 17-kDA, and rOmpA genes amplified by nested PCR from ticks in Japan. Microbiol Immunol 2003; 47:823-832.

Jado I, Oteo JA, Aldámiz M, Gil H, Escudero R, Ibarra V, Portu J, Portillo A, Lezaun MJ, García-Amil C, Rodríguez-Moreno I, Anda P. *Rickettsia monacensis* and human disease, Spain. Emerg Infect Dis. 2007; 13(9):1405-7.

Jansen A, Frank C, Koch J, Stark K. Surveillance of vector-borne diseases in Germany: trends and challenges in the view of disease emergence and climate change. Parasitol Res 2008; 103(1):11-7.

Johnson RC. Leptospira. In: Barron's Medical Microbiology. Edited by Barron S, Albrecht T, Castro G, Couch RB, Davis CP, Dianzani F, McGinnis MR, Niesel DW, Woods GW. Univ of Texas Medical Branch, 1996. (via NCBI Bookshelf)

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature 2008; 451(7181):990-993.

Kallio ER, Klingstrom J, Gustafsson E, Manni T, Vaheri A, Henttonen H, Vapalahti O, Lundkvist A. Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. J Gen Virol 2006; 87:2127-2134.

Kaysser P, Seibold E, Mätz-Rensing K, Pfeffer M, Essbauer S, Splettstoesser WD. Re-emergence of tularemia in Germany: presence of *Francisella tularensis* in different rodent species in endemic areas. BMC Infect Dis. 2008; 8:157.

Kiessling, JR. Untersuchung zum Vorkommen des Frühsommer-Meningo-Enzephalitis-Virus und *Borrelia burgdorferi* in ausgewählten Wildmaus- und Zeckenpopulationen Bayerns. Dissertation, LMU München: Tierärztliche Fakultät 2005.

Kurth A, Wibbelt G, Gerber HP, Petschaelis A, Pauli G, Nitsche A. Rat-to-elephant-to-human transmission of cowpox virus. Emerg Infect Dis 2008; 14(4): 670–671

Kuczka A, Nitsche A, Hoeveler R, Becker C, Kurth A. Kuhpocken - Seltene Zoonose vermehrt in Deutschland nachgewiesen. Deutsches Tierärzteblatt 2009; 3:316-319

Lambin X, Bretagnolle V, Yoccoz NG. Vole population cycles in northern and southern Europe: is there a need for different explanations for single pattern? J Anim Ecol 2006; 75(2):340-349.

La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. J Clin Microbiol 1997; 35(11):2715-27.

Le Gac P. Repercussions of myxomatosis on Mediterranean boutonneuse exanthematic fever [in French]. Bull World Health Organ 1966; 35:143–7.

Lee HW, Lee PW, Baek LJ, Song CK, Seong IW. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. Am J Trop Med Hyg 1981; 30:1106-12.

Lewellen RH, Vessey SH. The effect of density dependence and weather on population size of a polyvoltine species: the importance of time scale.

Ecol Mongr 1998; 68:571-594.

Lindgren E, Tälleklint L, Polfeldt T. Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. Environ Health Perspect. 2000; 108(2):119-23.

Little TWA, Naylor PF, Wilesmith JW. Laboratory study of *Mycobacterium bovis* infection in badgers and calves. Vet Rec 1982; 111:550-557.

Macaluso KR, Azad AF. Rocky Mountain Spotted Fever and other Spotted Fever Group Rickettsioses. In: Tick-Borne Diseases of Humans. Edited by Goodman JL, Dennis DT, Sonenshine D. Washington, 2005; 292-301.

Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus - a review of an emerging zoonosis. J Gen Virol 2009; 90:1781-94.

McDade JE, Newhouse VF. Natural History of *Rickettsia rickettsii*. Annu Rev Microbiol 1986; 40:287-309.

Mediannikov O, Matsumoto K, Samoylenko I, Drancourt M, Roux V, Rydkina E, Davoust B, Tarasevich I, Brouqui P, Fournier PE. *Rickettsia raoultii* sp. nov., a spotted fever group rickettsia associated with *Dermacentor* ticks in Europe and Russia. Int J Syst Evol Microbiol 2008; 58:1635-9.

Mehnert WH, Krause G. Surveillance of Lyme borreliosis in Germany, 2002 and 2003. Eurosurveillance 2005; 10:83–85.

Meng XJ, Lindsay DS, Sriranganathan N. Wild boars as sources for infectious diseases in livestock and humans. Philos Trans R Soc Lond B Biol Sci. 2009; 364(1530):2697-707.

Mills JN, Childs JE. Ecologic studies of rodent reservoirs: their relevance for human health. Emerg Infect Dis 1998; 4(4):529-37.

Morner T. The ecology of tularemia. Rev Sci Tech Off Int Epiz 1992; 11:1123–1130.

Muhldorfer K, Wibbelt G, Haensel J, Riehm J, Speck S. *Yersinia* species isolated from bats, Germany. Emerg Infect Dis. 2010; 16(3):578-80.

Munderloh UG, Kurtti TJ. Cellular and molecular interrelationships between ticks and prokaryotic tick-borne pathogens. Annu Rev Entomol 1995; 40:221–243.

Nichol ST, Beaty BJ, Elliott RM, Goldbach R, Plyusnin A, Schmaljohn CS, Tesh RB. Bunyaviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA [Eds.]. Classification and Nomenclature of Viruses. Eighth Report Of The International Committee on taxonomy of viruses. Elsevier Academic Press, San Diego, 2005; 695-716.

Nilsson K, Lindquist O, Pahlson C. Association of *Rickettsia helvetica* with chronic perimyocarditis in sudden cardiac death. Lancet 1999; 354:1169-1173.

Nilsson K, Påhlson C, Lukinius A, Eriksson L, Nilsson L, Lindquist O. Presence of *Rickettsia helvetica* in Granulomatous Tissue from Patients with Sarcoidosis. J Infs Dis 2002; 185:1128–1138.

Norment BR, Burgdorfer W. Susceptibility and reservoir potential of the dog to spotted fever-group rickettsiae. Am J Vet Res 1984; 45:1706-10.

Oliveira RP, Oliveira L, Dias C, Silva A Jr, Almeida M, Almada G, Bouyer D, Galvao M, Mafra C. Molecular identification of *Rickettsia felis* in ticks and fleas from an epidemic area for Brazilian spotted fever. Mem Inst Oswaldo Cruz 2008; 103:191-194.

Ormsbee R, Peacock M, Philip R, Casper E, Plorde J, Gabre-Kidan T, Wright L. Antigenic relationships between the typhus and spotted fever groups of rickettsiae. Am J Epidemiol 1978; 108:53-59.

Oteo JA, Portillo A, Santibanez S, Blanco JR, Perez-Martinez L, Ibarra V. Cluster of cases of human *Rickettsia felis* infection from Southern Europe (Spain) diagnosed by PCR. J Clin Microbiol 2006; 44:2669-2671.

Otis WL, Tarpley HL, Bain PJ, Latimer KS, LeRoy BE. Rocky Mountain Spotted Fever in Dogs. Veterinary Clinical Pathology Clerkship Program, 2004 URL: vet.uga.edu.

Palmer MV. Tuberculosis: A Reemerging Disease at the Interface of Domestic Animals and Wildlife. In: Wildlife and Emerging Zoonotic Diseases: The Biology, Circumstances and Consequences of Cross-Species Transmission. Editors: Childs JE, Mackenzie JS, Richt JA. 2007

Paddock CD, Greer PW, Ferebee TL, Singleton J Jr, McKechnie DB, Treadwell TA, Krebs JW, Clarke MJ, Holman RC, Olson JG, Childs JE, Zaki SR. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis 1999; 179:1469-1476.

Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 2005; 18(4):719-56.

Parola P, Raoult D. Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 2001; 32:897-928.

Pastoret PP, Brochier B. Epidemiology and control of fox rabies in Europe. Vaccine 1999; 17:1750–1754.

Peavy CA, Lane RS, Kleinjan JE. Role of small mammals in the ecology of *Borrelia burgdorferi* in a peri-urban park in north coastal California. Exp Appl Acarol 1997; 21:596- 84

Perlman SJ, Hunter MS, Zchori-Fein E. The emerging diversity of Rickettsia. Proc Biol Sci 2006; 273(1598):2097-106.

Pfeffer M, Essbauer S, Nöckler K, Jacob J, Ulrich RG. Aktueller Kenntnisstand zu Nagetier-übertragenen Zoonosen in Deutschland: Herausforderungen für die zukünftige Forschung. Rundschau für Fleischhygiene und Lebensmittelüberwachung 2010; 1:1-8.

Pichon B, Kahl O, Hammer B, Gray JS. Pathogens and host DNA in *Ixodes ricinus* nymphal ticks from a German forest. Vector Borne Zoonotic Dis 2006; 6(4):382-7.

Pluta S, Tewald F, Hartelt K, Oehme R, Kimmig P, Mackenstedt U. *Rickettsia slovaca* in *Dermacentor marginatus* ticks, Germany. Emerg Infect Dis 2009; 15(12):2077-2078.

Pluta S, Hartelt K, Oehme R, Mackenstedt U, Kimmig P. Prevalence of *Coxiella burnetii* and *Rickettsia* spp. in ticks and rodents in southern Germany. Ticks Tick Borne Dis 2010; Article in Press.

Pornwiroon W, Pourciau SS, Foil LD, Macaluso KR. *Rickettsia felis* from cat fleas: isolation and culture in a tick-derived cell line. Appl Environ Microbiol 2006; 72(8):5589-95.

Price WH. The epidemiology of Rocky Mountain spotted fever. II. Studies of the biological survival mechanism of *Rickettsia rickettsii*. Am J Hyg 1954; 60:292-319.

Randolph SE. Dynamics of tick-borne disease systems: minor role of climate change. Rev Sci Tech 2008; 27(2):367-81.

Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 1997; 10(4):694-719.

Raoult D, Tissot-Dupont H, Chicheportiche C, Peter O, Gilot B, Drancourt M. Mediterranean spotted fever in Marseille, France: correlation between prevalence of hospitalized patients, seroepidemiology, and prevalence of infected ticks in three different areas. Am J Trop Med Hyg 1993; 48:249–56.

Rehácek J, Liebisch A, Urvoelgyi J, Kovácová E. *Rickettsiae* of the spotted fever isolated from *Dermacentor marginatus* ticks in South Germany. Zentralbl Bakteriol Orig A 1977; 239(2):275-81.

Rehácek J, Urvoelgyi J, Kocianova E, Jedlicka L. Susceptibility of some species of rodents to rickettsiae. Fol Parasit 1992; 39:265-284.

Reif KE, Macaluso KR. Ecology of *Rickettsia felis*: A Review. J Med Entomol 2009; 46(4):723-736

Richter J, Fournier PE, Petridou J, Haussinger D, Raoult D. *Rickettsia felis* infection acquired in Europe and documented by polymerase chain reaction. Emerg Infect Dis 2002; 8:207-208

Ricketts HT. Contributions to Medical Science by Howard Taylor Ricketts, 1870-1910. Chicago: Univ Chicago Press. 1911; 497 pp.

Rovery C, Brouqui P, Raoult D. Questions on Mediterranean spotted fever a century after its discovery. Emerg Infect Dis 2008; 14(9):1360-7.

Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein *rOmpB* (*ompB*). Int J Syst Evol Microbiol 2000; 50(4):1449-55.

Ruiz Beltran R, Herrero-Herrero JI, Martin-Sanchez AM, Criado-Gutierrez LA. Role of lagomorpha in the wild cycle of *Rickettsia conorii* in Salamanca (Spain). Eur J Epidemiol 1992; 8:136–9.

Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis 1997; 3(2): 95-104.

Schmaljohn CS, Nichol ST. Bunyaviridae. Fields Virology 2007, 5th Ed, Vol 2, In: Knipe DM, Howley PM [Eds.]. Lippincott Williams & Wilkins, Philadelphia,1741–1789.

Sekeyova Z, Roux V, Xu WB, Rehacek J, Raoult D. *Rickettsia slovaca* sp.

nov., a member of the spotted fever group rickettsiae. Int J Syst Bacteriol 1998; 48:1455-1462.

Selbitz HJ. Bakterielle Krankheiten der Tiere. In: Medizinische Mikrobiologie, Infektions- und Seuchenlehre. Edited by Rolle M, Mayr A. 2002.

Shirai A, Bozeman FM, Humphries JW, Elisberg BL, Faber JE. Experimental infection of the cotton rat *Sigmodon hispidus* with *Rickettsia rickettsii*. J Bacteriol 1967; 94:1334-39.

Shpyonov S, Parola P, Rudakov N, Samoilenko I, Tankibaev M, Tarasevich I, Raoult D. Detection and identification of spotted fever group rickettsiae in *Dermacentor* ticks from Russia and central Kazakhstan. Eur J Clin Microbiol Infect Dis 2001; 20:903-905.

Silaghi C, Gilles J, Hoehle M, Pradel I, Just FT, Fingerle V, Kuechenhoff H, Pfister K. Prevalence of spotted fever group rickettsiae in *Ixodes ricinus* (Acari: Ixodidae) in southern Germany. J Med Entomol 2008; 45(5):948-55.

Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ, Munderloh UG. *Rickettsia monacensis* sp. nov., a spotted fever group Rickettsia, from ticks (*Ixodes ricinus*) collected in a European city park. Appl Environ Microbiol 2002; 68(9):4559-66.

Solano-Gallego L, Kidd L, Trotta M, Di Marco M, Caldin M, Furnanello T, Breitschwerdt E. Febrile illness associated with *Rickettsia conorii* infection in dogs in Sicily. Emerg Infect Dis 2006; 12:1985–8.

Stefancíková A, Bhide M, Pet'ko B, Stanko M, Mosanskỳ L, Fricova J, Derdáková M, Trávnicek M. Anti-Borrelia antibodies in rodents: important hosts in ecology of Lyme disease. Ann Agric Environ Med 2004; 11(2):209-13.

Takahashi M, Murata M, Machida K, Hori E, Kawamura A Jr, Tanaka H.

Aggregated distribution of infective spots composed of *Leptotrombidium pallidum*, highly prevalent with *Rickettsia tsutsugamushi*, demonstrated by sentinel voles, *Microtus montebelli*, on the ground. Jpn J Exp Med 1990; 60(6):325-35.

Tarnvik A, Sandstrom G, Sjostedt A. Epidemiological analysis of tularemia in Sweden, 1931–1993. FEMS Immunol Med Microbiol 1996; 13:201–204.

Telford SR 3rd, Dawson JE, Katavolos P, Warner CK, Kolbert CP, Persing DH. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. Proc Natl Acad Sci USA. 1996; 93(12):6209-14.

Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G, Leirs H. Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. Epidemiol Infect 2009; 137(2):250-6.

Tsui PY, Tsai KH, Wenig MH, Hung YW, Liu YT, Hu KY, Lien JC, Lin PR, Shaio MF, Wang HC, Ji DD. Molecular detection and characterisation of spotted fever group rickettsiae in Taiwan. Am J Trop Med Hyg 2007; 77:883-890.

Ulrich, RG, Meisel H, Schuett M, Schmidt J, Kunz A, Klempa B, Niedrig M, Kimmig P, Pauli G, Krueger DH, Koch J. Verbreitung von Hantavirusinfektionen in Deutschland. Bundesgesundheitsbl Gesundheitsforsch Gesundheitsschutz 2004; 47:661-670.

Ulrich RG, Schlegel M, Schmidt-Chanasit J, Jacob J, Freise J, Pelz HJ, Mertens M, Wenk M, Buechner T, Masur D, Sevke K, Meier M, Thiel J, Triebenbacher C, Buschmann A, Lang J, Loehr PW, Allgoewer R, Borkenhagen P, Schroeder T, Endepols S, Heidecke T, Stodian I, Hueppop O, Hornung M, Fiedler W, Krueger F, Ruehe F, Gerstengarbe FW, Pfeffer M, Wegener W, Bemmann M, Ohlmeyer L, Wolf R, Gehrke A, Heidecke D, Stubbe M, Zoller H, Koch J, Brockmann SO, Heckel G, Essbauer SS. Hantaviren und Nagetiere in Deutschland: Das Netzwerk „Nagetier-uebertragene Pathogene”. Mitt. Julius Kuehn-Inst 421, 2009; 37-92.

Van Bressem MF, Van Waerebeek K, Jepson PD, Raga JA, Duignan PJ, Nielsen O, Di Beneditto AP, Siciliano S, Ramos R, Kant W, Peddemors V, Kinoshita R, Ross PS, López-Fernandez A, Evans K, Crespo E, Barrett T. An insight into the epidemiology of dolphin morbillivirus worldwide. Vet Microbiol 2001; 81:287–304.

Varela AS. Tick-borne Ehrlichiae and Rickettsiae of dogs. In: DD Bowman, Companion and Exotic Animal Parasitology, International Veterinary Information Service, New York, 2003.

Vitale G, Di Stefano R, Damiani G, Mansueto S. Characterization of Sicilian strains of spotted fever group rickettsiae by using monoclonal antibodies. J Clin Microbiol 1989; 27:1081-1085

Vitullo AD, Hodara VL, Merani MS. Effect of persistent infection with Junín virus on growth and reproduction of its natural reservoir, *Calomys musculinus*. Am J Trop Med Hyg 1987; 37:663-9

Vorou RM, Papavassiliou VG, Tsiodras S. Emerging zoonoses and vector-borne infections affecting humans in Europe. Epidemiol Infect. 2007; 135(8):1231-47.

Walker DH. Rickettsiae. In: Barron's Medical Microbiology. Edited by Barron S, Albrecht T, Castro G, Couch RB, Davis CP, Dianzani F, McGinnis MR, Niesel DW, Woods GW. Univ of Texas Medical Branch, 1996. (via NCBI Bookshelf)

Walker DH, Ismail N, Olano JP, McBride JW, Yu XJ, Feng HM. *Ehrlichia chaffeensis*: a prevalent, life-threatening, emerging pathogen. Trans Am Clin Climatol Assoc. 2004; 115:375-82.

Warner RD, Marsh WW. Rocky Mountain spotted fever. J Am Vet Med Assoc. 2002; 221(10):1413-7

Wedincamp J Jr, Foil LD. Infection and seroconversion of cats exposed to cat fleas (*Ctenocephalides felis* Bouché) infected with *Rickettsia felis*. J

Vector Ecol 2000; 25(1):123-6.

Weinert LA, Werren JH, Aebi A, Stone GN, Jiggins FM. Evolution and diversity of *Rickettsia* bacteria. BMC Biol 2009; 7:6.

Williams SG, Sacci JB Jr, Schriefer ME, Andersen EM, Fujioka KK, Sorvillo FJ, Barr AR, Azad AF. Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. J Clin Microbiol 1992; 30(7):1758-62.

Wilson DE, Reeder DM. Mammal Species of the World. Johns Hopkins University Press, Baltimore 2005.

Woelfel R, Essbauer S, Dobler G. Diagnostics of tick-borne rickettsioses in Germany: A modern concept for a neglected disease. J Med Microbiol 2008; 298:368-374

Woelfel R, Terzioglu R, Kiessling J, Wilhelm S, Essbauer S, Pfeffer M, Dobler G. *Rickettsia* spp. in *Ixodes ricinus* ticks in Bavaria, Germany. Ann N Y Acad Sci 2006; 1078:509-11.

Woelfel S, Schmoldt S, Rieg S, Georgi E, Kern WV, Zoeller L, Dobler G. First description of TIBOLA (tick borne lymphadenopathy) in Germany. Medical Corps International Forum 2009; Suppl 28-29

World Animal Health Information Database (WAHID). Copyright: World Organisation for Animal Health (OIE) 2010. URL: www.oie.int/wahis/public.php?page=home.

Yabsley MJ, McKibben J, Macpherson CN, Cattan PF, Cherry NA, Hegarty BC, Breitschwerdt EB, O'Connor T, Chandrashekar R, Paterson T, Peream LM, Ball G, Friesen S, Goedde J, Henderson B, Sylvester W. Prevalence of *Ehrlichia canis*, *Anaplasma platys*, *Babesia canis vogeli*, *Hepatozoon canis*, *Bartonella vinsonii berkhoffii*, and *Rickettsia* spp. in dogs from Grenada. Vet Parasitol 2008; 151:279-285.

Zeier M, Handermann M, Bahr U, Rensch B, Müller S, Kehm R, Muranyi W, Darai G. New ecological aspects of hantavirus infection: a change of a paradigm and a challenge of prevention--a review. Virus Genes 2005; 30(2):157-80.

Zhu Y, Fournier PE, Eremeeva M, Raoult D. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol 2005; 5:11.

Zoeller, L. Leptospiren. In: Lexikon der Infektionskrankheiten des Menschen: Erreger, Symptome, Diagnose, Therapie und Prophylaxe. Edited by Darai G, Handermann M, Sonntag HG, Tidona CA, Zoeller L.Springer Berlin Heidelberg 2009 Part 12, 482-486.

X. ABBREVATIONS

ATL	animal tissue lysis
BSL	biosafety level
bp	base pair
°C	degree Celsius
CO_2	carbon dioxide
CSFV	Classical swine fever virus
CPXV	Cowpox virus
DEBONEL	*Dermacentor*-borne necrosis erythemalymphadenopathy
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
EID	emerging infectious disease
g	gram
gltA	citrate synthase gene
GPID	guinea pig infectious dosis
H_2O	water
HEV	hepatitis E virus
HFRS	hemorrhagic fever with renal syndrome
IFAT	Immun Fluorescence Assay Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
kDa	kiloDalton
kg	kilogram
LC	Light Cycler®
m^2	square meter
mg	milligram
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
ml	milliliter
mM	millimolar
MSF	Mediterranean spotted fever
µg	microgram

µl	microliter
µM	micromolar
NE	*Nephropathia epidemica*
ng	nanogram
NH_3	hydrogen nitride
ompA	outer membrane protein A
ompB	outer membrane protein B
PCR	polymerase chain reaction
PCV2	porcine circovirus type 2
pM	pikomolar
PPV	porcine parvovirus
PRV	pseudorabies virus
PRRSV	porcine reproductive and respiratory syndrome virus
PUUV	Puumala virus
RMSF	Rocky Mountain spotted fever
r*ompB*	rickettsial outer membrane protein B
rRNA	ribosomal ribonucleic acid
rt PCR	real-time polymerase chain reaction
SFG	spotted fever group
TAE	tris-acetate-EDTA
TBEV	tick-borne encephalitis virus
Taq	*Thermus aquaticus*
TIBOLA	tick-borne lymphadenitis
U	Unit
UDG	Uracil-DNA Glycosylase
UV	ultra violet

XI. FIGURES

XII. TABLES

XIII. ANNEX

1 Overview of investigations regarding rickettsiae

Table 12: Examples of *Rickettsia* spp. with or without vertebrate host

Species	arthropod vector	vertebrate host	disease	detection method	region	Ref.
R. conorii	*Rh. sanguineus*	n.d.	Mediterranean spotted fever	n.d.	Mediterranean	1), 2)
R. conorii ssp. israelensis spp. nov	*Rh. sanguineus*	n.d.	Israeli spotted fever	n.d.	Israel	1), 2)
R. conorii ssp. caspia ssp. nov	*Rh. sanguineus* *R. pumilio*	n.d.	Astrakhan fever	n.d.	Russia	1), 2)
R. sibirica	*D. auratus* *D. silvarum* *D. sinicus* *H.concinna* *H. wellingtoni* *H. yeni*	*A. agrarius* *M. fortis* *C. rufocanus* *O. zibethica* *R.flavipectus*	Siberian Tick typhus	PCR	Siberia Mongolia northern China	3), 4)
R. sibirica mongolitimonae	*Hyalomma asiaticum*	n.d.	lymphangitis associated rickettsiosis	cell culture PCR	Mongolia	1)
R. slovaca	*D. marginatus* *D. reticulatus*	n.d.	tick borne lymph-adenopathy	Serology Isolation (biopsy, tick)	Central Europe Portugal Slovakia Ukraine Armenia Siberia	1), 4), 5), 6)
R. aeschlimannii	*H. marginatum marginatum* *R. appendiculatus*	n.d.	n.d.	n.d.	Morocco South Africa	1)
R. massiliae	*Rhipicephalus* spp.	n.d.	n.d.	n.d.	Mediterranean France	1)
R. helvetica	*I. ricinus*	n.d.	n.d.	n.d.	N,O-Europe	1)
R. monacensis	*I. ricinus*	n.d.	n.d.	PCR (gltA, rOmpA) cell culture Westernblot	Europe	7), 8), 9)
R. helvetica	*I. ricinus*	n.d.	n.d.	PCR	Europe	10), 11), 12), 13
R. felis	*A. erinacei maura* *Ctenophtalmus sp.*	*Erinaceus europaeus* *R. norvegicus*	flea-borne spotted fever	PCR serology	n.d.	1),14) 15), 16), 17), 19),
R. typhi	*L. segnis*	*Rattus rattus*	endemic typhus	PCR	n.d.	15)
R. rickettsii	*D. andersoni* *D. variabilis*	*P. maniculatus* *Tamias amoenus* *Spermophilus lateralis* *T. speciosus* *S. beecheyi*	Rocky Mountain Spotted Fever	Serology, PCR	Sierra Nevada, USA	18)

References: 1) Brouqui et al., 2007; 2) Rovery et al., 2008; 3) Chen et al., 1998; 4) Chen et al., 2002; 4) Sekeyova et al., 1998; 5) Shpyonov et al., 2001; 6) Pluta et al., 2009; 7) Simser et al., 2002; 8) Jado et al., 2007; 9) Dobler et al., 2009; 10) Blanco & Oteo, 2006; 11) Fournier & Raoult, 2005; 12) Woelfel et al., 2006; 13) Dobler & Woelfel, 2009; 14) Ishikura et al., 2003; 15) De Sousa et al., 2006; 16) Tsui et al., 2007; 17) Oliveira et al., 2008; 18) Adjemian et al., 2008 19) Choi et al., 2007

2 Sequencing data

2.1 Alignment of partial 18S sequences

```
                                Univ18S fw
Mus musculus                ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Rattus rattus               ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTAGATACCG CAGCTAGGAA
Homo sapiens                ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Oryctolagus cuniculus       ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Cricetulus griseus          ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Equus caballus              ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Bos taurus                  ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Erinaceus europaeus         ATTAGAGTGT TCAAAGCAGG CCCGAGCCGC CTGGATACCG CAGCTAGGAA
Monodelphis domestica       ATTAGAGTGT TCAAAGCAGG CCCGAGTCGC CTGGATACCC CAGCTAGGAA
Didelphis virginiana        ATTAGAGTGT TCAAAGCAGG CCCGAGTCGC CTGGATACCC CAGCTAGGAA

Mus musculus                TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Rattus rattus               TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Homo sapiens                TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Oryctolagus cuniculus       TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Cricetulus griseus          TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Equus caballus              TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Bos taurus                  TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Erinaceus europaeus         TAATGGAATA GGACCGCGGT TCTATTTTGT TGGTTTTCGG AACTGAGGCC
Monodelphis domestica       TAATGGAATA GGACCCCGGT TCTATTTTGT TGGTTTTCGG AACTGGGGCC
Didelphis virginiana        TAATGGAATA GGACCCCGGT TCTATTTTGT TGGTTTTCGG AACTGGGGCC

Mus musculus                ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Rattus rattus               ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Homo sapiens                ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Oryctolagus cuniculus       ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Cricetulus griseus          ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Equus caballus              ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Bos taurus                  ATGATTAAGA GGGGCGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Erinaceus europaeus         ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGCGCCG CTAGAGGTGA
Monodelphis domestica       ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGTGCCG CTAGAGGTGA
Didelphis virginiana        ATGATTAAGA GGGACGGCCG GGGGCATTCG TATTGTGCCG CTAGAGGTGA

                                                        Univ18S rev
Mus musculus                AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Rattus rattus               AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Homo sapiens                AATTCCTTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Oryctolagus cuniculus       AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Cricetulus griseus          AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Equus caballus              AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Bos taurus                  AATTC-TTGG ATCGGCGCAA GACGGACCAG AGCGAAAGCA
Erinaceus europaeus         AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Monodelphis domestica       AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
Didelphis virginiana        AATTC-TTGG ACCGGCGCAA GACGGACCAG AGCGAAAGCA
```

2.2 *ompB* sequence comparison of *Rickettsia helvetica*

The following *R. helvetica* sequences were obtained in the present study and submitted to GenBank (accession numbers GU324464-65)

```
AF123725 R   GACTCTTAGC GGCGGTGTTC CTAATACCCC TGGTACAATT TACAGTTTAG GTATTGGTAA
GU324464 R   GACTCTTAGC GGCGGTGTTC CTAATACCCC TGGTACAATT TACAGTTTAG GTATTGGTAA
GU324465 R   GACTCTTAGC GGCGGTGTTC CTAATACCCC TGGTACAATT TACAGTTTAG GTATTGGTAA

AF123725 R   CGGTACACCA AAGTTAAAAC AAGTAACGTT TACTACAAAC TATAACAACT TAGGTAGTAT
GU324464 R   CGGTACACCA AAGTTAAAAC AAGTAACGTT TACTACAAAC TATAACAACT TAGGTAGTAT
GU324465 R   CGGTACACCA AAGTTAAAAC AAGTAACGTT TACTACAAAC TATAACAACT TAGGTAGTAT

AF123725 R   TATTGCAACC AACGCAACAA TTAATGACGG TTTAACTGTT ACTACAGGCG GTATAGCCGG
GU324464 R   TATTGCAACC AACGCAACAA TTAATGACGG TTTAACTGTT ACTACAGGCG GTATAGCCGG
GU324465 R   TATTGCAACC AACGCAACAA TTAATGACGG TTTAACTGTT ACTACAGGCG GTATAGCCGG
```

```
AF123725 R   AAAAGATTTC GACGGTAAAA TTACCCTTGG AAGTGCTAAT GGTAACTCTA ATGTAATTTT
GU324464 R   AAAAGATTTC GACGGTAAAA TTACCCTTGG AAGTGCTAAT GGTAACTCTA ATGTAATTTT
GU324465 R   AAAAGATTTC GACGGTAAAA TTACCCTTGG AAGTGCTAAT GGTAACTCTA ATGTAATTTT

AF123725 R   TGTTGACGGT ACAAACTCTA CTGCTACAAG TATGGTTGCT ACAGCTAAAG CTAATAACGG
GU324464 R   TGTTGACGGT ACAAACTCTA CTGCTACAAG TATGGTTGCT ACAGCTAAAG CTAATAACGG
GU324465 R   TGTTGACGGT ACAAACTCTA CTGCTACAAG TATGGTTGCT ACAGCTAAAG CTAATAACGG

AF123725 R   TACTGTAACT TATTTAGGTA GTGCAGCTGT AGGTAATATC GGTAGCTCTA ATGCTCTTGT
GU324464 R   TACTGTAACT TATTTAGGTA GTGCAGCTGT AGGTAATATC GGTAGCTCTA ATGCTCTTGT
GU324465 R   TACTGTAACT TATTTAGGTA GTGCAGCTGT AGGTAATATC GGTAGCTCTA ATGCTCTTGT

AF123725 R   AGCTTCTGTA AAATTTACGG GTCCTGCCGG TTCATTAGAA AAATTACAAG GAAATATATA
GU324464 R   AGCTTCTGTA AAATTTACGG GTCCTGCCGG TTCATTAGAA AAATTACAAG GAAATATATA
GU324465 R   AGCTTCTGTA AAATTTACGG GTCCTGCCGG TTCATTAGAA AAATTACAAG GAAATATATA

AF123725 R   TTCTACAGCC ACCAATTTTG GTAATGTTAA CTTAAATGTT GCTGGGTCGA ATATAATTTT
GU324464 R   TTCTACAGCC ACCAATTTTG GTAATGTTAA CTTAAATGTT GCTGGGTCGA ATATAATTTT
GU324465 R   TTCTACAGCC ACCAATTTTG GTAATGTTAA CTTAAATGTT GCTGGGTCGA ATATAATTTT

AF123725 R   AGGTGGCGAC ACAACTGCTA TTAATGGTAA TATTAATCTT GTAACAAATA CCTTAACATT
GU324464 R   AGGTGGCGAC ACAACTGCTA TTAATGGTAA TATTAATCTT GTAACAAATA CCTTAACATT
GU324465 R   AGGTGGCGAC ACAACTGCTA TTAATGGTAA TATTAATCTT GTAACAAATA CCTTAACATT

AF123725 R   TGAAAGCGGT ACTTCAACAT GGGGAAGTAA TACTTCTCTT AGCACAACGT TAACAGTATC
GU324464 R   TGAAAGCGGT ACTTCAACAT GGGGAAGTAA TACTTCTCTT AGCACAACGT TAACAGTATC
GU324465 R   TGAAAGCGGT ACTTCAACAT GGGGAAGTAA TACTTCTCTT AGCACAACGT TAACAGTATC

AF123725 R   AAACGGTAAT ATAGGTCATA TCGTTATTGC GGAAGGTGCT CAAGTTAATG TAACAACTAT
GU324464 R   AAACGGTAAT ATAGGTCATA TCGTTATTGC GGAAGGTGCT CAAGTTAATG TAACAACTAT
GU324465 R   AAACGGTAAT ATAGGTCATA TCGTTATTGC GGAAGGTGCT CAAGTTAATG TAACAACTAT

AF123725 R   AGGAACCACA ACCATTAACG TACAAGATAA TGCCAGTGCA AATTTCAGCG GTACAAAATA
GU324464 R   AGGAACCACA ACCATTAACG TACAAGATAA TGCCAGTGCA AATTTCAGCG GTACAAAATA
GU324465 R   AGGAACCACA ACCATTAACG TACAAGATAA TGCCAGTGCA AATTTCAGCG GTACAAAATA

AF123725 R   CTATACCTTA ATTGAAGGCG GTGCTCGTTT CAACGGTACT TTAAGAGATC CTA
GU324464 R   CTATACCTTA ATTGAAGGCG GTGCTCGTTT CAACGGTACT TTAAGAGATC CTA
GU324465 R   CTATACCTTA ATTGAAGGCG GTGCTCGTTT CAACGGTACT TTAAGAGATC CTA
```

2.3 *ompB* sequence comparison of *Rickettsia felis*

The following *R. felis* sequences were obtained in the present study and submitted to GenBank (accession numbers GU324466-70)

```
GQ329879 R   CACACTTAGC GGCGGTATTC CTAACACCCC AGGTACAATT TACGGTTTAG GTATAGAGAA
GQ385243 R   CACACTTAGC GGCGGTATTC CTAACACCCC AGGTACAATT TACGGTTTAG GTATAGAGAA
AF182279 R   CACACTTAGC GGCGGTATTC CTAACACCCC AGGTACAATT TACGGTTTAG GTATAGAGAA
GU324467 R   CACACTTAGC GGTGGTATTC CTAACACCCC TGGTACAATT TATGGCTTAG GTATAGAGAA
GU324468 R   CACACTTAGC GGTGGTATTC CTAACACCCC TGGTACAATT TATGGCTTAG GTATAGAGAA
GU324469 R   CACACTTAGC GGTGGTATTC CTAACACCCC TGGTACAATT TATGGCTTAG GTATAGAGAA
GU324470 R   CACACTTAGC GGTGGTATTC CTAACACCCC TGGTACAATT TATGGCTTAG GTATAGAGAA
GU324466 R   CACACTTAGC GGTGGTATTC CTAACACCCC TGGTACAATT TATGGCTTAG GTATAGAGAA

GQ329879 R   CGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GQ385243 R   CGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
AF182279 R   CGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GU324467 R   TGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GU324468 R   TGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GU324469 R   TGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GU324470 R   TGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT
GU324466 R   TGGTAGTCCG AAGTTAAAGC AAGTAACGTT TACTACAGAC TATAACAACT TAGGTAGTAT

GQ329879 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GQ385243 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
AF182279 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GU324467 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GU324468 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GU324469 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GU324470 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
GU324466 R   TATCGCAACT AACGCAACAA TTAATGACGG TGTAACTGTT ACTACAGGTG GTGTAGCCGG
```

```
GQ329879 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAT GGTAACGCTA ATGTAAGATT
GQ385243 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAT GGTAACGCTA ATGTAAGATT
AF182279 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAT GGTAACGCTA ATGTAAGATT
GU324467 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAC GGTAACGCTA ATGTAAGATT
GU324468 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAC GGTAACGCTA ATGTAAGATT
GU324469 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAC GGTAACGCTA ATGTAAGATT
GU324470 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAC GGTAACGCTA ATGTAAGATT
GU324466 R   AACAGATTTC GACGGTAAAA TTACCCTTGG AAGTGTTAAC GGTAACGCTA ATGTAAGATT

GQ329879 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GQ385243 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
AF182279 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GU324467 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GU324468 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GU324469 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GU324470 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG
GU324466 R   TGTTGACGGT ACATTTTCTG ATTCTACAAG TATGATTGTT ACTACTAAAG CTAATAACGG

GQ329879 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GQ385243 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
AF182279 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GU324467 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GU324468 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GU324469 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GU324470 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT
GU324466 R   TACTGTAACT TACTTAGGTA GTGCATTAGT CGGTAATATA GGTAGTTCAG ATACTCCTGT

GQ329879 R   AGCTTCTGTT AAATTTATAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GQ385243 R   AGCTTCTGTT AAATTTATAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
AF182279 R   AGCTTCTGTT AAATTTATAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GU324467 R   AGCTTCTGTT AAATTTACAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GU324468 R   AGCTTCTGTT AAATTTACAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GU324469 R   AGCTTCTGTT AAATTTACAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GU324470 R   AGCTTCTGTT AAATTTACAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC
GU324466 R   AGCTTCTGTT AAATTTACAG GTAGTGATGA TGGTGCAGGA TTACAAGGAA ATATTTATTC

GQ329879 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AAGTGTTTTA AATTCTAACG TAATTTTAGG
GQ385243 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AAGTGTTTTA AATTCTAACG TAATTTTAGG
AF182279 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AAGTGTTTTA AATTCTAACG TAATTTTAGG
GU324467 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AGGTGTTTTA AATTCTAACG TAATTTTAGG
GU324468 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AGGTGTTTTA AATTCTAACG TAATTTTAGG
GU324469 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AGGTGTTTTA AATTCTAACG TAATTTTAGG
GU324470 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AGGTGTTTTA AATTCTAACG TAATTTTAGG
GU324466 R   ACAAGTCACA GACTTTGGTA CTTATGACTT AGGTGTTTTA AATTCTAACG TAATTTTAGG

GQ329879 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GQ385243 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
AF182279 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GU324467 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GU324468 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GU324469 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GU324470 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC
GU324466 R   CGGCGGTACT ACTGCTATTA ACGGTGAAAT CGATCTTCTT ACAAATACCT TAACATTTGC

GQ329879 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GQ385243 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
AF182279 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GU324467 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GU324468 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GU324469 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GU324470 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA
GU324466 R   AAGCGGTACT TCAACATGGG GAAGCAATAC TTCTATTGAA ACTACTTTAA CAGTAGCAAA

GQ329879 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GQ385243 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
AF182279 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GU324467 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GU324468 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GU324469 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GU324470 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
GU324466 R   CGGTAATATA GGTCACATCG TTATTGCAGA AAATGCTCAA GTTAATGCAA CAACTACGGG
```

```
GQ329879 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGATA CGCAAACTTA
GQ385243 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGATA CGCAAACTTA
AF182279 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGATA CGCAAACTTA
GU324467 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGGTA CACAAACTTA
GU324468 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGGTA CACAAACTTA
GU324469 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGGTA CACAAACTTA
GU324470 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGGTA CACAAACTTA
GU324466 R    AACAACAACT ATTAACGTAC AAGATAATGC CAATGCAAAT TTCAGCGGTA CACAAACTTA

GQ329879 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GQ385243 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
AF182279 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GU324467 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GU324468 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GU324469 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GU324470 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA CGGTACTTTA GGAGGTCCTA
GU324466 R    TACTTTAATC CAAGGTGGTG CTAGATTTAA GGGTACTTTA GGAGGTCCTA
```

3 Trapping locations

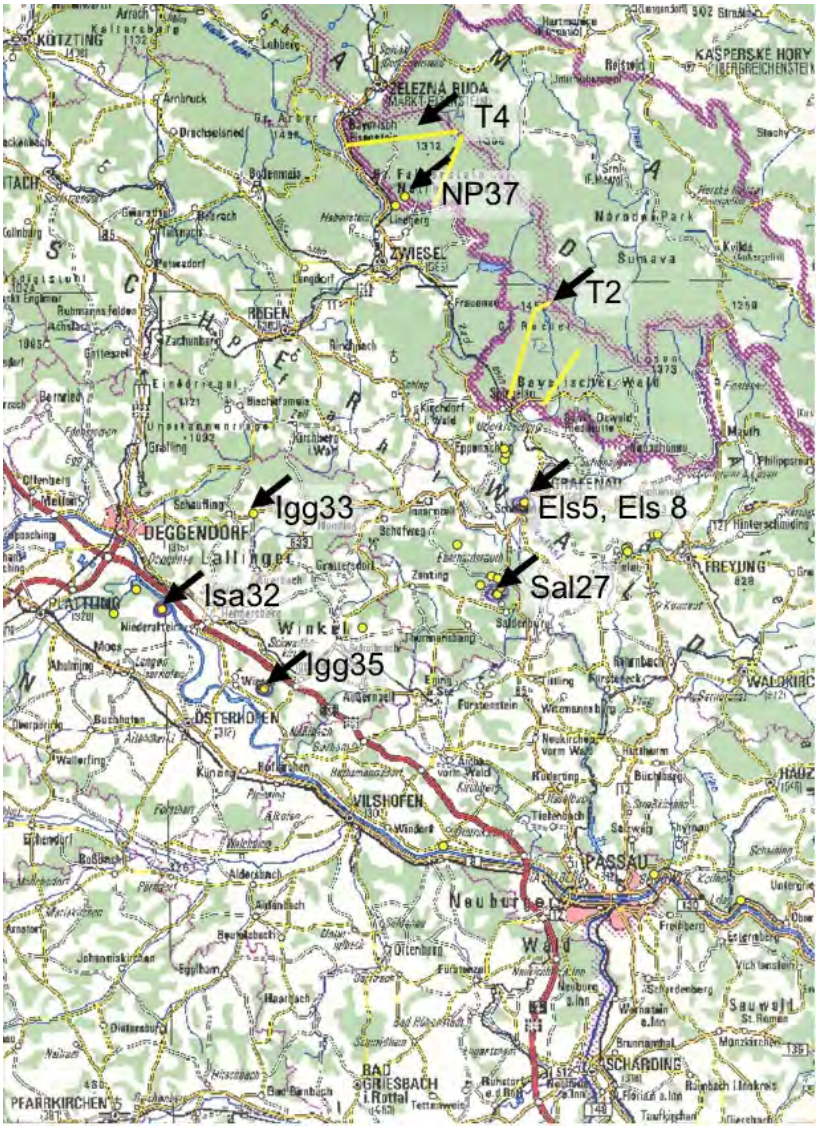

Figure 14: Topographic map of Lower Bavaria; trapping sites and transects from 2008 indicated with arrows

Table 13: Trapping sites and altitude in the National Park Bavarian Forest (2008)

m a.s.l.	trapping site
317	Isa 32
379	Igg 35
412	Igg 33
490	Sal 27
510	Els 5
578	Els 8
670	T4_29
707	T4_35
767	T4_39
827	T2_23
894	T4_47
949	T2_38
990	T2_44
1007	T4_51
1082	T2_50
1150	T4_59
1184	T2_54
1220	T4_72
1298	T4_78
1318	T2_73
1360	T2_Wsh
1420	T2_67

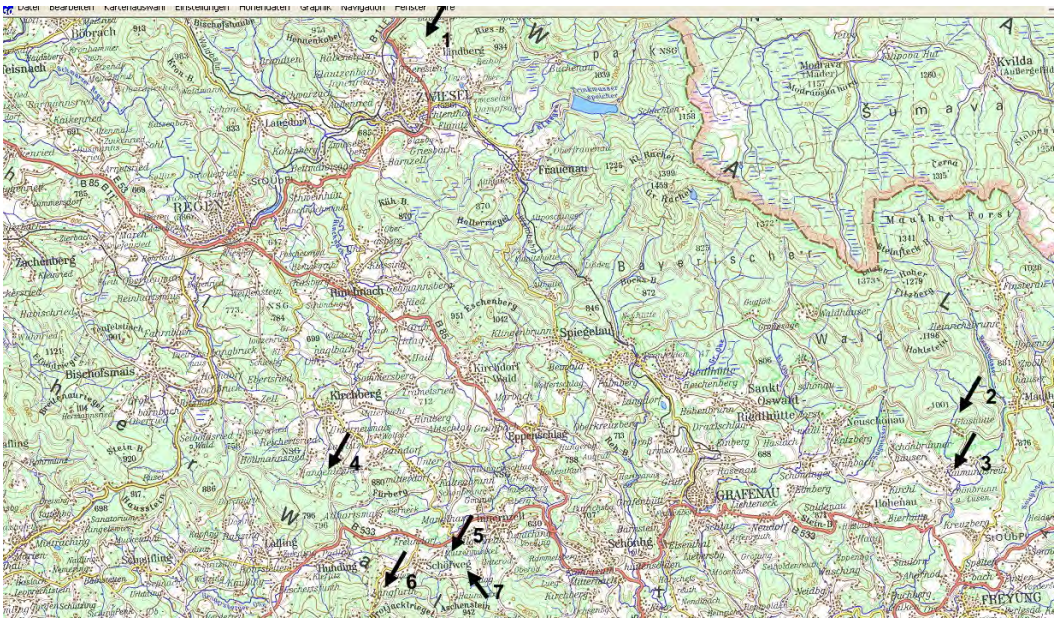

Figure 15: Topographic map (1:200000) of trapping sites from 2004 and 2005. 1: Falkenstein, 2: Glashütte, 3: Raimundsreuth, 4: Hangenleithen, 5: Mutzenwinkel 6: Langfurth, 7: Schöfweg (*generated with Top50, Version 3.0, Bayerisches Landesvermessungsamt Muenchen, 2001*)

4 Publication „Nagetier-übertragene Zoonosen: Beispiele aus Untersuchungen in Süd- und Westdeutschland“

Wirbeltierforschung in der Kulturlandschaft

Mitt. Julius Kühn-Inst. 421, 2009 37

Wirbeltierforschung in der Kulturlandschaft

Mitt. Julius Kühn-Inst. 421, 2009 37

Nagetier-übertragene Zoonosen: Beispiele aus Untersuchungen in Süd- und Westdeutschland

Essbauer, S.S.;[1] Schex, S.[1]; Splettstoesser, W.[1]; Pfeffer, M.[4]; Ulrich, R.G.[2]; Seibold, E.[1]; Dobler, G.[1]; Wölfel, R.[1]; Bäumler, W.[3]

[1]Institut für Mikrobiologie der Bundeswehr, München

[2]Friedrich-Loeffler-Institut, Institut für neue und neuartige Tierseuchenerreger, Greifswald - Insel Riems

[3]em. Studienfakultät für Forstwissenschaft & Ressourcenmanagement, Freising

[4]Institut für Tierhygiene und Öffentliches Veterinärwesen, Leipzig

Zusammenfassung

Nagetiere und andere Kleinsäuger können eine Vielzahl von Krankheitserregern, RNA- und DNA-Viren, Bakterien und Parasiten, auf den Menschen übertragen, die teilweise lebensbedrohliche Erkrankungen hervorrufen. In der folgenden Übersicht soll erstmals ein Überblick über Ergebnisse aus drei Untersuchungen in Deutschland gegeben werden: eine Studie in drei Landkreisen Bayerns von 2001-2004, Untersuchungen in einem Freilandgehege im Rahmen eines Tularämieausbruchs in Niedersachsen im Jahr 2005, und schließlich im Jahr 2007 eine Untersuchung an einem Truppenübungsplatz in Baden-Württemberg. Es wurde dabei exemplarisch die Verbreitung von Zoonoseerregern in Nagetieren und anderen Kleinsäugern näher untersucht, von drei Viren (Hantaviren, Kuhpockenvirus, Frühsommer-Meningo-Enzephalitis-Virus) und vier Bakterien (Leptospiren, Francisellen, Borrelien und Rickettsien). Die hier zusammengefassten Erkenntnisse sind ein erster wichtiger Schritt auf dem Weg zur Erstellung von Verbreitungskarten für die genannten humanpathogenen Zoonoseerreger in ihren Reservoirwirten und der Definition von entsprechenden Risikogebieten. Diese Arbeit soll zudem einen Beitrag leisten, einen Anstoß zu verstärkter Zusammenarbeit von Zoologen, Ökologen, Virologen, Human- und Veterinärmedizinern, Mikrobiologen, Parasitologen, Genetikern, Epidemiologen, Forstwissenschaftlern und Klimaforschern zu geben.

1. Nagetier-übertragene Krankheitserreger: eine Einleitung

Zoonosen sind Infektionskrankheiten, bei denen der Erreger vom oftmals nicht erkrankten Tierreservoirwirt auf den Menschen übertragen wird. Bei den mit Nagetieren und anderen Kleinsäugern assoziierten Zoonoseerregern handelt es sich um verschiedene RNA- und DNA-Viren, Bakterien und Parasiten. Sie unterscheiden sich nicht nur in ihrer genetischen Organisation, sondern vor allem in ihrer Assoziation mit spezifischen Reservoirwirten, ihrer geografischen Verbreitung und ihren Übertragungwegen. Diese Krankheitserreger können beim Menschen verschiedene, zum Teil lebensbedrohliche Erkrankungen hervorrufen.

Am Institut für Mikrobiologie der Bundeswehr (IMB) werden in Kooperation mit anderen Institutionen seit dem Jahr 2004 Untersuchungen zu Nagetier- und Kleinsäuger-übertragenen Krankheitserregern durchgeführt.

Diese Studien sind sowohl in Bundeswehr-internem Interesse, z.B. auf Anfragen von Kommandohygienikern, als auch im Rahmen einer zivil-militärischen Zusammenarbeit von großer Wichtigkeit für öffentliche Gesundheitsbehörden. Die bisherigen Felduntersuchungen wurden entweder aufgrund von Ausbruchsgeschehen (Hantaviren, Tularämie) initiiert oder dienten der Surveillance, um z.B. für Truppenübungsplätze eine Risikoabschätzung durchführen zu können.

1.1 Übertragungswege der Erreger

Der Mensch kann sich sowohl auf direktem als auch auf indirektem Wege mit Zoonoseerregern infizieren. Epidemiologisch gesehen stellt der Mensch meist einen Fehlwirt dar, d.h. die Infektion wird von ihm nicht auf weitere Personen übertragen. Bei den hier betrachteten Erregern sind Nagetiere (oder andere Kleinsäuger, wie Spitzmäuse oder Hasen) Reservoirwirt und Vektor.

Eine direkte Infektion kann über Urin, Speichel und Kot von Tieren erfolgen, wie es z.B. bei Infektionen mit Hantaviren, den Erregern der Tularämie (*Francisella tularensis*) und Leptospirose, Q-Fieber, und Arenavirus-Infektion bekannt ist. Eine Infektion über das Fell von toten oder lebenden Tieren kann bei Tularämie, bei der Lymphozytären Choriomeningitis (LCM) oder in manchen Fällen bei der Pest stattfinden. Durch Bisse werden Rattenbisskrankheit (*Spirillum minus, Streptobacillus moniliformis),* Leptospiren und Kuhpockenviren übertragen. Über die Aufnahme kontaminierter Nahrungsmittel, vor allem dem sogenannten „bush meat“, können, vornehmlich in Afrika, auch Menschen z.B. mit den Erregern der Affenpocken infiziert werden oder an Pest oder Lassa erkranken.

Eine indirekte Infektion kann über verschiedene blutsaugende Vektoren erfolgen. Einen wichtigen Vektor stellen Arthropoden dar, die durch Stiche oder Bisse beispielsweise Anaplasmen, Arenaviren, Alphaviren, Bunyaviren, Flaviviren wie das Frühsommer-Meningo-Enzephalitis-Virus (FSMEV), Borrelien und Francisellen vom Nagetier auf den Menschen übertragen können. Ein weiterer Infektionsweg ist das Einatmen von kontaminierten Stäuben, wodurch beim Menschen Infektionen mit Hantaviren, Tularämieerregern, Leptospiren oder Arenaviren entstehen können.

1.2 Kurzcharakteristik der untersuchten Erreger

Zu den am IMB und in Projekten am Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin der Ludwig-Maximilians-Universität untersuchten Nagetier- und Kleinsäuger- übertragenen Erregern zählen Hantaviren, Kuhpockenviren, FSMEV, Leptospiren, Francisellen, Borrelien, sowie Rickettsien.

1.2.1 Hantaviren

Hantaviren, Familie *Bunyaviridae*, sind behüllte Viren mit einem segmentierten RNA-Genom negativer Polarität. Die Infektion des Menschen erfolgt vornehmlich über mit Faeces, Urin und Speichel von infizierten Nagern kontaminierten Staub, oder auch über direkten Kontakt mit den Nagetieren. Die Wildnager sind persistent mit den Erregern infiziert, zeigen jedoch keine auffälligen Krankheitssymptome. Jede einzelne Hantavirus-Art ist in der Regel mit einer bestimmten Nagetierart oder nahe verwandten Arten assoziiert: die Rötelmaus *(Myodes glareolus*) überträgt ausschließlich das Puumalavirus, die Gelbhalsmaus (*Apodemus flavicollis*) eine genetische Linie des Dobrava-Belgrad- Virus (DOBV-Af), die Brandmaus (*Apodemus agrarius*) eine zweite genetische Linie des Dobrava- Belgrad-Virus (DOBV-Aa), die Feldmaus (*Microtus arvalis*) das Tulavirus und die Wanderratte (*Rattus norvegicus*) das Seoulvirus (Übersicht in Schönrich et al., 2008).

In Abhängigkeit von der Hantavirus-Art können beim Menschen grippeähnliche Erkrankungen bis hin zu Nephropathien (*Nephropathia endemica*) und hämorrhagischen Fiebern auftreten. Von den in Europa vorkommenden Hantaviren besitzt das DOBV-Af die höchste, das Tulavirus vermutlich die niedrigste

Virulenz. In Deutschland sind bisher vor allem Fälle von Puumalavirus-Infektionen (Süd- und Südwestdeutschland) und einige Fälle von DOBV-Aa-Infektionen (Nordostdeutschland) berichtet worden. Im Gegensatz dazu verursachen bestimmte nur in Amerika vorkommende Hantaviren, wie Sin Nombre-Virus und Andesvirus, weitaus schwerere Erkrankungen mit Lungenmanifestation, sogenanntes Hantavirales kardiopulmonales Syndrom, mit einer Letalität von bis zu 40% (Übersicht in Krüger et al., 2001).

Klinisch apparente Hantavirus-Infektionen sind in Deutschland meldepflichtig (Tabellen 1 und 2). Im Jahre 2007 kam es zu einem sehr starken Anstieg der Zahl der gemeldeten Fälle in Baden-Württemberg und Bayern was auch aus anderen Regionen Europas berichtet wurde (Ulrich et al., 2008a).

1.2.2 Kuhpockenviren

Kuhpockenviren, Gattung *Orthopoxvirus*, Familie *Poxviridae*, sind behüllte, komplex aufgebaute Viren. Sie besitzen ein Genom aus doppelsträngiger, linearer DNA von etwa 220-230 kbp Größe, das für etwa 200 Proteine kodiert.Kuhpockenviren werden zumeist durch freilaufende Katzen (Mäusefänger, „Schmusetiere“), manchmal auch durch erkrankte Elefanten oder Ratten auf den Menschen übertragen (Essbauer et al., 2006a). Die erhöhte Jagdaktivität der Katzen in den Sommermonaten führt zu einer saisonalen Verteilung der Erkrankungen mit Spitzen in den Monaten Juli bis Oktober. Während infizierte Nagetiere in der Regel symptomlos bleiben, entwickeln Katzen umschriebene, aber auch großflächige Wunden, über die Virus ausgeschieden wird. Viele Katzen verenden. Beim Menschen verursacht eine Infektion Pocken-ähnliche Hautausschläge (Exantheme) oft an Händen und Unterarmen, die bei falscher Behandlung auch wochenlang anhalten können und unter Umständen mit Narbenbildung verheilen. In Deutschland gab es bis zu der hier vorliegenden Studie keine Daten über Infektionen bei wildlebenden Reservoirtieren (Essbauer et al., 2004, 2006a).

Kuhpockenvirus-Infektionen sind in Deutschland bislang nicht meldepflichtig.

1.2.3 Frühsommer-Meningo-Enzephalitis-Virus

Das FSMEV ist ein behülltes, 40-50 nm großes RNA-Virus aus der Familie *Flaviviridae*. Dieses Arbovirus wird durch Zecken der Gattung Ixodes (vor allem *Ixodes ricinus, Ixodes persulcatus)* übertragen. Infektionen des Menschen können aber auch über orale Aufnahme von Rohmilchprodukten erfolgen. Infektionen mit dem europäischen Virustyp verlaufen in der Regel milder, während die mit den sibirischen und fernöstlichen Virustypen mit schweren Erkrankungen und hoher Letalität einhergehen können. In Westeuropa beträgt die Letalität 0,5-2%. Die Erkrankung verläuft meist biphasisch, mit unspezifischen Symptomen (Fieber, Myalgie, Kopfschmerz) in der ersten Phase, einem beschwerdefreien Intervall von etwa einer Woche und einer zweiten Fieberphase, wobei es zur Ausbildung einer aseptischen Meningitis, Meningo-Enzephalitis und im schlimmsten Fall zur Meningo-Enzephalomyelitis kommen kann. Das FSMEV ist der einzige Nagetier-assoziierte Erreger in Deutschland, gegen den ein zugelassener Impfstoff zur Anwendung beim Menschen verfügbar ist (Übersichten in Suess et al., 2004; Dobler et al., 2005)

Die Frühsommer-Meningo-Enzephalitis ist in Deutschland meldepflichtig, wobei die meisten Fälle in Süddeutschland in den Bundesländern Bayern und Baden-Württemberg registriert werden. (Tabellen 1 und 2).

Tab. 1 Auftreten von gemeldeten Erkrankungsfällen durch die in dieser Arbeit untersuchten Nagetier-übertragenen Erreger in Deutschland im Zeitraum von 2004 bis 2008 (Robert Koch-Institut: SurvStat, http://www3.rki.de/SurvStat, Datenstand: 01.04.09)

Erkrankung	Erreger	Anzahl gemeldeter Fälle (Inzidenz/100.000)				
		2004	2005	2006	2007	2008
Vektor-vermittelte Übertragung (Nagetiere oder andere Kleinsäuger als Reservoir)						
Viren						
Frühsommer-Meningo-Enzephalitis (FSME)	FSME-Virus	274 (0,3)	432 (0,5)	541 (0,7)	238 (0,29)	288
Bakterien						
Hasenpest (Tularämie)	*Francisella tularensis*	3 (<0,1)	15 (<0,1)	1 (<0,1)	20 (<0,1)	15
Lyme Borreliose[§]	*Borrelia* sp.	4.479	5.461	6.248	5.916	5717
Epidemisches Fleckfieber	*Rickettsia prowazekii*	0	0	0	0	0
B – Nagetiere als Reservoir oder Überträger						
Viren						
Hämorrhagisches Fieber mit renalem Syndrom/ Nephropathia epidemica	Hantaviren	242 (0,3)	448 (0,5)	73 (0,1)	1.687 (2,05)	243
Bakterien						
Leptospirose	*Leptospira interrogans*	58 (<0,1)	58 (<0,1)	45 (<0,1)	165 (0,2)	37

[§] nur in einigen Bundesländern meldepflichtig

Tab. 2 Auftreten der meldepflichtigen Erkrankungen in den Bundesländern, in denen Untersuchungen zu den Erregern in Nagetieren durchgeführt wurden, im Zeitraum von 2004 bis 2008 (Quelle: RKI, Survstat Datenstand: 01.04.09)

Jahr	Bundesland	Zahl der gemeldeten Erkrankungen in den Jahren 2004-2008				
	Regierungsbezirk/ Landkreis	FSME (FSME-Virus)	Tularämie (*Francisella tularensis*)	Fleckfieber (*Rickettsia* spp.)	HFRS/NE (Hantaviren)	Leptospirose (*Leptospira* spp)
2004/5/ 6/7/8	**Bayern**	**130/165/281/96/ 128**	**2/1/0/3/0**	**0/0/0/0/0**	**120/110/22/ 296/41**	**11/12/9/26/20**
	Fürstenfeldbruck	0/2/0/0/0	0/0/0/0/0	0/0/0/0/0	1/0/0/1/1	0/0/1/1/0
	Erlangen	2/0/0/1/3	0/0/0/0/0	0/0/0/0/0	0/0/0/0/1	0/0/0/1/0
	Traunstein	5/8/4/4/4	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/1/1
	Baden-Württemberg	**102/211/168/ 109/130**	**0/0/1/11/2**	**0/0/0/0/0**	**61/41/12/1089/ 74**	**7/10/8/35/13**
	Sigmaringen	1/2/3/0/1	0/0/0/0/0	0/0/0/0/0	0/0/1/49/1	0/0/0/1/1
	Niedersachsen	1/2/0/2/4	0/1/0/1/2	0/0/0/0/0	11/75/6/93/18	10/6/6/15/7
	Göttingen	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/11/0/1/0	0/0/0/0/0

HFRS, Hämorrhagisches Fieber mit renalem Syndrom; NE, Nephropathia epidemica

1.2.4 Leptospiren

Die Leptospirose wird durch eine Infektion mit Gram-negativen Bakterien der Gattung *Leptospira* aus der Familie der Spirochäten verursacht. Leptospiren kommen weltweit vor und werden gemäß der aktuellen Klassifizierung in 16 Arten unterteilt, wovon sieben Arten humanpathogen sind. Gegenwärtig findet sich allerdings häufig immer noch die frühere Einteilung in über 240 Serogruppen. Die Verbreitung des Erregers erfolgt über den Urin persistent infizierter Nager. Dabei kann der Erreger über mit Nagerurin verschmutztes Wasser in kleinste (Schleim-)Hautverletzungen des Menschen eindringen.

Auch ein direkter Kontakt zu Nagetieren spielt bei der Übertragung der Leptospiren eine Rolle. Außer dem Menschen erkranken auch Pferde, Hunde und Schweine an Leptospirose. Leptospiren sind bewegliche Bakterien, die aktiv in Organe invadieren können. Sie sind außerordentlich stabil und können im Wasser über

drei Monate infektiös bleiben. Leptospiren verursachen beim Menschen verschiedene Krankheitsbilder, wie z.B. die Weil-Krankheit, das sogenannte Feldfieber, die Schweinehüterkrankheit und das Batavia-Fieber. Die Symptome reichen von milden und unspezifischen, grippeähnlichen Symptomen mit Myalgien und Konjunktivitis, über ikterische Verläufe mit Fieber und Übelkeit, Meningitis bis zu Nieren- bzw. tödlichem Multiorganversagen (Palaniappan et al., 2007). Die Letalität schwankt unbehandelt zwischen 5 und 40% (eine Übersicht zu Erkankungen in Deutschland in Jansen et al., 2005).

Die Leptospirose ist in Deutschland meldepflichtig (Tabellen 1 und 2). Hier kam es im Jahr 2007 – vergleichbar mit dem Auftreten von Hantavirus-Infektionen – zu einem Anstieg der Erkrankungen in Bayern, Baden-Württemberg und Niedersachsen, den in den Studien untersuchten Regionen.

1.2.5 Francisellen

Auch die Tularämie (syn. Hasenpest) wird von einem bakteriellen Erreger, *Francisella tularensis*, verursacht. Francisellen sind kleine, Gram-negative, pleomorphe, unbewegliche Bakterien, die eine Kapsel bilden können und ein spezifisches Lipopolysaccharid (LPS) aufweisen. Francisellen werden heute in die Gruppe der γ-Proteobakterien eingeordnet und sind damit verwandt mit Legionellen und Coxiellen. Zwei Unterarten, *Francisella tularensis* ssp. *tularensis* (Nordamerika) und ssp. *holarctica* (Europa und Asien) spielen eine Rolle als humanpathogene Erreger. Sowohl zahlreiche Säugetierspezies (>150) als auch Menschen erkranken. Zecken, Stechmücken und Bremsen fungieren dabei als Vektoren.

Francisellen bleiben sehr lange infektiös, im Wasser und Boden über Monate hinweg, in gefrorenen Tierkörpern sogar bis zu 3 Jahre lang.

Drei bis zehn Bakterien über ein Aerosol aufgenommen, reichen bereits für eine Infektion des Menschen aus. Die Letalität beträgt abhängig von der Unterart 5-10% (Sjöstedt, 2007).

Beim Menschen unterscheidet man äußere lokalisierte (ulcero-glanduläre) Formen, die mit Geschwüren, Lymphadenitis und Konjunktivitis einhergehen, und innere (invasive) Formen, die sich meist in Pneumonien, aber auch in Abszessen und Magen-Darm-Symptomatik äußern. Betroffene Tiere sterben meist innerhalb von vier bis sechzehn Tagen, in protrahiert verlaufenden Fällen überleben sie bis zu 60 Tage. Krankheitszeichen sind äußerlich nicht immer erkennbar, was das Risiko gerade für Jäger noch erhöht.

Die Tularämie ist in Deutschland meldepflichtig (Tabellen 1 und 2). In den Jahren 2005 und 2007 trat die Erkrankung erstmals seit über 40 Jahren wieder gehäuft auf.

1.2.6 Borrelien

Borrelien gehören wie die Leptospiren zur Familie der Spirochäten. Die in Europa wichtigsten humanpathogenen Borrelien-Arten zählen zum *B. burgdorferi* sensu lato-Komplex. Sie werden durch Zecken der Gattung *Ixodes ricinus* übertragen, wobei Nagetiere und andere Kleinsäuger als Reservoir dienen. Eine Infektion beim Menschen kann zur Ausbildung der sogenannten Lyme-Borreliose führen.

Bei dieser Erkrankung unterscheidet man drei Phasen: eine Frühmanifestation meist an der Haut (bei ca. 50% der Fälle Hautrötung rund um die Stichstelle, *Erythema migrans*), eine frühe Allgemeinmanifestation vor allem an inneren Organen (z.B. als Neuroborreliose, Polyradikuloneuritis, Arthritis, Karditis, bei Kindern auch als lymphozytäre Meningitis), und eine späte Allgemeinmanifestation (chronische Arthritis, *Acrodermatitis chronica atrophicans*, chronische Enzephalomyelitis, chronische Polyradikulopathie). Erkrankungen sind auch bei Hund, Katze, Pferd und Rind nachgewiesen und äußern sich außer durch unspezifische Allgemeinsymptome auch durch Arthritiden (Fingerle & Wilske, 2006; Wilske et al., 2007).

In einigen Bundesländern Deutschlands (Berlin und die neuen Bundesländer) ist die Borreliose seit 2002 meldepflichtig (Tabelle 1).

1.2.7 Rickettsien

Rickettsien sind obligat intrazelluläre, Gram-negative Bakterien, deren Größe mit 0,3 × 0,7 µm im Bereich zwischen den kleinsten Bakterien und den größten Viren liegt. Man unterschiedet zwei Gruppen: die Zeckenbissfieber-Rickettsien (*Rickettsia conorii, R. helvetica. R. felis*), die durch verschiedene Zecken und eine Milbenart übertragen werden, und die Fleckfieber-Rickettsien (*R. prowazekii* und *R. typhi*), die durch Flöhe und Läuse übertragen werden. Als Reservoir dienen je nach Rickettsien-Spezies verschiedene Vögel, Reptilien und Säugetiere, und hier vor allem Nagetiere. Rickettsien befallen die Endothelzellen der kleinen Blutgefäße und verursachen eine Vaskulitis. Typischerweise äußern sich die Rickettsiosen durch Fieber, Kopfschmerzen und ein als „Eschar" bezeichnetes Ulkus an der Einstichstelle des übertragenden Ektoparasiten sowie ein generalisiertes Exanthem. Die Letalität schwankt je nach verursachender Rickettsien-Art, kann jedoch für das Epidemische Fleckfieber (*R. prowazekii*) oder das Rocky Mountain-Fleckfieber (*R. rickettsii*) unbehandelt bis zu 30% betragen (Parola et al., 2005; Wölfel et al., 2006).

Das Fleckfieber ist in Deutschland meldepflichtig (Tabellen 1 und 2). Seit 2003 wurden keine Fälle gemeldet.

1.3 Aktueller Wissensstand über die Verbreitung der hier vorgestellten Nagetier-assoziierten Erreger in Deutschland

Seit Inkrafttreten des Infektionsschutzgesetzes am 1. Januar 2001, in dem unter anderem auch die Meldepflicht für humane Infektionen durch bestimmte Zoonoseerreger geregelt ist, hat sich die Datenlage zur geographischen Verbreitung und Häufigkeit von durch diese Zoonoseerreger verursachten Krankheiten wesentlich verbessert. Vermutlich ist jedoch die Dunkelziffer für diese Infektionen immer noch sehr hoch, da viele der hier genannten Infektionen oft nur milde Verläufe und unspezifische Symptome verursachen und somit wahrscheinlich oft gar nicht oder nicht richtig diagnostiziert werden.

Die gemäß IfSG gemeldeten Daten (Tabellen 1 und 2) erlauben nur einen indirekten Einblick in die geographische Verbreitung der Erreger in Deutschland. Diesbezüglich aussagefähiger sind Untersuchungen in den Nagetier- und Kleinsäugerreservoirs.

Aus diesem Grunde wurden in den Jahren 2001-2004 in drei Landkreisen Bayerns, im Jahr 2005 in einem Landkreis in Niedersachsen und im Jahr 2007 in einem Landkreis in Baden-Württemberg Untersuchungen zum Vorkommen der oben beschriebenen Zoonoseerreger in den potentiellen Nagetierreservoirs durchgeführt, die hier vorgestellt werden sollen.

2. Material & Methoden

2.1. Untersuchungsgebiete

2.1.1 Longitudinalstudie in drei Landkreisen Bayerns während der Jahre 2001-2004

Von September 2001 bis März 2004 wurden in Grafrath bei München, Landkreis (LK) Fürstenfeldbruck, auf einem im Wald gelegenen Gartengrundstück Mäuse gefangen (Abb. 1). Im LK Erlangen wurden die Gebiete Frauenaurach (Mischwald), Klosterholz (Wasserschutzgebiet) und Dietenhofen (Hügelwald) von März bis September 2003 beprobt (Abb. 1). Im LK Traunstein wurden an den Orten Bürgerwald, Geißing, Burkhartsöd (Moorgebiet) und auf einer Wiese am Stadtrand im gleichen Zeitraum Mäuse gefangen (Abb. 1A, B). Alle Probeflächen befanden sich in der Nähe von Naherholungsgebieten mit Trimm-Dich-Pfaden und beliebten Spazierwegen. Bei den Probepunkten handelte es sich meist um Orte, die aufgrund ihrer Vegetation und Beschaffenheit ideale Lebensbedingungen für Mäuse bieten, z.B. Laubwälder mit dichtem Unterholz, Brombeer-/Himbeerbewuchs und dicht wachsendem Springkraut (*Impatiens glandulifera*). Hier fanden mittels PCR Untersuchungen auf Borrelien und Leptospiren statt.

Antikörper gegen Kuh(Ortho-)pockenviren wurden serologisch nachgewiesen mit einem

Plaquereduktionsneutralisationstest, Antikörper gegen FSMEV mittels einer *in house*-Immunfluoreszenz.

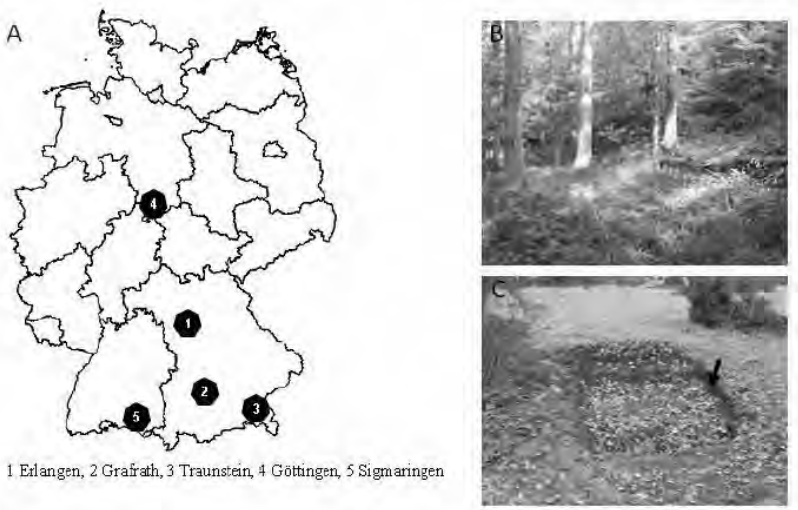

Abb.1 A Geographische Lage der untersuchten Regionen in Deutschland (1 Erlangen, 2 Grafrath, 3 Traunstein, 4 Göttingen, 5 Sigmaringen), B Hügel-Buchenwald mit krautigem Unterbewuchs als typisches Habitat für Rötelmäuse im Landkreis Erlangen, C Mäusegänge an Zeltgräben (Biwak) am Truppenübungsplatz Heuberg, Landkreis Sigmaringen.

2.1.2 Untersuchungen in Sennickerode, Landkreis Göttingen, Niedersachsen, 2005

Im Rahmen eines Tularämieausbruches wurden in einem Freilandgehege von Krallenäffchen (*Callithrix jacchus*) in Sennickerode Mäuse gefangen (Splettstoesser et al., 2007). Während der Fangaktion schien die Dichte der Nagerpopulation in diesem Freilandgehege sehr hoch zu sein, möglicherweise wegen des dichten Bewuchses mit hohem Gras und der Nutzung des für die Affen bestimmten Futters und Strohs. Außerdem wurde in den Gehegen keine Schadnagerbekämpfung durchgeführt, um eine indirekte Vergiftung der Affen durch Fangen und Fressen von kontaminierten Nagetieren zu vermeiden.

Fünf der verstorbenen Krallenäffchen wurden mithilfe eines Antigen-ELISA und einer Francisellen-spezifischen PCR positiv auf *Francisella tularensis holarctica* getestet, auch die Anzucht des Erregers war möglich.

2.1.3 Untersuchungen am Truppenübungsplatz Heuberg, Landkreis Sigmaringen, Baden-Württemberg 2007

Nach Auftreten von Hantavirus-Erkrankungsfällen wurden auf dem Truppenübungsplatz Heuberg, Landkreis Sigmaringen, Mäuse gefangen (Abb. 1A, C). Ziel dieser Untersuchungen war es, eine Risikoabschätzung für diesen Truppenübungsplatz leisten zu können. Die Tiere wurden anschließend serologisch und mittels RT-PCR auf Hantaviren und Rickettsien (Serologie anti-*R. conorii* und anti-*R. helvetica*) untersucht. Zur Untersuchung auf Kuhpocken wurden PCR und Anzucht verwendet.

2.2 Mäusefang

Beprobungsorte wurden zunächst „vorgeködert“, d.h. es wurden ausgewaschene 500 ml fassende große Joghurtbecher mit Apfelködern der Sorte Golden Delicious bestückt und in 10 m Abständen in parallel verlaufenden Reihen ausgebracht. Nach 24 Stunden wurden die Köderbecher auf Urin- und Kotspuren überprüft. Derart markierte Becher wurden durch sogenannte Sherman-Lebendfallen mit dem gleichen Köder ersetzt, welche zweimal täglich kontrolliert wurden. Die gefangenen Mäuse wurden tierschutzgerecht getötet, vermessen, gewogen und Blut und die benötigten Organe (Lunge, Niere, Leber, Milz, Gehirn, Ohren u.a.) unter aseptischen Bedingungen entnommen. Die Artbestimmung erfolgte bei allen Untersuchungen primär morphologisch, teilweise wurde die Artbestimmung zusätzlich durch Amplifikation des mitochondrialen

Cytochrom b-Gens mit anschließender Sequenzierung der PCR-Produkte verifiziert.

2.3 Untersuchung auf die Erreger

2.3.1 Direkter Erregernachweis

Kleine Organteile wurden mittels Schrotkugeln in der Mixermill (Studie von 2001-2004 in Bayern) oder mittels Fastprep-Gerät (alle anderen Studien) homogenisiert und Nukleinsäuren unter Verwendung von kommerziellen Kits manuell oder automatisert isoliert. Für die Untersuchungen wurden konventionelle PCRs bzw. RT-PCRs von Nukleinsäurepräparationen aus Ohr, Lunge, Milz und Gehirn zum Nachweis von Borrelien (Kießling, 2005), Hantaviren (Essbauer et al., 2006b) bzw. FSMEV (Kießling, 2005) verwendet.

Zur Untersuchung auf Leptospiren (Smythe et al., 2002), Rickettsien (Wölfel et al., 2008) und Francisellen (Splettstoesser et al., 2007) wurden jeweils Echtzeit-PCR-Verfahren mit extrahierter Nukleinsäure aus Niere, Ohren, Gehirn und Leber durchgeführt.

2.3.2 Indirekter Erregernachweis durch serologische Untersuchungen

Für den indirekten Nachweis von anti-FSMEV- und anti-Rickettsien-Antikörpern in Nagerseren wurden *in house*-Immunfluoreszenztests unter Verwendung infizierter Verozellen verwendet (Wölfel et al., 2006; Dobler unpubliziert). Orthopockenvirus-reaktive Antikörper wurden mittels eines Plaquereduktionsneutralisationstests bestimmt (Essbauer et al., 2004).

3. Ergebnisse

3.1 Nachweis von Infektionen mit Borrelien, Leptospiren, Kuhpockenviren und FSMEV in Nagern aus drei Landkreisen Bayerns während der Jahre 2001-2004

In den bayerischen Gebieten (siehe Abb. 1) wurden in dem angegebenen Zeitraum insgesamt 836 Mäuse und Kleinsäuger gefangen, wovon die meisten Tiere (606) auf das Gebiet Grafrath entfielen (Tab. 3).

Antikörper gegen Orthopockenviren wurden in insgesamt 39 Tieren (5,6%) nachgewiesen, wobei hier die Rötelmaus (*Myodes glareolus*) mit 18,8% im Gebiet Grafrath am stärksten betroffen war (Tab. 3).

In Traunstein wurden bei der Untersuchung von 67 Tieren keine Orthopockenviren-reaktiven Antikörper nachgewiesen.

FSMEV-reaktive Antikörper wurden in insgesamt 67 von 633 untersuchten Tieren (10,6%) gefunden (Tab. 3). Auch hier war die Prävalenz im Gebiet Grafrath jedoch bei der Gelbhalsmaus (*Apodemus flavicollis*) am höchsten (14,8%).

In insgesamt 17 von 266 getesteten Tieren (6,4%) wurde mittels PCR Leptospiren-spezifische DNA gefunden, wobei die Tiere aus Traunstein und Erlangen mit Ausnahme einer Gelbhalsmaus nicht auf Leptospiren getestet wurden. Auch hier waren Gelbhalsmäuse (*Apodemus flavicollis*; 11,3% in Grafrath, 25% an anderen Orten) die häufigsten Träger von Leptospiren.

Bei den PCR-Untersuchungen wurde bei insgesamt 91 Tieren (10,8%) aus vier verschiedenen Arten Borrelien-DNA nachgewiesen (Tab. 3). Insgesamt wurden Borrelien-Infektionen am häufigsten bei der Gelbhalsmaus (*Apodemus flavicollis*), Rötelmaus (*Myodes glareolus*) und Feldmaus (*Microtus arvalis*) gefunden, wobei sich die Häufigkeit der Borrelien-Infektionen in den einzelnen Nagetier-Arten von Fangort zu Fangort unterschied. Die höchste durchschnittliche Borrelien-Durchseuchung wurde mit 34,3% in Traunstein gefunden. Auch die Durchseuchung bei Rötelmäusen war in Traunstein am höchsten (44,1%).

Tab. 3 Nachweis von Infektionen mit Borrelien, Leptospiren, Kuhpockenviren und FSMEV in Wildmäusen von verschiedenen Beprobungspunkten in Bayern

	Untersuchte Tiere		Direkter oder indirekter Erregernachweis							
Fangort	**Familie***	**Spezies**	**Borrelien nPCR**		**Kuhpocken NT>1:24**		**FSMEV IFAT**		**Leptospiren PCR**	
			pos/ges	(%)	pos/ges	(%)	pos/ges	(%)	pos/ges	(%)
Erlangen	Muridae	*Apodemus flavicollis*	18/ 57	31,6	0/51	0	4/40	10,0	0/1	0
	Cricetidae	*Myodes glareolus*	11/56	19,6	1/47	2,1	3/45	6,6	0	0
	Cricetidae	Andere Arten[1]	0/9	0	1/1	k.A.	1/1	k.A.	0	0
		Gesamt	**29/122**	**23,7**	**2/112**	**1,8**	**8/86**	**9,3**	**0/1**	**0**
Grafrath	Muridae	*Apodemus flavicollis*	17/267	6,4	0/229	0	31/210	14,8	8/71	11,3
		Apodemus sylvaticus	2/43	4,7	1/33	2,9	1/35	2,9	1/12	8,3
	Cricetidae	*Myodes glareolus*	11/233	4,7	36/156	18,8	21/173	12,1	1/117	0,9
		Microtus arvalis	4/59	6,8	0/51	0	0/32	0	4/48	8,3
	Cricetidae	Andere Arten[2]	0/4	0	n.d.	k.A.	0/2	0	0/1	0
		Gesamt	**34/606**	**5,6**	**37/469**	**7,3**	**53/452**	**11,7**	**14/249**	**5,6**
Traunstein	Muridae	*Apodemus flavicollis*	2/8	25,0	0/5	0	0/6	0	0	-
	Cricetidae	*Myodes glareolus*	19/43	44,1	0/34	0	4/33	12,1	0	-
		Microtus arvalis	2/15	13.3	0/14	0	1/14	7,1	0	-
		Microtus agrestis	0/1	0	0/1	0	n.d.	k.A.	0	-
		Gesamt	**23/67**	**34,3**	**0/67**	**0**	**5/53**	**9,4**	**0**	-
Andere **	Muridae	*Apodemus flavicollis*	2/16	12,5	n.d.	k.A.	1/9	11,0	1/4	25,0
		Apodemus sylvaticus	0/11	0	0	0	0/10	0	0/1	-
	Cricetidae	*Myodes glareolus*	3/12	25,0	0	0	0/9	0	1/9	11,0
		Microtus sp	0/2	0	0	0	0/14	0	1/1	kA
		Gesamt	**5/41**	**12,1**	**0**	**0**	**1/42**	**2,4**	3/16	18,5
Gesamt			**91/836**	**10,8**	**39/699**	**5,6**	**67/633**	**10,6**	**17/266**	**6,4**

* die Taxonomie folgt der von Wilson und Reeder (2005): 1 *Microtus agrestis, Microtus arvalis, Arvicola amphibius*; 2 *Microtus agrestis, Arvicola amphibius;* ** Freising, Raitenhaslach, Stadtbereich München, Ulm, Breitasch, Ramersdorf, Oberschleißheim, Pommersfelden, Mehring, Singen; n.d., nicht durchgeführt; k.A., keine Angaben; nPCR, nested PCR

3.2 Nachweis von Infektionen mit Francisellen und Hantaviren in Nagetieren aus Sennickerode, Niedersachsen, 2005

In Sennickerode (Abb 1.) wurden insgesamt 144 Tiere gefangen, davon 28 Gelbhalsmäuse (*Apodemus flavicollis*), 62 Rötelmäuse (*Myodes glareolus*), 12 Feldmäuse (*Microtus arvalis*) und 37 Schermäuse (*Arvicola amphibius*, vormals *A. terrestris*).

Mittels PCR-Untersuchung aller Tiere wurde bei 10 Tieren (6,9%) *Francisella tularensis*–spezifische DNA nachgewiesen (Tab. 4). Der höchste Anteil entfiel dabei auf die Schermaus (*Arvicola amphibius*) mit 6 positiven Tieren (16,2%).

Insgesamt 134 der 144 gefangenen Tiere wurden mittels Hantavirus-S-Segment-spezifischer RT-PCR untersucht (Essbauer et al., unveröffentlichte Daten). Dabei zeigten 13 Tiere (9,7%) ein Amplifikat der erwarteten Größe (Tab. 4), wobei der höchste Prozentsatz (41%, 7 von 17 Tieren) auf die Feldmaus (*Microtus arvalis*) entfiel.

Tab. 4 Nachweis von Infektionen mit *Francisella tularensis* und Hantaviren in Wildnagern aus Sennickerode, Niedersachsen, 2005

Untersuchte Tiere		Erregernachweis			
Familie	Spezies	Tularämie - PCR		Hantavirus - RT-PCR	
		pos/ges	%	pos/ges	%
Muridae	*Apodemus flavicollis*	1/28	3,6	0/27	0
Cricetidae	*Myodes glareolus*	2/62	3,23	6/60	10,0
	Microtus arvalis	1/12	8,3	7/17	41,0
	Arvicola amphibius	6/37	16,2	n.d.	k.A.
k.A.	Andere*	0/5	0	0/30	0
Gesamt		10/144	6,9	13/134	9,7

n.d., nicht durchgeführt; k.A., keine Angaben; * *Sorex araneus, Apodemus agrarius*

3.3 Nachweis von Infektionen mit Hantaviren, Kuhpockenviren, Endoparasiten und Rickettsien bei Wildnagern vom Truppenübungsplatz Heuberg, Baden-Württemberg, 2007

Auf dem Truppenübungsplatz Heuberg wurden insgesamt 221 Tiere gefangen, darunter 98 Mäuse der Gattung *Apodemus*, 110 Rötelmäuse (*Myodes glareolus*), 10 Feldmäuse (*Microtus arvalis*) und drei Spitzmäuse.

Hantavirus-spezifische Nukleinsäure wurde mittels S-Segment-spezifischer RT-PCR bei 24 Tieren (10,9%) nachgewiesen (Tab. 5), am häufigsten bei der Feldmaus (*Microtus arvalis*) mit 50% (5 von 10 Tieren positiv), gefolgt von der Rötelmaus (*Myodes glareolus*) mit 19 von 110 Tieren (16%). Bei der Gelbhalsmaus wurden keine Hinweise auf Hantavirus-Infektionen gefunden (Essbauer et al., unveröffentlichte Daten).

Tab. 5 Nachweis von Infektionen mit Hantaviren, Kuhpockenviren, Endoparasiten und Rickettsien in Wildnagern vom Truppenübungsplatz Heuberg, 2007.

Untersuchte Spezies	Hantavirus RT-PCR		Endoparasiten makroskopisch		Rickettsien PCR		Rickettsien Serologie			
							anti-*R. conorii*		anti-*R. helvetica*	
	pos/ges	%	pos/ges	%	pos/ges	%	pos/ges	%	pos/ges	%
Apodemus sp[1].	0/98	0	1/98	1,0	1/98	1,0	11/98	11,2	0/98	0
Myodes glareolus	19/110	16,0	21/110	19,0	2/110	1,8	14/110	12,7	1/110	0,9
Microtus arvalis	5/10	50,0	1/10	10,0	0/10	0	0/10	0	0/10	0
Spitzmäuse[2]	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0
total	24/221	10,9	23/221	10,4	3/221	1,3	25/221	11,3	1/221	0,4

[1] *Apodemus flavicollis, Apodemus sylvaticus*; [2] *Sorex araneus, Sorex gemellus*

Bei einer Feldmaus konnte erfolgreich ein Orthopockenvirus angezüchtet werden, das gegenwärtig genauer charakterisiert wird (Meyer et al., unveröffentlichte Daten).

Endoparasiten wurden bei 23 von 221 Tieren (10,4%) angetroffen (Tab. 5), am stärksten war die Rötelmaus (*Myodes glareolus*) mit 19% (21 von 110 Tieren) betroffen (Essbauer et al., unveröffentlichte Daten).

Bei drei von 221 Tieren (1,3%) ließen sich Rickettsien mittels PCR nachweisen (Tab. 5), während 25 von 221 Tieren (11,3%) Antikörper gegen *R. conorii* und ein Tier (0,4%) Antikörper gegen *R. helvetica* gebildet hatten (Dobler et al., unveröffentlichte Daten). Dabei entfiel wiederum der größte Anteil positiver Tiere auf die Rötelmaus (*Myodes glareolus*) (14 von 110 Tieren, 12,7%), dicht gefolgt von *Apodemus sp*. (11 von 98 Tieren, 11,2%).

4. Diskussion und Ausblick

Erkrankungen des Menschen durch zoonotische Krankheitserreger haben in letzter Zeit in Deutschland erhöhte Aufmerksamkeit erlangt. Die Erfassung von humanen Infektionskrankheiten hat sich seit Einführung des Infektionsschutzgesetzes zwar insgesamt verbessert, aber gerade bei seltenen Zoonosen besteht ein

erhebliches Informationsdefizit. Als sehr hilfreich erweist sich hier insbesondere die öffentlich zugängliche Plattform „SurvStat“ des Robert-Koch-Institutes, die eine aktuelle Übersicht über die geographische Verbreitung und Häufigkeit meldepflichtiger Erkrankungen in Deutschland ermöglicht. Dennoch ist die Dunkelziffer der oft mit unspezifischen fieberhaften Symptomen einhergehenden Erkrankungen weiterhin als hoch einzuschätzen. Dies liegt zum einen an dem glücklicherweise vergleichsweise seltenen Auftreten dieser Erkrankungen und der damit verbundenen fehlenden Kenntnis über diese Erkrankungen innerhalb der Ärzteschaft. Desweiteren führt sicher auch die oft unspezifische und milde Symptomatik zu Fehldiagnosen und somit zu einem „underreporting. Insgesamt gibt es jedoch auch noch wesentlich zu wenige Untersuchungen zum Vorkommen seltener Zoonoseerreger beim Menschen und in den entsprechenden Wildnagerreservoiren.

Erste deutschlandweite Untersuchungen – wie die hier vorgestellten Untersuchungen - zeigen, dass Nagetiere in Deutschland in nicht unerheblichem Maße mit verschiedenen Krankheitserregern durchseucht sind (Ulrich et al., 2008a; hier vorgestellte Untersuchungen). So konnte beispielsweise eine breite geographische Verbreitung und ein stabiles Vorkommen des Puumalavirus in Populationen der Rötelmaus (*Myodes glareolus*) und des Tulavirus in *Microtus*-Populationen gezeigt werden (Ulrich et al., 2008a). Die hier beschriebenen Untersuchungen belegen diesen Befund für Gebiete in Niedersachsen und Baden-Württemberg. Diese Studien zeigen eine scheinbare Wirtspräferenz einzelner Zoonoseerreger für bestimmte Nagetier-Arten, die in weiterführenden Arbeiten zu prüfen wäre. So wurden Leptospiren, Borrelien und FSMEV besonders in der Gelbhalsmaus (*Apodemus flavicollis*), Francisellen vor allem in der Schermaus und Kuhpockenviren insbesondere in der Rötelmaus (*Myodes glareolus*) gefunden. Interessanterweise war die Rötelmaus auch in starkem Maße von Endoparasiten befallen, was möglicherweise auch die Durchseuchung mit anderen Erregern beeinflußt. Die hier zusammengefassten Erkenntnisse sind ein erster wichtiger Schritt auf dem Weg zur Erstellung von Verbreitungskarten für die genannten humanpathogenen Zoonoseerreger in ihren Reservoirwirten und der Definition von entsprechenden Risikogebieten.

Neben den genannten Forschungsaktivitäten muss die Öffentlichkeit auch für die Problematik der Nagetier- und anderen Vektor-übertragenen Zoonosen weiter sensibilisiert werden. In diesem Sinne sind einerseits die Gesamtbevölkerung, andererseits auch beruflich exponierte Risikogruppen, wie Forstarbeiter, Jäger, Beschäftigte in der Landwirtschaft und Soldaten auf die Gefährdungen hinzuweisen (Ulrich et al., 2007). Darüber hinaus müssen diese Personengruppen auch stärker mit den gegebenen- falls erforderlichen Vorsichts- und Hygienemaßnahmen vertraut gemacht werden.

Zusammenfassend ist festzuhalten, dass unsere Kenntnis von den Naturherden, den beteiligten Reseroirwirten und den entsprechenden Infektketten noch äußerst lückenhaft ist. Insbesondere zu den Ursachen für ein saisonal oder jährlich gehäuftes Auftreten bestimmter Humaninfektionen bestehen bestenfalls Arbeitshypothesen. Da an der Lösung dieser Probleme zwangsläufig Bereiche beteiligt sind, die weit über die Mikrobiologie hinausgehen, ist deren Bearbeitung nur in entsprechenden Netzwerken und Arbeitsgemeinschaften über Institutsgrenzen hinweg zielführend. Nur in einer synergistischen Zusammenarbeit von Zoologen, Ökologen, Virologen, Mikrobiologen, Parasitologen, Genetikern, Epidemiologen, Forstwissenschaftlern und Klimaforschern mit Klinikern der Human- und Veterinärmedizin können die komplexen Interaktionen zwischen Pathogenen, Reservoirwirten, Vektoren und Prädatoren im Zusammenhang mit dem Auftreten von Infektionen beim Menschen verstanden werden (Ulrich et al., 2008b).

Danksagung

Wir danken Frau Dr. Judith Kießling, Krystian Mistzela, Dr. Sonja Hartnack und den beteiligten Mitarbeitern des Instituts für Mikrobiologie der Bundeswehr für die tatkräftige Unterstützung bei der Beprobung der verschiedenen Punkte in Bayern, Sennickerode und dem Truppenübungsplatz Heuberg.

Herrn Prof. Hermann Meyer danken wir für die Übermittlung unpublizierter Ergebnisse und für die kritische Durchsicht des Manuskriptes.

Literatur

Dobler, G., Essbauer, S.S., Wölfel, R., Pfeffer. M. (2005): Interaktionen von Ökologie und Epidemiologie am Beispiel der Frühsommer-Menigoenzephalitis. Rundgespräche der Kommision für Ökologie, Bd. 29, „Zur Ökologie von Infektionskrankheiten“, Verlag Dr. Friedrich Pfeil, München, 43-52.

Essbauer, S.S., Friedewald, S., Hassler. D., Meyer. H., Pfeffer. M. (2006a): Von Ratten und Katzen auf den Menschen: Kuhpockenviren in Europa. DMW 131 (46), 2381-2382

Essbauer, S.S., Pfeffer, M., Wilhelm, S., Meyer, H. (2004): Zoonotische Pockenviren. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 47, 671-679

Essbauer, S.S., Schmidt, J., Conraths, F.J., Friedrich, R., Koch, J., Hautmann, W., Pfeffer, M., Wölfel, R., Finke, E. J., Dobler, G., Ulrich, R.G. (2006b): A new *Puumala hantavirus* subtype in rodents associated with an outbreak of severe *Nephropathia epidemica* in South-East Germany in 2004. Epidem. Infection 134, 1333- 1344

Fingerle, V., Wilske, B. (2006): Ticks, tick bites and how best to remove the tick. MMW Fortschr Med. 148(25), 30-2.

Jansen, A., Schöneberg, I., Frank, C., Alpers, K., Schneider, T., Stark, K. (2005): *Leptospirosis* in Germany, 1962- 2003. Emerg Infect Dis. 11(7), 1048-54.

Kießling, J. (2005): Untersuchung zum Vorkommen des Frühsommer-Meningo-Enzephalitis-Virus und *Borrelia burgdorferi* in ausgewählten Wildmaus- und Zeckenpopulationen Bayerns. Veterinärmedizinische Fakultät der LMU München, Dissertation

Krüger, D.H., Ulrich, R.G., Lundkvist, Å. (2001): Hantavirus infections and their prevention. Microbes Infect 3, 1129-1144

Palaniappan, R.U., Ramanujam, S., Chang, Y.F. (2007): *Leptospirosis: pathogenesis*, immunity, and diagnosis. Current Opinion in Infectious Diseases 20, 284-292.

Parola, P., Paddock, C. D., Raoult, D., 2005: Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev 18, 719-756.

Schönrich, G., Rang, A., Lütteke, N., Raftery, M.J., Charbonnel, N., Ulrich, R.G. (2008): Hantavirus-induced immunity in rodent reservoirs and humans. Immunol. Reviews (im Druck)

Sjöstedt, A. (2007): Tularemia: History, epidemiology, pathogen physiology, and clinical manifestations. Annals of the New York Acadamy of Sciences 1105, 1-29

Smythe, L.D., Smith, I.L., Smith, G.A., Dohnt, M.F., Symonds, M.L., Barnett, L.J., McKay, D.B. (2002): A quantitative PCR (TaqMan) assay for pathogenic Leptospira spp. BMC Infect Dis. 2, 13.

Splettstoesser, W.D., Mätz-Rensing, K., Seibold, E., Tomaso, H., Al Dahouk, S., Grunow, R., Essbauer, S.S., Buckendahl, A., Finke, E. J., Neubauerm H. (2007): Re-emergence of *Francisella tularensis* in Germany: fatal tularemia in a colony of semi-free living marmosets (*Callithrix jacchus*). Epidemiol. Infect. 135(8), 1256-1265

Süss, J., Scrader, C., Falk, U., Wohanka, N. (2004): Tick-borne encephalitis (TBE) in Germany – epidemiological data, development of risk areas and virus prevalence in field-collected ticks

and ticks removed from humans. Int J Med Microbiol 293 (Suppl 37), 69-79.

Ulrich, R.G., Heckel, G., Pelz, H.J., Wieler, L.H., 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., Hunfeld, K.P., Wölfel, R., Schares, G., Scholz, H.C., Brockmann, S., Pfeffer, M., Essbauer, S.S. (2008b): Nagetiere und Nagetier-assoziierte Krankheitserreger – das Netzwerk „Nagetier-übertragene Pathogene“ stellt sich vor. Bundesgesundheitsblatt 225,163-89.

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 9(1), 61-68.

Ulrich, R.G., Schlegel, M., Jacob, J., Pelz, H.J., Mertens, M., Schmidt-Chanasit, J., Wenk, M., Büchner, T., Masur, D., Sevke, K., Groschup, M.H., Gerstengarbe, F.W., Pfeffer, M., Oehme, R., Wegener, W., Bemmann, M., Ohlmeyer, L., Wolf, R., Zoller, H., Koch, J., Brockmann, S., Heckel, G., Essbauer, S.S. (2008a): Network „Rodent-borne pathogens“ in Germany: Longitudinal studies on the geographical distribution and prevalence of hantavirus infections. Parasitology Res. (im Druck)

Wilske, B., Fingerle, V., Schulte-Spechtel, U. (2007): Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol. 49(1), 13-21.

Wilson, D.E., Reeder, D.M. (2005): Mammal Species of the World. Johns Hopkins University Press.

Wölfel, R., Essbauer, S.S., Dobler, G. (2008): Diagnostics of tick-borne rickettsioses in Germany: A modern concept for a neglected disease. J Med Microbiol 298 (Suppl. 1), 368-374

Wölfel, R., Pfeffer, M., Essbauer, S.S., Dobler, G. (2006): Rickettsiosen in Einsatzgebieten der Bundeswehr – Aspekte zur Epidemiologie, Diagnostik und Therapie. Wehrmed. Mschr. 50(7), 185-187

XIV. ACKNOWLEDGEMENTS

First of all, I would like to thank PD Dr. Sandra Essbauer for supporting me not only technically, for giving me the opportunity to work on this thesis and also to attend many scientific conferences. Thank you for being a great mentor!

I also want to express my gratitude to Prof. Reinhard Straubinger for supervising this thesis, for constructive feedback and giving incisive advice.

Without the help of Dr. Jörg Müller, this work would not have been possible. Thank you for your immense support from the very beginning, for your help with field work and statistics and for being a friend!

Many thanks also to Dr. Claus Bässler and many more colleagues from the National Park Bavarian Forest Administration, for being always helpful and for the most entertaining Christmas and summer parties. Special thanks to Andreas Ebert for fabulous rodent photos!

I also wish to thank Dr. Stephanie Speck for very helpful advice and for providing most useful literature.

Dr. Julia Riehm, thank you not only for contribution to the publication but also for hosting cheerful lunch breaks.

Dr. Doblers technical support and willingness to take time to discuss my research is highly appreciated.

I wish to express my sincerest thanks to all the people that supported me in the field as well as in the lab, especially Daniela Eder, Robin Reiter, Mareike Pollaerts, Harald Weber, Rahime Terzioglu and many more. Thank you for being both a great help and entertaining company!

I also wish to thank Ricardo Castro César de Sá for great effort spent on the correction of this manuscript.

Finally, the deepest thanks go to my dear family for always supporting, encouraging and loving me. Without you, this dissertation would not have been possible.

édition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN: 978-3-8359-5736-7