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## Workshop Rodent-Borne Diseases

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### Ecology of Puumala hantavirus in Europe

**Heikki Henttonen<sup>1</sup>, Liina Voutilainen<sup>2</sup>, Eva Kallio<sup>3</sup>, Jukka Niemimaa<sup>1</sup>, Tarja Sironen<sup>4</sup>, Olli Vapalahti<sup>4</sup>**

<sup>1</sup>Natural Resources Institute Finland, Helsinki, Finland, Heikki.Henttonen@luke.fi

<sup>2</sup>Centre for Military Medicine, Helsinki, Finland

<sup>3</sup>University of Oulu, Oulu, Finland

<sup>4</sup>Virology, Medicum, University of Helsinki, Helsinki, Finland

Understanding the dynamics of zoonotic hosts and zoonotic pathogens in their reservoir host populations is a prerequisite for predicting and preventing disease epidemics. The infection risk of humans by Puumala hantavirus (PUUV) is high in northern Europe, where like in Finland bank voles (*Myodes glareolus*) undergo cyclic fluctuations. During 1995 - 2017 about 35,000 human cases of NE (nephropathia epidemica) were diagnosed in Finland. We analysed the annual NE incidence in various parts of Finland, from coastal areas to inland and from south to north, reflecting declining proportion of agricultural land and forest fragmentation. There was a trend for one year NE peaks in coastal and southern regions while two year peaks occurred inland, possibly indicating more restricted dispersal of host and virus in the increase phase in former ones. We review a detailed 7-year longitudinal capture-mark-recapture study, mostly at monthly intervals, on seasonal and multiannual patterns of the PUUV infection in the highly endemic area in Central Finland. Infected bank voles were most abundant in mid-winter months during years of increasing or peak host density. Seroprevalence of PUUV in bank voles exhibited a regular, seasonal pattern reflecting the annual population turnover and accumulation of infections within each cohort. In autumn, the PUUV transmission rate tracked increasing host abundance, suggesting a density-dependent transmission. However, prevalence of PUUV infection was similar during cyclic increase and peak years despite a twofold difference in maximum host density. This may result from the high proportion of young individuals carrying maternal antibodies in summer of the peak year delaying transmission during the cycle peak years. This increase/peak dilemma is reflected in the human NE incidence: even though the bank vole density is clearly higher in the peak year, the number of NE cases can often be similar or even higher in the increase year.

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### **Review of leptospirosis carriage in rodents worldwide and identification of key host species and knowledge gaps in the Asian-Pacific region**

**Vincent Sluydts<sup>1</sup>, Nyo Me Htwe<sup>2</sup>, Pyai Phyo Maw<sup>2</sup>, Sarathchandra Siriwardana<sup>3</sup>, Sudarmaji<sup>4</sup>, Grant R. Singleton<sup>5</sup>, Jens Jacob<sup>6</sup>**

<sup>1</sup>Evolutionary Ecology Group, Universiteit Antwerpen, Antwerpen, Belgium, vincent.sluydts@uantwerpen.be

<sup>2</sup>Plant Protection Division, Myanmar Agriculture Service, Yangon, Myanmar

<sup>3</sup>Entomology Division, Rice Research and Development Institute, Bathalagoda, Sri Lanka

<sup>4</sup>Assessment Institute for Agricultural Technology, Yogyakarta, Indonesia

<sup>5</sup>Crop and Environmental Sciences Division, International Rice Research Institute, Metro Manila, Philippines

<sup>6</sup>Institute for Plant Protection in Horticulture and Forests, Vertebrate Research, Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Münster, Germany

Some rodent species are well known to live in close proximity to human houses and storage facilities and can both consume and contaminate stored produce. Rodent borne diseases can also be transmitted to human and livestock through contaminated food and exposure to rodents feces and urine. Here we report on the epidemiological aspect of a joint research project; RAT-ADAPT (Rodent damage and transmission of rodent-borne zoonotic disease in households in Asian-Pacific territories). The project focuses on rodent-borne diseases and food security. In a first stage the SCOPUS database was screened with the keyword string "rodent\* OR rat\* AND leptospir\*" to identify the current state-of-the art knowledge on leptospirosis rodent reservoir hosts. Over 1,700 relevant English records over the past 20 years were found. These records were allocated to geographic regions using a text-mining approach and this information was combined with recent WHO maps on mortality and morbidity of leptospirosis worldwide to identify key knowledge gaps in the Asian-Pacific region. Grey literature reports and personal communication with health authorities in the region were used to complete the review. The primary rodent hosts were identified. Additionally, household surveys were conducted to gather farmers' knowledge on leptospirosis disease local rodent hosts. In a second stage and to fill some apparent knowledge gaps, rodent trapping and leptospira screening by PCR was carried out in selected countries of the Asian-Pacific region (Myanmar, Sri Lanka and Indonesia). These data will be used to identify the disease focal areas and implications of flooding on rodent host and disease epidemiology. Moreover, a better understanding of rodent dynamics will also assist in crop protection and conservation in the Asian-Pacific region.

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### **Leptospirosis in Madagascar: the epidemiology of multiple *Leptospira* species in diverse host communities**

**Sandra Telfer<sup>1</sup>, Mark Moseley<sup>1</sup>, Soanandrasana Rahelinirina<sup>2</sup>, Voahangy Soarimalala<sup>3</sup>, Steve Goodman<sup>3</sup>, Stuart Piertney<sup>1</sup>, Minoarisoa Rajerison<sup>2</sup>**

<sup>1</sup>University of Aberdeen, United Kingdom, s.telfer@abdn.ac.uk

<sup>2</sup>Institut Pasteur de Madagascar, Madagascar

<sup>3</sup>Association Vahatra, Antananarivo, Madagascar

*Leptospirosis* can be caused by a range of *Leptospira* species and is one of the most common, but neglected, zoonotic diseases in the world. Understanding the relative importance of different reservoir species and the distribution of infection across heterogeneous tropical landscapes is crucial for developing effective public health strategies. In a study of invasive and endemic small mammals inhabiting a forest-agricultural landscape in Madagascar, three of the four pathogenic *Leptospira* species found infected the invasive *Rattus rattus*, including species more commonly found in other sympatric hosts. Mixed infections were frequent, with strong evidence of facilitation between *Leptospira* species. Thus, due to their abundance and wide distribution, *Rattus rattus* could act as epidemiological bridges between forests and agricultural areas, as well as potentially enabling the evolution of new strains through recombination in coinfecting hosts. *Leptospira* infections were more common *Rattus rattus* from sites with more irrigated rice-fields. This pattern was largely driven by *Leptospira borgpetersenii* rather than *Leptospira interrogans*. Increased transmission of *Leptospira borgpetersenii* in rice-field dominated areas could be related to improved environmental persistence of leptospires, but could also be affected by the higher abundance of *Mus musculus* in these areas, a species with a high prevalence of intense *Leptospira borgpetersenii* infections. Thus, although *Rattus rattus* is a key reservoir host, other mammal species may play important roles in the complex epidemiology of leptospirosis.

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### **Bornaviruses as “novel” zoonotic pathogen**

#### **Martin Beer**

Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Südufer 10, Greifswald-Insel Riems, Germany, martin.beer@fli.de

Bornaviruses are known for a very long time in Germany and are named after the city of Borna in Saxony due to an outbreak of the “Borna disease” in horses. The causative agent is the classical Borna disease virus 1 (BoDV-1) which is most likely transmitted by shrews (*Crocidura leucodon*). Since 2015, four deaths of human encephalitis patients from infection by a novel squirrel bornavirus (variegated squirrel bornavirus 1; VSBV 1) were recorded in Germany. This unexpected incidence raised serious concerns about the zoonotic and pathogenic potential of VSBV 1 and related bornaviruses. The number of squirrels or humans currently infected with or exposed to potentially harmful bornaviruses is not known, and it is also unclear whether only squirrels can transmit such viruses or whether a reservoir in other hosts exists that needs to be identified. In addition, very recent findings also confirmed that the classical bornavirus BoDV-1 can induce lethal infections of humans. Therefore, more research data are urgently needed, and the BMBF-funded Zoonotic Bornavirus Consortium “ZooBoCo” started mid 2017 to provide data for a better understanding of the zoonotic potential of these mammalian bornaviruses, their distinctive features as well as their putative reservoir host species and the way of transmission. The consortium consists of members working in veterinary or human medicine, at universities, clinical research institutes and governmental institutions. Collaborations with international partners will also allow performing risk assessment studies in non-human primates. The main goal of this „one health“- approach is to provide a solid basis for improved public health measures and guidelines helping to identify and handle pathogenic bornaviruses and their reservoir species. This will be also a blueprint for effective measures and instruments concerning zoonotic infections originating from animal reservoirs like zoo and (exotic) pet animals, wild rodents or insectivores.

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### **The role of rodents in tick-borne viral diseases with special emphasis on tick-borne encephalitis**

**Gerhard Dobler**

Bundeswehr Institute of Microbiology, Germany, gerharddobler@bundeswehr.org

Tick-borne viruses are principally transmitted by ticks. However, beside ticks vertebrates play an important role for most tick-borne pathogens as reservoir hosts or as amplifying hosts. Tick-borne encephalitis (TBE) is the most important viral tick-borne disease in Eurasia. The tick-borne encephalitis virus circulates between ticks and several rodent species seem to play an important role as amplifying hosts. Rodents, mainly the bank vole (*Myodes glareolus*) and field mice (*Apodemus* spp.) play an important role for the amplification and sustaining of the natural transmission cycle. There is increasing evidence that TBE virus can be found in the brains of small rodents during winter time which let us hypothesize that the viral neurotropism of TBE virus in humans might be an erroneous ecological hibernating mechanism of TBE virus in a wrong host. The effect and impact of the amplifying host species on the pathogenicity of TBE virus remains to be clarified. Besides this, there is increasing evidence for a major role of rodents for the genetic stability of TBE virus. Multiple passages of TBE virus exclusively in ticks seem to degenerate the viral genome and cause extinction of the virus transmission cycle. These examples show that rodents beside a specific role as amplifying and reservoir hosts may play a more complex role in the viral natural maintenance cycle of tick-borne viruses.

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### Network “Rodent-borne pathogens”: looking into the rodent reservoirs

**Rainer G. Ulrich**

Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, and German Center for Infection Research (DZIF), Greifswald-Insel Riems, Germany, rainer.ulrich@fli.de

Rodents are important as pests in agriculture and forestry, as model organisms for biomedical studies and as pathogen reservoirs. These pathogens might be zoonotic and cause disease in domestic animals and humans. Alternatively, they might be rodent-specific and have no or still unknown zoonotic potential. The network “Rodent-borne pathogens” was established as a platform for an interdisciplinary collaboration of scientists working in mammalogy, ecology, genetics, immunology, toxicology, epidemiology, virology, microbiology, parasitology and human and veterinary medicine. The network was involved in the discovery of novel DNA and RNA viruses, some of them with potential to serve as models of human pathogens, such as bank vole hepacivirus, closely related to human hepatitis C virus or rat hepatitis E virus (HEV). Currently ongoing studies on the zoonotic variegated squirrel bornavirus 1 (VSBV-1) are dedicated to the identification of the geographic and host origin of this virus. Further studies of the network are focussed to pathogens in Norway rats from different habitats, including pest rats in zoos and from agricultural areas, and from breeding colonies. These studies detected high frequencies of infections with *Leptospira*, rat hepatitis E virus and *Rattus norvegicus* polyomavirus 1. Currently a workflow will be established including various pathogen-specific RT-PCR/PCR and serological assays, multiplex serology and open-view methods, including isolation approaches. A current interdisciplinary study within the RoBoPub consortium deals with the reservoir association and geographic distribution of Puumala orthohantavirus (PUUV) and *Leptospira* spp. in Germany and consequences of population bottlenecks on their molecular evolution. In conclusion, the network provides an important infrastructure for ongoing collaboration that is highly beneficial for interdisciplinary scientific work with high relevance in several fields of research. Additional targeted and non-targeted investigations within the network will help to increase our still very limited knowledge about the “virosphere” in rodents.

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### Who is the reservoir of Monkeypox? Work in progress

**Anne Laudisoit<sup>1</sup>, Erik Verheyen<sup>2</sup>, Mare Geeererts<sup>3</sup>, Tosca Van Roy<sup>3</sup>, Pascal Baelo<sup>4</sup>, Guy Crispin Gembu<sup>4</sup>**

<sup>1</sup>Ecohealth Alliance, New York, USA & University of Antwerp, Belgium, herwig.leirs@uantwerpen.be

<sup>2</sup>Royal Belgian Institute of Natural Sciences, Brussels, and University of Antwerp, Belgium

<sup>3</sup>University of Antwerp, Belgium

<sup>4</sup>Centre de Surveillance de la Biodiversité, Kisangani University, Democratic Republic of the Congo

Human Monkeypox is a disease that is known from central and west Africa and that is caused by the Monkeypox virus, an Orthopoxvirus. It is a zoonosis with symptoms similar to smallpox and increasing frequency of human-to-human transmission in central Africa. Two separate clades of the virus exist: the Congo basin clade with a mortality of about 15% and the west African clade that causes a milder disease. Human cases were frequently seen during localized outbreaks in DR Congo but in the last 12 months epidemics have been reported from Nigeria (where Monkeypox had not been reported since 1978), Central African Republic and Liberia. In none of these cases, the source of the virus or what caused the (re-)emergence of the disease was known. The natural reservoir is still unknown (despite the name, it is not primates) but an introduction of Monkeypox to the USA in 2003 was linked to the import of *Cricetomys gambianus* and *Graphiurus* sp. from Ghana. Also squirrels are often mentioned as potential hosts. Intensive field work in DRC, in areas where Monkeypox is endemic in humans but also in areas where it has not been reported, has yielded a number of sequences from different species of small mammals (rodents, shrews, bats, carnivores, ...) that showed traces of Orthopoxvirus DNA. Overall prevalence was between 10 and 20 percent. Yet no species stood out as one in which the infection is more common and the genetic distances between the observed viral material were not related to the phylogeny of the host. Similarly, a wide array of mammals have proven seropositive without pointing to a particular host species. Our screening work is currently continuing and by the time of the conference we hope to present more detailed results and to suggest working hypotheses for further research.

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### Optimal control model for rodent-borne leptospirosis in Salvador, Brazil

Hussein Khalil<sup>1</sup>, Amanda Minter<sup>2</sup>, Federica Costa<sup>3</sup>, Michael Begon<sup>1</sup>

<sup>1</sup>University of Liverpool, Liverpool, UK, hussein.khalil@liverpool.ac.uk

<sup>2</sup>London School of Hygiene and Tropical Medicine, London, UK

<sup>3</sup>Federal University of Bahia, Salvador, Brazil

Within the next three decades, two million people worldwide will live in urban slums, making up around 70% of the urban population in poor countries. Slum communities face a myriad of political, economic, and health challenges, yet often lack sufficient resources or central (governmental) planning to address them. In low to medium income countries, infectious diseases of poverty are common, including globally distributed zoonoses such as leptospirosis, which impart their greatest burden on the urban poor. In Brazil alone, 12,000 people annually are infected with *Leptospira*, a zoonotic bacteria carried and shed in urban settings mainly by brown rats (*Rattus norvegicus*). Few studies have evaluated the effectiveness of interventions, such as application of rodenticide or habitat modification. Rodenticides temporarily reduce rodent abundance while habitat modification such as closing of sewers reduces both carrying capacity of rat populations and survival of *Leptospira* in the environment. Given the scarcity of resources and need for intervention, it is important to evaluate the cost-effectiveness of different combinations of time-dependent intervention scenarios. We here present a mathematical model based on optimal control theory, using age-structured model for leptospire infection in a rat populations and parameters inferred from empirical studies in five slum communities in the city of Salvador, Brazil. An optimal control models optimizes time-dependent interventions through incorporating both the cost of an intervention and the cost of an infection. Our results suggest that habitat modification, despite being relatively more expensive; can reduce infection prevalence in rat populations. Continuous application of rodenticide reduces rat populations sufficiently to diminish human risk. The models presented here, using leptospirosis as a case study, can guide the optimal allocation of resources to reduce zoonotic risk.



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### Identification of potential endemic rodent hosts for zoonotic pathogens in South Africa using network analyses

Dina M. Fagir<sup>1</sup>, Ivan G. Horak<sup>2</sup>, Eddie A. Ueckermann<sup>3</sup>, Heike Lutermann<sup>1</sup>

<sup>1</sup>Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa, hlutermann@zoology.up.ac.za

<sup>2</sup>Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa

<sup>3</sup>School of Environmental Sciences and Development, North-West University, Potchefstroom, South Africa

The loss of biodiversity has been suggested to increase the risk of zoonotic spillovers of pathogens and parasites from wildlife posing a challenge to human and animal health. At the same time, predicting and identifying potential wildlife sources of zoonotic pathogens and/or parasites is difficult, particularly for poorly studied wildlife communities such as those of endemic rodents in many regions of Africa. Using field collections of ecological and parasitological data from eleven sympatric small mammal species (nine rodents, two insectivores) and their ectoparasitic arthropods (ten tick species, 14 mite species, 5 flea species and 4 louse species) we analysed network metrics including modularity and node centrality to evaluate the relative epidemiological importance of the rodent species captured in Telperion /Ezemvelo Nature Reserve, Gauteng Province, South Africa. Networks showed an intermediate level of nestedness but in both bipartite and unipartite networks two closely related murid rodents, *Micaelamys namaquensis* and *Aethomys ineptus*, emerged as sharing a large number of arthropod vectors with other rodents that also affect livestock and humans. These include ticks and fleas that may act as vectors for pathogens of medical (*Rickettsia conorii*, *Yersinia pestis*) and veterinary importance in the region (*Theileria parva*, *Anaplasma marginale*, *Babesia bigema* and *Ehrlichia bovi*). The two murids appear to be habitat generalists compared to other sympatric rodents and have been recorded as human commensals in parts of South Africa. As the geographic range of *Micaelamys namaquensis* includes most of sub-Saharan Africa and they are host to several *Bartonella* spp., including those of zoonotic concern, our findings suggest that this species may act as a source of zoonotic spillovers in the future. Thus, more detailed studies of its biology and pathogen/parasite community are need to prevent the emergence of zoonotic diseases from this host.

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### **An interdisciplinary approach to reduce leptospirosis in two slum communities in Salvador, Brazil**

**Ricardo L. Brito<sup>1</sup>, Whitney A. Howell<sup>2</sup>, Yeimi A. A. Lòpez<sup>1</sup>, Hussein Khalil<sup>3</sup>, Caio G. Zeppelini<sup>4</sup>, Ticiana Carvalho-Pereira<sup>1</sup>, Michael Begon<sup>3</sup>, Federico Costa<sup>1</sup>**

<sup>1</sup>Collective Health Institute of the Federal University of Bahia, Salvador, Brasil, [lustosaricardo@gmail.com](mailto:lustosaricardo@gmail.com)

<sup>2</sup>School of Public Health of the University of Michigan, Michigan, EUA

<sup>3</sup>Institute of Integrative Biology of University of Liverpool, Liverpool, England

<sup>4</sup>Institute of Biology of the Federal University of Bahia, Salvador, Brasil

One billion people worldwide reside in informal, slum communities, characterized by overcrowding, lack of infrastructure and basic sanitation, and inadequate access to potable water. Slum residents suffer disproportionately from infectious diseases including zoonosis. Leptospirosis, transmitted mainly by rats, is exacerbated by complex structural and tropical climate conditions that affect vulnerable populations. In low-and-middle-income countries, the majority of the slums lack support and action from the local and federal government agencies, in which community organizations fill the gap to address disparities within the current system. Our study aims to develop collaborative maps on a fine geographical scale using objective measures of *Leptospira* transmission risk, such as serological surveys and rodent trapping, which we combine with youths' assessment of the built and social environment. Perceived risk will be measured using participatory methods, such as Photovoice, to visualize and discuss communities' perceptions through the use of photography. To improve our spatial distribution of risk, we will integrate objective and perceived risk for human infection and infer relevant factors that were not originally considered. This collaborative effort can improve our assessment of risk through active engagement and dialogue with community members, who will also participate in the decision-making process to inform future local interventions.

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### **Assessing the effect of native forest replacement by exotic plantations on Andes hantavirus infection in wild rodents from central Chile**

**André V. Rubio<sup>1</sup>, Fernando Fredes<sup>2</sup>, Javier A. Simonetti<sup>1</sup>**

<sup>1</sup>Faculty of Sciences, University of Chile, Santiago, Chile, andre.rubio@gmail.com

<sup>2</sup>Faculty of Veterinary and Animal Sciences, University of Chile, Santiago, Chile

Land conversion for forestry/agricultural activities may increase hantavirus transmission in wild rodent hosts, sometimes creating conditions for outbreaks of hantavirus pulmonary syndrome (HPS) in humans. In Chile, a hantavirus strain named Andes virus (ANDV) causes HPS with mortality rate of 40%. One of the most important land-use change in central Chile is the replacement of native forests by exotic Monterey pine (*Pinus radiata*) plantations, which modifies the structure and species composition of rodent assemblages. Therefore, our aim was to study ANDV seroprevalence in wild rodents inhabiting native forest and pine plantations, and assess possible population and community parameters of small mammals that may influence ANDV infection. Rodents were sampled seasonally during 2016 and 2017 in a landscape that contains extensive stands of Monterey pine and interspersed remnants of Maulino forest, a native temperate forest from central Chile. Three types of habitats were sampled: native forest, adult pine plantation and young pine plantation. Blood samples were tested for antibodies against ANDV using a strip immunoblot assay. 1,630 blood samples from seven species were analyzed. Four species had seropositive samples and seropositive individuals were present across all sampling sites. ANDV seroprevalence in *Oligoryzomys longicaudatus* (the principal reservoir of ANDV) was significantly higher in native forest with an overall seroprevalence of 7.5%, compared to the other habitats in which the overall seroprevalence range from 0% to 2.8%. The abundance of the principal reservoir was the main predictor of ANDV infection. Our findings suggest that land conversion to Monterey pine plantations, including adult and young plantations would not increase ANDV risk exposure to humans.

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### **High disease transmission risk from occasionally synanthropic rodent reservoirs**

**Frauke Ecke<sup>1</sup>, Barbara A. Han<sup>2</sup>, Birger Hörnfeldt<sup>1</sup>, Hussein Khalil<sup>3</sup>, Magnus Magnusson<sup>1</sup>, Navinder Singh<sup>1</sup>, Richard S. Ostfeld<sup>2</sup>**

<sup>1</sup>Umeå, Sweden, frauke.ecke@slu.se

<sup>2</sup>Cary Institute of Ecosystem Studies, Millbrook, USA

<sup>3</sup>University of Liverpool, Liverpool, UK

The risk of transmitting pathogens from rodents to humans is determined by amongst others the contact zone in time and space. Transmission modes (indirect, direct) explain how pathogens are transferred to humans, but do not explain the conditions leading to the transfer of pathogens from rodents to humans. Here, we present the concept of "contact modes" between rodents and humans. We identified occasional synanthropy, viz. rodents occasionally entering human dwellings, as a major contact mode represented in 114 out of 216 reservoir rodent species and being a contact mode in 50 out of 67 zoonotic diseases spread by rodents. We revealed occasional synanthropy to be common in northern latitudes in North America, large parts of Europe, Russia and East Asia, while true synanthropy (species more or less exclusively found in and near human dwellings) to be more common in southern latitudes in South America, Africa and South-East Asia. Occasionally synanthropic rodents spend the majority of their life in other environments (e.g. forests, fields and wetlands). We analyzed intrinsic (e.g. population density) and extrinsic (e.g. weather conditions and food availability) factors potentially triggering occasional synanthropy. We discuss to which extent the timing of occasional synanthropy is predictable and how this knowledge can be used for alerting the public about transmission risk and for disease prevention and control.

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### **Dynamics of *Leptospira* and Tula orthohantavirus in small mammals: impact of landscape and biodiversity**

**Christian Imholt<sup>1</sup>, Kathrin Jeske<sup>2</sup>, Rainer G. Ulrich<sup>2</sup>, Jens Jacob<sup>1</sup>**

<sup>1</sup>Julius Kühn-Institut, Münster, Germany, christian.imholt@julius-kuehn.de

<sup>2</sup>Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

Rodents are important reservoirs for zoonotic pathogens that cause severe diseases in humans. Recent investigations revealed that factors like land-use and small mammal biodiversity have the potential to mitigate the underlying transmission processes on various levels. Biodiversity is hypothesized to be more closely related to pathogen prevalence on the population level, through direct mitigation of transmission rates while certain landscape features might also promote or hinder transmission. Although these factors are crucial to estimate potential future changes in human infection risk, they remain poorly understood. Here we present the first results of an ongoing large-scale field experiment. In Central Germany small mammals were trapped in 2017 and analysed for *Leptospira*, an ubiquitous bacterium, and *Tula orthohantavirus* prevalence in spring, summer and autumn. Trapping was conducted on woodlands and adjacent grasslands that differed in the intensity of land use. Prevalences ranged between 0-58% for *Tula orthohantavirus* and *Leptospira* prevalences reached 41% in *Microtus* species in autumn. First analysis revealed a strong impact of small mammal biodiversity on the growth rates of the common vole (*Microtus arvalis*) population. This study will generate a better understanding of natural pathogen-host dynamics, allowing public health recommendations and policy advice according to predicted future biodiversity scenarios.

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### **Temporal changes in rodent density and climatic factors as ecological drivers of tick-borne encephalitis (TBE) within a natural endemic foci**

**Valentina Tagliapietra<sup>1</sup>, Roberto Rosà<sup>1</sup>, Mattia Manica<sup>1</sup>, Heidi H.C. Hauffe<sup>1</sup>, Daniele Arnoldi<sup>1</sup>, Fausta Rosso<sup>1</sup>, Chiara Rossi<sup>1</sup>, Heikki Henttonen<sup>2</sup>, Annapaola Rizzoli<sup>1</sup>**

<sup>1</sup>Fondazione Edmund Mach, San Michele all'Adige, Italy, [valentina.tagliapietra@fmach.it](mailto:valentina.tagliapietra@fmach.it)

<sup>2</sup>University of Helsinki, Finland

Tick-borne encephalitis (TBE) is a severe neurological disease caused by the TBE virus (TBEV), a flavivirus transmitted mainly by the tick *Ixodes ricinus*. TBE has a patchy distribution in natural endemic hotspots. TBEV circulation in the natural environment occurs via three different modes of transmission: vertical transmission (from the infected female to the succeeding offspring), viraemic transmission (infectious animals are the source of the virus when ticks feed on them and vice versa) and non-viraemic transmission or co-feeding (NVT or COF, when ticks get infected while simultaneously feeding on susceptible or not susceptible/immune animals). NVT has been recognized as the most efficient so far. The rodent *Apodemus flavicollis* is the most important host in terms of supporting transmission of TBEV between feeding ticks. The co-occurrence of larvae and nymphs on rodent hosts is therefore essential for the NVT and is favoured by the seasonal synchronicity of their activity. We used a long term dataset from 2000 to 2014 on yellow-necked mouse (*Apodemus flavicollis*) population, feeding *Ixodes ricinus*, rodent TBE seroprevalence and climatic data to investigate the principal drivers of co-feeding ticks on rodents. In particular, climatic conditions (i.e. autumnal cooling) during the previous year of sampling affected COF occurrence in terms of ticks synchronicity. Larvae intensity, but not nymphs, was associated with rodent density only until a certain threshold, above which ticks bites on rodent hosts are wasted. Also individual features of rodents affected COF occurrence, i.e. heaviest males carried more COF groups and the overall number of COF groups positively affected TBEV infection prevalence in rodents the following year. In conclusion, climatic variables and rodent density could be used as early warning tools to determine the persistence of a TBE foci.